

Hepatitis B Virus and Liver Disease

Jia-Horng Kao
Ding-Shinn Chen
Editors

 Springer

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*To our mentor Professor Juei-Low Sung
Jia-Horng Kao
Ding-Shinn Chen
In memory of my parents Ping-Pei Chen
and Shiu-Chin Tseng Chen
By Ding-Shinn Chen
To my parents Shi-Yang Kao and
Wen-Shu Ho
By Jia-Horng Kao*

Foreword

This compact monograph represents a welcomed update on hepatitis B virus (HBV) and the disease it causes. The 17 chapters review the full spectrum of issues regarding HBV—its structure, molecular virology, replicative life cycle, immune pathogenesis, modes of transmission, epidemiology, natural history, complications, prevention, and treatment. Special chapters deal with the important issues of maternal-infant transmission; the disease in children, in immunosuppressed individuals, and in hepatitis C virus-, hepatitis D virus-, and human immunodeficiency virus-coinfected individuals; carcinogenesis; fibrosis progression; noninvasive means of assessment; and the needs for future basic and translational research. The authors are internationally recognized experts from Asia, Australia, the United States, and Europe, reflecting the global distribution and burden of HBV. Importantly, this book goes far beyond what can be covered in standard textbooks of medicine, hepatology, infectious diseases, and even virology.

There are several ways to view this monograph: a big book for a small topic, or a small book for a big topic, or perhaps both. For one thing, the book has two topics—one is small and one big—the hepatitis B virus (small) and the disease that it causes (big).

HBV is small. With its circular, partially double-stranded genome of only 3200 bases, it is the smallest known human DNA virus. This number of bases equates to about ten base pairs per page, or hundreds of words for each base. If HBV had a single open reading frame, it would produce a single moderately sized protein only. HBV, however, produces seven different polypeptides (pre-S1, pre-S2, small HBsAg, HBV polymerase, HBcAg, HBeAg, and X), each with a different structure and distinct function. In addition, the 7 HBV polypeptides contain far more amino acids than could be encoded by 3200 bases. How does this small DNA virus accomplish this big task?

The answer is that the HBV genome is small but efficient. The four open reading frames of HBV (S, C, P, and X) partially overlap each other, but produce different proteins because they are translated in different reading frames. The gene regions also have no introns. By frameshifting and not using introns, the same nucleic acid sequences can produce two or three different amino acid sequences, and each base pair in the genome can be used twice if not three times (particularly in view of the gene regulatory regions). In addition, some of the gene regions have several start sites so that polypeptides of different lengths are produced. The S gene region

possesses three start signals which allow it to encode three forms of HBsAg differing in their length and tertiary structure as well as their functions. The C region has two potential start sites. One start signal encodes the nucleocapsid core antigen (HBcAg) which serves as a structural component of the virus. The second C region start site includes a pre-core region and, after further posttranslational editing, produces HBeAg, a secreted, small molecular weight protein that circulates in the serum. The P region overlaps with the C, S, and X regions and produces a large multifunctional polymerase (both DNA and RNA dependent) and a separate ribonuclease activity. Finally, the small X region produces a polypeptide which is retained intracellularly and probably acts as a transcription factor. Each of the seven HBV polypeptides is essential; deletion of any of them results in a marked decline or termination of replication.

Thus, HBV is small in size but versatile in function and complex in structure. It also has a unique replicative strategy – through an RNA intermediate. Currently, the reasons for the complexity of structure and replicative cycle remain only partially understood. Why does HBV produce such excessive amounts of HBsAg that circulate as incomplete, non-virion forms in microgram amounts during acute and chronic infection? What is the function of HBeAg that circulates in patients with HBV infection with high levels of viral replication and seems necessary to produce chronic infection but not necessarily to sustain it? How does HBV blunt or circumvent the host innate and adaptive immune response to its presence? With its compact structure and multistep replicative cycle, how and when did this virus arise during human evolution?

In contrast to the virus itself, the disease that HBV produces in humans is a very large topic. When HBV was first discovered in the late 1960s, chronic infection with hepatitis B was found to affect 5–10% of the earth's population and to be the major cause of cirrhosis and hepatocellular carcinoma worldwide. Virtually, every human population, even those in the most remote areas of the world, harbored evidence of HBV infection. In China and Southeast Asia, with the highest rates, more than 200 million persons were believed to be chronically infected. In these areas, HBV was the most frequent cause of chronic liver disease and cirrhosis. In these areas and worldwide, hepatocellular carcinoma, the most dreaded long-term consequence of chronic HBV infection, ranked among the most common causes of cancer death. At that time, there was no means of prevention or treatment of this disease. This is not changed: all as a result of the discovery of HBV and the rapid subsequent advances in diagnosis, prevention, and now treatment.

The discovery of HBV was a major milestone of twentieth-century medicine. Quite aptly, Baruch Blumberg, the discoverer of the Australia antigen, which was later found to be the surface antigen of HBV and named HBsAg, was awarded the 1976 Nobel Prize in Medicine. The global implications of this discovery were immense. Once the Australia antigen was linked to HBV, it was rapidly found to be a reliable diagnostic marker for infection leading to means of screening donor blood and elimination of posttransfusion hepatitis B. More importantly, HBsAg could be purified in high quantities from serum, inactivated by heat and chemical treatment and used as the first, effective vaccine against this disease. Recombinant vaccines

(the first in humans) then followed and are now sensibly priced and used worldwide. Therapies for HBV followed means of prevention, but have now become clearly integral to any attempt to eradicate this disease worldwide. Current oral nucleoside analogues are highly effective in suppressing HBV replication and induce clinically significant remissions in disease in almost all patients. The combined effects of vaccination and treatment have begun to have major effects on the global burden of this disease. Eradication of HBsAg and all evidence of HBV replication by therapy is still limited, but new insights and innovative approaches are now zeroing in on this next step in HBV control.

These considerations make this small, compact monograph a welcome addition to our understanding of this small, compact virus and the very important disease that it causes.

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Preface

Hepatitis B virus (HBV) was identified more than 50 years ago, and it was soon found that the infection is among the most frequent and important in humans. It causes a wide spectrum of liver diseases, spanning from fulminant hepatitis to cirrhosis and hepatocellular carcinoma. In the last couple of decades, the understanding of HBV infection, especially the management of chronic infection, has evolved drastically. The pathogenesis of this virus has become clearer after basic, clinical, and epidemiological studies. More constructively, the infection can now be prevented effectively, and the chronic infection can be suppressed efficiently, shedding light at the end of the tunnel toward the elimination of HBV infection.

However, the rapid progresses are still not well taken by many people in the medical profession. And thus, it is timely and necessary to have a monograph on this subject. We edited a book *Hepatitis B Virus and Liver Disease* which is published by Springer Science + Business Media Singapore Pte Ltd. We aimed to provide a comprehensive, state-of-the-art review of HBV infection and liver disease.

The book updated the results of basic and translational medicine including hepatitis B viral life cycle, immunopathogenesis of HBV-induced chronic liver disease, viral and host genetic factors affecting disease progression, molecular mechanism of HBV-induced hepatocarcinogenesis, and the clinical implications. The clinical aspects of chronic HBV infection were elucidated by experts in epidemiology, natural history, hepatitis B vaccination, coinfection with hepatitis C or D viruses and human immunodeficiency virus, and management of special populations like children, pregnant women, and those under immunosuppressive therapy. The implications of occult HBV infection were also discussed. Finally, the advances and perspectives in the development of novel treatments for the cure of HBV infection were included.

We hope this book can serve as a useful resource for students, health-care providers, and researchers who are interested in the management and study of patients with hepatitis B.

Taipei, Taiwan
Taipei, Taiwan

Jia-Horng Kao, M.D., Ph.D.
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Darren J. Wong and Stephen A. Locarnini

Abstract

The hepatitis B virus is a prevalent human infection with no cure at present. It is a significant cause of global morbidity and mortality and has achieved its persistence in humans via its complex life cycle and ability to use its few protein products in a multifunctional manner to subvert and evade immune detection and clearance. These aspects of the virus are discussed in detail, as are the development of clinically important mutations in the viral genome that develop as a result of host immune selection, as well as those selected by the introduction of antiviral therapy or vaccination.

Keywords

Hepatitis B • Molecular Virology • Lifecycle • Antiviral Resistance

1 Classification

The hepatitis B virus (HBV) is the most well-known member of the virus family *Hepadnaviridae*. The species in this family are split amongst two genera—*Avihepadnavirus* and *Orthohepadnavirus*—with human HBV belonging to the latter. HBV has been further classified into ten genotypes, A to J, which are based

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on whole-genome nucleotide diversity of at least 8% (Norder et al. 1994; Stuyver et al. 2000; Kann and Gerlich 2005; Tatematsu et al. 2009; Olinger et al. 2008). There is distinct geographical variation amongst these genotypes (Table 1.1).

Table 1.1 Overview of the ten genotypes of HBV. Adapted from Locarnini et al. (2013). Note that genotype J has yet to be verified, as only one case has been isolated

Genotype	Subtype	Genome length (nt)	Frequency of mutation ^a		Global distribution
			PC	BCP	
A	A1	3221	Uncommon	Common	Africa, Asia
	A2				Northern Europe, North America
B	B1	3215	Common	Uncommon	Japan
	B2				Rest of Asia
	B3				Indonesia, China
	B4				Vietnam, Cambodia
C	C1	3215	Common	Common	Far East
	C2				Far East
	C3				Polynesia
	C4				Australian indigenous population
	C5				Philippines
D	D1	3182	Common	Common	Europe, Middle East, Egypt, India, Asia
	D2				Europe, Japan
	D3				Europe, Asia, South Africa, USA
	D4				Australia, Japan, Papua New Guinea
E		3212	NK	NK	Sub-Saharan Africa, UK, France
F	F1	3215	Uncommon	NK	Central and South America, Bolivia
	F2				Brazil, Venezuela, Nicaragua
	F3				Venezuela, Panama, Columbia
	F4				Argentina, Bolivia, France
G		3248	Very common	NK	USA, Germany, Japan, France
H		3215	NK	NK	USA, Japan, Nicaragua
I		3215	–	–	Vietnam, Cambodia
J		3182	–	–	Japan

Modified from Locarnini et al. (2013)

^aPC, precore mutations such as G1896A; BCP, basal core promoter mutations such as A1762T, G1764A; very common (most isolates); common (up to 50% of isolates); uncommon (less than 10% of isolates); NK, not known

Several of the genotypes (A through D, and F) can be further subclassified into subgenotypes, which are defined by a nucleotide diversity of between 4 and 8%.

The relevance of genotype classification emerges in clinical practice, where there are notable differences in pathogenic potential, and response to immunomodulatory antiviral therapy (Funk et al. 2002; Kao 2002). Genotype D is more pathogenic than genotype A2 and less responsive to interferon therapy, whereas genotype C is more pathogenic than genotype B (Kao and Chen 2003; Chan et al. 2002a, b; Orito et al. 2001).

2 Structure of the Hepatitis B Virus

2.1 Viral and Subviral Particles

Three types of viral particles are present in the blood of persons infected with HBV. The whole virion is the classical Dane particle, which is spherical in shape with a diameter of 42 nm. Virions have two layers—an outer envelope, coated with hepatitis B surface antigen (HBsAg) proteins—and an inner nucleocapsid, composed of dimers of hepatitis B core antigen (HBcAg). HBcAg exists as two distinct populations that exhibit T3 or T4 symmetry, consisting of 180 or 240 core proteins, resulting in sizes of 32 nm or 36 nm, respectively (Crowther et al. 1994). The nucleocapsid encloses the HBV genome and intimately associated, endogenous DNA polymerase. Two subviral particles are also present in the sera, both of which are composed solely of HBsAg and are not infectious. One is a smaller spherical structure of 17–25 nm diameter, and the other is filamentous, being approximately 20 nm in diameter and of variable length. The function of these subviral particles is not clear but is probably immune decoys.

2.2 Genome Organization and Viral Proteins

The genome of HBV is a circular, partially double-stranded, relaxed circular (RC) DNA molecule (Lok et al. 1994). The two DNA strands are held in a circular configuration by a 226 base pair overlap between the 5' ends that contain 11 nucleotide repeat sequences called DR1 and DR2 (Ganem and Schneider 2001). Genome length is between 3181 and 3221 bases, depending on the genotype. The minus DNA strand is not a closed circle and has a gap between its 3' and 5' ends, which is bridged by the plus DNA strand. The viral polymerase is covalently bound to the 5' end of the minus strand. The 5'-end of the plus strand is formed by an 18-base oligoribonucleotide, which is capped in the same manner as typical messenger RNA (mRNA), (Will et al. 1987). The 3' end of the plus strand is not at a fixed position, so there is single-stranded gap region of variable length, which can be from 20 to 80% of total genomic length, which can be filled in by the endogenous viral DNA polymerase. The minus strand encodes the complete viral genome and contains four overlapping open reading frames (ORF). The longest ORF encodes the viral

polymerase (Pol). The envelope ORF (Pre-S1, Pre-S2 and S) overlaps entirely with the Pol ORF, whilst the precore/core (Pre-C/C), and the X ORFs overlap partially with it. The ORFs overlap in a frameshifted manner, meaning that the virus strand is read approximately one and one-half times during transcription.

The transcriptional template of the virus is the covalently closed circular (ccc) DNA, which exists in the cell nucleus as a viral minichromosome. From this template, four mRNA transcripts are produced of varying kilobase (kb) length—the pregenomic (3.5 kb), the pre-S1 (2.4 kb), the pre-S2/S (2.1 kb) and the X (0.7 kb). Each transcript has its expression controlled by a different gene promoter, namely, the enhancer II/basal core, large surface antigen (Pre-S1), major surface antigen (S) and enhancer I/X, respectively (Ganem and Schneider 2001).

2.2.1 Pol ORF

The Pol gene spans approximately 80% of the genome length, and its product, the viral polymerase, is translated from pregenomic RNA. The polymerase is a 90 kDa, multifunctional protein that has four main domains required for viral replication (Ganem and Schneider 2001).

1. The N-terminal domain is known as the terminal protein and is responsible for linking the polymerase to the 5' end of the minus DNA strand. The terminal protein is also required for priming of minus strand synthesis. This is achieved by provision of a tyrosine substrate at amino acid 96 of the polymerase protein, which covalently binds to the first nucleotide of the minus strand DNA (Zoulim and Seeger 1994).
2. The second domain is the spacer or tether region, and has no specific known function, but overlaps pre-S1 and pre-S2.
3. The third and largest domain is the typical viral polymerase, responsible for RNA- and DNA-dependent reverse transcription. Reverse transcriptase activity is conferred by the essential YMDD sequence motif in domain C (Schlicht et al. 1991).
4. The fourth, C-terminal domain has ribonuclease H activity, which cleaves the RNA-DNA hybrids formed during the process of reverse transcription. It also has a role in viral packaging and in optimizing priming and elongation of minus DNA strand synthesis.

2.2.2 Pre-C/C ORF

The Pre-C/C ORF encodes two main protein end products: the core protein (HBc) P21, which is the major polypeptide of the nucleocapsid and expresses the HBV core antigen (HBcAg), and the soluble early antigen (HBeAg). The HBc protein varies in length depending on viral genotype and is either 183, 185 or 195 amino acids long. Upstream of the core ORF is a short, in-phase highly conserved ORF called the pre-core region, from which HBeAg is synthesized (Ganem and Schneider 2001).

HBc Protein

The HBc protein has two distinct domains. The first extends from amino acid residues 1–144 and is required for the assembly of the 32-nm nucleocapsid. The second

commences from approximately position 140 to the C-terminus and is rich in arginine clusters, forming a protamine-like domain that mediates nucleic acid binding and is involved in viral encapsidation and DNA replication (Ganem and Schneider 2001). This region includes a putative nuclear localization sequence. The core protein contains many hydrophilic and charged amino acids and becomes phosphorylated when expressed (Kann and Gerlich 2005). Phosphorylation of serine 170–172 between arginine cluster 3 and 4 may block nucleic acid binding, which negatively regulates nuclear localization of the core protein (Kann and Gerlich 2005). The HBc protein is translated from pregenomic RNA and carries the HBcAg epitopes, which are cross-reactive with the HBeAg epitopes (discussed below).

The HBc protein may also play an active role in regulating viral transcription. This hypothesis emerged following experiments showing that HBcAg aggregates in different cellular compartments in different phases of infection (Chu and Liaw 1987). The immunotolerant phase, in which high HBV DNA levels are seen, is associated with a predominant nuclear distribution of HBcAg. Conversely, in the immune clearance phase, HBcAg becomes predominantly cytoplasmic, in conjunction with decreasing viral load. Subsequently, HBcAg becomes undetectable in the immune control phase. In addition, HBcAg exhibits preferential binding for HBV cccDNA and is able to modulate viral transcription by altering nucleosomal packaging (Bock et al. 2001). These studies demonstrate the multifunctional nature of HBV proteins.

The predominant epitopes of HBcAg presented to the host immune system are discussed below in conjunction with the HBe protein.

HBc Protein

The HBeAg is an accessory protein of the virus and is essentially a soluble secretory form of the HBc protein. As mentioned, the precore sequence is upstream of core, and translation produces the precore protein. This protein contains the entire core amino acid sequence plus an additional 29 amino acids at the N-terminal end (P25) (Ganem and Schneider 2001). The bulk of translated precore protein undergoes extensive post-translational modification in the endoplasmic reticulum (ER). The first 19 amino acids of the pre-translational precore protein form a signal that allows translocation into the ER lumen. This signal is then cleaved off by a host cell signal peptidase, leaving the precore protein derivative P22. P22 is then secreted through the ER and Golgi apparatus, with further cleavage of up to 34 amino acids from the C-terminal end. The resulting proteins are a heterogeneous population of approximately 14–17 kDa, serologically defined as HBeAg, and are secreted into the serum (Ganem and Schneider 2001). This extensive modification renders the HBe protein quite different in almost all aspects from HBc protein despite the primary amino acid sequences being almost identical. A quantity of precore protein does not reach the ER lumen, however, and thus exists in an unmodified form. Additionally, the P25 HBe protein also expresses a nuclear transport signal (Kann and Gerlich 2005), meaning forms of HBeAg of variable length are found in practically all compartments of the cell, as well as being secreted. HBeAg and HBcAg are cross-reactive at the B cell level (see below) (Milich 1988).

The HBe protein has functions that target various arms of the host immune system in an effort to subvert and evade detection and elimination. Therefore, whilst not essential for viral replication, it is essential for the establishment of persistent infection (Hadziyannis 1995; Hunt et al. 2000). Mutations in the precore sequence are found commonly during chronic infection, some of which result in reduced or absent HBe production (discussed further below). Study of these mutants reveals the importance of the HBe proteins in chronic infection. As an example, an HBeAg-negative mutant of the woodchuck HBV is infectious for newborn woodchucks but is unable to establish persistent infection (Chen et al. 1992). Similar scenarios have been described in human infection (Hadziyannis and Vassilopoulos 2001), with many possible mechanisms of action including:

- Functioning as an immune tolerogen in vivo (Milich et al. 1990)
- Slowing of HBV DNA replication in vitro by reducing HBcAg dimerization, thereby reducing encapsidation of pgRNA (Scaglioni et al. 1997)
- Possessing anti-apoptotic properties in vitro (Locarnini et al. 2005)
- Downregulating TLR-2 expression on hepatocytes and on monocytes and CD86 on Kupffer cells (Visvanathan et al. 2007)

The HBeAg has two B cell epitopes, one of which is linear (HBe1) and the other conformational (HBe2). The HBe1 epitope is found at core amino acid positions 76–89, and the HBe2 epitope is at positions 130–138 (Salfeld et al. 1989). Two immunodominant core epitopes (HBc) are localized between residues 74 and 83 (HBc1) (Salfeld et al. 1989), which is co-linear with HBe1, and to residues 107–118 (HBc2) (Colucci et al. 1988). Given this overlap, HBcAg and HBeAg are highly cross-reactive at the T cell and B cell level (Chisari and Ferrari 1995; Milich 1987). Two cytotoxic T lymphocyte (CTL) epitopes of HBc can be mapped to residues 18–27 (human leukocyte antigen HLA-A2 restricted) (Bertoletti et al. 1993) and residues 141–151 (HLA-A31 and HLA-Aw68 restricted) (Missale et al. 1993). Three T-helper cell (T_H) epitopes have been found on the core protein at amino acid residues 1–20, 50–69 and 117–131 (Ferrari et al. 1991).

2.2.3 Pre-S/S ORF

The HBsAg contains small (SHBs), medium (MHBs) and large (LHBs) proteins, all of which exist in two forms that differ in the extent of glycosylation. N-linked glycosylation and glucosidase processing through the ESCRT pathway are necessary for virion secretion (Kann and Gerlich 2005; Stieler and Prange 2014; Bruss 2007). Conversely, subviral particles are packaged and released by the endoplasmic reticulum and Golgi complex, a process which is not dependent on glycosylation status.

SHBs

The SHBs is the most abundant of all three HBsAg particles. It is 226 amino acids long and is found in both glycosylated and non-glycosylated forms. It is this component of the HBs protein that houses the major antigenic determinant of HBsAg, the *a* determinant or antigenic loop (AGL) (Carman et al. 1990). The AGL is a conformational loop that contains a high number of cysteine residues that cross-link

with each other, spanning amino acids 99–169 of SHBs. Given its complexity, and conformational mature, a crystal structure of HBsAg has not been described to date. The AGL is highly conserved amongst all known HBsAg isolates, as it is responsible for initiating viral entry to target hepatocytes, by initially interacting with its low-affinity receptor, the cell surface heparan sulphate proteoglycans (HSPG) (Sureau and Salisse 2013). The AGL has the subdeterminants *d* or *y* and *w* or *r*. Determinant *d* has a lysine at position 122, whereas *y* has an arginine; likewise, determinant *w* has a lysine at position 160, but *r* is an arginine (Okamoto et al. 1994). These antigenic subtypes elicit cross-protecting anti-HBs following immunization. The *a* determinant has been renamed the major hydrophilic region (MHR) and is considered the main neutralization domain for anti-HBs in the context of prophylactic (protective) vaccination.

MHBs

The MHBs is constructed of the entire SHBs sequence with the addition of a 55-amino acid N-terminal extension (Ganem and Schneider 2001). This extension is known as the pre-S2 domain and is a minor component of the virion. MHBs can be either single or doubly glycosylated but is not essential for virus assembly and release. The immunogenic epitopes are not conformationally dependent, as is the case for SHBs.

The central part of the pre-S2 domain carries its major antigenic epitope. The region between amino acids 3 and 16 has the ability to bind polymerized human serum albumin (Kann and Gerlich 2005). The significance of this binding is unknown but has been postulated to act as a possible co-receptor or to mask the immunogenicity of this epitope (Sobotta et al. 2000). The MHBs is considerably more immunogenic than SHBs at the B cell level (Milich et al. 1985), and pre-S2-containing HBs particles generated from animal cell lines have been used in some countries as a prophylactic vaccine (Tron et al. 1989).

LHBs

The LHBs has a further N-terminal extension to the MHBs protein of either 108 or 119 amino acids (depending on the subtype/genotype). This extension is known as the pre-S1 domain, meaning that LHBs contains all three domains—pre-S1, pre-S2 and S. It exists solely in glycosylated form and is more prevalent than MHBs in virions and filaments but less prevalent in subviral spheres. In the mature virion and in HBs particles, the pre-S1 domain is exposed on the surface, where it covers both the S domain and parts of the pre-S2 domain (Kann and Gerlich 2005). In contrast to MHBs, the LHBs are essential for infection and viral morphogenesis, as the pre-S1 region encodes the entry receptor-binding domain (see Sect. 3.1 below) (Ni et al. 2014; Urban et al. 2014). The N-terminal end of the pre-S1 domain is myristoylated at glycine 2, a process that is essential to enable the virus particle to interact with the cellular plasma membrane (Urban et al. 2014). Amino acids 2–48 of the pre-S1 sequence specifically interact with the high-affinity entry receptor and sodium taurocholate co-transporting polypeptide (NTCP) and has a highly conserved motif (9-NPLGF(F/L)P-15) that is crucial for binding. Residues 49–75 are also required for infection, but the precise function of this region is not known (Urban et al. 2014).

The LHBs has important antigenic sites for both B and T cells that appear to play critical roles in recovery from viral infection or protection from infection (Milich et al. 1986). The major immunogenic epitopes within the pre-S1 coding region are at amino acids 27–35, 72–78 and 95–107. LHBs is also highly immunogenic for T cells in humans, at residues 21–48 and 81–108.

2.2.4 X ORF

The X ORF encodes a 154 amino acid polypeptide of 17 kDa known as the HBx protein. This is the second accessory protein of HBV and is conserved across all orthohepadnaviridae. The expression of full-length HBx protein is a critical component of the infectivity process in vivo (Lucifora et al. 2011) but can be dispensed of in in vitro culture. HBx acts as a transcriptional transactivator of a number of viral and cellular gene promoters through direct interaction with transcription factors such as the RPB5 subunit of RNA polymerase II, TATA-binding protein and ATB and is also involved in the activation of signal transduction pathways, such as the Ras/Raf/MAP kinase cascade (Kann and Gerlich 2005). It is also involved in epigenetic modification of the cccDNA template, resulting in upregulation of transcription (Levrero et al. 2009). In brief, epigenetic modification controls how tightly wound chromatin is to structural histone proteins. More tightly wound chromatin is not translated, whereas the converse is true; histone acetylation loosens chromatin, but methylation tightens it. This provides a mechanism to regulate gene transcription without acting directly on the coding sequences or their promoters. HBx recruits histone acetyltransferase and deacetylase enzymes in a fashion designed to prevent hypoacetylation (Belloni et al. 2009). Additionally, HBx in vitro inhibits protein arginine methyltransferase 1 (PRMT1), which increases histone methylation (Benhenda et al. 2013).

Another major target of HBx is the ubiquitin-proteasome system (UPS), which is a conserved host cellular pathway responsible for protein ubiquitination and proteolysis (Minor and Slagle 2014). Ubiquitin tagging marks cytosolic proteins for degradation via the proteasome. Many enzymes and proteins contribute to the function of this pathway, but of interest in the context of HBx is the damaged DNA-binding protein-1 (DDB1) and the Cullin4 (CUL4) proteins. HBx has been shown to bind DDB1, integrating itself into the DDB1-CUL4 complex, thus allowing regulation of its function (Guo et al. 2014). The importance of this interaction with regards to HBV replication has been demonstrated in vitro and in WHV models (Hodgson et al. 2012; Tang et al. 2008; Leupin et al. 2005). A particular target of HBx utilizing the DDB1-CUL4 machinery is the structural maintenance of chromosomes (Smc) 5/6 complex (Decorsiere et al. 2016). Smc5/6 acts as a restriction factor that selectively blocks extra-chromosomal DNA transcription. By destroying this complex, HBx relieves this inhibition, thereby allowing productive HBV gene transcription from the minichromosome.

The HBx protein is also a cofactor in HBV-mediated hepatocellular carcinoma (HCC) (Koike et al. 2002). The exact mechanism(s) of the contribution to hepatic carcinogenesis is unknown, although HBx-associated transactivation activity leads

to alterations in cellular gene expression that contributes to transformation (Rossner 1992). Findings in support of this hypothesis include:

- HBx suppression of p53-mediated upregulation of microRNA pathways that enhance the growth and metastatic potential of tumours in a mouse HCC model (Esteller 2011).
- Studies of epigenetic changes show that HBx induces hypermethylation of genes with tumour-suppressing activity (Tian et al. 2013).
- Disruption of signalling pathways critical to maintenance of cellular homeostasis (Zhang et al. 2014).
- Abrogation of p53-dependent apoptosis and cell cycle checkpoint deregulation (Wang et al. 1995).
- HBx targeting of the 26S proteasome complex has been implicated in hepatic carcinogenesis (Minor and Slagle 2014; Huang et al. 1996; Hu et al. 1999), suppressing viral antigen processing, and consequently presentation, thereby assisting evasion of immune detection (Wieland and Chisari 2005).
- Truncated HBx can be produced from integrated HBV DNA sequences (Martin-Vilchez et al. 2011).

3 Viral Life Cycle

There are multiple key events in the complete viral life cycle, which are described below. Detail of the molecular biology of HBV replication has been thoroughly reviewed previously and will not be repeated here (Beck and Nassal 2007).

3.1 Attachment and Penetration

The first stage of infection involves binding of the virion to its receptor on the hepatocyte surface membrane, with subsequent penetration of HBV into the cell cytoplasm. The AGL of HBsAg attaches to hepatocyte-associated heparan sulphate proteoglycans (HSPG) as the initial step in attachment, leading to cellular entry (Fig. 1.1) (Schulze et al. 2007). This is a co-receptor step, facilitating approximation of the pre-S1 receptor-binding domains to the sodium taurocholate co-transporting polypeptide (NTCP), which is the entry receptor for HBV (Ni et al. 2014; Yan et al. 2014). The NTCP normally functions to maintain bile acid homeostasis via the enterohepatic circulation. NTCP is encoded by the human *SLC10A1* gene, which is found on the long arm of chromosome 14. The pre-S1 domain of the HBV envelope protein contains two regions within 75 amino acids at the N-terminal end, which are responsible for binding to NTCP (Ni et al. 2014). These sequences must be myristoylated to permit virion infectivity (Meier et al. 2013; Gripon et al. 1995). After binding, receptor-mediated endocytosis is responsible for the delivery of viral nucleocapsids into the cytoplasm.

3.2 Conversion of Genomic RC DNA into cccDNA and Transcriptional Activation of the Viral Minichromosome

The nucleocapsids are transported to the nuclear membrane, where they uncoat and deliver their genetic load into the nucleus (Rabe et al. 2003). The RC DNA is released and converted into cccDNA using host cell enzymes, resulting in the formation of the viral minichromosome. This is the template of HBV that is used for the transcription of all viral mRNA and is also responsible for viral persistence (Bock et al. 1994; Newbold et al. 1995)(Fig. 1.1). The first step in this conversion is

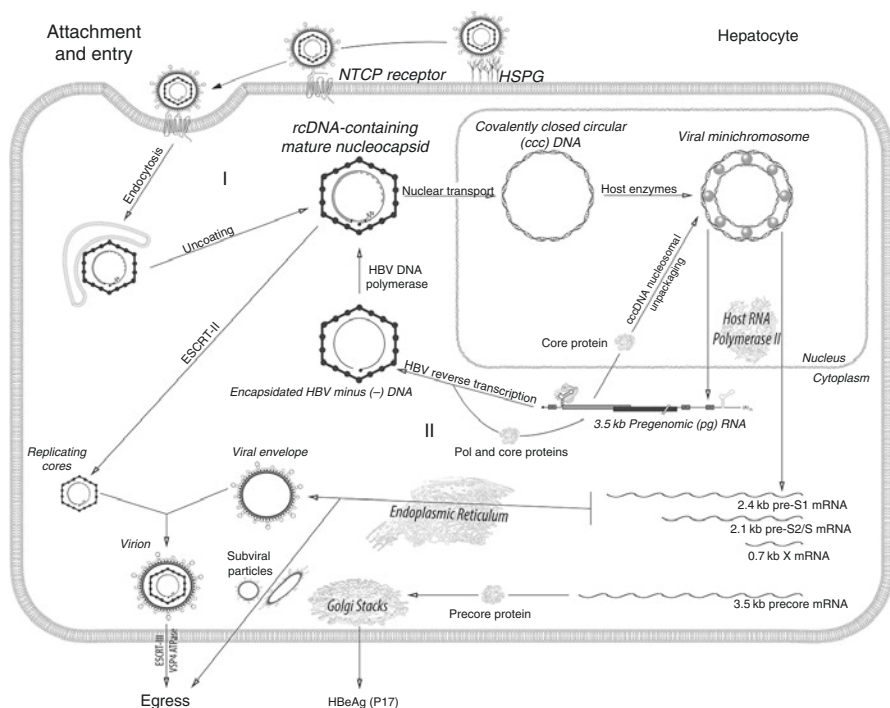


Fig. 1.1 Life cycle of HBV. Following attachment, penetration, and uncoating, the viral nucleocapsid is released into the cytosol and transported to the nuclear pore. The relaxed circular DNA is delivered into the nucleus where it is converted into cccDNA, and the viral minichromosome is generated. Transcription of the viral minichromosome produces the genomic and subgenomic HBV mRNA transcripts. Translation of the pregenomic RNA in the cytosol produces the Core and Pol proteins, and in association with Hsp-60, all are selectively packaged into a replication complex. Within the nucleocapsid, reverse transcription begins. The envelope proteins Pre-S1, Pre-S2 and S are translated at the rough endoplasmic reticulum (ER) and then bud into the lumen of the intermediate compartment. Approximately 50% of the Pre-S1-enriched ER-membrane areas envelope core particles. The HBV virions and subviral particles are then secreted into the extracellular space by usurping the cellular ESCRT pathway. The nucleocapsids can also be transported to the nucleus via an intracellular conversion pathway thereby increasing the number of nuclear cccDNA molecules

the release of the viral polymerase protein from the 5' end of the minus strand and is achieved by utilizing the host DNA repair enzyme tyrosyl-DNA-phosphodiesterase 2 (TDP2) (Koniger et al. 2014). The subsequent steps in cccDNA generation are yet to be elucidated but probably involve topoisomerase I and II and other host domain modification enzymes.

Genomic length and subgenomic length mRNA transcripts are synthesized from the HBV minichromosome utilizing host RNA polymerase II (Ganem and Schneider 2001). All transcripts are heterogenous, are of positive orientation and are capped at the 5' end and polyadenylated at the 3' end. The subgenomic transcripts function exclusively as mRNAs for translation of the envelope protein subunits and X protein. The two genomic transcripts are longer than genomic length and encode the precore, core and polymerase proteins. Generation of the pre-C/C mRNA is an early transcription/translation event and functions in translation of the precore protein, which is processed and then secreted as HBeAg (Ganem and Schneider 2001). It is not involved in reverse transcription. In contrast, the pregenomic RNA (pgRNA) is multifunctional, serving as the template for reverse transcription into the minus DNA strand and for translation of both HBcAg and HBV Pol (Fig. 1.1).

HBx production is needed to maintain the transcriptional activity of the HBV minichromosome, mainly by blocking its hyperchromatinization by Smc5/6 (see above).

3.3 Genomic Replication via Reverse Transcription

HBV genomic replication is initiated following packaging of the pgRNA together with newly translated viral polymerase into subviral core particles, forming replication complexes in the cytosol. Reverse transcription occurs within the HBV nucleocapsid. As the polymerase is being translated off the same pgRNA molecule that it will eventually be packaged with, its terminal protein domain specifically binds to the 'bulge' region of a unique RNA stem loop structure, known as the epsilon (ϵ) loop, at the 5' end of the pgRNA to prime reverse transcription (Bartenschlager and Schaller 1992) (Fig. 1.2). The epsilon loop is also the encapsidation signal around which cytoplasmic core protein dimers assemble into nucleocapsids. Following completion of translation, the polymerase undergoes a conformational change, which results in enzymatic activation, with the terminal protein domain now actively priming DNA synthesis (Zoulim and Seeger 1994). This Pol-oligonucleotide (Pol-G-A-A) complex then translocates to the complementary sequence of a direct repeat (DR-1) region located at the 3' end of the pgRNA. Minus DNA strand synthesis then continues until it reaches the 5' end of the pgRNA molecule (Will et al. 1987), generating a short terminal redundancy of approximately 8–9 nucleotides. Whilst reverse transcription is proceeding, the RNaseH activity of the polymerase degrades the pgRNA but leaves the 5'-capped terminal 18 nucleotides that contains the DR-1 sequence (Ganem and Schneider 2001). This fragment includes a six nucleotide homology to the direct repeat sequence, which allows circularization of the minus strand. The 18 nucleotide-capped RNA structure is then translocated to a second DR

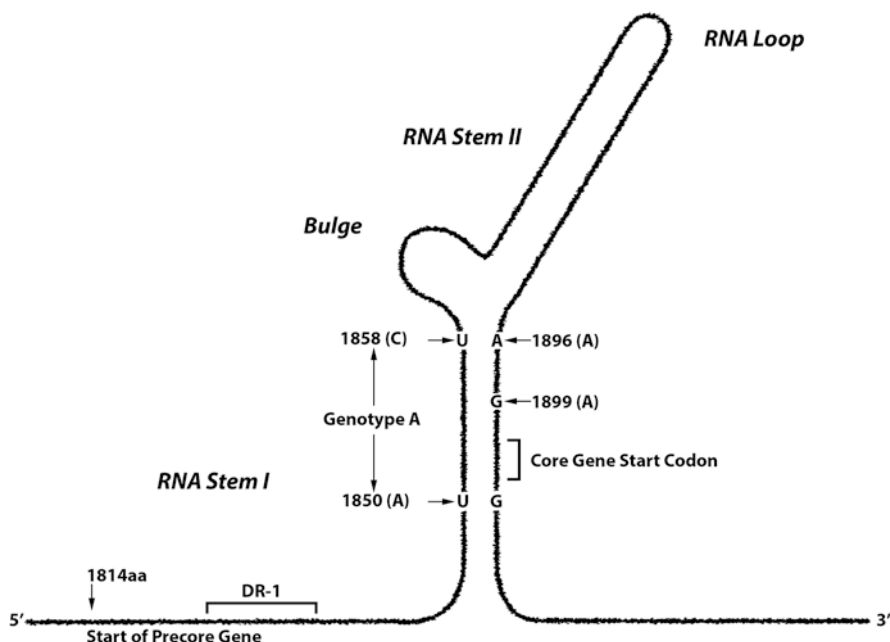


Fig. 1.2 Diagrammatic representation of the epsilon (ϵ) stem-loop structure of HBV. This is a highly conserved structure within the 10 genotypes of HBV, and the positions of base changes for genotype A2 are shown, as are the common translational precore mutations at G1896A (precore stop codon: UAG) and G1899A

sequence (DR-2) on the 5'-end of the newly made minus strand where it acts as primer for plus strand synthesis, using the minus strand as a template (Will et al. 1987). Synthesis of the plus strand continues until it reaches about 50–70% of the length of the minus strand.

3.4 Virion Assembly and Release

The viral envelope and HBsAg subviral particles are synthesized and assembled at the ER and then bud into its lumen. The HBc protein is synthesized in the cytosol and assembled independently of the envelope protein (Ganem and Schneider 2001). Immature nucleocapsids containing pgRNA-Pol can only commence envelopment once the nucleocapsid is phosphorylated, a process that is coupled to the initiation of minus strand synthesis (Ganem and Schneider 2001). The assembled nucleocapsids containing genomic RC DNA are then selectively enveloped before exiting the cell (Ganem and Schneider 2001). An export bias exists towards genomes that have completed minus strand synthesis and have started plus strand synthesis (Ganem and Schneider 2001). Envelopment of replicating cores requires a critical relative molar ratio of pre-S1 to S, without which abnormal virions result, hampering release (Ganem and Schneider 2001). As with many other enveloped viruses, HBV usurps

the endosomal sorting complex required for transport (ESCRT) network of proteins, which is a cellular network that functions to form multivesicular bodies and is involved in daughter cell cytokinesis (Stieler and Prange 2014; Bruss 2007; Votteler and Sundquist 2013; Lambert et al. 2007). Various components of this system have important roles in the HBV replication cycle. In particular, the formation of mature nucleocapsids requires the ESCRT-II protein (Stieler and Prange 2014), and virion budding and egress require ESCRT-III and the VPS4 ATPase (Lambert et al. 2007).

4 Variant Viruses

The replication strategy of HBV is error-prone due to a lack of proofreading capability. Additionally, all reverse transcription processes have an intrinsic G-to-A hypermutation rate largely attributable to host APOBEC enzymes (Noguchi et al. 2005). Accounting for approximations of daily base-pairing errors and the rate of virion production, it has been estimated that every 24 h, 10^{14} nucleotides are replicated with potentially 10^7 base-pairing errors (Sheldon et al. 2006). This rate equates to the potential for every nucleotide in the whole viral genome to be substituted daily. However, although mutations can occur randomly along the HBV genome, the overlapping open reading frames constrain the evolution rate, limiting the number and location of mutations that can result in viable virions. Because chronic HBV infection frequently persists for decades, many variants exist within the one host at any given time; the virus therefore exists as a quasispecies, with one variant typically dominating. There are three forms of variant HBV that are encountered commonly in clinical practice:

- HBeAg-negative mutants, which typically emerge after HBeAg seroconversion, are due to the development of mutations in the precore and basal core promoter regions.
- Polymerase mutants that emerge in the setting of nucleos(t)ide analogue (NA) therapy and are responsible for virological breakthrough and treatment failure.
- Envelope mutants, selected by hepatitis B immunoglobulin therapy in the post-transplantation setting, or by vaccines following prophylactic immunization.

4.1 Precore Mutants

The precore mutant results from a mutation at nucleotide position 1896 (codon 28: TGG; tryptophan) of the precore gene. A single-base substitution of G-to-A here gives rise to a translational stop codon (TGG to TAG; TAG = stop codon) in the second last codon (codon 28) of the precore gene located within the epsilon loop structure of the pgRNA. Ordinarily, the G1896 forms a base pair with nucleotide 1858 at the base of the loop. In HBV genotypes B, D, E, G and some strains of genotype C, the 1858 is a thymidine (T). Thus, the stop codon mutation created by G1896A (i.e., from T to A) stabilizes the structure based on conventional Watson-Crick pairing (Fig. 1.2). In contrast, HBV genotypes A, F and some strains of

genotype C have a cytidine (C) at position 1858, making the precore stop codon mutation rare in these genotypes. Other mutations have been found within the precore transcript that block HBeAg production, including abolition of the methionine initiation codon (Bartholomeusz and Schaefer 2004), and the development of stop codons at other positions within the precore/core region of genotype G, but these are far less common.

4.2 Basal Core Promoter Mutants

The basal core promoter (BCP) is the promoter for pre-C/C mRNA. Mutations within the BCP typically occur as a pair at nucleotides 1762 and 1764, resulting in a reduction in transcription rather than complete abolition (Hunt et al. 2000). These mutations, such as A1762T/G1764A, occur in isolation as well as in conjunction with precore mutations. The double mutation of A1762T plus G1764A results in a significant decrease in HBeAg levels and has been associated with an increase in viral load. This pattern of mutation is found in genotype A-infected individuals as the major cause of HBeAg loss (Hunt et al. 2000). A double mutant of BCP and precore is more common in the other, non-genotype A strains (Bartholomeusz et al. 2004). Importantly, these BCP mutations do not affect the transcription of HBV pgRNA or the translation of the core or polymerase protein. Thus, by removing the inhibitory effect of the precore protein on HBV replication, the BCP mutations appear to enhance viral replication by suppressing pre-C/C mRNA relative to pre-genomic RNA and may account for the increased pathogenicity of these mutant strains (Hunt et al. 2000). Longitudinal follow-up of a Taiwanese patient cohort infected with genotype B or C HBV confirmed this pathogenicity (Tseng et al. 2015). Patients infected with a BCP mutant virus were shown to have an independent risk factor for the development of cirrhosis. There also appeared to be a dose-response effect, with a significantly higher cumulative incidence if the BCP mutant population exceeded 45% of the quasispecies pool.

4.3 Polymerase Mutants

Drug resistance is associated with the loss of virological, biochemical and eventually histological therapeutic gain. In the setting of advanced liver disease, resistance may lead to hepatitis flares, hepatic decompensation and death (Liaw et al. 2004). Drug-resistance mutations also appear to be archived in the nuclear cccDNA template, highlighting the importance of prior antiviral therapy exposure when considering treatment, and first-line utilization of high-genetic barrier drugs (entecavir or tenofovir) (Zhou et al. 1999; Zoulim and Locarnini 2009).

Primary resistance mutations are those that directly alter drug binding and thus confer drug resistance. These mutations commonly result in reduced viral replicative ability when compared to wild-type virus (Zoulim and Locarnini 2009). Secondary resistance mutations arise in selected variants that have acquired primary resistance mutations and have the effect of causing a compensatory increase in viral replication levels.

4.3.1 L-Nucleoside Analogue Resistance

Primary resistance to lamivudine (LMV) and telbivudine (LdT) has been mapped to mutations in the YMDD locus in the catalytic domain of the HBV polymerase (Stuyver et al. 2001). These occur primarily at rtM204I/V/S $\pm$ rtL180M (Stuyver et al. 2001) and rtA181T/V (Yeh et al. 2000). The latter mutation confers cross-resistance to adefovir (ADV) and tenofovir (TDF) (Villet et al. 2008). Subsequent to these primary resistance mutations, multiple secondary resistance mutations can be selected for, which in combination may result in entecavir (ETV) resistance. When they occur individually, these secondary mutations do not significantly impact upon the clinical efficacy of ETV (Villet et al. 2007). The rtM204I substitution has been detected in isolation, but rtM204V and rtM204S are found only in association with other changes (Delaney et al. 2001). The major patterns of substitutions are (1) rtM204I, (2) rtL180M + rtM204V, (3) rtL180M + rtM204I, (4) rtV173L + rtL180M + rtM204V and (5) rtL80V/I $\pm$ rtL180M + rtM204I. Genotype influences the dominance of a particular mutation sequence (Zollner et al. 2004).

Resistance to LMV is a result of steric hindrance caused by the branched side group of valine or isoleucine amino acids colliding with the oxathiolane ring of L-nucleosides in the dNTP-binding site (Bartholomeusz et al. 2004). The in vitro sensitivity of such mutants to LMV decreases by between 100-fold and greater than 1000-fold. The rate of LMV resistance increases progressively with duration of therapy from between 14 and 32% per year (Lai et al. 2003). Whilst the rate of LdT resistance is lower than that seen with LMV, an exponential increase is still seen after the first year of therapy. Genotypic resistance was observed at 1 and 2 years of therapy in 4.4%/2.7% and 21.6%/8.6% in HBeAg-positive and -negative patients, respectively (Lok and McMahon 2009).

4.3.2 Acyclic Nucleoside Phosphonate Resistance

Resistance to ADV occurs less frequently than does resistance to L-nucleosides. The described mutations result in a three to eightfold increase in the IC₅₀ and confer partial cross-resistance to TDF in in vitro studies. This cross-resistance results from a similar molecular mechanism of resistance, with indirect perturbation of the triphosphate-binding site (Bartholomeusz et al. 2004). Mutations at rtA181T/V/S and rtN236T have been described (Angus et al. 2003). The rtN236T does not significantly affect sensitivity to LMV, LdT or ETV but reduces the in vitro efficacy of TDF (Angus et al. 2003; Brunelle et al. 2005). The rtA181T/V mutation is partially cross-resistant to LMV and LdT (Villet et al. 2008) and reduces sensitivity to TDF by threefold (Qi et al. 2007). The rtA181S mutation appears specifically to affect ADV (Liu et al. 2015).

Resistance to TDF in patients with HIV-HBV co-infection is conferred by the rtA194T mutation in combination with rtL180M and rtM204V (Sheldon et al. 2005). In vitro the presence of rtA194T results in partial resistance to TDF, but no cross-resistance with LdT or ETV. Additionally, these mutants had reduced replication efficiency compared to wild type, but this could be reversed by the introduction of either precore or BCP mutations (Amini-Bavil-Olyaei et al. 2009). Clinical studies have not confirmed an in vivo effect of this mutation. Further in vitro studies have shown a tenfold reduction in TDF sensitivity when the rtN236T and rtA181T/V mutations are found together. It should be noted that

these in vitro results show that TDF resistance is theoretically possible; however, there have been no reported cases of resistance in clinical practice due to these substitutions.

4.3.3 D-Cyclopentane Resistance

Genotypic resistance to ETV is uncommon in NA treatment naïve patients, seen in 1.2% of patients after 5 years of therapy (Tenney et al. 2009). However, patients with LMV resistance at commencement of ETV monotherapy develop resistance rapidly. This is because ETV resistance develops in a stepwise fashion, which means virus with substitutions in the YMDD domain need to develop fewer additional mutations to become ETV resistant (Villet et al. 2007). Therefore, ETV should not be used as monotherapy to treat LMV-resistant patients.

Mutations in the viral polymerase associated with the emergence of ETV resistance have been mapped to multiple domains in the viral polymerase. Thus, at least three mutations are required for resistance to develop, two of which are the YMDD substitutions rtM204V/I and rL180M (Tenney et al. 2004). In isolation, the rtM250V mutation causes a tenfold increase in IC_{50} , but no clinical resistance is seen (Levine et al. 2002). The molecular mechanism of resistance for the rtM250V change is exerted during RNA-directed DNA synthesis (Walsh et al. 2010). The mutation reduces ETV binding by shifting its target, the dNTP-binding site. The rtT184G + rtS202I ETV resistance-associated substitutions often occur together, but their in vitro effect is modest. The mechanism of resistance is an allosteric change with altered geometry of the nucleotide-binding pocket near the YMDD site (Walsh et al. 2010). A newly recognized mutation at rtA186T confers primary ETV resistance in combination with the LMV resistance substitutions (Hayashi et al. 2015). Importantly, ETV resistance-associated mutations are not cross-resistant with ADV or TDF.

4.3.4 Multidrug Resistance

Multidrug-resistant HBV occurs in patients who receive sequential NA monotherapy (Brunelle et al. 2005; Tenney et al. 2004; Fung et al. 2005; Mutimer et al. 2000; Yim et al. 2006). Such strains have been shown to develop if an ‘add-on’ therapeutic strategy to treat initial resistance to a single agent does not result in rapid and complete viral suppression (Zoulim and Locarnini 2009). Thus, incomplete viral suppression represents the most important risk factor for the development of antiviral resistance. The rtA181V/T mutation is a marker for multidrug resistance, as it is responsible for reduced susceptibility to both the L-nucleosides and the acyclic phosphonate nucleosides (Villet et al. 2008; Warner and Locarnini 2008).

4.4 Envelope Mutants

The current hepatitis B vaccine contains yeast-derived recombinant HBsAg. The subsequent anti-HBs response to the MHR of HBsAg between residues 137 and 148 induces protective immunity (Urban et al. 2014). Mutations within this

epitope have been selected during prophylactic vaccination (Carman et al. 1990) and following treatment of liver transplant recipients with hepatitis B immune globulin (HBIG) prophylaxis (Carman et al. 1996). Most vaccine-HBIG escape isolates have the substitution from glycine to arginine at residue 145 of HBsAg (sG145R) or aspartate to alanine at residue 144 (sD144A). The sG145R mutation has been associated with vaccine failure (Carman et al. 1990) and has been transmitted, resulting in persistent infection and disease. Similar vaccine-HBIG escape has been seen with the sP120T substitution, predominantly in the post-liver transplant setting (Bock et al. 2002). These two vaccine-HBIG escape mutants can also result in an HBsAg-negative phenotype. Clearly, this represents a significant risk of occult infection and reactivation, cases of which have been reported (Cheung et al. 2010). Such cases are fortunately rare. Other important mutations have been found at a MHC class I-restricted T cell epitope in HBsAg between codons 28 and 51 (Desmond et al. 2008).

Because of the complete overlap of the S ORF by the Pol ORF, mutants selected for during antiviral therapy can result in an altered HBsAg phenotype. The LMV resistance conferred by the rtV173L + rtL180M + rtM204V triple mutant results in two amino acid changes in HBsAg, sE164D + sI195M. This pair of envelope substitutions has a marked reduction in anti-HBs binding, similar to levels seen with the sG145R mutant (Matthews et al. 2006; Torresi 2002; Villet et al. 2006). Conversely, mutants selected by vaccine or HBIG-mediated immune pressure can result in alterations in the viral polymerase. As an example, sP120T and sG145R produce changes at rtT128N and rtW153Q, respectively. If these envelope variants develop in virus strains already resistant to LMV, replication fitness can be restored to wild-type levels (Torresi 2002).

Viral mutants that cannot synthesize pre-S1 and pre-S2 proteins have been found to occur frequently and are often the dominant virus populations in patients with CHB (Gunther et al. 1999). Pre-S1 mutants are associated with intracellular retention of the viral envelope proteins and the classical histological appearance of type I ground glass hepatocytes (GGH) (Wang et al. 2003). Type II GGH indicates the presence of pre-S2 mutants. The pre-S2 region overlaps the spacer region of the polymerase, which is not essential for enzyme activity; thus both envelope mutants replicate in their infected host but with differing histopathological sequelae. Presence of GGH has been associated with increased hepatocarcinogenic potential, in part attributable to a cellular growth advantage conferred by enhanced expression of vascular endothelial growth factor-A (Yang et al. 2009).

Conclusion

The complex life cycle of hepatitis B confers a significant survival advantage to the virus, particularly its ability to persist in the form of cccDNA and to evade immune elimination by secreting excess HBsAg. The nuclear location of this template provides a mechanism for reactivation and is a significant barrier to eliminating infection. As with any virus, it is able to mutate and adapt in response to factors that impact on its replication, from antiviral therapy and vaccination to attempts by the host immune system to affect clearance. Such mutations can

result in complex phenotypes with altered replication fitness and the ability to further evade detection. This can result in unsuspected progressive disease, including HCC, and highlights the need to develop new methods to progress towards a cure.

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Unmet Needs in Basic Research: In Vitro and In Vivo Models

2

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Abstract

Although hepatitis B virus (HBV) infection is preventable, there is a pressing need for new therapeutic approaches to treat the approximately 240 million people throughout the world with chronic HBV infection. Nucleoside analogs and peginterferon efficiently suppress HBV replication, but current HBV therapies are unable to clear the virus due to the long-term stability of HBV DNA present in the nucleus in the form of covalently closed circular DNA (cccDNA). The lack of suitable and practical in vivo and in vitro experimental models that accurately recapitulate key aspects of HBV infection in humans has complicated efforts to unravel HBV virology and hindered progress in development of new treatments. The identification of NTCP as the long-sought primary HBV receptor has helped to explain the species- and tissue-specificity of HBV infection as well as the rapid loss of infectivity of primary hepatocytes, and development of NTCP-overexpressing hepatoma lines has improved the usefulness of well-characterized cell lines in HBV research. At the same time, the development of new animal models, including tree shrew, macaque, and transgenic and chimeric mice have

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provided new in vivo models in lieu of the chimpanzee, as well as new sources of primary hepatocytes. New methods of improving primary hepatocyte culturing, including more structurally accurate 2D and 3D models and use of induced pluripotent stem cells, also begin to blur the line between in vitro and in vivo systems. Appropriate HBV model systems are now available to address a number of pressing research questions.

Keywords

Hepatitis B virus • HepaRG • HepG2 • NTCP • Human hepatocyte chimeric mice

Abbreviations

AAV	Adeno-associated virus
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
cccDNA	Covalently closed circular DNA
CPD	Carboxypeptidase D
DMSO	Dimethyl sulfoxide
HBsAg	HBV surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
iPS	Induced pluripotent stem
iPS-Heps	Differentiated hepatocyte-like cells
iPS-HPCs	Immature proliferating progenitor-like cell line
LHBsAg	Large hepatitis B surface antigen
NK cells	Natural killer
NOD	Nonobese diabetic
NTCP	Sodium taurocholate cotransporting polypeptide
uPA/SCID	Urokinase-type plasminogen activator/severe combined immunodeficiency

1 Introduction

Worldwide incidence of chronic hepatitis B virus (HBV) infection is estimated to exceed 240 million people. HBV is a preventable disease due to an effective vaccine that has been available since 1986, and even following exposure to the virus, most adults who develop acute HBV infection are able to clear the virus successfully. However, eradicating the virus in those individuals who develop chronic infections is difficult, and available treatment options focus on suppressing viral replication and preventing progression of liver disease rather than eliminating the virus

(Kwon and Lok 2011). Patients with chronic HBV may be treated with interferon or nucleoside analogs such as tenofovir disoproxil or entecavir, but long-term treatment is required to maintain viral suppression. Such long-term therapy is expensive and poses the risk of adverse events and emergence of escape mutants and drug resistance, whereas treatment discontinuation frequently leads to HBV reactivation. Current therapies act late in the HBV life cycle and are able to control but not eliminate HBV infection. Unlike viruses such as HCV that must replicate continuously in order to maintain chronic infection, HBV is able to persist in the liver almost indefinitely due to the presence of covalently closed circular DNA (cccDNA) that remains resident in the nucleus in a highly stable form and is periodically replaced through nucleocapsid recycling. Chronic HBV infection greatly increases the risk of hepatocellular carcinoma, and new therapeutic options are urgently needed, but the lack of suitable experimental models has long hindered development of new drugs and elucidation of the biology of the HBV life cycle. However, much of what is currently known about HBV was determined based on several in vivo and in vitro models. The advantages and disadvantages of these models will be reviewed below.

1.1 Hepatitis B Virus

Hepatitis B virus belongs to the genus *Orthohepadnavirus* in the *Hepadnaviridae* family. This genus includes three other viruses, the woodchuck hepatitis virus, the ground squirrel hepatitis virus, and the woolly monkey hepatitis B virus, but HBV is highly species-specific and is thought to fully infect only chimpanzees and humans (Wieland et al. 2004). Other viruses within the *Hepadnaviridae* infect rodents (*Orthohepadnaviruses*) and birds (*Avihepadnaviruses*). This pattern suggests an ancient origin for HBV, but differences in host receptors and patterns of cccDNA formation indicate important differences in the details of the life cycle among these viruses, limiting their usefulness as experimental models.

1.2 Woodchuck Hepatitis Virus

After the discovery of HBV, woodchuck hepatitis virus was the next member of the *Hepadnaviridae* to be characterized. Laboratory-maintained woodchuck colonies yield high rates of chronic HBV infection and develop hepatocellular carcinoma (HCC) (Tennant and Gerin 2001). As such, woodchucks have played an important role in the elucidation of HBV pathogenesis and the development of HCC, as well as an important model for drug toxicity (Allweiss and Dandri 2016).

1.3 Duck Hepatitis B Virus

Although distantly related to human HBV, the duck hepatitis B virus system successfully supports all stages of the HBV life cycle in duck hepatocyte tissue culture,

including the presence of cccDNA and DNA replication intermediates and production of HBc antigen and HBs antigen (Tuttleman et al. 1986). However, carboxypeptidase D (CPD) is critical for HBV binding in duck but not in human, indicating that host- and virus-specific differences must be taken into account (Spangenberg et al. 2001).

2 HBV In Vivo Experimental Models

Development of safe and effective therapies requires a suitable animal model that mimics HBV infection and response to therapy as closely as possible, and the lack of immunocompetent models has hindered progress in treatment of HBV. This difficulty stems from the high species- and tissue-specificity of HBV. While this suggests that factors specific to human hepatocytes are required for HBV entry and replication, it also complicates efforts to identify them. The advantages and disadvantages of several in vivo models are shown in Table 2.1.

2.1 Chimpanzee Model

Despite the practical and ethical drawbacks of their use, the chimpanzee (*Pan troglodytes*) has long remained an important model for HBV research as the only

Table 2.1 Advantages and disadvantages of in vivo HBV experimental models

Model	Advantages	Disadvantages
Chimpanzee	Fully infectious; immunocompetent; most similar to human infection	Ethical and practical limitations; potential differences in innate immune response
Macaque	Smaller, readily available, fewer restrictions; naturally occurring HBV infection; transferable	Not as well characterized as chimpanzee
<i>Tupaia</i>	Can be infected with patient sera	Transient, self-limited infection; animal handling difficulties
Transgenic mice	Consistent and well-characterized inbred lines; can achieve high replication rates	Does not support full HBV life cycle and not useful for analysis of drug resistance
Human hepatocyte chimeric mice	Supports full HBV life cycle; supports different host and viral genotypes; avoids confounding effect of adaptive immune response	Does not reflect adaptive immune response; animals are delicate and expensive
Hydrodynamic injection	Immunocompetent; can be used to analyze viral mutants	Transient expression
Adenovirus-mediated delivery	Persistent viremia	Low-level viremia; induces immune response
Adeno-associated virus delivery	Persistent viremia; useful for development of immune therapies	Suppresses immune response but can be overcome with agonist

primate fully susceptible to HBV infection (reviewed in Wieland 2015). Shortly after the virus was characterized, the HBV surface antigen (HBsAg), as well as antibodies against HBsAg, was detected in chimpanzee blood samples (Hirschman et al. 1969; Lichter 1969; Maynard et al. 1971), and it was soon shown that chimpanzees were susceptible to HBV isolated from human plasma (Barker et al. 1973). Chimpanzees may become infected with no more than one to three genome equivalents (GE) of HBV DNA (Komiya et al. 2008), leading to acute and chronic HBV infection similar to that of humans, including elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Tabor et al. 1983), although symptoms are typically less severe in chimpanzees than in humans (Barker et al. 1973). Importantly, the adaptive immune response in chimpanzees is similar to that of humans, making this animal model attractive for development of prophylactic and antiviral therapies. Chimpanzees played a central role in the development of the first-generation HBV vaccine, as well as in the testing of later recombinant vaccines (McAuliffe et al. 1980). While detailed analysis of the T-cell response in chimpanzee revealed a high degree of overlap in peptide-binding specificity between humans and chimpanzee (McKinney et al. 2000), analysis of gene expression during acute HBV infection failed to demonstrate induction of interferon-stimulated genes (ISGs), earning HBV a reputation as a stealth virus that initially remains undetected by innate immune surveillance (Wieland et al. 2004; Wieland and Chisari 2005). However, studies in other in vivo and in vitro models have begun to challenge this view, suggesting that HBV plays an active role in suppressing the innate immune response in humans (Shlomai et al. 2014; Luangsay et al. 2015; Yoneda et al. 2016) and indicating a potential difference between human and chimpanzee in the innate response to HBV infection.

2.2 Macaque Model

Efforts to find other primate model systems for HBV infection have largely been unsuccessful due to lack of susceptibility or persistence or inability to support serial passage. However, macaques (*Macaca fascicularis*) from Mauritius Island were shown to be positive for HBV DNA, HBsAg, and HBcAg with a strain of HBV closely related to HBV genotype D of probable human origin (Dupinay et al. 2013). This small Old World monkey might present an alternative to chimpanzees as a primate model for HBV infection (Bukh et al. 2013).

2.3 Tupaia Model

Although recently reclassified with respect to the primate lineage, tree shrews (*Tupaia belangeri*) are small squirrel-like mammals closely related to primates that can transiently support self-limited HBV infection in vivo, including from patient sera (Walter et al. 1996), and hepatocytes isolated from *Tupaia* can be infected with HBV or woolly monkey hepatitis B virus and secrete HBsAg and HBeAg (Walter

et al. 1996; Kock et al. 2001). As an in vitro HBV infection model, *Tupaia* played a critical role in the long-awaited identification of the primary HBV receptor, which was first identified in hepatocytes cultured from the tree shrew (Yan et al. 2012). In addition to HBV, tree shrews are also susceptible to herpes simplex virus 1 and 2 (Walter et al. 1996) and, along with chimpanzees, are the only experimental animal susceptible to both HBV and HCV infection, due in part to high sequence similarity with humans in the genes encoding the viral receptors NTCP (HBV) and CD81 (HCV) (Tsukiyama-Kohara and Kohara 2014). Although the tree shrew is a promising model for HBV infection, several problems with animal handling have so far limited wider adoption (Tsukiyama-Kohara and Kohara 2014).

2.4 Transgenic Mice

Use of inbred lineages is advantageous in the development of experimental systems because of limited understanding of the immunobiology of outbreeding organisms such as duck and woodchuck. To overcome this problem, transgenic mouse lineages were developed that expressed surface, core, precore, and X proteins, but these systems are limited by expression of only one viral gene at a time. Transgenic mice with linear constructs containing all gene products that can replicate in mouse hepatocytes were developed (Araki et al. 1989; Farza et al. 1988), but replication efficiency in these mice was low. To improve replication efficiency, transgenic mice bearing constructs with 1.3×-genome-length HBV sequences beginning with the region upstream of the X promoter and ending downstream of the shared polyadenylation site were developed and were able to achieve high HBV replication rates (10^7 – 10^8 copies per mL) without evidence of cytopathic effects. However, this approach prevents examination of the full HBV life cycle and cannot be used to study the effect of drug resistance. The mice have a high tolerance to the transgenic products, which makes them unsuitable for evaluating therapeutic vaccines, and the presence of integrated HBV DNA makes it difficult to evaluate the effects of treatment on viral clearance (Yang et al. 2014).

2.5 Human Hepatocyte Chimeric Mice

To overcome the host specificity of HBV, various attempts have been made to transplant human hepatocytes into mice. In trimera mice, human hepatocytes were transplanted into the kidneys of immunodeficient mice, but liver architecture was not established, and the repopulation rate was low, leading to low levels of viremia (10^5 IU/mL) even though the vast majority of mice (85%) became infected (Ilan et al. 1999). Improved replication rates were achieved by transplanting human hepatocytes into urokinase-type plasminogen activator/severe combined immunodeficiency (uPA/SCID) mice. Transgenic mice in which the urokinase gene is under the control of the human albumin promoter were mated with SCID mice (Heckel et al. 1990). These mice undergo liver failure due to accelerated

hepatocyte death, and the mouse liver is repopulated using transplanted human hepatocytes (Rhim et al. 1995), using the human albumin levels as a measure of the repopulation index. Mouse livers have also been successfully repopulated using *Tupaia* hepatocytes (Dandri et al. 2005). The human hepatocyte chimeric mouse model supports both HBV (Dandri et al. 2001) and HCV (Mercer et al. 2001) infections and serves as a model for drug metabolism. Although a small number of mouse hepatocytes remain, and efforts to remove residual cells using a herpes simplex virus type-1 thymidine kinase (HSVtk)/ganciclovir (GCV) system were unsuccessful (Douglas et al. 2010), several studies have shown that HBV retains infectivity after passage in mice and that HBV proteins are present in mouse livers but are not detectable in mouse hepatocytes (Tsuge et al. 2005; Meuleman et al. 2005; Sugiyama et al. 2006). The use of immunodeficient mice makes it possible to untangle host and innate immune interactions without the confounding influence of human adaptive immune cells. This model is particularly useful for evaluating therapeutic agents.

2.6 Immune-Competent Mouse Models

The nature of the adaptive immune response is essential to the outcome of HBV infection. Therefore, an immune-competent animal model is needed to study the immunology of HBV infection (Tzeng et al. 2013). Development of a system that can model chronic HBV in humans could be approached through hydrodynamic injection or through adenoviral-mediated delivery of HBV DNA.

2.7 Hydrodynamic Injection HBV Mouse Model

Hydrodynamic injection is a method for delivering genetic material into the liver by rapid injection of a large volume of DNA through the mouse tail vein, which increases the hydraulic pressure in the inferior vena cava and liver fenestrae, resulting in efficient passage through the hepatic vein and delivery to hepatocytes (Tzeng et al. 2013). Yang et al. demonstrated immune-mediated differences in HBV clearance in immunologically competent mice compared to immunocompromised mice through injection of a transposon delivery system containing greater than full-length HBV genomic DNA (pT-MCS-HBV1.3) into the tail vein of immune-competent mice as well as into nonobese diabetic (NOD)/SCID mice that lacked T cells, B cells, and natural killer (NK) cells (Yang et al. 2002). HBV gene expression was observed, and the virus was detected in the blood in both types of mice. Rapid disappearance of the virus coincided with appearance of CD8⁺ T cells in immunocompetent mice, but viral antigens remained detectable for up to 12 weeks in immunocompromised mice. Ketzinel-Gila reported similar results using a head-to-tail dimer of HBV serotype *adw* (pHBV*adw*HTD) and demonstrated suppression of HBV viremia after treatment with lamivudine or human monoclonal antibody (HBV-Ab17^{XTL}) (Ketzinel-Gilad et al. 2006).

2.8 Adenovirus-Mediated Delivery

An alternative delivery system to efficiently target genes to hepatocytes was developed in which HBV DNA was cloned into adenovirus or adeno-associated virus (AAV), resulting in persistent but low-level viremia (Tzeng et al. 2013; Bramson et al. 1995; Huang et al. 2006). While adenoviral delivery induces immune activation of cytokines, chemokines, and interferon-related genes, adeno-associated virus suppresses immune activation (Tzeng et al. 2013). Use of an improved recombinant form of AAV/HBV along with vaccination with HBsAg and the TLR9-agonist CpG as an adjuvant induced a strong T-cell and antibody response leading to suppression of viral replication and HBV clearance, providing a model system for development of immune therapies against HBV (Yang et al. 2014). Differences in clinical presentation, response to treatment, and risk of HCC among the ten characterized genotypes of HBV suggest that animal models should be able to support infection with various different genotypes of HBV (Liu and Kao 2013). In addition to HBV genotypes A and B (Huang et al. 2006; Li et al. 2013), a hydrodynamic infection model was recently developed for genotype C, the dominant genotype in Asia (pAAV-HBV1.3C) (Li et al. 2016). Although development of in vivo models for HBV infection has proven difficult, progress is increasingly being made toward the development of animal models that mimic most, if not yet all, aspects of human HBV infection.

3 HBV In Vitro Experimental Systems

While animal models are essential for unraveling the complex interactions between host and pathogen and in order to evaluate the safety and effectiveness of new therapies, a robust in vitro HBV infection model is also necessary in order to work out the molecular details of HBV infection and during the initial development of new antiviral drugs. Progress toward this goal has been hampered by the high specificity of HBV for human hepatocytes, due in part to uncertainty as to identify of the primary HBV receptor for HBV. Several systems have been developed that support various aspects of the HBV life cycle, but developing a model that supports the complete HBV life cycle with high titer has proven challenging. Advantages and disadvantages of several in vitro models are shown in Table 2.2.

3.1 Primary Human Hepatocytes

Fresh primary human hepatocytes are perhaps the gold standard for in vitro analysis of HBV infection (Shimizu et al. 1986; Gripon et al. 1988, 1993; Ochiya et al. 1989; Galle et al. 1994), but these cells are difficult to obtain, represent limited genetic variability, and quickly lose infectivity. Expression of hepatocyte-specific factors decreases over time, leading to phenotypic changes and a decrease in activity of cytochrome P450 and other liver-specific functions (Guillouzo et al. 2007; Wilkening and Bader 2003). Cellular metabolic activity slows, and cells fail to proliferate

Table 2.2 Advantages and disadvantages of in vitro HBV experimental models

Model	Advantages	Disadvantages
Primary human hepatocytes	Gold standard for in vitro analysis of HBV infection and analysis of drug toxicity	Difficult to obtain; limited genetic variability; rapid loss of infectivity
HepG2 cells	Produces HBV virions; polarized cells	Morphological and chromosomal differences from primary hepatocytes; do not support HBV entry
HepaRG cells	Supports replication of HBV and HDV; supports HBV entry	Requires time-consuming differentiation step; chromosomal differences
NTCP-expressing cells	Supports HBV entry and replication	Yield of HBs, HBe, and HBV DNA is low
Human hepatocytes isolated from chimeric mice	Benefits of human hepatocytes with larger number of available cells per donor; improved infectivity	Expensive; complex
2D/3D/microfluidic culture	More accurately reflect liver architecture and cell types	Complex; differences from in vivo
Induced pluripotent stem cells	Support HBV infection; innate immune response	Complex; heterogeneous; difficult to establish in culture

(Wilkening et al. 2003). These changes reduce HBV infectivity by reducing expression of liver-specific host factors required for HBV entry and replication as well as hepatocyte nuclear factors that bind to HBV promoter and enhancer elements and are required for transcription of the HBV genome, although treatment with dimethyl sulfoxide (DMSO) has been shown to improve infectivity of primary hepatocytes by promoting differentiation.

Cultures of primary hepatocytes from chimpanzees also support HBV infection but are also limited by availability and a short window of infectivity. However, primary hepatocytes from tree shrews (*Tupaia belangeri*) have also been shown to transiently support HBV infection (Walter et al. 1996) and played an important role in the identification of the HBV receptor (Yan et al. 2014). *Tupaia* hepatocytes also support infection with wooly monkey hepatitis B virus (Kock et al. 2001).

3.2 HepG2 Cells

To overcome the problems of limited availability and rapid loss of infectivity of primary hepatocytes, several hepatoma cell lines have been developed. The HepG2 cell line was derived from a well-differentiated hepatocellular carcinoma from a 15-year-old male. HBV DNA-transfected HepG2 cells are capable of producing HBV virions (Sells et al. 1987; Ladner et al. 1997), but HepG2 cells do not appear to support HBV entry even with addition of DMSO (Glebe and Urban 2007). Liver architecture and cell polarity is thought to play a role in HBV infection. In this respect HepG2 cells

serve as a useful *in vitro* model because they are polarized into basolateral and apical domains. However, HepG2 cells differ morphologically from primary hepatocytes by having a single nucleus with 48–55 chromosomes and epithelial morphology, whereas primary hepatocytes tend to have two nuclei and cubic morphology (Wilkening et al. 2003; Natarajan and Darroudi 1991). Gene expression also varies between HepG2 cells and primary hepatocytes. Both cell types express housekeeping genes at similar levels (Wilkening et al. 2003) and secrete plasma proteins and acute phase proteins including albumin, transferrin, fibrinogen, alpha 2-macroglobulin, alpha 1-antitrypsin, and plasminogen (Knowles et al. 1980). However, expression of C/EBP- α , CYP3A4, and other liver-specific transcription factors and enzymes is much lower in HepG2 cells (Rodriguez-Antona et al. 2002; Jover et al. 2001).

3.3 HepaRG Cells

Another hepatoma cell line, the HepaRG cell line, was derived from a hepatocellular carcinoma from a female with chronic HCV infection (Guillouzo et al. 2007). HepaRG cells are one of few cell lines able to HBV and HDV infection (Gripon et al. 2002; Hantz et al. 2009; Lucifora et al. 2010) and support HBV replication and secretion of infectious particles (Gripon et al. 2002; Hantz et al. 2009). However, HepaRG cells require a time-consuming differentiation step prior to becoming susceptible to HBV infection. HepaRG cells divide rapidly after seeding and maintain an elongated appearance. Differentiation into biliary-like cells and hepatocytes with one or two nuclei is triggered by addition of DMSO and hydrocortisone hemisuccinate. Removal of DMSO leads to reduced P450 expression (Kanebratt and Andersson 2008a), but expression of liver-specific transcription factors and transporters remains stable for 6 weeks or more at levels comparable to that of primary hepatocytes. However, as a hepatoma line, the cells contain an extra chromosome 7 and loss of the 12p region through a translocation between chromosomes 12 and 22 (Gripon et al. 2002). Albumin expression is variable among cells, and CYP3A4 and CYP7A1 expression is greatly increased (Kanebratt and Andersson 2008a, b).

3.4 HepCHLine-4 Cells

In order to combine the high infectivity of primary hepatocytes with the ability to culture hepatoma-derived cells, Jiang et al. created the HepCHLine-4 by fusing primary human hepatocytes with HepG2 cells (Jiang et al. 2009). HBs antigen, cccDNA, and viral particles were detected, and cells remained susceptible even after a year of subculturing.

3.5 Identification of the HBV Receptor

Although HBV is highly species-specific and hepatotropic, the chief hurdle to developing an experimental system remained the entry stage. A critical region

between amino acids 2 and 47 in the preS1 domain of the large S antigen (LHBsAg) was known to be involved in hepatocyte binding (Neurath et al. 1986), but the corresponding receptor on the hepatocyte membrane was unknown. CPD was found to serve a critical role in avian hepadnavirus infection but was not responsible for HBV entry in humans, highlighting a major limitation of reliance on model organisms to untangle host-pathogen interactions highly specific to humans. Several candidate receptors were proposed, including asialoglycoprotein receptor, transferrin receptor, and IL-6 receptor, and ferritin light chain (FTL) and squamous cell carcinoma antigen 1 (SCCA1) were proposed as co-receptors for HBV entry (Hao et al. 2012), but the primary receptor was not identified until 2012. Using near-zero-distance photo-cross-linking and tandem affinity purification, Yan et al. were able to isolate a single protein identified as sodium taurocholate cotransporting polypeptide (NTCP or SLC10A1) that facilitates HBV entry in primary human hepatocytes as well as in tree shrews (Yan et al. 2012). Ni et al. confirmed these results using RNA silencing in differentiated and undifferentiated HepaRG cells (Ni et al. 2014). Differences in the human and mouse NTCP sequence were shown to underlie species-specific differences in HBV binding (Ni et al. 2014; Elinger 2014; Watashi et al. 2014).

3.6 NTCP Expression as a Limiting Factor for HBV Infection

After NTCP was identified as the primary HBV receptor, it became clear that absence of this hepatocyte-specific factor was largely responsible for the poor success of in vitro HBV infection models, pointing the way to development of more effective systems (Watashi et al. 2014). The short window of infectivity in primary human hepatocytes was found to be followed by a steep decline in NTCP expression in cultured cells (Chen and Ye 2012), and lack of NTCP expression is primarily responsible for the poor infectivity of hepatoma cell lines. While differentiated HepaRG cells are able to maintain stable NTCP expression, the polarized orientation of the cells limits access to NTCP on the basolateral membrane. The infection rate of HepaRG cells was found to be improved by disrupting the epithelial barrier in order to expose the basolateral membrane (Schulze et al. 2012).

3.7 NTCP-Expressing Cell Lines

After the HBV receptor was identified, an effort was made to express NTCP in existing cell lines instead of developing new cell lines. Several cell lines have been developed that support stable transfection with the human NTCP gene, including hNTCP-HepaRG, hNTCP-Huh, hNTCP-HepG2, and hNTCP-HEK293 (Yan et al. 2012; Iwamoto et al. 2014). Iwamoto et al. transfected HepG2 cells with an NTCP expression plasmid to produce HepG2-NTCP-C4 cells that were susceptible to HBV derived from serum (Iwamoto et al. 2014). Knockdown or disruption of NTCP inhibited HBV infection. An infection rate approaching 50% was achieved by pretreating the cells with 3% DMSO. Similarly, Yan et al. developed the HepG2-NTCP12 line

by transfecting HepG2 cells with the pcDNA6-NTCP plasmid (Yan et al. 2015). The authors overcame a low infection rate using centrifugation (“spinoculation”). However, while overexpression of NTCP improved infection efficiency in hepatoma cell lines, a high titer is required to establish infection in these cells (6000–18,000 GEq/cell), and production of HBs antigen, HBe antigen, and HBV DNA is low (Yan et al. 2012; Ni et al. 2014; Iwamoto et al. 2013). While NTCP has been shown to be essential for HBV infection, other liver-specific host factors that have yet to be identified are probably required for robust HBV replication. For example, Verrier et al. reported that RNA silencing of glypican 5 (GPC5) inhibited HBV binding to hepatocytes and reduced levels of HBsAg and HBV pgRNA, suggesting a role in HBV entry as a co-receptor or accessory protein (Verrier et al. 2016). Development of an in vitro model capable of sustaining maximum replication efficiency might require expression of additional such host factors.

3.8 Human Hepatocytes Isolated from Humanized Mice

Despite properties that make them useful for cell culture, hepatoma cell lines lack key pathways that play an important role in HBV infection (Tnani and Bayard 1999). Another approach is to explore ways to improve the cell culture properties of primary hepatocytes. Noting that human hepatocytes explanted from human hepatocyte chimeric mice remain infective in culture longer than fresh primary hepatocytes, Ishida et al. developed an in vitro HBV infection system using the humanized mouse model as a source of primary human hepatocytes (Ishida et al. 2015) (Fig. 2.1). Although this approach is relatively expensive and technically difficult, it offers several advantages. In addition to the short window of infectivity, the use of primary human hepatocytes is usually limited by availability and excessive

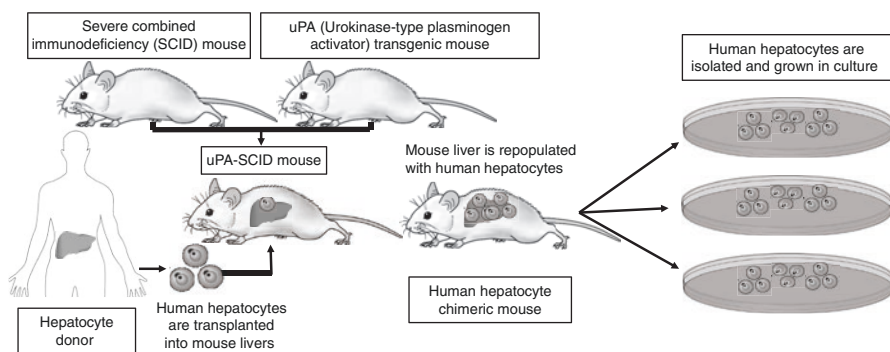


Fig. 2.1 Human hepatocyte chimeric mice as a source of primary human hepatocytes. Human hepatocyte chimeric mice are prepared by transplanting cryopreserved human hepatocytes from a donor into urokinase-type plasminogen activator-transgenic/severely combined immunodeficient (uPA/SCID) mice. Hepatocytes divide several times and repopulate the mouse liver. Human hepatocytes are then isolated from the mouse livers and grown in culture

heterogeneity among cells, whereas proliferated hepatocytes isolated from humanized mice yield a 500–1000× increase in the number of hepatocytes available from a single donor and have improved homogeneity and higher rates of HBV infection and HBV DNA production compared to fresh primary hepatocytes, HepaRG cells, or NTCP-expression HepG2 cell lines. The mice are prepared by transplanting cryopreserved hepatocytes from a single donor into uPA/SCID mice (Tateno et al. 2004), where they repopulate the mouse liver. The hepatocytes are isolated using a two-step collagenase perfusion method and then cultured in hepatocyte clonal growth medium with polyethylene glycol and DMSO.

3.9 Improvements to Primary Hepatocyte Culturing

While hepatocytes are the target of HBV infection and constitute about 80% of the cells in the liver, the remaining 20% of cells play a role in maintaining the highly structured morphology of the liver sinusoid. Based on the hypothesis that improved HBV infection rates can be achieved by more accurately reflecting this architecture in vitro, several attempts have been made to improve culture conditions through 2D, 3D, or microfluidic culture and by co-culturing hepatocytes with non-parenchymal cells (Shlomai et al. 2014; Godoy et al. 2013; Petropolis et al. 2016). Such approaches may also be useful for unraveling early steps in HBV infection that are not normally possible in in vitro studies, such as investigating how the virus crosses the sinusoidal endothelial barrier and examining the role of liver-resident immune cells such as Kupffer cells and hepatic stellate cells in HBV infection (Petropolis et al. 2016), although the complexity of such systems could increase rapidly.

3.10 Hepatic Cell Lines Derived from Human-Induced Pluripotent Stem Cells

One of the most pressing goals of HBV research is the development of therapies that target cccDNA. With this goal in mind, a new culture model of HBV infection was developed based on hepatocytes derived from human-induced pluripotent stem (iPS) cells (Kaneko et al. 2016). Instead of using primary hepatocytes from a donor, which are limited by availability and rapid loss of infectivity, somatic cells can be induced to become pluripotent and altered to develop hepatocytes through transient expression of factors involved in cell differentiation. Kaneko investigated iPS models of HBV infection using two approaches: immature proliferating progenitor-like cells (iPS-HPCs) and differentiated hepatocyte-like cells (iPS-Heps) (Kaneko et al. 2016). Both cell types supported HBV infection and induced innate immune responses similar to that of primary hepatocytes. Infection efficiency was higher in iPS-Hep cells than in iPS-HPC cells, but iPS-Hep cells were more heterogeneous and more difficult to establish in stable culture. Infection efficiency of iPS-HPC cells was improved through overexpression of NTCP. Long-term persistence of cccDNA was detected in these cells and was shown to be maintained through

inhibition of the Janus-kinase signaling pathway. While primary human hepatocytes remain the gold standard for in vitro analysis of HBV infection, iPS cell lines can be used to support large-scale approaches such as drug screening where primary hepatocytes are impractical (Kaneko et al. 2016).

Conclusions

Considering the historical difficulty of culturing HBV and the lack of suitable small animal models, it is remarkable how much has nonetheless been successfully learned about HBV virology and treatment, including the development of a highly effective vaccine. However, even now, functional differences among the *Hepadnaviruses*, as well as differences among animal hosts and cell lines, complicate efforts to resolve conflicting findings and identify key host factors. The discovery of NTCP helped to explain many of these differences, and NTCP over-expression has provided a partial solution to limited infectivity in cell culture, although differences between in vivo and in vitro viral output points to the presence of other as yet unidentified factors required for maximal replication. Despite the convenience of hepatoma cell lines, primary human hepatocytes have numerous advantages, and several recent advances have led to improvements in primary hepatocyte cell culture. Use of *Tupaia* or macaques have potential as small animal models, and human hepatocyte chimeric mice and non-transgenic mice with hydrodynamic or adenoviral-based delivery methods can be used to examine different aspects of the immune response in a murine model. Although each experimental system has specific advantages and disadvantages, and no single system is ideal, the current range of available methods provides a suitable framework for selecting an appropriate in vivo or in vitro system appropriate for a specific line of research. It is hoped that the stage is now set for new breakthroughs in the treatment of chronic HBV infection.

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Immunopathogenesis of Hepatitis B Virus (HBV) Infection

3

Fu-Sheng Wang and Ji-Jing Shi

Abstract

Though HBV itself is not cytopathic, the host immune responses associated with HBV infection determine the outcomes of HBV infection, either acute or chronic infection. Innate and adaptive arms of the immune system are usually involved in combating viral infection. During acute HBV infection, innate immunity is important for control of viral replication and dissemination at very early stage after HBV infection; subsequently, adaptive immunity, especially vigorous, multi-specific and long-lasting HBV-specific T cellular immunity can further efficiently control viral infection. However, dysfunction is the hallmark of adaptive immunity during chronic infection, including faint humoral immunity and exhausted T cellular immunity. Host immune responses induced by HBV infection not only substantially drive disease progression, but also significantly influence efficacy of antiviral treatments in chronic HBV-infected individuals. Therefore, it is important to fully understand the course of immune pathogenesis and to find efficient immunotherapy plus nucleoside analogue(NUC) or IFN- α treatment to completely eliminate or functionally cure HBV infection. In this chapter, we summarize the current progress in innate and adaptive immunities during acute or chronic HBV infection in humans.

Keywords

HBV • Immunopathogenesis • Infection • Treatment

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Hepatitis B virus (HBV) is one of the most prevalent viruses worldwide, which leads to liver injury and inflammation. Patients with chronic HBV infection carry a significant risk of developing severe liver diseases, including chronic hepatitis B (CHB), liver cirrhosis, liver failure, and hepatocellular carcinoma (HCC). Although the management of CHB patients has vastly improved within the last decade, approximately 240 million people worldwide are chronically infected, and approximately 600,000 people die annually of HBV-related liver diseases. Despite effective vaccines for HBV being available for more than 30 years, approximately 2% of vaccinated individuals develop chronic HBV infection in high-endemic areas (Koh and Bertolotti 2015).

The prognosis and severity of HBV-induced liver disease vary widely among patients. HBV itself is not cytopathic, and the host immune responses determine the outcome of HBV infection, which can be either acute or chronic. For example, a vigorous immune response is associated with rapidly spontaneous clearance of HBV in acute-resolved HBV infection. In neonates with an immature immune system, HBV leads to a 90% rate of chronic infection compared to 30% in children infected at 1–5 years of age and 5% in adults. Innate and adaptive responses of the immune system are generally involved in combating viral infection. Innate responses are important to control viral replication and dissemination very early after infection, as well as for the timely orchestration of virus-specific adaptive responses. During HBV infection, the adaptive response, especially vigorous, multi-specific, and long-lasting virus-specific T cellular immunity, is needed for efficient and persistent control of the infection (D'Antiga et al. 2006).

Host immune responses induced by HBV infection not only drive disease progression but also have a significant influence on the efficacy of antiviral treatments in HBV-infected individuals. Therefore, it is important to fully understand the course of immune pathogenesis and to find efficient immunotherapy to complement nucleoside analog (NUC) or IFN- α treatment to completely eliminate or functionally cure HBV infection.

Herein, we summarize the current progress in immunopathogenesis of HBV infection and associated mechanisms of antiviral effects and liver injury, which are induced by both innate and adaptive immune responses in humans.

1 Characteristics of Immune Response in Acute-Resolved HBV Infection

Outline of acute hepatitis B (AHB): In the early stage of infection (incubation phase), innate immune factors immediately recognize viral components and are activated concomitantly with peak HBV replication; however, viral factors can interfere with this process. As serum viral loads begin to decline, the adaptive immune system begins to respond, with the greatest incidence of liver injury occurring after virus resolution (acute hepatitis phase). A strong and effective adaptive immune response, especially HBV-specific CD8⁺ T cellular immunity, is required for virus elimination (Fig. 3.1) (Wang and Zhang 2009). After virus clearance and alanine aminotransferase (ALT)

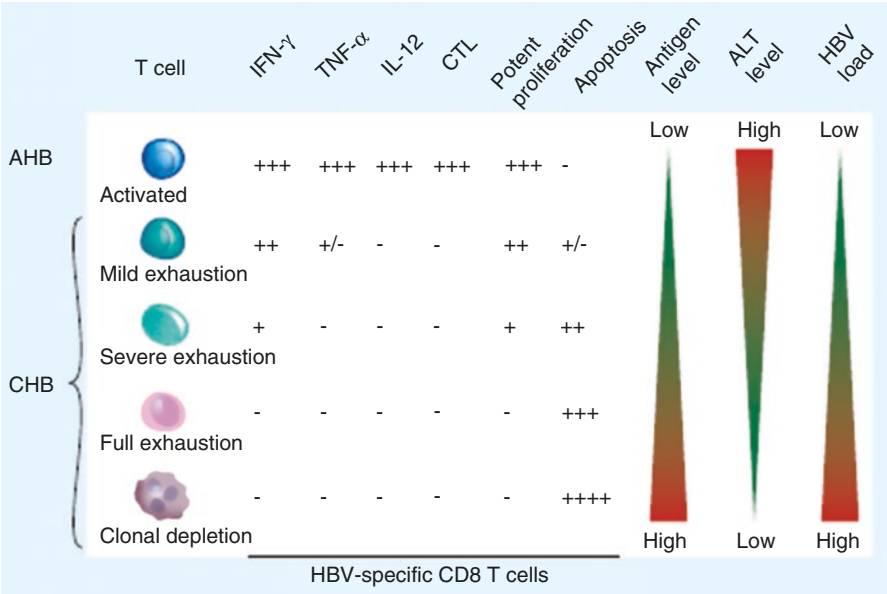


Fig. 3.1 Functional profiles of HBV-specific CD8 T cells in AHB and CHB infection. In the acute phase of HBV infection, HBV-specific CD8 T cells are capable of secreting cytokines and proliferating and are less susceptible to apoptosis. AHB patients generally exhibit a transient increase in ALT levels and viral resolution (serum HBV DNA negativity and HBsAg seroconversion). By contrast, CHB infection can lead to various functional impairments in HBV-specific CD8 T cells, which is often accompanied by persistently elevated HBV replication, a large amount of surface/envelope antigen release and liver injury. *AHB* acute hepatitis B, *ALT* alanine aminotransferase, *CHB* chronic hepatitis B, *CTL* cytotoxic T lymphocytes, *HBV* hepatitis B virus (Reprint with permission from Fu-Sheng Wang. “Host immunity influences disease progression and antiviral efficacy in humans infected with hepatitis B virus”, Expert Review of Gastroenterology and Hepatology, 10/2009)

normalization (resolution phase), HBV envelope-specific antibodies (HBsAbs), together with HBV-specific memory T cells, are generated to protect the infected individual from recurrence or reinfection with HBV.

1.1 The Innate Immune Response

Innate immunity comprises different components that sense the presence of invading pathogens and limit viral spread early during the course of infection. The anti-viral state mainly includes NK (natural killer) cells that kill infected cells and the production of IFN- α/β , pro-inflammatory cytokines, and chemokines, which contribute to the maturation and recruitment of adaptive immune responses (Bertoletti and Ferrari 2012). Although it is well known that HBV-specific CD4⁺ T cells and CD8⁺ T cells play a critical role in controlling HBV infection, the role of the innate immune response in the early stage of infection merits further elaboration.

1.1.1 HBV: Is It Sensed or Not by the Innate Immune System?

The innate immune system comprises a variety of cell surface or endosomal pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), NOD-like receptors (NLRs), and retinoic acid inducible gene I (RIG-I)-like receptors (RLRs), which can recognize specific structures presented on the invading pathogens, termed pathogen-associated molecular patterns (PAMPs). HBV is thought to be a “stealth virus” in the early phase of infection because it does not induce IFNs and IFN-stimulated genes (ISGs) in acute HBV-infected chimpanzees or patients. Whether HBV can be detected by the innate immune system remains controversial. However, some data demonstrated that HBV could be recognized via innate immunity, although viral factors can interfere with this; it appears that HBV is poor inducer of inflammatory cytokines.

TLRs, as the main PRRs, have been reported to recognize HBV proteins. TLR2 recognizes viral lipoproteins and glycoproteins, leading to the activation of immune cells. It has been reported that full-length HBcAg, with a unique arginine-rich C terminus, strongly induces the production of pro-inflammatory cytokines, such as TNF- α , IL-6, and IL-12p40, in THP-1 macrophages in a TLR2-dependent manner *in vitro* (Cooper et al. 2005). Hepatitis B surface antigen (HBsAg) has also been suggested potentially trigger TLR-4 activation through its association with CD14 (Maini and Gehring 2016). Recently, it was observed that intracellular nucleic acid sensors in infected hepatocytes could detect HBV. RIG-I was shown to bind the 5' ϵ region of the HBV pregenomic RNA and triggered strong expression of type III, but not type I, interferons in human primary hepatocytes. In addition, RIG-I could also counteract the interaction of HBV polymerase (P protein) with the 5' ϵ region in an RNA-binding dependent manner, which consistently suppressed viral replication. One study reported that the cytosolic DNA sensor, cGAS, could recognize HBV DNA when it is exposed in the host cell cytoplasm to trigger an innate immune response, which in turn modulates viral gene expression and replication (Cui et al. 2015).

However, HBV can evolve to escape the immune surveillance triggered by targeting PRR signal pathways. Experimental findings supporting the view that HBeAg, HBsAg, and HBV virions can reduce the expression of TLR2 or TLR9 on innate immune cells to inhibit the induction of inflammatory cytokines, such as IFN, after TLR stimulation. They also suppress activation of IRF-3, NF- κ B, and ERK1/2, which triggers the production of IL-10 to reduce the TLR3-mediated induction of inflammatory cytokines and IFN (Ma et al. 2015). Recently, it has been reported that HBV polymerase can suppress IFN- β production by interaction directly with the stimulator of interferon genes (STING) and subsequently disrupting its K63-linked ubiquitination (Liu et al. 2015). In addition, HBx, a multifunctional regulatory protein of HBV, reduces the expression of the mitochondrial antiviral signaling protein (MAVS) (Wei et al. 2010).

However, the above findings about recognition of HBV by the host innate immune system contradict the view established in certain experimental models. Therefore, we should be cautious when applying these results in the context of

natural HBV infection, because the majority of experiments were based on either conditions of overexpressing a single viral protein or in cells that are not normally infected by HBV.

1.1.2 NK Cells: The Main Force of Innate Immunity

Owing to HBV “stealth” characteristic, patients at the very early stage of HBV infection are almost unrecognizable, with no obvious clinical symptoms or liver damage. Therefore, studies of the role of NK cells during the early stages after infection in humans are limited and somewhat contradictory. Two HBV-seronegative blood donors who became HBsAg and HBV DNA positive but had persistently normal ALT were followed from a very early stage of HBV infection. In these patients, NK cells displayed an early and efficient response to HBV, and the innate immune system was able to sense HBV infection, which probably contributed to containing the HBV infection and allowed the timely induction of adaptive responses (Fisicaro et al. 2009). Another study demonstrated that in acute hepatitis B patients, NK cells expressing activation receptors are enriched preferentially in peripheral blood and display increased cytolytic activity and interferon- γ (IFN- γ) production, while the NK cells expressing inhibitory receptors are reduced. NK cell activation levels also correlated positively with serum ALT levels and negatively with plasma HBV DNA levels (Zhao et al. 2012). However, attenuation of NK cell function in acute hepatitis B patients has been reported. A study showed that NK cell activation and their capacity for IFN- γ production were reduced at peak viremia. Type I IFN, interleukin (IL)-15, and IFN-lambda1, which are essential activators of NK cells, were barely detectable (Dunn et al. 2009). In addition, the transient inhibition of NK cells immune response coincided with a surge in the immunosuppressive cytokine IL-10 accompanying HBV viremia. These findings were confirmed in a study based on AHB patients; the researchers observed a markedly impaired function of NK cells covering degranulation as well as cytokine and chemokine production (Lunemann et al. 2014). These results revealed the participation of NK cells in the early-stage response after HBV infection, although the data are inconsistent.

1.2 The Adaptive Immune Response

While the innate immune response may contribute to controlling viral replication, the presence of an efficient and timely adaptive response ultimately determines the outcome of HBV infection. During acute HBV infection, generating an abundant repertoire of HBV-specific B and T cells is considered very important.

1.2.1 B Cells

For a long time, there were few studies on the HBV-specific B cell response. In HBV infection, antibodies can be elicited by different HBV proteins (core, e, envelope, polymerase, and x), and the presence or absence of antibodies, especially anti-HBs, anti-HBc, and anti-HBe, have been used to distinguish different clinical phases

of HBV infection. Only anti-HBs have virus-neutralizing activity, and their role in controlling HBV infection, even with the persistence of covalently closed circular DNA (cccDNA), is associated with disease resolution and virus control. It has been demonstrated that anti-HBs develop in the course of spontaneously resolving infection, in addition to being induced by immunization with recombinant HBsAg. Anti-HBs-producing B cells are more commonly detected in the periphery of acute patients compared with CHB patients (Xu et al. 2015).

Recently, the discovery of sodium-taurocholate cotransporting polypeptide (NTCP) as the HBV receptor (Ni et al. 2014) enabled the precise mapping of HBV regions essential for infectivity to the preS1 domain and the antigenic loop region (also known as the “adeterminant”) of the HBs antigen. These data, together with the establishment of easily infectable cell lines *in vitro*, have made it possible to further investigate the protective ability of anti-HBV antibodies. Studies revealed that the preS1 domain (in particular amino acids 2–48) interacts directly with NTCP (Ni et al. 2014), whereas the HBs antigenic loop (residues 104–163, located between the HBs transmembrane regions I and II of the S protein) interacts with heparin sulfate proteoglycans on hepatocytes, increasing the concentration of HBV virions on the cell surface and aiding NTCP receptor interaction. Antibodies against these two regions are able to block HBV infection, while antibodies specific to other HBV regions not involved in HBV infectivity, such as the PreS2 region, do not show any neutralizing ability (Bertoletti and Ferrari 2016).

1.2.2 T Cells

In acute HBV infection, T cells have a crucial role in determining spontaneous resolution versus virus persistence, which is clearly supported by chimpanzee studies showing that *in vivo* depletion of either CD4⁺ or CD8⁺ T cells hampers HBV clearance and clinical recovery. In patients with resolved infection, vigorous, broad, and polyclonal HBV-specific T cell responses are detected, whereas in patients with persistent viremia, the T cell responses are relatively weak and narrow and induced later (Chisari et al. 2010).

The HBV-specific T cells immune response seems to be delayed during HBV infection. The kinetics of HBV-specific CD8⁺ T cells appearance and frequency have been well characterized in adult patients with acute HBV infection and in chimpanzees. Circulating HBV-specific CD8⁺ T cells are detected 6–8 weeks after infection, which is later than in HIV and HCV infection, where virus-specific CD8⁺ T cells are apparent within 1–2 weeks. The HBV-specific CD8⁺ T levels peak at the height of liver damage; however, the quantity of HBV-specific CD8⁺ T cells is very low, and the frequencies against any single HBV epitope are rare exceeding, representing 1–3% of the total CD8⁺ T lymphocyte pool. In addition, the peak of the HBV-specific T cell response is also concomitant with bystander CD8⁺ T cells activation against heterologous viruses (HCMV and Epstein-Barr virus), which might contribute to inflammatory events in the liver (Bertoletti and Ferrari 2016).

Unlike HCV infection, whether escape mutations accumulate in HBV-specific CD8⁺ T cell epitopes through selection pressure has not been determined. An early study revealed that mutational immune escape is rare in HBV infection (Rehermann

et al. 1995); however, a recent study showed that HLA class I-restricted epitopes in the HBV core protein are under reproducible selection pressure (Kefalakes et al. 2015).

Besides HBV-specific CD8⁺T cells, HBV-specific CD4⁺ T cells are also important in eliminating the virus in acute HBV infection. A vigorous, efficient, and multiple epitope HBV-specific CD4⁺ T cells immune response is detected in patients who clear acute HBV infection. Moreover, a study in chimpanzees showed that the depletion of CD4⁺ T cells abrogates the function of CD8⁺ T cells and leads to chronic HBV infection (Asabe et al. 2009).

Some mechanisms have been clarified that modulate the HBV-specific T cells response, such as IL-10, arginase, PD-1, T regulatory cells (Tregs), and myeloid-derived suppressor cells (MDSCs). After serum HBV clearance, the frequency of HBV-specific CD8⁺ and CD4⁺ T cell declines; however, a memory T cell response can be detected for up to 20–30 years after infection (Boettler et al. 2006).

2 Specialties of the Immune Response in Chronic HBV Infection

Outline of CHB. Typical chronic HBV infection in humans is characterized by progressive inflammatory liver damage and viral persistence. The exact mechanisms by which HBV and host immunity drive disease progression in CHB remain unknown; however, they are likely to be associated with an abnormal innate immune response, defects in the host HBV-specific T cell response, and viral interactions with the host immune system (Reprint with permission from Taylor & Francis).

2.1 The Innate Immune Response in CHB

In CHB, innate immunity changes correlate with the antiviral function and disease progression. In this section, we focus on NK cells, the main elements of the innate immune system. During this phase, the proportion, phenotype, and/or function of NK cells have been revealed, and the main defects in NK cells can be described as follows: (1) a dichotomy in the functional features of a conserved or even enhanced cytolytic activity that may be correlated to the severity of liver injury, and impaired cytokine production, such as IFN- γ and TNF- α ; (2) altered expression of activating or inhibitory receptors on NK cells; (3) upregulation of certain inhibitory molecules, such as T cell immunoglobulin and mucin domain-containing molecule-3 (Tim-3); and (4) the reduction of the percentages of hepatic and peripheral NK cells in immune-activated CHB patients, with or without changes in their subsets.

Notably, recent studies have emphasized that NK cells are capable of exerting immunoregulatory functions via death receptor pathways, especially killing of effector T cells (Maini and Peppas 2013). In CHB patients, NK cells can mediate a rapid, contact-dependent killing of HBV-specific T cells via the TNF-related apoptosis-inducing ligand (TRAIL)/TRAIL death receptor TRAIL-R2 pathway,

with high-level expression of TRAIL-R2 on HBV-specific CD8⁺ T cells. In addition, the death of HBV-specific CD8⁺ T cells can be partially rescued by blockade of this death receptor pathway (Peppia et al. 2013).

NK cell dysfunction is determined by multiple factors, as demonstrated by many studies:

1. Functional defects of mDCs (myeloid dendritic cells) and pDCs (plasmacytoid dendritic cells), which influence the cross talk with NK cells, making NK cells activation inefficient (Martinet et al. 2012).
2. The inhibitory effect of IL-10 and transforming growth factor- β 1 (TGF- β 1). Acting as an inhibitory cytokine, IL-10 can blunt NK cell activation, which restrains IFN- γ and TNF- α secretion, which has a more significant effect on cytotoxicity. Blocking the IL-10 pathway may partially restore the capacity of NK cells IFN- γ production (Peppia et al. 2010). TGF- β 1 may impair NK cell-mediated cytotoxic capacity and IFN- γ production by downregulating the expression of the activating receptors NKG2D and 2B4, as well as their intracellular adaptor proteins, DAP10, and SAP (Sun et al. 2012).
3. Pathological circumstances such as changes of TLR expression induced by HBV infection might interfere with NK cells function.

2.2 The Adaptive Immune Response in CHB

Dysfunction is the hallmark of the adaptive immune response during chronic infection, including faint humoral immunity and exhausted cellular immunity.

2.2.1 B Cells

Whether neutralizing antibodies can modulate the development of chronic HBV infection has not yet been fully investigated. Data show that few neutralizing antibody-producing B cells are detected during chronic HBV infection. Patients with CHB display a significantly higher portion of activated B cells of the memory and naïve phenotype, which are functionally impaired, without global defects in antibody production (Xu et al. 2015). In addition, T helper follicular cells are functionally impaired in CHB patients (Li et al. 2013). As antibodies exert long-term control of HBV infection, there is a potential to modify B cells function via some strategies to treat chronic HBV infection.

2.2.2 T Cells: Functional Exhaustion

HBV-specific T cell exhaustion, which represents functional impairment of T cells, is characterized by poor cytotoxic activity, impaired cytokine production, and sustained expression of multiple inhibitory receptors. HBV-specific T cell exhaustion has been underscored by large amounts of data in patients with chronic HBV infection and is the crucial element that maintains persistent viremia. This T cell impairment is even more pronounced in the liver compared with the blood in CHB patients (Fisicaro et al. 2010). What leads to the exhaustion of HBV-specific CD8⁺ T cells during persistent infection? Recently, studies have suggested that viral persistence,

elevated expression of inhibitory receptors, negative regulation of some cytokines (such as IL-10, TGF- β) and cells (such as Tregs and MDSCs), dysfunction of CD4⁺ T cells and DCs, and NK cells contact-dependent killing may contribute to the exhaustion of T cells (Fig. 3.2).

HBV Antigens: Persistent Stimulators of T Cells

Long-term exposure of T cells to HBV antigens is a crucial contribution to depressed T cell function. However, direct evidence of this T cell impairment has not been available in natural HBV infection settings, in contrast to LCMV infection, where a hierarchical loss of antiviral T cell functions was caused by viral persistence, with an initial general reduction in cytotoxicity and IL-2 production, followed by IFN- γ and TNF- α production, and ultimately, T cell deletion (Wherry et al. 2007). Recent studies have tried to correlate T cell responses with serum antigen levels but without reflecting the overall antigen expression in the liver environment (Loqqi et al. 2013; Boni et al. 2012).

Negative Co-inhibitory Molecules: PD-1, 2B4, Tim-3, LAG-3, and CTLA4

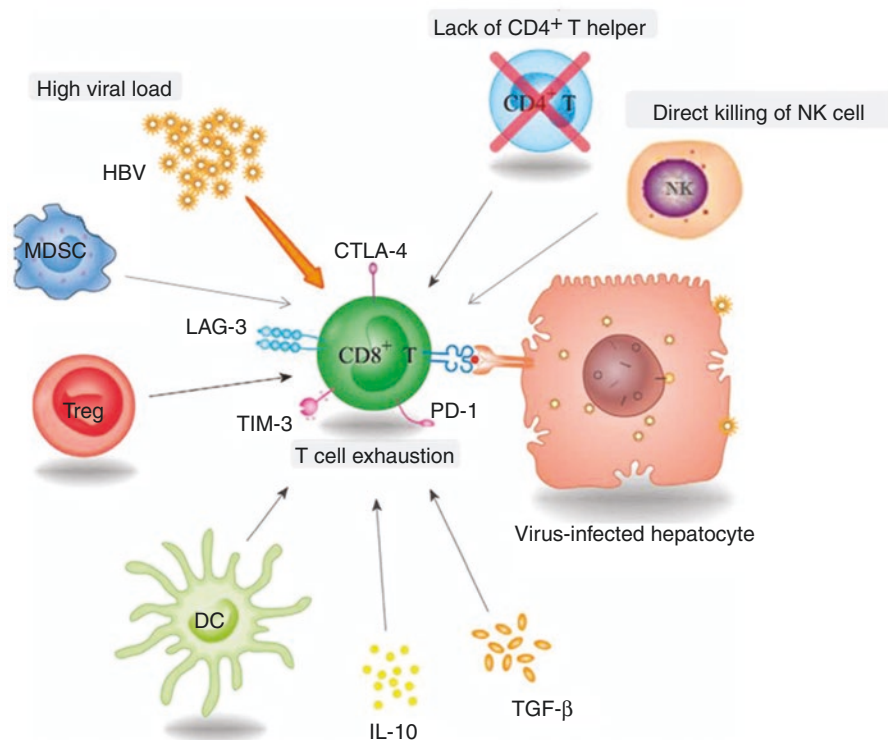


Fig. 3.2 Mechanisms of HBV-specific CD8⁺ T cells exhaustion. In chronic HBV infection, there are multiple mechanisms to establish the exhaustion of virus-specific CD8⁺ T cells, including high viral load, loss of CD4⁺ T help, direct killing of NK cells, regulations of immunosuppressive cells and cytokines (Reprint from (Ye et al. 2015))

Negative co-inhibitory molecules, including PD-1, CTLA4, Tim-3, 2B4, lymphocyte activation gene-3 (LAG-3), and CD160, are highly expressed on exhausted HBV-specific CD8⁺ T cells and are closely associated with their unresponsiveness (Chen and Flies 2013). As the main inhibitory receptor among these negative co-inhibitory molecules, PD-1 has been detected on exhausted HBV-specific CD8⁺ T cells, especially within the liver, where the vast majority are PD-1 positive. In addition, the expression of inhibitory receptors has a clear hierarchy. PD-1 and 2B4 are expressed on over 90% of liver-infiltrating HBV-specific CD8⁺ T cells, followed by LAG3 and CD160 (Bertoletti and Ferrari 2016).

Blocking the interaction of an inhibitory receptor and its ligand can restore the function of exhausted HBV-specific CD8⁺ T cells, which has been demonstrated extensively. Using anti-PD-L1 antibodies to block the PD-1/PD-L1 pathway can partially restore HBV-specific T cell function in vitro. Moreover the restoration is more efficient in intrahepatic T cells compared with peripheral T cells, although the restoration is always incomplete and detectable only in a proportion of chronic patients (Fisicaro et al. 2010). It has been suggested that the combination of PD-1/PD-L1 pathway blockade and CD137 activation could further restore the function of HBV-specific CD8⁺ T cells ex vivo (Fisicaro et al. 2012). Combining IL-12 with blockade of the PD-1 pathway further increased the function of exhausted HBV-specific CD8⁺ T cells in the majority of patients (Schurich et al. 2013). Furthermore, a recent study revealed a critical role of T-bet in increasing the capacity of HBV-specific CD8⁺ T cells to produce IFN- γ and proliferate, suggesting a new strategy to improve exhausted HBV-specific CD8⁺ T cells (Kurkschiev et al. 2014). The simultaneous expression of multiple co-inhibitory molecules suggests the need for therapeutic strategies that target multiple pathways simultaneously. A study reported that HBV-specific CD8⁺ T cell responses could be rescued by blocking Tim-3/galectin-9 interactions, as a complementary approach to PD-1 pathway inhibition (Nebbia et al. 2012).

Suppressive Cytokines and Cells

IL-10 and TGF- β have an inhibitory effect on T cells immune response. In a murine model, IL-10 and TGF- β , constitutively expressed by liver Kupffer cells, induced tolerance of liver-infiltrating lymphocytes. Moreover, these two cytokines can constrain the proliferative and survival abilities of T cells to attenuate viral control. In chronic HBV infection, a specific polymorphism of IL-10 has been correlated with disease severity (Bertoletti and Ferrari 2016). It has been reported that enhancement of effector T cell function correlates with decreased TGF- β expression (Liu et al. 2016). Multiple cells, including Tregs, B cells, and stellate cells (especially Tregs), can secrete IL-10 and TGF- β to regulate T cells effector function, contributing to maintaining HBV-specific T cells functional exhaustion (Bertoletti and Ferrari 2016).

CD4⁺CD25⁺Foxp3⁺Treg cells are involved in virus-specific T cells exhaustion during chronic HBV infection; whereas, the mechanisms that mediate the regulatory effect of Tregs require further study. In CHB patients, the numbers of Tregs increased in the peripheral blood and in the liver, which might significantly suppress

HBV-specific T cell responses (Zhang et al. 2012). However, one study showed that circulating Tregs modulate the function and expansion of HBV-specific CD8⁺ cells ex vivo in all patients without remarkable differences in their profiles of HBV infection, suggesting no link between Tregs and chronic HBV infection (Franzese et al. 2005). Shortly afterward, another study identified that chronic HBV patients with a high viral load have a higher proportion of regulatory T cells in their livers, but not in the blood, compared with patients with a low viral load, and the intrahepatic Tregs were phenotypically distinct from the peripheral blood Tregs (Stoop et al. 2008).

MDSCs, another type of suppressor cells, were studied recently in HBV infection. The frequency of arginase⁺ granulocytic MDSCs (gMDSCs) increased in chronic HBV infection, and these cells can suppress T cells function through arginase-dependent pathway, contributing to HBV-specific T cells functional exhaustion (Pallett et al. 2015).

NK Cells: A Direct Killer of HBV-Specific CD8⁺ T Cells

Accumulating data have revealed that NK cells participate in maintaining the exhausted state or even the deletion of HBV-specific CD8⁺ T cells in chronic HBV infection. The expression of apoptosis gene Bcl₂-interacting mediator, Bim, is consistently increased in HBV-specific CD8⁺ T cells from CHB patients compared with those in resolved patients. Thus, NK cells may induce the apoptosis of virus-specific CD8⁺ T cells via Bim (Lopes et al. 2008). In addition, a recent study showed that exhausted HBV-specific CD8⁺ T cells also upregulate the expression of TRAIL death receptor, TRAIL-2, rendering them susceptible to TRAIL-dependent NK cell lysis (Peppas et al. 2013).

CD4⁺ T Cells Exhaustion

In chronic HBV infection, CD4⁺ T cells also display functional exhaustion, and the loss of CD4⁺ T cell helper function may prevent the maturation of a functionally efficient CD8⁺ T cell response, leading to a more severe exhaustion of HBV-specific CD8⁺ T cells. As the dominant regulator of CD8⁺ T cells response to HBV infection, CD4⁺ T cells have multiple properties, including activating professional antigen-presenting cells (APCs), such as dendritic cells (DCs), through the CD40/CD40 ligand pathway, and producing cytokines, such as IL-2 and IL-21, as well as chemokines, which can introduce naïve T cells to the priming sites in secondary lymphoid organs and activate T cells to the infection location (Ye et al. 2015).

What causes the exhaustion of CD4⁺ T cells in chronic HBV infection? This question has been addressed by many studies, yet the mechanism of CD4⁺ T cell exhaustion remains poorly understood. Unlike exhausted CD8⁺ T cells, CD4⁺ T cells in CHB patients display a low expression of inhibitory receptors such as Tim-3, 2B4, CTLA4, and KLRG1 (killer cell lectin-like receptor subfamily G member 1). Blockade of the PD-1-PD-L1/2 signal pathway can improve the CD4⁺ T cells' capability to produce Th1 cytokines such as IL-2, IFN- γ , and TNF- α , with increasing T cell proliferation. However, CTLA4 or Tim-3 pathway blockade failed to achieve a similar effect to blocking the PD-1 pathway, which is distinct from the response of in CD8⁺ T cells, suggesting that CD4⁺ T cells exhaustion is different to that of CD8⁺ T cells (Raziorrouh et al. 2014).

3 Immune Response and Liver Injury

Persistent liver injury, or the continuous recruitment of inflammatory cells to the livers of CHB patients, ultimately results in fibrosis, cirrhosis, and possibly HCC. Although HBV is not cytopathogenic, it is generally accepted that host immune responses induced by viral persistence are responsible for the pathogenesis of liver disease. For example, HBV-specific T cell-mediated immune responses play an important role in inducing hepatocellular damage during chronic HBV infection (Zhang et al. 2012). However, recent studies have shown that the infiltration of non-virus-specific inflammatory cells within the liver might also participate actively in HBV-associated liver pathogenesis.

3.1 HBV-Specific T Cells

During acute HBV infection, the appearance of HBV-specific T cells in the liver tends to coincide with an increase in serum ALT levels, which suggests an important role of T cells in mediating liver injury. Studies have identified that HBV-specific CD8⁺ T cells contribute to virus control as well as liver injury in HBV infection. As a non-cytolytic antiviral cytokine, IFN- γ secreted by CD8⁺ T cells eliminates viruses without severe liver injury, whereas the cytotoxic properties of CD8⁺ T cell might cause liver injury. CD4⁺ T cells also have a role in liver injury. Furthermore, Th17 cells can cause liver injury in chronic HBV infection by producing IL-22, similar to HCV infection (Shin et al. 2016).

3.2 Non-HBV-Specific Cells

In addition to virus-specific T cells, many non-HBV-specific cells are significant contributors to liver injury. This was first examined in an HBV-transgenic mice model, in which acute necroinflammatory liver injury resembling human acute hepatitis B histologically occurred after the adoptive transfer of HBV-specific CD8⁺ T cells. Only hepatocytes in close proximity to the HBV-specific CD8⁺ T cells were induced to undergo apoptosis. The main liver injury was caused by nonspecific mononuclear cells, which are recruited to the liver by the HBV-specific CD8⁺ T cells through the production of chemokines (CXCL9 and CXCL10) and platelet activation (Ferrari 2015). In chronic HBV infection, HBV-specific T cells are functional exhausted; therefore, non-HBV-specific cells might play a more important role in liver injury (Iannacone et al. 2007).

NK cells. In addition to antiviral and immunoregulatory functions, NK cells also participate in the pathogenesis of liver injury in HBV infection. During the chronic infection phase, the cytolytic activity of NK cells increases, which positively correlates with the severity of liver damage. In addition to the enhanced cytolytic activity of NK cells, the transmission of apoptotic signals is another important factor causing hepatocyte death. Normally, NK cells express little or no TRAIL, and the

numbers of TRAIL receptors on the hepatocytes' surface are minimal. However, in patients with HBV infection and liver inflammation, TRAIL⁺NK cells are enriched in the liver, and hepatocytes express high levels of the TRAIL receptor, indicating that NK cells may mediate hepatocyte death via the TRAIL-TRAILR pathway, thereby contributing to liver inflammation. Moreover, activated NK cells are involved in disease progression of HBV-related acute-on-chronic liver failure (ACLF) via degenerating HBV-infected hepatocytes by the Fas/Fas ligand interaction (Wu et al. 2015).

Immunoregulatory cells. As well-known regulatory cells, Tregs not only suppress the function of HBV-specific T cells but also are involved in regulating liver injury. During the acute-resolved HBV infection, the numbers of circulating Tregs are low in the acute phase but significantly increase during the convalescent phase and then return to normal levels as the disease resolves. In CHB patients, the increased numbers of Tregs in both the peripheral blood and the liver might suppress HBV-specific T cell responses significantly, thereby constraining liver inflammation. Furthermore, the decreased frequency of HBcAg-specific Tregs, accompanied by increased HBcAg peptide-specific CTLs, might partially account for the acute exacerbation in CHB patients (Zhang et al. 2012).

MDSCs, as immature myeloid lineage cells, can modulate an expanding immune response by disrupting metabolic supplies of certain immunocytes (T cells and NK cells), thereby suppressing immune injury. A recent study showed that gMDSCs are expanded in acute HBV infection in parallel with viremia without liver inflammation. In addition, during the different clinical phases of CHB, the number of arginase⁺ gMDSCs was inversely correlated with liver inflammation, indicating that gMDSCs can ameliorate immune pathology in a partially arginase-dependent manner (Pallett et al. 2015). In addition to arginase, MDSCs can enlarge their effects through other metabolic pathways, such as indoleamine dioxygenase (IDO) or by inducing Tregs, which suggests that MDSC might inhibit pro-inflammatory responses through other components in the HBV-infected liver environment, such as monocytes, hepatic stellate cells, gamma-delta T cells, as well as HBsAg (Maini and Gehring 2016).

In addition to Tregs and MDSCs, B cells, Kupffer cells, liver resident DCs, and liver sinusoidal endothelial cells (LSECs) also have tolerogenic properties, exerting immunosuppressive effects to prevent an exaggerated immune response, thereby maintaining a tolerant liver microenvironment (Zhang et al. 2012).

4 Immune Response and Antiviral Treatment

CHB patients often exhibit varied responses to current antiviral therapies, and the antiviral responder types include complete responders, partial responders, and non-responders. In particular, in CHB patients undergoing successful antiviral treatment, three phases may develop: complete virus suppression (the viral load drops below a detectable level) with ALT normalization, HBeAg seroconversion, and HBsAg seroconversion (the “almost cured” clinical status, which is ideal, but difficult to attain via current therapies). Factors determining the response to antiviral

therapy remain unknown. Recently, increasing evidence has indicated that the efficiency of antiviral therapy is associated closely with the immune status of the host. The dynamic balance between virus production and viral clearance mediated by the host antiviral immune response generally leads to three outcomes *in vivo*: (1) the circulating viral load increases upon viral release if viral replication overcomes host-mediated elimination; (2) the serum viral load remains stable if viral replication keeps pace with viral elimination by the host; and (3) the serum viral load decreases when host immune elimination is more effective than viral replication and release (Reprint with permission from Taylor & Francis).

The ultimate goals of antiviral therapy are to fully suppress viral replication, to improve the host's immunomodulatory capability, to eliminate HBV DNA and viral antigens, and to attenuate liver inflammation *in vivo* so that disease progression to LC and HCC is hindered. On the basis of clinical observations and findings in animal models, we proposed a "three-step hypothesis" of antiviral immune restoration (Wang and Zhang 2009) (Reprint with permission from Taylor & Francis):

Step 1, a transient or reversible change comprising an enhanced immune response during the stage with complete virus suppression (the viral load drops below a detectable level) together with ALT normalization.

Step 2, partial restoration of host immune response with HBeAg seroconversion, but the restoration is not long lasting. Immune markers, such as T cell responses, pDC/mDC numbers, Treg numbers, PD-1 expression, IL-12, IL-10, and IL-21 are closely associated with HBeAg seroconversion.

Step 3, complete restoration of antiviral immunity in the host occurs when HBsAg seroconversion is attainable and when an efficient immune response is sustained for long time. However, it remains a lack of immune markers to predict the "almost cured" clinical status.

To better optimize antiviral therapy, we recently proposed a "climbing-slope hypothesis," a new model that attempts to explain the process of antiviral treatment in CHB patients (Wang and Zhang 2009). In the hypothesis, CHB patients cannot "stand up" but may have to "sit in a wheelchair" owing to the following three impediments: persistent viral replication, functional impairment of host immunity to HBV, and inflammatory, necrotic, and cirrhotic liver changes. To regain a healthy status, it is necessary that CHB patients overcome the aforementioned impediments. Although antiviral therapy in the narrow sense (nucleotide analogs, interferon) can suppress HBV DNA replication, it appears to be insufficient to help patients to reach the top because the immune system of these patients cannot generate complete antiviral responses against HBV. In such cases, broad-range antiviral therapy is proposed; this includes the combination of antiviral (nucleotide analogs and interferon) treatment, immunomodulatory therapy, and some drugs targeting elimination of HBsAg or cccDNA. This mode of antiviral therapy not only efficiently suppresses the viral DNA and antigen replication but also eliminates virions and HBV-infected cells. This suggests that immunomodulatory therapy is likely to improve the host antiviral immune responses to the level at which the individual attains a healthy status, thus, in combination with antiviral drug treatment, allowing some patients to "climb to the top" (Fig. 3.3). The hypothesis may help hepatologists to systemically evaluate the efficacy of antiviral therapies and design more

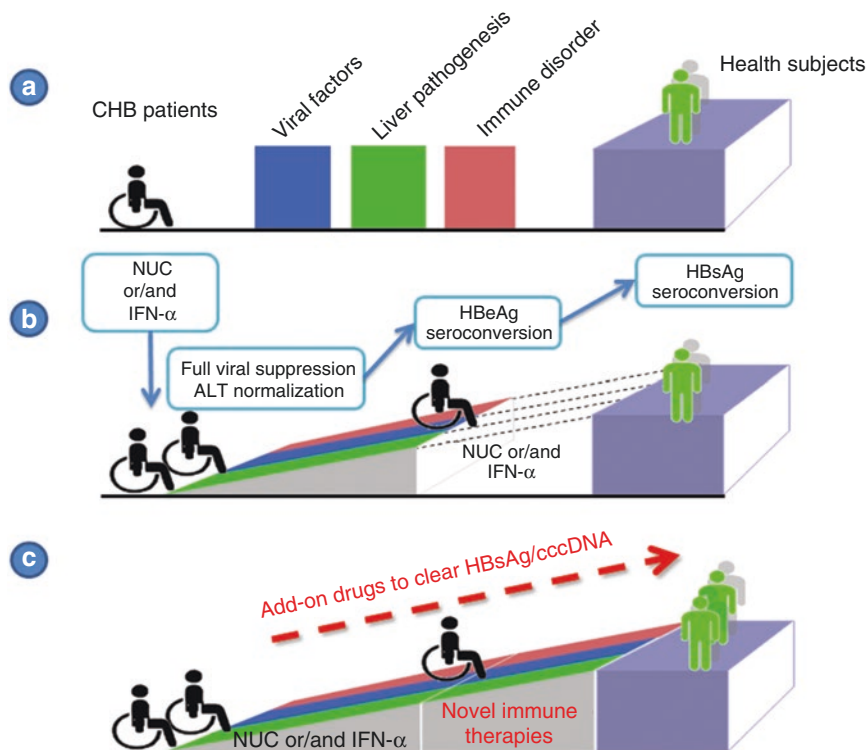


Fig. 3.3 Antiviral therapy for CHB patients: the climbing-slope hypothesis. **(a)** CHB patients face three obstacles: virological factors, liver pathogenesis, and immune disorders. **(b)** NUC or/and IFN- α therapy has the capacity to pull down these obstacles and establish an ascending slope, but there is still a “big gap” that exists in CHB patients who are often undergoing NUC or/and IFN- α therapy since it is very difficult for them to attain a HBsAg seroconversion. **(c)** Based on the nature of the climbing-slope hypothesis, the major task of management of CHB patients is to build up an ascending slope that may include both the full viral suppression/serum ALT normalization and HBsAg seroconversion and to bridge this “big gap.” Ideally, an adequate period of treatment with a combinational antiviral drug treatment and immunomodulation will be necessary to achieve successful immune restoration (including HBsAg seroconversion) with long-term control of the hepatitis B virus infection. *CHB* chronic hepatitis B, *ALT* alanine aminotransferase, *NUC* nucleoside analog (Reprint with permission from Fu-Sheng Wang. “Host immunity influences disease progression and antiviral efficacy in humans infected with hepatitis B virus”, *Expert Review of Gastroenterology and Hepatology*, 10/2009)

reasonable treatment regimens. However, the hypothesis remains to be validated by further clinical investigation (Reprint with permission from Taylor & Francis).

Conclusions

In general, it is considered that the host immune response against HBV infection mainly exerts an antiviral role but leads to simultaneous liver injury. Adaptive immunity, in particular HBV-specific CD8⁺ T cell immunity, plays a crucial role

in eliminating virus infection; however, the role of innate immunity should not be neglected. Novel therapeutic approaches have been developed on the basis of extensive research that clarified the immunopathogenesis of HBV infection. Prevention or elimination of the HBV-specific T cells functional exhaustion is the key element to restore patients' antiviral immunity, thereby eliminating HBV. In addition, the emergence of new biological immune agents (such as TLR agonists, anti-PD-1 ligand monoclonal antibodies, HBV-specific modified T cells, and therapeutic vaccines) has resulted in a vastly increased potential to cure HBV infection.

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Abstract

Hepatitis B virus (HBV) infection is a major public health issue worldwide despite the availability of an effective vaccine and the development of effective antiviral treatments. The World Health Organization estimates that 248 million people worldwide are chronically infected with HBV, with the highest prevalence in East Asia, sub-Saharan Africa, and the Pacific Islands. Complications of chronic HBV infection include hepatocellular carcinoma, end-stage liver disease, liver transplant, and death, with the risk of these complications varying by mode of transmission and disease duration. Annually, an estimated 650,000 people die from complications of chronic HBV, with 40% of these deaths occurring in Global Vaccine Alliance countries. Perinatal transmission is the leading cause of HBV transmission worldwide, accounting for more than 60% of all childhood-acquired HBV. Implementation of national vaccination programs has significantly decreased prevalence in multiple countries in both Asia and Africa. The initiation of antiviral therapy in pregnant mothers with detectable virus can also reduce the risk of perinatal HBV transmission when combined with vaccination and, where available, HBIG. Chronic HBV is defined as hepatic necroinflammation due to the persistent presence of infection (hepatitis B surface

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antigen-positive) beyond 6 months and can be divided into at least five major phases of infection. Each phase is associated with characteristic patterns of ALT, HBV DNA levels, hepatitis B e antigen (HBeAg) status, liver histology, and response to antiviral therapy. The age the infection is acquired largely dictates the immune response against acute HBV infection and the subsequent natural history of chronic infection.

Keywords

Hepatitis B virus • Natural History • Phases of Infection • Prevalence • Epidemiology • Interpretation of Serologies • Genotypes

1 Introduction

Hepatitis B virus (HBV) infection is a major public health issue worldwide despite the availability of an effective vaccine and the development of effective antiviral treatments. It is estimated that approximately 248 million people are chronically infected with HBV worldwide, with the highest prevalence seen predominantly in Asia, sub-Saharan Africa, and the Pacific Islands (Schweitzer et al. 2015). Approximately 15–40% of people with chronic HBV (CHB) will develop serious complications during their lifetime (Bosch et al. 2005), with an increased risk for cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC) (Beasley 1988). CHB is thought to be the cause of 45% of HCC cases and 30% of cirrhosis cases worldwide (Demma and Dusheiko 2016), with the risk of these complications varying by mode of transmission (Goldstein et al. 2005). Annually, an estimated 650,000 people die from complications of CHB, with 40% of these deaths occurring in persons living in Global Vaccine Alliance countries (Demma and Dusheiko 2016). The WHO has recently adopted the 2030 Agenda for Sustainable Development which outlines strategies to combat and eliminate chronic viral hepatitis (United Nations General Assembly 2016). The strategies include gathering information for focused action, formulating high-impact interventions, achieving equitable coverage, reducing costs for those requiring services, and promoting rapid progress and innovation. The ultimate goal of these strategies is to eliminate viral hepatitis as a major public health threat by the year 2030 by reducing the number of infections to under one million (down from six to ten million infections from all types of viral hepatitis viruses) and reducing the number of deaths to less than 500,000 (from 1.4 million) by 2030 (United Nations General Assembly 2016; World Health Organization 2012).

Several factors contribute to the high prevalence of CHB and its complications in Asian and African populations: the delayed implementation of effective programs for screening and vaccination of newborns (Goldstein et al. 2005), the lack of access to effective antiviral therapies, and the high risk of acute HBV exposure resulting in chronic infection with both mother-to-child (MTC) vertical transmission and early

childhood horizontal transmission (Lavanchy 2004). The risk of developing chronic infection after acute HBV exposure, defined as persistence of hepatitis B surface antigen (HBsAg) for at least 6 months, is correlated with age of initial infection, with earlier age of initial infection associated with higher risk of chronic infection (Goldstein et al. 2005). The importance of understanding the epidemiology and natural history of CHB lies in the opportunity to raise awareness of the need for expansion of screening and vaccination efforts and for early detection and management to prevent HBV-related complications and mortality. In this chapter, we will review the epidemiology and natural history of chronic HBV with a focus on high-prevalence regions.

2 Epidemiology

Up-to-date national level epidemiological information is crucial not only for assessing the burden of disease but also for identifying appropriate prevention and control strategies and determining their impact (Schweitzer et al. 2015). CHB endemicity levels have been defined based on the prevalence of HBsAg. Areas of low endemicity are defined as HBsAg <2%, low-intermediate areas as 2–4.99%, high-intermediate areas as 5–7.99%, and high-endemicity areas as ≥8% (Schweitzer et al. 2015) (see Fig. 4.1 (Schweitzer et al. 2015; Ott et al. 2012; MacLachlan et al. 2013; Robinson et al. 2005; Khan and Attaullah 2011; Mulwa 1994; McMahon et al. 1998; Musa et al. 2015; Paat et al. 2009; Chu et al. 2013; Cui and Jia 2013; Yano et al. 2015; Poorolajal and Majdzadeh 2009; Unnewehr and Stich 2015; Centre for Disease Prevention and Control of Latvia 2015; Rabenau et al. 2010; Mohamed et al. 2012; Chen et al. 2007)). An estimated 45% of the world's population resides in regions of high HBV endemicity. The greatest burden of disease lies in Asia where approximately 75% of infected persons reside (Mohamed et al. 2012). In China, certain islands in the Pacific, and regions of sub-Saharan Africa, up to 60–90% of the population has some serologic marker of HBV infection (Chen et al. 2007). One study estimating the worldwide prevalence of CHB based on data published between 1965 and 2013 estimated the global prevalence of HBsAg to be 3.61%, a total of 248 million people chronically infected, with a wide variation in prevalence between countries (Schweitzer et al. 2015). There is also substantial variation in prevalence between certain population groups within some countries.

2.1 CHB in Africa

The overall CHB prevalence in Africa is high (8.83%), with a total of over 75 million people estimated to be living with CHB (Schweitzer et al. 2015). Between 56 and 98% of the adult population of sub-Saharan Africa has some serologic marker of HBV infection (Kiire 1996). Out of the 54 countries in Africa, only 3 have low prevalence—Egypt (1.71%), Morocco (1.09%), and the Seychelles (0.48%)—and 5 low-intermediate prevalence—Algeria (2.89%), Botswana (3.8%), Eritrea (2.49%),

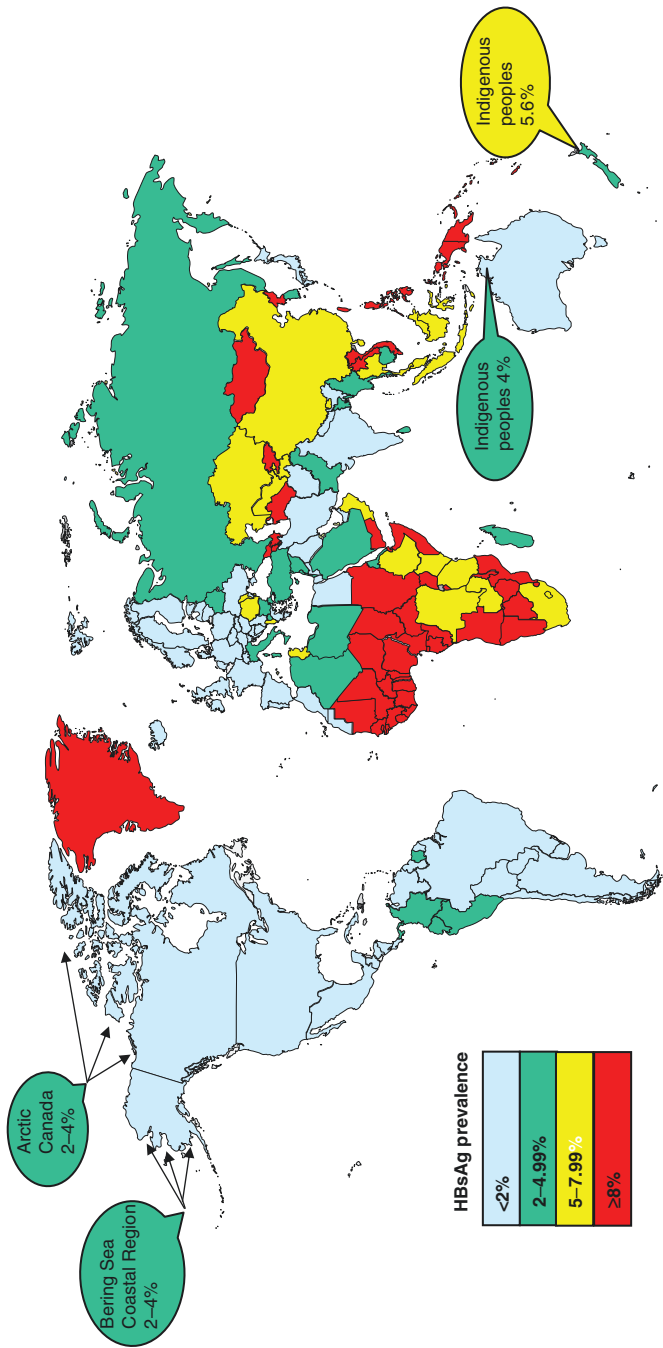


Fig. 4.1 Bering Sea coastal region: HBsAg prevalence is 2-4% in this region where the population is predominantly Alaska natives, compared to <2% in the rest of Alaska. Arctic Canada: HBsAg prevalence is 2-4% here compared to <2% in the rest of Canada. Australia and New Zealand: HBsAg prevalence is higher in indigenous peoples, 4% in Australia (compared to 0.9% in the nonindigenous) and 5.6% in New Zealand, predominantly on the North Island (compared to 4.1% in the nonindigenous). The estimated resident Aboriginal and Torres Strait Islander population of Australia is 669,900 people, 3% of the total Australian population. In New Zealand, 682,200 people identify as Maori, 15.4% of the population. In New Zealand, particularly high HBsAg prevalence has also been shown in other Pacific Islanders, including Tongan (13.1%), Niuean (8.6%), and Cook Islands (6.3%) peoples, as well as in Chinese (8.9%) and SE Asians (8.1%). Prevalence data compiled from multiple sources (Schweitzer et al. 2015; Gust 1996; Lin et al. 2005; Ott et al. 2012; MacLachlan et al. 2013; Robinson et al. 2005; Khan and Attallah 2011; Mulwa 1994; McMahon et al. 1998; Musa et al. 2015; Paat et al. 2009; Chu et al. 2013; Cui and Jia 2013; Yano et al. 2015; Poorolajal and Majdzadeh 2009; Unnewehr and Stich 2015; Centre for Disease Prevention and Control of Latvia 2015; Rabenau et al. 2010) by Gish RG, Locamini S, and McMahon, BJ. © 2017

Libya (2.16%), and Madagascar (4.6%) (Schweitzer et al. 2015; Matthews et al. 2015). Countries with high-intermediate prevalence are Cape Verde (7.26%), the Democratic Republic of the Congo (5.99%), Ethiopia (6.03%), Kenya (5.16%), Lesotho (5.5%), Rwanda (6.67%), South Africa (6.7%), Tanzania (7.17%), and Zambia (6.06%) (Schweitzer et al. 2015; Rabenau et al. 2010). All the other countries in Africa have high CHB prevalence (Schweitzer et al. 2015). In some of these, prevalence is extremely high, including Benin (15.57%), Guinea (15.06%), Liberia (17.55%), Mauritania (16.16%), Niger (15.48%), South Sudan (22.38%), and Swaziland (19.00%) (Schweitzer et al. 2015). Although improved screening of blood products and vaccination programs have resulted in an overall prevalence decrease between 1990 and 2005 in Western and Central sub-Saharan Africa, increases were actually seen during those years among children aged 0–14 in Southern sub-Saharan Africa and in children of the youngest ages in Eastern sub-Saharan Africa (Ott et al. 2012).

Throughout Africa, most CHB is the result of early childhood horizontal transmission (Kiire 1996; Hadziyannis 2011; Whittle et al. 1983), with most infections occurring between the ages of 1 and 5 years (Dumpis et al. 2001). In 1996, it was reported that in West Africa infection rates would increase rapidly beginning at 6 months of age, resulting in 40% of children being infected, 15% with CHB, by age 2, and 90% being infected, 20% with CHB, by age 10 (Kiire 1996). In a study of family members in Gambia, phylogenetic tree analysis of sequences from the pre-core and core regions showed a high likelihood of intrafamilial transmission of HBV in at least two-thirds of the families studied (Dumpis et al. 2001). Sibling to sibling transmission is common (Whittle et al. 1983). Several studies have shown that major risk factors for transmission in Africa include shared use of toiletries and sharp objects, tattooing, and tribal scarification, as well as medical procedures and injections (Kiire 1996; Jombo et al. 2005; Otegbayo et al. 2003; McCarthy et al. 1994). It has also been shown that sexual activity is a risk factor for HBV transmission in both urban and rural communities (Jombo et al. 2005; Komasa et al. 2013; Sirisena et al. 2002). The need for both improved screening and vaccination and for education of both the general public and health-care practitioners on risk factors for transmission is clear.

Although the national immunization programs of all sub-Saharan countries now include HBV vaccine, most countries schedule the initial dose at 6–8 weeks of age, in part because of the difficulties associated with vaccine delivery at birth in areas where home delivery is common and in part because only the pentavalent vaccine (DTP-HepB-Hib) is provided by the Global Alliance for Vaccines and Immunization (GAVI) and it is not licensed for birth administration (Miyahara et al. 2016). Thus, vaccine delivery program improvements are clearly needed.

Although *Bacillus Calmette-Guerin* (BCG) and oral polio vaccine are scheduled at birth in 48 and 39 of the 49 countries in sub-Saharan Africa, respectively, only 8 of these countries (Botswana, Cape Verde, Djibouti, the Gambia, Namibia, Nigeria, Mauritania, and Sao Tome and Principe) have introduced HBV vaccine at birth (World Health Organization 2015). In addition, only 77% of infants in the WHO Africa region receive the third dose of HBV vaccine (World Health

Organization-UNICEF 2015). WHO defines universal infant immunization as the most important strategy for reducing CHB prevalence, with the first vaccine dose given within 24 h of birth, followed by two doses, either given along with the first and third doses of DTP vaccine or, if preferred, by three doses coinciding with DTP or other infant vaccinations; the minimum interval between doses is 4 weeks. The first dose being given within 24 h of birth is considered crucial (World Health Organization 2010). This early administration of the vaccine is important to prevent both MTC and early horizontal HBV transmission (Miyahara et al. 2016). Although the former is not as common in Africa as in Asia, timely vaccination could substantially lessen the risk of both MTC transmission and the horizontal transmission at very young ages that is very common throughout Africa.

In a recent study carried out in the Gambia, it was found that although 93.1% of children had received the first dose of HBV vaccine by the age of 6 months, only 1.1% received the dose within 24 h of birth, 5.4% by day 7, and 58.4% by day 28 (Miyahara et al. 2016). Delayed vaccination was found to be associated with birth a long distance from vaccination delivery points, low maternal education, living in urban and peri-urban settings, and Fula ethnicity. Although the association between delayed vaccine delivery and living in an urban area might seem counterintuitive, it is thought to be the result of Gambia's primary health-care system in rural areas that includes village-based traditional birth attendants and health workers who are responsible for informing villagers about the outreach clinics where vaccines are delivered. Unfortunately, birth in a health facility was not associated with timely vaccination, pointing clearly to the need to educate health-care workers in both rural and urban areas on the importance of this and to integrate HBV vaccine delivery into maternal and neonatal services in all areas. In addition, education on the need for a timely birth dose of vaccine should be directed toward the traditional birth attendants who often attend home births and toward the general population and pregnant women so that knowledge about the need for this becomes widespread.

Maintaining constant refrigeration of the hepatitis B vaccine may be problematic in parts of both Africa and Asia and may reduce the likelihood of timely delivery of the birth vaccine, particularly in the case of home births. In general, it is recommended that hepatitis B vaccine be stored at +2 to +8 °C (35.6–46.4 °F), maintaining its potency for up to 4 years, and that freezing must categorically be avoided since it degrades potency. However, WHO reports that some yeast-derived recombinant DNA hepatitis B vaccines are apparently stable at increased temperatures, although data on vaccines from several manufacturers showed large differences in stability; at temperatures of 20–26 °C (68–78.8 °F), vaccine stability ranged from 1 month to 1 year and at 37 °C (98.6 °F), from 1 week to 6 months. The vaccine vial monitor (VVM) provides additional assurance that vaccines can be safely used. According to WHO, at the time of vaccine administration, the VVM indicates if the vaccine has been exposed to a combination of excessive temperature over time and thus is likely to have been damaged, thereby indicating to health-care workers whether a vaccine can be used; if health-care workers have been properly trained to interpret VVM readings correctly and to discard any vial that has reached its discard

point, vaccines with VVMs can be taken out of the cold chain (World Health Organization 2006). Careful use of this information might improve vaccine accessibility in resource-limited settings and thus improve timely vaccine delivery in many parts of Africa and Asia.

2.2 CHB in Asia and the Pacific Islands

Worldwide, more than 75% of people with CHB live in WHO's Western Pacific and Southeast Asia regions (Gust 1996). The home of 95 million people with CHB, the Western Pacific region is comprised of 37 countries and areas, including highly developed countries like Australia where HBsAg prevalence is the lowest in the region (0.37%) and much less developed island nations like Kiribati, the Solomon Islands, Nauru, Vanuatu, Tonga, Papua New Guinea, and Niue where prevalence is very high (22.7%, 18.83%, 17.55%, 17.54%, 14.81%, 14.59%, and 11.86%, respectively) (Schweitzer et al. 2015). In many countries in this region, prevalence varies substantially between population groups. For example, in Australia, HBsAg prevalence is substantially higher in indigenous peoples (4%) compared to the prevalence in the nonindigenous. The same is true in New Zealand where prevalence is 5.6% in indigenous peoples, located predominantly on the North Island, compared to 4.1% in the nonindigenous.

In addition to the small island nations already mentioned, several countries in the Western Pacific region have particularly high prevalence. In the Philippines, national HBsAg seroprevalence is estimated to be 16.7%, corresponding to an estimated 7.3 million adults with CHB in the Philippines (Wong et al. 2013). However, a wide variation has been reported there between ethnic groups, ranging from only 5.2% among Tagalogs in Oriental Mindoro to 18.2% among Mangyans (Evangelista et al. 2006). The factors that underlie the large prevalence discrepancy have not been fully established but are thought to relate to socioeconomic factors, including inadequate access to health services among the Mangyans (Gish et al. 2016). In Vietnam, overall national HBsAg prevalence is estimated to be 12%, corresponding to approximately ten million people with CHB (Gish et al. 2012), but prevalence varies between urban and rural areas, ranging from 10–14% in Ho Chi Minh City and Hanoi (Tran et al. 2003; Nakata et al. 1994) to 18.8% (Hipgrave et al. 2003) to 19% (Nguyen et al. 2007) in some rural areas. As with the Philippines, this prevalence discrepancy may be due, at least in part, to the reduced access to health-care services and screening programs in rural areas (Gish et al. 2012). Other high-endemicity countries in this region include Laos (8.74%) and Mongolia (9.07%) (Schweitzer et al. 2015).

Countries in the Western Pacific region with high-intermediate prevalence include China (5.49%), the Marshall Islands (7.80%), Samoa (5.53%), and Tuvalu (7.14%) (Schweitzer et al. 2015). Most other countries in this region have low-intermediate prevalence, including Brunei (4.06%), Cambodia (4.05%), Fiji (4.8%), Micronesia (3.5%), South Korea (4.36%), and Singapore (4.09%) (Schweitzer et al. 2015). The only countries with low prevalence other than Australia are Malaysia

(0.74%) and Japan (1.02%) (Schweitzer et al. 2015). Compared to the Western Pacific region, prevalence is generally lower in WHO's Southeast Asia region, with low HBsAg prevalence in Nepal (0.82%), India (1.46%), and Indonesia (1.86%); low-intermediate prevalence in Bangladesh (3.10%), Myanmar (3.40%), and Sri Lanka (2.51%); and high-intermediate prevalence only in Bhutan (5.84%) and Thailand (6.42%).

Effective infection control measures have reduced prevalence in some of the countries in these regions, particularly in people born after the widespread initiation of vaccination programs. Transmission of HBV in China has most commonly occurred through MTC vertical transmission and early childhood transmission (Lok 1992; Yao 1996a, b). Prior to implementation of universal HBV vaccination, the prevalence of HBsAg positivity among children in the 1–4 years age group equaled the level of the general population. The two most recent nationwide cross-sectional seroepidemiological surveys in China showed a reduction in HBsAg prevalence from 9.8% (high endemicity) in 1992 to 7.2% (high-intermediate) in 2006, with prevalence among children aged <5 years reduced to only 1.0% (Liang et al. 2009). The reduction was strongly associated with vaccination among all age groups. Since, historically, unsafe blood products were a major cause of HBV transmission in China, credit is also given to the improved screening of blood donors since 1999 (Yan et al. 2014). The universal infant vaccination program launched in Taiwan in 1984 has also been very successful, reducing HBsAg prevalence from 10% in 1984 to 0.9% in 2009 in people born after the initiation of the program (Ni et al. 2012). People vaccinated as infants were also shown to have significantly reduced hepatocellular carcinoma rates (Chang et al. 2009). Screening and vaccination programs have contributed to reduced prevalence in multiple other countries. Over the time periods of 1957–1989 and 1990–2013, decreases in prevalence were also noted in Thailand, India, South Korea, Malaysia, and Singapore (Schweitzer et al. 2015). In New Zealand and Australia, prevalence has increased in recent years due to immigrants from high-prevalence countries (Ott et al. 2012).

To fully achieve a reduction of CHB prevalence in Asia and the Pacific Islands, there are multiple factors that must be addressed. Although there has been an overall increase in timely birth dose coverage in this region, WHO estimates that it is only attained in 76% of the Western Pacific region overall, with multiple countries continuing to have much too low birth dose coverage (World Health Organization 2014). For example, some of the island nations noted above as having high CHB prevalence have much too low birth dose coverage, including Papua New Guinea (32%) and the Solomon Islands (65%) (World Health Organization 2016). WHO notes that in order to improve birth dose coverage in this region, it will be important to increase awareness about the importance of the birth dose, to increase deliveries that are attended by skilled birth attendants who have been educated about the need for the birth dose, and to ensure that vaccine is easily accessible (World Health Organization 2014). This also applies to other Asian countries, in some of which birth dose coverage is exceptionally low. For example, it is estimated that in India timely birth dose coverage is only 44%, in Iraq only 43%, in Afghanistan only 18%, and in Pakistan only 10% (World Health Organization 2016).

Although MTC transmission is the most common source of HBV infection throughout Asia and the Pacific Islands, it will also be important to address HBV transmission in the health-care setting. WHO estimates that in 2000 30% of injections were carried out with reused injection equipment in the Western Pacific Region (Hutin et al. 2003). Worldwide, it is thought that 21 million HBV infections annually (approximately 32% of new infections) are the result of contaminated injections (Hauri et al. 2004). In the Philippines, a 2007 survey of injection safety in 80 government health facilities discovered that in only approximately 80% of procedures were new sterile syringes and needles, lancets, or other devices taken from sterile packets or fitted caps used, including in only 65.7% of intravenous injections (World Health Organization 2007). Another study in the Philippines pointed to the need to address inadequately sterilized hospital equipment since the risk of developing HBV infection was found to be eightfold higher in patients with a history of hemodialysis and fourfold higher in patients with a history of blood donation (Tecson and Luna 2005). A meta-analysis of 32 studies has also shown that the risk of HBV infection is increased in people with a history of tattoo (Jafari et al. 2012). Because of the increasing popularity of tattoos in Asian Pacific countries, particularly among young people, it will be important both to educate the public on this and to provide guidance on eliminating unsafe practices by tattoo artists. Education of prisoners and institution-wide enforcement of guidelines and safer tattooing practices in prisons will also be important since the prevalence of CHB in correctional settings is high (Jafari et al. 2012). In addition, in many Asian Pacific countries, reuse of razors in barbershops to reduce costs is not uncommon so education of the public on this risk and guidance for barbers on unsafe practices are both needed (Gish et al. 2016).

2.3 CHB in the Americas

With the exception only of Haiti (13.55% prevalence), countries in the WHO region of the Americas are of low or low-intermediate endemicity levels (Schweitzer et al. 2015), although prevalence is substantially higher in certain population groups. Countries with low prevalence include the United States (USA), Canada, Mexico, the countries in Central America, and most of the countries in South America and the Caribbean (Schweitzer et al. 2015). Countries with low-intermediate prevalence include Belize (4.71%), the Dominican Republic (4.09%), Jamaica (3.76%), and several of the Amazon basin countries, including Colombia (2.29%), Ecuador (2.00%), Peru (2.10%), and Suriname (3.91%) (Schweitzer et al. 2015).

Although the US Centers for Disease Control and Prevention (CDC) estimates an overall prevalence in the United States of only 0.8–1.2% (805,000–1,405,000 persons) (Weinbaum et al. 2008), the basis for this estimate, the National Health and Nutrition Examination Survey (NHANES), excludes some populations with increased infection risk, including the incarcerated, the homeless, and institutionalized persons, and does not adequately represent multiple racial/ethnic groups with higher infection rates, including Native Americans, Alaska Natives, and Asians and Pacific Islanders

(APIs) (Gish et al. 2015a). HBsAg prevalence is estimated to be especially high (3.45%) in foreign-born US immigrants, a population estimated to equal 1.32 million persons (Gish et al. 2015a; Kowdley et al. 2012). A study that adjusted for this higher prevalence in the foreign-born estimated that up to 2.2 million US persons are living with CHB (Kowdley et al. 2012). In addition, the HBsAg prevalence in the Bering Sea coastal region of Alaska is estimated to be low-intermediate in the predominantly Alaska Native population there, compared to <2% in the rest of Alaska. In another area populated predominantly by indigenous peoples, Arctic Canada along the Arctic Ocean, the prevalence is also estimated to be low-intermediate.

2.4 CHB in Europe

The European WHO region is estimated to have low-intermediate endemicity overall (2.06%), but HBsAg prevalence varies widely between countries (European Centre for Disease Prevention and Control 2014) and is generally lower in Western Europe compared to Eastern Europe, with HBsAg prevalence estimated to be very low in the United Kingdom (0.01%), Norway (0.01%), Ireland (0.03%), Iceland (0.14%), and Switzerland (0.18%) and low or low-intermediate in most other European countries (Schweitzer et al. 2015). Prevalence is only estimated as high-intermediate in Albania (7.99%), Kazakhstan (6.05%), Moldova (7.38%), Romania (5.61%), Tajikistan (7.2%), and Uzbekistan (6.99%) and high in Kyrgyzstan (10.32%) (Schweitzer et al. 2015). Although considered part of the European region because it is a Danish territory and thus generally shown on prevalence maps as having low prevalence (Ott et al. 2012), Greenland actually has very high HBsAg prevalence in its population (16.6%), 89% of which is Inuit (McMahon et al. 1998).

Across all age groups, most CHB in Europe is the result of vertical MTC transmission (67%), but it is slightly more common among those aged under 30 years (70.1%) than among those aged 30 or over (65.2%) (European Centre for Disease Prevention and Control 2014). Inward migration of people with CHB from high-prevalence countries appears to account for a substantial proportion of European CHB cases, with data suggesting that most MTC infections in Europe were acquired in a country different from the reporting country (European Centre for Disease Prevention and Control 2014). The percentage of CHB cases reported as being due to injection drug use was estimated as 3.9% in 2012, while the percentage of cases classified as due to nosocomial transmission or as the result of blood or blood products is low, approximately 2% for each (European Centre for Disease Prevention and Control 2014).

3 HBV Genotypes

HBV has a number of genetic variants described as serotypes (adw, ayw, ayr, and adr) and genotypes (A, B, C, D, E, F, G, H, I, and J) (Lin and Kao 2015). Genotyping is specified by sequencing the HBV genome, using a line probe assay (capture hybridization), or by using serologically based antibody testing (Gish and Locarnini

Table 4.1 Geographic distribution of hepatitis B virus genotypes and subtypes

Genotypes	Serotypes	Subtypes	Geographic location
A	adw	A1	Sub-Saharan Africa and India
		A2	Northern Europe and India
		A3	Western Africa
B	adw, ayw	B1	Japan
		B2–5	East Asia, Taiwan, China, Indonesia, Vietnam, and the Philippines
		B6	Alaska, Northern Canada, and Greenland
C	adw, ayr, adr	C1–3	Taiwan, China, Korea, and Southeast Asia
		C4	Australia
		C5	The Philippines and Vietnam
		C6–11	Indonesia
D	ayw	D1–6	Africa, Europe, Mediterranean countries, India, and Indonesia
E	ayw		Restricted to West Africa
F	adw	F1–4	Central and South America
G	adw		France, Germany, and the United States
H	adw		Central America
I	adw		Vietnam and Laos
J			Japan

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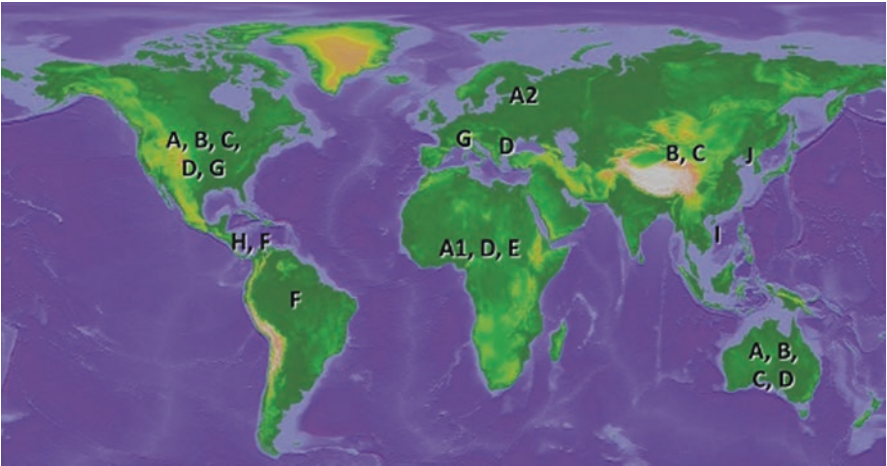


Fig. 4.2 Global distribution of HBV genotypes. Updated and adapted from Liaw YF, et al. Liver Int. 2005;25:472–489. Note: A1 is the African variant, and A2 is the European variant of HBV genotype A

2009). The distribution of HBV genotypes varies by world regions (Lin and Kao 2011) (Table 4.1 and Fig. 4.2). While genotyping of HBV is not routine practice in all settings, knowledge of HBV genotypes can provide important epidemiological and clinical information. Genotype assessment and nucleic acid sequence analysis may allow epidemiologists to track the transmission and movement of HBV strains through the world or regions of the world; to establish other epidemiological

behavior of HBV infection, including the investigation of common source outbreaks; and to establish clinical behaviors specific to each subgroup (Gish and Locarnini 2009; Lin and Kao 2011). Furthermore, HBV genotyping provides important data that help guide clinical management. For example, previous studies demonstrated that HBV patients with genotype A have higher response rates to treatment with interferon therapy (Lau et al. 2005). In addition, HBV genotype C is associated with higher risk of developing HCC (Chan et al. 2004; Kao et al. 2000, 2002), and HBV genotype D is associated with development of adefovir resistance (Gish and Locarnini 2009). While treatment outcomes with current first-line oral antiviral therapies and their success at HBV suppression and/or clearance are not well predicted by HBV genotype, knowledge of HBV genotype may be important with the advent of new HBV therapies in development. As previously discussed, mixed infections with two different genotypes can result in new recombinants (Zhou et al. 2012), but the survival of the recombinant strains varies. For instance, HBV/G is very slow to replicate as a monoinfection; however, after coinfection of mice with HBV/A, the replication of HBV/G was significantly increased (Sugiyama et al. 2007). Another study showed that coinfection with HBV/A was required for effective replication of HBV G genotype in a specific population in Japan (Tsuzuki et al. 2016). While many recombinants can potentially arise in individuals with coinfection with several genotypes, there is a strong selection power for elimination of hybrid genomes. A study of families in western China initially showed that many novel recombinants arose; however, after 18 months, only one recombinant variant had persisted (Zhou et al. 2012).

Genotypes in China have been studied extensively. While the predominant HBV genotypes in China are B2–5 and C1–3, within China there are region-specific variations. Genotype B is more prevalent in southern China, whereas genotype C is more prevalent in northern China (Lin and Kao 2011). The subgenotypes C1 and C2 are predominant in western China, and a high prevalence of CD1 and CD2 was found in a Tibetan population, indicating that the distribution of these two subgenotypes may be related to ethnic origin (Zhou et al. 2011). This is further supported by the study of recombinant hepatitis B viruses. Recombination contributes to the diversity in HBV genomes (Shi et al. 2013). A study of 15 families in which individuals were coinfecting with two different HBV genotypes showed naturally occurring HBV recombinants between C2, D1, and CD1 genotypes in 3 of the 15 families (Zhou et al. 2012). Some initial products of recombination may go undetected due to the selection to remove new, deleterious mutations. Thus the recombinant nature of HBV may preclude classification into genotypes, and a new classification for HBV sequences may be required (Zhou et al. 2011).

4 Natural History

The natural history of HBV and the risk of developing chronic infection after acute exposure is affected by age and mode of transmission. The modes of transmission vary substantially in different regions of the world and in the presence of high-risk

behaviors (Kuo and Gish 2012; McMahon 2005). Chronic HBV is defined as hepatic necroinflammation due to the persistent presence of HBV infection beyond 6 months. Patients are positive both for HBsAg and for HBV DNA with serum levels of HBV greater than 2000 to 20,000 IU/ml or have positive staining for the presence of hepatitis B core antigen (HBcAg) in the liver. Liver enzyme levels are elevated for over 6 months, either persistently or intermittently. The WHO case definition of chronic HBV requires two separate reactive serum test results for HBsAg over a 6-month period.

The risk of developing chronic HBV infection after acute exposure ranges from $\leq 5\%$ in adults to approximately 30% in children infected between ages 1 and 4 years to $>90\%$ in infants born to infected mothers who are positive for hepatitis B e antigen (HBeAg), a secreted protein that is detectable in the bloodstream when HBV is actively replicating (Hyams 1995; McMahon et al. 1985). Persistent and stable HBV DNA levels detected during the first 1–4 weeks following acute hepatitis B infection are strong predictors of not clearing HBV infection and progression to chronic HBV (Pan and Zhang 2005).

Perinatal transmission is a leading cause of HBV transmission, accounting for more than 60% of all childhood-acquired HBV worldwide (Gish and Locarnini 2009). However, the primary mode of transmission varies across regions. MTC vertical transmission is the most common source of infection in people with CHB in Asia and the Pacific Islands and in Europe, while early childhood horizontal transmission is most common in sub-Saharan Africa, the Mediterranean region, and Alaska (Kiire 1996; Hadziyannis 2011; Whittle et al. 1983; McMahon et al. 1985; Bortolotti et al. 2006; Dusheiko et al. 1989). In regions of low endemicity, infections most commonly occur among adolescents and adults through sexual and injection drug use exposures (Wasley et al. 2010).

For children born to CHB mothers, prevention of perinatal transmission with injection of hepatitis B immune globulin (HBIG) combined with vaccine is effective (Wong et al. 1984), but HBIG is neither available nor affordable in much of the developing world. The initiation of antiviral therapy in pregnant mothers, a much less costly approach, can also greatly reduce the risk of perinatal HBV transmission (Han et al. 2011; Shi et al. 2010; van Zonneveld et al. 2003; Xu et al. 2009; Pan et al. 2016). A randomized controlled study in which highly viremic pregnant women received antiviral therapy during the third trimester showed an 18% HBsAg seropositivity in infants born to lamivudine-treated mothers, compared with 39% seropositivity in infants born to untreated mothers (Xu et al. 2009). A study evaluating the efficacy of telbivudine for decreasing the rate of perinatal HBV transmission showed a 0% incidence of perinatal transmission at 7 months in infants born to mothers with high HBV viral load treated during weeks 20–32 of pregnancy, versus an 8% transmission rate in infants born to untreated mothers (Han et al. 2011). A recent study of HBeAg-positive pregnant women with an HBV DNA level of more than 200,000 IU/mL compared MTC transmission in women given tenofovir (300 mg daily) from 30 to 32 weeks of gestation until postpartum week 4 to women who did not receive antiviral therapy. The per protocol analysis showed transmission to none of the infants of mothers given tenofovir vs. 7% of infants of mothers who did not

receive antiviral therapy (Pan et al. 2016). Recently updated AASLD, EASL, and APASL guidelines recommend starting antiviral therapy in the third trimester in pregnant mothers with chronic HBV and viral load $\geq 200,000$ IU/mL (Terrault et al. 2016; Liaw et al. 2008; European Association for the Study of the Liver 2012).

Adult-acquired HBsAg positivity is more common in low- and intermediate-prevalence regions and results mainly from unprotected sexual contact or injection drug use (Pan and Zhang 2005). While the majority of adult-acquired HBV infections are asymptomatic, some patients will present with acute HBV with jaundice and elevated liver tests following a high-risk sexual exposure (Gish and Locarnini 2009). In populations that acquired HBV as adults, sexual contact between heterosexuals (42%) and men who have sex with men (15%) are common modes of acute transmission (Gish and Locarnini 2009). Injection drug use accounts for about 21% of all HBV infections acquired in adulthood (Gish and Locarnini 2009). Cognizant of these modes of transmission, public health interventions targeting high-risk behaviors can impact transmission rates and reduce morbidity and mortality. For example, elimination of the practice of reusing needles in developing countries may lead to a marked reduction in childhood-, adolescent-, and adult-acquired HBV infections (Yao 1996a; Ko and Chung 1991). Nosocomial/occupational exposure and transmission is also an important issue. Health-care workers exposed to blood of patients with chronic HBV through needlestick injuries or blood splash exposures are at risk of infection (Yao 1996a; Coppola et al. 2016). Worldwide, approximately three million health-care workers annually are exposed to blood or other potentially infectious biological materials via either a mucosal-cutaneous or percutaneous route, resulting in two million exposures to HBV (Coppola et al. 2016). The risk of HBV transmission with such exposures depends on the patient's HBeAg status and the HBV DNA viral load. In exposures to blood from patients who are HBeAg-positive or who have an HBV DNA load $>10^6$ IU/mL, the risk of HBV transmission is estimated at 19–30%, compared to 5% with exposures to blood from patients who are HBeAg-negative or who have $<10^6$ IU/mL viral load (Coppola et al. 2016). To prevent infection from such exposures, hepatitis B vaccination is strongly advised for all health-care personnel (Schillie et al. 2013).

Historically, renal dialysis patients have been considered a high-risk group for acquiring or transmitting HBV since many have had extensive blood exposure in the dialysis setting. A recent study of a large multiethnic hemodialysis cohort in London found that 2% of dialysis patients had chronic HBV infection (HBsAg-positive) and 20% were anti-HBc-positive but HBsAg-negative suggesting past exposure that was cleared from the blood, but not from the liver (Sowole et al. 2015). Of the patients with past infection, 2.2% had detectable HBV DNA, albeit very low levels, thus making transmission via blood exposure unlikely. The risks of HBV exposure from inadequately sterilized medical equipment are higher in the developing world. For example, in a study in the Philippines, it was shown that the odds of developing HBV infection was eightfold higher in patients who had a history of hemodialysis (Tecson and Luna 2005). Implementation of vaccination policies and infection control measures are necessary to decrease the risk of nosocomial transmission in dialysis patients.

Household transmission has also been clearly identified as a risk for acquiring HBV (Yao 1996a). The route of exposure is not clear, but HBV is a DNA virus that can exist outside the body on fomites for prolonged periods. Current recommendations state that the sharing of household utensils that may carry blood such as toothbrushes and razor blades must be avoided (Korean Association for the Study of the Liver 2012). The transmission of HBV with common kitchen items and food sharing has not been described. All household members living with an individual positive for HBsAg or known to be a person with chronic HBV should be tested for evidence of HBV infection and vaccinated if not exposed or immune (Yao 1996a; Weinbaum et al. 2009).

Blood transfusion is a very unlikely source of HBV in developed countries where effective screening of blood takes place. Screening of all blood units for HBV by HBsAg and anti-HBc, and in many countries HBV DNA by PCR, as well as pre-donation screening for risk behavior should become a standard practice throughout the world. The risk of acquiring HBV through a blood transfusion in the United States is less than 1:400,000 units. The best current test used to screen blood for HBV infection is the HBV DNA by nucleic acid testing (NAT), also termed “molecular testing,” as well as improved HBsAg and anti-HBc assays (Allain and Candotti 2009). The reason any risk is present from blood products relates to the fact that some patients donate blood before the onset of an immune response to HBV infection or the presence of clinical evidence of hepatitis. NAT is emerging as a method to make the blood supply even safer (Allain and Candotti 2009).

Finally, in some cases of adult-acquired HBV infection, the source of exposure is not clear. For example, approximately 20–35% of individuals with adult-acquired HBV infection in the United States have no known risk factor or easily identifiable risk factor (Gish and Locarnini 2009). Possible transmission through sexual contact, medical or dental treatment, injection drug use (IDU), sharing household utensils, or blood exposure in an occupational setting must all be considered.

5 Interpretation of Serologies

As a general principle of laboratory-based investigation for the virological cause of chronic liver disease, testing should include all the major etiological agents (HBV, HBV plus HDV, and HCV). The principal screening assay for acute and chronic hepatitis B, as well as for the screening of blood and organ donors, is detection of HBsAg in serum combined with anti-HBc (Shepard et al. 2006), although molecular testing with more advanced nucleic acid tests such as PCR and transcription-mediated amplification (TMA) is now being instituted in the United States and considered worldwide.

The serological profiles that are typically found in acute, chronic, or past HBV infection are shown in Table 4.2. The serological findings that are associated with the number of weeks post-exposure are shown in Fig. 4.3. In patients with CHB, hepatitis B core-specific IgG antibodies (IgG anti-HBc) are almost always present in serum. With MTC transmission, these antibodies may play a role in fetal and

Table 4.2 Interpretation of HBV serological tests

HBsAg	Total anti-HBc	IgM anti-HBc	Anti-HBs	Interpretation
–	–	–	–	Never infected and no evidence of immunization, needs vaccination
+	+	+	–	Acute infection
+	+	–	–	Chronic infection
–	+	–	–	Exposure with occult HBV, false positive (rare) or chronic infection
–	+	–	+	Exposure and clearance of HBV infection from the blood, with occult HBV

Modified from Weinbaum CM, et al. MMWR Recomm Rep. 2008;57:1–20

neonatal immunological responses to HBV (Gish and Locarnini 2009). Because these antibodies cross the placenta in utero, the neonate has high titers of maternal IgG anti-HBc at birth, but the antibodies are not neutralizing and do not appear to influence viral replication (Gish and Locarnini 2009).

Recent research has shown that with HBV exposure in utero, there are enhanced innate immune cell maturation, increased Th1 development, and lowered production of pro-inflammatory cytokines (IL-10, IL-6, IL-8, and TNF- α) along with higher production of IL-12p40, a state recently termed *trained immunity* by Hong and colleagues (Hong et al. 2015). Rather than the previous belief that HBV exploits the neonate's immature immune system and induces a state of immune tolerance in order to establish chronic infection, the findings of Hong et al. support the idea that defective priming of HBV-specific T cells may promote chronicity. Further countering the previously accepted idea of immune tolerance is the recent research by Vanwolleghem et al. showing that both B-cell responses and interferon responses are highly active during this phase of infection and that the immune system's antiviral sensing and effector machinery is working to control high levels of viral replication (Vanwolleghem et al. 2015).

The detection of IgM anti-HBc is generally diagnostic of acute HBV infection (Chau et al. 1983) (Fig. 4.3), but it is also seen in flares of HBV including reactivation (Yuan et al. 2015). Most patients with underlying inflammatory activity in the liver will show evidence of viral infection with one or more of the available tests. Relative levels of HBV DNA often correlate inversely with the degree of necroinflammatory activity in the liver, reflecting attempts by the host's immune response to control and eliminate the virus. The serum level of HBV DNA has been shown to be associated with the risk of developing HCC (Chen and Yang 2011; Chen et al. 2006; Tseng and Kao 2015). The REVEAL study, a prospective cohort study evaluating the risk factors for progression of chronic HBV, has helped researchers develop nomograms for the long-term risk of developing cirrhosis and HCC based on HBV DNA levels (Chen and Yang 2011).

HBeAg is detectable in the serum with active replication of wild-type virus or of mixed wild-type virus and HBV with core/precore mutations; however, mutant strains of HBV that replicate without producing HBeAg can be selected out (Frenette and Gish 2009). Thus, CHB patients can be divided into two subgroups,

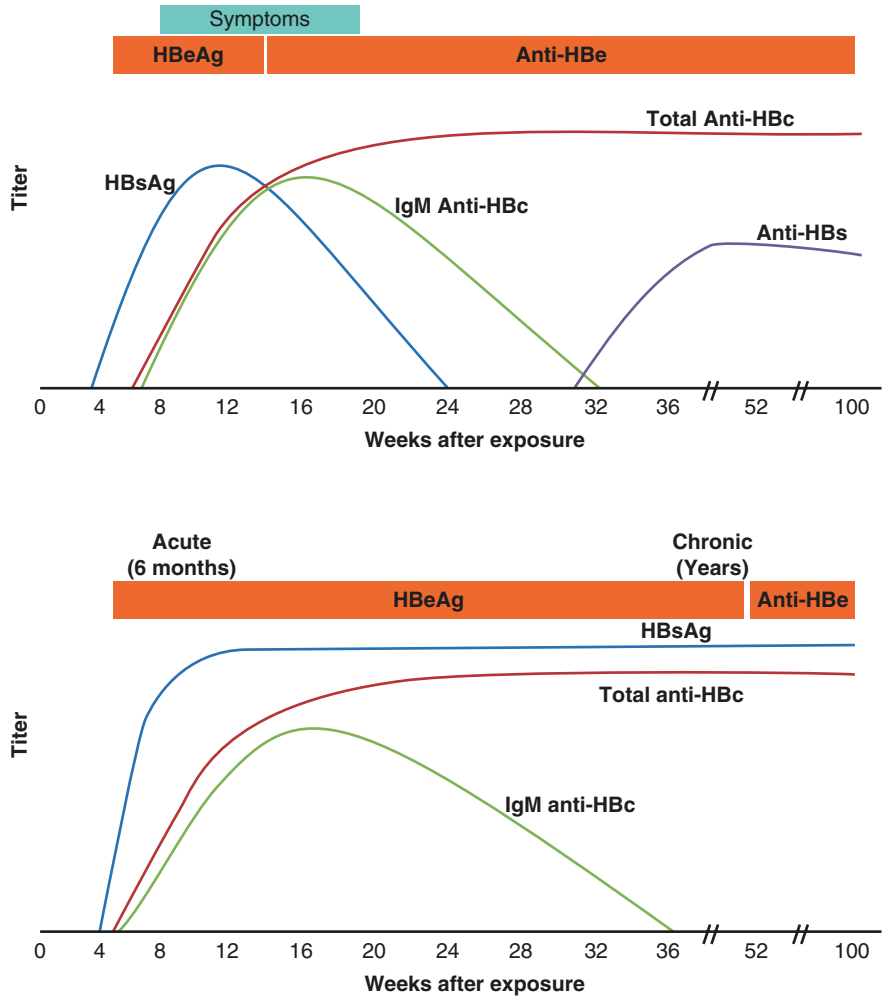


Fig. 4.3 Acute HBV infection with (a) recovery and (b) progression to chronic HBV. (a) Acute HBV infection with recovery: typical serologic course. (b) Acute HBV infection with progression to chronic HBV: typical serologic course. Adapted from Weinbaum CM, et al. MMWR Recomm Rep. 2008;57:1–20

HBeAg-positive (wild-type or mixed infection) and HBeAg-negative (basal core promoter and precore mutants) (Gish et al. 2015b). Interestingly, many adult patients who are HBeAg-positive have a mixed infection with both wild-type virus and virus with core promoter (CP) and/or, less commonly, precore (PC) mutations (Baqai et al. 2015; Takahashi et al. 1995). This may progress to a dominant (although not pure) HBeAg-negative disease. If infection is acquired from a person with mixed infection or HBeAg-negative disease, the person who becomes infected develops HBeAg-positive disease. Due to the high rate of mixed infection as well as HBeAg-negative disease, HBeAg seroconversion is no longer used as a marker for treatment

cessation (Frenette and Gish 2009). A team of researchers for the REVEAL study group found that seroclearance of HBeAg was not significantly associated with HCC risk (Liu et al. 2014). This may be associated with the high level of HBV DNA at HBeAg seroclearance. HBsAg clearance is the most favorable outcome in CHB (Yang and Kao 2016) and is the goal of treatment today (Frenette and Gish 2009). To further emphasize correct test interpretation, patients who are anti-HBs and anti-HBc-positive are not “naturally immune.” Rather, they carry a low level of HBV, similar to what is seen with herpes simplex virus, cytomegalovirus, and Epstein-Barr virus, and can have reactivation with immune suppression.

6 HBV Structure and Replication Cycle

HBV is an enveloped DNA virus with a partially double-stranded relaxed circular genome made up of 3200 base pairs. Four long open reading frames encode five proteins: HBsAg, HBcAg, HBeAg, DNA polymerase, and HBx protein (Demma and Dusheiko 2016). After internalization, the virus genome is converted into a covalently closed circular DNA (cccDNA) in the nucleus of the host hepatocytes and serves as the template for transcription of the four viral RNAs. cccDNA is a minichromosome in the nucleus of the infected hepatocytes and is multiplied by an amplification process during infection (Yan et al. 2012). Without cccDNA clearance from all cells, there is no HBV cure.

The multiple mRNAs produced from the cccDNA then produce a series of key viral proteins including the polymerase, hepatitis B x antigen (HBxAg), hepatitis B core-related antigen (HBcrAg), HBeAg, and HBsAg required for viral replication and full and partial virion formation. The capsid of HBV self-assembles into core particles that internalize the template mRNA required for the reverse transcription of HBV DNA. These capsid/DNA particles are then coated with HBsAg and liver cell membrane to form mature virus particles for release (Yan et al. 2012). This is measured in the blood with HBcrAg testing. Segments of HBV DNA are also incorporated into host DNA. This DNA incorporation is not a requirement for HBV replication; however, HBsAg is also produced from this pathway (Wooddell et al. 2015; Wooddell 2015). HBV DNA incorporation into the host genome in key areas of the genome is associated with later development of HCC (Kew 1981).

7 Phases of Infection

Chronic HBV can be divided into at least five major phases (Gish et al. 2015b) (Fig. 4.4). There are distinctive patterns of ALT, HBV DNA levels, HBeAg status, liver histology, and response to antiviral therapy that are associated with each phase (Gish et al. 2015b). The age of infection largely dictates the immune response against acute HBV infection and the subsequent natural history of chronic infection. These stages of infection are not static and may not be sequential, with movement

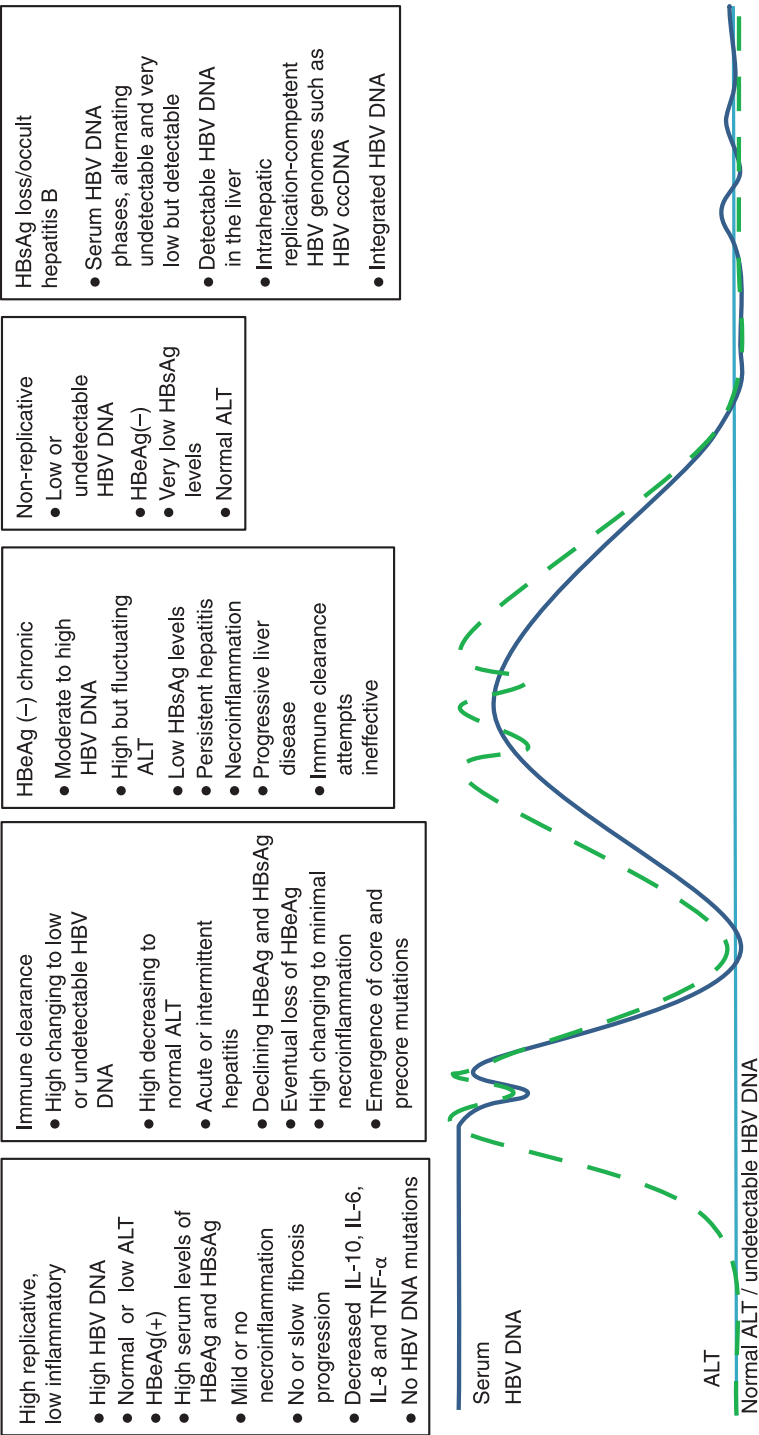


Fig. 4.4 Phases of hepatitis B infection

from one phase to another potentially occurring in any direction (Gish et al. 2015b). As recently described by Gish and colleagues, these phases can be defined as follows (Gish et al. 2015b):

- **High replicative, low inflammatory phase** (previously known as “immune tolerant”):
Characterized by high HBV DNA (>20,000 IU/mL), HBeAg-positive, normal or low levels of ALT, mild or no liver necroinflammation with lower production of IL-10 and pro-inflammatory cytokines (IL-6, IL-8, and TNF- α), and no or slow progression of fibrosis. In this phase the virus induces a state of trained immunity, enhancing the maturation of the innate immune cells (Hong et al. 2015).
- **Immune clearance:**
Characterized initially by wide fluctuations in serum HBV DNA and ALT, the latter resulting from acute changes in inflammation or intermittent episodes of hepatitis, with a decrease over time from high to low or undetectable DNA and from high to normal ALT (<30 IU/mL for men and <19 IU/mL for women), with eventual loss of HBeAg in wild-type infection. Liver histology ranges from high to minimal necroinflammation. In some patients, core and precore mutations and HBeAg-negative disease emerge.
- **HBeAg-negative chronic hepatitis:**
Characterized by persistent hepatitis with necroinflammation where attempts at immune clearance are ineffective, serum ALT levels can reach five times their normal value, HBV DNA levels are moderate to high, and liver disease is progressive.
- **Non-replicative** (previously known as “inactive carrier”):
Characterized by low or undetectable serum HBV DNA, HBeAg-negative, normal ALT, and the presence of HBsAg in serum. The clinical course of patients in this phase will be affected by the level of liver damage prior to HBeAg seroconversion to anti-HBe and the durability of the phase (Liaw et al. 2008). These patients are at risk of relapse to active liver disease and replication of virus as well as HBV DNA integration into the hepatocyte nuclear DNA (Fattovich 2003). They should be monitored at least every 6 months with serum tests for HBV replication, serology, liver enzymes, and synthetic tests (Demma and Dusheiko 2016).
- **Hepatitis B surface antigen (HBsAg) loss/occult hepatitis B phase:**
In occult hepatitis B infection (OBI), HBsAg is lost, but there is intrahepatic persistence of the entire, episomal, replication-competent HBV genomes such as HBV cccDNA chromatinized episomes. There is very low to undetectable HBV DNA in serum but detectable HBV DNA in the liver; in some patients all HBV serum markers are negative (Pollicino and Raimondo 2014). In a population of 1271 Alaska Natives with chronic HBV, the rate of HBsAg clearance was found to be 0.7% per year (McMahon et al. 2001; Simonetti et al. 2010). The incidence of HCC in patients who had cleared HBsAg was significantly lower than the rate in the HBsAg-positive individuals, but the risk of HCC was still present and periodic follow-up was warranted.

There are two additional proposed phases of HBV clearance which are theoretical and would be the ultimate goal of viral control (Gish et al. 2015b):

- Clearance of cccDNA
- Clearance of cells that have integrated HBV

In patients infected with HBV perinatally, the high replicative, low inflammatory phase is generally prolonged, with patients remaining in this phase for many years without disease progression (Lok 1992; Trepo et al. 2014). However, it is during this prolonged phase in perinatally infected persons that DNA integration into the hepatocyte genome most likely occurs, with its subsequent effect on oncogenicity. In HBeAg-positive children, seroconversion usually occurs around puberty (Lok and McMahon 2009).

In regions where HBV infection in adulthood generally occurs through sexual exposure or injection drug use, chronic infection is characterized by immediate entry into the immune clearance phase. In CHB patients with elevated ALT, the rate of spontaneous HBeAg seroconversion ranges from 8 to 12% per year (McMahon et al. 2001; Fattovich et al. 1986; Hoofnagle et al. 1981; Lok et al. 1987). Of those who achieve HBeAg seroconversion, up to 80% either transition to the non-replicative phase (with normal ALT and low or undetectable HBV DNA) or transition directly to HBeAg-negative chronic HBV (Bortolotti et al. 2006; Fattovich et al. 1986; Hoofnagle et al. 1981; Lok et al. 1987; Hadziyannis and Vassilopoulos 2001; Hsu et al. 2002). Among patients in the non-replicative phase, spontaneous reactivation has been shown to occur in 17% (defined as reversion from HBeAg-negative to HBeAg-positive) (McMahon et al. 2001) to 32% (defined as elevation in ALT and reappearance of markers of HBV replication as well as, in most cases, reversion to HBeAg-positive) (Davis et al. 1984) (Fig. 4.4).

Conclusion

Chronic hepatitis B virus continues to pose a very serious health-care problem all around the world and particularly in high-endemicity countries that have not yet fully implemented a national vaccination program beginning at birth. Widespread vaccination will most likely cause a shift in the most common modes of transmission, which will in turn affect the patterns of disease and infection phases. CHB is dynamic and the phases of infection may fluctuate. The next steps will be aimed at truly universal early vaccination for disease prevention, as well as targeting specific phases in order to prevent disease progression.

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John W. Ward and Pierre Van Damme

Abstract

Since 1982, safe and effective hepatitis B virus (HBV) vaccines have been commercially available first derived from plasma of HBV infected persons and later from yeast cells using recombinant DNA technology. Hepatitis B vaccines have an overall efficacy of 80–100% and provide virtually complete protection against acute and chronic hepatitis B infection among persons who complete the three dose vaccination series. Despite decrease or loss of serologic evidence of an effective immune response over time, immune protection from hepatitis B vaccination lasts for more than 25 years. New hepatitis B vaccines are in development to help achieve long-term protection in hepatitis B vaccine hyporesponsive populations (e.g., HIV-seropositive patients, the elderly) and to decrease the number of doses required to achieve an effective immune response. The greatest health impact is achieved through childhood vaccination, as children infected with HBV at birth or early childhood are at greatest risk for developing chronic HBV infection and HBV-related liver disease in later life. Globally, universal hepatitis B vaccination is recommended for all infants beginning preferably within 24 hours of birth, full immunization of infants by routine immunization programs in the first three years of life, and catch-up vaccination of unimmunized older children and adults. Evidence is growing that antiviral prophylaxis given in the last trimester of pregnancy to women with a high viral load of HBV can lower the risk for perinatal transmission of HBV. By 2010, as a result of multiple global initiatives, hepatitis B vaccination coverage among infants had

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reached an estimated 75% worldwide; coverage remains low in some countries and for newborns globally. Rates of HBV incidence, prevalence and liver cancer fall dramatically among vaccinated cohorts. As a result, hepatitis B vaccination is one of the most cost-effective public health interventions available, yielding a cost per life saved of \$4–\$36 in low-income countries. WHO has developed a framework for global action, with the goal of eliminating HBV and hepatitis C virus (HCV) as public health threats by 2030. Hepatitis B elimination can be achieved when countries implement comprehensive hepatitis B virus immunization programs including hepatitis B vaccine in national childhood immunization schedules, vaccinating newborns for hepatitis B and providing catch-up vaccination for children or adolescents, and adults.

Keywords

Hepatitis B • Hepatitis B virus (HBV) • Vaccination • Birth Dose • Immunization • Hepatocellular Carcinoma • Pre Exposure Prophylaxis • Post-Exposure Prophylaxis • Vaccine Efficacy • Adverse Events • Hepatitis B Elimination

1 Development of Hepatitis B Vaccines for Active Immunization

Safe and effective hepatitis B virus (HBV) vaccines have been commercially available since 1982. The American microbiologist [Maurice Hilleman](#) at [Merck](#) used three treatments ([pepsin](#), [urea](#), and [formaldehyde](#)) of blood serum together with rigorous filtration to yield a product that could be used as a safe vaccine. Hilleman hypothesized that he could make a hepatitis B vaccine by injecting hepatitis B surface protein. The first available vaccines were indeed produced by harvesting HBsAg (the 22-nm particle) from the plasma of persons with chronic HBV infection. Hepatitis B vaccine elicits development of antibody response to the *a* determinant, located in the surface antigen of HBV ([Coleman 2006](#)). Anti-HBs is the serologic marker of vaccine-induced protection. The efficacy of pre-exposure hepatitis B vaccination has been demonstrated in randomized, double-blind, placebo-controlled clinical trials involving high-risk groups such as men who have sex with men (MSM), healthcare workers, hemodialysis staff, and hemodialysis patients ([Crosnier et al. 1981](#); [Szmuness et al. 1982](#); [Desmyter et al. 1983](#); [Stevens et al. 1984](#); [Jack et al. 1999](#); [Szmuness et al. 1980](#); [Hadler et al. 1986](#); [Francis et al. 1982](#)). These studies demonstrated an overall efficacy of 80–100% and virtually complete protection against acute and chronic hepatitis B infection among persons who developed anti-HBs concentrations of 10 mIU/mL or greater, even if subsequently, over time, anti-HBs concentrations declined to less than 10 mIU/mL.

Subsequently, the development of recombinant DNA technology to express HBsAg in other organisms offered the potential to produce unlimited supplies of vaccine ([Sitrin et al. 1993](#); [Emini et al. 1986](#); [Stephenne 1990](#)), and nowadays recombinant DNA vaccines have completely replaced the plasma-derived vaccines

(Table 16.3). Hepatitis B vaccines are formulated to contain 2.5–40 μg of HBsAg protein and an aluminum phosphate or aluminum hydroxide adjuvant (World Health Organization 2001): 0.25 mg in pediatric-dosed vaccines and 0.5 mg in adult-dosed vaccines. A new recombinant hepatitis B vaccine that is intended for adult patients with renal insufficiency uses alum and lipid A as adjuvants (Beran 2008). Since 1999, hepatitis B vaccines available in industrialized countries have not contained thimerosal as a preservative because of ill-founded concerns about possible neuro-developmental effects of mercury (Centers for Disease Control and Prevention 1999). However, there is no evidence of any harmful effects from the small amounts of thimerosal in hepatitis B vaccine (Stratton et al. 2001). Thimerosal continues to be used as a preservative in multidose hepatitis B vaccine vials available in many countries and is particularly important in preventing bacterial contamination.

1.1 Recombinant DNA Vaccines

Most licensed recombinant DNA hepatitis B vaccines consist of a 226-amino-acid S gene product (HBsAg protein) (Sitrin et al. 1993). The yeast-produced vaccines, which are the most widely used, are manufactured by expression of HBsAg protein in genetically engineered yeast cells (*Saccharomyces cerevisiae*) that contain the S gene (McAleer et al. 1984). The expression plasmid generally contains only the 3' portion of the S gene, and only the major HBsAg protein, without pre-S epitopes, is produced. As a result of biochemical and biophysical purification, there is no detectable yeast DNA and only trace amounts of yeast proteins (1–5%) in the final vaccine products (DiMiceli et al. 2006). In the production of recombinant DNA hepatitis B vaccines, working seeds are derived from a master seed of the transformed yeast. Whenever a lot of vaccine is needed, yeast from the working seed is used to start the fermentation in large vessels. The HBsAg is then purified to eliminate yeast components by various physical separation techniques, including chromatography and filtration. The expressed HBsAg polypeptide self-assembles into immunogenic spherical particles closely resembling the natural 22-nm particles found in the serum of persons with chronic HBV infection. The *a* determinant that is responsible for the most important immune response is exposed on the surface of the artificial HBsAg particle, comparable to the natural particle. The artificial particles differ from natural particles only in the glycosylation of HBsAg.

In contrast to the production of yeast-derived non-glycosylated small envelope antigen, mammalian cells transfected with the gene coding for 2–3 of the HBV envelope proteins secrete the glycosylated viral surface antigen particles into the growth medium from which they are purified by physical means. Mammalian cell-derived vaccines (e.g., continuous mammalian cell line, like the mouse c127 clonal cell line) containing glycosylated pre-S1 and pre-S2 proteins, in addition to the major HBsAg protein, have also been produced in France, Germany, and Israel and are licensed in Israel, Hong Kong, India, the Philippines, and Vietnam (Shouval 2003; Zuckerman 2006). Evidence exists that vaccines containing the pre-S1 and pre-S2 proteins induce a faster and higher anti-HBs response as compared to yeast-derived S-containing vaccines and might be effective in persons who have genetic

nonresponse to the major HBsAg protein. However, the high cost of production for vaccines with pre-S1 and pre-S2 antigens limits their use and availability (Zuckerman et al. 2001; Rendi-Wagner et al. 2006; Shouval et al. 2015).

1.2 Combination Vaccines

Several vaccine manufacturers have produced combination vaccines containing a hepatitis B vaccine component, in particular pediatric vaccines including diphtheria and tetanus toxoids and whole-cell pertussis (DTwP) or acellular pertussis (DTPa), *Haemophilus influenzae* type b conjugate (Hib), or inactivated polio virus vaccine (IPV). For each of these combination vaccines, the manufacturer has shown that the components remain sufficiently immunogenic to elicit protective levels of anti-HBs (Diez-Delgado et al. 1997; Bruguera et al. 1996; West et al. 1997; Halperin et al. 2014).

2 Development of HBIG for Passive Immunization

The discovery that passively acquired anti-HBs can provide protection against acute hepatitis B and chronic HBV infection when administered soon after exposure led to the development and therapeutic use of HBIG, a specific immune globulin containing high concentrations of anti-HBs and later on to the development of hepatitis B vaccines. HBIG is prepared by fractionation from serum containing high concentrations of anti-HBs and that has been screened for HBsAg and antibodies to HIV and HCV. The process inactivates the potential presence of HIV and other viruses in the final product (Centers for Disease Control and Prevention 1986; Wells et al. 1986; Centers for Disease Control and Prevention 2011).

A major use of HBIG is as an adjunct to hepatitis B vaccine in preventing perinatal HBV transmission. Untreated, 70–90% of infants born to HBeAg-positive mothers become infected at birth and develop chronic HBV infection (Xu et al. 1985; Stevens et al. 1979). Immunoprophylaxis with both HBIG and hepatitis B vaccine increases the efficacy of preventing perinatal HBV transmission from 85 to 95% and provides long-term protection (Xu et al. 1985; Stevens et al. 1985; Schillie and Murphy 2013).

3 Vaccine Dosage, Schedule, and Duration of Protection

3.1 Vaccine Dosage

The quantity of HBsAg protein per dose that induces a protective immune response in infants and children varies by manufacturer (range, 2.5–10 µg) and by composition of the envelope protein(s) and is partially related to vaccine-production processes. In general, the vaccine dosage for infants and adolescents is 50% lower than that required for adults. There is no international standard of vaccine potency expressed in

micrograms of HBsAg protein, and the relative efficacy of different vaccines cannot be assessed only on the basis of differences in HBsAg content (Costa et al. 2011).

Given the differences in the manufacturing processes, the quantity of HBsAg protein per dose that will induce a protective immune response differs among the various vaccine products depending on the recipient's age. Vaccines produced by each manufacturer have been evaluated in clinical trials to determine the age-specific dosage that achieves the maximal seroprotection rate. Persons who respond to hepatitis B vaccine (complete and correct schedule) with concentrations of anti-HBs of 10 milli-international units (mIU)/mL or greater are protected against acute hepatitis B and chronic infection. Internationally marketed hepatitis B vaccines are considered immunologically comparable and can be used interchangeably (Seto et al. 1999).

Hepatitis B vaccine should be administered by intramuscular injection in the anterolateral aspect of the thigh of infants and children less than 24 months old and in the deltoid muscle of older children, adolescents, and adults. Administration in the buttock is not recommended because of an association with decreased protective antibody levels in some studies, probably because of inadvertent subcutaneous injection or injection into deep fat tissue (Shaw et al. 1989).

3.2 Vaccine Schedule

Historically, the standard three-dose hepatitis B vaccine series has consisted of two priming doses administered 1 month apart and a third dose administered 6 months after the first dose. Multiple schedules have been used successfully: at birth and at 1 and 6 months of age; at 2, 4, and 6 months of age; at 3, 5, and 11 months of age; at 8, 12, and 16 weeks and 12 or 15 months of age; and at 6, 10, and 14 weeks of age (in the World Health Organization's [WHO's] Expanded Program on Immunization [EPI] schedule) (Greenberg et al. 1996; Hadler and Margolis 1992; Coursaget and Kane 1993; Da Villa et al. 1997; Aspinall and Kocks 1998; Goldfarb et al. 1994; Yusuf et al. 2000). Increasing the interval between the first and second dose of hepatitis B vaccine has little effect on immunogenicity or final antibody concentration (Middleman et al. 2001; Jilg et al. 1989). Longer intervals between the last two doses result in higher final antibody concentrations but not seroconversion rates (Thisyakorn et al. 2011). An anti-HBs concentration of 10 mIU/mL or more measured 1–3 months after administration of the last dose of the primary vaccination series is considered a reliable marker of protection against infection. Protection persists even if anti-HBs concentration declines to less than 10 mIU/mL over time.

A variety of hepatitis B vaccine schedules have been shown to induce levels of seroprotection of >95% in infants. Hepatitis B vaccine schedules that have been demonstrated to induce seroprotection rates of >95% in adolescents include doses administered at 0, 1, and 6 months; 0, 2, and 4 months; and 0, 12, and 24 months (Jilg et al. 1989; Halsey et al. 1999; Cassidy et al. 2001; Schiff et al. 1995; Milne et al. 1988). In addition, for adolescents aged 11–15 years, the adult dose of hepatitis B vaccine can be used for administration at 0 and at 4–6 months (Centers for

Disease Control and Prevention 2000; Marsano et al. 1998). This two-dose schedule produces anti-HBs concentrations equivalent to those obtained with the pediatric dose administered on a three-dose schedule.

3.3 Duration of Protection

Hepatitis B vaccine induces a protective antibody response in approximately 30–55% of healthy adults aged <40 years after the first dose, in 75% after the second dose, and in more than 90% after the third dose (Zajac et al. 1986; Andre 1989). In adults older than 40 years, response rates decline with age; by age 60 years, protective levels of antibody develop in only 75% of vaccinated persons (Averhoff et al. 1998).

After primary immunization with hepatitis B vaccine, anti-HBs concentrations decline rapidly within the first year and more slowly thereafter. Among children who respond to a primary three-dose vaccination series with anti-HBs concentrations of 10 mIU/mL or greater, 15–50% have low or undetectable concentrations of anti-HBs 5–15 years after vaccination (Huang et al. 1999; Resti et al. 1997; Ding et al. 1993; Liao et al. 1999; Coursaget et al. 1994; Viviani et al. 1999; Mintai et al. 1993; Schönberger et al. 2013).

Among adult vaccinees, anti-HBs concentrations decline to less than 10 mIU/mL in 7–50% within 5 years after vaccination and in 30–60% within 9–11 years after vaccination (Hadler et al. 1991; Wainwright et al. 1997; Goh et al. 1995; Courouc   et al. 1988; Gibas et al. 1988). Despite decrease or loss of vaccine-induced anti-HBs over time, observational studies have shown that a primary series of hepatitis B vaccine can prevent infection for more than 25 years (Wu et al. 2011; But et al. 2008; Poovorawan et al. 2011; Roznovsky et al. 2010; Bialek et al. 2008; Fitzsimons et al. 2005; Kao et al. 2009; Su et al. 2007; Hammitt et al. 2007; van der Sande et al. 2007; van der Sande et al. 2006; Jafarzadeh and Montazerifar 2006; Alfaleh et al. 2008; FitzSimons et al. 2013; Gilca et al. 2013; Poovorawan et al. 2013; Spada et al. 2014; Zhu et al. 2011; Ni et al. 2012; Mendy et al. 2013; Shen et al. 2015; Bruce et al. 2016).

A recent follow-up and response to a hepatitis B vaccine challenge study in Alaska confirms earlier observation of long-term protection, showing evidence of protection (anti-HBs >10 IU/L or response to challenge dose) in 94% of persons and no chronic infections 30 years after vaccine administration (Bruce et al. 2016). This is similar to other long-term cohort studies conducted in other parts of the world carried out through 20–25 years of follow-up (Ni et al. 2012; Mendy et al. 2013). Indeed, in immunocompetent individuals, the specific immunity to HBsAg outlasts the presence of vaccine-induced antibodies, conferring effective long-term protection against acute disease and development of HBsAg carriage, even in those showing waning or disappearance of anti-HBs (Banatvala et al. 2000; Banatvala and Van Damme 2003; Jilg et al. 1990; West and Calandra 1996; Hall 2010). The mechanism for continued vaccine-induced protection is thought to be preservation of immune memory through selective expansion and differentiation of clones of antigen-specific B and T lymphocytes (Banatvala et al. 2000; Banatvala and Van Damme 2003).

To illustrate the prolonged duration of protection against hepatitis B even after disappearance of antibodies, an anamnestic response was measured shortly after offering a challenge dose (i.e., the “boostability”) in cohorts of vaccinees up to 30 years after primary vaccination (Hammitt et al. 2007; van der Sande et al. 2007; Jafarzadeh and Montazerifar 2006; Jan et al. 2010; Zanetti et al. 2005; Lu et al. 2004; Williams et al. 2003; Boxall et al. 2004; Gabbuti et al. 2007; Duval et al. 2005; Samandari et al. 2007; Lu et al. 2008; Zinke et al. 2009). Anamnestic responses have been demonstrated in 62% to more than 80% of subjects vaccinated 15–30 years earlier, indicating that a high proportion of vaccine recipients retain immune memory and would develop an anti-HBs response on exposure to HBV (Bruce et al. 2016).

Recently, increasing evidence suggests that the immune memory may begin to wane after the second decade of vaccination; however, this does not imply increased susceptibility to clinically significant HBV disease, as no acute or chronic HBV cases were reported in any of the ongoing follow-up studies (Bialek et al. 2008; Su et al. 2007; Jan et al. 2010; Samandari et al. 2007; Lu et al. 2008). Thus absence of an anamnestic response after such booster vaccination may not necessarily mean that individuals are susceptible to HBV infection (Hammitt et al. 2007; Leuridan and Van Damme 2011). Further research in this area is needed.

Some long-term studies have documented breakthrough infections illustrated by the seroconversion to anti-HBc, but virtually no clinically significant infections (acute diseases or carriage) were reported (Wainwright et al. 1997; van der Sande et al. 2006; Jafarzadeh and Montazerifar 2006; Lu et al. 2004; Boxall et al. 2004; Lu et al. 2008; Dentinger et al. 2005; McMahon et al. 2005; Mele et al. 2001; Young et al. 2003; Poovorawan et al. 2009). From a public health perspective, prevention of carriage remains of utmost importance: in a 20-year evaluation of the hepatitis B universal immunization program in the Gambia, Viviani and coworkers showed a vaccine efficacy of 67% against development of anti-HBc and 97% against HBsAg carriage (Viviani et al. 2008). In a more recent evaluation of the infant vaccination program in the Gambia, a 94% vaccine effectiveness was observed in fully vaccinated infants. Many of these fully vaccinated individuals had at some time been infected and experienced a nonsignificant breakthrough infection (anti-HBc positivity of 27%); chronic active hepatitis was not common and probably a consequence of perinatal infection. The study concluded that full infant HBV vaccination does provide strong protection against chronic HBV infection but less protection against ever having HBV infection (Peto et al. 2014). Persons who have received only one or two doses of vaccine can also have detectable anti-HBs. However, long-term protection has been demonstrated only for immunocompetent persons who have completed a licensed vaccination series and have ever had an anti-HBs concentration of 10 mIU/mL or greater (Leuridan and Van Damme 2011).

On the basis of currently available scientific evidence, WHO and advisory groups in the United States and Europe do not recommend routine booster doses of hepatitis B vaccine or periodic serologic testing to monitor anti-HBs concentrations for immunocompetent persons who have responded to vaccination or in universal immunization programs (Fitzsimons et al. 2005; CDC 1991; European Consensus Group on Hepatitis B Immunity 2000). For the next decade, additional data are

needed regarding the potential need for boosters and the duration of protection against infection and disease after hepatitis B vaccination, including information on the potential role of natural subclinical boosting.

4 Vaccine-Associated Adverse Events

Adverse events after immunization against hepatitis B are infrequent and generally mild. With the exception of localized pain, placebo-controlled studies have revealed that reported events (e.g., myalgia and transient fever) occur no more frequently among vaccinees than among persons receiving placebo (<10% among children, 30% among adults) (World Health Organization 2011). Data from numerous long-term studies fail to causally link serious adverse events to hepatitis B vaccination (Institute of Medicine 2002). Reports of severe anaphylactic reactions are very rare, and data do not indicate a causal association between hepatitis B vaccine and Guillain-Barré syndrome or demyelinating disorders, including multiple sclerosis. However, hepatitis B vaccine has been the subject of greatest concern regarding multiple sclerosis in France. In 1998, French media revealed possible occurrence of post-hepatitis B immunization multiple sclerosis. That same year the French health authorities abruptly ended routine school-based vaccination of adolescents, and adult vaccination began to be less widespread. Several epidemiologic studies conducted to evaluate the association between hepatitis B vaccination and multiple sclerosis have found no link. A recent nested case-control study using data from a US health maintenance organization (2008–2011) showed no longer-term association between hepatitis B vaccination and the risk of multiple sclerosis or other acquired central nervous system demyelinating syndromes, which argues against a causal association (Langer-Gould et al. 2014). Furthermore, no epidemiologic data support a causal association between hepatitis B vaccination and chronic fatigue syndrome, arthritis, autoimmune disorders, asthma, sudden infant death syndrome, alopecia, and diabetes (Mikaeloff et al. 2007; Yu et al. 2007; Duclos 2003; Schwalbe et al. 1998). WHO's Global Advisory Committee on Vaccine Safety and the recent report of the Institute of Medicine confirm the excellent safety profile of hepatitis B vaccine and continue to monitor the safety of this vaccine (Stratton et al. 2011).

5 Development of New Hepatitis B Vaccines

Existing hepatitis B vaccines are highly effective, and there is no evidence that new vaccines will be needed to eliminate HBV transmission in immunocompetent populations with recommended immunization strategies. Potential uses for new hepatitis B vaccines include enhancing seroprotection in nonresponders to existing vaccines (using pre-S1 and/or pre-S2 or adding new vaccine adjuvants instead of aluminum hydroxide) (Shouval et al. 2015; Rottinghaus et al. 2003; Jacques et al. 2002; Cooper and Mackie 2011; Leroux-Roels 2015; Janssen et al. 2015a; Said and Abdelwahab 2015), decreasing the number of doses required for seroprotection, and

providing protection against HBV infection with S-mutant viruses (Zuckerman 2006; Janssen et al. 2015a, b; Shapira et al. 2001).

The addition of CPG 7909 to hepatitis B vaccine is promising, as the immunostimulatory activity of this adjuvant can help in achieving long-term protection in hepatitis B vaccine hyporesponsive populations (e.g., HIV-seropositive patients, hemodialysis patients, oncologic patients, persons who are obese, and the elderly) (Janssen et al. 2015a, b; Janssen et al. 2013; Plotkin and Schaffner 2013). Such experimental vaccines—currently under examination for licensure in the United States—have been shown to be effective in patients with renal failure and on hemodialysis, nonresponders to conventional yeast-derived vaccines, and immunocompetent healthy individuals receiving only two vaccine doses instead of three (Zuckerman 2006; Shouval et al. 2015; Janssen et al. 2015a, b; Shapira et al. 2001). A two-dose schedule (4 weeks apart) was shown to trigger higher seroprotective rates compared with the classical three-dose schedule (0, 4, and 24 weeks) with a conventional hepatitis B vaccine (Heyward et al. 2013; Halperin et al. 2012). It has been hypothesized that a lack in vaccine efficacy might be explained by a mismatch between vaccine genotype and endemic HBV genotype (adw versus ayw); if this hypothesis is proven, the use of a modified HBV vaccine in specific regions could be considered (Davies et al. 2013; Littlejohn et al. 2014). Vaccines generating strong T-cell immunity with purified or recombinant vaccine antigens, along with intradermal, oral, and nasal hepatitis B vaccines that have potential to simplify administration and cost less than injections, are also under investigation (Langer-Gould et al. 2014; Filippelli et al. 2014; Streatfield 2005; Rajkannan et al. 2006; Coffman et al. 2010; Almeida and Borges 2015).

To date, chronic hepatitis B remains an incurable disease. Despite the tremendous progress made in developing nucleoside/nucleotide analogs capable of efficiently suppressing HBV replication, these agents do not eliminate the cccHBV-DNA residing in the nuclei of infected hepatocytes. Consequently, developing therapeutic immunization for persons with chronic HBV infection and restoring the defective immune tolerance to HBV remain important goals (Michel et al. 2015; Kosinska et al. 2015).

6 Recommendations for Hepatitis B Vaccination

HBV infection can occur at any age through multiple routes of transmission, and all persons at risk for HBV infection can benefit from immunization. However, the greatest health impact is achieved through childhood vaccination, as children infected with HBV at birth or early childhood are at greatest risk for developing chronic HBV infection and HBV-related liver disease in later life. Approximately 90% of HBV-infected infants <1 year of age develop chronic HBV infection compared with 30% of children infected between ages 1 and 4 years and <5% of persons infected as adults (Edmunds et al. 1993). Approximately 25% of persons who became chronically infected during childhood are at risk for death from HBV-associated liver cancer and cirrhosis (Beasley and Hwang 1991), a risk that is highest for children infected at birth. As a result, an estimated 21% and 48% of deaths from hepatitis are attributed to HBV infections occurring at birth or in early childhood, respectively (Goldstein et al. 2005).

Global recommendations for HBV prevention and control are set by WHO in consultation with the Strategic Advisory Group of Experts on Immunization (SAGE). In 2009, WHO recommended national programs for hepatitis B immunization (World Health Organization 2009) to include (a) universal vaccination of infants within 24 h of birth, (b) full immunization of infants by routine immunization programs, and (c) catch-up vaccination of unimmunized cohorts; WHO also recommended that individual countries develop goals for the control of HBV based on local prevalence, incidence, and epidemiology.

7 Recommendations for Pre- and Postexposure Prophylaxis

7.1 Infants Born to HBsAg-Positive Mothers

For infants born to HBsAg-positive mothers, protection increases to 95% when hepatitis B vaccination is augmented with a dose of hepatitis B immunoglobulin (HBIG) (Xu et al. 1985; Stevens et al. 1985; World Health Organization 2009; Lee et al. 2006; Schillie et al. 2015; Kubo et al. 2014). The United States and other countries recommend HBsAg screening of pregnant women and delivery of both HBIG and hepatitis B vaccination immediately after birth to infants of HBsAg-positive mothers. In the United States, newborns of HBsAg-positive women are recommended to receive both HBIG and hepatitis B vaccination within 12 h of birth.

7.2 HBeAg-Positive Pregnant Women

Evidence is growing that antiviral prophylaxis given in the last trimester of pregnancy to HBeAg-positive women or women with a high viral load of HBV can lower the risk for perinatal transmission of HBV (Pan and Lee 2013; Deng et al. 2012; Greenup et al. 2014; Pan et al. 2016; Shi et al. 2010; Bzowej 2010; Dionne-Odom et al. 2015; Bleich and Swenson 2014; Ayres et al. 2014). Infants born to these women are at greatest risk for “breakthrough” HBV infection despite receiving HBIG and hepatitis B vaccination. In a recent trial involving pregnant women with high viral loads (i.e., >200,000 IU/ml) and their infants (all of whom received HBIG/hepatitis B vaccine within 12 h of birth), HBV transmission was detected in only 5% of infants born to mothers receiving antiviral prophylaxis compared with 18% of infants who received HBIG/hepatitis B vaccination alone (Pan et al. 2016).

7.3 Persons with Percutaneous or Sexual Exposures

HBIG is indicated for postexposure prophylaxis (PEP) after needlestick or other percutaneous injuries (Seeff et al. 1978; Grady et al. 1978) and for preventing clinical hepatitis B or chronic HBV infection after sexual exposure to an acutely infected

partner when administered within 7 days of exposure (Redeker et al. 1975). Both passive-active PEP with HBIG and hepatitis B vaccine and active PEP with hepatitis B vaccine alone have been demonstrated to be highly effective in preventing transmission after exposure to HBV (Andre and Zuckerman 1994; Roumeliotou-Karayannis et al. 1985; Papaevangelou et al. 1988; Mitsui et al. 1989).

8 Recommendations for Pre- and Postvaccination Serologic Testing

Globally, pre-vaccination serologic testing is not recommended as routine practice. However, serologic screening with a single test (anti-HBc) or with a panel of tests (e.g., anti-HBs and HBsAg) can identify HBsAg-positive persons in need of evaluation for chronic HBV and related liver disease while also defraying costs by avoiding unnecessary vaccination of persons already infected with HBV. In the United States, pre-vaccination testing is considered cost-effective in adult populations with an HBV prevalence >20% (e.g., incarcerated persons) (Mast et al. 2006). In addition, CDC recommends HBsAg testing for persons of all ages born in countries or regions with high or moderate HBV endemicity and children with at least one parent from these areas; persons who have household, sex, and needle-sharing contacts of HBsAg-positive persons; MSM; and persons who inject drugs (Mast et al. 2006; Weinbaum et al. 2008).

Routine testing for HBV after hepatitis B vaccination is not typically recommended. Immunocompetent persons typically respond to hepatitis B vaccination, develop long-term protection, and do not need further periodic testing to assess anti-HBs levels. However, postvaccination testing is recommended for populations of immunosuppressed persons (e.g., chronic hemodialysis patients), for certain populations for whom knowledge of immune status is important for medical management (e.g., infants born to HBsAg-positive mothers), and for persons for whom documentation of protection is needed (e.g., healthcare workers) (European Consensus Group on Hepatitis B Immunity 2000; Mast et al. 2006).

9 Strategies for Increasing Hepatitis B Vaccination of Newborns

Given the strong association between risk for chronic HBV infection, liver disease, and age at infection, hepatitis B vaccination is most important for children aged <5 years, particularly newborns. Vaccination of children in this age group requires an immunization strategy unique to hepatitis B prevention: delivery of a “birth dose” immediately after birth of the child. Among infants born to HBsAg-positive mothers, a birth dose of hepatitis B vaccine reduces the risk for perinatal HBV transmission by at least 72% (Schillie and Murphy 2013); additional receipt of HBIG increases efficacy to 95%–97%. The timing of the delivery of hepatitis B vaccination is important, because protection afforded by vaccination wanes over time. Accordingly, WHO

recommends a dose of hepatitis B vaccine be provided to all infants as soon as possible after birth, preferably within 24 h (World Health Organization 2009).

Ensuring that all infants receive a dose of hepatitis B vaccine within the first 24 h of life requires integration of hepatitis B vaccination with other maternal and child health services provided by birth attendants. As such, delivery of a birth dose of hepatitis B vaccine is most readily achieved when deliveries are managed in birthing facilities. In Laos from 2011 to 2012, 70% of infants delivered in 31 birth facilities received a birth dose of hepatitis B vaccine, whereas only 41% of newborns delivered at home were vaccinated at birth despite provision of assistance by 17 healthcare facilities. Birth-dose coverage increases when (a) birth attendants are trained to deliver hepatitis B vaccination to infants within 24 h of birth, (b) standing orders are in place for the routine vaccination of newborns, and (c) a system of data collection is in place to enable monitoring and evaluation of immunization efforts (Liang et al. 2009; Mast et al. 2005).

In many areas of the world with high prevalence of HBV infection, most births occur at home rather than in healthcare settings. Interventions that improve birth-dose coverage for home births include training for birth attendants, systems to notify attendants of pending deliveries, and education to help communities understand the benefits and safety of hepatitis B vaccine (Creati et al. 2007; Nguyen et al. 2014; Wu et al. 2015; Levin et al. 2005). Because hepatitis B vaccines are heat stable, they can be used outside the cold chain for newborn vaccination in settings that lack refrigeration, including patient's homes (Averhoff et al. 1998).

10 Strategies for Increasing Hepatitis B Vaccination of Children, Adolescents, and Adults

WHO recommends all infants receive hepatitis B vaccine as part of the routine infant immunization schedule (see Dosage, Schedule, and Duration of Protection). Over time, as cohorts of vaccinated children age, an increasing proportion of the population is immune to HBV infection, further helping reduce new infections. "Catch-up" vaccination strategies can expedite this process by targeting older children, adolescents, and adults too old to receive vaccine when routine hepatitis B vaccination of infants began.

Mandatory hepatitis B vaccination for school and university entry can increase acceptance of hepatitis B vaccination and vaccination coverage among older children and adolescents. Vaccination programs also can target adolescents and adults with behavioral or occupational risks for HBV infection. Populations identified by WHO as possible targets for hepatitis B vaccination programs include recipients of blood or blood products, dialysis patients, recipients of solid organ transplantations, incarcerated persons, persons who inject drugs, household and sexual contacts of people with chronic HBV infection, people with multiple sexual partners, and healthcare workers and others with occupational exposures to blood and blood products. WHO also recommends that travelers to HBV-endemic areas complete the hepatitis B vaccination series before departure (World Health Organization 2009).

The integration of hepatitis B vaccination into settings where persons at risk for HBV infection receive care (e.g., HIV and correctional care settings) can improve vaccination coverage (Mast et al. 2006). Employee standards and mandates improve hepatitis B vaccination coverage among healthcare workers, first responders, and other groups at risk for occupational exposure (Lehman et al. 2012; Agerton et al. 1995; Roup 1993).

11 Hepatitis B Vaccine Coverage

Hepatitis B vaccine has been available since the early 1980s. However, the cost of vaccine, concerns about plasma-derived vaccines transmitting the agent causing AIDS, and the lack of global vaccine policies impeded efforts to immunize infants and children against hepatitis B. By 1991, only 20 countries had implemented routine infant immunization against hepatitis B (Kane 1998).

In 1992, WHO recommended all countries incorporate hepatitis B vaccination into their national childhood immunization services (Kane 1998; World Health Organization 1992), and by 1998, a total of 90 countries were routinely administering hepatitis B vaccine to infants. However, by the end of 2000, only 32% of the global birth cohort had received three doses of hepatitis B vaccine, with most unvaccinated infants living in developing countries (Beeching 2004).

In the following decade, hepatitis B vaccination coverage grew rapidly. By 2010, hepatitis B vaccination coverage among infants had reached an estimated 75% worldwide (CDC 2011a). Contributing to improvements in vaccination coverage was availability of low-cost recombinant vaccines, which decreased the price of hepatitis B vaccine from \$3 to \$6 per dose in the 1990s to \$0.25 per dose over the following decade (Zuber et al. 2011). Further, hepatitis B vaccine was originally available only in single-antigen formulations. By the early 2000s, HBV antigens were incorporated into combination vaccines (first as tetravalent [DTwP-HepB] and then pentavalent [DTwP-HepB-Hib] formulations), greatly simplifying vaccine delivery.

In 2000, the GAVI Alliance (previously the Global Alliance for Vaccines and Immunization) was formed to help low-income countries purchase and deliver childhood vaccinations. The first GAVI-supported vaccine initiative was hepatitis B vaccination. From 2000 through 2008, GAVI facilitated the immunization of 194 million (68%) of the 286 children fully immunized against hepatitis B during this period. By 2004, 50% of the low-income countries receiving GAVI Alliance support had included hepatitis B vaccines in their routine immunization program, and by the end of 2014, all 73 GAVI-eligible countries had done so (GAVI n.d.).

In China, GAVI Alliance collaborations resulted in administration of a birth dose of hepatitis B vaccine, free of charge, to more than 25 million newborns living in the poorest and most remote provinces of western and central China; this initiative was expanded in 2002 to provide free hepatitis B vaccine to all newborns born in China (Cui et al. 2013). Similar GAVI collaborations have occurred worldwide. It is anticipated that by 2020, hepatitis B vaccination in GAVI-eligible countries will have averted more than 4.8 million HBV-related deaths (Lee et al. 2013).

As of 2015, hepatitis B vaccine for infants had been introduced nationwide in 184 WHO Member States (World Health Organization 2015). Global coverage with three doses of hepatitis B vaccine is now estimated at 83% and is as high as 90% in the Western Pacific. In addition, 96 countries have implemented recommendations for a birth dose of vaccine, resulting in 39% of infants worldwide receiving a first dose of hepatitis B vaccine within 24 h of birth (<http://www.who.int/mediacentre/factsheets/fs204/en/>).

12 Impact of Vaccination

Hepatitis B vaccines are now affordable, and effective prevention strategies are being deployed worldwide. Hepatitis B vaccination is now one of the most cost-effective public health interventions available, yielding a cost per life saved of \$4–\$36 in low-income countries (Miller and McCann 2000). Indeed, in a report to the UN secretary-general, delivery of hepatitis B immunization was considered a “best buy” intervention because it is inexpensive, highly cost-effective, and culturally acceptable. Cost-benefit analysis has shown that perinatal hepatitis B prevention and routine infant hepatitis B immunization can save a net \$1 in medical and work-loss costs for each \$1 spent on immunization (Margolis et al. 1995). Other vaccine-based strategies, including universal adolescent vaccination and vaccination of at-risk adult populations, also have been found to be cost-effective or cost-saving (Hu et al. 2008; Beutels et al. 2002; Tu et al. 2009).

Many studies conducted in high-HBV-endemicity areas have demonstrated declines in the prevalence of chronic HBV and related disease after implementation of routine infant immunization (Chen 2009). For example, Taiwan, a country with previously high endemicity for hepatitis B, substantially decreased the burden of hepatitis B after the 1984 launch of routine hepatitis B vaccination of newborns. From 1984 to 2004, the HBsAg prevalence in persons <20 years of age decreased from 9.8 to 0.6% (Chen 2009; Ni et al. 2007; Chen et al. 1996). In 1984, the Gambia implemented a universal infant immunization program. By 2003, childhood HBsAg prevalence had decreased in that country from 10 to 0.5% (Da Villa et al. 1997; Viviani et al. 1999; Peto et al. 2014; Whittle et al. 2002). In Malaysia, after hepatitis B vaccination was implemented, HBsAg seroprevalence among children aged 7–12 years decreased from 1.6% (1997) to 0.3% (2003) (Ng et al. 2005).

China, another highly endemic country, has also dramatically reduced HBV prevalence through vaccination. By 2006, HBsAg prevalence in that country had declined to <1% among children 1–4 years of age (Liang et al. 2009; Hadler et al. 2013). During 1992–2009, hepatitis B vaccination in China prevented an estimated 24 million chronic HBV infections and 4.3 million future deaths due to cirrhosis, hepatocellular carcinoma, and acute hepatitis (Hadler et al. 2013).

Routine hepatitis B vaccination has also impacted rates of HBV infection in non-endemic countries. In 1991, Italy implemented universal infant and adolescent hepatitis B vaccination. Since introduction of routine hepatitis B vaccination, the overall incidence of acute hepatitis B declined from 5 cases per 100,000 in 1990 to 0.9 cases in 2010 (Romanò et al. 2012).

Routine hepatitis B vaccination for infants was implemented in the United States during 1991 (CDC 1991). In future years, this strategy was expanded to include recommendations for catch-up vaccination of older children and adolescents (1996), all newborns (2006), and persons with diabetes (2011) (Mast et al. 2006; Mast et al. 2005; CDC 2011b). These recommendations resulted in coverage of hepatitis B vaccination exceeding 90% among young children and adolescents (Hill et al. 2016; Reagan-Steiner et al. 2016). However, hepatitis B vaccination coverage remains low among adults, with less than one third of persons 19–49 years of age reporting receipt of hepatitis B vaccination (Williams et al. 2016).

Improvements in vaccination have contributed to large declines in the incidence of hepatitis B. From 1991 through 2014, cases of acute hepatitis B in the United States declined from an estimated 200,000–300,000 cases per year to approximately 19,000 acute hepatitis B cases in 2014, reflecting a 90% decline in HBV incidence (CDC 2014). National health surveys of the noninstitutionalized US population demonstrate increases in the number of persons with evidence of vaccine-induced seroprotection from 58 million in 1999–2006 to 69 million in 2007–2002 (Roberts et al. 2015). These surveys also found decreases in the prevalence of chronic hepatitis B among persons >20 years of age, reflecting improvements in hepatitis B vaccination coverage in younger age populations (Roberts et al. 2015). However, since 1988, the overall prevalence of persons infected with hepatitis B has not changed significantly (Roberts et al. 2015; McQuillan et al. 1999; Coleman et al. 1998). This trend in hepatitis B prevalence is a consequence of the migration of HBV-infected persons to the United States, particularly from Asia and Africa (Roberts et al. 2015; Kowdley et al. 2012; Mitchell et al. 2011). Improvements in hepatitis B vaccination not only impact countries with moderate-to-high prevalence of hepatitis B but benefit the United States and other low-endemic countries receiving migrants from areas of the world with higher HBV prevalence.

13 Targets and Goals for Eliminating New Cases of HBV

Elimination of hepatitis B virus transmission is an achievable public health goal given the long experience with hepatitis B vaccination and demonstrated effectiveness and safety of hepatitis B vaccine (CDC 1991, 1992, 1993, 1999; Ghendon 1990). Over 95% of persons who complete the three-dose vaccination series achieve immunoprotection (World Health Organization 2009; Mast et al. 2006), with levels of protection persisting for at least 23–30 years after vaccination; a booster dose is rarely recommended (Wu et al. 2011; But et al. 2008; Poovorawan et al. 2011; Roznovsky et al. 2010; Bialek et al. 2008; Fitzsimons et al. 2005; Kao et al. 2009; Su et al. 2007; Hammitt et al. 2007; van der Sande et al. 2006, 2007; Jafarzadeh and Montazerifar 2006; Alfaleh et al. 2008; FitzSimons et al. 2013; Gilca et al. 2013; Poovorawan et al. 2013; Spada et al. 2014; Zhu et al. 2011; Ni et al. 2012; Mendy et al. 2013; Shen et al. 2015; Bruce et al. 2016; Jan et al. 2010; Zanetti et al. 2005; Lu et al. 2004; Williams et al. 2003; Boxall et al. 2004; Gabbuti et al. 2007; Duval et al. 2005; Samandari et al. 2007; Lu et al. 2008; Zinke et al. 2009; Leuridan and Van Damme 2011; Dentinger et al. 2005; McMahon et al. 2005; Mele et al. 2001;

Young et al. 2003; Poovorawan et al. 2009; Viviani et al. 2008; Spradling et al. 2015; Middleman et al. 2014). Hundreds of millions of persons of all ages have been vaccinated over several decades with hepatitis B vaccines. Over successive generations, hepatitis B will become a progressively rare disease.

In September 2005, the WHO Regional Office for the Western Pacific Region (WPRO) became the first WHO region to adopt a goal of hepatitis B control. WPRO aimed to reduce the prevalence of chronic HBV infection to <2% in children at least 5 years of age by 2012, with an ultimate goal of an HBsAg seroprevalence of <1% in children ≤ 5 years of age (Rani et al. 2009). By 2014, WPRO member countries averaged 92% coverage rate for three doses of hepatitis B vaccine, and average birth-dose coverage was 81%. As a result, the prevalence of HBV infection has fallen in all countries in the region, resulting in an 89% decline in the regional prevalence of HBV infection (Wiesen et al. 2016).

Linking hepatitis B vaccination with other global health initiatives can help sustain existing hepatitis B vaccination programs. For example, because hepatitis B vaccination prevents liver cancer and cirrhosis, United Nations' immunization efforts have been linked to activities to reduce chronic, noncommunicable diseases. The UN General Assembly recognizes noncommunicable disease as an emerging global epidemic that is impeding achievement of UN Millennium Development Goals (United Nations 2011).

In 2010, WHO member states adopted Resolution WHA63.18 (World Health Organization 2010), designating July 28 as World Hepatitis Day and calling for comprehensive prevention and control of hepatitis B virus infection and development of time-specific immunization goals. Four years later, in response to a request from the World Health Assembly, WHO developed a framework for global action, with the goal of eliminating HBV and hepatitis C virus (HCV) as public health threats by 2030. This strategic framework encompasses a comprehensive set of interventions for eliminating HBV and HCV transmission and disease as public health threats. WHO set goals for a 90% reduction in HBV transmission and 65% reduction in HBV mortality by 2030, with interim goals of 30% and 10% reductions by 2020, respectively. To achieve these goals, WHO is calling on countries to implement a comprehensive hepatitis B virus immunization program with four attributes: (a) including hepatitis B vaccine in national childhood immunization schedules, (b) strengthening hepatitis B birth dose, (c) providing catch-up vaccination for children or adolescents with low coverage, and (d) offering hepatitis B virus vaccination to people who are at increased risk of HBV transmission (World Health Organization 2017). To reach reductions in HBV incidence and deaths, targets for service delivery include 90% and 50% coverage of a third dose and birth dose of hepatitis B vaccine, respectively, with an increase to 90% birth-dose coverage by 2030. In May 2016, the WHA approved the framework and the proposed hepatitis B elimination targets.

In the United States, the National Academies of Sciences, Engineering, and Medicine (NAS) convened a consensus committee to examine the feasibility of setting goals for the elimination of hepatitis B and C as public health threats and to propose a strategy for and actions needed to reach national elimination goals. In May 2016, the committee released preliminary conclusions that given political will and

sufficient resources, hepatitis B could be eliminated as a public health problem in the United States (National Academies of Sciences, Engineering, and Medicine 2016).

Universal infant vaccination beginning at birth on a global scale, along with targeted efforts to vaccinate all children and adults at risk for HBV infection, can reduce the risk for HBV infection to zero. Infant immunization programs successfully implemented by almost all countries provide the framework for such elimination, although continued high-vaccination coverage will be required for multiple generations to achieve this goal. Expanded access to diagnostic testing and HBV care and treatment provide additional opportunities to prevent both HBV transmission and related disease. These activities require commitment from public health organizations and expansion of public health resources. Only through continued recognition of the large global burden of disease, demonstration of the benefits of vaccination, and continued patience can the goals of vaccine-associated disease reduction, including the elimination of hepatitis B transmission, be realized.

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Abstract

The clinical outcomes of chronic hepatitis B virus (HBV) infection are heterogeneous, ranging from spontaneous seroconversion of hepatitis B surface antigen (HBsAg) to severe detrimental consequences, including hepatic failure, cirrhosis, and hepatocellular carcinoma (HCC). Four distinctive clinical phases are recognized in the natural course of chronic hepatitis B (CHB), namely, immune tolerance, immune clearance, inactive carrier, and HBV reactivation phases. Patients with prolonged immune active phases are prone to develop cirrhosis and HCC. Conventionally, serum HBV DNA level and HBeAg serostatus combined with serum ALT level are utilized to distinguish the disease states in the natural history of CHB. A number of other viral factors, including HBV genotype, several naturally occurring viral mutations as well as serum levels of HBsAg, HBV core-related antigen (HBcrAg), and total anti-HBc antibody, have been also associated with the long-term prognosis of CHB patients. All these qualitative and quantitative viral factors contribute to the disease progression. With the advance of technology, more new viral biomarkers may emerge in the future. Combination of all current and new viral factors may allow for more sophisticated delineation of disease states, the deeper mechanistic insight into the pathogenesis, and the more optimal management for patients.

Keywords

Hepatitis B virus • Chronic hepatitis B • Natural history • Viral factors

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1 The Natural History and Disease Progression of Chronic Hepatitis B

Chronic hepatitis B (CHB) undergoes prolonged and complex virus-host interactions and exhibits heterogeneous clinical outcomes, ranging from spontaneous seroconversion of hepatitis B surface antigen (HBsAg) to severe detrimental consequences, including hepatic failure, liver cirrhosis, and hepatocellular carcinoma (HCC). Conventionally, based on the degree of hepatic necroinflammation, the serostatus of hepatitis B e antigen (HBeAg), and the HBV DNA level, the natural history of CHB is characterized as four distinctive phases, including the immune tolerance, immune clearance, inactive carrier, and reactivation of HBV (Yang et al. 2002; Fattovich et al. 2008; Liaw and Chu 2009; McMahon 2009; Yang and Kao 2016). In the immune tolerance phase, patients have very high levels of HBV DNA, normal ALT, and positive HBeAg. Upon the entry to the immune clearance phase, HBsAg carriers experience elevation of ALT and decline of serum HBV DNA. HBeAg seroconversion, defined as HBeAg loss and appearance of anti-HBe antibody, is a milestone in the natural history of CHB and often signifies the transition from the immune clearance to an inactive carrier state. Early entry to an inactive carrier state usually confers a favorable clinical outcome (Hsu et al. 2002; Bortolotti et al. 2006; Ni et al. 2007). Some HBeAg seroconverters can finally achieve HBsAg loss or seroconversion, an indication of resolved hepatitis B or a state of functional cure. However, some patients with HBeAg seroconversion suffer from reactivation of HBV and recurrent hepatitis flares, named HBeAg-negative hepatitis. Although the majority of CHB patients have spontaneous HBeAg seroconversion in an early period of life and enjoy an uneventful clinical course, some of them have prolonged immune clearance phase or HBeAg-negative hepatitis and suffer from persistent or recurrent hepatitis flares, eventually leading to cirrhosis and HCC.

The lifetime risk of the end-stage liver disease, like cirrhosis and HCC, in HBsAg carriers is up to 15–40% (Kao 2007; Liaw and Chu 2009; Kao et al. 2010; El-Serag 2012). The annual incidence of cirrhosis is 3.5% in CHB patients with persistent HBeAg seropositivity and 2.9% in those with HBeAg-negative hepatitis (Liaw and Chu 2009). Cirrhotic patients have been shown to exhibit higher risk of HCC than those without cirrhosis, and the annual incidence of HCC is around 5–6% (Yang et al. 2002). A systemic review on the longitudinal studies estimated the incidence rates of HCC in HBsAg carriers in East Asian countries to be 0.2 per 100 person-years in inactive carriers, 0.6 in those with chronic HBV infection but without cirrhosis, and 3.2 in patients with compensated cirrhosis; the 5-year HCC accumulative incidence among cirrhotic patients is 15% (Fattovich et al. 2004). In contrast, the summary HCC incidence rate in individuals with chronic HBV infection in Europe was 0.02 per 100 person-years in inactive carriers, 0.1 in patients with chronic HBV but without cirrhosis, and 2.2 in subjects with compensated cirrhosis; the 5-year HCC accumulative incidence was 10% among those with cirrhosis.

Early identification of CHB patients at risk of cirrhosis and HCC for timely antiviral treatment is critical to prevent the disease progression and reverse the

detrimental outcomes. Therefore, the discovery of important risk factors that contribute to the disease progression and delineate the natural history of CHB should help to early diagnose patients at risk and provide the optimal care (Yang and Kao 2016). In this chapter, we will discuss the viral factors affecting the disease progression of CHB.

2 Viral Factors that Affect the Disease Progression of CHB

It has been known that HBV is non-cytopathogenic and causes the liver injury mainly through the immune-mediated mechanisms (Rehermann and Nascimbeni 2005; Guidotti and Chisari 2006). Nevertheless, a body of evidence has demonstrated that viral replication is the primary driving force for hepatitis flares and disease progression in CHB patients (Chen et al. 2006a). Because HBV replicates through the reverse replication which lacks the proofreading ability, emergence of viral mutants and rapid evolution within an infected individual often occur under the host immune pressure along the long course of CHB (Seeger and Mason 2015). In addition, diversification of viral genomes among different infected populations and across different geographic regions also results in different genotypes of HBV. Studies in the past decades have identified a number of qualitative and quantitative viral factors that affect the disease progression, including HBV DNA levels, HBeAg serostatus, HBsAg levels, HBV genotypes, naturally occurring mutants, quantitative anti-HBc antibody levels, and HBV core-related antigen (HBcrAg) levels. All these viral factors, to some extent, cause and/or reflect the complex virus-host interactions and liver injury and can serve as the predictive biomarkers for the clinical outcomes of CHB. We will describe and discuss these viral factors by emphasizing their impact on the disease progression and predictive roles in clinical outcomes.

3 HBV DNA Level

HBV viral load reflects the viral replicative capacity. Several prospective studies have demonstrated the risk of high HBV DNA levels in the development of HCC (Yu et al. 2005; Chen et al. 2006a, b, 2011). A large Taiwanese community-based REVEAL-HBV (Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus) cohort study that recruited mostly non-cirrhotic HBsAg carriers who were HBeAg negative and >30 years old reported that the risk of HCC is positively correlated with the increase of viral load at entry of study across a wide spectrum of HBV DNA levels. The cumulative incidence rates of HCC for patients with HBV levels ranging from 300 copies/mL to 1 million copies/mL were 1.3% and 14.9%, respectively (Chen et al. 2006a). Particularly, HBV DNA $\geq 10,000$ copies/mL is a strong predictor for the risk of HCC. Their following study also demonstrated that, among patients with HBV DNA $\geq 10,000$ copies/mL at entry of study, the long-term follow-up HBV DNA level was also a predictor for risk

of HCC (Chen et al. 2011). Compared with participants with HBV DNA <10,000 copies/mL at baseline, those with HBV DNA $\geq 10,000$ copies/mL at baseline but follow-up HBV DNA levels <10,000 copies/mL had only a slightly increased HCC risk (hazard ratio 2.25), but the hazard ratio for those with long-term HBV DNA that decreased to/persisted at 1,000,000–10,000,000 copies/mL was 16.78. HBV DNA levels were also correlated with the risk of cirrhosis and HBV-related mortality in a dose-dependent manner (Iloeje et al. 2006, 2007). In addition, the HBV DNA level also strongly predicted the progression to cirrhosis with the relative risk 2.5, 5.6, and 6.5 for HBV DNA levels $\geq 10^4$ –<10 (Fattovich et al. 2008), $\geq 10^5$ –<10 (Hsu et al. 2002), and $\geq 10^6$ copies/mL, respectively, as compared to the HBV DNA level <300 copies/mL (Iloeje et al. 2006).

Although above studies suggest that serum HBV DNA level is strongly associated with the development of cirrhosis and HCC independent of ALT levels, HBV genotype, and HBeAg serostatus, the long-term risk of HCC and cirrhosis in CHB patients in the immune-tolerant phase seems to be low. A prospective study evaluated the changes of liver histology within a period of 5 years in 57 immune-tolerant CHB patients with the median age of 31 and found that in 48 patients who had no ALT elevation and remained in the immune-tolerant phase, there was only minimal fibrosis progression. In contrast, 5 of 9 who had elevation of ALT and progressed to the immune clearance phase faced significant disease progression (Hui et al. 2007). In addition, the REVEAL-HBV study used ALT <45 U/L as the upper limits of normal (ULNs). By the use of more stringent ALT cutoffs (normal ALT levels of healthy adults are ≤ 30 U/L for males and ≤ 19 U/L for females (Lok and McMahon 2007)), only a minority (~20%) of HBeAg-positive patients with high HBV DNA ($>10^6$ IU/mL) showed significant histologic fibrosis and necroinflammation (Andreani et al. 2007; Lai et al. 2007). Therefore, despite their high HBV DNA levels, most of the immune-tolerant CHB patients have minimal liver necroinflammation and fibrosis. However, the long-term prognosis of CHB patients in a true immune-tolerant phase, particularly in those older than 40 years, requires to be determined in a large longitudinal cohort study in the future.

4 HBeAg Serostatus and Level

The biological role of HBeAg remains elusive because it is not required for viral assembly and replication (Milich and Liang 2003). Nevertheless, positivity of HBeAg indicates the active replication of HBV, whereas HBeAg seroconversion is often accompanied by the reduction of viral replication and remission of hepatic necroinflammation. The convincing evidence for the adverse effect of HBeAg positivity on the long-term outcomes of CHB patients came from the community-based REVEAL-HBV cohort study, which demonstrated the association between the positivity for HBeAg and the risk of HCC (Yang et al. 2002). After the adjustment of other confounding factors, the relative risk of HCC was 9.6 among men with HBsAg positivity alone and 60.2 among those who were positive for both HBsAg and HBeAg as compared to those who were negative for both. It has also been shown

that delayed HBeAg seroconversion or relapse of hepatitis after HBeAg seroconversion is an independent risk of cirrhosis (Chu et al. 2004).

Recently, quantitative HBeAg level has been proposed to guide antiviral therapy (Fried et al. 2008; Lee et al. 2011). In treatment-naïve CHB patients, HBeAg level is found to correlate with HBV DNA level, but the emergence of precore/basal core mutations can affect this relationship (Thompson et al. 2010). Nevertheless, the role of HBeAg levels in long-term clinical outcomes remains unknown.

5 HBsAg State and Level

HBsAg loss or seroconversion is a landmark in the natural history of CHB as well as the ultimate goal for antiviral treatment. Mechanistically, the HBsAg level can be considered a surrogate marker of HBV covalently closed circular DNA (cccDNA), the transcriptional and replicative template of HBV, although HBsAg can be also produced from the integrated HBV genomes (Thompson et al. 2010). HBsAg loss or seroconversion represents the resolution of hepatitis B or a state of functional cure. In the latter scenario, replication-competent latent viruses can still be recovered from patients (Rehermann et al. 1996; Lioriot et al. 1997; Marusawa et al. 2000). HBsAg loss or seroconversion used to be considered a rare event in HBsAg carriers. However, previous cohort studies revealed that HBsAg loss occurs in an annual rate from 0.5 to 2.3%, depending on the age at enrollment and disease status (McMahon et al. 2001; Chu and Liaw 2007; Kim et al. 2008; Liu et al. 2010; Simonetti et al. 2010; Tseng et al. 2011). HBsAg loss or seroconversion usually indicates a favorable clinical prognosis unless cirrhosis or HCC has already occurred (Chen et al. 2002; Yuen et al. 2004, 2008; Ahn et al. 2005; Arase et al. 2006; Chu and Liaw 2007).

Recently, HBsAg levels can be measured quantitatively. It has been found that HBsAg levels evolve along with distinct phases of CHB (Chan et al. 2010; Jaroszewicz et al. 2010; Nguyen et al. 2010; Matsumoto et al. 2012). The immune tolerance phase exhibits the highest HBsAg level, which subsequently decreases in the immune clearance phase and continues to decline in inactive carriers. However, HBsAg level rebounds upon HBV reactivation in HBeAg-negative hepatitis B patients. It has been shown that combination of HBsAg (<1000 IU/mL) and HBV DNA (≤ 2000 IU/mL) is able to accurately identify inactive carriers of genotype D (Brunetto et al. 2010).

HBsAg level has been demonstrated as a useful biomarker to predict the HBsAg loss and the risk of HCC. In a hospital-based cohort study that enrolled HBeAg seroconverters of genotype B and C in Taiwan, it was found that low serum HBsAg levels alone or combined with low HBV DNA levels, at 1 year after HBeAg seroconversion, are a predictor of HBsAg loss (Tseng et al. 2011). In addition, low HBV DNA level (<2000 IU/mL) and low HBsAg level <10 IU/mL, combined together, are able to predict HBsAg loss in HBeAg-negative HBV carriers (Tseng et al. 2012a). Furthermore, compared to individuals with HBsAg levels >1000 IU/mL, the rates of HBsAg loss in those with HBsAg levels of 100–999, 10–99, and <10 IU/

mL were significantly higher with hazard ratios of 2.5, 2.8, and 13.2, respectively. The HBsAg level of patients with low viremia (<2000 IU/mL) is also associated with the risk of disease progression (Tseng et al. 2013, 2014a). Recently, it has been found that in genotype B or C HBeAg-negative patients, the adjusted hazard ratio for HCC was 13.7 in those with HBsAg level ≥ 1000 IU/mL versus <1000 IU/mL. As a result, a combination of low HBV DNA (<2000 IU/mL) and low HBsAg (<1000 IU/mL) is a useful biomarker for identifying HBsAg carriers with the minimal risk of HCC (Tseng et al. 2012b).

6 HBV Genotype

Because HBV polymerase lacks the ability of proofreading, viral mutations often occur during the long course of chronic HBV infection. Accumulation of mutations drives the HBV evolution and phylogenetic diversification across different geographic regions. The genotypes of HBV are defined by an intergroup divergence of more than 8% in the complete genome sequence. Currently, at least ten genotypes of HBV, from A to J, have been discovered. Different HBV genotypes are associated with distinct virological and epidemiological properties (Schaefer 2007; Liu and Kao 2013; Kramvis 2014; Croagh et al. 2015). For example, genotype A is widespread in sub-Saharan Africa, Northern Europe, and Western Africa. Genotypes B and C are more common in Asia. Genotype D is primarily prevalent in Africa, Europe, the Mediterranean region, and India. Genotype E is found in West and Central Africa. Genotype F is distributed over the North and South America.

Since different genotypes of HBV are distributed in different geographic regions and ethnic groups, it is sometimes difficult to evaluate the risk potential of viral genotype on the long-term outcomes of CHB because of these confounding factors. Nevertheless, several lines of evidence have started to show that HBV genotype is associated with clinical outcomes. Previous studies found that the genotype influences the rate of HBeAg seroconversion. Compared to patients with genotype B infection, those with genotype C infection exhibited delayed or lower rate of spontaneous HBeAg seroconversion (Ni et al. 2004a). Furthermore, in a prospective cohort study that enrolled 1158 HBV carriers with the infection of genotypes A, B, C, D, and F throughout Alaska, it also found that genotype C infection had a delayed HBeAg seroclearance. Besides, individuals with genotypes C and F infection had a higher risk of HBeAg reversion after HBeAg seroclearance (Livingston et al. 2007). A longitudinal study that enrolled 258 Spanish patients with genotypes A, D, and F reported that, although the HBeAg seroconversion rate was unrelated to HBV genotype, genotype A patients exhibited a higher rate of sustained remission after HBeAg seroconversion than those with genotype D infection (Sanchez-Tapias et al. 2002). Furthermore, compared to patients with the infection of genotypes C and D, those with genotypes A and B infection had a higher rate of the HBsAg seroclearance (Sanchez-Tapias et al. 2002; Yuen et al. 2004).

Previously, it also has been shown that HBV genotypes are associated with the risk of cirrhosis and HCC (Liu et al. 2006). Patients with the infection of genotype

D have more active liver disease and advanced liver fibrosis compared to those with genotype A infection. In addition, compared to genotype B infection, genotype C infection is associated with higher risk of cirrhosis and HCC (Kao et al. 2000; Yang et al. 2008; Liu and Kao 2013, 2015). Interestingly, whereas genotype C infection is associated with the development of HCC at older age, genotype B infection is associated with HCC that occurs at younger age (Kao et al. 2000; Ni et al. 2004a).

The natural history of HBV carriers with genotype E infection has been unclear until recently. A longitudinal cohort study that recruited 405 HBV carriers primarily with genotype E infection (>95%) in Gambia, West Africa, reported that the genotype E infection has similar annual rates of HBeAg (7.4%) and HBsAg (1.0%) seroclearance with other genotypes. However, treatment-naïve male HBsAg carriers in Gambia had the HCC incidence of 55.5/100,000 carrier-years. The risk of HCC was higher than HBsAg carriers in Europe but lower than those in East Asia (Shimakawa et al. 2015).

7 HBV Mutants

Due to the error-prone nature of HBV polymerase, viral mutants often emerge during chronic HBV infection. Three naturally occurring HBV mutations, namely, G1896A precore premature stop codon mutation, A1762T, and G1764A dual basal core promoter (BCP) mutations and deletions in the pre-S/S genes, are commonly encountered in the natural course of CHB (Chu et al. 2003; Chotiyaputta and Lok 2009; Lin and Kao 2015). These HBV mutants have been suggested to affect the natural history of CHB, including HBeAg seroconversion and the risk of cirrhosis and HCC.

It has been shown that the precore and BCP mutations are associated with HBeAg seroconversion (Lok et al. 1995; Chang et al. 1998; Chan et al. 1999; Yuen et al. 2002; Chu et al. 2003; Ni et al. 2004b). The precore premature stop codon mutation results in failure of HBeAg production, whereas the dual BCP mutations are suggested to cause the reduction of the HBeAg expression (Chotiyaputta and Lok 2009). Previous studies found that precore mutation emerges before the occurrence of HBeAg seroconversion. In addition, following HBeAg seroconversion, the majority (>90%) of patients had core promoter or precore mutations (Chan et al. 1999). Interestingly, using the quantitative amplification-created restriction site assays for measurement of the precore mutant ratio, Chu et al. found that the higher ratio of the precore mutant is associated with earlier HBeAg seroconversion, but the patients who had significantly higher ratio of precore mutant tended to have high viremia and high ALT after HBeAg seroconversion (Chu et al. 2002). In addition, using a more quantitative SimpleProbe real-time PCR analysis of these mutations, Nie et al. discovered that the steady increase of the precore and/or BCP mutant frequencies usually occurs within 3 years before HBeAg seroconversion (Nie et al. 2012). Taken together, these observations on the temporal relationship between viral mutations and HBeAg seroconversion suggest that the emergence of precore and BCP mutations is likely to be the consequence of the immune selection pressure, rather than the causative driving force for HBeAg seroconversion.

The biological roles of precore and BCP mutations in chronic HBV infection remain elusive, and several studies have suggested that they may exert different effects on the outcomes of CHB. It has been shown that the dual A1762T/G1764A (TA) mutations in combination with T1753A, T1768A cause cell cycle dysregulation by the upregulation of SKP2 and downregulation of p21, leading to the development of HCC (Huang et al. 2011). BCP mutations have been associated with the risk of cirrhosis and HCC, but the role of precore mutation is somehow controversial (Baptista et al. 1999; Lindh et al. 1999; Kao et al. 2003; Liu et al. 2006, 2009; Tong et al. 2006). Moreover, in the community-based REVEAL-HBV cohort study in Taiwan, BCP mutations are associated with the increased risk of HCC (adjusted hazard ratio, 1.73), whereas the precore mutation is associated with the decreased risk of HCC (adjusted hazard ratio, 0.34) (Yang et al. 2008). A meta-analysis of 43 studies also demonstrated that BCP mutations, but not precore mutation, increase the risk of HCC with the odds ratio of 3.79 (Liu et al. 2009). A recent hospital-based cohort (ERADICATE-B) quantitatively analyzed the precore and BCP mutations and showed that higher percentage of the BCP mutation at 1 year after HBeAg seroconversion is associated with higher risk of cirrhosis (Tseng et al. 2014b). Recently, we also found that the percentage of the precore mutation increases during interferon-induced HBeAg seroconversion, whereas the percentage of BCP mutations does not change significantly (Yang et al. 2013). Taken together, the precore and BCP mutations are likely to play distinctive roles in the natural course of CHB.

The HBV pre-S/S deletion mutation is also commonly encountered in chronic HBV infection and is likely the consequence of the antiviral immune selection pressure (Fan et al. 2001; Chen et al. 2006c). The pre-S deletion has been suggested to cause endoplasmic reticulum (ER) stress and carcinogenesis of hepatocytes due to the accumulation of large surface protein ER. In addition, preS2 protein has been shown to activate hTERT transcription that leads to the development of HCC (Luan et al. 2009). Previous studies also reported that pre-S deletion is associated with the risk of advanced liver disease and HCC in HBeAg-negative patients (Chen et al. 2007; Pollicino et al. 2014).

It has also been shown that HBV mutations are associated with the HBV genotypes (Rodriguez-Frias et al. 1995). The precore stop codon mutation G1896A is rare in genotype A and more common in genotype D (Grandjacques et al. 2000). It has been shown that the precore mutation is restricted to the virus having T at nucleotide 1858 (Lok et al. 1995). In addition, patients with genotype D were more likely to have persistent HBV infection by selection of precore mutants. Core promoter changes were significantly more common in patients infected with HBV who have C at nucleotide 1858 (Chan et al. 1999; Lindh et al. 1999). Therefore, studies on the role of HBV mutants in disease progression should take into consideration the effect of viral genotype.

8 Quantitative HBcrAg

HBcrAg combines the antigenic reactivity against hepatitis B core antigen (HBcAg), HBeAg, and a 22-kDa precore protein without C-terminal arginine-rich domain (p22cr), so it can detect all these three antigens simultaneously (Kimura et al. 2002,

2005). HBcrAg levels have been shown to correlate well with serum HBV DNA levels as well as intrahepatic HBV DNA levels, particularly in HBeAg-positive patients (Rokuhara et al. 2005; Wong et al. 2007). More importantly, the HBcrAg level is positively correlated with the intrahepatic cccDNA level (Suzuki et al. 2009; Matsuzaki et al. 2013). Mechanistically, the HBcrAg level is an indicator of the gene expression of cccDNA, so it can serve an ideal marker for HBV replication or residual replicative-competent cccDNA. The cohort studies in Asian and European have shown that HBcrAg levels vary along the natural history of CHB. The immune tolerance and immune clearance phases have higher HBcrAg levels, which are followed by that in HBeAg-negative hepatitis. The HBeAg-negative quiescent/inactive carrier phase has lower HBcrAg levels (Seto et al. 2014; Maasoumy et al. 2015). The HBcrAg level is lowest in patients with HBsAg seroclearance. Of note, HBcrAg remains detectable in a portion of HBsAg seroconverters (Seto et al. 2014). Recently, HBcrAg has been also associated with the risk of liver inflammation, advanced fibrosis, and HCC. A longitudinal cohort study that enrolled 1031 treatment-naïve HBsAg carriers in Japan reported that HBcrAg >2.9 log U/ml is an independent risk factor of HCC (adjusted hazard ratio, 5.05) (Tada et al. 2016).

9 Total Anti-HBc Level

Anti-HBc antibody reacts against HBcAg and is a conventional serological marker for HBV infection. IgM anti-HBc represents an indicator of acute hepatitis B or flares during chronic HBV infection, whereas the IgG anti-HBc antibody lasts almost a lifetime in individuals with exposure to HBV. However, the evolution and dynamics of total anti-HBc levels in the natural history of CHB remain unclear. Recently, a quantitative anti-HBc (qAnti-HBc) has been developed and tested for its clinical utility (Li et al. 2010).

Quantitative qAnti-HBc levels were found to correlate well with ALT levels in CHB patients (Yuan et al. 2013, 2015; Jia et al. 2014; Song et al. 2015). In addition, a previous study also observed the variation of qAnti-HBc levels in different disease phases of HBV infection (Song et al. 2015). The levels of qAnti-HBc are higher in the immune clearance and reactivation phases than those in the immune tolerance and inactive carrier phases. Of note, qAnti-HBc levels of the immune-tolerant patients are similar to those of inactive carriers, despite that the immune-tolerant patients exhibit very high levels of HBV DNA. This further supports the association between ALT and qAnti-HBc levels. In addition, serum qAnti-HBc level of HBsAg-positive patients was higher than those of HBsAg-negative individuals. Interestingly, among HBsAg-negative persons, subjects with occult HBV infection had higher serum qAnti-HBc level than those with past HBV infection.

Taken together, given the correlation between qAnti-HBc and ALT, total qAnti-HBc level may serve as a serological marker for HBV-induced hepatic necroinflammation and is complementary to other quantitative viral markers, like HBsAg and HBV DNA levels. Nevertheless, previous studies on qAnti-HBc had small sample size and were cross-sectional. Therefore, the predictive role of qAnti-HBc level in the clinical outcomes of CHB needs to be validated in a large longitudinal cohort.

10 Development of Risk Scores for Cirrhosis and HCC by Integration of Viral and Host Factors in CHB Patients

Accurate prediction for the risk of cirrhosis and HCC is required for timely and appropriate treatment of CHB. Since several aforementioned viral factors that affect the disease progression have been identified, it allows to better calculate the risk of HCC by the incorporation of several viral and host factors in a risk scoring system in order to perform optimal and personalized surveillance for CHB patients (Yang et al. 2014; Wong and Janssen 2015). Previously, several risk scoring systems had been developed to predict the risk of HCC (Han and Ahn 2005; Yuen et al. 2009; Wong et al. 2010; Yang et al. 2010). Most of them were hospital-based cohort study except the community-based Taiwanese cohort (REVEAL-HBV). These risk scoring systems faced the challenges of lacking external validation. To solve this issue, these study groups cooperated to develop a 17-point risk scoring system for HCC (REACH-B), which was derived from non-cirrhotic naïve CHB patients in the REVEAL-HBV cohort and validated by a composite hospital-based cohort from Hong Kong and Korea. The REACH-B risk score integrates several host and viral factors including age, sex, ALT level, HBeAg serostatus, and serum HBV DNA level and accurately predicts the risk of HCC with a wide range, from 0.0 to 23.6% at 3 years, 0.0 to 47.4% at 5 years, and 0.0 to 81.6% at 10 years for patients with the lowest through the highest scores (Yang et al. 2011). Recently, the HBsAg level has been demonstrated to predict the risk of long-term adverse outcomes and HCC (Tseng et al. 2012b, 2013), so the original REVEAL risk scoring system was upgraded by the incorporation of HBsAg level and is internally validated (Lee et al. 2013). In addition to its use in prediction of HCC, this new risk calculator also accurately predicts the risk of cirrhosis. The sum risk scores ranged from 0 to 26 in the prediction model for cirrhosis and from 0 to 19 in the prediction model for HCC, respectively. The cirrhosis risk ranged from 0.08 to 43.15% at 3 years, 0.13 to 60.11% as 5 years, and 0.36 to 91.98% at 10 years. For the HCC risk, it ranged from 0.01 to 36.19% at 5 years, 0.03 to 79.72% at 10 years, and 0.07 to 98.16% at 15 years. Of note, external validation is still required for this new scoring system to prove its applicability in other CHB cohort patients.

Conclusions

Since the risk of long-term adverse outcomes in CHB patients is high, early diagnosis of patients at risk is critical to provide optimal and timely management for them. Previous studies, particularly the longitudinal cohort studies, have identified viral factors that affect the disease progression. Discovery of these viral factors not only allows to gain the mechanistic insight into the pathogenesis of CHB but also helps the risk stratification of patients in order to improve the quality of care. Integration of several viral and host factors into a simple risk scoring system is even more useful for the clinicians to evaluate the disease status of a patient. With the advance of quantitative and qualitative assays, more new viral biomarkers will emerge and help fine-tune the delineation of disease status and the risk stratification of CHB patients. Understanding the impact of viral factors

on disease progression and long-term outcomes of CHB will shed light on the complex virus-host interactions and eventually lead to optimal care of patients with the ultimate hope of cure.

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Hepatitis B Virus Genotype and Mutations Related to Clinical Outcome

7

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Abstract

Hepatitis B virus (HBV) infection is a high risk factor on severe liver diseases. HBV is currently divided into ten genotypes based on the genetic sequence of HBV, which has regional characteristics on clinical outcomes. Recently, cross-border incursion of HBV genotypes occurs worldwide. The nationwide epidemiological study in Japan showed that the number of acute hepatitis B patients was about 8000–8400 people per year. Interestingly, the European–US genotype HBV/A2 has made up an increasing proportion of acute HB cases over 50% in Japan. And 29% of acute HB patients infected with HBV/A became negative for HBsAg within 6–12 months, which were considered “prolonged,” rather than chronic hepatitis B. The acute infection of HBV/A2 is more likely to become chronic compared to HBV/B or C in Japanese population. The invasion of HBV genotypes into other region or country could provide new insights on clinical outcomes of HBV genotypes. The observation of long-term prognosis is needed to collect the clinical evidences on the infection of new genotype into a population.

Keywords

Hepatitis B virus • Genotype • Molecular phylogenetic analysis • Core promoter mutation

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1 Introduction

Hepatitis B is a global health problem. Nearly 350 million people are estimated to be infected with the hepatitis B virus (HBV) worldwide, with more than 600,000 people dying of hepatitis B each year (Centre and Hepatitis 2014). In Japan, the infection rate is thought to be about 1%, with about 1.5 million people infected nationwide, most of them elderly. Whereas mother-to-child infection is the primary route for HBV infections, such infections can be prevented by administering hepatitis B immune globulin or vaccines to infants born of mothers positive for the HBV surface antigen (HBsAg). Therefore, new mother-to-child infections are rare. Routes that have become problematic in recent years are sexually transmitted infections among young people and horizontal infections between drug addicts. Such patients exhibit acute hepatitis B and make up an increasing proportion of all HBV infections. In addition, specific HBV genotypes have been found to be involved in these acute hepatitis cases.

In this paper, we discuss the characteristics of HBV genotypes, and point mutations related to different genotypes are also described, and the newest topics in this area are introduced.

2 HBV and the Genotypes

Recently, the bat hepatitis virus (Bat-HV) has been identified and capable of infection into human hepatocytes (Drexler et al. 2013; He et al. 2013). The phylogenetic tree shows that the clusters of Bat-HV are located near mammalian, far from the cluster of bird (Fig. 7.1). As the habitat distribution of bat or squirrels is worldwide, host-switch infection between bat or squirrels and primates might be occurred.

The hepadnaviruses genome is small, at about 3 kb, but its genome replication process involves reverse transcription; therefore, mutations tend to be more common than in other DNA viruses. Moreover, since the main infection route has been mother-to-child, viruses of the same line succeed each other generation after generation. This creates species and regional characteristics in hepadnaviruses gene sequences. Molecular phylogenetic analysis is a type of analysis that makes use of differences in these sequences. Evolutionary analyses of gene sequences have been performed using the speed of mutations to show that genome sequences form clusters according to different regions (Miyakawa and Mizokami 2003; Mizokami et al. 1999). On hepatitis B virus study, these clusters were then used to categorize viruses by genotype. Genotype categories were created by examining the homology of gene sequences. Those with values of 8% or higher were categorized as a different genotype (Kato et al. 2005; Okamoto et al. 1988). A boundary at 8% creates two groups, with one population grouped at 8% or less (Kato et al. 2005). The HBV genotypes have affected clinical outcomes of the patients.

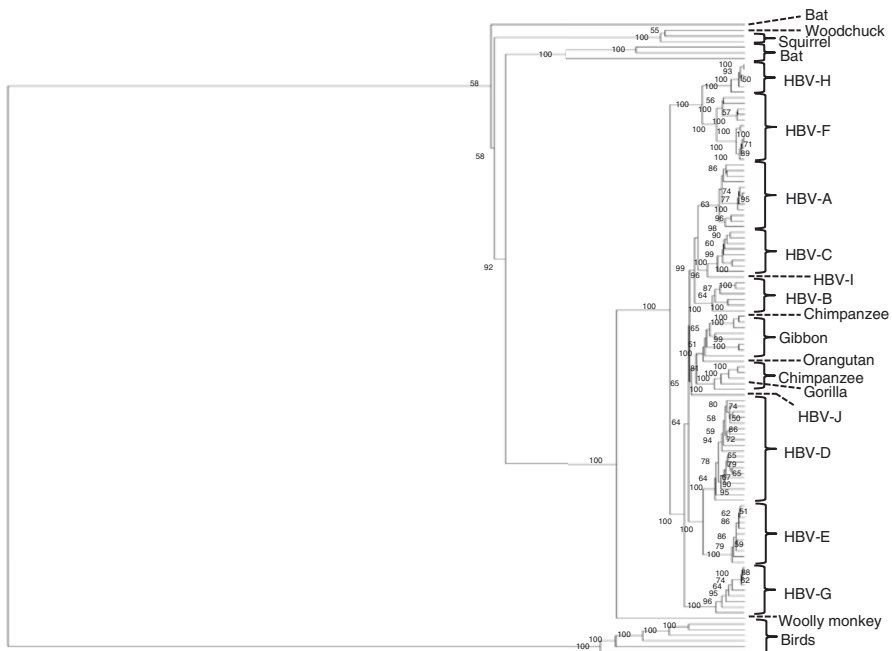


Fig. 7.1 Phylogenetic analysis of HBV and the related hepadnaviruses. Unweighted-pair group method using average linkages (UPGMA) tree of hepadnaviruses were constructed by MEGA 6 (Tamura et al. 2013)

3 HBV Genotypes and Global Distribution

There are currently ten categories of HBV genotypes, A to J, and there are subgenotypes within these genotypes. As described above, molecular phylogenetic analysis has shown that the distribution of genotypes and subgenotypes has regional characteristics (Fig. 7.2).

HBV/A is mainly distributed in Europe and the United States, with subgenotypes found in Asia and Africa (HBV/A1/Aa) and Europe and the United States (HBV/A2/Ae). HBV/B is often reported in Asia, with subgenotypes found in Japan (HBV/B1/Bj) and Asia (HBV/B2-B5/Ba). HBV/C is also mainly distributed in Asia, with subgenotypes found in Southeast Asia (HBV/C1/Cs) and the Far East (HBV/C2/Ce). HBV/D is distributed globally, but its subgenotypes are found in Europe and Central Asia (HBV/D1), Russia and Far Eastern Asia (HBV/D2), mainland Asia (HBV/D3), and Europe and Africa (HBV/D4). HBV/E is mainly distributed in West Africa. HBV/F and HBV/H have been confirmed primarily in Central and South America and Alaska. HBV/G has so far been found in North America, Europe, and Japan, but only a few cases have been reported, and



Fig. 7.2 Geographical map of HBV genotype distribution. The distribution of HBV genotypes was described in the map. The font size indicates the population size of the genotype in the country

much remains unclear about this genotype. HBV/J was discovered in Okinawa prefecture, Japan; however, only one case has been reported, and its characteristics remain unclear.

4 HBV Genotypes and Their Characteristics

Various studies have reported a relationship between genotype and clinical characteristics, which indicates that genotype classifications might be useful in the clinical setting. For instance, since many patients with hepatitis B are infected with genotypes HBV/A–D, much research has been performed on these types around the world. In this article, we focus on the situation in Japan in terms of the emerging problem of HBV/A2 infections. The clinical data of Japanese infected with HBV/A2 enable us to understand the viral factor because HBV/A2 was not prevalent in Japan before. We could compare the similarity or the differences between Japanese and Europeans.

5 Acute Hepatitis B and HBV Genotypes

According to a survey we conducted, only a small proportion of acute hepatitis B cases were found. Estimates based on the number of cases actually reported and other factors indicate that there are about 2000–3000 incidences of acute hepatitis B per year. Data from a 2010 Diagnosis Procedure Combination (DPC) report shows

that 2175 cases of acute hepatitis B were registered in 2007, and 2391 cases were registered in 2008. These are similar to the estimate made from the number of reported cases (Sako et al. 2011). In contrast, an estimate using a blood-donor cohort indicated that about 6000 people contract subclinical hepatitis B infections per year nationwide. This means that the number of people hospitalized due to acute hepatitis B plus the number of subclinical infections is about 8000–8400 people per year. This predicts that new hepatitis B infections are occurring via horizontal infections among adults.

What exactly are these new infections? In Japan, most cases of acute hepatitis B and chronic hepatitis have for a long time been due to HBV/B and HBV/C (Orito et al. 2001). Yet, in recent years, the European–US genotype HBV/A2 has made up an increasing proportion of acute hepatitis B cases (Suzuki et al. 2005). A research team from the Japanese Ministry of Health, Labor, and Welfare gathered data on and analyzed 1088 cases of acute hepatitis B from 1982 to 2010. They found no cases of HBV/A2 until around 1993. Cases of HBV/A2 started appearing in the mid-1990s, and their proportion has since increased to around 60% at the present in Japan. A comparison of the Tokyo metropolitan area to rural regions showed that while HBV/A2 appeared in Tokyo in 1994, it did not show up in the countryside until a few years later in the late 1990s. In Tokyo, HBV/A2 has replaced HBV/B and HBV/C as the main cause of infections, whereas in the countryside, about 50% of cases are now due to HBV/A2 (Fig. 7.3).

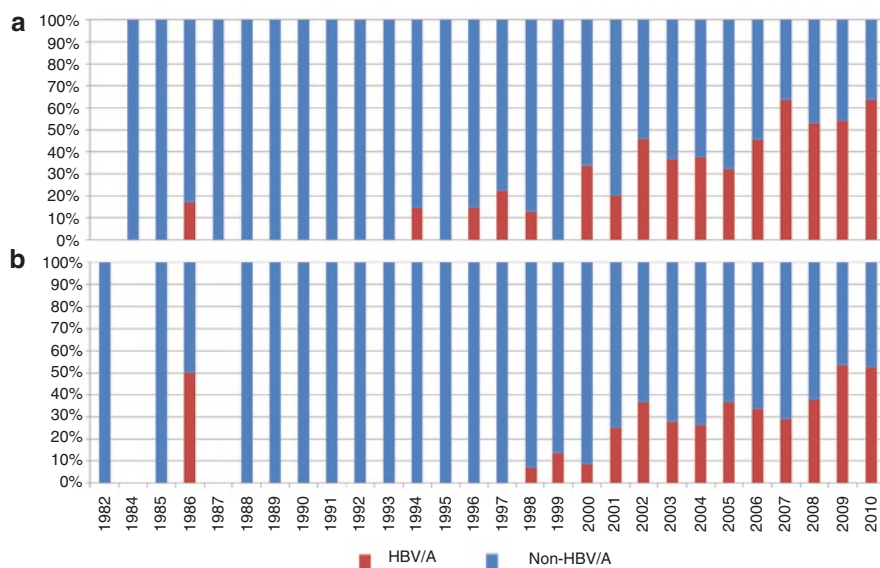


Fig. 7.3 Proportion of HBV/A2 on acute hepatitis B in Japan. (a) Urban area, (b) local city area

6 Acute Hepatitis B Is Becoming Chronic

In the conventional clinical course of acute hepatitis B, an initial infection in an adult resolves after a transient infection. That is, these patients quickly become negative for HBV DNA and HBsAg, and the history of the infection is evident in their being positive for HBe and HBs antibodies. The sources of infection in these cases are primarily HBV/B or HBV/C.

Previous analysis of the clinical characteristics of recent cases of acute hepatitis B has shown that coinfections with HIV occur in 11.3% of HBV/A2 cases and 2.5% of non-HBV/A2 cases, i.e., more frequently with HBV/A2. Moreover, patients are positive for HBsAg for 8.1 ± 10.7 months with HBV/A2, which is nearly twice as long as that for patients with non-HBV/A2 (4.3 ± 8.2 months). The definition of a case of acute hepatitis that becomes chronic is one in which hepatitis persists for 6 months or more and the patient continues to be positive for HBsAg. A case control study by the same researchers found that 23 of 80 (29.0%) HBV/A cases became negative for HBsAg within 6–12 months. These cases are considered “prolonged,” rather than chronic. On the other hand, no cases that continued to be HBsAg-positive for 12 months or more later became HBsAg-negative; therefore, these cases were considered chronic.

The above indicates that in Japan, cases of acute hepatitis B that used to be caused by HBV/B and HBV/C are transforming into acute cases, and cases that become prolonged are being caused by HBV/A2. A major characteristic of HBV/A2 infections is that HBsAg persists for a long period, and the infections are more likely to become chronic compared to other genotypes. Moreover, that they are more likely to occur with HIV infection should be taken note of during treatment.

7 Characteristic Mutations of HBV Genotypes

While HBV genotypes are determined by molecular phylogenetic analysis that consider mutations of the genome as a whole, mutations characteristic to each genotype can be found by close inspection of individual sequences. In particular, mutations around the sequences called core promoters can have a strong impact on the efficacy of viral replication. Here, we discuss mutations that have been observed in this region.

In HBV/A1, these characteristics can be found in the Kozak sequence and epsilon sequence, lying immediately before the ORF core, which translates into HBeAg (Tanaka et al. 2004). In basic research, it was found that a 1809T/12T mutation decreased antigen production and replication (Table 7.1). In addition, a 1862T/88A mutation was reported to suppress viral replication and reduce the production of core proteins (Guarnieri et al. 2006).

Table 7.1 Summary of popular mutation pattern on each genotype

HBV genotype	Feature	Mutation type
HBV/A	Kozak sequence	1809T/12T
	ε signal	1862T/88A
HBV/B	Core promoter	1762T/1764A
HBV/C	Core promoter	1762T/1764A
HBV/D	Core promoter	1764T/1766G

Similar characteristics have been found in HBV/B and HBV/C, which exhibit a well-known core promoter mutation (1762T/64A) (Ozasa et al. 2006). Mutations in this region are known to be dependent on long-lasting infections in the patients. In addition, new infections caused by viruses with this mutation are reportedly associated with a higher risk of fulminant hepatitis (Ozasa et al. 2006) and are linked to liver cancer (Kao et al. 2003). Basic research has shown that the core promoter mutation 1762T/64A accelerates viral replication.

Different HBV/D subtypes are known to exhibit different mutation patterns in the core promoter region. Core promoter mutations observed in HBV/D1 are only slightly different from those seen in HBV/B and HBV/C, with 1764T/66G being the main mutation (Elkady et al. 2008). In terms of viral attributes, this is not too different from 1762T/64A, in that it exhibits increased viral replication. However, unlike HBV/B and HBV/C, no evidence has established a connection with liver cancer. As with HBV/B and HBV/C, 1762T/64A is the primary mutation for HBV/D2 and other subtypes.

Conclusion

Research on HBV genotypes has yielded a wide range of findings and has clarified relationships between genotype and region. However, increasing globalization has watered down these characteristics. One particular example of this is the rapid spread of HBV/A2 in Japan. Nonetheless, for an infection to occur, both a host and a virus are needed; therefore, understanding human factors and pathology is also important. Since the influx of HBV/A has reduced differences from the viewpoint of viruses per se, examining how pathology in Japan differs from that in Europe and the United States could help enrich the understanding of hepatitis. Much is expected of analyses of the human genome and other such research.

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Valerie Fako and Xin W. Wang

Abstract

Primary liver cancer is the second most common cause of death due to cancer worldwide, and a vast majority of primary liver cancer is the hepatocellular carcinoma (HCC) subtype. Although a number of etiologies are recognized to cause HCC, HBV infection accounts for over 50% of diagnosed HCC cases. HBV infection leads to the development of HCC through a variety of mechanisms. Indirectly, viral infection leads to repetitive liver injury through oxidative stress, the immune response, and telomere shortening, eventually causing cellular transformation. Integration of viral DNA into the host genome can indirectly lead to carcinogenesis by inducing genomic instability, as well as directly causing oncogenesis by preferentially integrating into genomic loci of known oncogenes. The activity of the HBV X protein (HBx) has been demonstrated to directly promote transformation and hepatocarcinogenesis in a number of ways, including activating cancer-related pathways and altering cellular epigenetic and noncoding RNA expression. In this chapter, we will summarize the current scientific knowledge of the ways in which HBV infection can lead to HCC.

Keywords

Hepatocellular carcinoma • Hepatocarcinogenesis • Liver injury • Viral DNA integration • HBx protein

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1 Introduction

Globally, primary liver cancer represents the fifth most commonly diagnosed cancer and the second most common cause of death due to cancer (Theise 2014). Hepatocellular carcinoma (HCC) accounts for a majority (85–90%) of primary liver cancers and leads to an estimated 600,000 deaths per year (Jemal et al. 2011). Geographically, the incidence of HCC is not distributed equally, with greater than 80% of HCC cases occurring in East Asia or sub-Saharan Africa. China alone accounts for over 50% of global HCC cases (El-Serag 2012).

There are a number of recognized etiologies leading to the development of HCC including liver cirrhosis, alcoholic liver disease, exposure to aflatoxin B1, diabetes, and obesity. However, the main etiology of HCC is hepatitis B virus (HBV) or hepatitis C virus (HCV) infection (Sanyal et al. 2010). The association between HCC development and chronic HBV infection was first reported in 1975 (Blumberg et al. 1975). Chronic HBV infection is now recognized to account for over 50% of HCC cases worldwide. Over 350 million people are chronic HBV carriers, with 75% of these cases occurring in Asian countries. Prospective cohort studies have shown that people who are chronically infected with HBV have a 5- to 100-fold increased risk of developing HCC compared to non-infected persons. (El-Serag 2012).

Most cases of HCC caused by HBV occur in patients with cirrhosis (70–90%), which is a significant risk factor for HCC (Yang et al. 2011). Other factors also contribute to an increased risk of developing HBV-related HCC. For example, demographic factors, including male sex and advanced age, are associated with an increased risk of HCC. Exposure to aflatoxin B1 or alcohol consumption also raises the risk of developing HBV-related HCC. Patients infected with HBV have a 200-fold greater risk of HCC if they are obese (body mass index ≥ 30) and have diabetes mellitus, indicating an importance of metabolic factors. HCC risk is also dependent on viral factors. High levels of HBV DNA and HBV replication are associated with increased risk. The genotype of HBV also plays a role. At least eight genotypes of HBV have been identified, and increased risk of developing HCC is associated with HBV genotype C in Asian populations, genotype A in Africans, and genotype F in Alaskan natives (Kew 2010). In addition, seropositivity for hepatitis B surface antigen (HBsAg) remains one of the greatest risk factors for HCC, varying from a sevenfold risk increase in Japan to 60- to 98-fold risk increase in Taiwan. Seroclearance of HBsAg reduces the risk of HBV-related HCC (Burns and Thompson 2014).

Understanding the epidemiological characteristics of HBV-related HCC remains an important field of study, but it is also important to understand the cellular mechanisms by which HBV infection leads to the development of HCC. Molecular carcinogenesis of HBV-related HCC is caused by indirect mechanisms such as oxidative stress, the immune response, and telomere shortening, the “mixed” mechanism of DNA insertion leading to genomic instability and insertional mutagenesis, and direct oncogenesis caused by the HBV X protein (HBx) (Fig. 8.1). In this chapter,

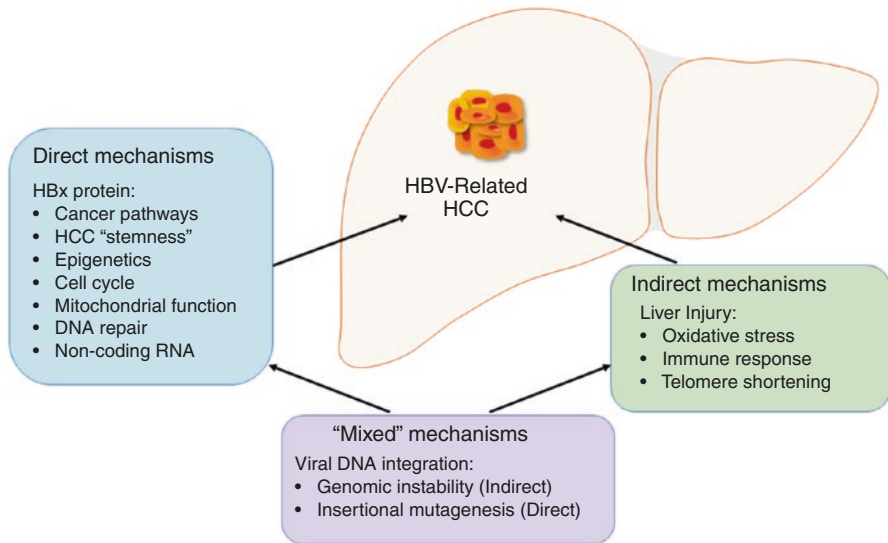


Fig. 8.1 Indirect and direct mechanisms contribute to HBV-related HCC carcinogenesis. Indirect mechanisms of carcinogenesis due to liver injury include oxidative stress, the immune response, and telomere shortening. Direct mechanisms of carcinogenesis are due to the oncogenic activities of HBx protein. Viral DNA integration into the genome promotes carcinogenesis indirectly by inducing genomic instability and directly via insertional mutagenesis

we will review the oncogenic mechanisms by which HBV viral infection contributes to the development and progression of HCC.

2 Indirect Oncogenic Roles of HBV: Inflammation and Liver Injury

Numerous studies have shown that HBV infection leads to inflammation and liver injury. A mouse model study that examined the progression of disease following HBV infection demonstrated that overproduction of the hepatitis B virus large envelope protein and high concentrations of HBsAg led to phenotypic changes within the liver, beginning with chronic hepatitis, followed by the development of regenerative nodules and oval cell hyperplasia, liver cell adenomas, and finally HCC (Dunsford et al. 1990). No histopathological changes were seen in age- and sex-matched controls. This study showed that HBV infection could directly lead to inflammation, regenerative hyperplasia, and injury to hepatocytes, with the potential to induce transforming mutations (Dunsford et al. 1990). There are several mechanisms by which HBV infection may lead to liver injury and HCC, including inducing oxidative stress, the immune response, and telomere length (Fig. 8.1).

2.1 Oxidative Stress

Oxidative stress represents an important mechanism by which HBV infection may lead to HCC. An overabundance of reactive oxygen species (ROS) can cause damage to cellular lipids, proteins, or DNA, which could potentially lead to chromosomal mutagenesis and carcinogenesis. ROS may also activate signaling pathways involved in cellular proliferation (Ha et al. 2010). DNA damage induced by oxidative stress and ROS has been observed within the livers of mice with chronic active hepatitis. The accumulation of 8-oxo-2'-deoxyguanosine, an adduct of deoxyguanosine that is modified by oxidation, increased as liver disease progressed. Livers exhibiting advanced disease including nodular hyperplasia, adenomas, and HCC showed the greatest increase in 8-oxo-2'-deoxyguanosine, indicating the highest amount of oxidative DNA damage (Hagen et al. 1994). DNA damage induced by ROS has also been seen in HBV-infected patients, which was measured by the accumulation of 8-hydroxydeoxyguanosine, a promutagenic DNA lesion induced by hydroxyl radicals (Fujita et al. 2008).

Several patient studies have examined changes in the liver due to HBV-related disease, with an increase in oxidative stress observed with increasing viral replication status and disease severity (Bolukbas et al. 2005). A study in Taiwan followed patients with HBV-associated HCC for over 20 years and found that these patients exhibited extensive oxidative damage during clinical disease progression (Tsai et al. 2009). Another study compared patients with inactive HBsAg carrier state (IHBCS) infection, patients with chronic HBV infection, and healthy control patients. The study found that patients with chronic HBV infection had significantly higher measured levels of total oxidative stress and lipid peroxidation compared to IHBCS patients and healthy controls. Antioxidant status was decreased significantly in chronic HBV patients (Duygu et al. 2012).

2.2 Immune Response

The association between the immune cell response and cancer is well established (Balkwill and Mantovani 2001). A decrease in the immune response can facilitate viral replication and tumor formation, whereas overactive immune response and inflammation can lead to changes in tissue architecture and tissue remodeling, apoptosis, and DNA alterations, which may also promote carcinogenesis (Budhu and Wang 2006). HBV infection is controlled by antiviral cytotoxic T cells. In patients with active hepatitis who ultimately clear the virus, the T cell response is vigorous. In patients with chronic hepatitis, the T cell response is weaker, destroying a portion of infected cells, leading to a continuous cycle of cellular destruction and regeneration, resulting in liver injury and HCC development. A murine model demonstrated that hepatocellular injury can be induced by overexpression of the HBV large envelope protein, leading to cellular regeneration, DNA damage, clonal expansion, and ultimately HCC (Nakamoto et al. 1998). Liver cell injury initiated

and mediated by HBV infection has the potential to sustain carcinogenesis in the absence of viral integration, HBx expression, or genotoxic agents. This study suggested that an ineffective T cell response may be a principal oncogenic factor in the development of HCC during chronic hepatitis infection (Nakamoto et al. 1998). Indeed, an impaired immune response has been detected in patients with chronic HBV infection. For example, circulating and liver-residing regulatory T cells (Tregs) were increased in HCC patients with HBV infection, which suppressed the immune response induced by the HBV antigen and the HCC tumor antigen, thus inhibiting tumor immunosurveillance and promoting tumor progression (Zhang et al. 2010).

2.3 Telomere Length

In normal cells, the length of telomeres, segments of short nucleotide repeats that cap the ends of chromosomes, become progressively shorter with each cell division. Too short telomeres can cause chromosomal instability; thus, healthy cells will enter cellular senescence when telomeres reach a certain length. Maintaining telomere length, thus avoiding cellular senescence, is a key process in the life cycle of tumor cells, leading to immortalization. A study examining patient samples with normal livers, chronic active hepatitis, liver cirrhosis, and HCC found a progressive shortening of telomeres with each stage. However, telomerase, an enzyme that stabilizes and lengthens telomeres by adding nucleotides onto the chromosomal ends, was also detected in high levels in all HCC patient samples examined. This study suggested that rapid cellular proliferation and turnover due to viral-induced liver injury causes a multistep process of HCC carcinogenesis, beginning with progressive telomere shortening and eventually leading to telomerase activation (Miura et al. 1997).

3 “Mixed” Mechanism of HBV-Related Oncogenesis: DNA Integration

After entering the nucleus, HBV DNA can integrate into host cell chromosomes, allowing the virus to continually replicate in the cell. HBV DNA integration likely occurs prior to HCC tumor development, as integration is found in patients during the acute infection stage (Murakami et al. 2004). Integrated HBV DNA is found in the hepatocyte genome in a vast majority (85–90%) of HBV-related HCC cases and is considered to be one of the most important ways in which HBV causes a pro-oncogenic effect (Minami et al. 2005). There are several mechanisms by which HBV DNA integration can cause a carcinogenic effect. Indirectly, HBV can lead to oncogenesis by inducing genomic instability, whereas causing alterations in oncogenic gene expression via insertional mutagenesis is considered to be a direct mechanism of HBV-related oncogenesis (Fig. 8.1).

3.1 Genomic Instability

Genomic instability is a hallmark of cancer. Chromosomal instability, in which chromosome structure progressively changes in cancer cells compared to normal cells, is common in many types of cancer (Negrini et al. 2010). The integration of HBV DNA into the genome has been shown to cause structural alternations in the chromosomal DNA of hepatocytes. Microdeletions in host DNA at HBV DNA integration sites are thought to be integral to the mechanism of integration. Macrodeletions, as well as amplification of chromosomal DNA at HBV DNA integration sites, have been reported. Chromosomal translocations have also been observed, in which the DNA from two chromosomes is joined to each end of an integrated viral DNA sequence (Idilman et al. 1998). HCC that is associated with HBV infection has been shown to contain more genomic instability via allelic deletions and amplifications, caused by HBV DNA integration, than HCC that is caused by other etiologies (Lee et al. 2008). These alterations could lead to alterations in expression of genes that are related to cancer such as tumor-suppressor genes or proto-oncogenes.

3.2 Insertional Mutagenesis

Initially, HBV DNA integration into the host genome was considered to occur at random, thus constituting an indirect mechanism of oncogenesis. However, with the implementation of next-generation sequencing, recent studies have shown that there are recurrent sites into which HBV DNA preferentially integrates. A number of these integration sites include genomic loci that code for genes that are highly well-known to regulate proliferation and cell survival in cancer, and the integration of viral DNA causes changes in gene expression at these loci. For example, multiple studies have shown that HBV DNA preferentially integrates into the gene encoding TERT, telomerase reverse transcriptase, the catalytic subunit of telomerase, the enzyme responsible for maintaining telomere length for inhibiting cellular senescence (*see* Sect. 2.3), allowing a cell to become immortal. Other common recurrent sites of integration include genes encoding MLL4, a histone methyltransferase known to critically regulate epigenetics and gene expression in cancer, as well as CCNE1, cyclin E1, which regulates the cell-cycle transition from G1 to S phase and is known to be overexpressed in cancer (Matsubara and Tokino 1990). It is possible that viral integration indeed starts as a random process but that certain integration sites with oncogenic potential can be selectively enriched during hepatocarcinogenesis.

It should also be noted that HBV DNA does not completely integrate into the host cell genome unaltered. The viral DNA itself is subject to rearrangements and genomic alterations such as deletions. Thus, the integration of viral DNA into the genome may also lead to functional or structural alterations of proteins via protein fusions containing protein coded from viral DNA and host cell DNA. These so-called chimeric proteins may have novel functions that promote carcinogenesis. The

cyclin A gene has been identified as a site of HBV viral DNA integration in which an HBV-cyclin A fusion protein was coded. This protein contained a substitution of 152 amino acids of the N-terminal portion of the cyclin A gene by 156 amino acids of the HBV middle surface protein. The resulting fusion protein demonstrated tumorigenic properties (Pollicino et al. 2011). In another case, HBV DNA integrated into the gene that encoded the retinoic acid receptor β , causing overexpression of the protein because its expression was under the control of the integrated HBV preS1 viral promotor. This fusion protein also demonstrated carcinogenic properties (Garcia et al. 1993).

4 Direct Oncogenic Roles of HBV: HBx Protein

The *HBx* gene of HBV encodes the 154 amino acid, 17 kDa HBx protein. This protein does not directly bind to DNA but acts as a transactivator of viral gene expression via protein-protein interactions and modulating signaling pathways. HBx protein is required for viral replication and is critical for protecting infected hepatocytes from immune destruction. HBx also enhances HCC carcinogenesis in a number of ways (Geng et al. 2015). Several studies have shown that HBx RNA and protein expression is found in HCC tumor cells without active viral replication (Su et al. 1998; Peng et al. 2005). HBx protein itself can also be mutated, and the C-terminal region of HBx, produced by truncation, has also been shown to contribute to HCC carcinogenesis.

HBx protein interacts with, and oftentimes enhances, many key proteins and pathways that are involved in HCC carcinogenesis. For example, transforming growth factor β (TGF- β) can act as a tumor suppressor by inhibiting epithelial cell growth. However, the TGF- β signaling pathway can also shift to promote oncogenesis, and it has been demonstrated that HBx can stimulate this change in the early stages of HCC carcinogenesis (Murata et al. 2009). In this section, we will discuss the various mechanisms by which HBx has been implicated in the maintenance and progression of HCC tumor cells (Fig. 8.1, Table 8.1).

4.1 HBx Affects Cancer-Related Pathways

It has been speculated that HBx can act as a growth factor for HCC tumor cells, by activating several pathways that have been implicated in the initiation and maintenance of cancer cells such as the Jak/STAT signaling pathway (Lee and Yun 1998) and the RAS/RAF/MAPK pathway (Benn and Schneider 1994). HBx also directly binds to the C-terminus of the tumor-suppressor p53. Inactivation mutations or deletion of p53 is one of the most common pan-cancer alterations. HBx inhibits the activity of p53, including p53-mediated apoptosis, by sequestering p53 in the cytoplasm and preventing it from entering the nucleus (Wang et al. 1994). HBx mutants, caused by the HBx gene overlapping with the HBV core promoter region, also indirectly modulate p53 by upregulating the S phase kinase-associated protein 2

Table 8.1 Summary of mechanisms of HBx-mediated HCC

Effect	Mechanism	Reference
Alterations of cancer pathways	TGF- β	Murata et al. (2009)
	Jak/STAT	Lee and Yun (1998)
	RAS/RAF/MAPK	Benn and Schneider (1994)
	p53	Wang et al. (1994)
	p53: SKP2	Yan et al. (2015)
	NF- κ B: MTA1	Bui-Nguyen et al. (2010)
	NF- κ B: IL6	Lee et al. (1998)
	NF- κ B: MMP, VEGF	Liu et al. (2010a)
	HIF-1 α	Moon et al. (2004)
	Ang2	Sanz-Cameno et al. (2006)
	Wnt/ β -catenin: APC	Hsieh et al. (2011)
	Wnt/ β -catenin: GSK3	Cha et al. (2004)
HCC cell “stemness”	Oct-4, Nanog, Klf-4, β -catenin, E-cadherin, EpCAM	Arzumanyan et al. (2011)
Epigenetic changes	DNMT1/DNMT3A: RAR- β (2)	Jung et al. (2010)
	DNMT1/DNMT3A: p16 ^{ink4a}	Zhu et al. (2010)
	DNMT1: E-cadherin	Lee et al. (2005)
	ASPP	Zhao et al. (2010)
Centrosome duplication and cell cycle	Crm1/Ran GTPase	Forgues et al. (2003)
	CDK1/2, cyclin A/B/E	Benn and Schneider (1995)
	p21 ^{waf1/cip1} , CDK2	Park et al. (2000)
	p15/p16/p21/p27, cyclin D1/E, CDK2/4	Gearhart and Bouchard (2010)
	hTERT	Liu et al. (2010b)
	p16/p21 via DNA methylation	Park et al. (2011)
Mitochondrial function	Abnormal aggregation	Takada et al. (1999)
	HVDAC3	Rahmani et al. (2000)
	Electron transport	Lee et al. (2004)
	Hsp60	Tanaka et al. (2004)
	Calcium signaling	Tan et al. (2009)
DNA repair	XAP-1/UVDDDB	Becker et al. (1998)
	p53/ERCC3	Wang et al. (1994)
	XPB/XPD	Jia et al. (1999)
	hMYH α	Chang et al. (2009)

Table 8.1 (continued)

Effect	Mechanism	Reference
Noncoding RNA	miR-15a/miR-16-1	Wang et al. (2013)
	Signal transduction, cell death, DNA damage, and recombination	Ura et al. (2009)
	NF- κ B: miR-143/FNDC3B	Zhang et al. (2009)
	Let7, STAT3	Wang et al. (2010)
	miR-148a: HPIP, Akt/ERK, mTOR	Xu et al. (2013)
	miR-21: IL6, STAT3	Li et al. (2014)
	miR-21: PDCD4	Qiu et al. (2013)
	miR-152: DNMT1	Huang et al. (2010)
	miR-122: PPAR γ	Song et al. (2013)
	miR-132 via DNA methylation	Wei et al. (2013)
	HULC: p18	Du et al. (2012)
	lncRNA-dreh: vimentin	Huang et al. (2013)
	DBH-AS1: MAPK	Huang et al. (2015)
	Evi1	Huang et al. (2016)
	UCA1: p27	Hu et al. (2016)
	HBx-LINE1 fusion: Wnt/ β -catenin	Lau et al. (2014)
	HBx-LINE1 fusion: miR-122	Liang et al. (2016)

(SKP2). SKP2 contributes to the downregulation of p53 via ubiquitination and degradation, thus contributing to tumorigenesis (Yan et al. 2015).

The nuclear factor (NF)- κ B protein complex is a key transcription factor in cancer biology, regulating inflammation and cell death. The activation of the NF- κ B pathway plays a known role in hepatocarcinogenesis (Luedde and Schwabe 2011). HBx stimulates the expression of metastasis-associated protein 1 (MTA1), via interactions with NF- κ B. MTA1 is a chromatin modulator that is involved in tumor initiation and the inflammatory response (Bui-Nguyen et al. 2010). The pleiotropic cytokine interleukin 6 (IL6), which is involved in immune and inflammatory response, is also upregulated due to the enhancement of two NF- κ B subunits to target gene DNA (Lee et al. 1998). HBx increases the expression of known NF- κ B target genes, including several matrix metalloproteinases (MMPs), which are involved in metastasis, and vascular endothelial growth factor (VEGF), a key regulator of angiogenesis (Liu et al. 2010a). HBx may also play a role in tumorigenesis by upregulating and stabilizing hypoxia-inducible factor 1 α (HIF-1 α), a key regulator of hypoxia, which stimulates angiogenesis (Moon et al. 2004). HBx also stimulates the upregulation of angiopoietin-2 (Ang2), a pro-angiogenic growth factor that is involved in vascular remodeling and development during carcinogenesis (Sanz-Cameno et al. 2006).

The Wnt/ β -catenin pathway plays an important role in embryonic development and also contributes to the homeostasis of stem cells. The transcription of downstream target genes by β -catenin is regulated by the β -catenin destruction complex, which modulates the amount of stabilized β -catenin that can enter the nucleus.

However, aberrant β -catenin signaling can lead to the improper transcription of target genes such as VEGF, oncoprotein c-Myc, and cyclin D1, an important cell-cycle regulator, leading to tumorigenesis. HBx competitively binds to the adenomatous polyposis coli (APC) protein, which is part of the β -catenin destruction complex, causing the release, stabilization, and nuclear localization of β -catenin, leading to the transcription of downstream Wnt pathway targets (Hsieh et al. 2011). β -catenin levels are also directly regulated by glycogen synthase kinase 3 (GSK3) activity, which directly phosphorylates β -catenin, priming β -catenin for ubiquitination and degradation. HBx can inhibit the activity of GSK3, leading to the Wnt/ β -catenin pathway activation (Cha et al. 2004).

4.2 HBx Promotes “Stemness” of HCC Cells

High tumor heterogeneity represents a hallmark of HCC. Tumor-initiating cancer stem cells (CSCs) are found within HCC tumors and are associated with aggressive disease and poor prognosis. It has been demonstrated that HBx protein promotes “stemness” characteristics in HCC cells. HBx activates the expression of classic CSC markers Oct-4, Nanog, and Klf-4, which are associated with self-renewal. HBx expression is also associated with decreased amounts of E-cadherin. Loss of E-cadherin leads to epithelial to mesenchymal transition and thus increased metastasis and invasion. In addition, the expression of epithelial cell adhesion molecule (EpCAM), a marker of HCC tumor-initiating stem cells, has been detected in the nuclei of patients who are infected with HBV-related HCC (Arzumanyan et al. 2011). EpCAM is also a direct transcriptional target of Wnt/ β -catenin signaling (Yamashita et al. 2009), which is known to be activated by HBx, as discussed in Sect. 4.1.

4.3 HBx Induces Epigenetic Changes

Epigenetics, in which gene expression is controlled by modifications to genetic DNA such as DNA methylation, are also associated with cancer. Tumors exhibit a global hypomethylation of DNA, yet silencing of tumor suppressors by hypermethylation (Esteller 2008). In addition to directly modifying cancer-related signaling pathways through signal activation and protein-protein interaction, HBx also causes changes in gene expression through epigenetic modifications of the promoter region of genes, causing the downregulation of gene expression. Aberrant hypermethylation of the retinoic acid receptor- $\beta(2)$ (RAR- $\beta(2)$) gene has been detected in HBV-induced HCC. HBx has been shown to upregulate the expression of DNA methyltransferases 1 and 3A (DNMT1, DNMT3A), which induces promoter hypermethylation of RAR- $\beta(2)$. Downregulation of RAR- $\beta(2)$ expression leads to the subsequent downregulation of G1-checkpoint regulators p16, p21, and p27, which desensitizes cells to growth inhibition signaled by retinoic acid (Jung et al. 2010). HBx can also induce the direct hypermethylation and downregulation of p16^{ink4a}

expression via DNMT1 and DNMT3A (Zhu et al. 2010). Activation of DNMT1 by HBx has also been associated with the downregulated expression of E-cadherin, a tumor suppressor that is associated with HCC CSCs (*see* Sect. 4.2) (Lee et al. 2005). Indirectly, HBx expression is associated with hypermethylation of the promoters for the genes encoding ankyrin-repeat-containing, SH3-domain-containing, and proline-rich-region-containing proteins (ASPP). The ASPP family of proteins is known to regulate cellular apoptosis via interaction with p53. ASPP downregulation was associated with increased tumor growth and decreased sensitivity to apoptotic stimuli (Zhao et al. 2010).

4.4 HBx Deregulates Centrosome Duplication and the Cell Cycle

The cell cycle is a highly regulated process in which DNA is replicated and cells divide to create daughter cells that are genetically identical to the parent cell. Centrosomes are key in regulating cell division and serve to organize and orient microtubules during interphase and direct the formation of bipolar spindles during mitosis. Centrosome duplication begins during the G₁/S phase of the cell cycle and is completed at S phase, occurring only once per cell cycle. However, centrosome amplification in cancer cells suggests that this process is deregulated and is not cell-cycle dependent (Budhu and Wang 2005). The nuclear export receptor Crm1 and Ran GTPase form a complex that is involved in the maintenance of centrosome duplication and assembly of the mitotic spindle. HBx binds and sequesters Crm1 in the cytoplasm, causing centrosome duplication, mitotic defects, and chromosome transmission errors. The genomic instability that results from centrosome amplification and loss of faithful cell division may contribute to carcinogenesis (Forgues et al. 2003).

The fidelity of cell-cycle progression is controlled by cyclins and the subsequent activation of cyclin-dependent kinases (CDK), which are responsible for regulating key transitions. Several studies have shown that HBx protein is capable of deregulating the cell cycle. One study demonstrated that HBx activated progression through the cell cycle, shortening the emergence from quiescence (G₀) and entry into S phase, accelerating through growth checkpoint controls at G₀/G₁ and G₂/M. The cyclin-dependent kinases CDK1 and CDK2, and their associations with cyclin A, cyclin B, and cyclin E, were activated at a quicker and increased rate by HBx (Benn and Schneider 1995). In another study, HBx induced growth arrest at the G₁ phase by activating the cyclin-dependent kinase inhibitor p21^{waf1/cip1}, inhibiting CDK2, and allowing HCC cells to evade apoptosis (Park et al. 2000). HBx induces quiescent hepatocytes to exit the G₀ phase by decreasing levels of p15 and p16 and increasing levels of active G₁ phase proteins cyclin D1, cyclin E, p21, p 27, and CDK4. The activation of CDK2, the CDK controlling late G₁/S phase, was also inhibited, causing cells to stall in the G₁ phase of the cell cycle. This study suggested that active HBV viral replication requires cells to maintain the G₁ phase of growth for the most efficient replication (Gearhart and Bouchard 2010).

HBx protein can also promote HCC tumor cell immortality by overcoming senescence, growth arrest, and apoptosis. HBx upregulates the transcriptional activation of TERT, increasing telomerase activity and leading to chromosomal telomere lengthening and preventing cells from becoming senescent (Liu et al. 2010b). HBx can also cause tumor cells to overcome senescence induced by all-trans retinoic acid by promoting the downregulation of p16 and p21 proteins via DNA methylation (Park et al. 2011).

4.5 HBx Affects Mitochondrial Function

HBx may induce cellular dysfunction in HCC cells through interactions with mitochondria. Mitochondrial structures aggregate abnormally in HBV-infected HCC cells, and HBx protein is associated with these structures, leading to a potential increase in cell death (Takada et al. 1999). HBx can alter the transmembrane potential of mitochondria by physically co-localizing with the voltage-dependent anion channel HVDAC3 (Rahmani et al. 2000). HBx has also been shown to downregulate key mitochondrial enzymes that are involved in electron transport for oxidative phosphorylation and increases oxidative injury via mitochondrial reactive oxygen species and the production of lipid peroxide. These changes sensitize cells to apoptosis signals (Lee et al. 2004). It has been found that the nuclear chaperone protein heat shock protein 60 (Hsp60) physically co-localizes with HBx in the mitochondria of HCC cells and enhances apoptosis (Tanaka et al. 2004). HBx protein also activates a kinase pathway that is essential for HBV replication. This pathway is calcium-dependent and can decrease mitochondrial calcium signaling, causing mitochondrial permeability transition (Tan et al. 2009).

4.6 HBx Affects DNA Repair

The ability of cancer cells to detect and repair DNA damage is another key hallmark of cancer. The accumulation of DNA damage leads to mutagenesis and genomic instability. Multiple studies have shown that HBx interferes with nucleotide excision repair (NER). One study demonstrated that HBx can interact with X-associated protein 1 (XAP-1), a human protein homologous to simian repair protein UV-damaged DNA-binding protein (UVDDDB), which is thought to be involved with the first step of NER by binding to damaged DNA, thus prohibiting effective NER (Becker et al. 1998). HBx also affects NER by complexing with p53 protein and inhibiting the association of p53 with the NER transcription factor ERCC3 (Wang et al. 1994). HBx can also directly bind to NER factors XPB and XPD DNA helicases and inhibit NER (Jia et al. 1999). Another study indicated that HBx affects the expression of NER DNA repair enzyme DNA glycosylase α (hMYH α), leading to an increase in the level of 8-hydroxy-2 deoxyguanosine (8-OHdG), a mutagenic DNA adduct that serves as a cellular indicator of oxidative stress (Cheng et al. 2010).

4.7 HBx Causes Changes in Noncoding RNA

A majority of the DNA in the human genome is transcribed into RNA transcripts that are not translated into protein products. However, these noncoded transcripts still have an important function within the cell, controlling cellular pathways by modulating the expression of other noncoding RNA and mRNA from genes encoding proteins or directly interacting with proteins. There are two general classes of noncoding RNA based on size: short noncoding RNAs, including microRNAs (miRNA), with a length of under 200 nucleotides, and long noncoding RNAs (lncRNA) that are greater than 200 nucleotides in length (Ghidini and Braconi 2015). Expression of HBx in HCC cells deregulates the expression of numerous miRNAs and lncRNAs. The HBx transcript directly downregulates the expression of two well-known tumor-suppressor microRNAs, miR-15a and miR-16-1, via viral RNA sequences that target the microRNA (Wang et al. 2013). A number of other studies have demonstrated that HBx protein indirectly affects microRNA expression. For example, one study showed that in tumor samples from patients infected with HBV, deregulated miRNAs targeted genes in pathways associated with signal transduction, cell death, and DNA damage and recombination (Ura et al. 2009). NF- κ B, upregulated by HBx protein, transcribes an increased amount of miR-143, causing the repression of fibronectin type III domain-containing 3B (FNDC3B), leading to an increase in cellular invasion and migration, promoting tumor metastasis (Zhang et al. 2009). HBx expression downregulated members of the let-7 family of miRNAs, leading to an upregulation of signal transducer and activator of transcription 3 (STAT3) and an increase in cellular proliferation (Wang et al. 2010). Another study found that HBx enhances tumorigenesis by downregulating the p53-mediated activation of miR-148a, which reduces growth, invasion, and metastasis of HCC cells. A decrease in miR-148a led to the increased expression of hematopoietic pre-B-cell leukemia transcription factor-interacting protein (HPIP), which regulates cancer cell growth through the Akt/extracellular-related kinase (ERK) and mammalian target of rapamycin (mTOR) signaling pathway (Xu et al. 2013). The microRNA miR-21 has been shown in several studies to promote HCC carcinogenesis. A C-terminal-truncated HBx protein upregulated miR-21 through IL6 and STAT3 activation. This study demonstrated that miR-21 overexpression plays a critical role in hepatocyte carcinogenesis following HBV infection (Li et al. 2014). HBx downregulated the expression of programmed cell death 4 (PDCD4) through miR-21. PDCD4 may induce apoptosis in cells; thus, a decrease in PDCD4 may lead to decreased apoptosis in HCC cells (Qiu et al. 2013).

Epigenetics and microRNAs are interrelated in the progression of HCC. For example, HBx can lead to epigenetic aberration via downregulation of tumor-suppressing miR-152, which causes an increase in DNMT1, leading to hypermethylation and silencing of target genes (Huang et al. 2010). Another microRNA, miR-122, is the most highly expressed liver microRNA, and reduced expression of miR-122 is associated with the progression of HCC. Peroxisome

proliferator-activated receptor-gamma (PPAR γ) is part of a nuclear complex that modifies gene transcription, via epigenetic changes through the recruitment of histone deacetylases or histone methyltransferases, and promotes the expression of miR-122. PPAR γ is directly bound by HBx in HBV-infected hepatocytes, leading to decreased miR-122 expression and cancer progression (Song et al. 2013). MiR-132, a tumor-suppressive microRNA, represses tumor growth by inhibiting the Akt-signaling pathway. HBx epigenetically represses the expression of MiR-132 by inducing DNA methylation of the MiR-132 promoter (Wei et al. 2013).

LncRNAs have been implicated in cancer progression as well. The lncRNA highly upregulated in liver cancer (HULC) is positively associated with HCC. HBx promotes the expression of HULC by associating with the cAMP-responsive element-binding protein and activating the HULC promoter. Increased expression of HULC was also found to downregulate the expression of the tumor-suppressor protein p18, leading to increased hepatocarcinogenesis (Du et al. 2012). Another lncRNA, downregulated expression by HBx (termed lncRNA-dreh), acts as a tumor suppressor by interacting with and reducing the expression of the intermediate filament protein vimentin, preventing tumor metastasis. A decrease in lncRNA-dreh is associated with poor prognosis in HCC patients (Huang et al. 2013). The expression of a novel lncRNA, DBH-AS1, was discovered to be positively associated with HBsAg expression, as well as HCC tumor size. DBH-AS1 promotes tumorigenesis by inducing cell-cycle progression, inhibiting apoptosis, and activating the MAPK pathway. The expression of DBH-AS1 is significantly increased by HBx protein (Huang et al. 2015). HBx protein also affects lncRNA expression through the transcription regulator ecotropic viral integration site 1 (Evi1). HBx expression causes an increase in Evi1, which deregulates a cluster of lncRNAs involved in HCC progression (Huang et al. 2016). HBx expression also induces the upregulation of the lncRNA UCA1, which promotes cell growth by enhancing cell-cycle progression via the downregulation of p27 genes (Hu et al. 2016).

As discussed in Sect. 3, HBV viral DNA inserts into host DNA, causing insertional mutagenesis. HBV insertion often occurs near or within DNA that is noncoding, such as long interspersed nuclear elements (LINEs), leading to the creation of chimeric HBx-LINE fusions. One study demonstrated that the HBx-LINE1 chimeric fusion is found in 23.3% of HCC tumors that are associated with HBV and is correlated with poor survival. The authors of the study also demonstrated that HBx-LINE1 expression could affect the activity of β -catenin and activate Wnt pathway signaling, thus acting like a lncRNA with HCC promoting properties (Lau et al. 2014). The HBx-LINE1 fusion transcript also contains six binding site for the tumor-suppressive miR-122, thus sequestering and depleting miR-122 in HCC cells. The reduction of miR-122 promotes tumorigenesis by activating epithelial-mesenchymal transition (EMT), leading to changes such as activation of the Wnt/ β -catenin pathway, downregulation of E-cadherin, and increased cell migration (Liang et al. 2016).

5 Concluding Remarks

HBV infection constitutes a major etiological factor for the development of primary liver cancer and remains a public health issue worldwide. HBV contributes to the carcinogenesis of HCC by various indirect and direct mechanisms (Fig. 8.1). HBV infection triggers cellular damage through oxidative stress, reactive oxygen species, the inflammatory response, and telomere shortening, leading to potential mutation and cellular transformation following repeated cycles of liver cell injury. HBV integration into the genome contributes to oncogenesis indirectly by inducing genomic instability and directly through insertional mutagenesis in or near genomic loci that code for genes that regulate cellular proliferation and survival. The integration of viral DNA also allows for the expression of the HBx protein, which directly contributes to the molecular carcinogenesis of HCC by a number of mechanisms including affecting cancer-related pathways and altering cellular epigenetics and noncoding RNA expression (Table 8.1), which promotes cellular transformation.

To date, the most effective treatment for HBV-related HCC is prevention through vaccination programs. In Taiwan, a universal nationwide childhood vaccination program was implemented in the 1980s. A 20-year follow-up revealed a significant reduction of both the number of HBV carriers, as well as the incidence of childhood HCC, with benefits being maintained into adulthood (Chang et al. 2009). For people who are already carriers of HBV, continued surveillance of disease progression is an important preventative strategy, and early interventional antiviral therapy is key for reducing the incidence of HCC. Antiviral therapy, in addition to reducing serum viral load, controlling viral replication, and promoting the serum conversion of hepatitis B e antigen, may also block the carcinogenic properties of HBx. The immunomodulator interferon alpha (INF- α) may provide a benefit to patients with cirrhosis, whereas treatment with nucleoside analogs has been shown to reduce the risk of HCC development and delay disease progression in patients with HBV. However, the development of resistance continues to be a challenge (Sanyal et al. 2010; Lai and Yuen 2013).

Despite our current understanding of the molecular mechanisms of HCC carcinogenesis that are promoted by HBV infection, there are still a number of important questions that remain to be answered. For example, there is a disconnect between the vast amount of information we have gathered regarding the various mechanisms of HBV-induced HCC carcinogenesis and the true contribution that each plays in cellular transformation. It is unknown whether the amount of HBV replication or viral integration in each patient is directly correlated to increased clonal expansion, cellular transformation, and advanced tumor progression. Additionally, it remains to be answered whether there are distinct subtypes of HBV-related HCC or how HBV may interact with other etiological factors in HCC, which may lead to unique molecular pathogenesis. Thus, the discovery of biomarkers and new therapeutic targets for the treatment of HBV-related HCC will continue to be an important field of study, which will be necessary for the development of prevention strategies and personalized treatment modalities for patients with HBV infection (Levrero and Zucman-Rossi 2016).

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Non-invasive Assessment of Liver Disease

9

Henry Lik-Yuen Chan and Vincent Wai-Sun Wong

Abstract

Liver fibrosis or cirrhosis is one of the strongest risk factors of hepatocellular carcinoma and liver-related complications in patients with chronic hepatitis B. Because current therapies for chronic hepatitis B are safe and highly effective, routine liver biopsy for prognostication and selection of patients for treatment cannot be justified. Instead, non-invasive tests of liver fibrosis such as physical measurement of liver stiffness (e.g. transient elastography, acoustic radiation force impulse, shear wave elastography and magnetic resonance elastography) and serum tests of fibrosis (including both proprietary and generic markers) have largely replaced liver biopsy as the initial assessment of disease severity. This chapter focuses on the nature and diagnostic performance of these non-invasive tests. We also discuss their role in detecting portal hypertension and predicting adverse outcomes.

Keywords

Transient elastography • Liver stiffness measurement • Portal hypertension • Hepatocellular carcinoma • Liver fibrosis • Cirrhosis

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1 Introduction

Chronic hepatitis B is characterized by a complex natural history with variable bouts of hepatitic activities ranging from subclinical fluctuation in aminotransferase levels to acute-on-chronic liver failure. Disease progression depends on the frequency and severity of these hepatitis flares (see Chap. 4 for details). Patients who enter the inactive chronic hepatitis B phase or achieve hepatitis B surface antigen seroclearance early in their lives have a benign clinical course and low risk of liver-related complications. In contrast, patients who continue to have active disease are at a high risk of developing cirrhosis and hepatocellular carcinoma (HCC).

In any case, liver fibrosis is central in the natural history of chronic hepatitis B. Like other chronic liver diseases, hepatic necroinflammation triggers a wound healing process with deposition of the fibrous tissue. While fibrosis limits the degree of injury during acute liver insult, it becomes maladaptive when liver injury is chronic. With accumulating fibrous tissue and distortion of liver architecture, cirrhosis develops and sets the stage for different cirrhotic complications and HCC. Moreover, as chronic hepatitis B is a dynamic disease, it is difficult to determine the phase of disease accurately based on one single set of laboratory results. For example, a patient with positive hepatitis B e antigen (HBeAg) and high hepatitis B virus DNA level can be in the immune-tolerant phase or HBeAg-positive immune-active phase. A patient with negative HBeAg can be in the inactive chronic hepatitis B phase or HBeAg-negative immune reactivation phase. A normal aminotransferase level cannot be used to distinguish among the phases because hepatic necroinflammation may fluctuate with time. In this situation, the detection of significant fibrosis can inform doctors whether the patient has already experienced liver injury. Therefore, assessing the degree of liver fibrosis allows doctors to understand the phase of the disease and predict the prognosis of patients with chronic hepatitis B. On the management side, the presence of significant fibrosis is an important indication for antiviral therapy. The severity of fibrosis and cirrhosis determines if screening for HCC and varices is needed. Serial evaluation of fibrosis also reflects disease progression and response to treatment.

The diagnosis of cirrhosis is obvious when the liver is already shrunken with regenerative nodules or in the presence of clinical or radiological features of portal hypertension. However, routine imaging techniques like abdominal ultrasonography and computed tomography cannot reliably diagnose early cirrhosis, not to mention fibrosis. Traditionally, liver biopsy was considered the gold standard for fibrosis staging. Nevertheless, liver biopsy is invasive and poorly accepted by patients. It is also undesirable to perform repeated liver biopsy to assess disease progression. Finally, liver biopsy is an imperfect “gold standard.” It is important to remember that only a small portion of the liver is sampled during biopsy. Sampling variability involving the less severe part of the liver would lead to underestimation of the

fibrosis stage. For all these reasons, reliable and accurate non-invasive tests of fibrosis are required.

In this chapter, we will first introduce different non-invasive tests of fibrosis. This is followed by a discussion on the use of these tests in fibrosis staging and estimation of portal hypertension and HCC risk. Although most data on non-invasive tests of fibrosis came from studies involving other liver diseases, we will refer to studies on chronic hepatitis B whenever possible and highlight cases when the data were from other liver diseases.

2 Non-invasive Tests of Liver Fibrosis

Non invasive tests of fibrosis can be divided into serum tests and physical measurements of liver stiffness or elasticity. Serum tests can be done in virtually all patients at the outpatient setting. The cost depends on the biomarkers involved. In comparison, physical measurements are probably more accurate overall. On the other hand, they can only be done in centers equipped with the machines. Measurements may also fail in some patients and be influenced by factors unrelated to fibrosis.

2.1 Serum Tests

Serum biomarkers of fibrosis can be divided into class I biomarkers, which directly measure fibrogenesis and fibrinolysis, and class II biomarkers, which are parameters that correlate with fibrosis (Table 9.1). Class II biomarkers are often routinely performed in clinical practice and are therefore inexpensive, but they are also expected to be less accurate. Because no single biomarker has been shown to be sufficiently accurate, most serum tests are formulae from a mixture of biochemical and/or clinical parameters. It should be noted that these formulae were modeled against liver histology. As a result, the reliability of liver histology would be the ceiling for the accuracy of serum tests (Mehta et al. 2009). This may explain why serum tests often perform less well in independent validation cohorts and also appear less accurate than physical measurements of liver stiffness. For the same reason, the results of serum tests must be validated independently before they can be considered reliable.

2.1.1 Class I Biomarkers

FibroTest (BioPredictive, Paris, France), also known as FibroSure in the USA, is a patented biomarker panel comprising of α 2-macroglobulin, gamma-glutamyl transpeptidase, apolipoprotein A1, haptoglobin, total bilirubin, age, and gender. The test was first developed using liver histology as the reference standard in patients with chronic hepatitis C (Imbert-Bismut et al. 2001; Poynard et al. 2003). When applied

Table 9.1 Serum tests of fibrosis for chronic hepatitis B

Tests	Formulae	AUROC (histologic system)	Limitations
<i>Class I biomarkers</i>			
FibroTest (Myers et al. 2003; Poynard et al. 2009; Kim et al. 2012)	Patented formula including $\alpha 2$ -macroglobulin, gamma-glutamyl transpeptidase, apolipoprotein A1, haptoglobin, total bilirubin, age, and gender	0.78–0.90 for F2–F4, 0.87 for F4 (METAVIR)	- Some of the tests are expensive and not routinely performed - Haptoglobin affected by hemolysis - Total bilirubin affected by hemolysis and Gilbert's syndrome
FibroMeter VIRUS (Cales et al. 2005, 2008; Leroy et al. 2014)	Patented formula including platelet count, $\alpha 2$ -macroglobulin, ALT, urea, prothrombin index, gamma-glutamyl transpeptidase, AST, age, and gender	0.91 for F2–F4, 0.85 for F3–F4, 0.87 for F4 (METAVIR)	- Some of the tests are expensive and not routinely performed - Platelet count affected by immune thrombocytopenia purpura - Both AST and ALT affected by hepatitis flare
Enhanced liver fibrosis panel (Lichtinghagen et al. 2013)	Patented formula including age, hyaluronate, matrix metalloproteinase-3, tissue inhibitor of metalloproteinase-1	0.90 for F3–F6, 0.95 for F5–F6 (Ishak)	- The tests are expensive and not routinely performed
Zeng score (Zeng et al. 2005)	$-13.995 + 3.220 \log (\alpha 2\text{-macroglobulin [g/l]}) + 3.096 \log (\text{age [years]}) + 2.254 \log (\text{gamma-glutamyl transpeptidase [IU/l]}) + 2.437 \log (\text{hyaluronic acid [\mu g/l]})$	0.77 for F2–F4 (Scheuer)	- Some of the tests are expensive and not routinely performed
<i>Class II biomarkers</i>			
AST-to-ALT ratio (Wai et al. 2006)	AST (IU/l)/ALT (IU/l)	0.58 for F3–F6 (Ishak)	- Both AST and ALT affected by hepatitis flare
AST-to-platelet ratio index (Wong et al. 2010a)	$(\text{AST [IU/l]}/\text{upper limit of normal for AST [IU/l]})/(\text{platelet count} (\times 10^9/\text{l}) \times 100$	0.55 for F3–F4 (METAVIR)	- AST affected by hepatitis flare - Platelet count affected by immune thrombocytopenia purpura
FIB-4 index (Wong et al. 2010a)	$\text{Age (years)} \times \text{AST (IU/l)}/(\text{platelet count} (\times 10^9/\text{l})/\sqrt{\text{ALT (IU/l)}})$	0.64 for F3–F4 (METAVIR)	- Both AST and ALT affected by hepatitis flare - Platelet count affected by immune thrombocytopenia purpura

Table 9.1 (continued)

Tests	Formulae	AUROC (histologic system)	Limitations
Forns index (Forns et al. 2002)	$7.811 - 3.131 \ln(\text{platelet count } [\times 10^9/\text{l}]) + 0.781 \ln(\text{gamma-glutamyl transpeptidase } [\text{IU/l}]) + 3.467 \ln(\text{age } [\text{years}]) - 0.014 \times \text{cholesterol } (\text{mg/dl})$	0.72 for F3–F4 (METAVIR)	- Platelet count affected by immune thrombocytopenia purpura - Cholesterol affected by lipid-lowering drugs
HUI score (Hui et al. 2005)	$3.148 + 0.167 \times \text{body mass index } (\text{kg/m}^2) + 0.088 \times \text{total bilirubin } (\mu\text{mol/l}) - 0.151 \times \text{albumin } (\text{g/l}) - 0.019 \times \text{platelet count } (\times 10^9/\text{l})$	0.77 for F3–F6 (Ishak)	- Total bilirubin affected by hemolysis and Gilbert's syndrome

ALT Alanine aminotransferase, AST aspartate aminotransferase, AUROC area under the receiver operating characteristic curve

to patients with chronic hepatitis B, FibroTest has an area under the receiver operating characteristic (AUROC) curve of around 0.78–0.90 in diagnosing significant fibrosis (F2) and 0.87 in diagnosing cirrhosis (Myers et al. 2003; Poynard et al. 2009; Kim et al. 2012). When alanine aminotransferase (ALT) is added to the parameters of FibroTest, the combined formula of ActiTest can also be used to diagnose significant necroinflammation. FibroTest is reported on a scale of 0–1.0, with increasing values suggestive of higher fibrosis stages. In the report, the FibroTest result is also translated into corresponding fibrosis stages by the METAVIR system (F0–F4), though in reality there is some overlap in FibroTest values in patients with different fibrosis stages.

In patients receiving antiviral therapy for chronic hepatitis B, FibroTest values decrease with time (Poynard et al. 2009, 2005). While some of the changes may reflect fibrosis regression, this may also be contributed by reduced hepatic necroinflammation. At present, the accuracy of FibroTest in diagnosing changes in histological fibrosis has not been formally evaluated.

FibroMeter (Echosens, Paris, France) is another patented biomarker panel comprising of platelet count, α 2-macroglobulin, ALT, urea, prothrombin index, gamma-glutamyl transpeptidase, aspartate aminotransferase (AST), age, and gender. Separate formulae were developed for viral hepatitis, nonalcoholic fatty liver disease, and alcoholic liver disease, with FibroMeter VIRUS designed for chronic hepatitis B and C. All reported results are on a scale of 0–1, which indicate the probability of significant fibrosis (fibrosis score/FibroMeter) and cirrhosis (cirrhosis score/CirrhoMeter) and significant necroinflammation (activity grade/InflaMeter). In early works involving patients with chronic viral hepatitis (mainly hepatitis C but some hepatitis B), FibroMeter VIRUS had AUROC of 0.84–0.91 for significant fibrosis (F2), 0.85 for advanced fibrosis (F3), and 0.87 for cirrhosis (Cales et al. 2005, 2008; Leroy et al. 2014). The performance of both FibroTest and

FibroMeter appears to be similar in patients with chronic hepatitis B and chronic hepatitis C (Leroy et al. 2014).

The enhanced liver fibrosis (ELF) is a patented panel of three class I biomarkers, hyaluronate, matrix metalloproteinase-3, and tissue inhibitor of metalloproteinase-1, adjusted for age. The normal range and optimal cutoffs of ELF were derived from 400 healthy controls and 79 patients with chronic hepatitis C in Germany (Lichtinghagen et al. 2013). The AUROC was 0.90 for significant fibrosis (F3–F6 by the Ishak system) and 0.95 for cirrhosis (F5–F6). Importantly, the diagnostic performance of ELF was higher than that of its individual components. Based on this study, the recommended interpretation of the ELF score was <7.7 = none to mild fibrosis, 7.7 – 9.7 = moderate fibrosis, and ≥ 9.8 = severe fibrosis. The test has been validated in Asian patients with chronic hepatitis B (Kim et al. 2012; Wong et al. 2014a).

In addition, a study from China showed that the combination of $\alpha 2$ -macroglobulin, age, gamma-glutamyl transpeptidase, and hyaluronic acid could achieve moderate accuracy in detecting significant fibrosis (F2–F4 by Scheuer's system) (Zeng et al. 2005).

2.1.2 Class II Biomarkers

Models involving Class II biomarkers utilize parameters associated with fibrosis and cirrhosis (Table 9.1). Although the formulae differ, similar clinical and laboratory parameters have been chosen. For example, since fibrosis develops over a long period in patients with chronic viral hepatitis, age is an important factor associated with fibrosis. Similarly, hepatic necroinflammation is the driver toward fibrosis progression and cirrhosis, and therefore, patients with elevated aminotransferases are more likely to have fibrosis. Patients with cirrhosis may also have decreased protein synthesis (reflected by serum albumin), increased bilirubin level, and hypersplenism (with thrombocytopenia). The latter markers may reflect cirrhosis better than earlier stages of fibrosis.

The AST/ALT ratio, AST-to-platelet ratio index (APRI), and FIB-4 index were first evaluated in patients with chronic hepatitis C and/or HIV coinfection (Williams and Hoofnagle 1988; Wai et al. 2003, 2006). While easy to calculate, the accuracy of these generic formulae is modest when applied to patients with chronic hepatitis B (Wai et al. 2006; Wong et al. 2010a).

Apart from liver biochemistry and markers of liver dysfunction, the Forns index and the HUI score also include metabolic parameters (cholesterol for the Forns index and body mass index for the HUI score). By head-to-head comparison, both scores performed better than the AST/ALT ratio and APRI (Wong et al. 2010a). This underscores the fact that there are numerous metabolic changes in patients with cirrhosis. Besides, metabolic syndrome is a risk factor for the development of cirrhosis and HCC in patients with chronic hepatitis B (Wong et al. 2009a; Chen et al. 2008; Yu et al. 2008).

Some of the biomarkers may be affected by factors other than fibrosis. The limitations and potential source of misinterpretation are listed in Table 9.1.

2.2 Physical Measurements

Table 9.2 summarizes the characteristics of the machines for physical measurement of liver stiffness or elasticity. Other than the listed machines, some other ultrasound machines also have built-in technologies to measure tissue elasticity. However, they will not be discussed further because they are not specifically designed for liver assessment and performance data are limited.

For transient elastography (FibroScan, Echosens, Paris, France), shear wave elastography (SuperSonic Imagine, Aix-en-Provence, France) and magnetic resonance elastography, shear waves, or vibrations are generated externally by a probe or transducer and propagated across the liver parenchyma. Based on physical principles, waves travel faster in a stiffer medium. It is therefore possible to estimate liver stiffness and thereby fibrosis by measuring the velocity of the shear waves. Because of potential interferences, ultrasound techniques use a single transient pulse to generate mechanical vibration. In contrast, continuous mechanical excitation is used in magnetic resonance elastography. Whether this accounts for superior diagnostic accuracy is currently unknown. On the other hand, acoustic radiation force impulse uses acoustic pulses to “push” tissue internally and capture tissue motion.

When an ultrasound machine or magnetic resonance imaging is used, it is possible to visualize the liver parenchyma. One may therefore combine fibrosis assessment and HCC screening during the same examination. In comparison, only M mode ultrasound can be done with transient elastography (FibroScan); a separate examination is needed for anatomical evaluation.

For historical reasons, transient elastography has been most extensively evaluated (Table 9.3). Overall, it has moderate accuracy to diagnose significant fibrosis and high accuracy in diagnosing cirrhosis. One limitation of transient elastography is the low success rate of measurement in obese patients (Wong et al. 2011a). The development of the XL probe has largely but not completely solved this problem (de Ledinghen et al. 2012; Wong et al. 2012). The XL probe generates a more forceful mechanical impulse to produce shear waves, and it uses lower-frequency ultrasound (2.5 MHz instead of 3.5 MHz) to gain access to deeper tissues.

Table 9.2 Physical measurement of liver stiffness/elasticity

Tests	Mode of examination	Stress or vibration	Visualization of liver parenchyma
Transient elastography (FibroScan)	Ultrasound	External	No
Acoustic radiation force impulse	Ultrasound	Internal “push”	Yes
Shear wave elastography	Ultrasound	External	Yes
Magnetic resonance elastography	Magnetic resonance imaging	External, continuous	Yes

Table 9.3 Diagnostic performance of transient elastography in chronic hepatitis B

Study	N	AUROC	Cutoff (kPa)	Sensitivity (%)	Specificity (%)
<i>F2-4</i>					
Marcellin (2009)	173	0.81	7.2	70	83
Wang (2009)	88	0.86	8.0	80	77
Degos (2010)	284	0.78	5.2	89	38
Sporea (2010)	140	0.65	7.0	59	70
Viganò (2011)	217	0.85	6.2–9.4	94	96
Cardoso (2012)	202	0.87	7.2	74	88
Goyal (2013)	357	0.84	6.0	82	67
Wong (2015a)	1051	0.77	7.2	70	70
<i>F4</i>					
Chan (2009)	161	0.93	12.0–13.4	98	75
Marcellin (2009)	173	0.93	11.0	93	87
Kim (2009)	91	0.80	9.7	82	59
Wang (2009)	88	0.89	10.0	85	88
Degos (2010)	284	0.85	12.9	52	93
Sporea (2010)	140	0.97	13.6	86	99
Viganò (2011)	217	0.94	9.4–13.1	98	95
Cardoso (2012)	202	0.93	11.0	75	90
Goyal (2013)	357	0.93	11.0	81	95
Wong (2015a)	1051	0.81	10.0	70	76

There are only limited data for the use of the other techniques in patients with chronic hepatitis B, and head-to-head comparisons are rare. Based on studies on chronic hepatitis B and other liver diseases, it appears that acoustic radiation force impulse and shear wave elastography have at least similar diagnostic performance as transient elastography and the success rate of examination may be higher (Friedrich-Rust et al. 2009; Rizzo et al. 2011; Leung et al. 2013).

Magnetic resonance elastography can examine the whole liver and is not affected by obesity and ascites. Its diagnostic accuracy is higher than that of ultrasound-based techniques, with AUROC approaching 95–100% for the diagnosis of significant fibrosis and cirrhosis (Huwart et al. 2008; Wang et al. 2012; Venkatesh et al. 2014). The main hurdle to widespread use of magnetic resonance elastography is the availability of the technology and cost of examination. A few patients may also have contraindications to magnetic resonance imaging such as metallic implants and claustrophobia. In addition, while it is possible to perform ultrasound-based elastography as a point-of-care test, patients need a separate appointment for magnetic resonance elastography.

It is important to understand that liver stiffness can be affected by factors other than fibrosis. Although most data in this area came from studies of transient elastography, other physical measurements should be similarly affected. The single most important confounding factor is active hepatic necroinflammation (Table 9.4). Patients with ALT above five to ten times the upper limit of normal often have grossly elevated liver stiffness in the range of cirrhosis; the stiffness typically falls with the resolution of hepatitis. This may occur in patients with

Table 9.4 Conditions leading to false-positive liver stiffness measurement

Conditions	Clinical implications
Active hepatitis	- Use higher liver stiffness cutoffs in patients with elevated alanine aminotransferase - Avoid liver stiffness measurement in patients with very high alanine aminotransferase
Food intake	- Fast for at least 2 h (preferably 6 h) before liver stiffness measurement
Biliary obstruction	- Avoid liver stiffness measurement in patients with mechanical biliary obstruction
Congestive heart failure	- Avoid liver stiffness measurement in patients with active heart failure
Amyloidosis	- Avoid liver stiffness measurement in patients with amyloidosis involving the liver
Severe steatosis	- The influence of steatosis on liver stiffness remains controversial - Caution in the interpretation of liver stiffness in patients with severe steatosis and morbid obesity

acute exacerbation of chronic hepatitis B (Wong et al. 2009b). Furthermore, a milder degree of inflammation (ALT one to five times the upper limit of normal) has also been shown to increase liver stiffness (Chan et al. 2009). An ALT-based algorithm for the interpretation of liver stiffness has thus been proposed. Besides, food intake increases portal blood flow and can raise liver stiffness by 1–2 kPa (Mederacke et al. 2009). While congestive heart failure, mechanical biliary obstruction, and amyloidosis can also substantially increase liver stiffness, these conditions should be clinically apparent. The more controversial issue is the impact of severe steatosis. Theoretically, hepatic steatosis should make the liver softer, but a few but not all studies showed that patients with severe steatosis had higher liver stiffness (Petta et al. 2015). This may alternatively be due to increased liver stiffness in patients with extreme body mass index (Das et al. 2012; Wong et al. 2013).

Apart from elastography-based assessment, a multiparametric magnetic resonance model has been developed to assess different aspects of the liver (LiverMultiScan, Perspectum Diagnostics, Oxford, UK). Using T1 and T2* mapping and proton spectroscopy, hepatic steatosis, iron content, inflammation, and fibrosis may be measured in a relatively short time (Banerjee et al. 2014). The combined liver inflammation and fibrosis (LIF) score has been shown to predict HCC and cirrhotic complications in a small prospective cohort (Pavlidis et al. 2016). Data in chronic hepatitis B are limited.

3 Combination of Serum Tests and Physical Measurements of Liver Stiffness

Because serum tests and physical measurements work by different mechanisms, it is reasonable to use them together to improve the diagnostic accuracy. One approach is to do both tests together as initial assessment. If both tests agree with each other,

the estimation of fibrosis stage is reliable, and treatment decisions can be made. In case the tests show conflicting results, one should explore for factors that may affect the performance of the tests and consider repeating the tests later. Liver biopsy can be reserved for cases with indeterminate results after the above workup. This approach is endorsed by the European Association for the Study of the Liver and Asociación Latinoamericana para el Estudio del Hígado for patients with chronic hepatitis C, but the algorithm has not been adequately tested in chronic hepatitis B (European Association for Study of the Liver and Asociación Latinoamericana para el Estudio del Hígado 2015). A few studies have tested the possibility of adding a serum test like the Forns index and enhanced liver fibrosis panel to transient elastography to improve the confidence of ruling in advanced fibrosis in patients with chronic hepatitis B (Wong et al. 2010a, 2014a).

Since routine use of two non-invasive tests of fibrosis would invariably increase healthcare costs, an alternative approach is to perform a second test only when the diagnosis is uncertain. After all, although recommended cutoffs are used to facilitate interpretation, the results of the non-invasive tests are continuous variables. The confidence of excluding and ruling in different fibrosis stages increases when the non-invasive test results are more extreme (Wong et al. 2015a).

4 Portal Hypertension

Portal hypertension accounts for most of the complications of cirrhosis such as variceal bleeding, ascites, hepatic encephalopathy, and hepatorenal syndrome. The gold standard for the measurement of portal pressure is hepatic vein pressure gradient (HVPG). The normal HVPG is below 5 mmHg; a level of 10 mmHg or more is described as clinically significant portal hypertension, when varices start to develop. There is a strong mechanistic basis for non-invasive tests of fibrosis to predict portal hypertension. First, both serum test results and liver stiffness increase from early to more advanced cirrhosis. They can therefore be used to diagnose not only cirrhosis but also advanced cirrhosis. Second, elastography-based assessments can also reflect portal blood flow, which is a major mechanism leading to portal hypertension. While studies on portal hypertension were seldom limited to patients with chronic hepatitis B, it is reasonable to extrapolate the findings to this population.

Serum tests such as the FIB-4 index have moderate correlation with HVPG (Park et al. 2009). Liver stiffness measurement also has moderate to good correlation with HVPG and the presence of large varices (Wong et al. 2015b). In the latest Baveno VI guidelines, a liver stiffness of ≥ 20 –25 mmHg is considered sufficient to rule in clinically significant portal hypertension (de Franchis and Baveno 2015). In contrast, patients with liver stiffness < 20 mmHg and normal platelet count $> 150 \times 10^9/l$ have minimal risk of having varices that require treatment and may be spared from screening endoscopy. They should however undergo yearly assessment for disease progression.

Splenomegaly is a feature of portal hypertension, and therefore, platelet count (reflecting hypersplenism) and spleen size by abdominal ultrasonography have been used to identify patients for varices screening. For the same reason, spleen stiffness may reflect portal hypertension more directly than liver stiffness. A few studies have confirmed the association between spleen stiffness and HVP or large varices (Colecchia et al. 2012; Stefanescu et al. 2011; Takuma et al. 2013). Nevertheless, it is important to note that transient elastography is not designed for the measurement of spleen stiffness. Because stiffness is measured in a core of tissue that is 4 cm in length and 1 cm in diameter, it may be impossible to find an optimal window to measure spleen stiffness in some patients, particularly when the spleen size is relatively normal. This difficulty can be overcome by using acoustic radiation force impulse, shear wave elastography, or magnetic resonance elastography. There is currently a pragmatic randomized controlled trial to test transient elastography versus endoscopy as the initial assessment in patients with radiological cirrhosis with final results expected by the end of 2016 ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02024347) identifier NCT02024347).

5 Hepatocellular Carcinoma and Mortality

Cirrhosis is the single most important risk factor of HCC development in patients with chronic hepatitis B (Wong et al. 2010b). It is therefore not surprising that patients with abnormal non-invasive tests of fibrosis have increased risk of HCC. While HCC risk increases in a stepwise manner as cirrhosis becomes more advanced (Jung et al. 2011), such cases are clinically apparent and do not need additional workup by non-invasive tests. Instead, the main role of non-invasive tests is to detect subclinical advanced fibrosis and cirrhosis that would otherwise be missed by routine imaging and blood tests (Kim et al. 2015).

In a multicenter French study of 1312 patients with chronic hepatitis B, FibroTest was associated with HCC risk in a dose-response manner (Poynard et al. 2014). Similarly, ELF has been shown to predict liver-related complications in Korean patients with chronic hepatitis B, with HCC accounting for most of the events (Kim et al. 2014).

The incidence of HCC increases with increasing liver stiffness measurement by transient elastography (Jung et al. 2011). Since HCC can develop in a non-cirrhotic liver and there are other important risk factors of HCC in patients with chronic hepatitis B, liver stiffness has been combined with other risk factors in some HCC risk scores (Table 9.5). On the whole, the LSM-HCC and mREACH-B scores, both incorporating liver stiffness, perform better than similar scores based on clinical characteristics alone in predicting HCC development in the next 3–5 years (Wong et al. 2014b; Jung et al. 2015). Likewise, liver stiffness and serum tests of fibrosis are at least as good as histological fibrosis staging in predicting mortality in chronic hepatitis B patients (de Ledinghen et al. 2013).

Table 9.5 Liver stiffness-based hepatocellular cellular prediction scores for chronic hepatitis B

Score	Calculation			HCC risk		
					3 years	5 years
LSM-HCC score (Wong et al. 2014b)	Age (years)	>50	10 points	0–10 points	0%	0.3%
	Albumin (g/l)	≤35	1 point	11–20 points	2%	5%
	HBV DNA (IU/ml)	>200,000	5 points	21–30 points	11%	12%
	Liver stiffness (kPa)	8.1–12.0	8 points			
		>12.0	14 points			
	Total		0–30 points			
mREACH-B score (Jung et al. 2015)	Age (years)	30–34	0 point	0–6 points	0.6%	0.9%
		35–39	1 point	7–9 points	1.9%	3.7%
		40–44	2 points	10–11 points	5.6%	11.6%
		45–49	3 points	12–13 points	16.0%	21.6%
		50–54	4 points	14–16 points	23.3%	30.0%
		55–59	5 points			
		60–65	6 points			
	Male sex		2 points			
	ALT (U/l)	<15	0 point			
		15–44	1 point			
		≥45	2 points			
	Hepatitis B e antigen	Positive	2 points			
	Liver stiffness (kPa)	8.0–13.0	2 points			
		>13.0	4 points			
	Total		0–16 points			

HBV Hepatitis B virus, HCC hepatocellular carcinoma

6 Unresolved Questions

Most studies to date validated non-invasive tests of fibrosis against liver histology or portal hypertension or used a single baseline result to predict clinical outcomes. In clinical practice, however, doctors do not see a patient once but have to evaluate

him/her repeatedly over time. It is unclear how well changes in non-invasive tests correlate with histological changes. For example, APRI and the FIB-4 index have been shown to decline during antiviral therapy even in patients without fibrosis regression (Ray Kim et al. 2016). This is because AST and ALT are integral components of these scores. Antiviral therapy can reduce aminotransferases rapidly well before fibrosis regresses. The same problem occurs with transient elastography because an improvement in hepatic inflammation also reduces liver stiffness (Wong et al. 2011b).

Along the same line, it is now established that patients can achieve reversal of cirrhosis with prolonged antiviral therapy (Marcellin et al. 2013). Incident varices are rare in patients receiving antiviral therapy (Lampertico et al. 2015). It is important to evaluate if non-invasive tests can be used to diagnose reversal of cirrhosis and change the practice of HCC and varices screening in some patients.

Conclusion

Fibrosis assessment is an important part of the management of chronic hepatitis B. Serum- and image-based non-invasive tests of fibrosis can diagnose advanced fibrosis and cirrhosis with reasonable accuracy and have already changed clinical practice and reduced the need for liver biopsy in some patients. These tests also help doctors predict prognosis and select patients for HCC and varices screening. Developing tools to monitor disease progression in treated and untreated patients should be the next research priority.

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Chronic HBV Infection: Interferon Therapy and Long-Term Outcomes

10

Tarik Asselah and Patrick Marcellin

Abstract

Chronic hepatitis B infection affects around 240 million people worldwide, with long-term morbidity such as cirrhosis and hepatocellular carcinoma. Interferon treatment enhanced HBsAg seroclearance. Pegylated interferon induced a 10-15% yearly rate of HBsAg seroclearance in patients who developed sustained virological response in clinical trials. By contrast, treatment with nucleos(t)ides analogues did not significantly affect the rate of HBsAg seroclearance, especially in patients with hepatitis B e antigen (HBeAg) - negative disease. Recently, it has been shown that a significantly greater proportion of patients receiving tenofovir plus pegylated-interferon for 48 weeks had HBsAg loss than those receiving tenofovir or pegylated-interferon alone. HBsAg clearance is the closest to cure outcome, and there is increasing interest in HBsAg quantification. Quantification of serum HBsAg has also been recently shown to be a promising tool for monitoring virologic response in HBeAg-negative patients treated with pegylated interferon. This chapter reviews Interferon therapy and long-term outcomes.

Keywords

HBV cure • Immune therapy • cccDNA • HBs quantification

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Abbreviations

AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
cccDNA	Covalently closed circular DNA
CHB	Chronic hepatitis B
ETV	Entecavir
HBeAg	HBe antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
IFN	Interferon
NAs	Nucleos(t)ide analogues
NI	Nucleoside inhibitors
NNI	Non-nucleoside inhibitors
PEG-IFN	Pegylated interferon
QD	Once daily
TDF	Tenofovir

1 Introduction

Chronic hepatitis B (CHB) infection affects over 240 million people worldwide, with long-term morbidity such as cirrhosis and hepatocellular carcinoma (HCC) accounting for around 600,000 deaths annually (World Health Organization 2012). HCC is one of the most frequent cancers in many countries, especially Africa, the Middle East, and Asia. Fibrosis is the most important prognosis predictor of survival (Marcellin et al. 2002; Asselah et al. 2014). HBV research and development programs aim to achieve an HBV cure. Interferon is the only immune-modulator treatment approved for HBV infection. This review will summarize results from interferon therapies, prediction of treatment response, and long-term outcomes.

2 HBV Current Therapy

The goal of therapy for CHB is to improve quality of life and survival by preventing progression of the disease (European Association for the Study of the Liver 2012; Sarin et al. 2016; Terrault et al. 2016). Previously, HBV DNA suppression with long-term lamivudine (LAM) has been associated with a reduction in the incidence of hepatic decompensation and HCC (Lai et al. 1998; Liaw et al. 2004). Treatment goals can be achieved by sustained suppression of HBV replication, thereby reducing necro-inflammation and reducing the risk of fibrosis progression. Suppression of HBV replication is critical and has to be maintained. Currently, there are two

Table 10.1 Advantages and limits of pegylated-interferons and analogue therapies

	PEG-IFN	Nucs
Advantages	• Finite duration • Absence of resistance • Higher rates of anti-HBe and anti-HBs seroconversion with 12 months of therapy	• Potent antiviral effect • Good tolerance • Oral administration
Disadvantages	• Moderate antiviral effect • Inferior tolerability • Risk of adverse events • Subcutaneous injections	• Duration • Risk of resistance • Unknown long-term safety

main treatment strategies for both hepatitis “e” (HBe) antigen positive (HBeAg+ve) and antigen negative (HBeAg–ve) (Table 10.1).

A finite treatment course of interferon (IFN) alpha/pegylated IFN (PEG-IFN) and long-term therapy with nucleoside/nucleotide analogues (NAs) are the currently approved treatments for chronic hepatitis B. A 1-year treatment with PEG-IFN offers the potential for immune-mediated control of HBV infection, with higher rates of HBe seroconversion and the possibility of off-treatment viral suppression and with loss of hepatitis B surface antigen (HBsAg) in a proportion of patients who maintain undetectable HBV DNA. However, PEG-IFN needs to be administered by subcutaneous injection and is associated with frequent side effects such as depression; it is also contraindicated in patients with decompensated cirrhosis or relevant autoimmune disease, during pregnancy.

In contrast, NAs suppress HBV via direct antiviral activity, and if compliance to treatment is good, more than 95% of patients treated with the newer, highly potent NAs tenofovir (TDF) and entecavir (ETV) achieve virological remission. NAs are administered orally, and tolerance is favorable, although the safety of these drugs over lifelong therapy is unknown. Regarding the risk of drug resistance, although common with earlier less potent NAs such as LAM and adefovir (ADV), resistance has become considerably less of a problem with the highly potent NAs TDF and ETV.

Long-term clinical data up to 6 years and beyond are emerging for the newer NAs that are providing reassuring data on their efficacy and safety. There is cumulative evidence that complete long-term suppression of HBV replication by the most potent drugs (ETV and TDF) results in an improved long-term outcome with a decreased risk of progression to cirrhosis and complications such as liver failure, HCC, and improved survival. In addition, a recent study assessing liver histology in patients treated with TDF for 5 years demonstrated that fibrosis regressed in most patients (Marcellin et al. 2013a; Marcellin and Asselah 2013). Moreover, unlike what is generally believed, the reversal of cirrhosis was observed during treatment in 75% of patients with cirrhosis, probably associated with a decreased risk of HCC and improved survival. Further long-term data are emerging from studies using newer potent NAs in routine clinical practice confirming safety and efficacy of these agents in the “real-world” setting.

3 The Importance of Fibrosis as Prognosis Factor

Fibrosis is the most important prognosis predictor of survival (Marcellin et al. 2002; Asselah et al. 2014). Therefore, patients with advanced fibrosis may be prioritized for treatment. Several new markers are developed to assess fibrosis.

For instance, we developed a simple scoring system to determine the severity of fibrosis in patients with genotype B or C HBV infection who are hepatitis B e antigen positive (Marcellin et al. 2015). We developed two prediction scoring systems (PSs). PS1 analyzed data on HBV genotype (B vs. C), patient age (<30 years vs. ≥ 30 years), level of hepatitis B surface antigen ($\leq 17,500$ IU/mL vs. $> 17,500$ IU/mL), and level of alanine aminotransferase (≤ 3 -fold vs. > 3 -fold the upper limit of normal). PS2 analyzed data on only age and level of hepatitis B surface antigen. Our system differentiated patients with no or mild fibrosis (F0–F1) from those with marked or severe (F2–F4) fibrosis with a high PPV. The high level of specificity for the identification of nonsevere fibrosis (F0–F2) limits the risk of overlooking patients with severe fibrosis (F3–F4).

In another study, the expression of 13 fibrosis-related microRNAs (miRNAs) (miR-20a, miR-21, miR-27a, miR-27b, miR-29a, miR-29c, miR-92a, miR-122, miR-146a, miR-155, miR-221, miR-222, and miR-224) was analyzed in 194 serums and 177 liver biopsies of patients with either CHB or CHC to develop models to diagnose advanced fibrosis and cirrhosis (Metavir F3–F4) (Appourchaux et al. 2016). In CHB patients, the model (serum miR-122, serum miR-222, platelet count, and alkaline phosphatase) was more accurate than APRI and FIB-4 to discriminate in between mild and moderate fibrosis (F1–F2) and F3–F4 (AUC of CHB model, 0.85, vs. APRI, 0.70, and FIB-4, 0.81). In CHC patients, the model (hepatic miR-122, hepatic miR-224, platelet count, albumin, and alanine aminotransferase) was more accurate than both APRI and FIB-4 to discriminate in between patients with F3–F4 and F1–F2 (AUC of the CHC model = 0.93 vs. APRI, 0.86, and FIB-4, 0.79). Most of the miRNAs tested were differentially expressed in patients with CHB and CHC. In particular, serum miR-122 was 28-fold higher in patients with CHB than in those with CHC. Both CHB and CHC models may help for the diagnosis of advanced fibrosis and cirrhosis (F3–F4).

4 Pegylated IFN (PEG-IFN): Results

The efficacy of PEG-IFN- $\alpha 2a$ in HBeAg-positive and HBeAg-negative patients has been established in two large pivotal trials including 814 and 552 patients, respectively (Lau et al. 2005; Marcellin et al. 2004), and similar efficacy has been reported with the use of PEG-IFN- $\alpha 2b$ (Buster et al. 2008). In both studies, PEG-IFN- $\alpha 2a$ monotherapy, the combination of PEG-IFN- $\alpha 2a$ plus lamivudine, and lamivudine monotherapy were compared during a 48-week treatment course. At the end of a 6-month posttreatment follow-up period, HBeAg seroconversion rates were 32%, 27%, and 19%, respectively, in the HBeAg-positive study (Lau et al. 2005), and serum HBV DNA levels were < 400 copies/mL in 19%, 20%, and 7% of patients, respectively, in the HBeAg-negative study (Marcellin et al. 2004).

Interestingly, HBsAg seroconversion is achieved with a high steady rate in patients responding to interferon and associated with excellent outcome (Moucari et al. 2009a).

More recently, long-term follow-up data were reported for a subgroup of patients enrolled in the HBeAg-negative trial (Marcellin et al. 2009, 2013b). Interestingly, sustained off-treatment response (HBV DNA < 400 copies/mL) was maintained in 18% of patients treated with PEG-IFN- α 2a. Importantly, HBsAg clearance occurred with a high steady rate (>10% per year) in sustained virological responders—reaching 64% at 5-year posttreatment.

5 PEG-IFN: Pretreatment Prediction

Selecting patients with the highest probability of achieving a response to PEG-IFN is essential to optimize its use and help the clinician decide whether to begin a finite course of 48 weeks of PEG-IFN.

6 HBV Genotype

HBV genotype A responds better to IFN when compared to other genotypes. A significant correlation between viral genotype and sustained HBeAg loss was found in HBeAg-positive patients in large multicenter trials of PEG-IFN, with the highest rates of HBeAg clearance at the end of follow-up in genotype A (47%), followed by B (44%), C (28%), and D (25%) (Flink et al. 2006). The meta-analysis clearly shows that genotype A is the most responsive to treatment in HBeAg-positive patients (Wiegand et al. 2008). Similar results have been reported in HBeAg-negative patients. The multicenter study by Bonino et al. including 518 HBeAg-negative patients has shown that genotype is significantly predictive of the efficacy in patients treated with IFN (Bonino et al. 2007).

7 HBV Viral Load

A low serum HBV DNA is predictive of HBe seroconversion. In HBeAg-positive patients, a low HBV DNA load (below 2×10^8 IU/mL) is predictive of anti-HBe seroconversion (Lau et al. 2005).

8 HBsAg Level (Quantification)

A low HBsAg level is predictive of response. Baseline HBsAg levels are reliable predictors of SVR, and current data confirm that baseline levels are good predictors of response. Baseline HBsAg levels are significantly lower in patients who achieve an SVR than in non-responders, both in HBeAg-positive and HBeAg-negative patients (Chan et al. 2010; Martinot-Peignoux et al. 2015).

9 Quantitative Hepatitis B Core-Related Antigen (qHBcrAg)

Quantitative hepatitis B core-related antigen (qHBcrAg) has been proposed as an additional marker to quantitative HBsAg (qHBsAg), for the management of chronic hepatitis B (Martinot-Peignoux et al. 2016). A recent study aimed to evaluate baseline combination of qHBsAg and qHBcrAg for the identification of patients that could benefit from pegylated interferon (PEG-IFN) alpha-2a-based therapy. Baseline qHBsAg is predictive of HBsAg loss. Both markers could be used, separately or in combination, for PEG-IFN-based “precision therapy.” Our results emphasize that the combination of PEG-IFN alpha-2a plus TDF with 53% of SR might be an alternative to finite therapy.

10 Other Predictors: Genomics

The impact of IFNL3 (IL28B) polymorphism on response to interferon (IFN) treatment in patients infected with hepatitis B virus (HBV) is controversial (Lampertico et al. 2013; Holmes et al. 2013). We aimed to investigate whether IFNL3 polymorphism (rs12979860) influences the long-term response of chronic hepatitis B (CHB) treatment to conventional IFN (Zhang et al. 2014). No significant relationship between IFNL3 rs12979860 and fibrosis stage was observed ($P = 0.85$). IFNL3 genotype was neither associated with SVR nor with HBeAg seroconversion and long-term HBsAg seroconversion in HBeAg-positive CHB patients responding to IFN therapy.

Recently, Wu et al. investigated liver gene expression profiles to reveal the molecular basis associated with chronic hepatitis B and IFN α treatment response in CHB patients (Wu et al. 2016). Expression profiles were compared between seven paired liver biopsy samples taken before and 6 months after successful IFN α treatment or between pretreatment biopsy samples of 11 IFN α responders and 11 nonresponders. A total of 132 differentially upregulated and 39 downregulated genes were identified in the pretreated livers of CHB patients. The upregulated genes were mainly related to cell proliferation and immune response, with IFN γ and B cell signatures significantly enriched. Lower intrahepatic HBV pregenomic RNA levels and 25 predictive genes were identified in IFN α responders. The predictive gene set in responders significantly overlapped with the upregulated genes associated with the pretreated livers of CHB patients. The mechanisms responsible for IFN α treatment responses are different between HBV and HCV patients. HBV infection evokes significant immune responses even in chronic infection. The upregulated genes were predictive of responsiveness to IFN α therapy, as are lower intrahepatic levels of HBV pregenomic RNA and pre-activated host immune responses.

HBV DNA levels at weeks 12 and 24 cannot be used to develop a stopping rule similar to that for hepatitis C. A lack of decrease in HBsAg and a serum HBV DNA decrease of less than 2 log₁₀ IU/mL have a strong negative predictive value for SVR

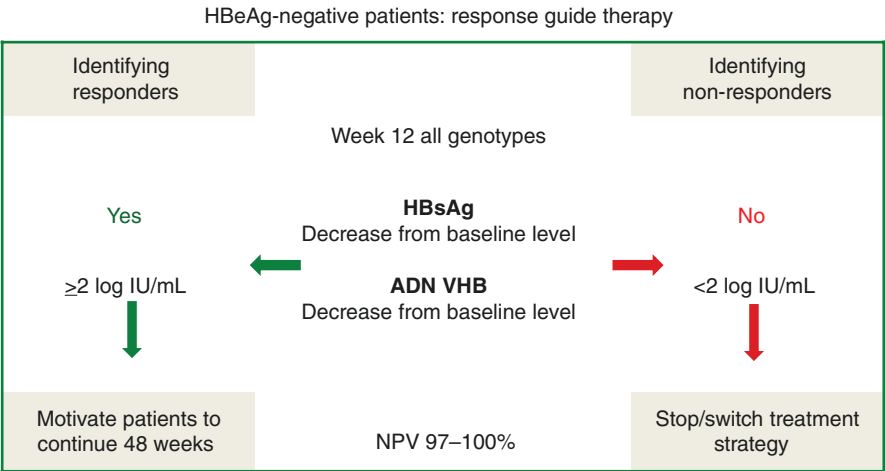


Fig. 10.1 Week-12 stopping rule for HBeAg-negative patients receiving PEG-IFN therapy

(Chan et al. 2010; Martinot-Peignoux et al. 2015; Moucari et al. 2009b; Brunetto et al. 2009). A week-12 response guide algorithm based on HBsAg kinetics has been proposed. Treatment should be discontinued or switched in patients without or with a limited decrease in HBsAg and/or a decrease in HBV DNA < 2 log₁₀ IU/mL at week 12 (Fig. 10.1).

11 Major Recent Advances

11.1 Combination Therapy (TDF Plus PEG-IFN)

In a recent study, HBsAg loss was evaluated in patients receiving the combination of TDF and PEG-IFN for a finite duration (Marcellin et al. 2016). In an open-label, active-controlled study, 740 patients with CHB were randomly assigned to receive TDF plus PEG-IFN for 48 weeks (group A), TDF plus PEG-IFN for 16 weeks followed by TDF for 32 weeks (group B), TDF for 120 weeks (group C), or PEG-IFN for 48 weeks (group D) (Fig. 10.2a–c). At week 72, 9.1% of subjects in group A had HBsAg loss compared with 2.8% of subjects in group B, none of the subjects in group C, and 2.8% of subjects in group D. A significantly higher proportion of subjects in group A had HBsAg loss than in group C (*P* < 0.001) or group D (*P* = 0.003). However, the proportions of subjects with HBsAg loss did not differ significantly between group B and group C (*P* = 0.466) or group D (*P* = 0.883). HBsAg loss in group A occurred in hepatitis B e antigen-positive and hepatitis B e antigen-negative patients with all major viral genotypes.

Finally, a significantly greater proportion of patients receiving TDF plus PEG-IFN for 48 weeks had HBsAg loss than those receiving TDF or PEG-IFN alone.

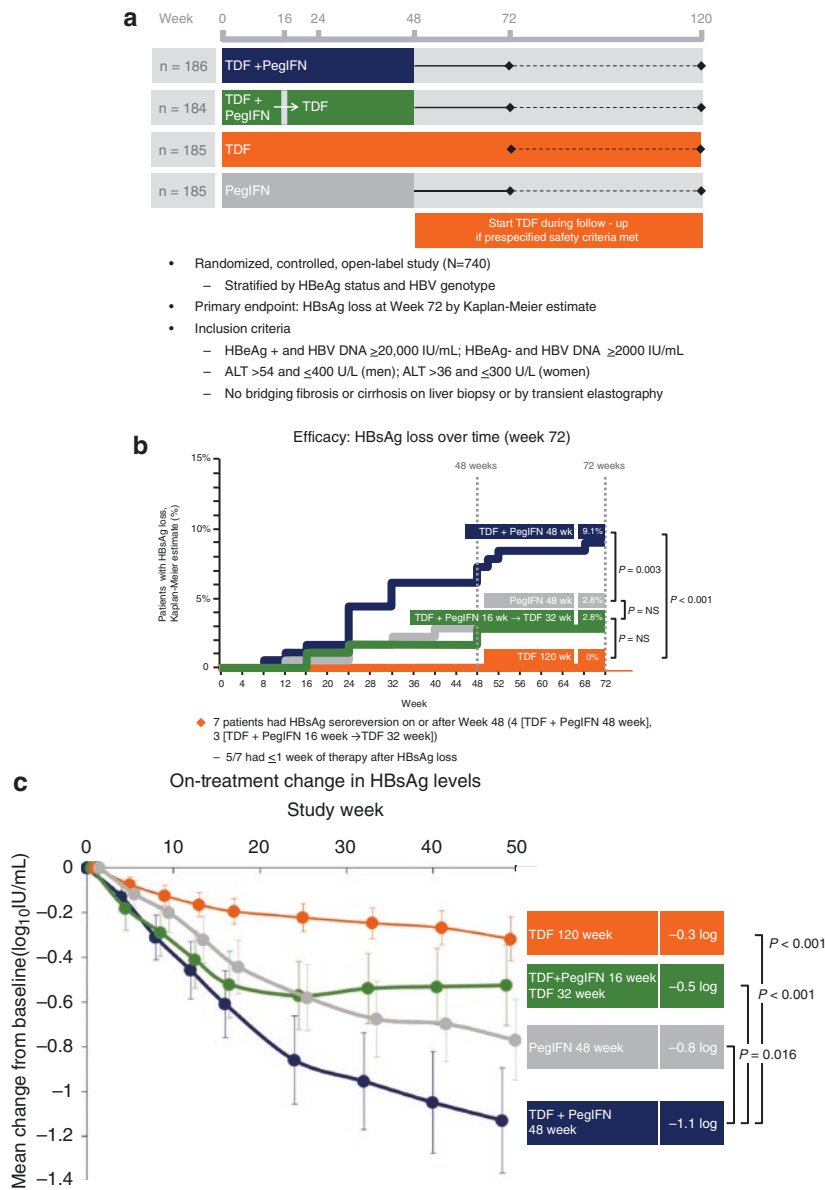


Fig. 10.2 Combination therapy with pegylated interferons plus tenofovir. **(a)** Trial design. **(b and c)** Results. At week 72, 9.1% of subjects in group A had HBsAg loss compared with 2.8% of subjects in group B, none of the subjects in group C, and 2.8% of subjects in group D. A significantly higher proportion of subjects in group A had HBsAg loss than in group C ($P < 0.001$) or group D ($P = 0.003$). However, the proportions of subjects with HBsAg loss did not differ significantly between group B and group C ($P = 0.466$) or group D ($P = 0.883$). Error bars represent 95% confidence intervals

11.2 HBsAg Quantification: A New Tool for HBV Monitoring

Quantifying HBsAg is certainly an important new tool for predicting the severity of disease, to distinguish inactive carriers from patients with HBeAg-negative chronic active hepatitis and thus help tailor follow-up and treatment management (Martinot-Peignoux et al. 2014, 2013). In addition, a decline in quantitative HBsAg during therapy is a strong predictor of SVR after PEG-IFN therapy and of the probability of HBsAg loss, which is the ultimate goal of therapy. In patients treated with analogues, a decline in HBsAg levels is also a predictor of HBsAg loss, allowing therapy to be discontinued.

Conclusion

HBsAg clearance is the closest to cure outcome as one can expect to achieve in hepatitis B. Support for this comes from natural history studies demonstrating increased length of survival, lower rates of hepatic decompensation, reduction in incidence of hepatocellular carcinoma, and regression of liver fibrosis. HBsAg seroclearance may occur spontaneously at a yearly incidence of 1–2%, preceded usually by a long period of inactive disease. Interferon treatment enhanced HBsAg seroclearance by approximately threefold in western studies and sixfold in Asian studies compared with non-treated patients. Pegylated interferon induced a 10–15% yearly rate of HBsAg seroclearance in patients who developed sustained virological response in clinical trials. By contrast, treatment with nucleos(t)ides analogues did not significantly affect the rate of HBsAg seroclearance, especially in patients with hepatitis B e antigen (HBeAg)-negative disease. Quantification of serum HBsAg has also been recently shown to be a promising tool for monitoring virological response in HBeAg-negative patients treated with pegylated interferon.

There is increasing research in the field of HBV infection. The new goal of HBV therapy is to achieve “functional cure” or even “absolute cure” with HBsAg loss/seroconversion and clearance of cccDNA. New drugs aimed to decrease or eliminate cccDNA and/or HBsAg.

Conflicts of Interest Tarik Asselah is a speaker and investigator for BMS, Boehringer Ingelheim, Tibotec, Janssen, Gilead, Roche, and MSD.

Patrick Marcellin is a speaker and investigator for BMS, Boehringer Ingelheim, Tibotec, Janssen, Gilead, Roche, and MSD.

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Abstract

The goal of therapy for chronic hepatitis B is to decrease the risk of liver-related complications including progression to cirrhosis, decompensated cirrhosis, hepatocellular carcinoma, and death. Given that complete elimination of hepatitis B virus (HBV) from host is not possible with current available treatment owing to persistence of covalently closed circular DNA inside hepatocyte, the primary target for treatment should be to suppress HBV replication and reduce serum HBV DNA at the lowest possible levels to achieve the goals. Currently, five approved nucleos(t)ide analogues (NUC) are available for treating chronic hepatitis B in most countries: L-nucleosides (lamivudine and telbivudine), deoxyguanosine analogue (entecavir), and acyclic nucleoside phosphonates (adefovir dipivoxil and tenofovir). These NUCs act primarily by inhibiting the reverse transcription of the pregenomic HBV RNA to the first strand of HBV DNA. The American Association for the Study of Liver Diseases, European Association for the Study of the Liver, and Asian Pacific Association for the Study of the Liver practice guidelines recommend entecavir or TDF as preferred first-line monotherapies due to their superior efficacy and high barrier to resistance over comparable drugs. NUCs are administered orally and have favorable safety profiles over the course of several years. Many data have consistently shown that long-term suppression of HBV DNA replication by NUCs leads to the improvement in hepatic inflammation and fibrosis, hepatic function, and survival of the patients. However, it has been controversial whether the decrease in the risk of hepatocellular carcinoma could be achieved by the direct effects of NUCs apart from that by decrease in hepatic inflammation.

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Keywords

Nucleos(t)ide analogues • Chronic hepatitis B virus infection • Hepatocellular carcinoma

1 Nucleos(t)ide Analogues

1.1 Lamivudine

Lamivudine is the first oral nucleos(t)ide analogue (NUC) for treating chronic hepatitis B (CHB). It is an analogue of cytidine and is phosphorylated to its active metabolite which acts as a chain terminator after competing for incorporation into viral DNA (Fung et al. 2011). Lamivudine is not presently considered a first-line monotherapy for CHB due to its high rate of resistance and inferior efficacy compared with entecavir and tenofovir disoproxil fumarate (TDF) by all international guidelines (EASL 2012; Sarin et al. 2015; Terrault et al. 2016).

1.1.1 Short-Term Responses

(a) *HBeAg-positive patient*

In a randomized placebo-controlled trial, lamivudine-treated group, compared with placebo group, had a higher rate of histologic improvement (52% versus 23%, $P < 0.001$), loss of HBeAg (32% versus 11%, $P = 0.003$), sustained suppression of HBV DNA to undetectable levels (44% versus 16%, $P < 0.001$), and ALT normalization (41% versus 7%, $P < 0.001$) after 52 weeks of treatment (Dienstag et al. 1999).

(b) *HBeAg-negative patient*

A 1-year course of lamivudine therapy has been reported to achieve ALT normalization in 96% and undetectable levels of HBV DNA in 68% of HBeAg-negative patients (Hadziyannis et al. 2000). The lower limit of detection for serum HBV DNA has changed over years and was 1×10^5 copies/mL for most of the studies for lamivudine.

1.1.2 Long-Term Outcomes

(a) *Histologic improvement*

A 3-year follow-up study investigating histologic outcome during lamivudine therapy reported that lamivudine therapy reduced necroinflammatory activity and reversed fibrosis (including cirrhosis) in most patients. However, the emergence of lamivudine resistance mutations could offset histologic improvement throughout treatment period (Dienstag et al. 2003).

(b) *Clinical trial*

The effectiveness of lamivudine therapy in preventing liver-related complications has been demonstrated in a large randomized controlled study with a mean duration of 32 months. Lamivudine-received patients had significantly lower rates of disease progression, defined as an increase in Child-Pugh score by ≥ 2 points, renal insufficiency, bleeding varices, spontaneous bacterial peritonitis, liver-related death, and hepatocellular carcinoma (HCC) compared with pla-

cebo-received patients (7.8% versus 17.7%, $P = 0.001$). HCC occurred in 3.9% of lamivudine group and 7.4% of placebo group (hazard ratio [HR], 0.49; $P = 0.047$) (Liaw et al. 2004).

(c) *Observational study*

In a 5-year long-term follow-up study with lamivudine (median follow-up 3.8 ± 1.4 years), virological and biochemical remission rate at 48 months was 34% and 36%, respectively. In this study, long-term lamivudine therapy significantly improved survival and reduced the risk of major complications, compared with untreated patients (Papatheodoridis et al. 2005). In patients with decompensated cirrhosis defined as a Child-Pugh score of ≥ 10 , lamivudine significantly improved hepatic function in 60.9% of treated patients versus none of the controls ($P < 0.001$). Time to death or orthotopic liver transplantation was longer in lamivudine-treated patients than in controls ($P < 0.001$) (Yao 2001). In a meta-analysis, lamivudine treatment versus no treatment reduced the risk of HCC (four observational studies, relative risk [RR] = 0.6, 95% confidence interval [CI] 0.4–0.96, $P = 49.9\%$), all-cause mortality (one study, RR = 0.4, 95% CI 0.3–0.6), and decompensated liver disease (one study, RR = 0.3, 95% CI 0.3–0.5) (Lok et al. 2016).

1.1.3 Resistance

The major drawback of lamivudine is the high rate of emerging drug resistance throughout treatment period. The overall incidence of lamivudine-resistant mutation increased from 23% in 1 year of lamivudine treatment to 46%, 55%, 71%, and 65% in 2, 3, 4, and 5 years, respectively. Moreover, patients with lamivudine-resistant mutations experienced more hepatitis flares than those without lamivudine-resistant mutations ($P < 0.005$) (Lok et al. 2003).

The main mechanism for lamivudine resistance includes mutations in rtM204V/I (a methionine to valine or isoleucine substitution) alone or rtM204V/I along with rtL180M mutation (Allen et al. 1998). Antiviral drug resistance could manifest as virological breakthrough which may be followed by biochemical breakthrough, hepatitis flares, and hepatic decompensation. Once drug-resistant HBV mutants develop, they do not disappear even after the discontinuation of the treatment, but retained in the viral archive permanently causing resistance to subsequent multiple drugs, such as telbivudine, entecavir, and adefovir (Lok and McMahon 2009; Zoulim and Locarnini 2012).

1.1.4 Dosage, Safety, and Side Effects

Lamivudine is administered orally at a dosage of 100 mg daily in patients with normal renal function. In general, lamivudine is a very safe drug. Pancreatitis and lactic acidosis were reported in patients who received lamivudine treating for CHB. Lamivudine is classified as pregnancy category C.

1.2 Adefovir

Adefovir, a nucleotide analogue of adenosine monophosphate, was approved in 2002 for treating CHB (Fung et al. 2011). Considering its inferiority to TDF in terms of viral suppression and resistance profile, adefovir is no longer a first-line drug.

1.2.1 Short-Term Responses

(a) *HBeAg-positive patient*

In patients with HBeAg-positive CHB, adefovir treatment group than placebo group had more histologic improvement (53% versus 25%, respectively, $P < 0.001$) after 48 weeks of treatment in a phase 3 randomized controlled trial (Marcellin et al. 2003).

(b) *HBeAg-negative patient*

In a placebo-controlled randomized study with HBeAg-negative CHB patients, adefovir group had histologic improvement compared with placebo group (64% versus 33%, $P < 0.001$). Adefovir treatment resulted in greater decrease in the median serum HBV DNA (-3.91 versus -1.35 $\log_{10}$ copies/mL, $P < 0.001$). ALT normalization occurred in 72% of adefovir and 29% of placebo (Hadziyannis et al. 2003).

1.2.2 Long-Term Outcomes

(a) *Histologic improvement*

In an open-label phase study with adefovir for HBeAg-negative CHB for up to 5 years, 73% of patients had improvement in fibrosis, and 83% had improvement in necroinflammation. Compared with baseline, Ishak fibrosis score improved in 35%, 55%, and 71% of patients after 48, 192, and 240 weeks of adefovir treatment, respectively (Hadziyannis et al. 2006).

(b) *Clinical trial*

A 5-year long-term follow-up study including a subset of the above trial demonstrated that -4.05 $\log_{10}$ copies/mL of the median changes from baseline in serum HBV DNA, 48% of HBeAg seroconversion, and 67% of histologic improvement with adefovir treatment (Marcellin et al. 2008a). Treatment with adefovir for up to 240 weeks led ALT normalization in 69%, improvement in fibrosis in 73%, and HBV DNA suppression (HBV DNA levels <1000 copies/mL) in 67% of patients (Hadziyannis et al. 2006). In an open-label, international study involving 128 patients with decompensated cirrhosis who failed lamivudine therapy, 81% and 76% of the patients achieved undetectable HBV DNA (HBV DNA <400 copies/mL) and ALT normalization, respectively. Additionally, the Child-Pugh score improved in 92% of patients at 48 weeks of adefovir treatment (Schiff et al. 2003).

(c) *Observational study*

A long-term follow-up study with adefovir for wait-listed liver transplantation patients with lamivudine-resistant CHB evaluated the efficacy after 96 weeks of treatment with adefovir. Sixty-five percent of patients had undetectable HBV DNA levels (<1000 copies/mL), and a median decrease in Child-Pugh score was 2 (Schiff et al. 2007).

1.2.3 Resistance

The main mutations developing adefovir resistance are rtA181V/T and rtN236T (Angus Gastro 2003). Adefovir resistance mutations rtA181V or rtN236T developed in 20% of patients with HBeAg positive at week 195 (Marcellin et al. 2008a).

In patients with HBeAg negative, cumulative adefovir resistance rates at 1, 2, 3, 4, and 5 years are 0%, 3%, 11%, 18%, and 29%, respectively (Hadziyannis et al. 2006). However, adefovir resistance appeared to present earlier and more frequently in lamivudine-resistant patients compared with those who were treatment-naïve. In a study comparing lamivudine-resistant and treatment-naïve patients, 18% of lamivudine-resistant patients were found to have developed adefovir-resistant mutations, whereas none of treatment-naïve patients developed after 48 weeks (Lee et al. 2006).

1.2.4 Dosage, Safety, and Side Effects

For patients with normal renal function, the dosage of adefovir is 100 mg daily. However, increasing interval for dose is required for patients with renal impairment. A long-term follow-up study with adefovir for up to 5 years reported 3% of renal toxicity from adefovir treatment, defined as an increase of serum creatinine ≥ 0.5 mg/dL above the pretreatment value (Hadziyannis et al. 2006). Therefore, a close monitoring of serum creatinine and phosphate at least 3-month intervals is required for patients who is likely to progress renal impairment or receives adefovir for more than 1 year.

1.3 Telbivudine

Telbivudine, a synthetic L-nucleoside analogue of thymidine, has been used for the treatment of CHB since 2006. Telbivudine undergoes phosphorylation to its triphosphate form by cellular kinases, which can inhibit HBV DNA polymerase by competing with thymidine 5'-triphosphate. Incorporation of telbivudine leads to premature chain termination (Fung et al. 2011). Telbivudine has been demonstrated to be more potent than lamivudine against HBV. However, telbivudine has an intermediate rate of resistance and inferiority to TDF in its efficacy. Thus, telbivudine is not a first-line monotherapy for CHB by international guidelines.

1.3.1 Short-Term Responses

(a) HBeAg-positive patient

In a double-blind phase 3 trial (GLOBE trial) comparing between telbivudine and lamivudine, telbivudine showed a higher rate of therapeutic response (75% versus 67%, $P = 0.005$) and histologic response (65% versus 56%, $P = 0.01$) at 1 year. With regard to the mean reduction in the HBV DNA levels from baseline and the proportion of patients with a reduction in HBV DNA to undetectable levels, telbivudine was superior to lamivudine (Lai et al. 2007). Another 2-year follow-up study including HBeAg-positive patients treated with telbivudine or lamivudine demonstrated that telbivudine-treated patients had a higher rate in therapeutic response (63% versus 48%, $P < 0.001$) and undetectable levels of HBV DNA (56% versus 39%, $P < 0.001$), compared with lamivudine-treated patients. However, in regard to HBeAg seroconversion, both groups were comparable (30% in telbivudine versus 25% in lamivudine, $P = 0.056$) (Liaw et al.

2009). A randomized study conducted with Chinese CHB treatment-naïve patients did not show any difference between telbivudine and entecavir in treatment effectiveness including the mean reductions from baseline in serum HBV DNA, the rate of undetectable levels of HBV DNA, HBeAg seroconversion rate, and ALT normalization at 24 weeks (Zheng et al. 2010).

(b) *HBeAg-negative patient*

In one of two abovementioned trials comparing between telbivudine and lamivudine, telbivudine was shown a greater decrease in the mean HBV DNA levels (difference $-0.83 \log_{10}$ copies/mL, $P < 0.001$), a higher rate of HBV DNA undetectability than lamivudine (88% in telbivudine versus 71% in lamivudine, $P < 0.001$) at week 104. However, there were no significant differences in histologic response and ALT normalization (Lai et al. 2007). Another study demonstrated superior efficacy of telbivudine to lamivudine in therapeutic response, HBV DNA undetectability but ALT normalization (Liaw et al. 2009).

1.3.2 Long-Term Outcomes

(a) *Histologic improvement*

Study data showing histologic improvement after a long-term telbivudine therapy are sparse.

(b) *Clinical trial*

In a noninferiority, double-blind randomized 2-year study evaluating the efficacy between telbivudine and lamivudine in treatment-naïve patients with decompensated cirrhosis, telbivudine was associated with a higher rate of patients with clinical response (HBV DNA < 300 copies/mL and ALT normalization) compared with lamivudine (45.6% versus 32.9%, $P = 0.093$, respectively). In this study, improvement in Child-Pugh score (decrease ≥ 2) occurred in 38.6% of telbivudine group and in 40.4% of lamivudine group ($P = 0.83$) (Chan et al. 2012).

(c) *Observational study*

A 3-year extension of GLOBE study consisting of 484 telbivudine-treated patients reported two cases (0.5%) of HCC occurrence during study period (Gane et al. 2011). Patients with cirrhosis in the GLOBE trial showed a sustained improvement of renal function with a long-term telbivudine therapy (4–6 years), particularly among patients with increased risk of renal impairment (Gane et al. 2014).

1.3.3 Resistance

The resistance rate of telbivudine is relatively high at 4.4%/2.7% and 25%/11% at 1 year and 2 years for HBeAg-positive/HBeAg-negative patients, respectively (Lai et al. 2007; Liaw et al. 2009). The main mutation responsible for telbivudine resistance is rtM204I with or without concomitant rtL80I/V and rtL180M mutations.

1.3.4 Dosage, Safety, and Side Effects

Telbivudine is administered orally at a dosage of 600 mg daily. Myopathy has been reported in patients who are treated with telbivudine. Data of 667 patient in the GLOBE trial reported that 13% of patients receiving telbivudine had an

elevation of creatine kinase (CK) level compared with 4% of patients receiving lamivudine (Liaw et al. 2009). Telbivudine has also been reported to be associated with peripheral neuropathy, particularly when used along with pegylated interferon. In a randomized trial, of the 50 patients who had received pegylated interferon and telbivudine, 7 (14%) patients developed peripheral neuropathy (Marcellin et al. 2015).

1.4 Entecavir

Entecavir, a deoxyguanosine analogue, is a potent inhibitor of HBV replication and converts into its active form of entecavir-triphosphate in vivo. Entecavir is a competitive inhibitor of HBV polymerase negative-strand synthesis from pregenomic RNA and positive-strand replication. It also acts by inhibiting the HBV priming reaction (Levine et al. 2002). In vitro, entecavir demonstrated a 30- to 2200-fold efficacy in reducing viral DNA replication compared to lamivudine (Fung et al. 2011). Entecavir has been available since 2005 at a dose of 0.5 mg/day and 1 mg/day for treatment of treatment-naïve and lamivudine-resistant CHB, respectively. By all international guidelines, entecavir is preferred as a first-line monotherapy for CHB because of its high potency and low rate of drug resistance.

1.4.1 Short-Term Responses

(a) *HBeAg-positive patient*

A phase 3, double-blind randomized trial including 715 nucleos(t)ide-naïve, HBeAg-positive patients with compensated liver disease compared the efficacy between entecavir 0.5 mg/day and lamivudine 100 mg/day for 48 weeks (Chang and Lai 2006). After 48 weeks, more patients in the entecavir group than in the lamivudine group had histologic improvement (72% versus 62%, $P = 0.009$), undetectable serum HBV DNA levels (67% versus 36%, $P < 0.001$), and ALT normalization (68% versus 60%, $P = 0.02$). HBeAg seroconversion rates were comparable with entecavir group (21%) and lamivudine (18%) ($P = 0.33$) (Chang and Lai 2006). In a follow-up to the above study for up to 96 weeks, higher proportions of entecavir-treated than lamivudine-treated patients achieved cumulative confirmed HBV DNA <300 copies/mL (80% versus 39%, $P < 0.001$) and ALT normalization (87% versus 79%, $P = 0.006$) (Gish et al. 2007). However, cumulative HBeAg seroconversion occurred in 31% of entecavir-treated versus 25% of lamivudine-treated patients through 96 weeks ($P = \text{NS}$) (Gish et al. 2007). In a study of open-label treatment with entecavir 0.5 mg/day or adefovir 10 mg/day for a minimum of 52 weeks treatment including 69 nucleos(t)ide-naïve HBeAg-positive patients, the mean reduction in serum HBV DNA level at week 48 was greater in entecavir-treated patients compared with adefovir-treated patients (-7.28 versus -5.08 $\log_{10}$ copies/mL, $P < 0.001$). At 48 weeks, higher proportion in entecavir-treated patients achieved HBV DNA <300 copies/mL compared with adefovir-treated patients (58% versus 19%). HBeAg seroconversion rates were similar in both groups (Leung et al. 2008).

(b) *HBeAg-negative patients*

In a double-blind, randomized, phase 3 trial, entecavir was shown to be superior to lamivudine in histologic improvement (70% versus 61%, $P = 0.01$), the rate of undetectable HBV DNA levels (90% versus 72%, $P < 0.001$), and ALT normalization (78% versus 71%, $P = 0.045$) at week 48 (Lai et al. 2006).

1.4.2 Long-Term Outcomes

(a) *Histologic improvement*

The efficacy of entecavir and lamivudine were compared in 245 biopsy-proven cirrhotic patients, which demonstrated that entecavir group achieved improvement in Ishak fibrosis at week 48 in 57%/59% of nucleos(t)ide-naïve HBeAg-positive/HBeAg-negative patients. Whereas, among HBeAg-positive patients, histologic improvement (defined as a ≥ 2 -point improvement in the Knodell necroinflammatory score and no worsening of fibrosis) was occurred in 80% and 64% of entecavir and lamivudine group at 48 weeks, respectively. In HBeAg-negative patients, 75% of entecavir group demonstrated histologic improvement compared with 60% of lamivudine group (Schiff et al. 2008).

(b) *Clinical trial*

A randomized open-label comparative study of entecavir (1.0 mg/day) versus adefovir (10 mg/day) therapy in CHB patients with hepatic decompensation (Child-Pugh score ≥ 7) demonstrated the superiority of entecavir to adefovir in the mean reduction in HBV DNA at week 24 (treatment difference 1.74 $\log_{10}$ copies/mL). About 2/3 of subjects in both groups showed improvement in Child-Pugh score, and a decrease in Model for End-Stage Liver Disease (MELD) score was 2.6 for entecavir and 1.7 for adefovir at week 48. Cumulative HCC incidence rates were 12% for entecavir and 20% for adefovir. Cumulative death rates, moreover, were 23% for entecavir and 33% for adefovir (Liaw et al. 2011a).

(c) *Observational study*

Hosaka et al. reported that the incidence of HCC was lower in entecavir-treated patients than non-treated patients among 316 propensity score matching cohort and the suppression of HCC development was greater in patients at higher risk of HCC (Hosaka et al. 2013). In a cohort study of 1980 patients with cirrhosis (a mean duration of follow-up, 52 months), treatment with entecavir compared with controls reduced the risk of HCC (RR = 0.3, 95% CI 0.1–0.5) and death (RR = 0.6, 95% CI 0.3–0.98) (Wong et al. 2013). A recently published retrospective-prospective cohort study in Taiwan showed that entecavir was associated with a 60% HCC risk reduction (HR = 0.40, 95% CI: 0.28–0.57) compared with untreated cohort (Su et al. 2016). A large retrospective study including 5374 patients with CHB treated with entecavir or lamivudine has demonstrated that entecavir therapy was associated with a lower risk of death or transplantation (HR = 0.49, $P < 0.001$) but a similar risk of HCC (HR = 1.08, $P = 0.48$) compared with lamivudine. Entecavir also reduced the risk of death or transplantation in cirrhosis subset (HR = 0.42, $P < 0.001$), but the risk for HCC did not show difference (HR = 1.00, $P = 0.999$) when comparing with lamivudine (Lim et al. 2014).

1.4.3 Resistance

Entecavir has a relatively low incidence of drug resistance as it requires a combination of three mutations before resistance develops. In addition to the rtM204V and rtL180M mutations being responsible for lamivudine resistance, an additional mutation at rtI169T, rtT184G, rtS202I, or rtM250V is required for entecavir resistance (two-hit mechanism) (Tenney et al. 2004). The rate of entecavir resistance is 1.2% at 5 years in treatment-naïve patients. On the contrary, the rate of entecavir resistance was as high as 51% at 5 years in lamivudine-refractory patients (Tenney et al. 2009).

1.4.4 Dosage, Safety, and Side Effects

For treatment-naïve patients with normal renal function, entecavir dosage is 0.5 mg daily whereas 1.0 mg daily for those who experienced lamivudine or telbivudine or has decompensated cirrhosis. Entecavir dosage should be adjusted according to the creatinine clearance of patients. Common adverse events (AEs) from entecavir were myalgia (5%) and neuropathy (4%) in a long-term cumulative safety results, and 1% of patients discontinued entecavir due to AEs (Manns et al. 2012). A safety concern with entecavir relates to its possible carcinogenic potential based on the rodent carcinogenicity studies. In two epidemiological studies, subjects with entecavir were followed for 8 years in the US study and for 11 years in the Taiwan study; the rates of malignancy development were comparable between entecavir and lamivudine group. However, the observational period might be short to exclude carcinogenicity risk, and long-term follow-up studies may be required.

1.5 Tenofovir Disoproxil Fumarate (TDF)

TDF is a prodrug of tenofovir that undergoes phosphorylation to competitively inhibit the natural substrate deoxyadenosine 5'-triphosphate. Once incorporated into HBV DNA polymerase, it functions as a chain terminator (Fung et al. 2011). Currently, TDF is a preferred first-line antiviral drug for CHB due to its high potency and high barrier to resistance.

1.5.1 Short-Term Responses

(a) *HBeAg-positive patients*

A phase 3 double-blind study compared the efficacy in patients with HBeAg positive between TDF 300 mg and adefovir 10 mg for 48 weeks. Seventy-six percent of patients receiving TDF had an HBV DNA level of less than 400 copies/mL, and 13% of patients receiving adefovir showed HBV DNA suppression (<400 copies/mL). With regard to ALT normalization, a higher proportion of patients with TDF than adefovir achieved ALT normalization (68% versus 54%, $P = 0.03$). However, there were no significant differences in the rate of HBeAg seroconversion in both groups (24% in TDF versus 18% in adefovir, $P = 0.36$) (Marcellin et al. 2008b). In an extension of the above study, 60% of patients achieved HBV DNA suppression (HBV DNA <69 IU/mL), and 47% of patients

showed ALT normalization after 5 years of treatment. HBeAg seroconversion was achieved in 40% of patients, and HBsAg seroconversion occurred in 9.7% of patients with TDF treatment for 7-year follow-up. In the on-treatment analysis, defined as excluding missing data and patients with emtricitabine added to their treatment regimen, HBV DNA suppression was maintained up to 99% of patients, and ALT normalization was detected in 74% at year 7 (Buti et al. 2015).

(b) *HBeAg-negative patient*

In a phase 3 randomized trial, TDF was superior to adefovir with respect to primary endpoint of antiviral efficacy (defined as HBV DNA <400 copies/mL and ≥ 2 -point reduction in Knodell inflammatory score without worsening of fibrosis at 48 weeks). Among 250 patients with TDF and 125 patients with adefovir, 71% of patients receiving TDF achieved primary endpoint compared with 49% of patients receiving adefovir ($P < 0.001$). However, regarding the rate of ALT normalization, both treatment groups were comparable (Marcellin et al. 2008b). After the completion of the above trial, patients enrolled a 7-year open-label study where all patients continued or switched to TDF, for a total duration of up to 8 years. Seventy-seven percent of patients achieved HBV DNA suppression (HBV DNA <69 IU/mL), and 65% of patients achieved ALT normalization at year 7. On the other hand, in the on-treatment analysis, 99% and 84% of patients had HBV DNA suppression and ALT normalization in the same treatment period (Buti et al. 2015).

1.5.2 Long-Term Outcome

(a) *Histologic improvement*

A study of investigating improvement of liver histology after TDF treatment for 5 years has demonstrated that long-term suppression of HBV with TDF can lead to regression of fibrosis and cirrhosis. In this study, 348 patients (54%) had biopsy results at both baseline and week 240. Three hundred and four (87%) of the 348 had histologic improvement (defined as ≥ 2 -point reduction in Knodell necroinflammatory score with no worsening of fibrosis), and 176 (51%) had regression of fibrosis (defined as ≥ 1 unit decrease by Ishak scoring system) at week 240 ($P < 0.001$). Of the 96 (28%) patients with cirrhosis (Ishak score 5 or 6) at baseline, 74% no longer had cirrhosis (≥ 1 unit decrease in score) at year 5 (Marcellin et al. 2013).

(b) *Clinical trial*

TDF has been shown to be effective in patients with decompensated cirrhosis (a CTP score of 7–12 or prior CTP score ≥ 7 and CTP ≤ 12 at screen) in phase 2 double-blind randomized study. At week 48, in this trial, HBV DNA was <400 copies/mL in 71%, the rate of ALT normalization was in 57%, and HBeAg seroconversion was 21% of TDF treatment group. In addition to virological and biochemical improvement, all patients improved in Child-Pugh and MELD scores (Liaw et al. 2011b). Furthermore, TDF appears to reduce mortality and improve liver function in a small study of 27 patients who developed acute-on-chronic liver failure compared to placebo (probability of survival, 57% in TDF versus 15% in placebo, $P = 0.03$) (Garg et al. 2011).

(c) *Observational study*

TDF was associated with a reduced incidence of HCC among patients without cirrhosis compared with those who were not receiving antiviral therapy in a well-validated prediction model. With the Risk Estimation for Hepatocellular Carcinoma in Chronic Hepatitis B (REACH-B) model, which estimates HCC incidence for up to 10 years based on age, sex, ALT level, HBeAg status, and HBV DNA, the standardized incidence ratio of HCC was 0.40 (95% confidence interval, 0.20–0.80), representing a 60% reduction in the incidence of HCC in patients receiving TDF treatment at the end of the 384 weeks (Kim et al. 2015). In another large European retrospective cohort study of 1666 CHB patients with entecavir or TDF, the cumulative probability of HCC was 1.3%, 3.4%, and 8.7% at 1, 3, and 5 years after treatment initiation. However, virological remission with entecavir or TDF was not associated with the HCC development (Papatheodoridis et al. 2015b). These findings are not different from those being at risk of HCC among published untreated or lamivudine-treated cohorts of patients (Rapti and Hadziyannis 2015).

1.5.3 Resistance

In an in vitro study, mutation in rtA194T, along with lamivudine resistance-associated mutations, was reported to confer a resistance to tenofovir in HBV-/HIV-coinfected patients (Sheldon et al. 2005). Another in vitro study has demonstrated that rtA194T polymerase mutation is associated with partial tenofovir resistance and negatively impacts viral replication, especially when it harbors lamivudine resistance mutations (rt180M + rtM204V), precore mutations, and basal core promoter mutations, suggesting patients with HBeAg negative may be at particular risk for developing tenofovir resistance (Amini-Bavil-Olyaei et al. 2009). However, in the following study, rtA194T mutation (with or without rtL180M + rtM204V), regardless of precore or basal core promoter mutations, remained susceptible to tenofovir, and rtA194T alone is susceptible to lamivudine (Zhu et al. 2011). In a long-term follow-up study of a clinical trial, no resistance to TDF was detected for 7 years (Buti et al. 2015). So far, no clinical case of TDF resistance has been reported in patients with CHB mono-infection (Lampertico et al. 2015).

1.5.4 Dosage, Safety, and Side Effects

Standard dose for TDF is 300 mg daily for patients with normal renal function. No dose adjustment is necessary based on renal function, but dose interval should be adjusted according to patient's renal function. TDF is not eliminated by hepatic metabolism; thus, it is not affected by hepatic impairment, so it can be safely administered to patients with poor liver function. With regard to the drug-related AEs of TDF, more evidences have been accumulated comparing with other NUCs because it has been used for one of elements of antiviral therapy for HIV-infected patients prior to using for CHB treatment. Three major AEs that should be paid attention to are mitochondrial toxicity, proximal renal tubular damage, and osteomalacia. Mitochondrial toxicity can manifest as lactic acidosis, fat redistribution syndrome, hepatic steatosis, acute pancreatitis, or peripheral neuropathy (Duarte-Rojo and Heathcote 2010). TDF has been reported to cause proximal tubular dysfunction,

e.g., Fanconi syndrome, and also other related nephrotoxicities including diabetes insipidus and calcium and phosphorus dysregulation with bone disease have been reported (Gupta 2008). Fanconi syndrome may lead to severe complications such as loss of calcium and phosphorus due to proximal tubular dysfunction. However, this report was from majority of the patients with concomitant use of other HIV drugs which also could cause renal dysfunction (Gupta 2008). Renal function, however, should be assessed before and during treatment periodically, in particular, in patients at high risk for renal dysfunction (old age, hypertension, diabetes, chronic kidney disease, and renal calculi) (Terrault et al. 2016).

In a study of HIV-infected patients, patients with TDF including regimen had a high urinary excretion of phosphate. Although TDF was not associated with hypophosphatemia, this report addressed the possible concern for chronic demineralization of bone due to TDF (Labarga et al. 2009). The long-term significance of TDF effect on the kidney and bone remains uncertain. Annual bone mineral density assessment in a 7-year long-term follow-up study with TDF showed no significant changes at years 4 and 7. Renal AEs developed infrequently (1.7%), were generally mild, and improved with dose modification (Buti et al. 2015).

In pregnancy, the FDA category assigned to TDF is B. Studies on carcinogenesis, mutagenesis, and fertility, whether in vitro or in animals, with doses over five times that seen in humans on standard doses of TDF have not shown any consistent adverse findings that raise concern for humans (Duarte-Rojas and Heathcote 2010). Therefore, TDF is a preferred choice for pregnant patients considering its high potency, low resistance rate, and safety data of use during pregnancy (Terrault et al. 2016; EASL 2012). In a cohort of HBeAg-positive mothers with an HBV DNA level of more than 200,000 IU/mL during the third trimester, the rate of mother-to-child transmission was lower among those who received TDF therapy than among those who received usual care without antiviral therapy (Pan et al. 2016). In this study, the maternal and infant safety profiles were similar in the TDF group and the control group.

2 Treatment Strategies

2.1 Definition of Treatment Response

Definitions of terms for treatment response are shown in Table 11.1.

2.2 On-Treatment Monitoring

Serum HBV DNA levels may be checked every 3 months by sensitive PCR method until HBV DNA is undetectable. HBeAg, anti-HBe (in patients with HBeAg positive), and ALT may be measured every 3–6 months during NUC therapy. Thereafter, every 3–6 months of serial monitoring is required for early detection of virological and biochemical breakthrough. Renal safety measurements including serum

Table 11.1 Definitions of terms related to HBV infection, oral antiviral therapy, and resistance to nucleos(t)ide therapy

Terminology	Definition
HBeAg clearance	Loss of HBeAg in a person who was previously HBeAg positive
HBeAg seroconversion	Loss of HBeAg and detection of anti-HBe in a person who was previously HBeAg positive and anti-HBe negative
HBeAg reversion	Reappearance of HBeAg in a person who was previously HBeAg negative and usually associated with increased HBV replication
HBsAg seroconversion	Loss of HBsAg and development of anti-HBs
Acute exacerbation or flare of hepatitis B	Intermittent elevation of aminotransferase to more than 5 times the upper limit of normal and more than twice the baseline value
Reactivation of hepatitis B	Reappearance of active necroinflammatory disease of liver in a patient known to have the inactive chronic HBV infection state or resolved hepatitis B infection
Resolved hepatitis B infection	Previous HBV infection, but now HBsAg negative and anti-HBs positive
Hepatic decompensation	Defined as significant liver dysfunction as indicated by raised serum bilirubin (more than 2.5 times the upper limit of normal) and prolonged prothrombin time (prolonged by more than 3 s) or INR [1.5 or occurrence of complications such as ascites and hepatic encephalopathy
Biochemical response	Normalization of serum ALT level
Virological response on NUC therapy	
Primary nonresponse	Reduction of serum HBV DNA <1 log IU/mL at 12 weeks of oral antiviral therapy in an adherent patient
Suboptimal or partial virological response	Reduction of serum HBV DNA >1 log IU/mL but still detectable at 24 weeks of oral antiviral therapy in an adherent patient
Virological response	Undetectable serum HBV DNA during therapy
Virological breakthrough	Increase of serum HBV DNA [1 log IU/mL from nadir of initial response during therapy, as confirmed 1 month later
Sustained off-treatment virological response	No clinical relapse during follow-up after stopping therapy
Complete response	Sustained virological response with HBsAg seroclearance
Viral relapse	Serum HBV DNA [2000 IU/mL after stopping treatment in patients with virological response
Clinical relapse	Viral relapse along with ALT >2× ALT
Histologic response	Decrease in histology activity index by at least 2 points and no worsening of fibrosis score compared to pretreatment liver biopsy or fibrosis reduction by at least 1 point by Metavir staging
Drug resistance	
Genotypic resistance	Detection of mutations in the HBV genome that are known to confer resistance and develop during antiviral therapy
Phenotypic resistance	Decreased susceptibility (in vitro testing) to inhibition by antiviral drugs; associated with genotypic resistance
Cross-resistance	Mutation selected by 1 antiviral agent that also confers resistance to other antiviral agents

creatinine, phosphorus, urine glucose, and urine protein and bone profile should be assessed before treatment commencement and during treatment periodically if treated with adefovir or TDF. Muscle weakness and development of neuropathy should be monitored during telbivudine therapy. In patients receiving long-term therapy with NUCs, checking patient's compliance and antiviral resistance testing should be assessed when virological breakthrough occurs during therapy, especially in patients who are taking low genetic barrier drugs.

2.3 Treatment Failure

Primary nonresponse in treatment-naïve patients is relatively low for all available NUCs; adefovir seems to have a higher rate of primary nonresponse compared with other NUCs. The strategies for partial virological response depend on types of prior NUC. In patients on drug with low genetic barrier to resistance (lamivudine, telbivudine, or adefovir), change to a more potent drug (entecavir or TDF) is recommended considering their high rate of long-term antiviral resistance (EASL 2012). Patients with primary nonresponse or partial response on entecavir or TDF can continue monotherapy regardless of ALT even though it is still controversial. Testing for genotypic resistance may be performed in patients with virological breakthrough if the patient is complaining with treatment. Identification of drug resistance during entecavir therapy is time to change treatment strategies: switch to TDF monotherapy is the recommended rescue therapy (Terrault et al. 2016).

2.4 Endpoint of Treatment with NAs

The ideal endpoint of treatment for CHB is HBsAg loss following anti-HB seroconversion. However, given the rarity of HBsAg seroconversion rate with current NUC therapies, long-term, life-long treatment is required in most of the patients regardless of the virological and/or serologic responses (Su and Kao 2015).

In HBeAg-positive patients, HBeAg seroconversion with undetectable HBV DNA might be considered criteria to discontinue antiviral treatment after at least 12 months of consolidation therapy. However, the long-term durability of HBeAg seroconversion induced by NUCs is still less satisfactory. Virological relapse with or without HBeAg reversion is common after cessation of treatment with a recurrence rate of 40–50% in 2 years. In HBeAg-negative patients, virological relapse rate is >90% after cessation of antiviral treatment (Seto et al. 2015). Among CHB patients who discontinue NUC treatment, the combination of serum HBV DNA and quantitative HBsAg levels may predict the risk of clinical relapse (Wang et al. 2016). Several aspects should be considered prior to deciding cessation of antiviral treatment: risk for virological relapse, hepatic decompensation, development of HCC, liver-related death, and burden of sustained antiviral therapy (long-term monitoring, adherence, and potential for drug resistance).

Without an evidence of cirrhosis, antiviral treatment can be considered to withdraw after HBsAg loss (Kim et al. 2014). In patients with compensated cirrhosis, HBsAg loss is regarded as an endpoint of antiviral treatment even though the HBsAg seroconversion rate is very low. After patients achieve HBsAg loss, cessation of treatment may be considered. However, periodic monitoring is required for early detection of virological relapse and hepatic flare to ensure retreatment as soon as possible. In patients with decompensated cirrhosis, antiviral treatment is recommended indefinitely irrespective of HBV DNA level. Even with effective antiviral treatment, serial HCC surveillance is mandatory for all patients with cirrhosis.

3 Treatment for Antiviral-Resistant Cases

The rate of resistance to NUCs can vary with duration of treatment, pretreatment HBV DNA levels, potency of antiviral agent, and prior exposure to NUCs. The long-term resistance rates are presented in Table 11.2. Once genotypic resistance is evident, a rescue therapy with drugs that does not share cross-resistance should be initiated to minimize emergence of multiple drug-resistant strains (European Association for the Study of the Liver 2012). Cross-resistance data for resistant HBV mutants are presented in Table 11.3.

3.1 Lamivudine Resistance

Current evidence advocates that switching to TDF monotherapy is preferred. In a randomized prospective trial, patients with lamivudine resistance were treated with either TDF or TDF/emtricitabine. In this study, 89.4% of patients in the TDF and 86.3% of TDF/emtricitabine had undetectable HBV DNA levels (HBV DNA <69 IU/mL, $P = 0.43$) at week 96 (Fung et al. 2014).

3.2 Adefovir Resistance

It has been reported that adefovir resistance is more likely developed in patients with previous exposure to lamivudine (Lee et al. 2006). Adefovir monotherapy in patients with lamivudine resistance had a 22% of cumulative adefovir resistance at 2 years (Fung et al. 2006). In a study with patients who were resistant to both lamivudine/adevovir or lamivudine alone, entecavir was less effective in reducing serum HBV DNA levels in lamivudine/adevovir group at week 48 (10% versus 34%, $P = 0.006$, respectively) (Shim et al. 2009). In a prospective study of 60 patients who failed to treat with both lamivudine and adefovir, TDF monotherapy has been shown to be effective in reducing HBV DNA levels (Patterson et al. 2011). In a case of treatment failure or resistance to adefovir, treatment with TDF achieved long-term viral suppression (HBV DNA <400 copies/mL) in 84% of patients at week 168

Table 11.2 Approved oral nucleos(t)ide analogue treatment in adults

	Lamivudine (%)	Adefovir (%)	Telbivudine (%)	Entecavir (%)	TDF (%)
Dose	100 mg daily	10 mg daily	600 mg daily	0.5 or 1.0 mg daily	300 mg daily
Pregnancy category	C	C	B	C	B
Potential side effects	Pancreatitis Lactic acidosis	Acute renal failure Fanconi syndrome Nephrogenic diabetes insipidus	Myopathy Peripheral neuropathy	Lactic acidosis	Nephropathy Fanconi syndrome Osteomalacia
HBsAg-positive patients					
Undetectable HBV DNA ^a	36–44	13–21	60	67	76
ALT normalization ^b	41–72	48–54	77	68	68
HBsAg seroconversion	16–18	12–18	22	21	21
HBsAg loss	0–1	0	0.5	2–3/4–5 (1 year/3 years)	3/8 (1 year/3 years)
References	Lau et al. (2005), Dienstag et al. (1999), Chang et al. (2006), Lai et al. (2007))	Marcellin et al. (2003, 2008b)	Lai et al. (2007)	Chang and Lai (2006), Gish et al. (2007), Lok et al. (2012)	Marcellin et al. (2008b), Heathcote et al. (2011)
HBsAg-negative patients					
Undetectable HBV DNA ^a	72–73	51–63	88	90	93
ALT normalization ^b	71–79	72–77	74	78	76
HBsAg loss	0	0	0	<1	0
References	Lai et al. (2006, 2007), Marcellin et al. (2004)	Marcellin et al. (2008b), Hadziyannis et al. (2003)	Lai et al. (2007)	Lai et al. (2006)	Marcellin et al. (2008b)
Resistance					
At 1 year	24	0/4.4–18	2.7–4.4	0.2/6	0
At 3 years	53	11/34.3		1.2/36	0
At 5 years	>70	29/65.6		1.2/51	0
References	Lai et al. (2003), Lok et al. (2003)	Hadziyannis et al. (2006), Lee et al. (2006, 2010), Yoon et al. (2006)	Lai et al. (2007), Liaw et al. (2009)	Tenney et al. (2009)	Lampertico et al. (2015)

^aHBV DNA <60–80 IU/mL

^bALT normalization defined by laboratory normal

Table 11.3 In vitro cross-resistance for resistant HBV mutants

HBV mutants	Level of susceptibility				
	Lamivudine	Telbivudine	Entecavir	Adefovir	TDF
Wild type	S	S	S	S	S
M204V	R	S	I	S	S
M204I	R	R	I	S	S
L180M + M204V	R	R	I	S	S
A181T/V	R	R	S	R	I
N236T	S	S	S	R	I
A181T/V + N236T	R	R	S	R	I/R
L180M + M204V/I $\pm$ I169T $\pm$ V173L $\pm$ M250V	R	R	R	S	S
L180M + M204V/I $\pm$ T184G $\pm$ S202I/G	R	R	R	S	S

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(Berg et al. 2014). In a prospective randomized trial (Lim et al. 2016b), patients who had adefovir-resistant HBV mutations with serum HBV DNA levels >60 IU/mL were treated with either TDF monotherapy or TDF and entecavir combination therapy for 48 weeks. TDF monotherapy achieved a virological response comparable to that of TDF/entecavir combination therapy at 48 weeks. In this trial, the extension of TDF monotherapy for 96 weeks showed durable viral suppression. Currently, switch to TDF is the recommended therapy for adefovir resistance treatment by international guidelines (Sarin et al. 2015; Terrault et al. 2016; WHO 2015).

3.3 Telbivudine Resistance

There is few evidence to support optimal treatment regimen for telbivudine resistance. Given similar profile of antiviral resistance with lamivudine, telbivudine resistance could be treated in a similar way of lamivudine resistance.

3.4 Entecavir Resistance

Entecavir resistance can develop in patients with prior resistance to lamivudine. The 5-year cumulative probability of emerging entecavir resistance is 51% in lamivudine-resistant patients (Tenney et al. 2009). In a randomized study of 90 patients with entecavir resistance treated with TDF alone or TDF/entecavir combination therapy (Lim et al. 2016a), the primary efficacy endpoints (HBV DNA <15 IU/mL) were not significantly different between TDF monotherapy and TDF/entecavir combination therapy (71% versus 73%, respectively, $P > 0.99$) at week 48. In addition, none developed additional resistance mutations. In case of entecavir resistance, switch to TDF monotherapy is a preferred option (Sarin et al. 2015; Terrault et al. 2016; WHO 2015).

3.5 Tenofovir Resistance

To date, no tenofovir resistance has been reported in clinical setting. A long-term follow-up study of tenofovir-treated patients showed an effective serum HBV DNA suppression with no evidence of tenofovir resistance up to 6 years of treatment (Kitrinos et al. 2014).

4 Risk of HCC Development with Oral Antiviral Treatment

With the advent of NUCs, mounting evidence has been accumulated supporting that HCC incidence has decreased with oral NUC treatment. The first data with lamivudine by Liaw et al. demonstrated that HCC risk was reduced with antiviral treatment compared to placebo in patients with cirrhosis or advanced fibrosis and active liver disease ($HR = 0.49$; $P = 0.047$) (Liaw et al. 2004). The annual incidence of HCC in patients treated with entecavir or TDF which are the preferred agents for first-line therapy is presented in Figs. 11.1 and 11.2. The observed HCC risk with entecavir or TDF ranges from 0.9 to 5.4% and 0.01 to 1.4% in patients with and without cirrhosis, respectively (Papatheodoridis et al. 2015a). Compared to untreated patients, treatment with entecavir or TDF showed a lower risk of HCC development ranging from a HR of 0.27 to 0.41 (Hosaka et al. 2013; Kumada et al. 2013; Wu et al. 2014; Su et al. 2014). However, it is still controversial whether the risk reduction for HCC from entecavir or TDF is greater than other NUCs (i.e., lamivudine, adefovir, and telbivudine) (Lim et al. 2014; Papatheodoridis et al. 2015a).

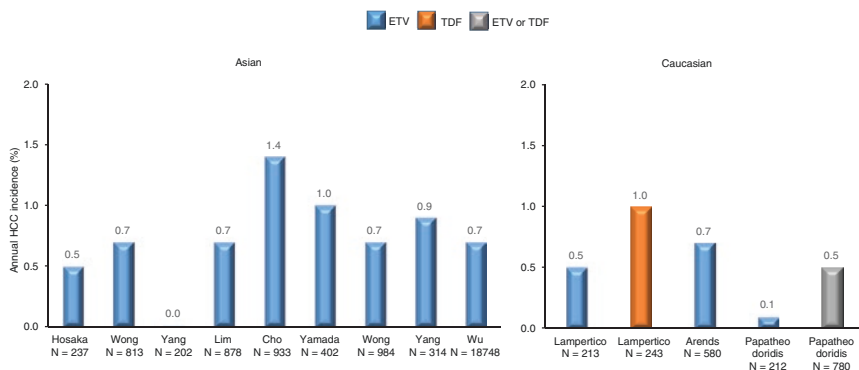


Fig. 11.1 Annual HCC incidence rates with entecavir or tenofovir in CHB without cirrhosis (Reprint with permission from Papatheodoridis et al. 2015a)

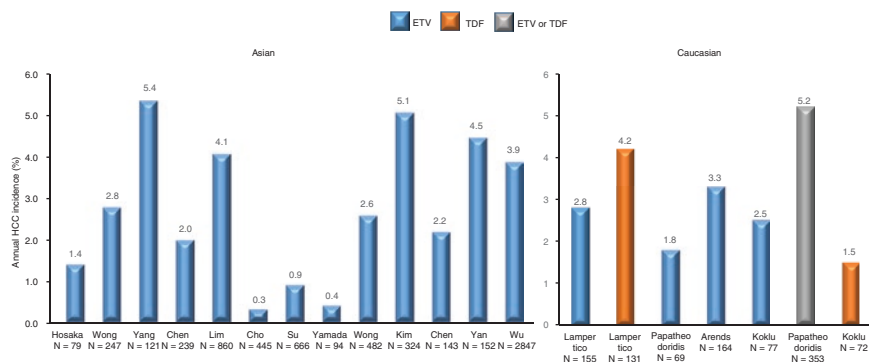


Fig. 11.2 Annual HCC incidence rates with entecavir or tenofovir in CHB with cirrhosis (Reprint with permission from Papatheodoridis et al. 2015a)

5 Summary and Conclusions

The goal of CHB treatment is to prevent liver-related morbidity and mortality through the persistent suppression of HBV viral replication with current available oral NUCs. Entecavir and TDF are the preferred choices for first-line antiviral treatment by all international practice guidelines due to their superior efficacy and high barrier to resistance over comparable drugs. However, currently available NUCs cannot completely eradicate HBV owing to persistence of cccDNA in the hepatocytes. Mounting evidence suggests that NUCs can reduce the risk of development of HCC but not eliminate the risk.

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Di Wu and Qin Ning

Abstract

Chronic hepatitis B virus (HBV) infection may progress to liver failure, cirrhosis, and hepatocellular carcinoma (HCC). Current approved antiviral treatments include nucleos(t)ide analogues (NUCs) and immunomodulators, such as pegylated interferon alpha (Peg-IFN). NUCs are safe and generally well-tolerated agents with direct antiviral activities. However, off-treatment durability of response to NUC therapy is low, requiring a long-term or lifelong course of treatment. Peg-IFN treatment has the advantage of a finite duration and a good chance of achieving sustained off-treatment response. However, only a minority of patients has a success response to IFN alone, and high rates of side effects limit its clinical use. Elimination (complete cure) of HBV is hard to achieve with either therapy alone, given that the covalently closed circular DNA (cccDNA) persists stably in the nuclei of infected hepatocytes. Hepatitis B surface antigen (HBsAg) seroclearance is therefore considered the optimal endpoint and a “functional/clinical cure” for HBV infection, despite the lack of HBV complete clearance. Theoretically, combination of Peg-IFN and NUC with differential mechanisms of actions on HBV is an alternative strategy to treat chronic hepatitis B. Recent studies demonstrated virological or serological benefits of de novo combination therapy with Peg-IFN and NUC, or addition of Peg-IFN (add-on or switch) to an ongoing NUC therapy, but few data exist about the long-term outcomes in patients receiving combination therapy. Currently, several new antiviral or immunomodulatory agents are being explored in experimental models or have reached clinical testing, which may have the potential to complement NUC or

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IFN-based therapy. This chapter summarizes current combination therapy and novel therapeutic approaches developed to accomplish a cure of chronic HBV infection.

Keywords

HBV • CHB • Peg-IFN • NUC • Combination therapy • Functional cure

Abbreviations

ADV	Adefovir dipivoxil
cccDNA	Covalently closed circular DNA
CHB	Chronic hepatitis B
DAAs	Direct-acting antivirals
ETV	Entecavir
FTC	Emtricitabine
HAPs	Heteroaryldihydropyrimidines
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B s antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
LAM	Lamivudine
LdT	Telbivudine
NTCP	Sodium-taurocholate cotransporting polypeptide
NUC	Nucleos(t)ide analogue
Peg-IFN	Pegylated interferon
PPAs	Phenylpropenamides
TDF	Tenofovir disoproxil fumarate
TLR	Toll-like receptor

1 Goals of Therapy and Definition of “Functional Cure” for HBV Infection

Hepatitis B virus (HBV) infection remains a worldwide health burden. The World Health Organization (WHO) estimates that 240 million people are chronic carriers of hepatitis B surface antigen (HBsAg) globally, and around 650,000 people die annually from the complications of chronic hepatitis B (CHB). Overall, HBV accounts for around 30% of cases of cirrhosis and 45% of hepatocellular carcinoma (HCC) (Ott et al. 2012; WHO Guidelines Approved by the Guidelines Review Committee 2015). Both viral and host factors are involved in the chronicity of HBV infection. The HBV genome forms covalently closed circular DNA (cccDNA), a stable minichromosome, within the nuclei of hepatocytes, which enables the infection to persist (Levero et al. 2009a). Data suggests that HBV is potentially capable

of suppressing innate immunity (Op den Brouw et al. 2009; Lang et al. 2011), and persistent exposure to high concentrations of viral antigen may cause functional exhaustion of T cells in CHB patients (Das et al. 2008; Boni et al. 2007). The ultimate goal of antiviral therapy is to prevent or significantly delay the progression of HBV-related liver disease. This goal may be achieved firstly through sustained immunological control of HBV infection and, eventually, by the means of complete elimination of HBV (European Association For The Study Of The Liver 2012; Lampertico and Liaw 2012; Scaglione and Lok 2012). But elimination of HBV (complete cure) is rarely experienced, given that HBV cccDNA persists stably, at very low level, even after the seroclearance of HBsAg. Serum HBsAg represents a surrogate marker for intrahepatic cccDNA, and loss of HBsAg is thought to be associated with a functional remission and improved long-term outcomes in CHB patients, even if HBV genome may not be eliminated and the few persisting infected hepatocytes are controlled by the host immune system (Chan et al. 2007; Moucari et al. 2009; Martinot-Peignoux et al. 2014). HBsAg loss with or without HBsAb seroconversion is therefore considered the optimal endpoint and a “clinical or functional cure” for HBV infection and represents the complete suppression of viral replication with sustained immunological control of HBV infection (European Association For The Study Of The Liver 2012; Lampertico and Liaw 2012; Scaglione and Lok 2012). This ideal endpoint could be achieved, although relatively rarely, after therapy with available antiviral drugs. The suboptimal and satisfactory endpoint is defined by sustained off-therapy virological/serological response with biochemical and histological improvement and prevention of complications; under this circumstance, the antigen-specific immune response might be sufficient to suppress HBV replication.

2 Advantages and Disadvantages of Current Anti-HBV Therapies

Currently approved treatment options remain limited to two classes of antiviral agents: nucleos(t)ide analogues (NUCs) including lamivudine (LAM), telbivudine (LdT), adefovir dipivoxil (ADV), entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide fumarate (TAF), which suppress HBV replication effectively through directly targeting the reverse transcription functions of polymerase (European Association For The Study Of The Liver 2012; Sarin et al. 2016; Terrault et al. 2016), and immunomodulatory agents including interferon- α (IFN) and pegylated interferon alpha (Peg-IFN), which are found to have dual effects, immunomodulatory action and modest antiviral activity (European Association For The Study Of The Liver 2012; Sarin et al. 2016; Terrault et al. 2016; Sadler and Williams 2008; Samuel 2001). Both treatment strategies have advantages and limitations. NUCs have been widely used due to their convenience, well tolerability, and potent antiviral activity. Recent studies have shown significant reductions in cccDNA levels after long-term NUC treatment (Boni et al. 2012). However, even third-generation NUCs (ETV and TDF), recognized as

first-line treatment options, have only small effects on the expression of viral antigens since NUCs do not directly inhibit the transcriptional abilities of cccDNA (Dandri and Locarnini 2012; Brunetto et al. 2009), thus sustained immunological control is hard to achieve, and high rates of relapse after NUCs withdrawal require these oral antiviral treatments to be lifetime commitments, especially in HBeAg-negative patients. As a consequence, indefinite duration can increase the likelihood of viral resistance, poor adherence, and late adverse effects. Furthermore, several studies indicate that NUC treatments reduce but cannot completely eliminate the risk of HCC (Arends et al. 2015). Unlike NUC, IFN has synergic effect, which can induce a complex network of intracellular signaling and the production of antiviral proteins encoded by IFN-stimulated genes (ISGs), and enhances the activity of immune cells and expression of cytokines for control of viral replication (Sadler and Williams 2008; Samuel 2001). Moreover, recent studies report that IFN is capable of promoting degradation of pregenomic RNA and core particle and inhibiting HBV transcription by inducing epigenetic regulation of the nuclear cccDNA minichromosome (Wieland et al. 2005; Xu et al. 2010; Belloni et al. 2012), thus directly reducing the production of viral antigen. IFN therapy is associated with low rates of drug resistance, a finite duration, a marked reduction in serum HBsAg, and sustained off-treatment responses; nevertheless, only a minority of patients has a success response to IFN alone, such as younger individuals, patients with lower baseline HBV DNA levels and HBsAg titer and higher alanine aminotransferase (ALT) levels. Moreover, IFN therapy has relatively high rates of adverse effects and is therefore often not well tolerated (Brunetto and Bonino 2014; Kim et al. 2011).

Despite being the optimal treatment endpoint for chronic hepatitis B, HBsAg loss is only attained in less than 10% of patients after long-term NUC or 1-year Peg-IFN therapy (Marcellin et al. 2004, 2008; Lau et al. 2005). Therefore, novel therapeutic concepts such as combination therapy with the approved agents are needed to achieve higher rates of HBsAg clearance and, ideally, HBsAb seroconversion.

3 Combining Different NUCs as Rescue Therapy

Combining antiviral drugs with similar mechanisms of action, such as different NUCs, may not have additional therapeutic effects. Therefore, current guidelines (European Association For The Study Of The Liver 2012; Sarin et al. 2016; Terrault et al. 2016) recommend combination therapy of NUCs with nonoverlapping resistance profiles only as rescue regimen under highly selected circumstances such as for suboptimal responses to NUC monotherapy and for patients infected with multidrug-resistant (MDR) strains of HBV. It is uncertain whether de novo combination therapy offers advantages over NUC monotherapy in treatment-naïve CHB patients, especially in patients with high baseline viral load, thus more studies are required. Combination treatment with LAM plus ADV as rescue therapy in patients with resistance to LAM or ADV leads to greater virological suppression and lower risk of emergent LAM or ADV resistance than monotherapy (Gaia et al. 2008;

Kim et al. 2016a). Recently, several studies investigated combination therapy with ETV and TDF in primary non-responders, partial responders, or patients with virological breakthrough or MDR CHB. One of these studies has shown that combination therapy with ETV plus TDF can provide high rates of viral suppression without treatment-emergent resistance to either agent in patients with previous NUC treatment failure (Zoulim et al. 2016). However, unfavorable respects of rescue combination therapy include higher treatment costs and potentially harmful side effects. A recent study reported that virological responses were durable after withdrawal of LAM in a majority of patients with LAM resistance who had achieved complete virological response with LAM and ADV combination therapy (Kim et al. 2016b). In a follow-up study of MDR CHB patients, after achieving virological response, TDF was discontinued in ADV-resistant patients, and ETV was discontinued in LAM-resistant patients with ETV plus TDF combination therapy, and the results demonstrated that switching to a monotherapy from combination therapy appeared to be efficient and safe (Petersen et al. 2014). However, only a few studies have been published on this adapting step-down strategy, and most of them have involved small sample sizes; it requires larger comparative, prospective trials to investigate its safety and efficacy.

4 Interferon-Based Combination Therapy

Since IFN and oral antivirals have different mechanisms of action, it has been hypothesized that combination treatment with NUC and Peg-IFN may enhance chances of serological response and sustained off-treatment response, thereby eventually facilitating the discontinuation of NUCs, through harnessing both direct antiviral and immunomodulatory mechanisms (Thimme and Dandri 2013; Wu et al. 2015). The updated Chinese guidelines for the treatment of HBV recommend that sequential therapy with additional Peg-IFN or switching to Peg-IFN in patients who have achieved virological suppression on long-term NUC treatment may be advantageous to obtain higher rate of HBeAg seroconversion and greater HBsAg decline than continuous NUC monotherapy (Chinese Society of Hepatology et al. 2015). The 2015 updated Asian-Pacific guidelines recommend that the combination treatment with NUC and Peg-IFN could be considered the ideal treatment for chronic hepatitis B (Sarin et al. 2016). Sequential therapy with ETV followed by IFN is recognized as a first-line treatment for young HBeAg-negative patients with high viral load in the Japanese guidelines (Kumada et al. 2010). Currently, IFN and NUC combination treatment is not recommended either by American guidelines or by European guidelines (European Association For The Study Of The Liver 2012; Terrault et al. 2016). Clinical trials evaluated the de novo combination, as well as add-on or switch strategies with Peg-IFN and oral antivirals for CHB patients, but the results are inconclusive.

Early observation studies which evaluated Peg-IFN in combination with LAM or ADV started simultaneously demonstrated higher rates of on-treatment virological response, but not improved posttreatment response rates (Marcellin et al. 2004; Lau et al. 2005; Piccolo et al. 2009; Janssen et al. 2005). Previous studies reported that

Peg-IFN plus ADV combination therapies contributed to remarkable reductions in levels of HBV DNA, intrahepatic cccDNA, and serum HBsAg titer (Wursthorn et al. 2006). A randomized, multicentered study investigated the outcome of HBeAg-positive CHB patients treated with LdT plus Peg-IFN combination therapy and demonstrated that even though the combination therapy led to greater declines in HBV DNA and HBsAg levels, it carried an increased risk of unexpected severe peripheral neuropathy, thus should not be used (Marcellin et al. 2015). Recently, a large prospective, active-controlled randomized trial evaluated loss of HBsAg in patients receiving treatment with either TDF or Peg-IFN, or simultaneously combining TDF and Peg-IFN for 16 or 48 weeks. This study showed that, at 24 weeks posttreatment, 48 weeks of combination therapy with TDF plus Peg-IFN led to a higher rate of HBsAg loss than either therapy given alone. But the overall rate of HBsAg clearance remained low (9.1%), with the highest rate occurring in genotype A patients (Marcellin et al. 2016). It is notable that a prolonged follow-up of these subgroups of patients is necessary to determine sustained off-treatment responses and the long-term benefits.

Several studies have shown that host genetic background was associated with HBsAg loss during combination therapy (Jansen et al. 2014; Stelma et al. 2016; Tangkijvanich et al. 2016). One recent genome-wide association study in patients treated with ADV and Peg-IFN identified one single nucleotide polymorphism (SNP), rs12356193 located in the SLC16A9 gene, was strongly associated with HBsAg seroclearance (Jansen et al. 2014). A randomized clinical trial demonstrated that the combination therapy with Peg-IFN plus ETV in treatment-naïve HBeAg-negative patients led to higher rates of undetectable HBV DNA at week 48 but did not increase HBsAg clearance and decline compared with Peg-IFN monotherapy. Interestingly, patients who carried the SNP rs3077 genotype GG with baseline HBsAg <1000 IU/ml had a good chance of attaining virological response and HBsAg loss. These studies indicate that the viral and host genetic characteristics may be beneficial to individualize decision-making before initiating Peg-IFN therapy (Tangkijvanich et al. 2016). Although simultaneous administrations of Peg-IFN and NUC other than TDF might not provide additional benefits, the optimal approaches for conducting IFN-based combination therapy remain to be explored, and the time schedule of drug administration should be taken into consideration.

Late-breaking clinical studies demonstrate that sequential combination therapy with IFN and NUC contributes to a better chance of HBsAg loss over NUC monotherapy. A prospective, multicentered, randomized, and controlled trial (OSST study) reported that HBeAg-positive patients who did not seroconvert to HBeAb during 9–36 months of ETV treatment were randomly assigned to switch to Peg-IFN treatment or to receive continuous ETV monotherapy for 48 weeks; as compared to continuous ETV monotherapy, sequential combination therapy using ETV and Peg-IFN led to significantly increased rates of HBeAg seroconversion and HBsAg seroclearance (8.5%) (Ning et al. 2014). During untreated follow-up in those patients who switched from ETV to Peg-IFN therapy, rates of HBeAg seroconversion improved from 17.7% at the end of therapy to 38.7% 1 year posttreatment; moreover, HBsAg seroclearance was sustained in six of seven patients (Han et al. 2016). Results from

OSST cohort study were in line with previous studies in which patients received sequential combination therapy with NUC and IFN; however, these studies included a very limited number of patients (Moucari et al. 2011; Sarin et al. 2005). An exploratory study demonstrated that sequential treatment, specifically, 12 weeks of ETV alone, then ETV plus Peg-IFN for 12 weeks, and lastly only Peg-IFN for 36 weeks, led to significantly higher rates of HBeAg and HBsAg seroconversion in CHB patients with non-D genotypes and high HBV viremia, as compared with 48 weeks of Peg-IFN monotherapy (Boglione et al. 2013). The interim results from NEW SWITCH study also suggested that sequential combination therapy with ETV and Peg-IFN in NUC-experienced CHB patients with HBV DNA suppression and HBeAg loss achieved 17.3% HBsAg loss at week 48 (Hu et al. 2015).

Recent studies demonstrate that quantitative HBsAg is a useful marker for stopping antiviral therapy or initiating Peg-IFN sequential therapy (Höner Zu Siederdisen and Cornberg 2014). More studies are required to identify patients with the greatest chance of achieving favorable outcome with sequential therapy. The OSST study has shown that patients undergoing long-term ETV therapy with HBeAg clearance and low HBsAg titers (<1500 IU/ml) are recommended to receive sequential therapy with Peg-IFN because they have a high probability of HBeAg seroconversion (33.3%) and HBsAg clearance (22.2%). Patients with HBsAg titers declining to 200 IU/ml at week 12 have the highest rates of successful response. Patients with HBsAg titers >1500 IU/ml at week 12 should consider discontinuing Peg-IFN treatment because they have a minimal chance of attaining HBeAg seroconversion and HBsAg loss (Ning et al. 2014). This data is consistent with previous reports and interim results from the NEW SWITCH study which demonstrate that serum HBsAg levels at baseline, week 12, or week 24 seem to be strong predictors of response in IFN-based combination therapy and allow response-guided treatment (Hu et al. 2015; Höner Zu Siederdisen and Cornberg 2014; Piratvisuth et al. 2013). However, the exploratory analyses assessing predictors associated with response were performed only in a small group of patients. Hence, the results should be interpreted cautiously, and large-scale prospective trials are needed to validate these findings.

Another novel combination treatment approach, by adding Peg-IFN to NUC therapy, has recently been shown to be beneficial in improving the response rates. An observation study demonstrated that in CHB patients undergoing a stable oral therapy with undetected HBV DNA, the addition of Peg-IFN led to HBsAg seroconversion in 2 out of 12 patients (Kittner et al. 2012). Another prospective study showed that in HBeAg-negative patients on a long-term NUC treatment with HBV DNA undetectable, add-on of Peg-IFN induced sustained HBsAg clearance and cessation of NUC therapy in six out of ten patients (Ouzan et al. 2013). A global multicentered, randomized, controlled trial (ARES study) investigated the effectiveness of Peg-IFN add-on therapy for 24 weeks in HBeAg-positive patients after 24 weeks of ETV monotherapy; when compared to continuous ETV monotherapy, this combination strategy of adding Peg-IFN to ETV did not improve response rates, which defined as HBV DNA <200 IU/ml with HBeAg clearance at week 48, but resulted in a higher rate of HBeAg seroconversion and more decline in HBV

DNA, HBeAg, and HBsAg and seemed to prevent posttreatment relapse, thereby allowing for NUC discontinuation (Brouwer et al. 2015). A multicenter randomized trial demonstrated that among treatment-naïve HBeAg-positive patients, a combination therapy regimen of ETV pretreatment or ETV add-on to Peg-IFN significantly increased rates of on-treatment virological response but failed to improve sustained off-treatment response, as compared to Peg-IFN monotherapy (Xie et al. 2014).

Due to differences in case-control selection and study designs, at this stage, it is difficult to determine the optimal use of combination therapy with NUC and Peg-IFN to obtain the best treatment outcomes in CHB patients. However, it is conceivable that once suppression of HBV replication has been achieved with NUC treatment, the addition of Peg-IFN would be more advantageous and effective. The above clinical trials provide novel therapeutic concepts which warrant further investigation before being introduced into routine clinical practice.

5 The Mechanisms Involved in Immune Restoration Induced by Peg-IFN and NUC Combination Therapy

During persistent HBV infection, the impaired antiviral immune responses were reported through virus-mediated mechanisms, including the suppression of natural killer (NK) cell activity, the exhaustion of viral specific cytotoxic lymphocytes (CTLs), and the activation of regulatory T cells (Tregs). Current data emphasizes the critical role of both innate and adaptive immune response in resolution of HBV infection (Maini and Schurich 2010; Thimme et al. 2003; Billerbeck et al. 2007). Further studies on the mechanism underlying enhanced immune response induced by IFN-based combination therapy may help guide future clinical trial design.

Accumulating data suggests that the host immune response can be impacted by NUC and Peg-IFN in different ways. It is widely accepted that Peg-IFN mediates significant augmentation of innate immunity especially NK cells. Micco et al. investigated the immunomodulatory effects of Peg-IFN on NK cells and CD8+ T cells, and the results showed that Peg-IFN was able to induce IL-15 and Nkp46, accompanied by increases in expansion, activation, and antiviral ability of CD56bright NK cells with upregulated expressions of TRAIL and IFN- γ ; in contrast, Peg-IFN resulted in a sustained depletion of effector CD8+ T cells and had a limited capacity to restore HBV-specific T cell functions (Micco et al. 2013). Studies conducted by Huang et al. revealed that significantly reduced expressions of Toll-like receptors (TLR) 3 and TLR9 on peripheral CD14+ monocytes in CHB patients could be restored by effective Peg-IFN therapy (Huang et al. 2013, 2014). However, different effects of Peg-IFN were observed on T cells. Consistent with the results obtained by Micco et al., Penna et al. reported that in HBeAg-negative CHB patients, Peg-IFN did not improve early circulating HBV-specific T cell responses (Penna et al. 2012), which demonstrated a contrasting impact of Peg-IFN on innate and adaptive antiviral immune responses. Contrarily, potent NUC treatment was not able to restore the antiviral capacity of NK cells (Peppas et al. 2010). But effect of NUC on T cell

response seems different from IFN. Several studies demonstrated that the defective T cell function could be partially and transiently restored by NUC treatment. A recent study demonstrated that following in vitro culture, the dysfunctional HBV-specific T cells from patients achieving viral suppression with long-term NUC treatment had a significant functional recovery (Boni et al. 2012).

Accumulating evidence emphasizes the crucial role of immune restoration in achieving clinical cure of chronic hepatitis B. The different impacts of Peg-IFN and NUC on innate and adaptive immune response, as well as the findings that suppression of HBV replication by NUC prolongs innate immune response to Peg-IFN, give the mechanistic rationale for combination of these two agents in chronic HBV infection (Allweiss et al. 2012; de Niet et al. 2016; Tan et al. 2014). A recent study evaluated HBV-specific T cell function from patients with high HBV DNA level receiving Peg-IFN plus ADV combination therapy and demonstrated a partial restoration of HBV-specific T cells in patients who achieved HBsAg clearance (de Niet et al. 2016). Control of HBV replication by prior long-term NUC treatment could contribute to partial functional restoration of HBV-specific T cell; subsequent administration of immunomodulatory drug such as Peg-IFN might further augment the immune response and increase the possibility of treatment success. According to this scenario, it is conceivable that sequential therapy with Peg-IFN on long-term NUC treatment might be an alternative strategy leading to higher rate of HBsAg clearance. In line with this theory are intriguing immunology research findings based on OSST study. The OSST immunological study showed that successful serological responses to sequential Peg-IFN-a therapy were associated with significant restoration of an impaired immune response before week 24. The restoration was manifested by increased proportions of NKG2C+ NK cells, higher proportions of TLR2+ CD14+ monocytes, and decreased proportions as well as diminished inhibitory function of Tregs (Yan et al. 2015a). This data suggests that, during the early phase of IFN-based combination treatment, the activation and recovery of immune system will be beneficial for CHB patients to obtain satisfactory endpoints. Obviously, comprehensive analyses of innate and adaptive immune system in parallel with large prospective clinical trials are needed to further investigate the combination treatment strategies.

6 Novel Antiviral Strategies and Possible Combination Therapies Toward a Cure of HBV Infection

Combination therapies with drugs acting on novel targets may help achieve a cure of HBV infection. It is suggested that complete HBV control does not only depend on the reduction in viral burden but also the induction of effective antiviral immune response (Lin and Kao 2016). Currently, several strategies including small molecules targeting various stages of HBV life cycle (HBV entry, HBV cccDNA production and processing, viral replication and viral protein expression, etc.) as well as immunotherapeutic approaches are being explored in experimental models or have reached clinical testing, which may have the potential to complement NUC or IFN-based therapy (Fig. 12.1).

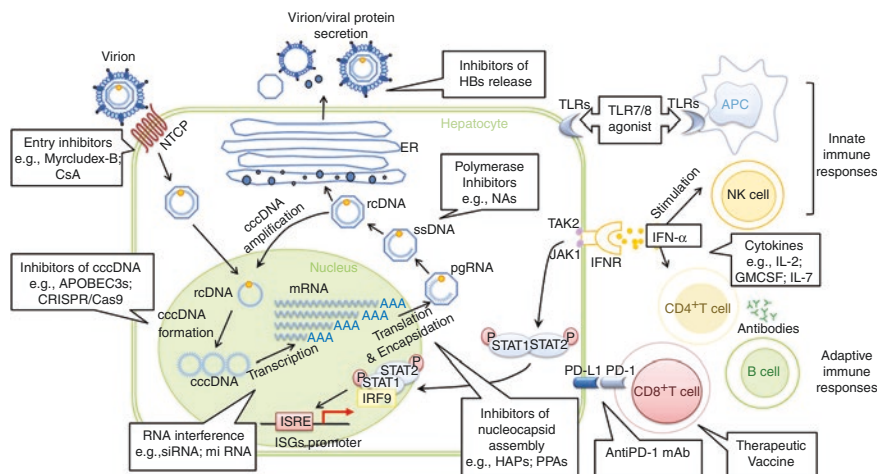


Fig. 12.1 Schematic representation of current treatment options and newly developed therapeutic strategies against HBV. Current treatment options involve NAs, which efficiently inhibit the HBV DNA polymerase and thus block the viral replication pathway, and IFN which has both direct antiviral and immunomodulatory effects. Several strategies will appear in the near future, including direct-acting antivirals (DAAs) targeting different stages of the life cycle of the virus (HBV entry, HBV cccDNA production and processing, viral replication and viral protein expression, etc.) as well as immunotherapeutic approaches including Toll-like receptors (TLRs) agonist, pleiotropic cytokines (IL-2, GM-CSF, IL-7), programmed cell death-1 (PD-1)/PD-L1 blockage, therapeutic vaccines, etc., to revive host immunity and restore the function of the HBV-specific T cell. It is conceivable that the use of these additional strategies combined with NAs and IFN might be of synergic benefit in the restoration of innate and adaptive immune responses in CHB patients. Abbreviations: NA, nucleos(t)ide analogues, IFN interferon, cccDNA covalently closed circular DNA, ER endoplasmic reticulum, hNTCP human sodium-taurocholate cotransporting polypeptide, pgRNA pregenomic RNA, rcDNA relaxed circular DNA, CsA cyclosporine A, HAPs hetero-aryldihydropyrimidines, PPAs phenylpropenamides, TLR Toll-like receptors

The sodium-taurocholate cotransporting polypeptide (NTCP), a newly identified cellular receptor for HBV entry and a bile salt transporter, is a promising target enabling the development of entry inhibitors and new research possibilities (Yan et al. 2012). Cyclosporine A or B is able to inhibit NTCP and prevents HBV entry in cell culture models (Nkongolo et al. 2014; Watashi et al. 2014). Myrcludex B can block HBV/hepatitis D virus (HDV) entry and has entered in clinical trial (Volz et al. 2013). These agents may prevent new infection (Kaneko et al. 2015; Yan et al. 2015b) but do not eliminate the preexisting HBV infection or directly target on cccDNA. Therefore, the combination regimens of NTCP inhibitors with other antivirals seem to be superior to their use as monotherapy, which need to be further investigated.

Therapeutic strategies targeting the cccDNA for HBV cure aim to inhibit cccDNA formation, degrade cccDNA, or silence cccDNA transcription. Genome-editing approaches using vectors to deliver DNA cleavage enzymes into the

hepatocytes, such as the clustered regularly interspaced short palindromic repeats (CRISPR)/CAS (Kennedy et al. 2015; Ramanan et al. 2015), might be promising approaches to degrade cccDNA. Epigenetic modification of histone, inhibiting cccDNA transcription, provides the proof of concept for silencing of cccDNA (Levrero et al. 2009b; Pollicino et al. 2006). Activation of lymphotoxin- β receptor (LT β R) and IFN- α increased the APOBEC3 cytidine deaminases and eventually contributed to degradation of cccDNA but did not affect genomic DNA, thereby allowing the development of these novel strategies combined with other antiviral agents to cure CHB (Lucifora et al. 2014).

Several attempts are also made to develop nucleocapsid protein inhibitors. AT-61 and AT-130 (Delaney et al. 2002; Feld et al. 2007) and phenylpropenamides (PPAs) derivatives can interfere with the pregenomic RNA packaging, and BAY41-4109 (Wu et al. 2008), a heteroaryldihydropyrimidines (HAPs) antiviral compound, can decrease the stability of capsids. Both HAPs and PPAs synergize with nucleoside reverse transcriptase inhibitors (NRTIs) and are active against NRTI-resistant strains in vitro (Delaney et al. 2002; Billioud et al. 2011), highlighting the potential for combination therapy with other antivirals such as NUC.

RNA interference (RNAi) is a transcriptional process in which the introduction of microRNA (miRNA) or small interfering RNA (siRNA) contributes to gene silencing in a sequence-specific way (Bernstein et al. 2001; Hammond et al. 2000). Currently, several drug candidates have entered clinical development for CHB including ARC-520, ALN-HBV, and TKM-HBV (Wooddell et al. 2013; Petersen et al. 2016). Although the RNAi-based therapeutics are promising, there are lingering concerns about its long-term effects.

HBsAg is capable of suppressing host immunity permitting viral persistence. Nucleic acid polymer (NAP) can block HBsAg release from infected cells. REP 9AC, the first-generation NAP, induced rapid clearance of HBsAg from the serum in HBeAg-positive patients and thus allowed sustained restoration of immune control of HBV infection (Mahtab et al. 2011a). Recent studies on REP 2139AC, the second-generation NAP, demonstrated that the add-on of immunomodulator such as Peg-IFN to REP 2139 after HBsAg clearance showed a synergistic antiviral effect (Mahtab et al. 2011b; Bazinet et al. 2016). Another study revealed that a combination regimen of REP 2139AC plus ETV or TDF improved antiviral outcomes and allowed reducing the duration of treatment (Petersen et al. 2016).

Studies on the immunopathogenesis of HBV infection pave the way for the development of potential novel approaches, including Toll-like receptors (TLRs) agonist (Lanford et al. 2013; Steuer et al. 2015), pleiotropic cytokines (Dahmen et al. 2002; Guptan et al. 2002; Shih et al. 2016), programmed cell death-1 (PD-1) and its ligand PD-L1 blockages (Liu et al. 2014), therapeutic vaccines (Gaggar et al. 2014; Martin et al. 2015), etc. GS-9620, a recently developed orally active TLR-7 agonist, was able to enhance IFN- α and ISG expression and activate NK cells, T cells, and B cells in animal models (Lanford et al. 2013; Steuer et al. 2015). Therapeutic cytokines can also be potentially used for immunotherapy (Dahmen et al. 2002; Guptan et al. 2002; Shih et al. 2016). Several studies evaluated the

antiviral activities and therapeutic potential of cytokines including IL-2, granulocyte-macrophage colony-stimulating factor (GMCSF), IL-7, etc. An open-labeled trial demonstrated that combination treatment with HBsAg vaccine plus LAM or IL-2 could induce antiviral immune response and viral elimination may be consequently obtained in CHB patients (Dahmen et al. 2002). A prospective study investigated whether the immunomodulatory effect of IFN could be enhanced by adding GMCSF, and the result showed that the combination therapy with GMCSF and IFN was effective in patients who had previously failed IFN monotherapy (Guptan et al. 2002).

Given that the elimination of cccDNA may still be challenging even with the promising antivirals targeting the virus, combination strategies involving immunotherapies aiming at boosting the host immune response may be necessary. The difficulties in breaking the immune tolerance and eliminating cccDNA constitute the main obstacles for a cure of HBV infection. It is conceivable that combination of potent DAA with immunotherapeutic approach is encouraging and may ultimately help overcome these difficulties.

Conclusions

The combination treatment of NUC and Peg-IFN is an alternative strategy to optimize treatment efficacy and improve the chance of clinical cure. Although it is not possible to determine at this stage which antivirals or which combinations will be most beneficial, it can be reasonably assumed that sequential combination therapy with NUC and IFN might achieve better treatment outcomes than either treatment given alone because NUC reduces the viral load and thus subsequently enhances the immune response to IFN (Yang and Kao 2015). Novel therapeutic approaches will be approved for clinical use in the near future, including compounds targeting various stages of HBV life cycle and immunomodulators. We can speculate that combination of these new strategies with current available antivirals will synergistically help enhance the host immune responses and eliminate cccDNA, ultimately leading to a complete cure of HBV infection. Several large prospective randomized trials (e.g., Endeavor and Anchor study) investigating the effectiveness of sequential combination treatment with NUC, IFN, and immunotherapeutic agents (IL-2, GMCSF and therapeutic vaccine) are currently carried out to verify this hypothesis.

Conflict of Interest The authors declare no conflicts of interest.

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Abstract

Of the 240 million persons infected with chronic hepatitis B worldwide, an estimated 15–20 million are coinfecting with HDV, 2–9 million are coinfecting with HCV, and 24–48 million coinfecting with HIV. Triple infections (i.e., HIV/HBV/HDV, HIV/HBV/HCV, or HBV/HCV/HDV) are less common and most frequently identified in high-intermediate HBV endemic regions, especially in high-risk individuals, i.e., persons who inject drugs (PWID) and men who have sex with men (MSM). The importance of recognizing HBV patients with HCV and HDV coinfections lies in the altered natural history and the higher risk of progressing to cirrhosis and/or developing HCC. Additionally, treatment of HBV in patients with coinfection is more complex, as treatment of HBV may have an influence on the viral activity of the other viral co-pathogen. As well as special considerations with therapy.

Keywords

Screening • Antivirals • Interferon • Transplantation

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1 Introduction

Due to common routes of transmission, persons with chronic hepatitis B virus (HBV) infection are at risk for coinfections with hepatitis D virus (HDV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). Of the 240 million persons infected with chronic hepatitis B worldwide, an estimated 15–20 million are coinfecting with HDV, 2–9 million are coinfecting with HCV, and 24–48 million coinfecting with HIV (Alter 2006; Coffin and Terrault 2008). Epidemiologic data on the frequency of triple infection (i.e., HIV/HBV/HDV, HIV/HBV/HCV, or HBV/HCV/HDV) are less abundant; however, triple infections are most frequently reported in high-intermediate endemic regions, especially in high-risk individuals, i.e., persons who inject drugs (PWID) and men who have sex with men (MSM) (Zhou et al. 2012; Bagheri Amiri et al. 2016; Hung et al. 2014). The importance of recognizing HBV patients with coinfections lies in the altered natural history and risk of cirrhosis and HCC, as well as special considerations with therapy.

2 HBV and HIV Coinfection

2.1 Epidemiology of HBV and HIV-1 Coinfection

There are approximately 33 million persons living with HIV worldwide. Among HIV-positive individuals, an estimated 4 million (10%–20%) of them are coinfecting with HBV (Hall, 2009 #2214; Alter, 2006 #1768) (Alter 2006; Coffin and Terrault 2008). In HIV monoinfected persons, the advent of highly active antiretroviral therapy (ART) has delayed progression of HIV type 1 (HIV-1)-related disease to complications of the acquired immunodeficiency syndrome (AIDS) and dramatically increased the life expectancy of patients (Mocroft et al. 2003; Thio 2009; Thio et al. 2002; Mallet et al. 2011; Palella et al. 1998, 2006). Thus, liver-related morbidity and mortality due to viral hepatitis are now the leading cause of death in HIV-positive individuals living in areas where antiretroviral therapy is available (Weber et al. 2006).

2.2 Virology of HBV and HIV-1

Both the HIV and the HBV reverse transcriptase lack proofreading function, and both viruses exist as a pool of distinct, albeit closely related variants or quasispecies (Bartholomeusz and Locarnini 2006; Norder et al. 2004). HIV-1 quasispecies show enormous diversity that increases with disease progression and are affected by viral fitness, availability of target cells, antivirals, and the immune response (Wolinsky et al. 1996). For HBV, viral diversity is constrained by the overlapping ORFs, which limit the fitness of mutants that are generated (Locarnini 2004, 2005; Locarnini et al. 2003). There are eight major HBV genotypes (A–H) with ~8% nucleotide

sequence divergence within the entire genome. HBV genotypes have been linked to disease progression, drug resistance, and response to antiviral therapy (Norder et al. 2004; Livingston et al. 2007; Chou et al. 2008; Osiowy and Giles 2003; Osiowy et al. 2008). HBV genotype A (HBV-A) or HBV-G is frequently reported in HIV coinfection, particularly in MSM. HBV-G has core gene changes preventing expression of HBeAg and almost exclusively occurs along with another genotype, usually HBV-A (Osiowy et al. 2008; Kato et al. 2002). Further, the organization of the HBV genome into overlapping ORFs has unique implications with respect to antiviral effects on its genome. Not only can long-term therapy targeting the HBV polymerase result in drug-resistant polymerase mutants, but due to the overlap with surface (S), such polymerase mutations can cause concomitant changes in the HBV S gene and vice versa. Studies of HIV/HBV-coinfected patients with anti-HBV therapy and clearance of HBsAg showed persistent HBV DNA (Sheldon et al. 2005, 2007; Sheldon and Soriano 2008), but a number had a premature S gene stop codon that impaired HBsAg secretion (Sheldon et al. 2005). Other studies of coinfecting patients on nucleos(t)ide analogue (NA) therapy, i.e., lamivudine (LMV) and tenofovir, found S gene mutants with reduced anti-HBs binding or a truncated HBsAg (Sheldon et al. 2007; Amini-Bavil-Olyaei et al. 2009). These HBV drug-resistant and vaccine escape mutants have potential public health implications (Delaney et al. 2003; Teo and Locarnini 2010).

2.3 Natural History of HBV and HIV Coinfection

HIV coinfection increases the risk of liver decompensation, cirrhosis, and hepatocellular carcinoma (HCC). Hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) seroconversion rates are reduced, and HBV DNA levels are higher (Bodsworth et al. 1991; Table 13.1). HBV viremia is associated with higher HIV RNA loads, lower CD4⁺ T helper (Th) cell counts, and lower alanine aminotransferase (ALT) levels (Thio 2009). The lower ALT levels indicate less immune-mediated hepatocyte damage, possibly due to depressed HBV-specific CD4⁺ T cell responses given that CD4⁺ T cells are preferentially infected with HIV-1 (Chang et al. 2005). As well, HIV antiviral treatment and immune restitution may worsen immune-mediated hepatitis (Lascar et al. 2005; Drake et al. 2004; Carr and Cooper 1997). The Multicenter AIDS Cohort Study reported a 19-fold increase in liver-related mortality, and HBV coinfection was an independent risk factor for HAART-related hepatotoxicity (Thio et al. 2002). Liver disease is second only to AIDS-related complications as a cause of death in HIV-infected patients (Falade-Nwulia and Thio 2011; Joshi et al. 2011). The Antiretroviral Therapy Cohort Collaboration examined specific causes of mortality in HIV-1-infected patients who initiated HAART from 1996 to 2006. Liver disease accounted for 113/792 or 7.0% of all deaths, of which 3.9% were viral hepatitis related and ~3.1% were “other liver related.” The rates of liver-related death were six times higher in PWIDs often due to drug dependency, alcohol abuse, or coinfection with HCV (Antiretroviral Therapy Cohort Collaboration 2010). Also, HIV infection and

Table 13.1 Impact of HIV coinfection on the natural history of HBV infection

	HIV negative	HIV positive
Acute infection	>95% of healthy adults spontaneously clear HBsAg	Six times more likely to progress to chronic infection More likely to lose protective HBV surface antibody (i.e., reactivation) especially if low CD4 ⁺ T cell counts
Chronic infection: spontaneous HBeAg loss/seroconversion	Varies depending on baseline clinical factors, from 0.8 to 15% per year	Lower spontaneous seroconversion rates, especially if low CD4 ⁺ T cell counts
Response to potent anti-HBV NA therapy	Sustained HBV DNA suppression	Up to 25% show incomplete viral suppression, slower decline (may be related to adherence or treatment interruption)
Cirrhosis/HCC	Variable history based on clinical factors (i.e., male sex, HBV DNA levels, family history) with 20–30% risk of cirrhosis, and 5% risk of HCC	Accelerated fibrosis progression, threefold higher mortality, especially in pre-HAART era, those with low CD4 ⁺ T cell count, or triple infection (HCV, HBV, and HIV)

alcohol abuse were strongly associated with fibrosis progression and severity of chronic hepatitis due to HCV and HBV (Antiretroviral Therapy Cohort Collaboration 2010; Klein et al. 2012).

Compared to HIV monoinfection, HIV/HBV coinfection is associated with lower CD4⁺ T cells, but the underlying mechanisms are unknown (Nikolopoulos et al. 2009). The depletion of CD4⁺ T cells could result in hepatic injury through induction of fibrogenesis or by enhancing the negative effect of opportunistic infections, alcohol abuse, or hepatotoxic drugs. Along with the CD4⁺ T cell receptor, two co-receptors, CCR5 and CXCR4, mediate HIV entry. Iser et al. showed that HIV enters human hepatic cell lines via CCR5 or CXCR4 and that HBV/HIV coinfection of hepatocytes increased intracellular HBsAg levels and accelerated liver disease (Iser et al. 2010). It may also be due to HIV-induced upregulation of TNF-related apoptosis-inducing ligand receptor 2 (TRAIL R2) leading to hepatocyte apoptosis (Babu et al. 2009). In both HBV and HBV/HIV coinfection, HBV-specific Th+ responses are reduced, suggesting that HIV-1 infection has a significant effect on HBV T cell immunity (Chang et al. 2005). Data from the Swiss HIV Cohort Study reported that HBsAg-positive patients had significantly impaired CD4 recovery during the first 3 years of HAART despite similar virologic effectiveness of HAART compared to HBsAg-negative patients [504 cells/ μ L (95%CI: 496–511) vs 449 cells/ μ L (95%CI: 428–469)] (Wandeler et al. 2013). Chun et al. examined the interactions of HBV and HIV using the composite endpoint of AIDS-defining illnesses and death among HIV-infected individuals and reported that the risk for an AIDS or death event was almost double (adjusted hazards ratio, 1.80; 95%CI: 1.20–2.69) for those with HBV coinfection (Chun et al. 2012).

2.4 Screening in Populations at Risk for HIV and HBV

According to the Centers for Disease Control and Prevention (CDC) and the Public Health Agency of Canada, HIV testing should be offered to individuals aged 13–64 (at least once), if symptoms or signs of HIV infection, illness associated with a weakened immune system, those with TB or another sexually transmitted disease, any one engaged in unprotected anal or vaginal intercourse or shared drug equipment with a partner known to be HIV positive, pregnancy, and victims of sexual assault (Centres for Disease Control and Prevention 2016). The World Health Organization has identified key populations and vulnerable groups that are disproportionately affected by HIV; thus, all countries should consider the importance of reaching those populations, i.e., men who have sex with men, people in prison or other closed settings, people who inject drugs, sex workers, and transgender people (WHO 2016).

Screening for past or active hepatitis B virus infection is recommended for all HIV individuals, using HBsAg, HBV surface antibody (anti-HBs), and HBV core antibody (anti-HBc). HBsAg-negative individuals, who lack protective anti-HBs, should receive the complete HBV vaccine series. HIV-positive patients may require a different HBV immunization schedule to achieve the same degree of protective immunity (Whitaker et al. 2012). In HIV-positive patients, four doses of double-strength HBV vaccine were found to be more effective in producing serological response without added side effects (Launay et al. 2011). Others have showed that a double dose of the vaccine was more effective in previous HBV nonresponders. Although data are limited, it is also recommended that HBsAg-negative/anti-HBc-positive individuals (with or without anti-HBs, indicating previous exposure and resolved HBV infection) undergo annual testing for HBsAg, given reports of reverse seroconversion in HIV-positive persons.

2.5 Baseline Clinical Evaluation and Indications for Antiviral Therapy (Fig. 13.1, Table 13.1)

Initial clinical evaluation should include assessment for factors that may modify the natural history of liver disease or the effectiveness of antiviral therapy including lifestyle habits (e.g., smoking and alcohol usage), injection drug use socioeconomic status including homelessness, aboriginal status, and immigration to North America; other non-liver comorbidities such as diabetes and cardiovascular disease; non-HIV-related malignancies and neurological conditions; and HIV-related factors including viral load and CD4⁺ T cell counts. All HIV-positive individuals should undergo screening for chronic liver disease, including evaluation of liver enzymes such as ALT, aspartate aminotransferase (AST), alkaline phosphatase, liver synthetic function tests (albumin, direct bilirubin, internationalized normalized ration or INR), and routine screening tests for other causes of chronic liver disease (i.e., hepatitis C, autoimmune hepatitis, and metabolic liver disease, as deemed appropriate). The complete blood

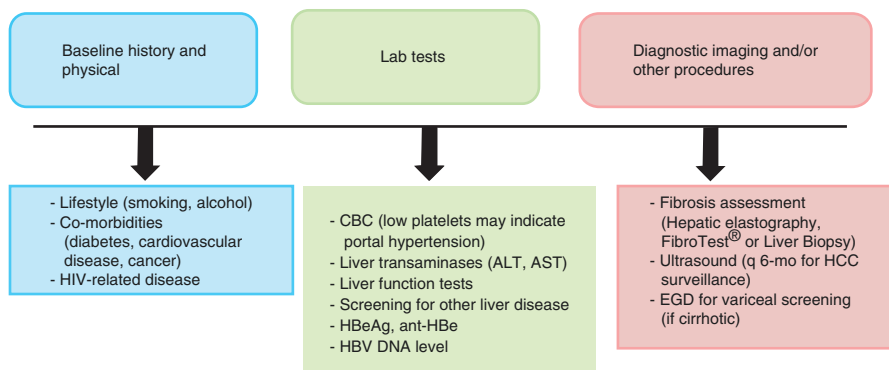


Fig. 13.1 Summary of recommended clinical evaluation and follow-up for hepatitis B activity and/or liver-related disease in HIV- and HBV-coinfected Individuals. All evaluations should be done in addition to usual HIV standard of care management

count, including platelet count, may be an indicator of portal hypertension due to cirrhosis; however, thrombocytopenia may also be due to HIV infection. Ultrasound of the liver should be used to evaluate for cirrhosis, splenomegaly, and portal hypertension. A liver biopsy or transient elastography (or assessment of liver stiffness) (e.g., FibroScan®) can be used to evaluate for the degree of fibrosis and cirrhosis. All cirrhotic patients should undergo upper endoscopy for screening for esophageal varices. Finally, all HBV/HIV-coinfected individuals should be enrolled into a surveillance program for hepatocellular carcinoma with liver ultrasound every 6 months.

2.6 Overview of Recommended Management for Hepatitis B and HIV Coinfection

Current guidelines recommend that all HIV-positive individuals initiate HAART regardless of CD4 count or HIV viral load. HBV-HIV infected patients require a drug regimen antivirals with dual anti-HBV/HIV activity (Table 13.2). Currently approved anti-HBV drugs include NA such as LMV, emtricitabine (FTC), adefovir (ADF), tenofovir disoproxil fumarate (TDF) and amlafenamide (TAF), ETV, or interferons (IFN, PEGylated-IFN)(Terrault, 2016). Although IFN and NA are approved as first-line therapies for management of chronic hepatitis B, IFN is rarely used in HIV-positive individuals due to adverse side effect profile and immunomodulatory mode of action. In resource-constrained settings, treatment for HBV is not readily accessible, and for HIV the only treatment available often includes LMV (Soriano et al. 2008; Wiersma et al. 2011). However, this treatment is suboptimal especially in HIV-coinfected persons on long-term LMV (without a second anti-HBV drug); the rate of LMV resistance (LMVr) HBV is ~90% leading to severe hepatitis and fatalities (Bruno et al. 2001; Benhamou et al. 1999; Ramos et al. 2007).

Table 13.2 Summary of treatment indications and anti-HBV therapy in HBV-monoinfected vs. HBV/HIV coinfectd, according to major expert guidelines

	HBV monoinfected	HBV/HIV coinfectd
Treatment indications	Active hepatitis (ALT > 2× upper limit of normal for >6 months) HBeAg negative: HBV DNA > 2000/mL HBeAg positive: HBV DNA > 20,000 IU/mL Advanced fibrosis (>stage 2) to prevent progression All persons with cirrhosis	All patients at initiation of antiretroviral therapy, include drugs with dual anti-HBV/HIV activity
Recommended first-line therapy	Tenofovir (TDF or TAF) Entecavir	Tenofovir (in combination with emtricitabine or lamivudine)
Treatment endpoints	Continue indefinitely in all with cirrhosis or HCC <i>Non-cirrhotic:</i> In HBeAg positive: consolidation period after HBeAg/anti-HBe seroconversion In HBeAg negative: indefinitely or consider cessation after undetectable HBV DNA/normal ALT × 3 years Hepatitis B surface antigen loss (New studies showing potential role of quantitative HBsAg levels, i.e., 2 log < IU/mL, may be okay to stop NA)	Anti-HBV treatment continued indefinitely, along with anti-HIV therapy If anti-HIV drug interruption needed, important to continue anti-HBV monotherapy to prevent HBV flares or reactivation

Definitions of Treatment response: *viral relapse*, HBV DNA > 2000 IU/ ml after stopping Rx in patients with virological response; *vlinical relapse*, viral relapse along with ALT> 2 x ULN or baseline ALT; *sustained response*, HBV DNA <200 IU/ml and ALT normal after stopping NA; *complete response*, sustained off-treatment virological response, together with loss of HBsAg

In contrast, the second-generation NAs for HBV (TDF/TAF and ETV) are safe and potent with high genetic barriers to resistance. ETV only has weak anti-HIV activity and can increase risk of HIV resistance; hence, it is not recommended in HBV/HIV coinfection, unless HIV is well controlled with alternative anti-HIV therapy. Thus, most coinfectd patients receive TDF/TAF-inclusive therapy due to its high antiviral potency and low genetic barrier to resistance and can be used in HAART due to dual anti-HBV and HIV mode of action. One study of 43 HBV/HIV-coinfectd patients reported a potential TDF-resistant mutant (rtA194T) in vitro in two patients, but ~50% had persistent HBV viremia despite adherence to TDF in association with LMVr (Sheldon et al. 2005). TDF/TAF plus either LMV or FTC (i.e., Truvada) is recommended in HAART.

TDF is reported to have rare but significant potential side effects such as renal toxicity and metabolic bone disease, especially in HIV- and HBV-coinfectd patients. Although the exact mechanism of TDF-related toxicity is unclear,

affected individuals present with decreased glomerular filtration rate (GFR) and proximal tubular dysfunction. In both HBV monoinfected and HBV/HIV coinfecting, a higher risk of renal dysfunction was associated with older age, low body weight, lower baseline estimated glomerular filtration rate (GFR), and other comorbidities (i.e., diabetes, hypertension, concomitant nephrotoxic medications). Additionally certain ABCC2 gene (encoding the outward tenofovir transporter MRP2) polymorphisms were associated with TDF-induced renal dysfunction. After discontinuation of the drug, renal function usually recovers (Rodriguez-Novoa et al. 2009).

TAF is a next-generation prodrug of tenofovir that is FDA approved for treatment of HIV and HBV. There are three approved fixed-dose TAF-containing combination (i.e., elvitegravir/cobicistat/emtricitabine/TAF (brand name: Genvoya), emtricitabine/rilpivirine/TAF (brand name: Odefsey), and (Zhou et al. 2012) emtricitabine/TAF (brand name: Descovy)). Compared with those receiving TDF, HIV-positive patients on TAF experienced significantly smaller changes in estimated creatinine clearance, renal tubular proteinuria, and bone mineral density (Sax et al. 2014).

2.7 Monitoring of Treatment Response in HBV and HIV Coinfection

In both HBeAg-positive and HBeAg-negative CHB, clinicians assess on treatment response to NA by monitoring serum HBV DNA (i.e., suppression of viral replication), HBeAg/anti-HBe seroconversion (in HBeAg-positive patients), normalization of serum liver transaminases, and improvement in liver stiffness or histology. Large randomized clinical trials have demonstrated that achievement of these surrogate clinical endpoints lead to reduced risk of liver disease progression, HCC development, and even fibrosis regression in HBV mono-infection (Marcellin et al. 2013). According to expert guidelines after achievement of surrogate endpoints (i.e., HBeAg seroconversion, HBV DNA suppression), stopping NA therapy in some HBV-mono-infected patients may be considered (Table 13.2). Although most of these studies excluded HBV/HIV-coinfecting individuals, improvement in these surrogate clinical endpoints is still achievable on long-term potent NA therapy with dual anti-HBV/HIV activity. Risk factors for incomplete HBV DNA suppression on antiviral therapy include high baseline HBV DNA as well as persistent HIV viremia (Hafkin et al. 2014). However, HBV therapy should be continued in HBV/HIV-coinfecting patients, and if any interruption in HIV therapy occurs, care providers should ensure alternative anti-HBV therapy is continued or patients undergo close monitoring for HBV flares.

The ultimate goal in HBV treatment is clearance of HBsAg and seroconversion to hepatitis B surface antibody (anti-HBs). Quantitative HBsAg (qHBsAg) titers have been suggested as a tool to predict HBsAg loss, as a reflection of intrahepatic levels of cccDNA. HBsAg levels have been proposed as a marker of treatment

efficacy when HBV DNA load becomes undetectable (Jaroszewicz et al. 2010; Sonneveld et al. 2011; Chan et al. 2011; Brunetto et al. 2010). Recent studies of qHBsAg levels in HBV/HIV coinfection documented that HIV-induced immunosuppression influenced qHBsAg kinetics and a slow decline occurred despite suppression of HBV replication (Thibault et al. 2011; Maylin et al. 2012; Jaroszewicz et al. 2012; Zoutendijk et al. 2012). Other studies have noted that the accuracy of qHBsAg may be affected by the presence of circulating S gene variants leading to diminished anti-HBs binding or decreased HBsAg secretion (Simon et al. 2012; Pollicino et al. 2012).

Although current antiviral therapy for HBV and HIV is effective and life prolonging, it is not curative and does not eradicate either viral infection. In persons with HBV/HIV coinfection, liver disease is the primary cause of death, and despite suppressive therapy many develop virological breakthrough and/or have low-level HBV viremia, mandating lifelong treatment. Additionally, patients who did achieve HBeAg seroconversion or HBsAg loss had significantly higher CD4⁺ T cell counts, indicating that host immunological factors and/or HBV/HIV viral interactions play an important role in HBV and HIV-1-related disease progression. Future studies are needed to enhance our understanding of CHB and HIV-related disease pathogenesis during coinfection, ultimately leading to the development of novel therapeutic strategies.

2.8 Transplantation for Complications of HBV/HIV Coinfection

HIV was historically considered a contraindication for liver transplantation (LT) due to concerns of immunosuppression and accelerated HIV disease, especially in the pre-HAART era. However, several large cohort studies have demonstrated acceptable posttransplant outcomes with no increased risk of HIV complications compared to HBV-monoinfected patients undergoing LT for HBV infection. Thus, all HBV/HIV-coinfected individuals with end-stage liver disease and/or HCC should be offered LT as a potentially lifesaving and curative therapy.

3 Hepatitis D Virus Infection

3.1 Epidemiology

The prevalence of HDV coinfection varies geographically and within populations. Countries with high rates of HDV coinfection include Taiwan, Mongolia, Italy, Spain, Turkey, Amazon Basin, and Central Africa, and clustered outbreaks have been described in other countries (Hughes et al. 2011). While HDV prevalence rates generally declined with introduction of HBV vaccination in the 1980s, studies suggest a plateau in more recent years and even increases among some HBV-infected populations, owing to immigration from areas of high endemicity, continued

transmission within high-risk groups and the survival benefits achieved with antiretroviral therapy in HIV-infected persons. For example, in Germany, the prevalence of HDV and the prevalence of anti-HDV antibody decreased from 18.6% in 1992 to 6.8% in 1997 but between 1998 and 2006 increased to between 8% and 14% (Wedemeyer et al. 2007), and the majority of cases were among immigrants from Turkey and Eastern Europe. In Taiwan, the prevalence of HDV coinfection increased from 29.4% in 2001–2004 to 62.5% in 2009–2012 among injection drug users and from 38% to 89.8% among HIV-positive injection drug users, contrasting with rates of 1.9% and 4.1% in the general population of HBsAg-positive individuals (Lin et al. 2015). Such studies serve as a reminder of the global impact of HDV infection.

3.2 Virology of HDV

HDV is a small defective RNA virus that is dependent on hepatitis B virus for completion of its life cycle. Classified under the *Deltavirus* genus, HDV is more closely related to plant viroids than to human viral pathogens. There are eight reported genotypes of HDV with unexplained variations in their geographical distribution and pathogenicity (Hughes et al. 2011). HDV utilizes the hepatitis B surface proteins in its lipid envelop and uses the same receptor for entry into hepatocytes as HBV. HDV binds to this receptor, the human sodium/taurocholate cotransporting polypeptide (NTCP), encoded by SLC10A1 (Lempp et al. 2016). Inhibitors of NTCP block both entry of HBV and HDV into hepatocytes and may represent a novel future approach to treatment of HDV (see below). Once internalized, the HDV genome enters the nucleus, and genome replication occurs through RNA intermediates. The HDV has a single-stranded circular RNA genome that encodes one protein, the HDAg, which exists in small (s-HDAg) and large forms (L-HDAg). This protein has both structural and regulatory functions and replicates using predominantly host proteins. Farnesylation of L-HDAg has the effect of negatively regulating HDV RNA replication and simultaneously promoting viral assembly at the level of the endoplasmic reticulum and release via the secretory pathway. Prenylation inhibitors are another novel therapeutic drug class currently being studied. Thus, an enhanced knowledge of the HDV life cycle has paved the way for new developments in HDV therapeutics.

3.3 Natural History HBV and HDV Coinfection

Acute hepatitis delta may occur in the setting of simultaneous HBV/HDV infection (coinfection) or as superinfection in person with chronic hepatitis B (superinfection). Coinfection occurs much less frequently than superinfection but is associated with a higher risk for fulminant liver failure (Smedile et al. 1982). Spontaneous resolution of HDV is also more common with coinfection than with superinfection.

For the majority of patients with superinfection, chronic HDV will develop with a variable course of viremia. Cross-sectional studies report that HDV typically predominates—with more patients being HBeAg negative and with low or undetectable levels of HBV DNA compared to HBV-monoinfected patients (Mumtaz et al. 2011). Indeed, the typical clinical scenario in which HDV testing has been typically recommended is when ALT levels are elevated (suggesting active liver disease) and HBV DNA levels are low or undetectable. However, longitudinal studies reveal a more complex and dynamic picture. In a study of 25 HDV-infected patients followed with quantitative levels of HBV DNA, HDV RNA, and HBsAg over a period of 4–8 years (Schaper et al. 2010), seven different replication profiles were seen, with fluctuating activity of one or both viruses, including alternating periods of viral predominance. This study highlights the need for repeat evaluation of viral levels over time to guide decisions regarding therapy.

Most studies report more rapid progression to cirrhosis and/or rates of cirrhosis and HCC among patients with HDV infection compared to those with HBV alone. In the Swiss HIV Cohort study, HBV/HIV-infected patients with HDV (triply infected), had a higher risk of liver-related death (HR = 7.71, 95% CI: 3.1–19.0) and HCC (HR 9.3; 95% CI: 3.0–28.6), compared to HBV/HIV patients without HDV (Beguelin et al. 2016). Cross-sectional studies of HDV RNA levels and liver necro-inflammatory activity and fibrosis do not show any clear association (Zachou et al. 2010). Some studies found HDV RNA levels to be associated with risk of progression to cirrhosis and HCC, but pathogenesis of accelerated liver disease development due to HDV coinfection remains unclear (Romeo et al. 2014). Collectively, findings indicate HDV is not directly cytopathic and that immunologic, genetic, or other factors are responsible. HDV was most prevalent among HBV/HIV patients with history of injection drug use and HCV coinfection.

3.4 Diagnosis and Screening

The European Association for the Study of Liver Diseases and the Asian Pacific Association for the Study of Liver Disease recommend routinely screening for HDV infection in all HBsAg-positive persons (European Association for the Study of the Liver 2012; Sarin et al. 2016). The American Association for the Study of Liver Diseases recommends testing in select patient groups (Terrault et al. 2016), including PWIDs, HIV infected and men who have sex with men (MSMs), immigrants from areas of high-HDV endemicity, as well as HBsAg-positive patients with low or undetectable HBV DNA levels but high ALT levels. The rate of HDV screening in clinical practice appears to be highly variable (Kushner et al. 2015; El Bouzidi et al. 2015), and given the importance of the diagnosis of HDV to the long-term management of HBV, universal screening of HBsAg-positive patients would seem appropriate in most clinical settings.

The recommended screening test for hepatitis delta infection is the antibody to the delta antigen (anti-HDV). In the appropriate setting (i.e., acute hepatitis), testing

for anti-HBc IgM will assist with differentiating coinfection from superinfection, the latter confirmed with positive anti-HDV with negative anti-HBc IgM. For anti-HDV-positive patients, HDV RNA testing is needed to determine whether the antibody reflects active HDV infection. Two major challenges lie in reliance on HDV RNA testing as marker of active HDV infection. First, the assays are not standardized resulting in variability in detection rates (Le Gal et al. 2016). The World Health Organization (WHO) has established a HDV international standard (WHO-HDV-IS), which is the first international external quality control for HDV viral load quantification. A recent study evaluating the sensitivity of 28 clinical labs in 17 countries worldwide highlighted the high heterogeneity in results, with 16 laboratories (57.1%) unable to confirm HDV RNA positivity in 1 up to 10 of the 20 clinical samples tested.

Lack of accuracy in detecting and quantifying HDV RNA has been attributed to the high genetic variability of HDV and, possibly, its complex secondary structure. Second, as highlighted by longitudinal studies, the levels of HDV RNA may fluctuate over time, so serial testing is essential to establish whether HDV infection contributes to clinical outcomes or warrants consideration of treatment. Finally, access to HDV RNA testing may be limited in some geographical areas of the world, so alternative means of defining patients with active HDV infection and/or those in need of treatment are needed. Anti-HDV IgM has a poor correlation with HDV RNA levels but appears to correlate with histologic inflammatory activity and ALT in one study (Wranke et al. 2014). HDVAg can be measured but is present transiently in serum; hence, it is not a useful marker of active infection.

3.5 Indications for Antiviral Therapy

As with any patient with chronic hepatitis B, the patient coinfecting with delta virus requires repeat testing of ALT levels and viral activity over time to determine the need for antiviral therapy. For patients with elevated ALT levels, measurement of HBV DNA and HDV RNA will allow determination of the need for NA alone, IFN, or combination therapy (Fig. 13.2). Treatment decisions should be directed by the virologic results. For patients who do meet criteria for treatment and have normal liver enzymes, monitoring ALT activity every 6 months and repeated HBV DNA and HDV RNA levels if ALT levels increase would be a reasonable and low-cost approach. The presence of underlying cirrhosis may further modify treatment decisions, as is the case in HBV monotherapy. For patients with cirrhosis, treatment of HBV when HBV DNA levels are lower than the usual treatment threshold (<2000 IU/mL) and anti-HBV treatment should be started to minimize any contribution of active HBV infection to HDV progression (Terrault et al. 2016). Since many clinicians lack access to quantitative HDV RNA levels, the decisions to treatment HDV infection are typically based on a positive qualitative HDV RNA result.

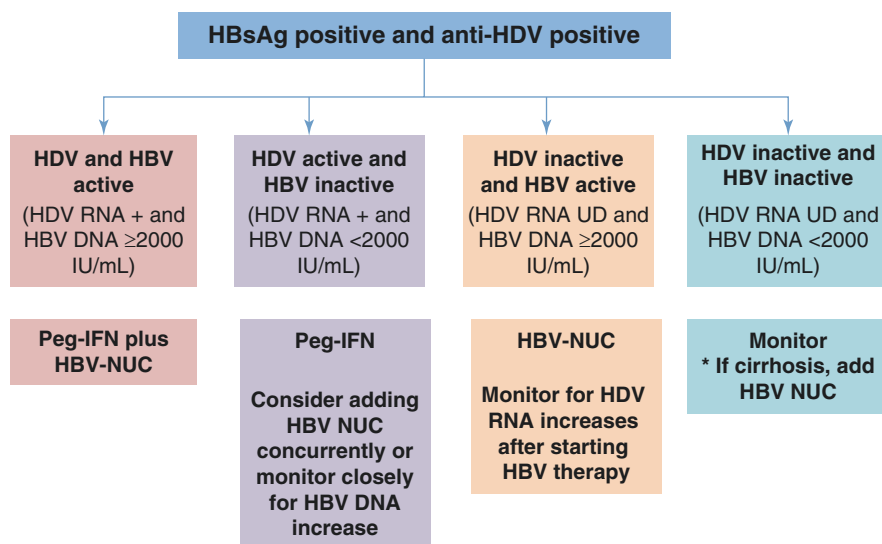


Fig. 13.2 Suggested treatment approach for chronic HBV (HBsAg positive)- and HDV-coinfected patients. Shown are the possible serologic/virologic profiles among coinfecting patients and below each the suggested treatment approach. *NUC* nucleos(t)ide analogue, *UD* undetectable

3.6 Overview of Approved Antiviral Therapy for Hepatitis D Infection

Interferon (IFN) is the only approved antiviral therapy shown to have any efficacy in HDV infection. Treatment success in clinical studies is typically defined by an undetectable HDV RNA level 24 weeks after completion of therapy. This is an arbitrary time point, since this does not reflect the long-term absence of HDV RNA. Late relapses occur leading to very low rates of HDV RNA undetectability with off-treatment follow-up period beyond 5 years. In the multicenter HIDIT-1 study of peginterferon (Peg-IFN) for 48 weeks with or without adefovir, 40% of patients achieved an undetectable HDV RNA level 24 weeks after completing therapy, but at a mean follow-up 4.3 years later, only 12% were still undetectable (Heidrich et al. 2014). Thus, a complete virologic response, defined by loss of HBsAg plus sustained suppression of HDV RNA, is the better endpoint of therapy but occurs rarely with treatment periods of 1 year. In one study of treatment of Peg-IFN for up to 5 years, HBsAg loss occurred in 3 of 13 (23%) patients after 24, 37, and 202 weeks of treatment (Heller et al. 2014).

Initial studies of standard interferon used doses ranging from 3 to 18 MU thrice weekly. Rates of response, defined by undetectable HDV RNA levels, were only ~20% with similar rates of biochemical (ALT normalization) (Abbas et al. 2011). Currently, Peg-IFN is the drug of choice without apparent differences in efficacy between Peg-IFN alpha-2a (180 ug weekly) and alpha-2b

(1.5 µg/kg weekly) (Bahcecioglu et al. 2015; Castelnau et al. 2006; Gheorghe et al. 2011; Wedemeyer et al. 2011; Niro et al. 2006; Erhardt et al. 2006). The reported virologic responses (HDV RNA undetectable) 6 months after completing 1 year of treatment range from 17% to 43% (Castelnau et al. 2006; Erhardt et al. 2006). Normalization of ALT levels parallel the virologic responses. The combination of NA with IFN does not increase the likelihood of an off-treatment virologic response (Wedemeyer et al. 2011; Abbas et al. 2016; Yurdaydin et al. 2008). Limited data on treatment of HIV infected patients with delta hepatitis suggest tolerability and efficacy are similar to HIV-uninfected patients (Puoti et al. 1998). Identifying patients likely to achieve a sustained virologic response (defined by undetectable HDV RNA 24 weeks after treatment end) would be desirable, and several studies suggest that those with HDV RNA undetectable at 24 weeks of treatment have a high chance of achieving this endpoint (Castelnau et al. 2006; Abbas et al. 2014; Keskin et al. 2015). In contrast, if the decline in HDV RNA is less than 2-log copies/mL after 24 weeks, only 5% achieve a sustained off-treatment response (Keskin et al. 2015). These prediction rules need to be validated in large numbers of patients, across different HDV genotypes and using standardized HDV RNA assays.

IFN therapy has been associated with reduced rates of liver complications, including death. In a single center study of 136 patients (39% with cirrhosis at entry), the rate of liver-related complications (decompensation, HCC, liver transplantation, or death) over a median follow-up of 5.2 years was fourfold higher in untreated and 2.2-fold higher in NA-treated HDV/HBV patients compared to IFN-treated patients (Wranke et al. 2017). The benefits of IFN on disease progression and clinical outcomes have been most closely associated with undetectability of HDV RNA during follow-up (Wranke et al. 2017).

Nucleos(t)ide analogues have no efficacy against HDV infection and are not recommended in patients with suppressed or low HBV replication, except patients with cirrhosis (Terrault et al. 2016). However, as the HBV DNA levels may change over time, including during treatment of HDV infection, NA should be used if HBV DNA levels are elevated. Regular monitoring of HBV DNA levels and HDV RNA levels, with an individualized approach to treatment of the one or both viruses, is necessary to minimize liver disease progression. Long-term suppression of active HBV infection may be expected to reduce quantitative HBsAg levels, in turn, having a beneficial effect on HDV coinfection (Sheldon et al. 2008).

The duration of therapy for HDV is typically at least 1 year, although longer duration has been used. Treatment of HBV should follow treatment guidelines. Ultimately the achievement of HBsAg loss and acquisition of anti-HBs would be the best marker of successful treatment and allow discontinuation of therapy. However, the latter is very infrequently achieved, so the approach of HDV treatment with interferon for 1–2 years initially and then stopping and reassessing need for retreatment might be a strategy that balances the patient's quality of life and need to prevent liver disease progression.

3.7 Novel Therapeutic Approaches Under Study

There are several new therapies on the horizon. Prenylation of the HDV is an essential step in the envelopment and release of the virus, and, in a proof-of-concept study, the small molecule prenylation inhibitor, lonafarnib, was evaluated for 28 days in 14 patients with HDV infection. After 28 days, the mean log HDV RNA declined from baseline by -0.73 log IU/mL and -1.54 log IU/mL in lonafarnib 100 and 200 mg twice daily groups, respectively, compared to -0.13 log IU/mL in placebo-treated patients (Koh et al. 2015). As the prenylation inhibitor targets a host rather than a viral enzyme, as expected, no virological resistance was seen. The most common side effects were gastrointestinal (diarrhea, nausea, and bloating). Additional studies of this novel drug therapy are now underway, including studies of ritonavir-boosted lonafarnib with and without peginterferon ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02430194): NCT02430194).

Another novel target is the entry inhibitor, myrcludex B[®], which is a synthetic peptide of the HBV pre-surface (S1) protein that specifically binds to the hepatocyte NCTP receptor. Myrcludex B[®] competitively binds to NCTP and blocks HBV and HDV entry. In a pilot study, HDV RNA and HBsAg levels were evaluated in 24 HDV-infected patients randomized to myrcludex B[®], 2 mg subcutaneously daily, myrcludex B[®], and Peg-IFN or Peg-IFN alone (Bogomolov et al. 2016). HBsAg did not change significantly in any group, but a decline in HDV RNA levels was evident in all groups with HDV RNA becoming negative in 2/7 patients each in the myrcludex B[®] and Peg-IFN α -2a cohorts and in 5/7 patients of the myrcludex B[®] + Peg-IFN α -2a cohort. Synergistic antiviral effects on HDV RNA and HBV DNA were shown in the combination arm. Treatment was generally well tolerated, and the only side effects attributed to myrcludex B[®] were slight and transient cytopenias. Additional dose-ranging studies are planned.

Finally, yet another novel therapeutic approach being evaluated is the effect of highly negatively charged nucleic acid polymers that interfere with the attachment of HBV/HDV to the viral co-receptors on the surface of hepatocytes, heparin sulfate proteoglycans, which may block viral entry and the formation of intracellular subviral particles. Preliminary data show that these nucleic acid polymers can clear HBsAg from the serum and the nucleic acid polymer, REP 2139-Ca given intravenously once a week, is being studied in combination with Peg-IFN in patients with chronic HDV infection to treatment of HDV infection ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02233075): NCT02233075).

While in early phase of study, these new therapies offer optimism for the millions affected by HDV/HBV coinfection, for which effective and well-tolerated therapies have been lacking.

3.8 Transplantation for Complications of HDV Coinfection

Historically, prior to the use of prophylactic therapies, HDV patients were shown to have a better prognosis after liver transplantation (LT) than patients with HBV infection alone (Samuel et al. 1993). This was presumably due to the lower HBV

DNA levels present pre-LT in patients with HDV coinfection. However, recurrent HDV infection leading to graft loss did occur, and prophylaxis for HBV infection with HBIG alone and later combined with NA prevented HBV and HDV infections. Given the importance of prevention HBsAg reappearance post-LT, patients with HDV infection are recommended to remain on long-term HBIG as part of their prophylaxis (Fox and Terrault 2011).

4 Hepatitis C and B Virus Coinfection

4.1 Epidemiology

The global prevalence of HCV coinfection among HBsAg-positive persons is largely unknown. Countries endemic for hepatitis B are predicted to have highest numbers of affected individuals. Specific studies from Spain, Italy, Taiwan, and Japan report anti-HCV positivity rates of 7–15% among HBV carriers (Konstantinou and Deutsch 2015). Even within countries, specific risk groups with chronic HBV will be at higher risk of HCV coinfection than others, notably PWIDs, those on hemodialysis, hemophiliacs, and other groups who received multiple blood transfusions and HIV-infected persons. Based upon the global anti-HCV positivity rate of 1–4% in the general population, it is estimated that 2.4–9.6 million of HBV-infected persons are potentially HCV coinfecting (Liu and Chen 2014).

4.2 Natural History HBV and HCV Coinfection

Acute coinfection of HBV and HCV is rare but described among PWIDs. Spontaneous clearance of both, one and neither virus is possible, and biphasic hepatitis has been observed. Acute HCV superinfection in patients with chronic hepatitis B frequently presents with icteric hepatitis and can be severe with one study finding that 34% of patients develop decompensation, 11% acute on chronic hepatitis leading to liver failure, and 10% died (Liaw et al. 2004). Historically, HBV/HCV coinfecting patients also had a higher incidence of cirrhosis (48% at 10 years) and HCC (14% at 10 years, 21% at 15 years, and 32% at 20 years) than patients with chronic active hepatitis B without HCV coinfection. Interestingly, these patients were also found to achieve earlier loss of HBsAg. There is less information on the outcome of HBV when acutely acquired in a patient with chronic hepatitis C, and overall this scenario appears less frequent. Acute liver failure has been described in a patient with chronic HBV acutely infected with HCV (Liaw et al. 2000).

Among those with chronic HBV/HCV coinfection, different profiles may be seen, reflecting the predominance of HBV, HCV, or both. Patients who have both HBV DNA and HCV RNA detectable in blood appear to be at highest risk of progression to cirrhosis and liver decompensation. Patients with active HCV infection (HCV RNA+) in the setting of inactive HBsAg (HBsAg+/HBV DNA-) seem to behave similarly to patients with HCV mono-infection, at least until HCV

clearance is achieved. Finally, HBsAg-positive patients who have antibody to HCV but have undetectable HCV RNA, indicating prior HCV exposure and spontaneously HCV clearance, have a natural history similar to patients with chronic HBV alone. Several cross-sectional studies have reported an inverse relationship in the viral levels when coinfection is present, suggesting direct or indirect viral interference (Alberti et al. 1995; Liaw 1995); however, longitudinal studies in coinfecting patients show more variable patterns, with viral levels seeming to be independent of each other (Raimondo et al. 2006; Nguyen et al. 2011). That being said, studies of HCV treatment in HBV/HCV-coinfecting patients have described reappearance of HBV DNA in patients achieving HCV clearance, lending support to the concept of viral interference (Potthoff et al. 2008). Likewise, HBV reactivation has been reported in some HCV/HBV-coinfecting patients after successful treatment of HCV. The latter has led to a recommendation to test all HCV patients with markers of HBV infection (HBsAg, anti-HBc, and anti-HBs) (AASLD/IDSA/IAS–USA 2017).

Consistently, studies have shown that patients with dual HBV and HCV infection are at higher risk of progressive disease and have higher rates of cirrhosis and HCC than patients with hepatitis B or hepatitis C alone (Zampino et al. 2015; Benvegnu et al. 1994; Yang et al. 2016). A meta-analysis of 22 studies of patients with HBV/HCV coinfection demonstrated an additive risk of HCC but with more recent studies showing a subadditive effect of the copathogens compared to older (prior 2000) studies (Cho et al. 2011).

4.3 Indications for Antiviral Therapy

As with any patient with chronic HBV infection, the treatment goals are to reduce liver injury and progression to cirrhosis and HCC and, when possible, eliminate infection. In the case of HBV/HCV coinfecting patients, eradication of HCV should be possible in most patients with currently available direct antiviral drugs. Indeed, HBV/HCV-coinfecting patients are considered a high-priority group for HCV treatment (Sarin et al. 2016; AASLD/IDSA/IAS–USA 2017). Elimination of the risk of progression from HBV requires sustained suppression of HBV DNA to low levels (<2000 IU/mL) and, ultimately, achievement of HBsAg loss. Achievement of sustained suppression of HBV DNA is possible with current HBV therapies, but HBsAg loss is a very infrequent event. Thus, the interim endpoints of treatment of HBV/HCV coinfecting patients are elimination of HCV (defined by undetectable HCV RNA at least 12 weeks after completing antiviral therapy), low to undetectable levels of HBV DNA, and normalization of ALT levels.

The viral activity responsible for liver disease is assessed by determining the viral loads of HCV and HBV. If active viral replication is detected for one virus but not the other, treatment should focus on the active virus and utilize the guidelines for treatment of that specific virus. If both viruses are shown to be active, then treatment should be directed against both pathogens (Fig. 13.3). Importantly, treatment of one virus may lead to changes in the activity of the other virus, and thus monitoring during and after treatment is necessary to assess for viral activity.

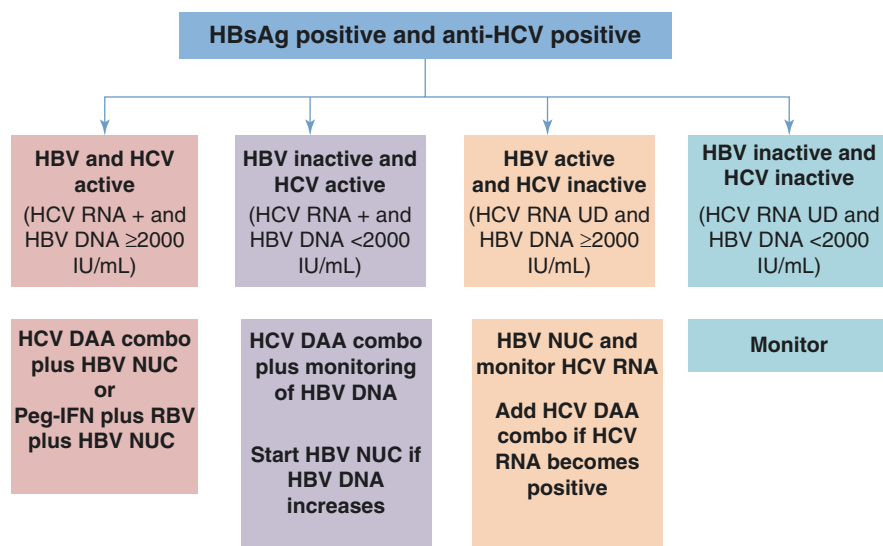


Fig. 13.3 Suggested treatment approach for chronic HBV (HBsAg positive)- and HCV-coinfected patients. Shown are the possible serologic/virologic profiles among coinfecting patients and below each the suggested treatment approach. *DAA* direct acting antiviral drugs, *NUC* nucleos(t)ide analogue, *UD* undetectable

4.4 Approach to Antiviral Therapy for HBV/HCV Coinfection

Antiviral therapy should be tailored to the virus that is “active.” Based on published data, the most common clinical scenario is active HCV with inactive HBV infections, although other combinations can occur (Fig. 13.3). In the IFN era, the treatment of choice for patients with HCV RNA detectable (with or without active HBV) was Peg-IFN and ribavirin (RBV) for 24–48 weeks, depending on HCV genotype. Moderate to high rates of HCV eradication and HBV suppression were reported with this combination (Potthoff et al. 2008; Kim et al. 2011; Liu et al. 2009; Uyanikoglu et al. 2013). In the largest study conducted, 161 HBV/HCV-coinfected Taiwanese patients (HCV genotypes 1, 2, or 3) were treated with Peg-IFN alfa-2a (180 μ g) weekly for 24 (genotype 2 and 3) or 48 weeks (genotype 1) and ribavirin and compared to 160 with HCV-monoinfected controls (Liu et al. 2009). The SVR rate for genotype 1 patients was 72% in dually infected patients compared with 77% in monoinfected patients and 83% and 84 for genotypes 2/3 patients. Serum HBV DNA did not correlate with achievement of SVR for HCV. Pretreatment, HBV DNA was undetectable in 53%, and among these patients, reappearance of HBV DNA occurred in 36% at the end of follow-up (Liu et al. 2009). Of the 47% with detectable HBV DNA pretreatment, 56% has undetectable HBV DNA at last follow-up. Moreover, posttreatment HBsAg clearance was seen in 11% of dually infected patients. This study highlights the importance of monitoring each virus during treatment and points to the potentially important benefit of HBsAg loss with use of Peg-IFN therapy in these coinfecting patients.

In the era of HCV direct acting antivirals (DAA), IFN-based therapy has a rapidly diminishing role in the treatment of HBV/HCV dually infected patients. Though no clinical trials of DAA therapy have been done in HCV/HBV-coinfected patients, the high patient and provider acceptability coupled with high efficacy of DAAs in HCV-monoinfected patients makes DAA therapy the first choice if HCV infection is active. Based on case reports of increasing HBV DNA levels in coinfecting patients treated with DAAs, monitoring of HBV DNA levels during treatment is recommended, with the addition of ETV, TDF or TAF if HBV DNA levels are rising. In resource-constrained areas, lacking access to DAA combinations for all HCV genotypes, Peg-IFN with ribavirin may still be needed.

Drug-drug interactions need always to be considered. Based on current knowledge, there is no *a priori* expectation of interactions between TDF, TAF or ETV and approved HCV DAAs. For triply infected patients with HIV/HBV and HCV, more opportunities for drug interactions exist, and careful review of antiretroviral therapy pre-initiation of HCV or HBV therapy is recommended.

4.5 Summary

All HBsAg-positive persons should be tested for HCV, HDV, and HIV so that these important coinfections are not missed. Coinfecting persons typically have a more accelerated natural history and higher risk of cirrhosis and HCC than HBV-monoinfected patients, and monitoring for these complications is critical to preventing liver-related deaths. Treatment paradigms are more complex in coinfecting patients with viral activity varying over time and in response to antiviral therapy. Long-term monitoring of viral activity is useful in guiding the timing of interventions.

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Management of Chronic Hepatitis B Virus Infection in Children and Pregnant Women

14

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Abstract

Chronic hepatitis B virus (HBV) infection is a major global health burden. Despite successful immunoprophylaxis program and advancement in antiviral therapies in the past decades, there are still an enormous load of afflicted people, mostly adults born before HBV universal vaccination program and children with hepatitis B immunization failure. Infected children, like adults, may experience hepatitis activity and chronic consequences without awareness and thus require screening, followed by close monitoring and proper treatment. Pregnant women with HBV infection are a special group that may need screening to identify as

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well as monitor disease activity and viral loads, not only during pregnancy but also postpartum, to implement proper management for either maternal or fetal health and/or for prevention of mother-to-infant transmission of HBV. Although there are approved antiviral therapies for adults with chronic hepatitis B, the best practice and long-term impacts in children, and pregnant women/offspring are still evolving. To eradicate global HBV endemics, preventive vaccination is the frontline program; screening, monitoring, antiviral therapy, and surveillance of complications, such as cirrhosis and hepatocellular carcinoma, are the backup strategies which should include children and pregnant women as a whole.

Keywords

Hepatitis B vaccines • vertical transmission • mother-to-infant transmission • nucleos(t)ide analogues • pegylated interferons

1 Introduction

Chronic hepatitis B virus (HBV) infection affects more than 240 million people around the world and may cause hepatic necroinflammation, fibrosis, and advancement into cirrhosis, end-stage liver failure, hepatocellular carcinoma, and death in 15–40% of infected patients (Hepatitis 2015; Lok 2002). Most of these infections developed before the launch of hepatitis B vaccination. Since the implementation of the world's first universal infant vaccination program in Taiwan in 1984, universal hepatitis B vaccination has been adopted in 184 countries with three-dose coverage estimated at 82% among one-year-olds (Immunization surveillance, assessment and monitoring 2015; Immunization coverage 2016) and has achieved 62–100% effectiveness in prevention from hepatitis B surface antigen (HBsAg) carriage, especially in hyperendemic areas like Africa and Asia-Pacific regions (Harpaz et al. 2000; Mendy et al. 2013; Chen 2009; Ni et al. 2016). However, around 10% infants of hepatitis B e antigen (HBeAg) -positive or high viral load carrier mothers still get infection despite passive-active immunoprophylaxis in infancy (Chen et al. 2012; Wen et al. 2013).

Through the advancement of antiviral therapy (AVT) in the past three decades and screening campaigns promoted by liver associations/health organizations, a better management of chronic HBV-infected patients can be applied toward a goal to reduce detrimental outcomes. Major international liver associations have established guidelines to the management of chronic HBV infection and updated the recommendations according to new evidence. These guidelines are mostly based on prospective studies or randomized clinical trials aiming at adult populations, the major disease-ridden groups. Although HBV-infected children are usually experiencing an immunotolerant course with mild inflammation but very high viral replication, a small proportion indeed develops liver cancer even in the vaccination era ($0.27\text{--}0.4/10^5$ person-year, 6–19 years of age, vaccinated birth cohort) (Chang et al. 2016). However, therapeutic agents, indications, duration, resistance, end points,

etc., in the pediatric ages are restricted due to limitation of approved antivirals and long-term follow-up. Due to vulnerability and small numbers of pediatric patients, clinical trials of antivirals which have been already licensed for adults usually take longer time in the process of board reviewing and participant recruitment. Pediatric hepatologists often need to treat patients with hepatitis activity according to experiences extrapolated from adults' series or experts' opinion.

Another special group is pregnant women with hepatitis B carriage, who may experience active hepatitis and need treatment or be asymptomatic but transmit HBV to their babies; the latter would contribute to the HBV reservoir across generations. The proper management of these two special groups, children, and pregnant women, constitutes essential jigsaws in the global elimination of hepatitis B virus burden.

2 Management of Children with Chronic Hepatitis B

2.1 Identifying Infected Children

2.1.1 Postvaccination Serologic Testing in High-Risk Infants

Identification of the infected persons is the first step to monitor or treat them. For children born after the launch of universal hepatitis B vaccination, the most important risk factors for breakthrough infection are maternal HBsAg carriage and incomplete vaccination. Currently, there are four main strategies of hepatitis B vaccination:

1. Universal maternal screening of HBsAg→universal neonatal vaccine with additional hepatitis B immunoglobulin (HBIG) in infants of carrier mothers (USA, Italy, Korea, etc.)
2. Universal maternal screening of HBsAg and HBeAg→universal neonatal vaccine and additional HBIG in infants of HBsAg-positive/HBeAg-positive mothers (Taiwan, Singapore)
3. No maternal screening→universal neonatal vaccine (most middle-income and developing countries)
4. Universal maternal screening of HBsAg→selective neonatal active and passive immunization only to infants of carrier mothers (UK, Denmark, etc.) (Chen et al. 2012; Centre for Disease Prevention and Control 2010)

Not only would different strategies affect infant immunization policy but also surveillance target on children at risk of infection. Postvaccination serologic testing of HBsAg/anti-HBs in infants of carrier mothers with/without HBeAg at 9–12 months of age or 1–2 months after final dose is recommended to identify infected infants and has been adopted by many countries (USA, UK, Taiwan, etc.) that include maternal screening as part of universal neonatal immunization program (Schillie et al. 2015a; O'Flanagan et al. 2013). This step is critical in understanding the global chronic HBV infection burden in next generations and whether the elimination of HBV is achieved through universal immunization in the long run.

2.1.2 Screening Immunocompromised Patients

Another risk group that may potentially acquire hepatitis B infection even after vaccination is immunocompromised patients (human immunodeficiency virus (HIV)-infected, hemodialysis patients, under chemotherapy, recipients of organ transplant or stem cell transplant). Vaccine-induced immune memory may last for more than 20–30 years and a booster dose is not recommended in immune-competent individuals with waning anti-HBs below 10 mIU/mL (West and Calandra 1996; Leuridan and Van Damme 2011; Bruce et al. 2016). In immunocompromised pediatric patients, whose immune response is impaired, monitoring for HBV infection markers [HBsAg, antibody to core antigen (anti-HBc), and/or HBV DNA] and vaccine booster during combination antiretroviral therapy (for HIV), before initiation of immunosuppressant/chemotherapy, or pretransplantation is recommended if anti-HBs decline below 10 mIU/mL. In hematopoietic stem cell transplant or organ transplant, seromarkers of the donors should also be checked, especially in high endemic areas (Hui et al. 2005). In liver transplantation from anti-HBc-positive donors, anti-HBs should be boosted to >200 mIU/mL (or to >1000mIU/mL if possible) to prevent de novo hepatitis B (Su et al. 2009; Lin et al. 2015). Close monitoring liver function profiles and hepatitis B markers in potential occult HBV-infected patients (e.g., HBsAg-negative, anti-HBc-positive, or high titer anti-HBs without previous vaccine booster (Shahmoradi et al. 2012; Liu et al. 2006a; Hsu et al. 2014)) are mandatory to apply preemptive or therapeutic antivirals (Hui et al. 2006; Uhm et al. 2007).

2.1.3 Other Risk Groups

Household contacts or sexual partners of HBsAg carriers, injection drug users, or men who have sex with men are usually protected by complete primary vaccination if immunity is not impaired (Leuridan and Van Damme 2011).

2.2 Antiviral Therapy in Children with Chronic Hepatitis B Virus Infection

2.2.1 Natural History of CHB Infection and Its Clinical Implication in Children

Most children were infected by HBV at the neonatal period (mother-to-infant transmission, the main route of transmission in Asia) or before 6 years of age (horizontal infection in Africa) and have a 90% and ~30% probabilities of chronicity, respectively (Hyams 1995). Along the long-term natural history of chronic infection, most children (<18 years old as defined by the United Nations) are in the immunotolerance phase, characterized by normal aminotransferases, minimal liver histology, HBeAg seropositivity, and high HBV DNA levels. Mechanisms of immunotolerance have been elucidated from clinical and experimental studies. Maternal transplacental transfer of HBeAg may induce in utero Th-cell tolerance and guarantee persistent perinatal infection (Milich et al. 1990; Hsu et al. 1992a), while secreted HBeAg in chronic stage may function as an age-dependent immunoregulator,

tolerogenic or anti-inflammatory, in neonates and children (Milich and Liang 2003). Defective viral antigen processing and transport to major histocompatibility complex class I molecules due to low expression of transporter associated with antigen processing (TAP) 1 and low molecular weight protein (LMP) 2 may play an important role in immune tolerance phase of CHB patients (Sukriti et al. 2010).

After a variable period (generally lasting 10–30 years) of immunotolerance, the breakpoint commences, and immune clearance begins with an elevation of aminotransferases, HBV DNA fluctuation, and necroinflammation activity in liver histology. If the immune activity is appropriate, alanine aminotransferase (ALT) returns to normal, HBV DNA level decreases, and HBeAg loses with the development of anti-HBe (i.e., e seroconversion), which indicates a transition into inactive carrier or low replicative phase.

Several host and viral factors affect the initiation of immune clearance: HLA class I and class II types, cytokine IL-10 and IL-12 polymorphism and phenotype, toll-like receptors 4 and 5 polymorphism, early onset of puberty or menarche, and genotype B and A (versus genotype C and D) are correlated with earlier or higher rate/sustenance of HBeAg seroconversion (Wu et al. 2006, 2010a, b, 2012a, 2014; Kao et al. 2004; Sánchez-Tapias et al. 2002).

Spontaneous clearance of HBeAg in children is uncommon in comparison with adults. The expected annual rates of e seroconversion in genotype B and C infections were 15.5% and 7.9%, respectively, in HBeAg-positive adults followed up for 52 months (Kao et al. 2004). In an Alaska adult cohort (>17.8 years old), the age of the 50th percentile of persons at the time of HBeAg clearance by genotype was as follows: A, 19.4; B, 19.5; C, 47.8; D, 18.0; and F, 16.1 (Livingston et al. 2007). On the contrary, the annual HBeAg clearance rate was <2% in children younger than 3 years of age and approximately 5% in those beyond (Chang et al. 1989). Spontaneous HBeAg seroconversion generally takes 2–7 years to happen, mostly asymptomatic in the seroconverters. Unless the children are identified by risk screening policy or symptomatic, and regularly monitored, elevation of ALT would be unnoticed. The appropriate cutoff levels of ALT to diagnose chronic liver disease in children are reevaluated in SAFETY (screening ALT for elevation in today's youth) study, considering only healthy weight, metabolically normal, liver disease-free pediatric participants in the USA. The 95th percentile levels for ALT for boys and girls are 25.8 and 22.1 U/L, respectively, which are much lower than the usual laboratory cutoff (40 U/L for male and 35 U/L for female) (Schwimmer et al. 2010). Serial monitoring of ALT levels in a group of HBeAg-positive children also disclosed the predictable span of 8.35, 5.14, 4.25, 3.95, and 2.80 years after the ALT levels over 20, 30, 40, 60, and 150 U/L, respectively. Therefore, ALT >30 U/L may serve as a more suitable cutoff in children to detect the beginning of immune clearance phase and to guide AVT in real practice or clinical trials (Wu et al. 2012b).

HBeAg seroconversion is regarded as a turning point in chronic hepatitis B, indicating the breakdown of host immunotolerance and viral replication, which is usually considered as an advantage to the host and set as one of the endpoints in antiviral therapies (Liaw 2009). Most cases after HBeAg seroconversion will continue to stay in inactive status. However, those who experience an eventful course of immune clearance

(multiple acute exacerbations) or fail to sustain HBeAg seroconversion or experience HBeAg seroreversion or HBeAg-negative hepatitis afterward will suffer from repetitive hepatic inflammation/fibrosis and may progress to cirrhosis or even hepatocellular carcinoma. Two Italian pediatric series with longitudinal observation for 24 and 29 years, respectively, found 1.7 and 4.5% had cirrhosis, and two of the four cirrhotic children in the longer series (HBeAg-positive at enrollment) had HCC 9 and 16 years after HBeAg seroconversion, one even with HBsAg clearance (Bortolotti et al. 2006; Iorio et al. 2007). In addition, early HBeAg seroconversion not necessarily designates a benign process with nearly half (14/30 liver biopsy at the anti-HBe-positive stage) showing various degrees of fibrosis/cirrhosis and an early converter before 3 years of age developed HCC at 11 years of age (Chang et al. 1995). After universal vaccination for 30 years in Taiwan, the first country initiating nationwide vaccine program, the HCC incidence in vaccinees 6–26 years of age has reduced to more than 70 percent (0.11 ~ 0.41 per 10⁵ person-year; relative risk 0.24, in reference to pre-vaccination birth cohort). However, HCC preventive failure in vaccinees still exists, mainly in carriers born to HBeAg-positive carrier mothers (Chang et al. 2016). Infected children need continuous monitoring for hepatitis activity, viral activity, and complication of chronic hepatitis B while they grow into adulthood.

Spontaneous HBsAg clearance in chronic hepatitis B is a rare event with an annual incidence of about 0.12–2.38% in Asian countries and 0.54–1.98% in Western countries in different cohorts (Chu and Liaw 2010). The mean age of clearance in chronic hepatitis B Taiwanese patients enrolled at 16–76 years old is 47.8 ± 9.6 years of age (Chu and Liaw 2007). None of the 56 cases less than 20 years of age had HBsAg clearance in a total of 946 HBsAg carriers living in Okinawa (Furusyo et al. 1999). Hsu et al. found an average HBsAg loss rate of 0.6%/year in 420 carrier children enrolled at 1 month to 17 years old and followed for 1–12 years in Taiwan (Hsu et al. 1992b). The higher HBsAg clearance rate was associated with HBeAg negativity (inactive carriers) (Furusyo et al. 1999; Hsu et al. 1992b) and older age at enrollment (Chu and Liaw 2007; Hsu et al. 1992b; Kato et al. 2000), HBsAg-negative mother (non-perinatal infection) (Hsu et al. 1992b), genotype C (Tseng et al. 2015), and more severe histology change or ALT elevation when they were HBeAg-positive (Hsu et al. 1992b; Tseng et al. 2015). Although HBsAg loss or seroconversion usually indicates a better prognosis, some may still possess HBV DNA in serum (0–18%, (Hsu et al. 1992b; Kato et al. 2000)) or liver (91%, (Fong et al. 1993)). HCC risk is reduced but still present with an incidence of 36.8 (HBsAg loss) vs. 195.7 (HBsAg persistence) per 100,000 per year (Hsu et al. 1992b; Kato et al. 2000; Liaw et al. 1991; Simonetti et al. 2010).

2.2.2 Current Treatment for Children with Chronic Hepatitis B

The ideal therapy for chronic HBV infection would eradicate the virus with HBsAg seroconversion or loss and undetectable viral DNA without substantial liver injury. Currently, there is no cure for chronic HBV infection. The treatment goal is to suppress disease activity and avoid the morbidity and mortality of cirrhosis or HCC. At present, the decision to start antiviral therapy (AVT) in children is dependent on ALT elevation, HBeAg positivity, HBV DNA levels, the severity of liver disease by

histology or noninvasive method, and family history of HCC. There are two main antiviral treatment regimens, interferon (IFN) -based (conventional or PegINF) and nucleos(t)ide analogues (NUCs). Although two IFNs and five NUCs are available in the market for chronic hepatitis B therapy, the approved usage in children is limited to conventional IFN- α (≥ 12 months old), lamivudine (LAM) (≥ 3 years old), adefovir dipivoxil (ADV) (≥ 12 years old), telbivudine (LdT) (≥ 16 years old), tenofovir disoproxil fumarate (TDF) (≥ 12 years old), and entecavir (ETV) (≥ 2 years old) until 2016. In adult series, IFN-based therapy holds the benefit of immune control, finite course; sustained off-therapy response (HBeAg seroconversion, low HBV DNA, and normal ALT) occurs 20% more often in treated than in controls (Wong et al. 1993). In those who achieved HBeAg seroconversion, 80% cleared HBsAg in a decade after therapy (Korenman et al. 1991). However, IFN needs an injection and has more adverse effects (flu-like syndrome, cytopenia that needs dose reduction or interruption, hair loss, depression, etc.). Pegylated IFN (PegINF) has replaced conventional IFN in adult patients due to more convenient, once per week injection, but usage in CHB children is still under clinical trial. NUCs are given in oral route and have a good tolerance. In adult series, HBeAg seroconversion, undetectable HBV DNA, and normal ALT occur in 10–20% treated cases compared to about 6% in controls after 1 year of therapy (Dienstag 2008; Bedre et al. 2016). However, a prolonged or indefinite course of therapy is necessary to maintain virological control. Prolonged usage of NUCs with low genetic barriers takes a high risk of emergence of drug-resistant mutations in adults. Consequently, ETV and TDF with infrequent resistant mutants after prolonged use have mostly replaced LAM and ADV in adults.

Interferon (Table 14.1)

Interferon inhibits HBV replication by blocking certain step in the pregenomic RNA-primed assembly of core particles (Hayashi and Koike 1989), suppression of cccDNA transcription by reduction of acetylated histone H3 lysine 9 (H3K9) and 27 (H3K27) in cccDNA minichromosomes (Liu et al. 2013), as well as enhancement of expression of HBsAg/preS2 on hepatocytes to augment immune recognition (Lau et al. 1991). Conventional interferon has been applied in children since the 1990s. Several clinical trials were conducted in Chinese or Caucasian children with HBeAg-positive, ALT normal or $>1.5 \times \text{ULN}$, high viral DNA with or without prednisolone priming. The doses ranged from 5 to 10 MU subcutaneous injection three times per week for a duration of 16 weeks to 1 year. At the end of treatment and after 6–24 months of follow-up, HBeAg loss/seroconversion occurred in 7–61%, and HBV DNA clearance ranged from 10 to 61% in the treated groups, which were higher than the untreated group (0–33% and 0–44%, respectively). HBsAg loss occurred in 2–19% in the treated but 0–< 1% in the untreated. ALT normalization was 11–67% in treated but 7–44% in the untreated (Table 14.1) (Lai et al. 1991; Ruiz-Moreno et al. 1991; Utili et al. 1991; Gregorio et al. 1996; Vajro et al. 1996; Sokal et al. 1998). Several factors including lower baseline HBV DNA levels, higher baseline ALT, moderate to severe histology, female sex, and age < 5 -year-old may predict a better treatment response. (Utili et al. 1991; Gregorio et al. 1996; Vajro et al. 1996; Sokal et al. 1998; Barbera et al. 1994; Kobak et al. 2004). The

Table 14.1 Antiviral therapies in randomized control trials in children

Intervention	Age N (total) N (group)	ALT normalization (%)	HBsAg loss/ seroconversion (%)	HBV DNA suppression (%)	HBsAg loss	Predictors in responders
<i>IFN-α, FDA approved in 1997 for children ≥ 1 year old</i>						
Ruiz-Moreno et al. (1991), 5 or 10 MU tiw 24 weeks, FU 15 months	1.5–15.5 years N = 36 I ₁₀ = 12 I ₅ = 12 C = 12	I: 42 C: 8	I: 46/46 C: 17/17	I ₁₀ : 58 I ₅ : 42 C: 17	—	Not significant (ALT, HBV DNA, etc.)
Utili et al. (1991), 3 MU tiw 12 months, FU 6 months	6–14 years N ₀ = 20 I = 10 C = 10	I: 30 C: 10	I: 20/20 C: 10/10	I: 30 C: 10	—	↑ Baseline ALT ↑ Histological activity
Lai et al. (1991), Prednisolone (+/–) 6 weeks + 5 MU/m ² tiw for 16 weeks, FU 24 months	2–17 years N = 90 I = 29 P+I = 31 C = 30	*Persistently raised ALT before entry only in 1 case (P+I) and 1 case (C)	I: 3/3 P+I: 13/3 C: 0/0	I: 7 P+I: 16 C: 0	I: 3 P+I: 3 C: 0	—
Barbera et al. (1994), 7.5 or 3 MU/m ² tiw 6 months, FU 18 months	Mean 8 years N = 77 I _{7.5} = 21 I ₃ = 19 C = 37	I: 41 C: 32 (EOT)	I: 26/— C: 14/—	I: 36 C: 22	—	↑ Baseline ALT
Vajro et al. (1996), 10 MU/m ² , tiw one year, FU 24 months	7.8±2.7 years N = 31 I = 13 P+I = 9 C = 9	I: 67 P+I: 67 C: 44	I: 67/67 P+I: 56/56 C: 33/33	I: 67 P+I: 56 C: 44	I: 16 P+I: 22 C: 0	ALT > 120 U/L ↑ Histology activity, DNA < 175 pg/mL
Gregorio et al. (1996), 5 MU/m ² tiw 12 weeks, FU 12–18 months	2–16 years N = 95 I = 30 P+I = 34 C = 31	—	I: 39/38 C: 16 ^a /13 ^a	I: 39 C: 26	—	Baseline DNA < 1000 pg/mL

Sokol et al. (1998), 6 MU/m ² tiw 24 weeks, FU 24 weeks	1–17 years N = 149 I = 72 C = 77	I: 11 C: 7	I: 26/— C: 11 ^a /— (EOT) I: 33/— C: 11 ^a /—	I: 33 C: 11 ^a	I: 10 C: 1 ^a	Female
<i>Lamivudine (LAM), FDA approved in 2000 for children 2–17 years old</i>						
Jonas et al. (2002), 3 mg/kg once daily for 52 weeks, EOT	2–17 years N = 288 L = 191 C = 97	L: 55 C: 12 ^a	L: 26/22 C: 15 ^a /13	L: 61 C: 16 ^a	L: 2 C: 0	Baseline ALT >2x, ↑ Histology activity, DNA < 800 meq/mL
Sokol et al. (2006), 24 months extension of the above trial, HBeAg(+) Tx, HBeAg(–) Ob	2–18 years N = 276 L3y = 134 Pl-L2y = 79 L1y-Ob = 49 Pl-Ob = 14	L3y: 39 L2y: 54 L1y-Ob: 71 Pl-Ob: 93	L3y: 26/26 L2y: 35/34 L1y-Ob: 85/84 Pl-Ob 100/100	L3y: 21 L2y: 30 L1y-Ob: 86 Pl-Ob: 92	L3y: 2 L2y: 1	VR at 24 m: YMDD (+) 5 % YMDD(–) 54 % [†]
<i>Adefovir Dipivoxil (ADV), FDA approved in May 2010 for children 12 to <18 years old</i>						
Jonas et al. (2008) 2–7 years 0.3 mg/kg; 7–<12 0.25 mg/kg 12–<18 y 10 mg; once daily for 48 weeks; EOT	2–6 years N = 35 7–11 years N = 55 12–17 years N = 83 ADV = 118 C = 58	2–6 years ADV: 30 C: 25 7–11 years ADV: 58 C: 16 ^a 12–17 years ADV: 64 C: 22 ^a Total ADV: 56 C: 21 ^a	Total ADV: 16/— C: 5/—	2–6 years ADV: 17 C: 8 7–11 years ADV: 19 C: 0 12–17 years ADV: 29 C: 0 ^a Total ADV: 23 C: 2 ^a	ADV: 0.9 C: 0	Baseline ALT >90; DNA ≤ 8.8 log ₁₀ copies/mL

(continued)

Table 14.1 (continued)

Intervention	Age no	ALT normalization (%)	HBeAg loss/seroconversion (%)	HBV DNA suppression (%)	HBsAg loss	Predictors in responders
Entecavir (ETV), FDA approved in Mar 2014 for children 2 to <18 years old						
Jonas et al. (2016), 0.015 mg/kg up to 0.5 mg/d for minimal 48 weeks	2–6 years N = 41	E TV: 68	ETV: 24/24	ETV: 49	ETV: 1.7	HBV DNA < 8 log ₁₀ IU/mL non-D genotype
	6–12 years N = 46	C: 23 ^a	C: 10 ^b /10 ^a	C: 3 ^a	C: 1.7	
	12–<18 years N = 93				(week 48) ETV: 5.8	
	ETV = 120				C: 0	
	PI = 60				(week 96)	
Tenofovir disoproxil fumarate (TDF), FDA approved in 2010 for children 12 to <18 years old						
Murray et al. (2012), 300 mg daily for 72 weeks, open-label 120 weeks	12–18 years: N = 106	TDF: 77 C: 39 ^a	TDF: 21/— C: 15/—	TDF: 89 C: 0	TDF: 2 C: 0	—
	TDF = 52 PI = 54	(72 week)				

Interferon: I₁₀, interferon 10 MU; I₅, interferon 5 MU; C, control; P+I, prednisolone priming + interferon; I_{7.5}, interferon 7.5MU; I₃, interferon 3MU; EOT, end of treatment

Lamivudine: L3y, lamivudine for 3 years; PI-L2y, placebo then Lam for 2 years; L1y-Ob, lam for one year then observation

PI-Ob, placebo then observation; VR, virological response (HBeAg loss and HBV DNA negativity)

^a*p*< 0.05 statistics between control and antivirals

—: not available

treatment benefit was challenged by no significant difference in cumulative HBeAg or HBsAg seroconversion rates in long-term (5–7 years after stopping therapy) comparison between IFN-treated and untreated children with elevated ALT despite an accelerated course of 1–3 years in treated children (Bortolotti et al. 2000; Vo Thi Diem et al. 2005; Hsu et al. 2008). Beneficial short-term and long-term virological outcomes were noted only in those children with pretreatment HBV DNA $< 2 \times 10^8$ copies/ml (Hsu et al. 2008). In summary, conventional IFN 5–10 MU three injections per week for 6–12 months in ALT-elevated, HBeAg-positive children may accelerate HBeAg seroconversion without hazard of resistance.

Nucleos(t)ide Analogues (NUCs) (Table 14.1)

Although NUCs are potent HBV polymerase inhibitors, due to the persistence of cccDNA in the nucleus, prolonged treatment to maintain viral suppression is necessary; therefore, the emergence of polymerase-resistant mutants is a major concern, especially low genetic barrier first-generation agents, which were by now licensed for children.

Lamivudine. A multicenter 52-week RCT showed 23% of treated children cleared HBV DNA and had HBeAg seroconversion compared to 13% of the placebo group. ALT > 2 times ULN, higher histology activity, and lower HBV DNA loads at baseline predict virological response (Jonas et al. 2002). Subsequent 24-month open-label extension trial showed the durability of virological response in around 90% of responsive cases. The prevalence of resistant mutants during LAM therapy was 19, 49, and 64% in children treated for 52, 96, and 144 weeks, which were similar to cumulative incidence of resistant mutants in adult (23%, 46%, and 55%) (Jonas et al. 2002; Sokal et al. 2006; Zoulim and Locarnini 2009). Virological response decreased to 5% in those with YMDD (tyrosine, methionine, aspartate, aspartate) mutants compared to 54% in those without (Sokal et al. 2006). In a Korean study, 2-year treatment induced 65% HBeAg seroconversion with 89% in those younger than 7 years old in compared to 43% in those beyond. Notably, 42% of cases younger than 7 years old also cleared HBsAg compared to none of those beyond 7 (Choe et al. 2007). In summary, LAM 3 mg/kg orally once daily for ≥ 1 year may result in around 20–65% HBeAg clearance and undetectable HBV DNA in children ≥ 3 years old, but a high incidence of resistant mutants has precluded it as first-line therapy when newer agents with higher genetic barrier were licensed.

Adefovir dipivoxil. ADV was approved in the year 2000 for children ≥ 12 years old after a 48-week RCT showed significant (23%) virological response in adolescent subgroup compared to 0% of the placebo group (Jonas et al. 2008). An extended open-label phase was conducted in HBeAg-positive children up to 192 additional weeks (total 240 weeks), either monotherapy or combined with LAM (in LAM-experienced cases). Nearly 40% cases who were HBV DNA-positive at 24 weeks discontinued ADV (virological failure). HBeAg seroconversion occurred in 55 of 101 continuous therapy group (54% or 34% of intention-to-treat analysis) and five cases cleared HBsAg (5%). ADV-associated rtN236T mutation only developed in one monotherapy case at 240 weeks, and rtA181T mutant, which is dually resistant to ADV and LAM, developed in one case with LAM added on at

144 weeks. The incidence of ADV-resistant mutants was much lower than that in adult series (cumulative incidence of 0, 3, 11, 18, and 29% at 1, 2, 3, 4, 5 years (EASL 2012)).

Entecavir. Due to high mutation profiles in the prolonged usage of LAM, ADV, and LdT, ETV and TDF have become the first-line therapy in adults with CHB (Sarin et al. 2016; Terrault et al. 2016). ETV has been approved in children 2–18 years of age in March 2014. In the clinical trials with a minimum duration of 48 weeks, HBeAg seroconversion and undetectable HBV DNA (<50 IU/mL) were achieved in 24% at 48 weeks, significantly higher than the control group (3%). In the ETV-treated group, HBV DNA suppression occurred in 49% compared to 3% in control, HBeAg seroconversion in 24% compared to 10% in control, and ALT normalization in 68% compared to 23% in control. The entire efficacy end points continued to increase after 96-week therapy with virological response to 36%. Resistant mutants (M204 V, L180 M, S202G) were found in one (0.8% of 120 ETV-treated) at week 48 and three more at week 96 (cumulatively 2.6% at year 2) (Jonas et al. 2016). The incidence was higher than those in adult series (cumulative incidence 0.2, 0.5, 1.2, 1.2, 1.2 at year 1, 2, 3, 4, 5 (EASL 2012)).

Tenofovir disoproxil fumarate. A 72-week RCT of TDF in adolescents 12 to <18 years of age (85% antiviral-experienced, 90% HBeAg-positive, 27% normal ALT, 52 TDF, and 54 placebo) showed high viral suppression (HBV DNA <400 copies/mL; TDF, 89%, versus placebo, 0%) in either naïve or LAM-experienced cases with no resistant mutant at 72 weeks nor increased serious safety issues. In HBeAg-positive cases, 21% (10/48) of patients in the TDF group and 15% (7/48) in by week 72 ($p > 0.05$). A decrease in bone mineral density did not reach the safety endpoint of more than 6% in TDF group at week 72 (Murray et al. 2012).

Combined IFN and NUC. Immunotolerant children with normal ALT and high viral loads did not respond to monotherapy with IFN- α 2b in previous clinical studies (Lai et al. 1987, 1991; Sokal et al. 1998) or may develop drug resistance on the prolonged usage of NUCs. Accordingly, they are not good candidates to apply antiviral therapies. However, a pilot open study with 8-week LAM followed by combined LAM with IFN for 44 weeks in 23 immunotolerant children showed promising results of 78% HBV DNA negativity, 22% HBeAg seroconversion, and 17% HBsAg seroconversion (D'Antiga et al. 2006). Based on this study, two clinical trials (NIDDK/NCT01368497, ETV/+PegIFN; Roche/NV25361, LAM or ETV/+PegIFN) are underway to elucidate the effectiveness in RCT.

2.2.3 Who, When, and How to Treat in Children with Chronic HBV Infection

Based on the results of these trials and clinical data, the recommendations of administration of AVT in CHB children are as follows (Fig. 14.1):

Who and When

- In chronic HBV-infected children with liver cirrhosis, either decompensated or compensated, or severe acute hepatitis flares, AVT therapy is indicated. Liver transplantation may be considered if condition deteriorates.

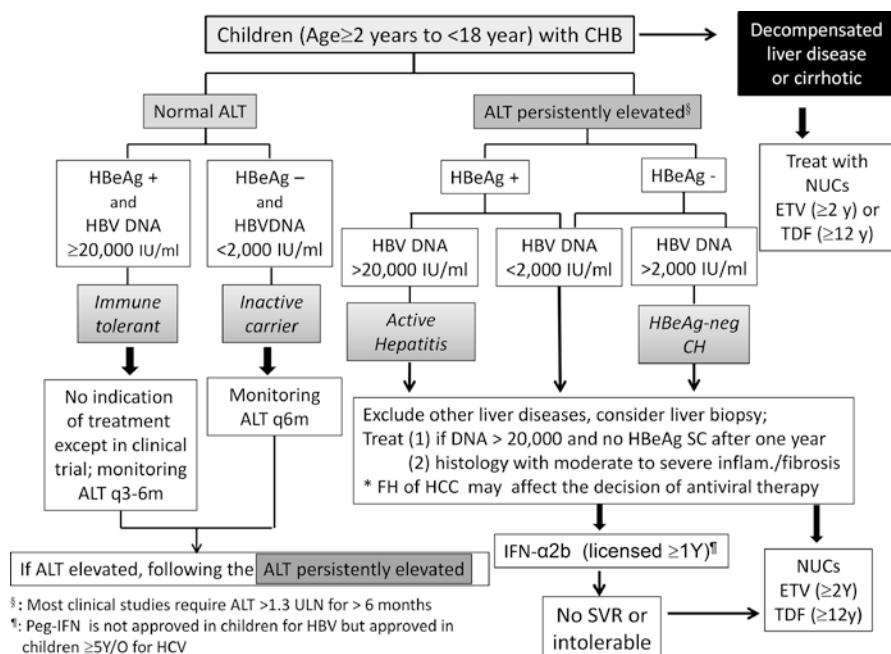


Fig. 14.1 The algorithm of management of children with chronic hepatitis B virus infection

- In non-cirrhotic HBeAg-positive children entering immune-active phase with ALT levels persistently elevating to >1.3 – 1.5 folds upper limit of normal (> 34 U/L by SAFETY study, >39 U/L by AASLD, >60 U/L by EPSGHAN) for more than 6 months without spontaneous HBeAg seroconversion after observation for up to 1 year, consider antiviral treatment. HBV DNA levels should be measured and are mostly well above 20,000 IU/mL. Other causes of hepatic inflammation should be excluded. There is no consensus of a lower limit of HBV DNA with respect to treatment in children. In those with HBV DNA 2000–20,000 IU/mL or even lower than 2000 IU/mL, if significant inflammation or fibrosis is documented by histology or noninvasive methods (such as Fibroscan or elastography) or presence of family history of HBV-related cirrhosis or HCC, AVT may be considered if ALT is persistently elevated. Without significant fibrosis, therapy may be deferred to see whether spontaneous HBeAg seroconversion emerges if viral loads are below 10,000 IU/mL (AASLD).
- HBeAg-negative children with persistently elevated ALT for more than 12 months indicate reactivation. Usually, HBV DNA levels in these cases are over 2000 IU/mL. If viral loads are <2000 IU/mL, other causes of hepatitis inflammation should be differentiated.
- In those with normal ALT levels in immunotolerant phase, regular monitoring of ALT/HBeAg/or HBV DNA status is suggested. AVT is not recommended unless in clinical trial condition.

Table 14.2 Risk of HBV reactivation in different HBsAg status in various immunocompromised scenarios

	HBsAg-positive	HBsAg-negative/ anti-HBc-positive
Anti-CD20 Hematopoietic stem cell transplantation	Very high risk	Moderate risk
High-dose steroids (>20 mg for ≥ 4 weeks)	Moderate risk	Low risk
Cytotoxic chemotherapy without steroids Anti-TNF therapy Antirejection therapy for solid organ transplantation	Low risk	Rare
Methotrexate or azathioprine	Very low risk	Rare

- In children with evidence of chronic HBV infection (HBsAg-positive or anti-HBc-positive) who are expected to receive immunosuppressant therapy, baseline HBV DNA level should be measured and the risk of reactivation should be assessed (Table 14.2). The risk of reactivation is higher in those who are HBsAg-positive than HBsAg-negative and depends on different immunosuppressants/clinical condition. HBV reactivation is diagnosed when a patient with serologic evidence of HBV has (1) a detectable HBV DNA level when they previously had an undetectable level and (2) a rise in HBV DNA of more than 2 log₁₀ IU/mL above baseline. AVT is recommended for all patients who develop HBV reactivation. AVT concurrently or prior to immunosuppressive therapy, especially in moderate- to very high-risk patients, can decrease the risk of HBV reactivation. Among those at low risk or very low risk of reactivation, early detection of HBV reactivation by frequent monitoring and timely AVT are appropriate. AVT should be maintained for at least 6 months after withdrawal of immunosuppression but should be maintained for at least 12 months after discontinuing anti-CD20 (Bisceglie et al. 2015).

How

- The choice of initial AVT depends on age, hepatitis activity, drug availability/price, and tolerability after thorough discussion with the children and parents. IFN may be considered due to a finite course. In adults, conventional IFN is replaced by PegIFN unless it is not available. However, PegIFN has not yet been approved for children with chronic HBV infection but has been approved for children 5 years of age or older with HCV infection. Therefore, conventional IFN- α (6 MU per m² body surface area, maximum 10 MU three injections per week) may be applied in children ≥ 1 -year-old and PegIFN (180 μ g/1.73 m² to maximum 180 μ g once weekly) may be provided as a choice for children ≥ 5 years of age. The standard treatment course is 6 months for HBeAg-positive cases and 12 months for HBeAg-negative cases from adults' experience. IFN is contraindicated in patients with severe flares (ALT > 5 $\times$ ULN) or hepatic decompensation (jaundice or coagulopathy), because in-therapy flares may precipitate

liver failure. IFN is also contraindicated in children with cytopenia, autoimmune disorders, cardiac or renal failure, and serious neuropsychiatric disease and in transplanted patients (Jara and Bortolotti 1999; Shah et al. 2009).

- If NUCs are considered as AVT, ETV may be applied in children ≥ 2 years of age and weight ≥ 10 kg (weight-based dose to 10–30 kg, above 30 kg 0.5 mg daily in treatment-naïve cases and double dose in LAM-experienced cases). TDF (300 mg daily) may be a choice in children ≥ 12 years of age. These two NUCs are potent in viral suppression and good in safety profiles in children. LAM and ADV are now rarely used for the treatment of chronic HBV infection due to limited efficacy and emergence of resistant mutants. The optimal duration of NUCs is not defined and may be influenced by HBeAg status, duration of HBV DNA suppression, and presence of cirrhosis/decompensation. Based on the kinetics of HBsAg change during NUC therapy, finite treatment course seems unlikely (Chevaliez et al. 2013). In non-cirrhotic HBeAg-positive children who seroconvert to anti-HBe, a period of consolidation for at least 12 months of persistently normal ALT and undetectable HBV DNA is recommended. In cirrhotic cases with HBeAg seroconversion on NUC therapy, indefinite treatment is suggested based on adults' studies. In those who stop NUCs, careful monitoring monthly for initial 3 months and then 3 monthly for at least 1 year is recommended for recurrent viremia, ALT flares, HBeAg seroreversion, or decompensation (Sarin et al. 2016; Terrault et al. 2016). In NUC nonresponder or virological breakthrough cases, genotypic mutation should be checked. Either ETV (for ADV-resistant and LAM-naïve patients) or TDF (for ≥ 12 years old, LAM-resistant patients or ADV-resistant patients previously treated with LAM) may be substituted. Conventional IFN- α or PegIFN (≥ 5 years or when approved) can be a possibility if no other NUCs are available (Zoulim and Locarnini 2009; Sokal et al. 2013).
- HBeAg-negative chronic hepatitis is a progressive disease and difficult to cure in adult patients, whereas it is less prevalent in pediatric patients (4.8% in IFN-treated or naïve children (Iorio et al. 2007)). In adults, two strategies are applied in HBeAg-negative hepatitis: a finite course (48 weeks) of PegIFN or long-term administration of the more potent and less resistance-prone NUCs, i.e., ETV and TDF. PegIFN alone for 48 weeks achieved a significantly higher rate of sustained response than LAM alone without additional benefit when combined with LAM (Marcellin et al. 2004). Higher sustained response may be predicted from baseline ALT (higher), HBV DNA levels (lower), viral genotypes (B > C > D), IL-28B (rs12979860 genotype CC vs. non-CC), and early on-treatment HBsAg kinetics (a decrease of 0.5 and 1 Log₁₀IU/mL HBsAg levels at weeks 12 and 24 of therapy) (Bonino et al. 2007; Lampertico et al. 2013a; Moucari et al. 2009; Lin and Kao 2013). The ideal end point of therapy in HBeAg-negative hepatitis is HBsAg clearance, which may be better achieved via PegIFN than NUC (4% vs. 0%, off-therapy 6 months; 8% in off-therapy year 3–5) (Chotiyaputta and Lok 2010; Lampertico et al. 2013b). If NUC is selected, an alternative stopping rule is at least three results of undetectable HBV DNA 6 months apart after a minimum of 24 months of treatment (Sarin et al. 2016).

2.2.4 HCC Surveillance in Children with Chronic HBV Infection

Most children with chronic HBV infections present with mild or minimal liver inflammatory activity, yet 0.01–0.03% of these carriers may develop HCC before adulthood (Bortolotti et al. 2006; Wen et al. 2004). The incidence of HCC birth cohort born before HBV vaccination program was 0.51–0.60 per 100,000 person-years and reduced to 0.15–0.19 per 100,000 person-years in birth cohort after the vaccination program (Chang et al. 2009). The risk of HCC in adults with CHB is predictable by age, gender, HBeAg serostatus, viral loads, ALT levels, quantitative HBsAg levels, and HBV genotypes (Chen et al. 2011; Lee et al. 2013). The risk is increased in those with precore/basal core promoter mutants (Yuen 2004; Liu et al. 2006b; Kao et al. 2003). Although a landmark study showed HBeAg seropositivity predicted high risk for HCC in the era that DNA quantification was not available (Yang et al. 2002), a later study found that HBeAg seroclearance was not sufficient to reduce HCC risk unless it was followed by DNA seroclearance/HBsAg clearance (Liu et al. 2014). HCC has been described in children who had undergone early (< 2 years of age) HBeAg seroconversion, indicating that there is still a risk for HCC after HBeAg seroconversion if DNA levels are not persistently low (<2000 IU/mL) (Wen et al. 2004; Chen et al. 2011). HBV genotype may influence HCC development. Compared to genotype B infection, genotype C with distinctive features of delayed HBeAg seroclearance, higher frequency of basal core promoter mutation, and higher DNA levels are associated with cirrhosis and HCC in older patients in Taiwan (Kao 2003; Ni et al. 2004). The risk model to stratify HCC screening policy in adult carriers may not be practical in children. HCC surveillance in children using liver ultrasound should be done every 6–12 months, depending on the stage of fibrosis and family history of HCC (Bruix and Sherman 2011; Yu et al. 2000). Alpha-fetoprotein alone was recently shown to offer insufficient sensitivity and specificity for cost-effective HCC surveillance in adults (Singal et al. 2009; Lok et al. 2010). Regardless of whether the virus is actively replicating, HBV infection is the fundamental risk of HCC. Continuous surveillance for HCC in all ages and phases of HBV infection, even after HBeAg seroclearance or HBsAg clearance, is mandatory (Bortolotti et al. 2006; Wen et al. 2004; Hsu et al. 2002).

3 Management of Pregnant Women with HBV Infection

3.1 Acute Hepatitis B in Pregnancy

Acute hepatitis B during pregnancy is usually mild without increased mortality or teratogenicity (Sookoian 2006; Lobstein et al. 2011). Some reported prematurity or low birth weight in the neonates of pregnant mothers with acute hepatitis B infection (Hieber et al. 1977; Jonas 2009). AVT will be considered only if an impending acute liver failure develops (Degertekin and Lok 2009). The mother-to-infant transmission rate of HBV depends on the gestational ages at acute infection, about 10% in early pregnancy to as high as 60% when near delivery (Sookoian 2006; Jonas 2009).

Monitoring of maternal seromarkers and HBV DNA is necessary to adopt preventive tasks for mother-to-infant transmission (MTIT). If the mother is HBsAg-positive with detectable HBV DNA near delivery, HBIG needs to be given to the neonate in addition to standard vaccination. If high maternal DNA levels are detected at late pregnancy, AVT may be considered to reduce MTIT. Otherwise, supportive management is generally acceptable with careful monitoring of liver biochemical and coagulation profiles. The choice of AVT in pregnant women will be based on safety, accessibility, and expected duration of therapy and will be discussed below.

3.2 Chronic Hepatitis B Virus Infection in Pregnancy

3.2.1 Effect of Pregnancy on Maternal Chronic HBV Infection

Most HBsAg carrier mothers can tolerate pregnancy well unless advanced liver disease already exists. However, pregnancy itself is a hormone-induced immunotolerant status (to the fetus) with high levels of adrenal corticosteroid that may modulate immune responses (Trowsdale and Betz 2006).

Acute flares. Due to a pregnancy-related immunotolerant status, ALT decreases significantly in a pairwise comparison between three trimesters (ALT in the first trimester > in the second trimester > in the third trimester, $P = 0.01$ and 0.02 , respectively), while serum HBV DNA levels do not change significantly in three trimesters. ALT flares in the range of 38–1654 U/L were reported in 25% postpartum in contrast to 1.6% during pregnancy. HBeAg positivity at baseline may predict postpartum flares. Most flares with ALT <5× ULN returned to normal within 9–12 months after delivery. Those with ALT >10× ULN may take >12 months and antivirals are indicated (Giles et al. 2015). In those mothers who received AVT in the third trimester for prevention of MTIT, there was no difference in the occurrence or onset of flares no matter whether the antivirals stopped at 2 weeks (50%, 8 weeks) or 12 weeks after delivery (49%, 10 weeks) or there are no antivirals (29%, 9 weeks). Extending antiviral therapy does not protect against postpartum flares or affect HBeAg seroconversion rates (Nguyen et al. 2014).

3.2.2 Effect of Chronic HBV Infection on Pregnancy Outcomes

Although some reported an increased risk of gestational diabetes mellitus (Tse et al. 2005; Lao et al. 2007), antepartum hemorrhage (Tse et al. 2005; Safir et al. 2010), preterm labor (Tse et al. 2005), premature birth (Hieber et al. 1977; Safir et al. 2010), and perinatal morbidity (Safir et al. 2010), other studies could not show any significant adverse effects on pregnancy (Lobstein et al. 2011; Livadas et al. 1979; Pastorek et al. 1988; Wong et al. 1999). The outcomes of pregnancy in patients with cirrhosis were described in a population-based study. Antenatal and delivery admissions in 339 cirrhotic pregnant women in comparison with 6625 matched control showed that cirrhotic mothers were more likely to deliver by cesarean section, to have maternal and fetal mortality, antepartum admission, and maternal and fetal complications, including gestational hypertension, placental abruption, and uterovaginal hemorrhage in mothers and prematurity and growth restriction in

babies. Hepatic decompensation occurred in 15%, which resulted in 6 and 12% maternal and fetal mortality, respectively (Shaheen and Myers 2010).

3.3 Management of Pregnant Women with Chronic HBV Infection

Various factors need to be considered when encountering the management of pregnant women with chronic HBV, including the indications for antivirals, potential influences to the fetus, expected duration of therapy, the risk of developing drug resistance, and accessibility and cost of the antivirals.

3.3.1 Women Who Become Pregnant When Under Antiviral Therapy

Women should notify their clinician immediately if they get pregnant while taking AVT, and the risks and benefits of continuing treatment should be discussed. Interrupting treatment may pose a risk of hepatitis flare for the mother, while continuing treatment may affect the fetus. Discontinuing treatment can be a choice if the patient is non-cirrhotic. Most safety data of NUCs are from HIV-infected pregnant women. TDF and LdT are classified as pregnancy category B, while the rest are rated as pregnancy category C. Conventional IFN is contraindicated in pregnancy, and PegIFN is category C, but not recommended due to its antiproliferative effect. Although lamivudine is considered category C, there is a long history of safety data in HIV-infected women. Clinical trials evaluating the efficacy of LAM, LdT, and TDF to reduce the risk of mother-to-infant transmission also support the safety of these agents during pregnancy; however, long-term outcomes are still under surveillance. Therefore, women receiving ETV, ADV, or IFNs can be shifted to pregnancy category B agents, TDF or LdT, if continuing treatment is favored. They should be carefully monitored during the switching to ensure no flare-up.

3.3.2 Indications for Initiating Antiviral Therapy During Pregnancy

The decision to initiate AVT complies with the EASL, AASLD, and APASL guidelines in the management of adult chronic HBV infection (EASL 2012; Sarin et al. 2016; Terrault et al. 2016). However, some scenarios may need consideration:

- Although AVT is recommended for most patients with an ALT $>2\times$ ULN, women without evidence of cirrhosis may choose to defer therapy until after completion of childbearing if they have low viral loads and mild disease activity.
- Women with high viral loads should consider initiating therapy in the third trimester to prevent transmission to their child, even if ALT levels are normal (see 14.3.4 AVT to reduce mother-to-infant transmission).
- Management of cirrhosis in pregnant women does not differ from that of nonpregnant patients (Tran et al. 2016).
- In women with childbearing potential, indications for AVT are the same as other adults. However, several issues may be addressed. Those with mild disease, who are planning to conceive soon, may elect to defer treatment until they have com-

pleted childbearing. Those who are willing to receive treatment before pregnancy may choose PegIFN because of its finite duration (48 weeks) under contraception during treatment. If the patient chooses treatment with a NUC, TDF is preferred due to its potency, safety data in pregnancy, and low risk of drug resistance.

3.4 Antiviral Therapy to Reduce Mother-to-Infant Transmission of HBV

3.4.1 Risk of Transmission

Although “mother-to-child-transmission (MTCT)” or vertical transmission has been commonly used to describe this transmission route, we consider it more appropriate to use “mother-to-infant transmission (MTIT)” of HBV, because the timing of transmission most likely occurs in the perinatal or intrauterine period. The prophylaxis strategies including maternal antivirals and neonatal immunization both focus on preventing perinatal transmission. Postnatal transmission (or known as horizontal transmission) may occur but is a rare event in children under appropriate immunoprophylaxis. The rate of MTIT of HBV from HBsAg-positive mothers was reported to be 40 ~ 50% in the pre-immunization era (Stevens et al. 1975). The risk is highest in HBsAg-positive/HBeAg-positive/anti-HBe-negative mothers (transmission rate, 70%–90%), lower for HBsA-positive/HBeAg-negative/anti-HBe-negative mothers (transmission rate, 25–40%), and lowest in HBsAg-positive/HBeAg-negative/anti-HBe-positive (0–12%) (Beasley et al. 1977; Stevens et al. 1979; Degli Esposti and Shah 2011; Borgia et al. 2012; Wong et al. 1984). Transmission can occur in utero (intrauterine), at birth (perinatal), or after birth (Lin et al. 1987; Tang et al. 1998). The MTIT has been reduced significantly after universal vaccination program to around 1% in the USA (Schillie et al. 2015b) or 2.46% in Taiwan (Chen et al. 2012) with different maternal HBV seroprevalence (6% in Asian ethnic, 0.14 ~ 1% in non-Asian ethnic in the USA, versus 3.1 ~ 15.5% different birth cohorts in Taiwan) (Euler et al. 2003; Lin et al. 2008) and immunoprophylaxis policy (hepatitis B immunoglobulin (HBIG) and vaccination to all infants born to HBsAg-positive mothers in the US, HBIG to infants born to HBsAg-positive/HBeAg-positive mothers and vaccine to all infants in Taiwan).

3.4.2 Risk Factors of Mother-to-Infant Transmission

The common risk factors for transmission are HBeAg-positive mothers, high maternal viral load, or younger maternal age; transmission was also associated with infants receiving <3 doses of the hepatitis B vaccine or delayed immunoprophylaxis (Wen et al. 2013; Schillie et al. 2015b). Among those factors, the most important determinant for the propensity of MTIT is maternal HBV viral loads.

Maternal Viral Loads

An early study by dot hybridization assay for HBV DNA showed that high maternal serum HBV DNA (>8000 pg/mL) at delivery predicted 100% MTIT despite immunoprophylaxis (Lee et al. 1986). A more clear correlation or cutoff of maternal viral titers to transmission rate has been documented by recent studies applying more sensitive

Table 14.3 Maternal viral loads or seromarker status and infant transmission rates in different studies

Study	Mother-infant pair	HBIG/vaccine	Definition of infant transmission	Maternal seromarkers or viral loads/transmission rate (%)	
Wiseman et al. (2009) (Australia)	138 infants of HBV DNA (+) mothers	<12h ^a / <12 h, 2, 4, 6 months	HBsAg (+) at 9 months	DNA (+)	3
				HBeAg (+)	7
				DNA ($\log_{10}$ copies/ml)	
				> 8	9
				< 8	0
Zou et al. (2012) (China)	869 infants of HBsAg (+) mothers	<12 h/ <12 h, another two doses <6 months	HBsAg (+) at 7–12 months	DNA ($\log_{10}$ copies/mL)	
				<6	0
				6–6.99	3.2
				7–7.99	6.7
				>8	7.6
Wen et al. (2013) (Taiwan)	303 infants of HBsAg (+) mothers	≤ 24 h/0, 1, 6 months	HBsAg (+) at 4–8 months and/or 1–3 years	HBsAg (+)	3.3
				HBeAg (+)	12.3
				DNA ($\log_{10}$ copies/mL)	
				5	0.9
				6	2.6
				7	6.6
Kubo et al. (2014) (US)	4446 infants of HBsAg (+) mothers	<12 h/ <12 h, at 24–67, 64–214 days	HBsAg (+) at 9–15 months	8	14.6
				9	27.7
				HBsAg (+)	0.75
				HBeAg (+)	3.37
				DNA (IU/mL)	
				$\geq 5 \times 10^7$	3.61
				$< 5 \times 10^7$	0

^aOne of the infected infants inadvertently missed HBIG administration

quantitative PCR methods (Table 14.3). In HBV high endemic areas (Asia), higher maternal viral loads (from 6 to $>8 \log_{10}$ copies/ml) are correlated with higher infant transmission rates despite passive and active immunoprophylaxis (Wen et al. 2013; Zou et al. 2012). In low-endemic countries (Australia and USA), the transmission threshold may be higher ($> 8 \log_{10}$ copies/ml) with lower infant infection rate in the same cutoff levels (Wiseman et al. 2009; Kubo et al. 2014). There is still no consensus on the threshold of maternal viral loads to target for preventive therapy for MTIT. Some advocates lower threshold ($>200,000$ IU/mL, $\sim 5.3 \log_{10}$ IU/mL, or $>6 \log_{10}$ copies/mL) to target, aiming to prevent all possible MTIT (Zou et al. 2012; Zhang et al. 2014). However, lower target levels may render a larger number of pregnant women under AVT exposure, some with a borderline risk of MTIT.

Maternal HBeAg Status

Transplacental maternal HBeAg may play an important role in establishing a chronic infection in infants through induced immunological tolerance in transgenic mice model and human study (Milich et al. 1990; Hsu et al. 1992a). The significant

difference in MTIT rates between HBeAg-positive and HBeAg-negative mothers has been well-documented in many studies before (85 vs. 31% (Beasley et al. 1977)) or after immunoprophylaxis programs (9.3 vs. 0.23% (Chen et al. 2012), 1.8 vs. 0% (Kubo et al. 2014), 3.2 vs. 0% (Schillie et al. 2015b), 9.8 vs. 0.7% (Xu et al. 2002)). HBeAg-positive CHB correlates with higher HBV DNA levels (25th to 75th centile, 6.8–8.3 log₁₀ IU/mL) compared to HBeAg-negative CHB (25th to 75th centile, 3.2–5.8 log₁₀ IU/mL, $p < 0.0001$) unless BCP/PC variants are present, which may result in dissociated HBeAg levels with viral replication (Thompson et al. 2010). In HBeAg-positive mothers, serum HBV DNA is significantly higher than HBeAg-negative mothers (7.4 ± 1.9 vs. 2.7 ± 1.4 log₁₀ copies/mL, $p < 0.0001$) (Wen et al. 2013). Therefore, the link between maternal HBeAg seropositivity and MTIT is considered mainly a reflection of maternal viral loads with MTIT based on clinical studies (Kubo et al. 2014; Burk et al. 1994). The roles of HBeAg-induced immunological tolerance are to be validated in clinical settings.

Maternal HBsAg Levels

Although maternal HBV DNA levels are the best predictors of MTIT of HBV, universal screening of HBsAg-positive mothers with quantitative PCR is hindered by its high cost. Due to correlation of HBsAg levels with HBV DNA, especially at high DNA levels or HBeAg-positive status (Thompson et al. 2010; Su et al. 2010), several studies evaluate the option of using maternal HBsAg level as a surrogate predictor of MTIT (Sun et al. 2012; Wen et al. 2016). Sun et al. found HBV DNA level was positively correlated with HBsAg in the HBeAg-positive group but not in the HBeAg-negative group. The optimum cutoff point HBsAg titer above 4.1 log₁₀ IU/mL could predict HBV DNA levels of ≥ 7.0 log IU/mL with favorable sensitivity (85%) and specificity (97%) (Sun et al. 2012). Wen et al. showed maternal HBsAg was positively correlated with maternal viral load and accurately predicted maternal viral load above 6–8 log₁₀ IU/mL with an area under curve (AUC) of 0.95 to 0.98. The estimated rates of transmission at maternal HBsAg levels of 4, 4.5, and 5 log₁₀ IU/mL were 2.4, 8.6, and 26.4% (Wen et al. 2016).

Amniocentesis and Other Factors Causing Maternal-Fetal Hemorrhage

Obstetric procedures or complications that may cause maternal-fetal hemorrhage, such as threatened abortion, chorionic villus sampling, amniocentesis, threatened preterm labor, emergent cesarean section after any period of labor, and forceps/vacuum delivery, have been reported to increase the risk of MTIT (Lin et al. 1987; Xu et al. 2002). A study showed, in comparison to infants without amniocentesis, significant higher transmission rate was noted in HBsAg-positive mothers with high viral loads > 7 log₁₀ copies/mL (4.5% vs. 50%), but no difference in those with viral loads < 6.99 log₁₀ IU/mL (1.5% vs. 1.8%) (Yi et al. 2014). Another statistics from Canada shows MTIT attributable to amniocentesis in HBsAg-positive mothers ranges up to 1.4%. However, the rate may be as high as 16% in HBeAg-positive mothers (Gagnon et al. 2014). Therefore, noninvasive methods of prenatal risk screening that provide the highest sensitivity with the lowest false-positive rate should be used to minimize the number of amniocenteses performed. Knowledge of

the viral loads or HBeAg status is important in counseling high-risk mothers about the risk of transmission associated with amniocentesis.

Different modes of delivery also influence the transmission rate. A study compared 1409 infant-carrier mother pairs delivered vaginally (VD) ($n = 673$), elective cesarean section (ECS) ($n = 496$), or urgent cesarean section (UCS) ($n = 240$) with active and passive infant immunoprophylaxis. A significantly lower rate of MTIT was noted by ECS (1.4%), compared to vaginal delivery (3.4%, $p < 0.032$) or by UCS (4.2%, $p < 0.02$). Based on this study, authors suggested that ECS for HBeAg-positive mothers with predelivery HBV DNA $>6 \log_{10}$ copies/mL (or $>200,000$ IU/mL) may reduce MTIT (Pan et al. 2013). However, a review from Australian, UK, and New Zealand experts suggests there has been no sufficient evidence to modify the mode of delivery for MTIT reasons if neonatal immunization is used (Visvanathan et al. 2016).

Breastfeeding

Although HBsAg, HBeAg, and HBV DNA are present in breast milk, no differences in the rates of HBV infection have been reported between breastfed infants versus formula-fed infants even before the era of immunoprophylaxis (Beasley et al. 1975; Hill et al. 2002; Montoya-Ferrer et al. 2015). Infants who received HBIG and the birth dose of hepatitis B vaccine can be breastfed (Dionne-Odom et al. 2016). However, HBsAg-positive mothers should also avoid bleeding from cracked nipples. Carrier mothers should not donate breast milk and not breastfeed during AVT.

3.4.3 Algorithm to Prevent Mother-to-Infant Transmission

(Fig. 14.2)

- Pregnant women should have HBsAg testing at the first prenatal visit. Those who are HBsAg-positive are recommended to test for HBeAg, HBV DNA, and aminotransferase levels.
- Women who have a high HBV DNA level (i.e., $>200,000$ IU/mL) and/or a positive HBeAg should be referred to a hepatologist to evaluate the indication of AVT.
- Women with low HBV DNA levels in the first trimester should have viral load testing again around weeks 20 to 28. If the levels are over the threshold of indication, AVT should be considered.
- Antiviral treatment for prevention of MTIT should be started at the beginning of the third trimester, so there is sufficient time for the HBV viral load to decrease, considering some women may have preterm labor.
- Women who are HBsAg-negative and are at high risk for HBV infection (e.g., injection drug user, sexual partner, or household contacts of HBsAg carriers) should be vaccinated if she is also negative for anti-HBs and anti-HBc. If she is still susceptible, HBsAg testing should be repeated in late pregnancy (approximately 28 weeks).

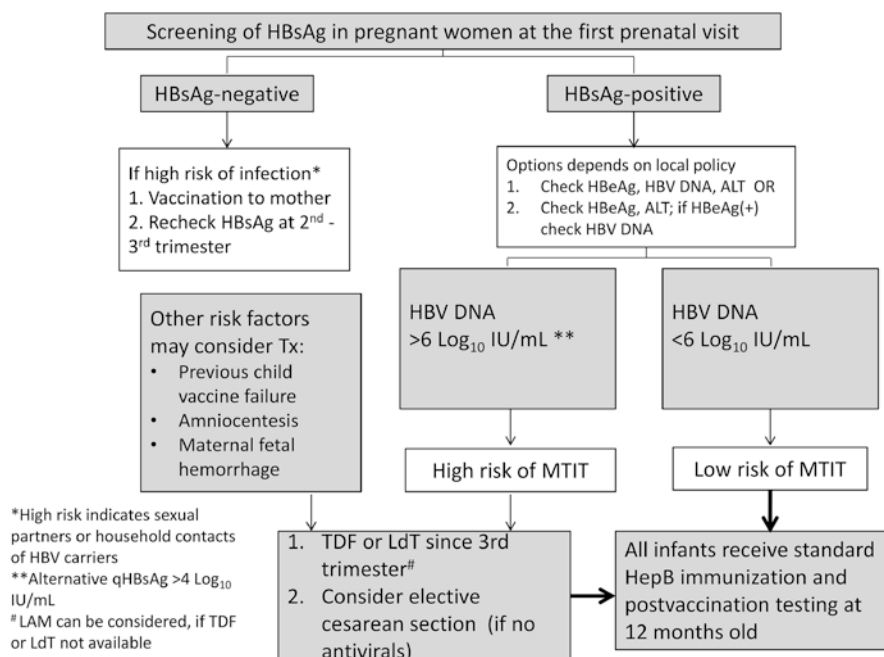


Fig. 14.2 Algorithm of management of pregnant women to prevent mother-to-infant transmission of HBV

3.4.4 Choice of Antiviral Agents

For those who require treatment, TDF is the first choice due to its high potency and low resistance mutation. This is important since many of these young mothers may require antiviral treatment for their liver disease in the future. In addition, this agent appears to be safe for use in pregnancy and has been evaluated in several prospective clinical trials (Chen et al. 2015; Brown et al. 2016; Pan et al. 2016). Although other agents (e.g., LAM and LdT) also reduce mother-to-infant transmission and appear to be safe when administered during pregnancy (Han et al. 2011; Pan et al. 2012; Shi et al. 2010), they are associated with higher rates of antiviral resistance. Lamivudine may be a reasonable alternative if cost is a barrier to obtaining AVT or category B medication is not available, and treatment is going to be administered for a short duration (i.e., ≤ 3 months). However, it is important that patients have not received lamivudine in the past, since such patients are at risk of having lamivudine-resistant virus; and also consider the fact that maternal viral load reduction is slower compared to newer AVTs.

3.4.5 Efficacy and Safety of Antiviral Therapy in Preventing of Mother-to-Infant Transmission of HBV

A growing number of studies using AVTs to prevent MTIT have been published in recent years showing promising results. A systemic review and meta-analysis

included 26 controlled trials from 1988 to 2014 that enrolled 3622 pregnant women. Eleven compared LAM vs. control, nine compared LdT vs. control, three compared TDF vs. control, two compared LAM vs. LdT, and another compared TDF vs. LAM. Treatments started in the second or third trimester with an average baseline HBV DNA level of 7.63 log₁₀ IU/mL and ALT level of 37.7 U/L. All infants (except one study) received passive-active immunization. The infant outcome is defined by seropositivity of HBsAg or HBV DNA at 6–12 months of age. The meta-analysis concludes use of any AVT compared to control in pregnant women which reduces the likelihood of MTIT (HBsAg seropositivity, risk ratio (RR) = 0.26; HBV DNA positivity, RR = 0.31). LdT, LAM, and TDF appear to be safe in pregnancy with no increased adverse maternal or fetal outcome (Brown et al. 2016). The study designs were highly variable in earlier trials and lacked sufficient controls.

Recently, a well-controlled prospective trial in Taiwan enrolled 118 pregnant women with HBV DNA ≥ 7.5 log₁₀ IU/mL. Sixty-two mothers received TDF from 30–32 weeks of gestation until 1 month postpartum. Compared to untreated group, infants from the TDF group had lower seropositivity rate of HBV DNA at birth (6.15% vs. 31.48%, $P = 0.0003$) and lower HBsAg seropositivity rate at 6 months of age (1.54% vs. 10.71%, $P = 0.0481$). Mothers in the TDF group experienced less incidence of ALT $>2 \times$ ULN lasting more than 3 months, less extent of ALT elevation, and less severe flares ($>5 \times$) of ALT elevation at postpartum week 8. An infant in the TDF group who was seronegative for HBsAg at 6 months old became seropositive at 12 months old, which was attributed to low anti-HBs response (11.1 mIU/mL at 6 months old) even after passive and active immunoprophylaxis. Thus, it illustrates the significance of adequate vaccine-induced immune response to protect infants at risk and the necessity of seromarker monitoring in children of carrier mothers (Chen et al. 2015). An open-label RCT included 200 Chinese HBsAg-positive pregnant mothers with HBV DNA level $>200,000$ IU/mL, who were 1:1 randomly assigned to control or TDF from 30 to 32 weeks of gestation until postpartum week 4. At postpartum week 28, the rate of MTIT was significantly lower in the TDF group than in the control group, both in the intention-to-treat analysis (5% vs. 18%, $P = 0.007$) and the per-protocol analysis (0 vs. 7%, $P = 0.01$). After discontinuation of TDF, ALT elevations above the normal range occurred more frequently in mothers in the TDF group than in those in the control group (46% vs. 30%, $P = 0.03$) (Pan et al. 2016). From the incubation period of HBV infection (average 75 days, range 30–180 days), surveillance of HBsAg or HBV DNA of children at risk when they are 6 months of age is appropriate to check MTIT status. Ongoing studies have been conducted regarding different target populations, different treatment durations, when to stop the AVT to facilitate breastfeeding without increasing risk of maternal ALT flare, and long-term follow-up of mothers and children.

3.4.6 When to Stop AVT Postpartum

If the ALT levels are normal during pregnancy and the goal of AVT is exclusively for preventing MTIT, mothers may stop antivirals soon after delivery, especially if they decide to breastfeed. Most clinical studies continued treatment until postpartum weeks 4–12 to reduce the risk of postpartum flares (Visvanathan et al. 2016). A

prospective study showed extending AVT (10–12 weeks versus 2 weeks versus no AVT) beyond delivery did not appear to reduce the frequency of HBV flares (40% versus 50%; no AVT, 29%, $p = 0.33$) over a median of 48 weeks of follow-up (Nguyen et al. 2014). However, if maternal ALT levels are elevated during pregnancy, continuation of AVT postpartum until the virological endpoints are achieved is recommended. A prospective observational study involved 241 pregnant women receiving LdT since the 24th or 28th week of gestation and opting cessation of therapy ($N = 143$) at postpartum week (PPW) 12 or continuation ($N = 98$, shifting to ETV) after PPW12. Stratified by ALT elevation or not during pregnancy, in those who stopped AVT at PPW12, hepatic flares were significantly higher in those who had ALT elevation during pregnancy (25% vs. 6.8%, $p = 0.003$). In those who continued AVT after PPW12, no virological or ALT flares developed at PPW24–52, while those with ALT elevation during pregnancy had higher HBeAg seroconversion rate (36.6% vs. 8.8%, $p = 0.001$ (Liu et al. 2016). It is suggested that all mothers receiving AVT treatment during pregnancy should be closely monitored for ALT elevations during treatment and in the first 6 months after delivery.

3.4.7 Breastfeeding Issue

The risk of HBV transmission from breastfeeding is negligible if infants are receiving timely neonatal immunoprophylaxis as recommended. For mothers under AVT, it is recommended not to breastfeed when taking the drug, mainly due to insufficient safety data regarding potential exposure of the drug to infants. LAM may be excreted into breast milk from HIV-infected women treated with 300–600 mg/day in very low concentration, 0.183 mg/kg/day, which is much lower than the therapeutic dose of 4–8 mg/kg/day for an HIV-positive infant and a very low mean serum concentration in infants (0.03 µg/mL), indicating very low intake amount of lamivudine from the GI tract of infants (Moodley et al. 1998; Shapiro et al. 2005). The median concentration of TDF measured in breast milk is approximately 3% of the median maximum concentration measured in mother's serum, around 0.03% of infant proposed therapeutic dose, and such low amounts of active drug found in breast milk are not absorbed through the baby's GI tract (Visvanathan et al. 2016; Benaboud et al. 2011). There is no data on the excretion of LdT into human milk. LdT is excreted into rat milk. Although expert opinion recommends LAM or TDF should not be contraindicated in nursing mothers with HBV monoinfection (Visvanathan et al. 2016), the manufacturers recommend not to breastfeed when taking the drug.

4 Conclusions and Future Scope

Pregnant women and children with chronic HBV infection are two special populations in the global burden of HBV infection, both serving as patients themselves as well as infectious sources and, through mother-to-infant transmission, perpetuating the reservoir of HBV. To combat HBV infection before it can cause any damage to the host or transmission to vulnerable people, we should treat it

early if primary prevention by immunoprophylaxis fails. However, due to the vulnerability of these subjects, most studies implement an observation scheme for disease courses in children or pregnant women. Thanks to the advent of more potent antivirals and safety profiles, therapies in children and pregnant women have entered a new era after the accomplishment of critical trials. However, there are still many unsolved issues, including whether or not to treat children in the immunotolerant phase, insufficient approved antivirals in children, the evolution between AVT and AVT-induced mutants, the threshold target of viral loads in children and in pregnant mothers, the long-term impact of AVT on children or mothers/offspring, and, finally, lack of ccc DNA eradication effect for HBV. No matter how much armamentarium is, the foundation to all measures for HBV eradication is the maximization of hepatitis B immunization programs worldwide. Additionally, universal screening of high-risk populations, timely application of antivirals, close monitoring the disease course, and surveillance of HCC are essential in the successful management of CHB. Development of new antiviral therapies to eradicate HBV infection in childhood is highly anticipated.

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Man-Fung Yuen

Abstract

Occult hepatitis B infection is a unique disease entity defined by detectable hepatitis B virus DNA in the sera and/or livers in subjects who are negative for HBsAg. OBI can be classified by serology. It can also be classified according to the medical history of the subjects. Majority of subjects with OBI have had chronic hepatitis B infection with subsequent loss of HBsAg during the natural history of disease or as a result of antiviral treatment. There are still challenges in diagnosing this condition in many service-based laboratories. The prevalence of OBI was estimated to be ranging from <1 to 18% in general population. The HBV usually replicates at an extremely low level due to various postulated mechanisms. The clinical significance of OBI is particularly prominent in three main areas: (1) transmission of HBV through blood and organ donations, (2) development of chronic liver diseases and liver-related mortality and (3) HBV reactivation during immunosuppressive therapy. With the use of more potent immunosuppressive therapies including B-cell depleting agents, the disease manifestations become more apparent which require more research to establish clinical management guidelines.

Keywords

Hepatitis B virus • Occult hepatitis B infection • Clinical implication • Hepatitis B virus replication • Hepatocellular carcinoma • Hepatitis B reactivation

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1 Introduction

Hepatitis B virus (HBV) infection is typically classified into acute infection with full recovery and chronic infection with life-long carriage state. It is generally believed that the former has no long-term clinical sequelae, whereas the latter is associated with a considerable risk of development of long-term liver-related complications. These include disease reactivation with hepatitis flares, cirrhosis leading to liver decompensation and/or hepatocellular carcinoma (HCC). To distinguish these two prognostically distinct conditions, the presence or absence of HBsAg of more than 6 months after the initial infection is the criterion. In other words, disappearance of HBsAg measured by conventional assays within 6 months signifies acute HBV infection, whereas persistence of HBsAg beyond this duration signifies chronic HBV infection. However, challenges were brought out by studies showing that HBV DNA were still present in serum and/or liver of subjects after decades of documented acute HBV infection (Raimondo et al. 2008a, 2013; Michalak et al. 1994; Yotsuyanagi et al. 1998; Yuki et al. 2003; Rehmann et al. 1996; Bläckberg and Kidd-Ljunggren 2000). One of these studies also documented the presence of HBV DNA in the peripheral blood mononuclear cells of subjects up to 70 months after recovery of acute hepatitis B infection (Michalak et al. 1994). This special condition is under the category of “occult hepatitis B infection” (OBI) which was consensually defined in a meeting held in Taormina 2008 as HBsAg-negative subjects with detectable HBV DNA either in serum or the liver (Raimondo et al. 2008b). In fact, this entity of OBI has been firstly described back in 1979 (Tabor and Gerety 1979).

It is noteworthy that majority of patients with OBI are not subjects with acute HBV with persistent viraemia. Patients with chronic hepatitis B (CHB) who lost HBsAg (HBsAg seroclearance) either naturally or through drug treatment indeed attribute for the majority of this population. There is also a small proportion of patients with CHB in which the HBV has hepatitis B surface gene mutations altering the epitopes of HBsAg leading to non-reactivity to the conventional HBsAg assays (Yamamoto et al. 1994; Hou et al. 1995; Carman et al. 1997). This is considered to be the minor group because studies have shown that the HBV in most of the OBI subjects do not have corresponding mutations in the HBsAg genome (Pollicino et al. 2004, 2007; Huang et al. 2014;).

2 Classifications of OBI Patients

OBI patients can be classified by two different ways. First, patients can be classified according to the serology of HBV. Second, patients can be classified according to their medical histories of HBV infection.

2.1 HBV Serology

Sero-positive OBI refers to the presence of anti-HBs and/or anti-HBc in the serum. On the contrary, absence of detectable serum anti-HBs and anti-HBc indicates sero-negative OBI. The hypothesis to explain the total absence of antibodies in

sero-negative OBI is the insufficient specific immune response in the presence of a very minute level of hepatitis B viraemia. According to several studies, up to 20% of OBI subjects are sero-negative (Morales-Romero et al. 2014; Raimondo et al. 2007). At present, there are no detailed studies to compare sero-positive with sero-negative OBI in terms of the disease underlying mechanisms and the clinical disease profiles.

2.2 Medical History of HBV Infection

2.2.1 Subjects with Documented History of Acute HBV Infection

More than 95% adult subjects contracting HBV is associated with “clinical” recovery without HBV chronicity. They would have anti-HBs and anti-HBc positivity with or without clinical symptoms of malaise, loss of appetite and jaundice. Although it is believed that these subjects are free of developing long-term clinical sequelae, it has been shown that HBV DNA was detectable in some subjects even after 70 months of acute HBV infection (Michalak et al. 1994). The findings were subsequently confirmed by two other studies in which HBV DNA were still detectable 6 months to 13 years after the acute HBV infection (Yotsuyanagi et al. 1998; Penna et al. 1996). In spite of the presence of low-grade viraemia in these subjects, their livers are usually with minimal necroinflammation (Yuki et al. 2003; Bläckberg and Kidd-Ljunggren 2000). As such, nearly all these subjects would have normal liver biochemistry. These are compatible with the findings in studies of woodchucks recovering from acute HBV infection with detectable viraemia in that the degree of liver inflammation was low (Mulrooney-Cousins and Michalak 2007).

The clinical significance of OBI subjects after acute HBV infection remains obscure. It is however expected that the chance of developing long-term liver-related complications is low basing on the fact that these subjects have very low viraemic levels, normal histology and alanine aminotransferase levels. However, the clinical implications become important in the aspect of HBV transmission to uninfected subjects through blood product donations. In addition, with the more profound immunosuppressive effects exerted by B-cell depleting monoclonal antibodies for various malignant and immunological diseases, the problem of HBV reactivation as a result of immune reconstitution in these subjects has been increasingly recognized (Yuen 2016). These topics will be discussed below.

2.2.2 Subjects with Chronic Hepatitis B Infection

As mentioned above, majority of subjects with OBI are CHB patients who undergo an uncommon event of HBsAg seroclearance. This event happens either spontaneously or with the assistance of antiviral therapy. In the former situation, patients undergo the different phase of CHB infection from immunotolerance through immune clearance before entering into the residual phase where HBsAg seroclearance may occur (Fig. 15.1). It has been shown that HBsAg seroclearance occurs in these subjects at an annual rate of 0.1–2% (Lai and Yuen 2009; Liu et al. 2010). In patients receiving antiviral therapy, the rate of HBsAg seroclearance is around 10% in long-term follow-up studies (Yuen et al. 2016).

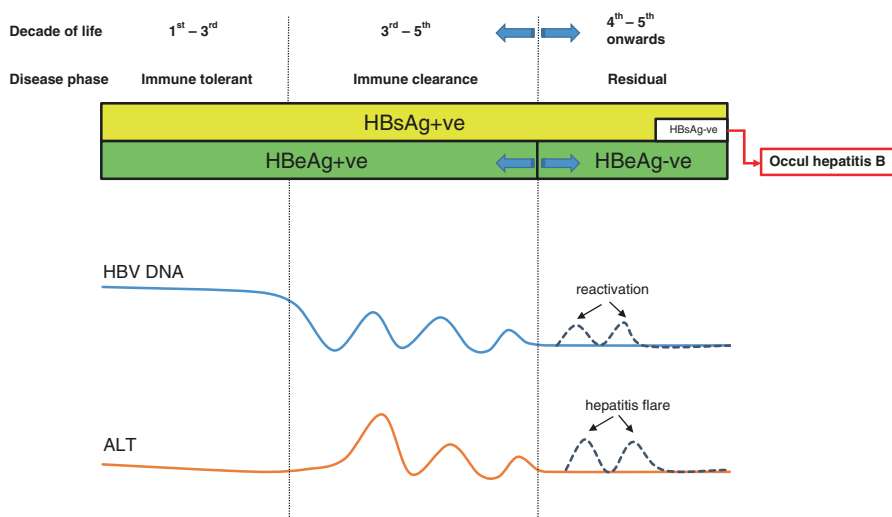


Fig. 15.1 Different phases of chronic hepatitis B infection

Around 60% of CHB patients with HBsAg seroclearance would develop anti-HBs at the time of HBsAg seroclearance or at the subsequent follow-up after HBsAg seroclearance (Yuen et al. 2008). CHB patients with HBsAg seroclearance usually have undetectable HBV DNA in the serum. The serum HBV DNA detection rate inversely correlates with the time of measurement after HBsAg seroclearance. For instance, serum HBV DNA was detectable in 13%, 6% and 3.7% when the measurements were carried out at 1, 5–10 and >10 years after HBsAg seroclearance (Yuen et al. 2008). It is worth to note that the intrahepatic HBV DNA was detectable (although at low levels) practically in all subjects with HBsAg seroclearance at all times (Yuen et al. 2008; Ahn et al. 2005). The HBV transcriptional activities however are minimal as indicated by negligible mRNA expression of the core, surface and X genes (Yuen et al. 2008; Kuhns et al. 1992). It is therefore an expected observation among 29 patients with liver biopsies that two-third of them had no necroinflammation and fibrosis and the remaining one-third only had mild necroinflammation or fibrosis (Yuen et al. 2008). It has further been shown that for those with early HBsAg seroclearance, e.g. <40 years old, none of these subjects had fibrosis in the liver (Yuen et al. 2004).

With regard to the chance of the development of long-term liver-related complications in CHB patients with HBsAg seroclearance, it largely depends on the clinical status at the time of HBsAg seroclearance. According to an early study (Huo et al. 1998), HBsAg seroclearance did not reduce the risk of development of complications as the cumulative probability of developing them was as high as 30% after 4 years of HBsAg seroclearance. Nevertheless, a subsequent study showed that this risk would be lower if there were no other viral co-infection (e.g. HCV infection) and no pre-existing cirrhosis (Chen et al. 2002). A study conducted in Hong

Kong showed that those who achieved HBsAg seroclearance before the age of 50 years had a significantly lower risk of development of complications compared with patients who had the HBsAg seroclearance after the age of 50 years (Yuen et al. 2008). None of the former group developed HCC compared to 10% ($p = 0.004$) of patients developing HCC in the latter group at the median follow-up of 3 years after HBsAg seroclearance. It is likely related to the lower proportion of patients having severe fibrosis or cirrhosis when the HBsAg seroclearance occurring at a younger age.

2.2.3 Subjects with Unknown HBV History

There are subjects in whom OBI is diagnosed by routine screening especially during the blood donation surveillance. There are many blood donation centres worldwide routinely performing nuclear acid testing either in pooled or individual samples which are firstly tested negative for HBsAg. The absence of the medical history of HBV infection in these subjects makes it impossible to differentiate unnoticed clinically silent acute HBV subjects from CHB patients with HBsAg seroclearance who are never known to themselves or their physicians.

In the context of OBI subjects diagnosed incidentally in the blood donation services, a study with 40 OBI blood donors showed that the liver biochemistry was normal in all subjects. They also had negligible liver necroinflammation (median necroinflammatory score: 1 out of 18) and fibrosis (median Ishak's fibrosis score: 1 out of 6) (Wong et al. 2016). The viral replicative status was extremely low. Only 13% of OBI subjects had detectable pre-genomic RNA, and the median intrahepatic HBV DNA was 0.22 copies/cell compared to 260 copies/cell ($p < 0.0001$) in age- and sex-matched HBsAg-positive CHB patients. Only 45% of OBI subjects had serum HBV DNA > 1.1 IU/mL.

3 Prevalence of OBI

The true prevalence of OBI worldwide has not been well studied because of several unresolved issues. First, the HBV DNA level in serum is notoriously low for it to be measurable by standard commercial assays. Although the HBV DNA level was described to be usually less than 200 IU/mL (Morales-Romero et al. 2014), studies have shown that the HBV DNA levels were typically less than 20 IU/mL (Yuen et al. 2010, 2011). Second, the fluctuations of low HBV DNA levels in OBI subjects often cast doubts on the reliability of the sensitive HBV DNA assays. These two difficulties may be partly overcome by using large-volume samples for testing. Finally, although the yield of detectable HBV DNA can be greatly enhanced by measuring it in the liver tissues, invasive nature of liver biopsy makes it an unpopular methodology in many studies. With all these issues to be considered, prevalence of OBI was estimated to be <1 to 18% (Yuen et al. 2008, 2010; Song et al. 2009; Bhatti et al. 2007; Werle-Lapostolle et al. 2004; Reesink et al. 2008; Georgiadou et al. 2004; Fang et al. 2009; Minuk et al. 2005; Kim et al. 2007; Svicher et al. 2012). According to a large-scale study in

Hong Kong with 9990 blood donors, the prevalence of OBI was found to be 0.11% (Yuen et al. 2010). It suggests that the prevalence of OBI may not be as high as one would expect even in region with high CHB endemicity.

4 Pathogenesis of OBI

There are several mechanistic postulations for the existence of OBI. These include the viral and host factors. It has long been proposed that mutations over the major surface gene, e.g. G145R, may lead to detection escape from the standard HBsAg assays (Yamamoto et al. 1994; Hou et al. 1995; Carman et al. 1997). It is however not supported by three studies in which corresponding mutations as suggested were not found in OBI subjects (Pollicino et al. 2004, 2007; Huang et al. 2014). In addition, a study also showed that most of the standard assays were able to detect various mutated HBsAg epitopes (Yuen and Lai 2006). Another hypothesis is the persistently low viral replication in OBI subjects because of two possible but interlinking factors. The HBV may be intrinsically replicate at an extremely low level because of multiple genomic mutations or rearrangements in splice donor sites of pre-S1, pre-S2, S genes as well as their regulatory genomic regions (Candotti et al. 2012; El Chaar et al. 2010). This is supported by studies showing greater sequence diversities in both nucleoside and amino acid levels in subjects with OBI compared with HBsAg-positive CHB subjects (Huang et al. 2014; Pollicino et al. 2004; Lai and Yuen 2009). The viral replication capacity may be much restrained with all these changes which may be additive or synergistic. At the post-transcriptional level, it was found that Pre-S2/S RNA splicing may also reduce the pre-S2/S and HBsAg transcripts (Candotti et al. 2012; Hass et al. 2005). Epigenetic factor exerting on the virus has also been postulated. It has been shown that the cccDNA-bound histones in OBI subjects were highly hypoacetylated which may result in low HBV replication (Pollicino et al. 2006).

The other factor interlinking with viral replication is the more intense immune control of the HBV in OBI subjects. There is an indirect but very strong evidence from the phenomenon of HBV reactivation in HBsAg-negative, anti-HBc-positive subjects (potential OBI subjects) under immunosuppressive therapy especially with potent anti-B-cell depletive effect, e.g. anti-CD 20 (Larrubia 2011; Bes et al. 2012). It suggests that OBI status is successfully kept by adequate immune control and the virus reactivates upon withdrawal of this controlling factor under immunosuppression. To explore this, studies to compare the HBV-specific T-cell responses between OBI and CHB subjects are deemed to be important. In fact, intense immune response has been shown to be detectable many years after the recovery of acute HBV infection (Rehermann et al. 1996; Penna et al. 1996).

Finally, there may be external factors inhibiting HBV replication, e.g. co-infection with other viruses, leading to the OBI state. In HCV-HBV co-infection, HBV replication is often diminished because HCV core protein has been shown to exert inhibitory effects on HBV replication (Chen et al. 2003; Raimondo et al. 2005; Schüttler et al. 2002; Shih et al. 1995). It seems to be in accordance to the observation

that OBI is apparently more commonly found in HCV patients than other populations. Therefore, HCV may play a role in inducing OBI status (Pollicino and Raimondo 2014).

5 Clinical Significance of OBI

There are three major clinical areas where OBI plays an important role (Fig. 15.2). They include (1) the possibility of transmission of HBV disease through blood and organ donations, (2) the pathologic role on chronic liver disease and liver-related complications and (3) the HBV reactivation during immunosuppressive therapy.

5.1 Transmission of HBV

OBI donors, although usually having very low HBV viraemia, may transmit the HBV to other individuals in the setting of blood donation and organ transplantation. It is because any products containing full HBV viral particles are considered to be potentially infectious. According to a chimpanzee study, HBV DNA of only ten copies can already achieve the minimum 50% infectious dose of HBV (Komiya et al. 2008). In another study using severe immunodeficient chimeric mice with human hepatocytes, serum HBV DNA, intrahepatic HBV DNA as well as covalently closed circular (ccc) DNA (the viral replication template) were detectable in one of the four mice inoculated with the sera from OBI subjects (Yuen et al. 2011). The mouse hepatocytes were also stained positive for HBcAg. Whether these findings are applicable to human would require actual human studies to verify as the immune components in animals are different and the mice are basically immunodeficient. In addition, there are other confounding factors in human situations influencing the chance of HBV transmission from OBI subjects. These

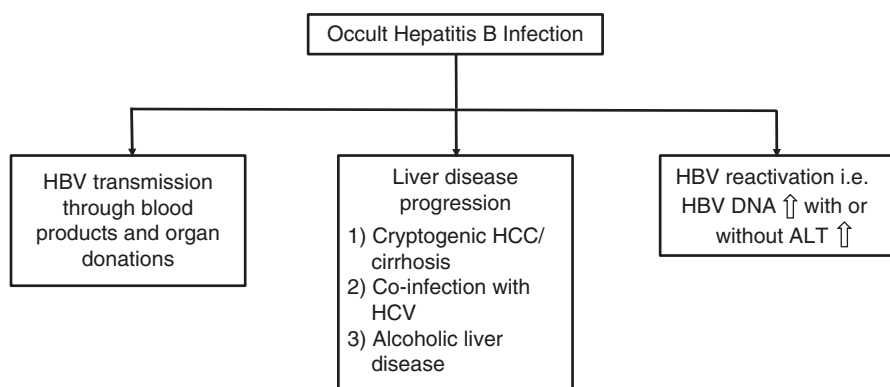


Fig. 15.2 Clinical scenarios of occult hepatitis B infection

include anti-HBs status of both the donors and the recipients, the kind and the volume of the blood products being transfused to the recipients and the immunological status of the recipients (Raimondo et al. 2013; Mosley et al. 1995; Satake et al. 2007). Finally, the actual circulating viral load (which may fluctuate with times) of the OBI donors at the time of donation may also affect the outcome (Chemin et al. 2009).

Although there are already many human studies examining the transmissibility rate of HBV from OBI blood donors, most of these studies are retrospective in nature. This greatly limits the effectiveness of contact tracing to the recipients. In areas where HBV is of high endemicity, meticulous phylogenetic analysis of the viral sequence between the donors and recipients which are especially essential to establish the HBV transmission is required. This is however difficult to achieve in context of the retrospective nature of the studies. In addition, the difficulty in obtaining good quality of viral sequence (from amplification of the low HBV DNA levels) also impinges the study accuracy.

Nevertheless, even with all these problematic issues, there are several studies performed to determine the HBV transmission rate from OBI donors. It was found that the transmission rate was low at around 1–3% (Yuen et al. 2011; Mosley et al. 1995; Candotti and Allain 2009). One the studies further showed that the risk was greatly reduced if the donor serum was anti-HBs positive (Mosley et al. 1995). From a study conducted in Japan, of the identified 12 subjects with transfusion-related hepatitis B, all received the blood products from donors who were negative for both anti-HBs and anti-HBc (Satake et al. 2007). It is likely that these donors were at the window period of the acute HBV infection when all the antibodies were yet to be detectable. This group of donors may also be sero-negative OBI donors if their sera remain to be negative for both anti-HBs and anti-HBc.

There are studies showing that the HBV transmission is possible from anti-HBc-positive donors (Hoofnagle et al. 1978; Lander et al. 1978; Koziol et al. 1986). The transmissible rate is around 2.4–3.0% according to one study (Lai and Yuen 2009). It is compatible with another study conducted in Hong Kong using detailed phylogenetic relatedness methodology. This study showed that the transmission rate of 2.2% (Yuen et al. 2011).

In the context of organ transplantation in which the recipients receive organs from OBI donors, HBV transmission may also be of concern. Unlike the situation in blood product donations given to usually immunocompetent recipients. Patients receiving organ transplantation have an additional risk factor of acquiring the HBV from OBI donors because of the immunosuppressive status rendered by the immunosuppressive therapy for prevention of organ rejection. In the setting of liver transplantation, it has been estimated that 22–100% of liver-transplanted recipients would have de novo HBV infection of the liver grafts from HBsAg-negative, anti-HBc-positive donors (Dickson et al. 1997; Muñoz 2002). It seems that recipients positive for both anti-HBs and anti-HBc are at a relatively low risk for de novo HBV infection. It should also be noted that HBV reactivation from pre-transplanted, “hidden” HBV of the OBI recipients is also possible especially after the use of intensive immunosuppressive therapy for the liver transplantation.

Finally, unlike the situation in liver transplantation, the risk of HBV transmission from OBI donors to recipients of kidneys, hearts and bone marrows seems to be lower (Wachs et al. 1995; De Feo et al. 2005). It is now a routine procedure in many centres that prophylactic antiviral therapy would be given to patients who are going to receive organs from OBI donors. Some centres are also extending this prophylactic measure to recipients receiving anti-HBc-positive donors with undetectable HBV DNA.

5.2 Role on Chronic Liver Disease and Complications

For chronic liver diseases, the initial activities can be assessed by the liver biochemistry and the liver histology, the more reliable parameter for assessment of liver damage. According to various studies (Yuen et al. 2010; Fung et al. 2010; Wong et al. 2014), OBI subjects usually have normal liver biochemistry as well as very minimal histological inflammation and fibrosis. However, it has been shown in early 1980s that HBV DNA were detectable in HBsAg-negative patients with chronic liver disease implying a possible pathologic role of OBI (Bréchet et al. 1981, 1985). It is therefore not surprising to observe that around 5–50% of cirrhotic patients without identifiable cause have documented presence of OBI (Pollicino et al. 2004; Hou et al. 2001; Bréchet et al. 2001; Alavian et al. 2012).

The pathologic roles of OBI are firstly described in HBsAg-negative alcoholic patients with HCC (Bréchet et al. 1982). This suggests a possible synergism between these two factors leading to the development of HCC. According to a recent study, 56% of HBsAg-negative alcoholic patients with HCC had the presence of HBV DNA in the liver (Wong et al. 2011). There are even more studies examining the role of OBI in patients with chronic hepatitis C virus (HCV) infection. They have shown the pathological component of OBI in causing cirrhosis and HCC in patients co-infected with HCV (Bréchet et al. 1985; Cacciola et al. 1999; Fukuda et al. 1999; Tanaka et al. 2004; Squadrito et al. 2006; Shetty et al. 2008; Adachi et al. 2008; Shi et al. 2012). For instance, HCV patients with OBI had more advanced fibrosis compared to HCV patients without OBI (Matsuoka et al. 2008). Liver cirrhosis was found in 33% of HCV patients with OBI compared to 19% of HCV mono-infected HCV patients (Cacciola et al. 1999). In addition, HBV DNA and anti-HBc positivity were found to be two independent risk factors for the development of HCC in HCV patients (Adachi et al. 2008; Matsuoka et al. 2008; Ikeda et al. 2007). All these findings suggest that HCV patients with OBI carry a more progressive course for the development of cirrhosis and HCC. A recent meta-analysis study showed that HCV patients with OBI had a 2.8-folds higher risk of development of HCC compared to mono-infected HCV patients (Shi et al. 2012). However, there are studies which did not show an additively or synergistically increased risk for the development of liver complication by OBI (Sagnelli et al. 2008; Kao et al. 2002; Tsubouchi et al. 2013). As these studies are all cross-sectional and also of small scale, the best way to confirm whether OBI has additive or synergistic effects on development of liver complications in HCV patients is to have large-scale longitudinal studies.

Apart from the possible role of OBI in patients with alcoholic liver disease and patients with HCV infection, OBI may also be the primary aetiological cause for liver disease patients with apparently no identifiable cause, i.e. cryptogenic cirrhosis or HCC. It has been shown by very early studies that HBV DNA was detected in HBsAg-negative patients with HCC (Bréchet et al. 1981; Shafritz et al. 1981). The proportion of patients with cryptogenic HCC having OBI is in a range between 48% and 73% according to two studies (Wong et al. 2011; Yotsuyanagi et al. 2000). In fact, OBI was shown to be an independent risk factor for liver carcinogenesis with the hazard ratio of 8.3 (Ikeda et al. 2007). According to a longitudinal study performed in Japan, there was an association between OBI and HCC. In a meta-analysis study including 16 retrospective and prospective studies, OBI was confirmed to be a risk factor for the development of HCC with the hazard ratio of three when the analyses were limited to the five prospective studies (Shi et al. 2012). Another meta-analysis revealed a ninefold increased risk of development of HCC in OBI subjects (Covolo et al. 2013).

Concerning the best methodology for detection, different studies use different experimental protocols targeting for different genomic regions of HBV. In general, the detection rate is higher in non-tumorous parts compared to tumorous parts of the liver. In addition, sequencing the X gene compared to other genomic regions also yields a higher positive rate (Wong et al. 2011).

Similar to the oncologic mechanisms of overt CHB disease, mechanisms of OBI causing HCC include the following:

1. Persistently low-grade necroinflammation leading to cirrhosis of the liver (it may be possible that there are unrecognized high-grade necroinflammation during the overt stage of CHB disease before entering into the OBI state) (Yuki et al. 2003; Bläckberg and Kidd-Ljunggren 2000).
2. Integration of viral sequence from OBI into the human genome triggering the oncogenic cascade (Pollicino et al. 2004; Bréchet et al. 1981, 2000; Shafritz et al. 1981; Tamori et al. 1999; Paterlini et al. 1995). Integration of the HBV gene into the human genomes is also commonly found in OBI patients (Bréchet et al. 1981). It has also been shown that the process of HBV DNA integration can happen even at the time of acute HBV infection (Murakami et al. 2004; Kimbi et al. 2005).
3. OBI may have HBV transcriptional activities producing oncogenic proteins, namely, protein X and truncated pre-S-S protein (Cougot et al. 2005).

5.3 HBV Reactivation

HBV reactivation can be associated with significant liver-related morbidity and mortality. Under normal circumstance, spontaneous HBV reactivation from OBI is rare. In most of the OBI subjects, the serum HBV DNA level remains undetectable, and if it is detectable at baseline, it usually remains very low throughout subsequent follow-up. However, there are circumstances where HBV may

reactivate from this low viraemic state. It is well known that HBsAg-positive CHB patients undergoing chemotherapeutic treatment and immunosuppressive therapy, in particular steroid containing regimes, have a considerable risk of HBV reactivation (Yuen 2016; Cheung et al. 2016). As such, it is already firmly established that all HBsAg-positive CHB patients before receiving immunosuppressive therapy are recommended to receive antiviral prophylaxis to prevent HBV reactivation. However, this recommendation has not been universally implemented in OBI subjects yet.

As a result of the increasing use of biologics and monoclonal antibodies with potent immunosuppressive effects for autoimmune diseases and haematological malignancies, HBV reactivation from OBI subjects have emerged to be a more serious problem nowadays. It was found that there was no HBV reactivation from HBsAg-negative, anti-HBc-negative subjects with lymphoma receiving chemotherapy (Yeo et al. 2009). However, approximately 24% of anti-HBc-positive subjects receiving cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP) plus anti-CD 20 (rituximab) had HBV reactivation resulting in one death. On the other hand, there was no HBV reactivation in anti-HBc-positive subjects if they received rituximab-free treatment regime. It strongly suggests that rituximab significantly increases the risk of HBV reactivation in OBI subjects. This issue was studied in detail by a prospective study recently (Seto et al. 2014). Of the 63 HBsAg-negative, anti-HBc-positive subjects (all with undetectable serum HBV DNA) receiving rituximab, 42% of subjects develop HBV reactivation (Seto et al. 2014). It was also found that the rate of HBV reactivation was lower in anti-HBs-positive subjects compared with anti-HBs-negative subjects (34% vs. 68%, respectively, $p = 0.012$). Prompt treatment using entecavir at the time of HBV reactivation was able to control the HBV DNA level to undetectable level in all patients, and therefore no hepatitis flares were observed.

Concerning the biologics, the rate of HBV reactivation from OBI subjects was lower than that of using rituximab. According to a large-scale study with HBsAg-negative and anti-HBc-positive, the use of anti-tumour necrosis factor was associated with the HBV reactivation rate of 1.7% (Lee et al. 2013). The rate of HBV reactivation is however, considerably higher (15–35%) in HBsAg-positive subjects receiving anti-tumour necrosis factor (Perez-Alvarez et al. 2011).

Patients with haematological malignancy are usually at a higher risk of HBV reactivation because of the high degree of bone marrow suppression by intense immunosuppressive therapy. Among those, patients receiving haematopoietic stem cell transplantation (HSCT) are having the highest rate of HBV reactivation. The rate of HBV reactivation in HSCT patients who were HBsAg-negative and anti-HBc-positive is in a range of 3 and 43% as reported by different studies (Vigano et al. 2011; Hammond et al. 2009; Nakamoto et al. 2014). A recent prospective study with 62 HSCT patients showed that the HBV reactivation was as high as 40% with 2 years of follow-up (Seto et al. 2017). In addition, patients with chronic graft-versus-host disease (requiring more intense and long-term immunosuppressive therapy) and patients older than 50 years old would have a higher rate of HBV reactivation compared to subjects without these factors.

The underlying mechanisms involved in the HBV reactivation in OBI subjects are believed to be the same as for CHB subjects. These include direct increase in HBV replication related to the agents of the therapy. It has been well known that steroid can increase HBV replication as it can act through the glucocorticoid-responsive element in the HBV genome. As the immune control may be further jeopardized by the B-cell depleting agent, e.g. rituximab, HBV may reactivate whenever under a less intensive immune control environment. Finally, at the later stage upon cessation of the immunosuppressive therapy, the immune reconstitution may exert an even more overwhelming immune-mediated attack to the hepatocytes where the HBV load is high.

Because of the potential mortality from HBV reactivation, prevention using prophylactic antiviral agents is generally recommended. It is now a universally accepted protocol to prescribe antiviral agents for HBsAg-positive patients undergoing chemotherapy or immunotherapy. For OBI subjects, this practice is also widely implemented for those with detectable HBV DNA. A controversy, however, exists in HBsAg-negative, anti-HBc-positive patients with undetectable HBV DNA. Some clinicians advocate the prophylactic approach, whereas others adopt a close surveillance strategy. The latter approach recommends HBV DNA monitoring at a 4–12 weekly interval. According to a study of lymphoma patients receiving rituximab, prompt administration of entecavir at the time of detectable HBV DNA checked at 4 weekly intervals is able to render HBV DNA undetectable without hepatitis flare in all patients (Seto et al. 2014). This strategy is also recommended by treatment guidelines (European Association for the Study of the Liver 2012). At present, there are no studies to determine the best monitoring intervals for HBV DNA. Practical logistics, manpower and costs are the major factors for the consideration. Another issue is whether the “interval” of monitoring would be the same for all kinds of chemotherapy and malignancy. It is because the risk of HBV reactivation would largely be determined by the type of chemotherapy and to a lesser extent the specific malignancy.

In summary, OBI is now a well-recognized disease entity with increasing attention in various aspects of liver diseases. The prevalence is yet to be studied in greater detail. The diagnosis of OBI is greatly dependent on the clinicians’ awareness and the availability of standard and accurate HBV DNA testing. The presence of anti-HBc would trigger off the investigative steps. Subjects with OBI are potentially infectious with a low transmissibility rate in which the level of viraemia and the immune status of the recipients are the two major determinants. OBI can lead to end-stage liver disease and HCC, and this potential aetiology should be actively sought for in patients with cryptogenic cirrhosis and/or HCC. HBV reactivation from OBI is now being increasingly recognized in the present era of using potent B-cell depleting therapy for various malignant and immunological conditions. While effective antiviral therapy reducing the morbidity and mortality from the HBV reactivation is already in place, the best strategy of prevention of HBV reactivation in these subjects remains to be defined.

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Hepatitis B Virus Reactivation and Management of Patients Undergoing Immunosuppression

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Prowpanga Udompap and W. Ray Kim

Abstract

Hepatitis B reactivation (HBR) occurs in patients with inactive or resolved hepatitis B virus (HBV) infection, in whom the host immune response is overtaken by the replicative drive of the virus. While HBR may be clinically silent, there is abrupt reappearance or rise of HBV DNA in the serum (usually > 2 log) with or without a flare of alanine aminotransferase (ALT) activities. HBR commonly occurs in the setting of B-cell depleting agents and corticosteroids although cytotoxic chemotherapeutic and other immunosuppressant and immunomodulant agents may precipitate it. The risk of HBR may be determined by host characteristics, viral factors, and the type of medical therapy. For an optimal outcome, screening for patients at risk of HBR is critical. For patients identified to be at risk, strategic utilization of antiviral prophylaxis has been shown to improve outcomes.

Keywords

Hepatitis B • Reactivation • Immunosuppression

1 Introduction

Hepatitis B reactivation (HBR) is characterized by an abrupt reappearance or rise of hepatitis B virus (HBV) DNA in the serum of a patient with previously inactive or apparently resolved HBV infection (Lok et al. 1991). Compared to their baseline status of minimal or inactive disease activity, patients experiencing HBR may end up with recrudescence or flare of hepatitis activity. The most severe end of the spectrum may be symptomatic acute hepatitis, which may progress to fulminant liver failure.

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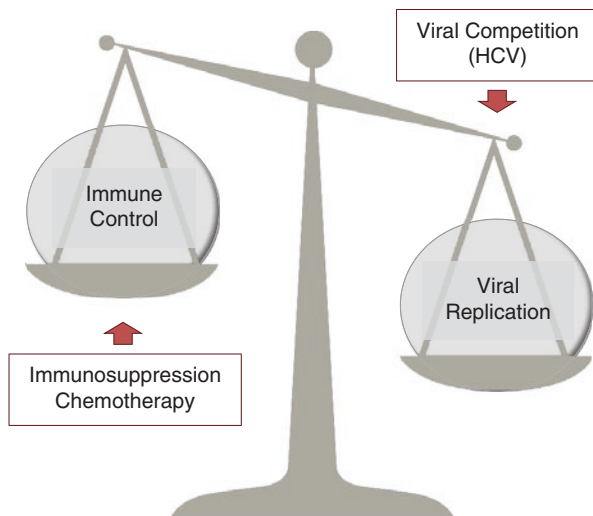
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Fig. 16.1 The occurrence of HBR depends on the balance between the replicative drive of the virus and the immune response from the host



In the background of HBR is the balance between the replicative drive of the virus and the immune response from the host (Fig. 16.1) (Lok et al. 1991; Yeo et al. 2004a). In patients with inactive HBV infection, viral replication is inhibited as a result of the control by the host immune system. HBR occurs when this balance is perturbed by an environmental agent such as an immunosuppressive or cancer therapeutic compound which lifts the immune control allowing HBV replication to resume (Hoofnagle 2009; Keam et al. 2011). More recently, there may be another mechanism to HBR when there is a coexistent hepatotropic virus such as hepatitis C virus (HCV). Successful eradication of HCV with recently available direct-acting antiviral agents has been associated with HBR.

Although HBR is often temporary and clinically silent, it may cause a symptomatic flare of hepatitis. While the flare in and of itself may evolve into a serious condition incurring morbidity and even mortality, another major clinical consequence of HBR is the need for interruption of immunosuppressive or chemotherapy. Moreover, in patients who are not suspected to have HBV infection, HBR may be a source of confusion and misdiagnosis, leading to a delay in appropriate clinical management. Hence, preventing HBR protects the patient from experiencing potentially dangerous flares and from failing to achieve the intended goals of the immunosuppressive therapy.

In this chapter, we will review the current literature on HBR and discuss its epidemiology, risk factors and mechanisms, manifestations and diagnosis, and clinical management in terms of prevention and therapy. We will also discuss challenges and future research direction and devote a section on the recent topic of HBR in the setting of HBV-HCV dual infection.

2 Mechanisms of HBR

HBV genome is able to persist in the liver for long periods of time, even when HBsAg is cleared in the serum. In patients in whom HBV infection is inactive, viral replication is suppressed by immune control of HBV infection, which is mediated through HBV-specific cytotoxic T cells (Rehermann et al. 1996; Zhang et al. 2012), while B cells also play a role in antigen presentation and viral clearance (Chang and Lewin 2007). Administration of an immunosuppressive drug to chronic HBV patients may potentially allow the virus to escape the immune control, leading to increased HBV replication and a marked increase in expression of HBV transcription intermediaries and products within hepatocytes (Keam et al. 2011).

2.1 Corticosteroids

Corticosteroids have a number of ways to promote HBV replication. The HBV genome has a glucocorticoid-responsive element which enhances replication of the virus (Tur-Kaspa et al. 1986; Calabrese et al. 2006; Tur-Kaspa et al. 1988). In a recent prospective study, half of the study patients had increased HBV DNA levels within 2 weeks of starting a corticosteroid-containing chemotherapy regimen. This occurred well before the development of neutropenia, suggesting a direct stimulatory effect of corticosteroids on HBV DNA transcription (Cheng et al. 2003). Indirectly, corticosteroids have a number of immunosuppressive effects including inhibition of cytotoxic T-cell function (Tur-Kaspa et al. 1988). The risk of HBR among those treated with corticosteroid varies by the dosages, duration of treatment, and HBV serologic status of the host.

2.2 B-Cell-Depleting Agents

Rituximab and ofatumumab are the two licensed monoclonal antibodies against CD20 of B cells in the USA, which possess potent immunosuppressive effects. They are used to treat hematologic malignancies and, less commonly, severe autoimmune diseases such as rheumatoid arthritis and vasculitis. While the mechanism of rituximab/ofatumumab-associated HBV reactivation is not completely understood, the putative mechanisms are that depletion of B cells and the resulting disruption of antigen presentation impair CD8+ cytotoxic T cell's ability to kill HBV-infected hepatocytes. Anti-CD20 antibodies reduce the number of CD4 memory T cells, increase Th1/Th2 and Tc1/Tc2 ratios, and upregulate Fas ligand on Th1 and Th2 cells, further impairing the host immune control against the virus (Evens et al. 2011; Misumi and Whitmire 2014; Tsutsumi et al. 2015). B-cell depletion may lead to loss of anti-HBs (Pei et al. 2012).

2.3 Cytotoxic Chemotherapeutic and Immunosuppressant Agents

Cytotoxic cancer chemotherapeutic agents disrupt cell cycles, leading to DNA destruction. Subsequent activation of chemotherapy-induced DNA repair mechanisms results in a cascade of responses including upregulation of promyelocytic leukemia protein (PML) and PML nuclear body (PML-NB), which have been linked with increased HBV pregenomic transcription, HBV-core expression, and HBV DNA replication (Chung and Tsai 2009). Traditional immunosuppressants such as methotrexate, azathioprine, and 6-mercaptopurine also disrupt DNA synthesis. However, these are apparently not as detrimental from the standpoint of HBR as other chemotherapeutic agents (Calabrese et al. 2006; Droz et al. 2013; Flowers et al. 1990).

2.4 Biological Immunomodulants

HBV-specific cytotoxic T cells inhibit hepatocellular HBV gene expression and replication through a noncytotoxic mechanism, which is mediated in part by TNF- α and interferon- α (Kasahara et al. 2003; Tzeng et al. 2014). Inhibition of TNF- α activity may lead to enhanced viral replication (Carroll and Forgione 2010). Currently, there are three commonly used TNF- α inhibitors, etanercept, infliximab, and adalimumab, all of which have been implicated in HBR.

Cytokine or integrin inhibitors have been introduced in practice only in the past few years, and reported experience of HBR related to these agents is limited. Abatacept blocks costimulation of T lymphocytes (Herrero-Beaumont et al. 2012). Imatinib and other tyrosine kinase inhibitors can inhibit T-cell activation and proliferation (Seggewiss et al. 2005; Dietz et al. 2004). Ustekinumab is a human monoclonal antibody that is directed to interleukin-12 and interleukin-23 (Cingoz 2009). Natalizumab and vedolizumab are recently developed inhibitors to the cell adhesion molecule α 4 integrin found on lymphocytes (Hutchinson 2007; Rezaie 2014). While empirical data are lacking, the direct effects of these agents on T-cell immunity raise concerns that the risk of HBR may be significant (Perrillo et al. 2015).

3 Incidence of HBR

The accurate incidence of HBR among patients with immunosuppressive therapy is poorly defined for a number of reasons. First, the settings in which HBR occurs are heterogeneous depending upon host characteristics, baseline HBV status, types of immunosuppressive therapy, and the underlying disease that requires the immunosuppressive therapy. Second, most studies are conducted in retrospective fashion and would be enriched with patients with severe HBR requiring medical attention. Finally, the criteria to diagnose HBR are not uniformly defined, creating further

heterogeneity in study results. With these caveats, we summarize data regarding the incidence of HBR according to the clinical scenario.

3.1 Patients Undergoing Cancer Chemotherapy

HBR was initially described in cancer patients undergoing chemotherapy. Today, the most common scenario for HBR to occur in cancer therapy is patients with hematological malignancies receiving anti-CD20 antibodies. The incidence of HBR is much lower with cytotoxic chemotherapy for solid tumors, with breast cancer being one of the most frequently tumors associated with HBR. The incidence of HBR in HBsAg+ patients being treated for cancer has been reported to be 14–72%, whereas it is much lower (<3%) among patients who are HBsAg–/anti-HBc+ (Lok et al. 1991; Kim et al. 2007; Kumagai et al. 1997; Yeo et al. 2000a, b; 2003; 2004b; Kusumoto et al. 2009; Vento et al. 2002). In patients receiving a regimen including anti-CD20 antibodies, the incidence of HBR may be as high as 60% in those with HBsAg+ (Abramson and Chung 2014; Mendez-Navarro et al. 2011; Mozessohn et al. 2015). Even in patients who are HBsAg–/anti-HBc+, HBR may be seen in 8–42%.

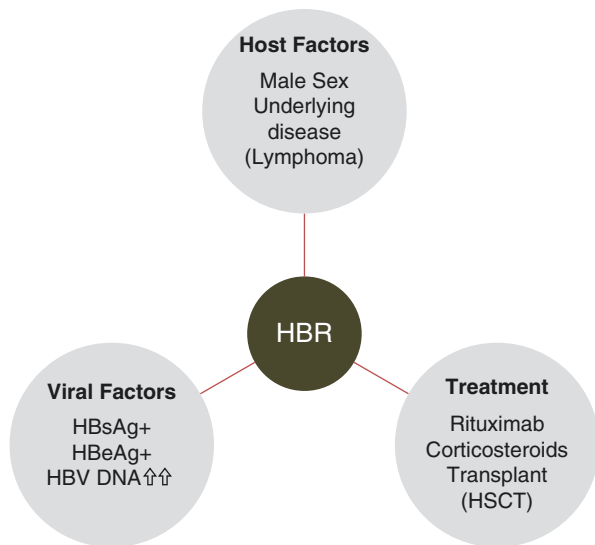
In addition to systemic chemotherapy, HBR may also occur in patients undergoing regional therapy such as transarterial chemoembolization (TACE) for hepatocellular carcinoma (HCC). Although the incidence of HBR in those settings has not been accurately defined, it may be as high as 34% (Jang et al. 2004).

3.2 Patients Undergoing Organ/Cell Transplantation

3.2.1 Hematopoietic Stem Cell Transplantation (HSCT)

Patients undergoing allogeneic HSCT tend to be heavily immunosuppressed, including immunoablative therapy applied prior to the infusion of the donor marrow. The incidence of HBR after HSCT is almost universal among HBsAg+ patients (Lalazar et al. 2007; Lau et al. 1997; Martin et al. 1995) and may be up to 50% in HBsAg–/anti-HBc+ patients (Hammond et al. 2009; Park et al. 2011; Seth et al. 2002; Vigano et al. 2011; Ramos et al. 2010; Knoll et al. 2004; Onozawa et al. 2005). Reverse HBsAg seroconversion describes the latter scenario in which a patient who initially lacked HBsAg becomes HBsAg positive. In HSCT patients, the risk of reverse seroconversion persists for many years because of the delay in reconstitution of the recipient's immune response to HBV. Reverse seroconversion may occur in patients who are initially anti-HBs positive: in studies measuring the anti-HBs titer serially, HSCT recipients gradually lost anti-HBs to become undetectable 1–3 years after transplantation. Meanwhile, HBV DNA increased, and HBsAg reappeared in the serum. In one retrospective study of HBsAg–/anti-HBc+ HSCT recipients, the cumulative probability of reverse seroconversion was 9% at the end of 1st year, which more than quadrupled to 43% at the end of 4th year (Hoofnagle 2009; Hammond et al. 2009).

Fig. 16.2 Three important factors affecting the risk for HBR



3.2.2 Solid Organ Transplantation (SOT)

The effect of HBV infection on the outcome of SOT has been studied most in kidney transplantation (KT). Patients with HBsAg+ have an increased risk of graft loss and mortality (Reddy et al. 2011; Fabrizi et al. 2005). The risk of HBR is higher in the HBsAg+ recipient, especially in those with detectable HBV DNA or HBeAg+ compared with HBsAg-recipients (Reddy et al. 2011). For the HBsAg+ recipients, Degos (Degos et al. 1988) demonstrated that HBR occurred in 11 of 12 (92%) initially HBsAg+ recipients. A study by Fornairon also described that 85% of HBsAg+ KT recipients developed histological progression, leading to cirrhosis and hepatocellular carcinoma in some patients (Fornairon et al. 1996). The reported HBR incidence among KT recipients with isolated anti-HBc+ was lower, varying from 0 to 6.5% (Chen et al. 2013; Kanaan et al. 2012).

4 Risk Assessment for HBR

The risk of HBR depends on three important factors: host, baseline status of HBV infection and the type of treatment (Fig. 16.2). Clearly, presence of HBsAg is a predominant determinant of HBR. In a study by Lau, individuals who were HBsAg+ carried a greater risk for HBV reactivation compared with those who are HBsAg- (HR 33.3, 95%CI 7.4–142.9, $p < 0.01$). Among those with HBsAg+, the risk of HBR correlates with markers of viral replication status, namely, HBeAg and serum HBV DNA. In particular, HBV DNA levels exceeding 10^5 copies/mL were associated with the highest risk of HBR (Lau et al. 2002). Compared to undetectable HBV DNA, detectable viremia was associated with a HR of 9.35 (95%CI 1.65–52.6, $p = 0.01$). Another study by Yeo evaluated risk for HBR among cancer patients treated with chemotherapy. They found that HBeAg positivity ($p < 0.01$), male

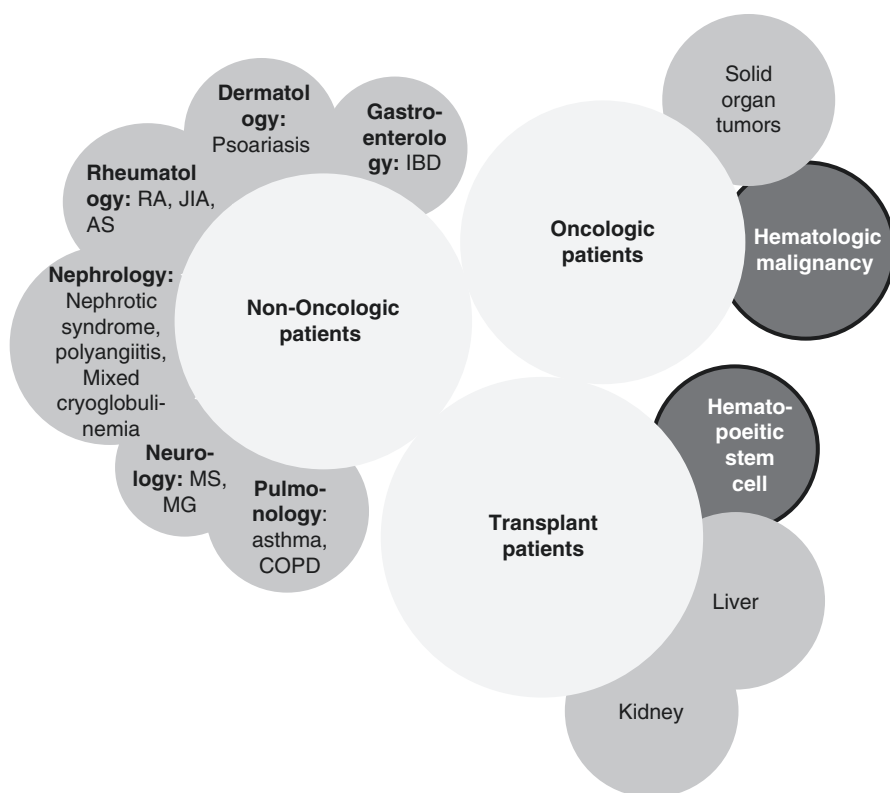


Fig. 16.3 Patients with conditions that may need immunosuppressive treatment. *IBD* inflammatory bowel disease, *RA* rheumatoid arthritis, *JIA* juvenile idiopathic arthritis, *AS* ankylosing spondylitis, *MS* multiple sclerosis, *MG* myasthenia gravis, *COPD* chronic obstructive pulmonary disease

gender ($p = 0.045$), and diagnosis of lymphoma ($p = 0.03$) were associated with HBR (Yeo et al. 2000a). Other studies showed that among patients with solid organ tumors, HBR occurs more commonly in breast cancer patients (41%) compared with other organs (7–29%) (Yeo et al. 2003, 2004a). The presence of anti-HBs is in general protective, depending on the host and treatment factors. The role of quantitative anti-HBs titers in the prediction of HBR in these settings is not clear.

In addition to the viral characteristics, host and treatment factors play an important role in determining the risk of HBR. Figure 16.3 shows categories of patients requiring immunosuppressive treatment. Organ transplantation carries an immense risk of HBR, especially HSCT, which affects the host immune function most profoundly. The risk of HBR in patients receiving immunosuppression in settings other than transplantation correlates with the type and level of immunosuppression. For example, the risk is highest when the regimens contain rituximab or high-dose corticosteroid (Cheng et al. 2003; Abramson and Chung 2014; Mendez-Navarro et al. 2011; Mozessohn et al. 2015; Kim et al. 2010; Yeo et al. 2009).

Table 16.1 Risk stratification for hepatitis B reactivation (HBR) based on the types of immunosuppressive agents and HBV serologic status

Risk of HBR	HBsAg+/anti-HBc+	HBsAg–/anti-HBc+
High	– B-cell-depleting agents, e.g. rituximab, ofatumumab – Anthracycline derivatives, e.g. doxorubicin, epirubicin – Moderate- or high-dose corticosteroids^a daily for ≥ 4 weeks	– B-cell-depleting agents, e.g. rituximab, ofatumumab
Moderate	– TNF-α inhibitors, e.g. etanercept, adalimumab, certolizumab, infliximab – Cytokine or integrin inhibitors, e.g. abatacept, ustekinumab, natalizumab, vedolizumab – Tyrosine kinase inhibitors, e.g. imatinib, nilotinib – Low-dose corticosteroids daily for ≥ 4 weeks	– TNF-α inhibitors, e.g. etanercept, adalimumab, certolizumab, infliximab – Cytokine or integrin inhibitors, e.g. abatacept, ustekinumab, natalizumab, vedolizumab – Tyrosine kinase inhibitors, e.g. imatinib, nilotinib – Moderate- or high-dose corticosteroids daily for ≥ 4 weeks – Anthracycline derivatives, e.g. doxorubicin, epirubicin
Low	– Traditional immunosuppressive agents, e.g. azathioprine, 6-mercaptopurine, methotrexate – Intra-articular corticosteroids – Any dose of oral corticosteroids daily for ≤ 1 week	– Traditional immunosuppressive agents, e.g. azathioprine, 6-mercaptopurine, methotrexate – Low-dose corticosteroids daily for ≥ 4 weeks – Intra-articular corticosteroids – Any dose of oral corticosteroids daily for ≤ 1 week

^aDefinitions of corticosteroid doses

High dose: >20 mg prednisone daily or equivalent

Moderate dose: 10–20 mg prednisone daily or equivalent

Low dose: <10 mg prednisone daily or equivalent

Table 16.1 categorizes the risk of HBR based on the types of immunosuppressive agents and HBV serologic status of the patients. Three strata in HBR risk may be defined, high-, intermediate-, and low-risk groups, corresponding to anticipated incidence of $>10\%$, 1–10%, and $<1\%$ of cases, respectively. These categories inform patient management, as described later in this chapter.

5 Diagnosis for HBR

Uniform, standardized nomenclature and definitions for HBR are unavailable. The most recent publication aimed toward a consensus was put forth as proceedings of the 2013 Emerging Trends Conference organized by the American Association for the Study of Liver Diseases (AASLD) on the topic of reactivation of hepatitis B (American Association for the Study of Liver Diseases 2013). Table 16.2 summarizes diagnostic criteria that we propose in part based on the AASLD report.

Table 16.2 Diagnostic criteria for HBV reactivation

Baseline status	HBsAg+ HBeAg+/-	HBeAg-	HBsAg-
Criteria	HBV DNA ↑ ≥ 2 log regardless of ALT	HBeAg+ (e seroreversion)	HBsAg+ (s seroreversion)
	HBV DNA ↑ ≥ 1 log in association with ALT ↑		
	De novo appearance of HBV DNA ≥ 100 IU/ml		

In patients with chronic HBV infection (HBsAg + and anti-HBc +), HBR is most commonly diagnosed by an increase in HBV DNA in the setting of immunosuppression. A consensus is lacking regarding the threshold in the rise of HBV DNA. In our view, an abrupt increase of ≥2 log in HBV DNA would be sufficient to diagnose HBR, regardless of changes in ALT. Similarly, in patients who were previously undetectable, a new appearance of HBV DNA at titers above 100 IU/ml would correspond to a 2 log rise. In patients with a clinically significant rise in ALT, a smaller degree of HBV DNA rise (≥ 1 log) should trigger management actions (Di Bisceglie et al. 2015; Hwang and Lok 2014).

There is even less clarity regarding what degree of ALT changes is needed to define HBR. Possible proposals have included (1) multiples of the baseline ALT (e.g., threefold increase), (2) multiples of the upper limit of normal (e.g., three times the upper limit of normal), or (3) an absolute cutoff (e.g., 100 IU/mL). The difficulty is compounded by the lack of uniform definition of normal ALT values and poor standardization across laboratories for ALT assays. In our practice, a twofold increase from the patient’s prior baseline is sufficient to prompt a diagnostic investigation.

Changes in the serological profile are easier to define and likely to connote more serious consequences of HBR. HBe seroreversion, namely, reappearance of HBeAg with or without the loss of anti-HBe, signals a significant shift in the immune status of the patient, corresponding to a diagnosis of HBR. HBs seroreversion is even more serious (Hwang and Lok 2014). Most patients experiencing HBs seroreversion have the baseline profile of HBsAg- and anti-HBc+; however, in patients receiving profound levels of immunosuppression (e.g., allogeneic HSCT), HBs seroreversion from anti-HBs-positive to HBsAg-positive status may occur and herald a severe degree of HBR.

6 Clinical Manifestations of HBR

The clinical features of HBR vary from asymptomatic changes in the laboratories to fulminant hepatic failure leading to death. The majority of patients present with mild degree of HBR.

The course of HBR has been described in three phases (Fig. 16.4). The first phase is mainly a virological event, characterized by an abrupt increase in viral replication soon after immunosuppressive therapy is initiated. There are no apparent hepatitis symptoms, and serum aminotransferase levels are usually unchanged from

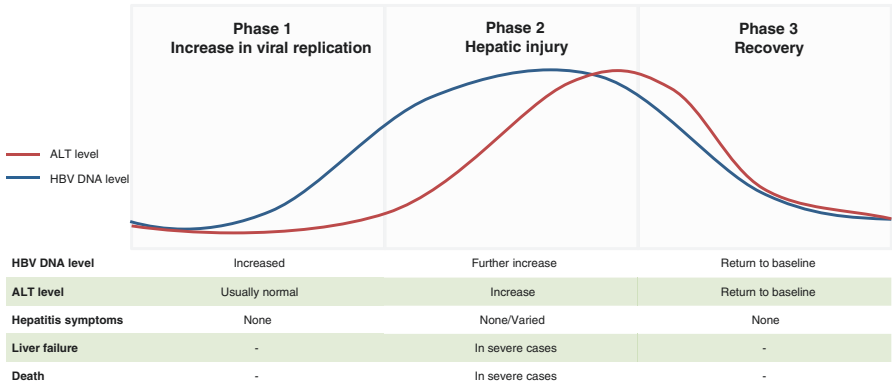


Fig. 16.4 Phases of HBV reactivation (Reprint with permission from Hwang JP, Lok ASF. Management of patients with hepatitis B who require immunosuppressive therapy. *Nat Rev Gastroenterol Hepatol* 2013;11(4))

baseline. HBV DNA levels continue to rise during the second phase and may be accompanied by elevation in serum aminotransferases with or without symptoms such as fatigue. In severe cases, hepatitis activities may be severe enough to result in liver failure. As expected, these poor outcomes tend to occur more frequently in cirrhotic patients. In the third phase, HBV DNA levels and serum aminotransferases levels start to decrease, and HBV markers may return to the baseline. Not all HBR patients go through these three phases.

7 Management Strategies for Hepatitis B Reactivation

The goal in the management of HBR is two-fold: (1) prevent liver-related morbidity and mortality and (2) allow the immunosuppressive therapy to continue unperturbed. In achieving these goals, the most effective strategy is to prevent HBR to begin with. This principle is best demonstrated in randomized controlled trials that compared prophylactic antiviral therapy in patients considered to be at high risk versus withholding antiviral treatment until a diagnosis of HBR is established. Figure 16.5 summarizes the results of trials in which lamivudine was used to prevent HBR (Lau et al. 2003; Hsu et al. 2008; Jang et al. 2006; Long et al. 2011). In patients who did not receive prophylactic antivirals, HBR occurred in 30–50%—more frequently in lymphoma patients. Prophylactic lamivudine was able to virtually eliminate HBR. While lamivudine may not be the ideal agent today, these data are convincing that in high-risk patients, prevention is a preferred strategy than reactive treatment of HBR once it has occurred.

Recently, American Gastroenterological Association (AGA) released a technical review and guideline on the prevention and treatment of HBR during immunosuppressive drug therapy (Perrillo et al. 2015; Reddy et al. 2015). Table 16.3 summarizes recommendations addressed in the document.

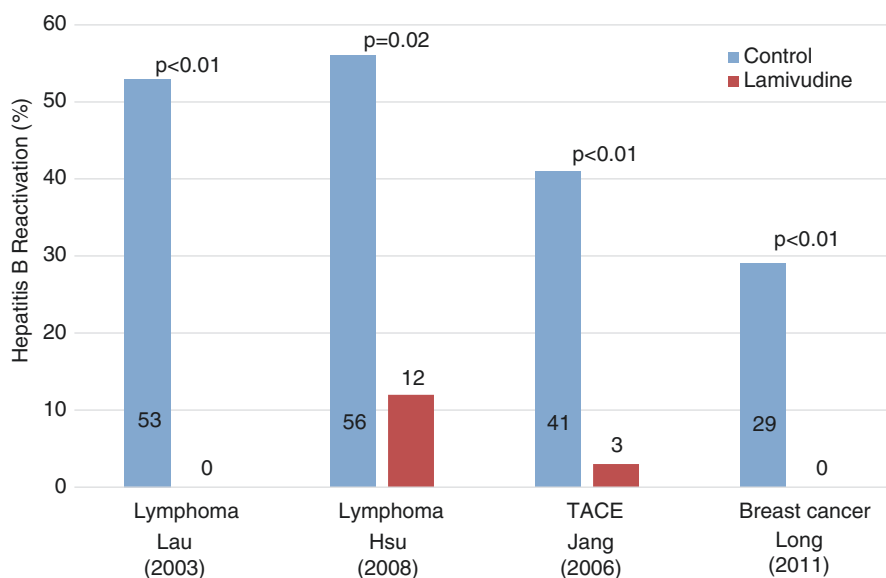


Fig. 16.5 Results from multiple trials showing the effect of lamivudine in the prevention of HBR (Lau et al. 2003; Hsu et al. 2008; Jang et al. 2006; Long et al. 2011)

7.1 Screening and Risk Stratification

A crucial element in HBR management is to identify patients with HBV infection prior to initiation of immunosuppressive therapy. Various governmental and professional organizations have published guidelines about screening as shown in Table 16.4 (Reddy et al. 2015; Weinbaum et al. 2008; Lok and McMahon 2009; Baden et al. 2012; European Association for the Study of the Liver 2012; LeFevre 2014; Sarin et al. 2016; Hwang et al. 2015). Although these guidelines vary in some of the details in their recommendations, they all agree that initial screening should be performed with HBsAg and anti-HBc. Regarding anti-HBc testing, it can be either total anti-HBc or anti-HBc immunoglobulin G, but not immunoglobulin M.

An approach to diagnose all patients at risk of HBR would be universal screening—namely testing every patient for HBV infection before immunosuppressant therapy is instituted. For example, the US Food and Drug Administration (FDA) recommends all healthcare providers to routinely screen all patients for HBV infection prior to the initiation of chemotherapy or immunosuppressive therapy. A study by Hwang found that case identification was substantially improved through universal screening rather compared to the usual practice (Hwang et al. 2012). While it would detect the most number of patients with HBV infection and optimize patient management, this approach is not widely practiced because most oncologists do not perceive the benefit of universal screening to be large enough to justify the efforts and expenses needed, particularly in low HBV prevalence settings such as the US general practice. Presumably, if HBV prevalence is high enough, universal

Table 16.3 AGA recommendations on the prevention and treatment of HBR in patients undergoing immunosuppressive therapy (Reddy et al. 2015)

Questions	Recommendations	Levels of recommendations
Screening and risk stratification		
• Should patients who will undergo long-term immunosuppressive drug therapy be screened for HBV before starting treatment?	High and moderate risk: Screen with HBsAg and anti-HBc, followed by a sensitive HBV DNA test if positive	Strong recommendation; moderate-quality evidence
	Low risk: No screening	Weak recommendation; moderate-quality evidence
• Does the presence of HBsAb in HBsAg–/HBcAb+ patients confer additional protection against HBR?	Anti-HBs status not to be used to guide antiviral prophylaxis for all risk groups	Weak recommendation; very-low-quality evidence
Antiviral prophylaxis		
• Is antiviral prophylaxis needed for HBsAg+ patients undergoing immunosuppressive drug therapy? • Is antiviral prophylaxis needed for HBsAg–/HBcAb+ patients undergoing immunosuppressive drug therapy?	High risk: Recommend antiviral prophylaxis	Strong recommendation, moderate-quality evidence
	Moderate risk: Recommend antiviral prophylaxis	Weak recommendation; moderate-quality evidence
	Low risk: Not recommend routinely using antiviral prophylaxis	Weak recommendation; moderate-quality evidence
• Is prophylactic treatment with third-generation nucleos(t)ide analogues (NAs) more effective than first- or second-generation NAs?	Use antiviral drugs with a high barrier to resistance over lamivudine for prophylaxis	Weak recommendation; moderate-quality evidence
Rescue therapy for established HBR		
• Is HBV DNA monitoring followed by on-demand antiviral therapy as effective as prophylactic antiviral therapy?	No recommendation for a strategy of HBV DNA monitoring followed by rescue treatment as an alternative to antiviral prophylaxis	No recommendation—knowledge gap
• Is treatment of established HBR with third-generation NAs more effective than first- or second-generation drugs?	Use antiviral drugs with a high barrier to resistance over lamivudine	Strong recommendation; moderate-quality evidence

screening may prove to be cost-effective. To date, available cost-effectiveness analyses suggested that universal screening with HBsAg and anti-HBc is not cost-effective in palliative or adjuvant setting for solid tumors but it is cost-saving for lymphoma patients undergoing chemotherapy with a rituximab-containing regimen (Day et al. 2011; Zurawska et al. 2012).

An alternate strategy in screening for HBR prophylaxis candidates is to stratify individual patients according to their risk of HBR. The recommendations by AGA in Table 16.3 utilize such a risk stratification scheme. Based on the immunosuppressive regimen and the serologic profile (see Table 16.1), the patient may be classified as

Table 16.4 Recommendation for HBV screening in order to prevent HBR from various organizations

Organizations	Population to be screened	Screening tests	References
CDC ^a (2008)	All patients before immunosuppression	HBsAg, anti-HBc, anti-HBs	Weinbaum et al. (2008)
AASLD ^b (2009)	All patients with high risk for HBV infection	HBsAg, anti-HBc	Lok and McMahon (2009)
NCCN ^c (2012)	All patients with B-cell lymphoid malignancies, high risk for HBV infection, intensive immunosuppression especially anti-CD20 monoclonal antibody	HBsAg, anti-HBc, anti-HBs	Baden et al. (2012)
EASL ^d (2012)	All patients before immunosuppression	HBsAg, anti-HBc	European Association for the Study of the Liver (2012)
USPSTF ^e (2014)	All patients with high risk for HBV infection	HBsAg, anti-HBc, anti-HBs	LeFevre (2014)
APASL ^f (2015)	All patients before immunosuppression	HBsAg; anti-HBc if needs anti-CD20 monoclonal antibody or anti-TNF	Sarin et al. (2016)
AGA ^g (2015)	All patients before immunosuppressive therapy at moderate/high risk of HBV reactivation, but not before low-risk therapy	HBsAg, anti-HBc	Reddy et al. (2015)
ASCO ^h (2015)	All patients with high risk for HBV infection and before starting anti-CD20 therapy or hematopoietic cell transplantation	HBsAg, anti-HBc	Hwang et al. (2015)

^aCDC = Centers for Disease Control and Prevention^bAASLD = American Association for the Study of Liver Diseases^cNCCN = National Comprehensive Cancer Network^dEASL = European Association for the Study of the Liver^eUSPSTF = US Preventive Services Task Force^fAPASL = Asian Pacific Association for the Study of the Liver^gAGA = American Gastroenterological Association^hASCO = American Society of Clinical Oncology

high, moderate, and low risk. High- and moderate-risk patients should receive HBV screening, whereas screening may be reserved for certain low-risk patients who meet the screening criteria recommended for the general population according to the CDC and the US Preventive Service Task Force. Patients are screened for HBsAg and anti-HBc, followed by HBV DNA, if either is positive. The advantage of this risk stratification strategy is that it is more likely to be cost-effective than universal screening and reduce the potential harm of false-positive results. However, it is limited by the complexity in its application. In clinical scenarios where care is being planned for a

cancer patient requiring chemotherapy or a patient with an immunological disorder in need of immunosuppressive therapy, applying the HBR risk rules may not be considered high priority, and screening may not be performed at all.

Finally, the guideline addresses the utility of anti-HBs in the management of HBR. It is often believed that the presence of anti-HBs makes it less likely that the patient will experience HBR. However, HBR may occur despite anti-HBs, particularly in patients undergoing the deepest level of immunosuppression (e.g., HSCT), in whom HBR may occur in conjunction with HBs seroreversion. The guideline recommends against using anti-HBs status in determining the need for antiviral prophylaxis regardless of the risk level.

7.2 Antiviral Prophylaxis Algorithm

The next set of questions in the management of patients at risk of HBR addresses (1) who are candidates for antiviral prophylaxis and (2) what antiviral regimen should be used. Figure 16.6 represents an algorithm that we propose, based on the AGA guideline for the management of patients undergoing immunosuppressive therapy to optimize their outcomes with regard to HBR. Once a decision is made how screening is performed (universal versus risk-stratified), the patient will undergo testing for a minimum of HBsAg and anti-HBc. Depending on the patient's risk profile, additional testing for hepatitis C, human immunodeficiency virus or hepatitis D (if HBsAg is positive) may also be considered.

In a patient who is negative for both HBsAg and anti-HBc, there is no need for antiviral prophylaxis. If, however, the patient is either HBsAg+ or anti-HBc+, the risk of HBR needs to be assessed. If the patient meets the high-risk criteria (Table 16.1), prophylactic antiviral is indicated, whereas in a patient who is at low risk, prophylaxis is not recommended. In patients who are at moderate risk, the guideline expresses preference for antiviral prophylaxis. However, the evidence to support the recommendation is not very robust, and an alternate approach may be to monitor HBV DNA levels for early detection and prompt treatment for HBR. However, there is no consensus about optimal ways to monitor for HBR both during and after cessation of immunosuppressive therapy, although some have suggested a monitoring interval of 3 months (Hwang and Lok 2014). In our opinion, upfront institution of antiviral prophylaxis obviates the cost and inconvenience of repeated HBV DNA testing, especially if the antiviral therapy can be delivered inexpensively. We do concur with the AGA guideline which recommended that in patients who place a higher value on avoiding any long-term use of antiviral therapy and costs associated with its use and consider avoiding the small risk of reactivation less important, it may be reasonable to choose no prophylaxis over antiviral prophylaxis, particularly if HBsAg is negative.

With regard to the choice of prophylactic antiviral agent, lamivudine has been most widely studied (Yeo et al. 2004b, c; Lau et al. 2003; Hsu et al. 2008; Jang et al.

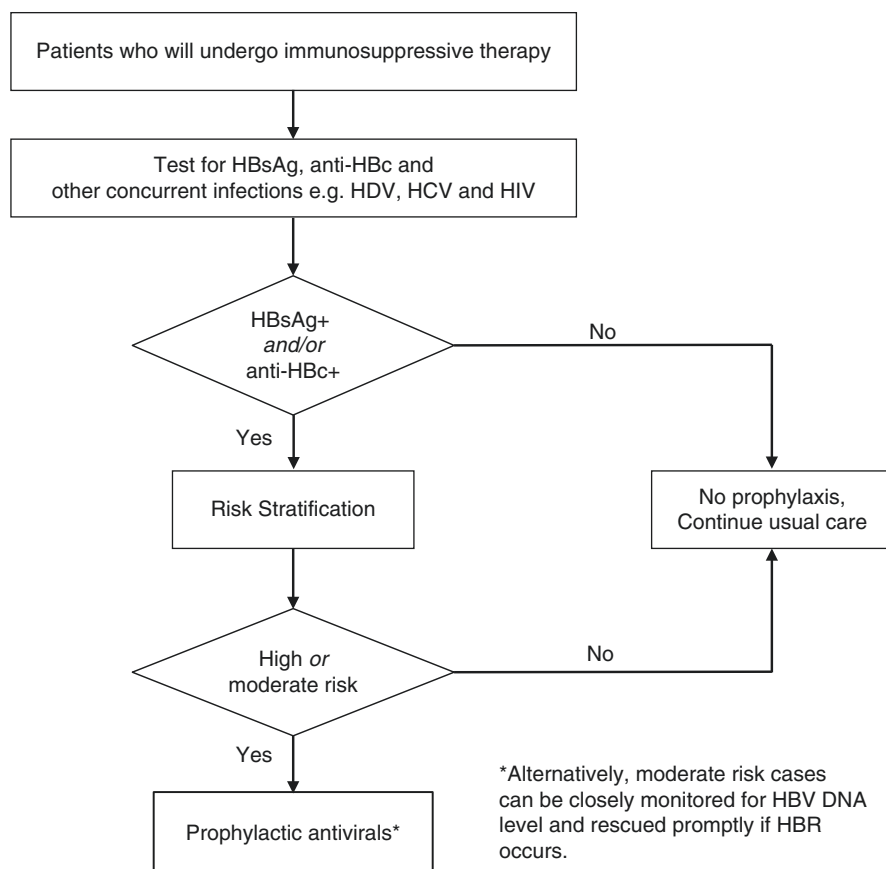


Fig. 16.6 Algorithm for HBV screening and antiviral prophylaxis to prevent HBR in non-transplant patients

2006; Loomba et al. 2008; Ahmed and Keeffe 1999; Kohrt et al. 2006; Li et al. 2006; Rossi et al. 2001; Nagamatsu et al. 2004; Dai et al. 2004). Those studies showed that lamivudine improved outcome of patients with respect to HBR, including the occurrence of HBR, the need to delay or interrupt chemotherapy, and ultimately the outcome of the cancer therapy. While lamivudine was effective in proving the concept of antiviral prophylaxis of HBR, it has fallen out of favor in the treatment of chronic HBV infection in general, due in part to its susceptibility to viral mutations that negate its efficacy and lower potency compared to more modern agents. The AGA guideline prefers a third-generation NA over lamivudine for HBR prophylaxis. A randomized controlled trial showed the superiority of entecavir over lamivudine in decreasing the risk of HBR, hepatitis B flare, and interruption of

immunosuppressive therapy (Huang et al. 2013). There are other studies with a similar conclusion, although the quality of those studies is not as robust.

A counterargument in favor of lamivudine is that most patients receiving antiviral prophylaxis have low or undetectable levels of HBV DNA at baseline and lamivudine failure is expected to be infrequent. The AGA guideline acknowledges this trade-off: it suggests that in patients who put a higher value on cost of antiviral therapy and a lower value on avoiding the potentially small risk of resistance development, it may be reasonable to select the least expensive anti-HBV medication over more expensive antiviral drugs with a higher barrier to resistance. In patients with undetectable viral load with expected duration of prophylaxis for 6 months or less, lamivudine may be acceptable.

Data are sparse as to the optimal timing of the initiation and discontinuation of antiviral prophylaxis. In our practice, we try to start HBR prophylaxis as soon as the need is determined. For patients with low or undetectable viremia, prophylaxis initiation concurrent to immunosuppressive therapy would be sufficient. In patients with higher levels of HBV DNA, we expect it to be advantageous if HBR prophylaxis can precede the onset of immunosuppression. However, immunosuppressive therapy, especially cancer chemotherapy, should not be delayed on account of achieving viral suppression. With regard to the duration of therapy, the AGA guideline recommends the prophylaxis to continue for at least 6 months after discontinuation of immunosuppressive therapy, with the exception of patients receiving B-cell-depleting agents, in whom prophylaxis is extended to 12 months.

7.3 Treatment of Established HBR

Any abnormalities in liver test of patients undergoing immunosuppressive therapy or chemotherapy need to be carefully investigated. It is necessary to differentiate HBR from various potential causes including infections from other hepatitis virus (A, C, D, and E), opportunistic pathogens (e.g., cytomegalovirus), drug-induced liver injury, or other causes (e.g., graft-versus-host disease). In patients who have been screened for HBV infection and deemed to be at moderate risk for HBR and elect to be monitored without prophylactic antiviral therapy, HBR may be diagnosed early by rising HBV DNA levels before biochemical or clinical evidence of hepatitis activities emerges. Whether employing the so-called on-demand rescue therapy in that setting is inferior to upfront prophylaxis remains uncertain, and the AGA guideline makes no recommendation about such a strategy. In patients who were not screened initially and develop active hepatitis B, HBR may be misdiagnosed as acute HBV infection since anti-HBc IgM may be detected in severe hepatitis B flare (Law et al. 2016).

Once the diagnosis of HBR is established, the treatment goal is to prevent severe hepatitis and hepatic failure. This may be achieved by (1) effective and expeditious viral control and (2) monitoring and supportive treatment for hepatic insufficiency. To achieve viral control, potent oral NAs must be initiated as soon as possible, although high-quality evidence demonstrating the efficacy of antiviral therapy in

reducing morbidity and mortality in patients with HBR is lacking (Liao et al. 2002). Delay in institution of antiviral therapy may lead to hepatic failure, liver transplantation, and death, and interferon-based therapy is inappropriate in this setting (Lok et al. 1991; Lau et al. 2003; Jindal et al. 2013; Hsu et al. 2014).

With regard to the choice of antiviral agents, there have been no randomized studies of the clinical effectiveness comparing third-generation NAs with earlier generation agents. In part based on data in immunocompetent patients, the AGA guideline recommends entecavir or tenofovir in this setting (Perrillo et al. 2015). There are little data to define the optimal duration of therapy—it may take patients with established HBR longer to bring HBV replication under control compared to patients with low viral burden undergoing antiviral prophylaxis (Hwang and Lok 2014). In patients with satisfactory viral control, we believe it would be reasonable to apply the same rule for therapy discontinuation as that for prophylaxis. In most patients, antiviral therapy should be continued for at least 6 months after discontinuation of immunosuppressive therapy. In population treated with a B-cell-depleting regimen, consideration should be given to continue the therapy for at least 12 months after discontinuation of immunosuppressant.

In patients whose HBR progresses to symptomatic hepatitis and develop signs of hepatic insufficiency, rapid cessation of ongoing necro-inflammation and loss of functioning hepatocyte mass is even more important. While the definitive treatment for liver failure would be liver transplantation, rarely patients with HBR are candidates for liver transplantation because of their underlying disease (Noterdaeme et al. 2011). However, we believe that these patients should be cared for by a team of healthcare providers with hepatology expertise to maximize support and afford a chance for recovery.

7.4 Management of Transplant Recipients

In addition to being subjected to immunosuppression, organ transplant recipients may develop HBR as a result of transmission of donor-derived HBV. The risk of HBV transmission is highest in liver transplantation, since hepatocytes are the primary site of HBV infection. However, recipients of other organs may also be at risk for HBV infection. Several management guidelines have been published in order to enhance the quality of care and improve the efficiency of HBR prevention in transplanted patients (Tomblyn et al. 2009; Kasiske et al. 2010). Care of liver transplantation patients is discussed elsewhere.

In general, both donors and recipients should be tested for HBsAg, anti-HBs, and anti-HBc. If the recipient is either HBsAg+ or anti-HBc+, he/she should be tested for HBV DNA. Non-HSCT candidates who are HBsAg+ or HBV DNA+ should receive antiviral prophylaxis. Whenever possible, HBsAg- candidates should be immunized against HBV and the response to vaccination be confirmed. Transplant recipients who are HBsAg-/anti-HBc+ may be managed in a similar fashion as immunosuppressed patients at moderate risk—they may be given antiviral prophylaxis or monitored for HBV DNA level for early detection of HBR followed by

preemptive treatment. All candidates with evidence of active HBV DNA replication (either HBsAg+ or detectable HBV DNA) should be evaluated for the degree of liver fibrosis, preferably by a liver biopsy, prior to the transplantation, since advanced fibrosis/cirrhosis can increase treatment-related morbidity and mortality.

A special consideration is given for HSCT patients. HSCT candidates should be immunized prior to chemotherapy with the initial two doses given 3–4 weeks apart, followed by the third dose 6 months later. If this schedule cannot be met, the third dose may be administered a few months after completion of chemotherapy. If anti-HBs titer after vaccination is <10 IU/L or pre-transplant vaccination is impractical, hepatitis B immune globulins (HBIG) at a dose of 0.06 ml/kg should be administered immediately prior to infusion of stem cells.

HSCT donors with detectable HBV DNA should be treated with antivirals for at least 4 weeks or until HBV DNA becomes undetectable. The cell volume from HBsAg+ and/or anti-HBc+ should be minimized and all cell products be tested for HBV DNA at the time of harvest. If HBV DNA is detectable at harvest either in the donor or harvested cells, the recipients should receive antiviral prophylaxis and optionally HBIG for 4 weeks after transplantation. If HBV is undetectable in the donor and harvested cells, recipients may be monitored with monthly ALT for the first 6 months. If ALT increases, HBV DNA and HBsAg should be tested. If there is detectable HBV DNA or HBsAg+, preemptive therapy is needed (Tomblyn et al. 2009).

With regard to kidney transplantation, a kidney from an HBsAg+ donor should not be transplanted into an HBsAg- recipient as there is a significantly high risk for HBV transmission. A kidney transplant from a donor with isolated anti-HBc+ possesses a relatively low risk of transmission and may be considered for candidates with anti-HBs+ (Abrao et al. 2014).

According to the 2009 Kidney Disease: Improving Global Outcomes (KDIGO) clinical practice guideline on the monitoring, management, and treatment of kidney transplant recipients, if KT candidates have postvaccination anti-HBs titer <10 IU/L, booster vaccination should be administered in an attempt to raise the titer to ≥ 100 IU/L. In those with ongoing HBV infection, adequate suppression of HBV must precede the transplant. Prior to the availability of oral antiviral agents, recipients with HBsAg+ had 2.5-fold increased risk of death and 1.4-fold increased risk of allograft loss compared to HBsAg- recipients (Fabrizi et al. 2005). More recent data in the era of oral antiviral agents indicate improved survival of KT recipients with HBV infection. Five-year survival rates of KT recipients with and without HBV infection were 85% versus 86%, respectively. Graft survival was also similar approximately at 75% (Reddy et al. 2011). These support KDIGO recommendations that recipients with HBsAg+ must be given antiviral therapy. For recipients with isolated anti-HBc+, there is insufficient evidence to recommend routine antiviral prophylaxis. However, we suggest to follow AGA guideline to judge the need for prophylactic treatment. Regarding the choice of antivirals, entecavir is a preferable choice for KT recipients due to its high barrier of resistance and non-nephrotoxic property. Interferon should be avoided as it is associated with the increased risk of acute rejection (Kasiske et al. 2010).

HBsAg-positive KTx recipients receiving prophylaxis must be monitored closely, although the optimal monitoring strategy to detect HBR remains to be

defined. For patient with high viral load prior to the prophylactic treatment, HBV DNA may be checked every 1–3 months until it becomes undetectable before initiation of immunosuppression. During the period of immunosuppression, HBV DNA should be checked every 3–6 months to ensure viral suppression. For those who are not on prophylactic antiviral therapy, ALT may be checked every other week for the first 16 weeks then every 3–4 weeks for the first years. HBV DNA should be monitored every month for the first year. If there is an increase in ALT level, evaluation of HBR should be performed, and preemptive therapy is needed when HBR is established (Chan and Lok 2016).

8 Emerging Trend: HBV Reactivation After Successful Treatment of Hepatitis C

HBV/HCV dual infection is not uncommon especially in the endemic areas of HBV and among high-risk population as these two viruses share similar routes of transmission. The prevalence of dual infection with HBV has been reported from 5% to 20% of individuals with HCV infection (Chu and Lee 2008). In addition, occult HBV infection, defined by the presence of HBV DNA in the absence of HBsAg, may be found in 12–44% of HCV-infected patients (Fukuda et al. 1999). As commonly seen in patients with infection with multiple hepatotropic viruses, in HBV/HCV dual infection, one of the viruses predominates (as measured by the viral load), which tends to be HCV.

Direct-acting antivirals against HCV available today afford high rates of cure. Recently, the US FDA has warned of the risk of HBR in patients with HBV/HCV dual infection treated with DAAs. Although HBR has been previously reported with interferon and ribavirin, HBR may occur much more rapidly during DAA treatment. The mechanism behind HBR after DAA therapy remains unclear. In experimental conditions, HBV and HCV could replicate in the same hepatocyte without evidence of interference, suggesting that HCV may suppress HBV replication via an indirect mechanism (Yang et al. 2014)—relatively abrupt elimination of HCV from hepatocytes would disinhibit HBV replication. It has also been postulated that the clearance of HCV may mitigate the immune control (e.g., downregulation of previously overexpressed IFN-stimulated genes).

To date, there have been 24 cases with HBR identified in the FDA Adverse Event Reporting System (FAERS) database and/or reported in the published medical literature. HBR has been reported in various DAA regimens including simeprevir and sofosbuvir (Collins et al. 2015), daclatasvir and asunaprevir (Hayashi et al. 2016; Takayama et al. 2016), and ledipasvir and sofosbuvir (De Monte et al. 2016). As expected, HBR has been reported more commonly in patients with HBsAg-positive dual infection compared to those with isolated anti-HBc positivity. In an observational study in Chinese patients treated with DAAs, 3/10 HBsAg+ patients developed HBR, compared to none of 124 who were HBsAg negative and anti-HBc positive. In most cases, HBR occurred within 4–8 weeks after starting DAA therapy. These patients were heterogeneous in terms of HCV genotype and baseline HBV

Table 16.5 Approach to prevent HBR in patients with dual HBV/HCV infection

Goal	Candidate	Action
Screen	All HCV patients being considered for DAA therapy	HBsAg, anti-HBc, and anti-HBs
	If HBsAg or anti-HBc-positive	HBV DNA
Prophylaxis	HBsAg positive and/or detectable HBV DNA	Assess fibrosis and determine HBV therapy candidacy and endpoints Anti-HBV therapy concurrent to DAA
	Isolated anti-HBc and undetectable HBV DNA	Monitor HBV DNA at week 4 of DAA therapy; anti-HBV prophylaxis if increasing
	Anti-HBs positive	No action
Prevention	Anti-HBs negative	Vaccinate

status. The severity of HBR in those patients varied from no symptoms to severe hepatic failure or death (U.S. Food and Drug Administration 2016).

There is insufficient data based on which to incorporate HBV/HCV dual infection patients into our management algorithm (Fig. 16.6). Table 16.5 describes our proposed approach to these patients. Patients undergoing DAA therapy must be screened for HBsAg, anti-HBc, and anti-HBs, followed by HBV DNA in patients who are either HBsAg negative or anti-HBc positive. We propose that anti-HBV prophylaxis be started concurrent with the DAA therapy in patients who are HBsAg positive or HBV DNA detectable, whereas in patients who are anti-HBc positive and HBV DNA undetectable, prophylaxis may not be necessary. In the former category of patients, it is important to determine whether the patient would have been a candidate for HBV therapy regardless of the HCV dual infection and define the HBV treatment endpoint, such as HBe seroconversion. We believe it is also important to assess their liver fibrosis status, which is commonly performed in preparation of the HCV therapy. For patients with isolated anti-HBc without detectable HBV DNA, monitoring for a rise in HBV DNA may be reasonable, perhaps at week 4 of therapy, as patients are often tested for HCV RNA at the same time.

There is no data available to inform the optimal duration of HBV therapy in dual infection patients being treated for HCV. To the degree that there may be host immunological shift that underlie the development of HBR, in our practice, we continue the prophylaxis for 3 months after discontinuation of DAA. These patients are monitored for another 3 months to ensure absence of HBR off anti-HBV prophylaxis. Obviously, in patients determined to be candidates for HBV therapy independent of HCV, therapy should be continued until the planned endpoint is met. Care must be taken in discontinuing anti-HBV prophylaxis in patients with cirrhosis, which may precipitate hepatic decompensation.

9 Current Challenges and Future Directions

HBR leading to a poor patient outcome such as liver failure or disruption of cancer chemotherapy represents an unnecessary clinical tragedy, which is eminently preventable by appropriate screening and prophylaxis. Despite a multitude of guidelines to inform clinicians caring for patients undergoing cancer

treatment, transplantation, and immunomodulatory therapy, HBR continues to occur (Patel et al. 2016; Yuen 2016). Survey studies conducted in practicing physicians indicate that adherence to routine HBV screening prior to the immunosuppressive therapy is unacceptably low—approximately 20–40% of oncologists, 40% of dermatologists, and 70% of rheumatologists follow a guideline in some fashion (Hwang et al. 2012; Stine et al. 2011, 2010; Tran et al. 2010; Kawsar et al. 2012).

This problem may be partly attributable to the inconsistency among the guidelines. Clearly, multi-society collaboration to develop a broadly applicable consensus is an essential step. Secondly, efforts to disseminate the consensus guideline to all practitioners are needed. For healthcare providers that are not routinely involved in the care of patients at risk of HBR, electronic mechanisms may be helpful to alert them of candidates for screening and to guide them to initiate appropriate prophylaxis. Such a proactive measure may be even more important in the future, as increasingly more complex and potent immunosuppressive and chemotherapeutic regimens are being developed.

Finally, as investigators strive toward gaining more biological insight and immunopathogenetical knowledge of HBV infection, deeper understanding of the basic mechanisms of HBV reactivation may help better inform clinical decisions for HBR. This is particularly true of the HBV/HCV dual infection cases. In addition, the effect of new therapeutic agents that interact with the immune system in a non-conventional manner on the occurrence and course of HBR remains to be studied. Finally, as new diagnostic biomarkers and therapeutic agents are being actively developed for the goal of “cure” of HBV, additional tools may become available to provide more accurate risk stratification and then inactivate, if not cure, HBV in a sustainable fashion in patients undergoing increasingly sophisticated regimens that have a diverse effect on the immune system.

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Exploring New Therapies for a Serological Cure of Chronic Hepatitis B

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Jau-Hau Horng, Chang-Ru Wu, and Pei-Jer Chen

Abstract

Chronic hepatitis B is a global health issue, as WHO estimates about 240 millions in the world. Without antiviral therapies, a quarter of the HBV carriers will eventually succumb to cirrhosis or hepatocellular carcinoma (Fattovich et al. 2004; Di Bisceglie 2009; Beasley 1988; Liaw et al. 1988). Active therapies have been developed, such as pegylated interferon and antiviral nucleos(t)ide analogs, in the last 20 years (Belloni et al. 2012; Papatheodoridis et al. 2002; Lau et al. 2005). Despite of an effective control of HBV replication by long-term NA treatments, the success of eliminating intrahepatic HBV or infected hepatocytes remains low (about 1–10% of treated patients). A search for novel, curative antiviral therapies has been actively taken up in many institutes. Practically, current NA therapies effectively eliminate extracellular virions, and so the next step will be to eliminate the intracellular viral RNAs, viral proteins, or eventually viral DNAs. The theme has become a more and more important medical task, especially after the advent of highly effective direct antiviral agents curing essentially hepatitis C. Therefore, there are many excellent reviews on the HBV life cycle or immunology that point out or summarize possible targets for future antiviral development (Lanford et al. 2013; Isogawa et al. 2005; Ma et al. 2015; Tang et al. 2016; Petersen et al. 2016). This chapter will skip these overlapping parts but only describe certain exploratory experiments toward novel targets ongoing. The main approach is to develop antiviral immunotherapies which can eradicate intrahepatic HBV DNAs or the HBV-infected hepatocytes in the near future.

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Keywords

HBV • Curative antiviral therapy • Antiviral agent development • Core Protein Allosteric Modulators • Kinase inhibitors • Immune Checkpoint Blockers

1 Introduction

Chronic hepatitis B is a global health issue, as WHO estimates about 240 millions in the world. Without antiviral therapies, a quarter of the HBV carriers will eventually succumb to cirrhosis or hepatocellular carcinoma (Fattovich et al. 2004; Di Bisceglie 2009; Beasley 1988; Liaw et al. 1988). Active therapies have been developed, such as pegylated interferon and antiviral nucleos(t)ide analogs, in the last 20 years (Belloni et al. 2012; Papatheodoridis et al. 2002; Lau et al. 2005). Despite of an effective control of HBV replication by long-term NA treatments, the success of eliminating intrahepatic HBV or infected hepatocytes remains low (about 1–10% of treated patients). A search for novel, curative antiviral therapies has been actively taken up in many institutes. Practically, current NA therapies effectively eliminate extracellular virions, and so the next step will be to eliminate the intracellular viral RNAs, viral proteins, or eventually viral DNAs. The theme has become a more and more important medical task, especially after the advent of highly effective direct antiviral agents curing essentially hepatitis C. Therefore, there are many excellent reviews on the HBV life cycle or immunology that point out or summarize possible targets for future antiviral development (Lanford et al. 2013; Isogawa et al. 2005; Ma et al. 2015; Tang et al. 2016; Petersen et al. 2016). This chapter will skip these overlapping parts but only describe certain exploratory experiments toward novel targets ongoing. The main approach is to develop antiviral immunotherapies which can eradicate intrahepatic HBV DNAs or the HBV-infected hepatocytes in the near future.

2 Unique Innate Immunity Toward HBV Infection

Traditionally, HBV is known as a stealth virus because it does not induce strong innate immune response like other virus, even though the virus spreads throughout the liver (Wieland et al. 2004; Wieland and Chisari 2005). Moreover, during viremia, HBV induces more immunosuppressive cytokine IL-10 than other hepatitis viruses (Das et al. 2012; Dunn et al. 2009). After HBV exposure, adults are usually capable to mount strong adaptive immune response against the virus and develop self-limited infection (Milich and Liang 2003). In contrast, after infection about 30% children under 5 years of age or 95% infants cannot clear the virus effectively and become chronic carriers (Mahoney 1999; Hyams 1995; McMahon et al. 1985). To this date, little is known about how HBV is recognized by host innate immune system and stimulates subsequent adaptive immunity.

In vivo HBV research is highly restrained because HBV has very limited host range and cannot infect common laboratory animals, including mice (Dupinay et al. 2013; Purcell et al. 1976; von Weizsacker et al. 2004). The recent identification of NTCP as one HBV receptor moves the field forward, but the infection experiments are still deemed not very efficiently. To overcome this issue, our previously described hydrodynamic injection (HDI) mouse model can achieve transient HBV transfection to mice liver and persists for weeks and even months (Huang et al. 2006). Compared with other HBV mouse models, the advantage of this one is that it allows one round of HBV replication, producing HBV replication intermediate or viral particles, which then stimulate strong T-cell and B-cell responses in an immunocompetent host. This model provided us an efficient tool to investigate HBV-related immunity in the small rodent model. Also, we found some specific mice strains, such as CBA/CaJ or C3H/HeN, and concede HBV to persist for >6 months after hydrodynamic HBV transfection (Chou et al. 2015; Peng et al. 2015). These strains show higher tolerance to HBV replication and do not trigger strong adaptive immune response even though they are immunocompetent. Hence, such strains mimic immune-tolerant state of chronic HBV carriers and can thus serve as a platform for HBV therapeutic study. The model has been adopted to understand the host immunity against HBV and used for exploring new therapeutic regimens. Some recent progresses are summarized below.

3 HBV Capsid as One Pro-Inflammatory Pathogen-Associated Molecular Pattern (PAMP): The Immunomodulatory Effect of CpAM (Core Protein Allosteric Modulators)

The main reason for HBV failing in induce innate immunity after infections may come from our ignorance of viral PAMPs (pathogen-associated molecular pattern) and how HBV PAMPs interact with cellular PRR (PAMP recognition receptors). Our previous findings show that HBV core antigen is essential for HBV immune clearance in both C57BL/6 and Balb/c mice (Lin et al. 2010). Furthermore, we found the HBV capsid, not just core protein alone, as one important PAMP to trigger effective anti-HBV immunity. To understand whether capsid conformation of HBcAg will affect HBV immune clearance in mice, we constructed a capsid assembling deficient mutant, HBc-Y132A in pAAV-HBV1.2 backbone. This mutant HBV core forms core dimer but cannot assemble into viral capsid and do not deplete total HBcAg or HBV RNA levels in vivo. After hydrodynamic injection, mice receiving mutant plasmid showed delayed viral clearance and compromised antiviral adaptive immunity (Lin et al. 2012). These data suggested that only HBcAg capsid form, but not free dimer form, is able to stimulate anti-HBV adaptive immunity.

Several viral nucleocapsids have been reported to interact with host factors and to affect antiviral innate immunity. For example, HIV capsid interacts with host factor CPSF6 and CypA to stabilize its capsid. Stabilized capsid prevents premature

viral genome exposure to cytosolic nucleic acid sensors such as cGAS to suppress interferon activation (Rasaiyaah et al. 2013). Moreover, sequestering CypA which forms HIV capsid by a non-immunosuppressive cyclosporine analogue, SmBz-CsA, restores the host innate immune sensor activity and induces IFN- β production in macrophage. The HIV case demonstrated the feasibility in restoring host immunity by the viral capsid modulators.

3.1 The Possible Immunomodulatory Effects of CpAM: More than Antiviral

According to the data described previously, we consider HBV capsid conformation as a pathogen-associated molecular pattern (PAMP). HBV capsid modulators (or core protein allosteric modulator, CpAM) have become a new and major direction for anti-HBV development. As HBV capsid is considered a PAMP triggering host immune responses, these CpAM may not only exert direct antiviral effect but also modulate innate immunity. However, certain class of capsid modulators (such as conventional capsid disruptors) suppresses HBV replication but also inhibit immune responses. Novel classes of HBV capsid modulators that can suppress viral replication and also activate immune responses deserve exploration. Thus, we proposed the following scheme:

HBV capsid can serve as a pathogen-associated molecular pattern (PAMP) and be recognized by hepatic intrinsic receptors to induce antiviral immunity. HBV capsid modifier has dual role in anti-HBV immune regulation (Fig. 17.1).

Ongoing Approaches

To validate activity of known/novel capsid modulators on inhibiting viral replication in cell culture and HDI mouse model.

To preliminarily screen possible capsid modulators which possess immune-regulatory activity, we would like to validate its direct antiviral effect and type I interferon (IFN-I) production in cell culture model. HepG2.2.15 cell will be treated with different capsid modulator and assay their HBV-inhibiting activity (cellular HBV DNA), capsid-modulating activity (Native agarose gel capsid assay), and IFN-I or TNF-alpha production (ELISA and real-time PCR). Based on their regulatory effect on innate immunity, these capsid modulators will be categorized into two different types: (1) negative regulators have negative or no effect and (2) positive regulators have enhancing effect on IFN-I/TNF-alpha production.

According to their immune-regulatory type, we plan to use HBV hydrodynamic injection model to test them separately in different model.

For those negative regulators, we would like to test whether they can inhibit host immune response or attenuate liver inflammation in the rapid clearance Balb/c HDI model. GLS4 or its similar compound can serve as a positive control, and capsid enhancer is negative control. Also, we are interested whether host immunity will recover after drug withdrawing. For the positive regulators, we will inject wild-type HBV plasmid (genotype A) into CBA/CaJ mice. After injection for 4 weeks, serum HBsAg will be censored to check HBV persistency, and then candidate

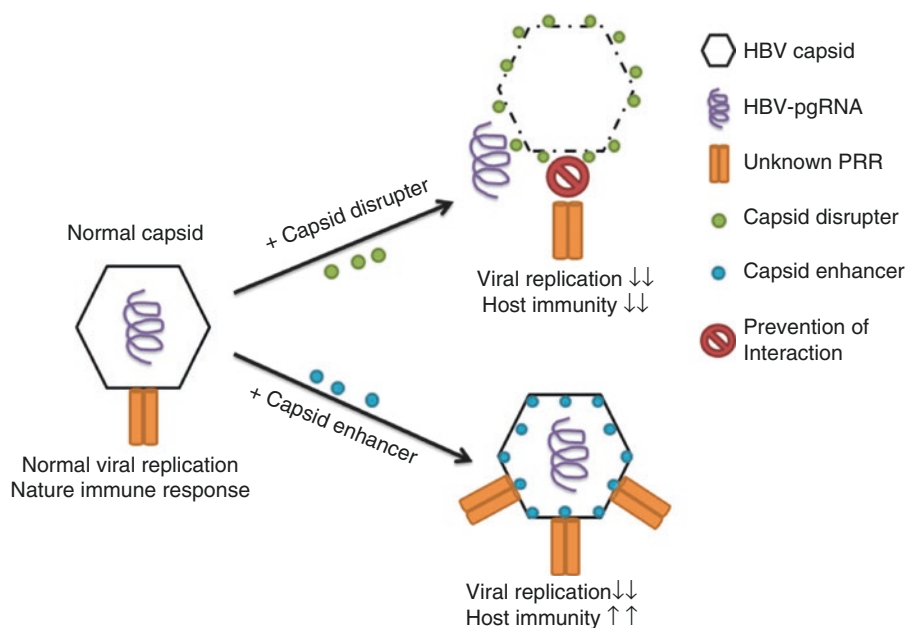


Fig. 17.1 A hypothetical model of the interaction among HBV capsid, capsid modulator, and host PRR. (1) Capsid modulators alter capsid conformation to inhibit HBV replication but also destroys the PAMP and then results in the inhibition of host immunity and viral clearance. (2) Capsid enhancer inhibits HBV replication through other mechanism. Meanwhile it maintains capsid structure and stabilizes the interaction between capsid and its corresponding cellular PRR to enhance host immunity

CpAMs will be administered for 4 weeks with the dose. The sample collection and assay protocols are the following: serological study, 100 μ L serum sample will be collected weekly and subject to HBsAg, anti-HBc, anti-HBs, HBV DNA, and sALT analysis. Liver sample will be collected at the day of sacrifice and subject to Western blot (HBc), Northern blot, and IHC (HBcAg) analysis. Total splenocytes will be isolated and perform ELISPOT assay (stimulate by recombinant HBcAg or HBcAg peptide pool).

4 Inhibition of Core Protein Phosphorylation as the New Target: In Search for Cellular Kinase Inhibitors

HBV core protein (HBc) is a 183- to 185-aa-long polypeptide of MW 21 kd and is divided into two domains due to distinct functions. The first 149 amino acids at N-terminus are sufficient for capsid formation. The C-terminal domain (CTD, residue 150–183) containing three serine-proline (Ser-Pro) rich motifs, plays a critical role of nucleic acid binding (Wynne et al. 1999). Lack of CTD does not influence the capsid formation but blocks the viral RNA encapsidation (Nassal 1992). It has been

documented that HBc is a phosphoprotein, with putative phosphorylation sites at Ser155, Thr160, Ser162, Ser168, Ser170, and Ser176 within CTD (Jung et al. 2014; Liao and Ou 1995). However these sites have not been conclusively validated by the mass spectrometry analysis. Moreover, the phosphorylation status of Ser or Thr residues in CTD during different replication stage has not yet been reported in HBV. Phosphorylation of these residues within CTD modulates HBV replication at multiple stages. Mutagenesis data suggested that replacement of Thr160, Ser162, and Ser170 to Ala decreased the RNA encapsidated into the capsid (Jung et al. 2014; Gazina et al. 2000; Lan et al. 1999; Lewellyn and Loeb 2011; Melegari et al. 2005). Interestingly, packaging-competent mutants mimicking phosphorylation were impaired significantly in further DNA synthesis (Gazina et al. 2000; Lewellyn and Loeb, 2011; Basagoudanavar et al. 2007). It has been suggested that dephosphorylation of HBc may be important for viral DNA replication (Lan et al. 1999; Basagoudanavar et al. 2007).

Several kinases were reported in association with HBc phosphorylation, including a 46-kd serine kinase, cyclin-dependent kinase 2 (CDK2), protein kinase C (PKC), and SR protein-specific 1 and 2 (Daub et al. 2002; Kann and Gerlich 1994; Kau and Ting 1998; Ludgate et al. 2012). However, none of them provides the *in vivo* evidence to supporting their functional role in regulation the phosphorylation at specific serine residues of HBc. To date, no further evidence showed any functional significance of these kinase in modulating RNA encapsidation and DNA synthesis. On the other hand, DHBV has shown that the immature nucleocapsid undergoes a dephosphorylation change to become mature nucleocapsid (Perlman et al. 2005). However the putative phosphatases involved in viral replication of HBV or DHBV have not been identified.

	RNA encapsidation	First DNA synthesis	Second DNA synthesis	References
S155	No effect	No effect	Lack RC DNA	Lan et al. (1999), Gazina et al. (2000), Melegari et al. (2005), and Lewellyn and Loeb (2011)
T160	No effect	Reduced	Lack RC DNA	Jung et al. (2014)
S162	Reduced	Reduced	Lack RC DNA	Lan et al. (1999), Gazina et al. (2000), Melegari et al. (2005), and Lewellyn and Loeb (2011)
S168	No effect	No effect	No effect	Jung et al. (2014)
S170	Reduced	Reduced	Lack RC DNA	Lan et al. (1999), Gazina et al. (2000), Melegari et al. (2005), and Lewellyn and Loeb (2011)
S176	No effect	No effect	No effect	Jung et al. (2014)

To date, many efforts have been put into identifying the role of core phosphorylation in viral replication; nevertheless, the mutagenesis strategy causing defective in viral replication limits investigation regarding the function for the phosphorylation of specific residues in CTD of HBc in regulation the viral replication. The

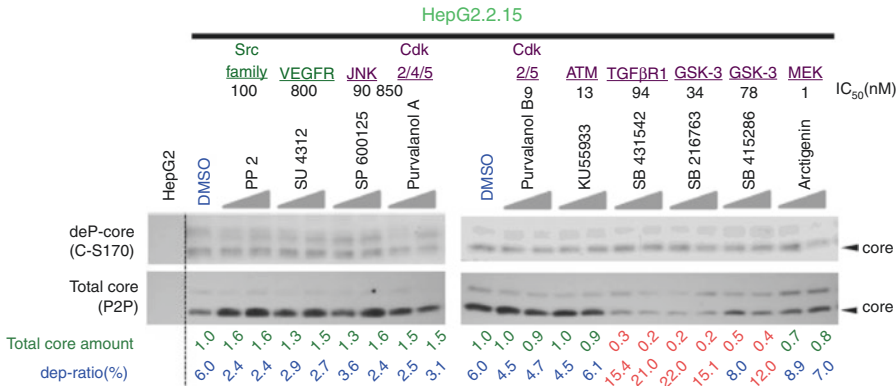


Fig. 17.2 Preliminary screening for known kinase inhibitors on phosphorylation of HBV core protein

identification of cellular kinase(s) responsible for HBc phosphorylation will pave the way to discovering potent and specific kinase inhibitors, which, in the long run, may become a new class of anti-HBV regimen.

Ongoing Approaches
Some known kinase inhibitors were shown to regulate HBV replications (Haitao Guo et al. 2007) (Fig. 17.2a, b).

5 A New Combination of Immunomodulators: Interferon and Immune-Checkpoint Blocker

Chronic hepatitis B patients exhibit a residual but exhausting cellular immunity to HBV. Conventionally the patients receive interferon to boost their immune activity, with modest effect. Now it becomes clear that the HBV-specific T cells in many of CHB patients are exhausted because of an overexpression of immune-checkpoint molecules, especially PD-1 (Boni et al. 2007; Protzer et al. 2012). In the mice model, we actually demonstrated that blocking PD-1 in the T cells accelerated the clearance of HBV in the acute HBV exposure model (Tzeng et al. 2012). Therefore, it is feasible that a single or combination treatment of anti-PD-1 and/or interferon may restore the residual anti-HBV immunity and then improve the HBV clearance. The concept can be tested in the HDI-HBV persistence model.

At the beginning, we tested the effect of HBsAg titers in CBA mice carrying HBV by interferon or anti-PD-1 alone. The results showed a quite limited HBsAg reduction in the mice models (Fig. 17.3a, b).

Ongoing Approaches
Therefore, we plan to test the combination effect but in different orders. The study may provide important information regarding the optimal application of interferon and anti-PD-1 in CHB patients.

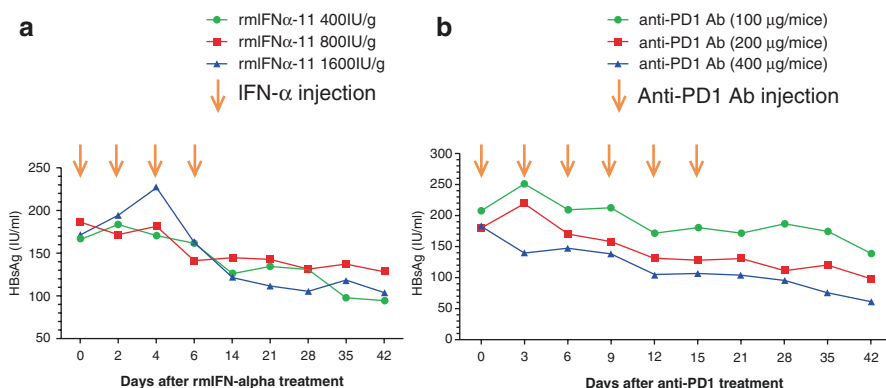


Fig. 17.3 CBA mice carried with HBV for more than 20 weeks were treated with IFN- α (a) or anti-PD-1 (b), respectively. The serum HBsAg titer was determined at the indicated time points by EIA (Abbott Diagnostics)

6 Depletion of Excessive, Tolerizing Viral Protein from Circulation: The Potential Effect of Anti-HBs Treatment

Other than PD-1, the other well-known tolerizing factor has been the excessive viral HBsAg in the circulation (Wieland and Chisari 2005; Bertoletti et al. 2009). Many previous studies suggested these HBsAg likely undermining the host innate immunity, especially, interferon responses (Shi et al. 2012; Xu et al. 2009). Depletion of HBsAg from the CHB patients may remove the viral suppressors and leads to restoring of host immunity. Toward this end, we collaborated with Xiamen University team who developed several highly potent anti-HBs monoclonal antibodies. One such mAb, E6F6, was then tested in the HDI-HBV persistence model for their immunomodulatory effect.

Ongoing Approaches

Initially the single-dose injection of E6F6 into the HBV-carrying mice cleared HBsAg from circulation in 1 day. Though HBsAg disappeared from serum for 1 or 2 weeks, it eventually rebounds to level before treatment (Fig. 17.4a, b). Apparently, a multiple dosing is needed. Therefore, our team conducted the E6F6 dosing every 2–3 months in HBV-carrying mice—giving the new injection when HBsAg rebounding from previous treatment. Interestingly, after four dosages, the HBsAg level in the treated mice declined and almost disappeared. Moreover, the immunological assays indicated a recovery of HBV-specific T cells responses in the treated mice (Zhang et al. 2016). These encouraging findings suggested the long-term removal of HBsAg from circulation might lead to a recovery of HBV-specific cellular immune responses and to clear the HBV reservoir in the liver.

CBA mice were first injected hydrodynamically with 10 μ g of HBV plasmid pAAV/HBV1.2, and anti-HBsAg antibody E6F6 was injected 24 h thereafter. The HBsAg titer in the mouse serum was determined at the indicated time points

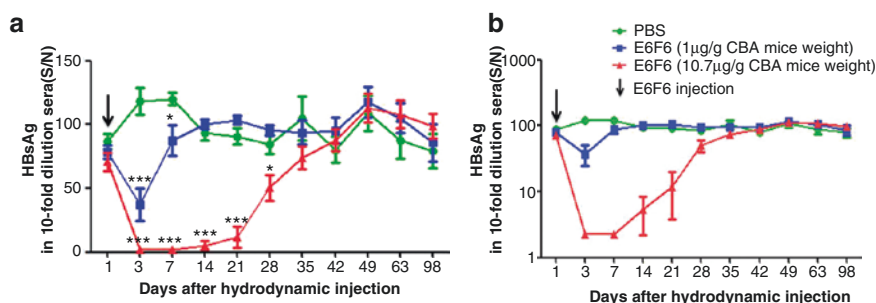


Fig. 17.4 Neutralization of extracellular HBsAg with one dose of anti-HBsAg antibody E6F6 did not affect HBV persistence in CBA mice transfected by hydrodynamic injection of HBV plasmids

by an EIA (Abbott Diagnostics). (a) HBsAg expression in CBA serum after plasmid pAAV/HBV1.2 injection. The detection limitation is 2.0 in S/N ratio. (b) HBsAg expression in CBA serum after plasmid pAAV/HBV1.2 injection. The S/N ratio were expressed in logarithmic form by applying a standard curve with known concentrations of HBsAg.

7 Prospect

Chronic hepatitis B establishes viral DNA persistence in the infected hepatocytes. The only way to cure HBV infection is to eliminate intracellular viral DNA or to eliminate the infected cells carrying viral DNAs. The former possibility is daunting despite recent data suggesting interferon or lymphotoxin-beta able to partially degrade intracellular DNA without cytotoxicities. Whether CRISPR-cas9 system could be adopted for target-degrading HBV DNA is under studying bit, the safety remains to be resolved. In contrast, the latter approach is more likely and natural as many adoptive transfers of anti-HBV cellular immunity by bone marrow transplantation into HBV carrier recipients have documented the feasibility of HBV cure. One key question is to develop a universal platform that can activate the HBV-specific cellular immune responses to reach a serological cure of CHB in all patients. The hope appears rise in the horizon now.

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