Diagnostic and Clinical Relevance of HBV Mutations

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Abstract and Introduction

Abstract

Despite vaccinations, hepatitis B virus (HBV) infections are still very common worldwide. The virus replicates by reverse transcription using a viral polymerase lacking proof-reading ability. This results in the emergence of mutant viruses that can be selected out by host immunity or viral therapeutic agents. Several well-characterized HBV variants have been identified that challenge the effectiveness of the current vaccines. Other mutations result in a change within the HBV surface antigen, resulting in a loss of detection by some diagnostic assays. Additionally, a number of mutations have evolved in response to antiviral therapy. This report is an overview of the HBV mutations leading to vaccine failure, loss of HBV detection by diagnostic assays, increased viral replication, and resistance to antiviral agents.

Introduction

Hepatitis B virus (HBV) infection continues to be a global public health issue. The World Health Organization (WHO) reports that approximately 2 billion people worldwide have been infected with HBV and approximately 350 million individuals suffer from HBV-induced chronic liver disease.[1] Without intervention, 15% to 40% of chronic HBV-infected individuals will develop cirrhosis, end-stage liver disease, hepatocellular carcinoma (HCC), or will require liver transplantation.[1,2]

Chronic disease is the most common presentation of HBV infection. The chronic nature of HBV results in individuals producing a high level of virus with few symptoms.[3] Hepatitis B virus infects and replicates in the host hepatocyte cells producing high levels of virus. During HBV replication, the virus uses reverse transcription to copy its DNA genome. However, this HBV polymerase lacks proof-reading ability, allowing mutations to occur. This leads to a heterogeneous population of HBV with altered genomes. Under selective pressure from the host immune response and/or antiviral therapy, viruses with mutations emerge as the dominant viral population. This review presents an overview of HBV biology, diagnostic laboratory assays, vaccines, antiviral agents, and the association with specific HBV mutations.

Epidemiology

Humans are the only reservoir for HBV, which is 50 to 100 times more infectious than HIV. In high endemic areas, most HBV infections occur at birth or during early childhood. Hepatitis B virus infection is spread through contact with infected serum, sexual activity, and vertical transmission from mother to infant. Childhood infections with HBV are usually asymptomatic. These infections may not be recognized for many years. The prevalence of HBV infection varies in different parts of the world, with most of the disease burden occurring in Asia and Africa.[4] Hepatitis B virus is naturally heterogeneous with 8 genotypes (A-H) that differ genetically by greater than 8%.[5] This diversity is driven by viral replication and the survival of virions that exhibit an advantage. Recently some patients have been identified with HBV infections evading vaccine-induced immunity. It has also been noted that some HBV infections cannot be detected by laboratory diagnostic assays.[6,7] It appears that these variant HBV have been able to evade specific human immunity due to a natural selection of mutants during viral replication.

Description of Virus

Hepatitis B virus is in the family *Hepadnaviridae* and was first identified by Blumberg and colleagues in 1965.[8,9] Since that time there has been a large amount of work on the nature of the virus and disease in humans.

The HBV genome is organized as a partially double-stranded, circular DNA molecule of approximately 3200 bases encased within viral-specific proteins (Figure 1).[10] The longer strand of HBV DNA (L strand) is a complete circle with a nick. The complementary strand is shorter (S strand) than the L strand.[11,4] This virus is unique among human viral pathogens as it is a DNA virus that replicates via an RNA intermediate.
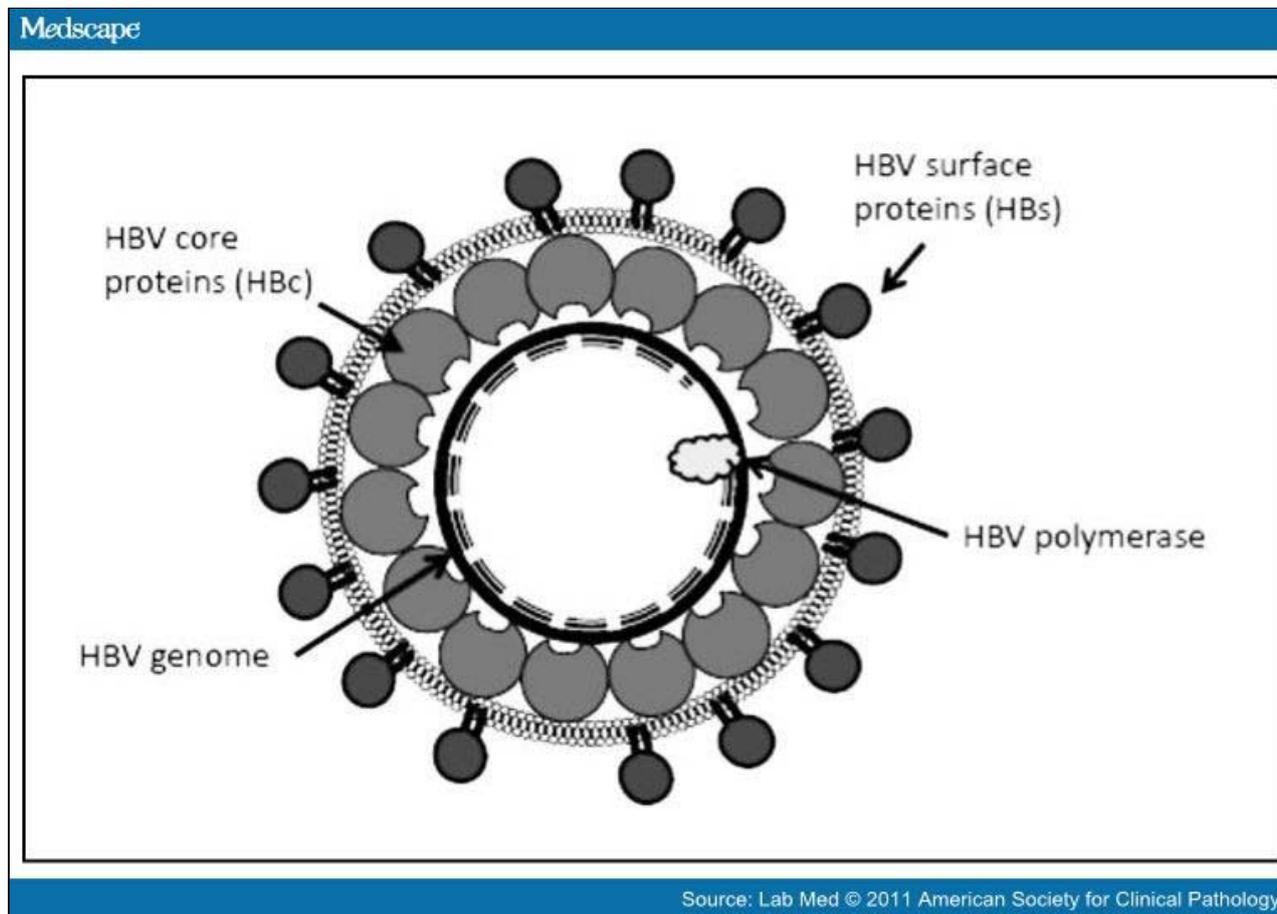
Figure 1.

Schematic of HBV intact virion. HBV is a DNA virus that is approximately 42 nm in diameter and consists of an inner protein core (HBc) and an outer protein envelope (HBs). The nucleocapsid is arranged into an icosahedral formation and contains the hepatitis B genome and at least 1 HBV polymerase protein. Figure was drawn from information in references 4, 10, and 11.

Hepatitis B virus is a very efficient virus with every nucleotide in at least 1 coding region and many times 2 coding regions. The HBV DNA contains genes that overlap and are transcribed in different open reading frames (ORFs) (Figure 2).[10,12,13] There are 4 ORFs directing the transcription and translation of all HBV proteins by using different start codons (Figure 2). Consequently, a relatively small genome generates 7 distinct proteins. These proteins are the polymerase protein (Pol gene), which is the largest HBV protein; core antigen and e antigen (both from the C gene); large, medium, and small surface-antigen proteins (S gene); and the X protein (X gene) (Figure 2). The overlap in the ORFs does not appear to limit variability since all HBV genes have variants.[11]
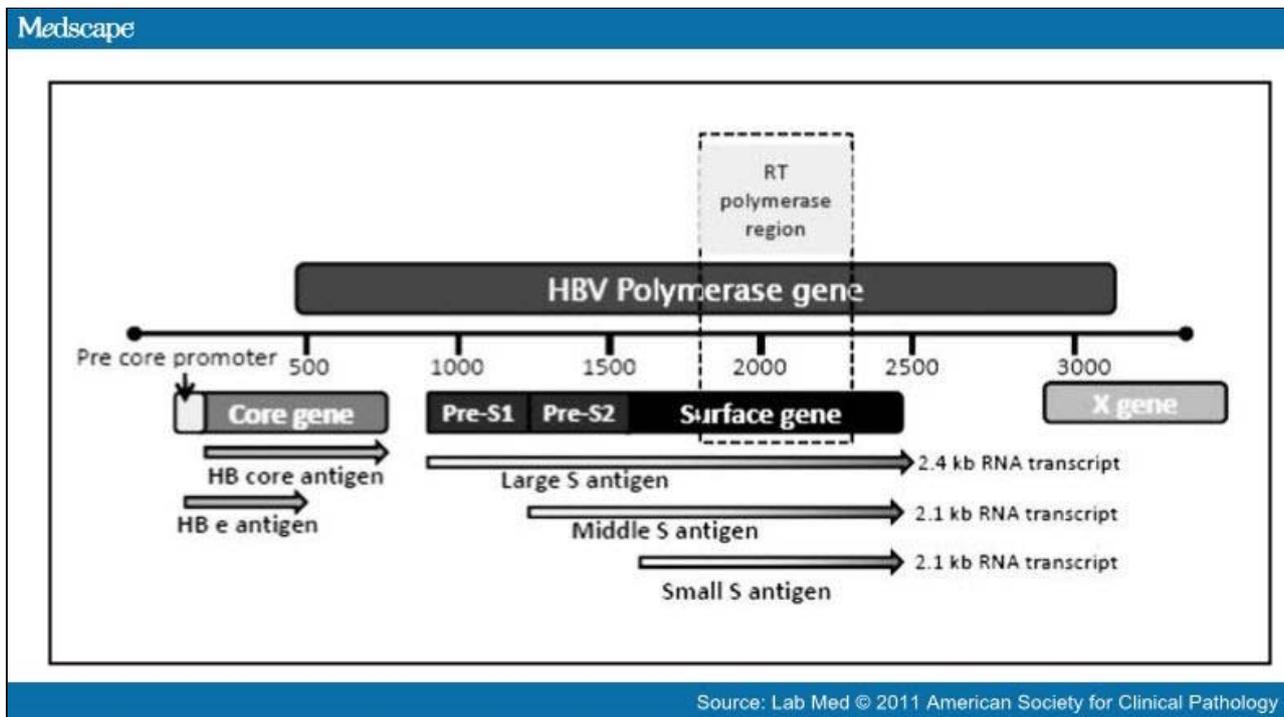
Figure 2.

The HBV polymerase gene overlaps completely with the genes of the 3 surface (S) antigen proteins. The area boxed by dashed lines indicates the area of the HBsAg immunodominant area, and the HBV reverse transcription area indicates the genome area most likely to produce mutants in both genes. Figure was drawn from information in references 10, 11, 12, 13, and 46.

During infection the HBV virion in the blood moves to the liver where it binds specifically to hepatocytes. After binding and entering the cell, the virus is uncoated in the host cell cytoplasm. The partially double-stranded viral genome is converted to a covalently closed, circular DNA (cccDNA) form by the host cell enzymes. This circular DNA is used as the template for transcription of all the HBV mRNAs.[2,14] The infectious virions are produced and packaged with an RNA transcript of the entire genome (3.5 kb), which is then translated into the circular DNA molecule. This long RNA transcript and the HBV polymerase are encased first in the HBV core proteins. At this time the reverse transcriptase (RT) enzyme (HBV polymerase) synthesizes a new viral DNA genome. This particle then becomes enclosed in the HB surface antigen (HBsAg) during passage through the cytoplasm during the process of leaving the hepatocyte as an intact virion (Figure 1). The viral particles are released from the host cells into the tissue and blood stream. A large excess of the HBsAg is made during this replication process and is also released from infected cells as empty spherical and filamentous particles. These HBsAg particles can be detected in serum as a diagnostic marker of active HBV replication.

Disease

Hepatitis B virus disease can occur as an acute or chronic disease. Acute disease typically occurs in infected adolescents or adults who have not been vaccinated. This acute presentation can be life threatening due to massive liver damage from the host immune reactions. Recovering patients eventually contain the virus and reduce the immune damage in infected tissue. This recovery phase is detected by the loss of HBV DNA and HBsAg in serum and the production of HBV-specific antibodies.[15]

Chronic HBV is the consequence of an infection acquired in early childhood in which the immune system tolerates the virus. These chronic HBV infections can be associated with high levels of viral replication with few or no clinical symptoms. Patients will have high levels of HBV DNA and HBsAg in serum. In a few patients, the HBsAg will disappear from the circulation, followed by the appearance of antibody to HBsAg (anti-HBs) and HBV core antigen (anti-HBc).[11,16,17] This stage of disease is referred to as a chronic inactive HBV infection.

An HBV infection passed from mother to child poses a higher probability that the child will become a chronic HBV carrier. These infections are associated with the high risk of eventually developing severe liver damage or HCC.[11,17] These patients have very high HBV viral levels often for a lifetime leading to a significant chance of passing the virus on to other individuals.[11,17]

Diagnostic Assays

A number of sensitive and specific diagnostic tests have led to a deeper understanding of the natural history of this disease.[16] These tests establish the HBV disease stage as well as monitor for effective vaccination and treatment ().

Table 1. Diagnostic Assays for HBV

Assay Type	Name	Use
HBV antigen	HBs Antigen	Detected in active HBV infection
·	HBe Antigen	Detected during high level of HBV replication
HBV antibody	Anti-HBs	Detected in immune individuals due to immunization and/or infection
·	Anti-HBc IgM	Detected in a new HB infection
·	Anti-HBc total	Detected in individuals with a current HBV infection or past infection
·	Anti-HBe total	Detected after recovery from an acute infection
HBV molecular	Quantitative HBV DNA	Detected in an early HBV infection and used to monitor HBV-infected patients during antiviral treatment

There are 2 HBV-specific proteins that can be detected directly in the serum of an infected patient. These are the HBsAg and the HBV e antigen (HBeAg). The HBsAg is a major viral protein inducing protective immune responses in humans. This antigen is found on the surface of the viral envelope and is also found in high concentrations in the serum of infected patients. The presence of HBsAg in serum for more than 6 months indicates a chronic HBV infection.[1,18]

The presence of HBeAg indicates a high level of viral replication. Some variants of HBV do not produce the HBeAg; however, they continue to have high levels of HBsAg. These HBV variants are associated with a poor clinical outcome as discussed below.[19]

Several specific antibodies can also be detected in both active and chronic HBV infections. The presence of antibody to HBsAg (anti-HBs) indicates either a past infection with HBV or an individual who has been vaccinated.[11,20] A protective antibody response to vaccine is defined as the presence of >10 IU/mL of anti-HBs. In many countries the presence of anti-HBs is used to monitor for effective vaccination. The presence of antibody to HB core antigen (anti-HBc) is used to determine infection with HBV.[11,16,20] The vaccine does not contain the HBc antigen and thus will not induce HBc antibody. Immunoglobulin M (IgM) specific antibody to HBc can also be detected by clinical laboratories and indicates recent HBV exposure. The patient may present with an acute HBV infection.[3,20]

The anti-HBc develops first after HBV infection while anti-HBs antibody is detected later.[11,16,20] The delay in detection of anti HBs is likely due to the rapid binding of the antibody to the HBsAg present at high levels in infected patients. This binding of antibody decreases the circulating anti-HBs, leading to the loss of detection in laboratory assays. The anti-HBs bind to a major epitope on the HBs proteins on intact virions as well as infected cells and are effective in preventing HBV infection. This is the basis for using HBsAg in all the HBV vaccines. It is also the protective antibody found in irradiated HBV immune globulin (HBIG), which is used to prevent infection in newly infected patients.[21]

The antibody to the hepatitis B e antigen (anti-HBe) is detected only during acute HBV infections. It does not directly neutralize the HBV virion because intact virus particles do not contain HBeAg. However, it has been noted that anti-HBe levels decline when viremia declines, indicating this immune response may have a protective nature.[20]

The presence of HBV in human serum can also be detected using molecular methods. Currently, there are several reliable molecular assays that quantitate HBV DNA levels.[22] These assays are used for both the initial evaluation of HBV infections and the monitoring of patients before and during therapy.[23,24] Additionally, blood donors in the United States and some European countries are routinely screened for HBV DNA using a qualitative assay to detect donors in the earliest stage of HBV infection.[22] All of the molecular assays have wide dynamic ranges from 5 copies/mL and 10 copies/mL to levels greater than 1 million copies/mL. This allows monitoring HBV DNA during infection and identifying HBV infections that become resistant to antiviral therapy.[22,25] In 2001 a high-titer HBV genotype A (code 97/746) was established as an international HBV DNA standard by the WHO. It was assigned a potency of 106 international units/milliliter (IU)/mL and now provides a reliable way to evaluate HBV DNA quantitative values.[1,26] The standard also established that 1 IU of HBV is equivalent to 5.[4] genome equivalents/copy.[1,26]

The available HBV DNA assays use conversion factors based on this standard material. Commercial assays have different conversion factors demonstrating their variability. As a result, the best practice is the consistent monitoring of patients using the same assay in the same laboratory.[22]

With the use of these diagnostic assays it is possible to detect new infections, determine protective vaccine efficacy, and monitor HBV-infected individuals on antiviral therapy. In spite of these assays there are now some new challenges for HBV disease. The HBV has the ability to change in response to antiviral therapy as well as vaccinations. There is evidence that some new HBV variants evade the current diagnostic assays as well as vaccine-induced immunity.

HBV Vaccines

The basis for prevention of HBV infections is the use of effective vaccines.[2,4] Such HBV vaccines have been available since 1982.[2] Their success in preventing disease has been proven both in the United States as well as other countries monitored by the WHO.[27,28] In the United States there has been a decline of new HBV infections since 1991, when a national HBV vaccination program was implemented.[29] The WHO reports that 164 countries have HBV vaccination programs for infants, and since 1982 more than 1 billion doses of HBV vaccine have been administered worldwide.[28] The complete HBV vaccination series is protective in >95% of infants, children, and adults.[28,30,31] Vaccine-induced protection lasts at least 20 years and may be lifelong, with an exceptional record of safety.[2,31]

In the United States, 2 single-antigen vaccines and 3 combination vaccines for HBV are available (). All vaccines contain the recombinant HBsAg produced in yeast cultures. The 2 single antigen vaccines are Energix-B (GlaxoSmithKline, Rixensart, Belgium) and Recombivax HB (Merck, Whitehouse Station, NJ). The first combination vaccine (Comvax [Merck]) contains a Haemophilus b conjugate antigen with the recombinant HBsAg. It is given as a series beginning at 2 months of age with boosters given at 4 months and between the ages of 12 to 15 months. The CDC recommends that newborns receive the monovalent HBV vaccine at birth followed by 3 additional doses of the combined Haemophilus b and HBsAg vaccine The second combination vaccine (Pediarix, GlaxoSmithKline) consists of recombinant HBsAg, diphtheria, tetanus, and acellular pertussis (DTaP), and inactivated poliovirus (IPV). This vaccine has not been approved for children younger than 6 weeks of age or over the age of 7 years.

Table 2. HBV Vaccines Approved in the United States

Trade Name	Manufacturer	AntigensIncluded	Patient Population	Dosage	Schedule
Comvax	Merck	*Haemophilus influenzae* type b capsular polysaccharide + recombinant HBsAg	Infants 6 weeks to 15 months born of HBsAg-negative mothers	7.5 mcg Hemophilus b polysaccharide 5 mcg HBsAg	2, 4, and 12–14 months
Engerix-B	GlaxoSmith Kline Biologicals	Purified recombinant HBsAg from *Saccharomyces cerevisiae*	Infants-adults	Infant-19 yrs=10 µg/dose	0, 1, and 6 months
.	.	.	.	Adults (>19 years) =20 µg/dose	.
.	.	.	.	Adult hemodialysis=20 µg/dose	.
Pediarix	GlaxoSmith Kline Biologicals	Diphtheria and tetanus toxoids (DT); acellular pertussis toxoid, filamentous hemagglutinin; HBsAg and inactivated poliovirus (IPV) types 1, 2, and 3	Infants born of HBsAg-negative mothers.	6 weeks-6 years	2, 4, and 6 months
Recombivax HB	Merck	HBsAg produced in yeast	Infants-adults	Infant-19 yrs=10 µg/dose	0, 1, 6 months
.	.	.	.	Adults (>19 years) =10 µg/dose	.
.	.	.	.	Adult hemodialysis=40 µg/dose	.
Twinrix	GlaxoSmith Kline Biologicals	Inactivated hepatitis A (strain HM175) grown in MRC-5 cells with purified HBsAg	Persons >18 years	720 units of inactivated hepatitis A virus and 20 µg of HBsAg/dose	0, 1, 6 months Alternative dosing dose 4 doses on days 0, 7, and 21 –30 followed by a booster dose at 12 months

The third combination vaccine includes inactivated hepatitis A virus (strain HM175) and recombinant HBsAg (Twinrix, GlaxoSmithKline). This vaccine is recommended for individuals 18 years of age or older who are at an increased risk for hepatitis A virus and HBV infections.

The vaccination schedule for monovalent HBV vaccines for children and adults is 3 intramuscular injections with the second and third doses administered at 1 and 6 months, respectively, after the first dose.[2,4] If this schedule is interrupted, subsequent vaccinations can be given even if they are not at the intervals noted above. The second and third dose should still be separated by at least 8 weeks. If the third dose is the only 1 delayed, then it can be given at any time. An extra dose of HBV vaccine does not appear to be harmful. The vaccine is also effective when given to immunocompromised patients, such as hemodialysis or HIV-infected individuals;21 however, these patients may require a larger vaccine dose to induce

protective immunity (). Post vaccination testing for anti-HBs should be done on all vaccinated individuals to determine the effectiveness of the vaccine.[2,4] This testing should occur at least 6 weeks after the final vaccine dose is administered.

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Engerix-B	GlaxoSmith Kline Biologicals	Purified recombinant HBsAg from *Saccharomyces cerevisiae*	Infants-adults	Infant-19 yrs=10 µg/dose	0, 1, and 6 months
.	.	.	.	Adults (>19 years) =20 µg/dose	.
.	.	.	.	Adult hemodialysis=20 µg/dose	.
Pediarix	GlaxoSmith Kline Biologicals	Diphtheria and tetanus toxoids (DT); acellular pertussis toxoid, filamentous hemagglutinin; HBsAg and inactivated poliovirus (IPV) types 1, 2, and 3	Infants born of HBsAg-negative mothers.	6 weeks-6 years	2, 4, and 6 months
Recombivax HB	Merck	HBsAg produced in yeast	Infants-adults	Infant-19 yrs=10 µg/dose	0, 1, 6 months
.	.	.	.	Adults (>19 years) =10 µg/dose	.
.	.	.	.	Adult hemodialysis=40 µg/dose	.
Twinrix	GlaxoSmith Kline Biologicals	Inactivated hepatitis A (strain HM175) grown in MRC-5 cells with purified HBsAg	Persons >18 years	720 units of inactivated hepatitis A virus and 20 µg of HBsAg/dose	0, 1, 6 months Alternative dosing dose 4 doses on days 0, 7, and 21 –30 followed by a booster dose at 12 months

The HBsAg used in all vaccines is from the *adw* subtype of HBsAg. Thus, the immune response induced by vaccines made by different manufacturers does not appear to be significantly different. Therefore, a patient can receive the first dose of vaccine from 1 manufacturer and additional vaccine doses made by a different manufacturer.[28] Anyone who has an allergy to yeast should not receive vaccinations with any of the HBV vaccines since they are all prepared in yeast cultures.

Persons with a known or suspected exposure to HBV should be given the HBV vaccine as soon as possible after the exposure in addition to HBIG. Administration of vaccine and HBIG within 24 hours has been shown to prevent HBV infection.[21] Guidance on HBV vaccination is available from the Advisory Committee on Immunization Practices.

There is hope that the use of HBV vaccines will control and eradicate HBV infection. However, several reports have described the presence of HBV mutants, which have been detected in vaccinated individuals.[32–35] These viruses have an altered HBsAg resulting in HBV infections that escaped vaccine induced immunity. Additionally, some individuals had an HBsAg that was undetectable with the current HBsAg assays.[36–38] Thus, the virus can escape both the vaccine induced immunity as well as the detection methods used by laboratory assays. These strains are now known as HBV "escape mutants."[6,33,34]

Variability in HBV Mutants

The HBV genome is arranged as 4 overlapping ORFs (Figure 2). These ORFs encode the HBsAg (*S* gene) with 3 different start codons resulting in 3 different HBsAg proteins. These are the large, medium, and small HBsAg proteins. The largest ORF is the *P* gene, which codes for the DNA polymerase. The *X* gene encodes the regulatory protein, and the *C* gene codes for both the HBeAg and HBc proteins. The reproduction of HBV DNA occurs through an RNA copy that is then converted into a DNA genome by a HBV RT (polymerase). The RT activity of the HBV polymerase enzyme does not have proof-reading ability, resulting in frequent mistakes that are incorporated into a new DNA strand. The number of viral particles generated in infected persons can be as high as 1011 viral particles per day. Because the polymerase reverse transcription error rate is high (~1 per 100 bases), a large number of errors are generated during a highly active infection.[6] During the replication of HBV, the polymerase error rate is estimated at approximately 1 error per 107 bases.[6,13] Thus, the only condition needed to produce mutations is the replication of HBV.

The replication of the 3200 nucleotides of the HBV genome results in approximately 107 errors per day.[6,39] Individuals with chronic diseases often have 108 to 1011 HBV viral particles/mL per day. Thus, a large number of HBV variants are generated. Many of these mutants do not survive the genomic change. However, some modifications to the HBsAg will provide the virus with an advantage. One advantage is the ability to escape HBV specific immunity induced by the vaccine. This would allow the new virus strain to replicate in the presence of a vaccine-induced antibody.

Currently, HBV mutants have been recognized in previously vaccinated individuals as well as in neonates treated with HBIG.[6,33,39] Because of the unique character of overlapping genes in the HBV genome, the reading frames of a single nucleotide can potentially lead to amino acid changes in 2 different viral proteins. An example of this is the RT gene and the HBsAg gene (Figure 2).

HBV Mutants Effecting Detection and Vaccination

The HBsAg is a peptide with 226 amino acids with a single major antigenic determinant. Within this protein there are highly conserved areas defining the genotype of the virus. Substitutions in these sites change the antigenic determinants essential for inducing protective antibody.

The 1 major antigenic determinant is called the "a" determinant and is located in the amino acid positions between 100 and 16040 (Figure 2, boxed area). Mutations inducing a conformational change within the "a" determinant result in a protein with significant 3 dimensional changes in the antigenic epitope. These conformational changes result in HBsAg that is not detected by diagnostic assays and/or by vaccine induced immunity.[10,36,41]

The first recognition of an escape mutant was in a child who acquired HBV from the mother. The HBV was transmitted to the young baby in spite of the rapid administration of HBIG and HBV vaccination.[33] Further studies showed that the HBV transmitted to the baby had a HBsAg mutation at the amino acid position 145 that translated into a change from a glycine to an arginine. This single alteration changed the conformation of the protein resulting in an antigenic epitope not recognized by the anti-HBs in the HBIG. This allowed the altered virus to establish infection by escaping the HBV antibodies. The patient remained positive for HBV DNA with the mutated HBsAg for at least 12 years.[32] Subsequently other mutations within the "a" determinant region of HBsAg have been recognized, raising concerns that this could impact the success of the vaccination program.[6,34,42] Recent publications have estimated the prevalence of HBsAg variants among random chronic carriers to be between 6% to 12%.[43] Of great concern is the possibility that escape HBV mutants will spread to other successfully vaccinated individuals.[44]

As shown above, mutations in the "a" determinant of the *S* gene result in virions that escape the neutralizing anti-HBs induced by vaccination leading to HBV infections in vaccinated individuals.[32,33] The most common HBV mutants associated with immune escape are those with a change in the 145 location. However, there are also other mutations in the "a" determinant of the *S* genes resulting in an occult HBV infection (). These infections are characterized by the presence of HBV DNA in the absence of HBsAg.[45]

Table 3. Vaccine and Diagnostic Mutants in HBV

Vaccine Escape	HBsAG Assay Escape	Precore (PC)	Basal Core Promoter (BCP)
Gly145Arg	Thr 126 Ser	Gly1896Ala	Ala1762Thr
Asp144Ala	Gln 129 His	Cys1858Thr	Gly1764Ala
	Met 133 Leu	.	Thr1753Cys

Insertion of Asn-Ser-Thr-Gly-Pro-Cys-Thr-Thr between Thr-123 and Cys-124				
▫	Asp144Ala	▫	Ala1762 Thr	
▫	Gly145Arg	▫	Gly1764Ala	
▫	Thr126Ser	▫	Cys1766Thr	

References 13, 32, 33, 34, 35.International Union of Biochemistry (IUB) 3-letter code for amino acid: Ala, Alanine; Arg, Arginine; Asn, Asparagine; Asp, Aspartic acid; Cys, Cysteine; Gly, Glyceine; Gln, Glutamine; Ile, Isoleucine; Leu, Leucin; Met, Methionine; Phe, Phenylalanine; Pro, Proline; Ser, Serine; Thr, Threonine; Val, Valine.

The related issue is whether these escape HBsAg mutants can be detected by diagnostic assays. The HBV mutants with conformational changes in the "a" determinant may produce false-negative or low reactive results in some diagnostic assays. Certain variations in HBs codons between 121–124 have reduced immunogenicity, whereas substitution of cysteines, especially with serine, results in reduced or complete loss of immunoreactivity in laboratory assays[46] ().

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Insertion of Asn-Ser-Thr-Gly-Pro-Cys-Thr-Thr between Thr-123 and Cys-124	Met 133 Leu	·	Thr1753Cys
·	Asp144Ala	▫	Ala1762 Thr
·	Gly145Arg	·	Gly1764Ala
·	Thr126Ser	▫	Cys1766Thr

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A number of studies have evaluated the available HBsAg diagnostic assays to determine their ability to detect well-defined HBsAg mutant proteins.[6,36,41] The majority of diagnostic HBsAg assays use monoclonal antibodies directed to the immunodominant "a" determinant of the HBsAg (amino acid residues 100 thru 160) in order to capture the protein.[47] Mutations within this region change the ability of the protein to be detected by monoclonal antibodies. Thus, the use of monoclonal antibody to capture HBsAg may not be sufficient to detect altered HBsAg associated with mutations. A more robust approach would be the use of a pool of well-characterized polyclonal antibodies directed to HBsAg. This has been adopted by some manufacturers, and the assays appear to detect mutant HBsAg.[6] However, the consistency of polyclonal antibodies in any commercial diagnostic assay may be difficult to sustain.[6,39] Consequently, due to these problems the HBsAg assays should not be used as the only test for detecting HBV infection. As a result, anti-HBc screening of blood donors was introduced in several countries to ensure that blood donors who are HBsAg negative are not infected with escape mutants.[48,49] In addition, several European countries and the United States now screen for HBV DNA using molecular tests.[49,50] Donors with positive tests for HBV DNA and anti-HBc and negative for HBsAg should be checked for possible infection with an escape mutant.

A list of documented mutations associated with occult HBV infection is found in . Several studies have shown the site of a mutation and not the number of mutations appears to be important in determining non-reactivity in an assay.[47] Other mutations are associated with occult HBV infection. These mutations usually occur in the PreS regions of the HBV genome (Figure 2). The peptides coded in the PreS1 region contain the hepatocyte binding site (amino acids 21–47). This peptide section is essential for virion assembly and the transport of intact virions out of the host cell. These mutations result in the inactivation of a promoter within the PreS2/S region resulting in the interference with HBsAg secretion.[51,46]

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·		▫	

			Asp144Ala			Ala1762 Thr
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HBV Mutations Associated With Disease Progression

The detection of HBeAg in serum indicates active disease with a high level of viral replication. The HBeAg is transcribed just upstream from the start of the HBc gene (Figure 2). There are 2 classes of mutants in the HB core promoter region affecting HBeAg expression, basal core promoter (BCP) mutants and precore (PC) mutants.[46,52,53] The transcription of HBV PC mRNA and pregenomic RNA (whole HBV genome) occurs on the same large mRNA molecule. This combined core protein RNA and viral polymerase RNA molecule is also used as the template for new viral replication. The HB core promoter is regulated by an enhancer protein controlling the transcription of PC mRNA and pregenomic RNA.

The BCP mutations result in changes in the transcriptional mechanism, leading to a reduction in HBeAg levels with a consequent increase in viral replication.[46,52,54] The PC HBV mutants are a result of a translational stop codon eliminating HBeAg production, resulting in HBeAg-negative disease.[19,53,54] Over time these mutants become the dominant viral population during late stages of HBV infection.[46,52] It has also been recognized that increased viral replication with little or no HBeAg is associated with increased liver damage.[53] This is due to an increase in HBV replication within the cell with reduced secretion of virions. This increase of virus within the liver cells triggers immune-mediated liver damage. The damage to the liver increases hepatocyte turnover, leading to fibrosis. This then leads to increased chances of hepatocyte transformation and malignancy.[53]

The BCP mutation most frequently detected is a double mutation at Ala1762Thr and Gly1764Ala. However, other mutations within the BCP/Pre core region have been identified ().[46,52,53] These mutations result in decreased HBeAg expression with enhanced viral replication. The prevalence of core promoter mutation is approximately 40% and can be detected in all major HBV genotypes. Studies show that these mutations are associated with an increased risk of HCC.[55]

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References 13, 32, 33, 34, 35.International Union of Biochemistry (IUB) 3-letter code for amino acid: Ala, Alanine; Arg, Arginine; Asn, Asparagine; Asp, Aspartic acid; Cys, Cysteine; Gly, Glyceine; Gln, Glutamine; Ile, Isoleucine; Leu, Leucin; Met, Methionine; Phe, Phenylalanine; Pro, Proline; Ser, Serine; Thr, Threonine; Val, Valine.

Mutations Associated With Drug Resistance

Antiviral treatment for chronic HBV has been used for more than 20 years to reduce HBV levels in patients. This reduction in viral levels also reduced the long-term damage to the liver. The antiviral agents successful in inhibiting HBV include nucleotide and nucleoside analogs such as adefovir, entecavir, famciclovir, lamivudine, lobucavir, and tenofovir, which inhibit polymerase activity. In many patients these drugs have been able to successfully reduce HBV to undetectable levels for many years.[23,56,57] On the other hand, these drugs apply selective pressure on HBV, leading to mutations

conferring antiviral resistance (). The failure of antiviral treatment is defined as the absence of a 1 $\log_{10}$ decrease in HBV DNA within 3 months of starting therapy.[1] This usually indicates the presence of antiviral resistance. However, other reasons, such as lack of adherence to therapy or metabolic causes, should also be considered.[57,58] The HBV polymerase gene is the largest ORF in the viral genome (Figure 2). It is divided into 4 domains referred to as the following: 1) primer region, 2) spacer region, 3) RT, and 4) RNase H region.[46] The active site of this polymerase is within a conserved region of the gene known as the Tyr-Met-Asp-Asp (YMDD) motif (Figure 2, boxed area).[13]

Table 4. HBsAg Mutants Associated With Antiviral Resistance

	Antiviral Agents				
Lamivudine	**Telbivudine**	**Entecavir**	**Emtricitabine**	**Adefovir**	**Tenofovir**
Met204Val	▫	▫	Met204Val	▪	▫
Met204Ile	Met204Ile	▪	Met204Ile	▪	▪
Ala181Val	Ala181Val	▫	▫	Ala181Val	Ala181Val
Ala181Thr	Ala181Thr	▪	▪	Ala181Thr	Ala181Thr
Ser202Ile	▫	Ser202Ile	▫	▪	▫
Ser202Gly	▪	Ser202Gly	▪	▪	▪
▪	▫	▫	▫	Asn236Thr	Asn236Thr
Val173Leu	▪	Thr184Als/Phe/Met/Cys/Ser/Gly/Ile/Leu	▪	▪	Ala194Thr
Met204Ser	▫	Met250Val/Ile/	▫	▪	▫
▪	▪	Leu	▪	▪	▪
Leu180Met	▫	Ser202Cys	▪	▪	▪

References 60, 61, 62, 64. International Union of Biochemistry (IUB) 3-letter code for amino acid: Ala, Alanine; Arg, Arginine; Asn, Asparagine; Cys, Cysteine; Gly, Glyceine; Ile, Isoleucine; Leu, Leucin; Met, Methionine; Phe, Phenylalanine; Ser, Serine; Thr, Threonine; Val, Valine.

The use of nucleoside analogues to inhibit HBV replication has resulted in the development of several resistant mutants within this conserved region. These mutations translate into specific amino acid changes in the YMDD motif. shows the different point mutations in the C domain of the RT, resulting in specific amino acid alterations conferring antiviral resistance.[13,46] The antiviral agent lamivudine has exerted pressure on this region for a longer time than the newer antiviral agents. However, mutations induced by lamivudine often confer resistance to the newer antiviral agents. Of concern is the finding that HBV-infected patients who have not received any antiviral therapy can have HBV with antiviral resistance mutations.[46] Adefovir Dipivoxil (ADV) treatment, which is an alternative therapy for lamivudine-resistant HBV, can also result in the selection of ADV-resistant variants.[59] Mutations associated with entecavir resistance occur within the background of lamivudine resistance. When patients receive entecavir for lamivudine failure, various substitutions result in mutations, which cause an increased level of entecavir resistance.[60] shows that some resistance mutations predict resistance to more than 1 antiviral agent. Several reports have documented naïve HBV patients infected with adefovir-resistance mutants.[61,62] Thus, drug-resistant mutants can be transmitted to a naïve subject and establish a stable HBV infection.[46,59]

Table 4. HBsAg Mutants Associated With Antiviral Resistance

	Antiviral Agents				
Lamivudine	Telbivudine	Entecavir	Emtricitabine	Adefovir	Tenofovir
Met204Val			Met204Val		
Met204Ile	Met204Ile		Met204Ile		
Ala181Val	Ala181Val			Ala181Val	Ala181Val
Ala181Thr	Ala181Thr			Ala181Thr	Ala181Thr
Ser202Ile		Ser202Ile			
Ser202Gly		Ser202Gly			
				Asn236Thr	Asn236Thr
Val173Leu		Thr184Als/Phe/Met/Cys/Ser/Gly/Ile/Leu			Ala194Thr
Met204Ser		Met250Val/Ile/			
		Leu			
Leu180Met		Ser202Cys			

References 60, 61, 62, 64.International Union of Biochemistry (IUB) 3-letter code for amino acid: Ala, Alanine; Arg, Arginine; Asn, Asparagine; Cys, Cysteine; Gly, Glyceine; Ile, Isoleucine; Leu, Leucin; Met, Methionine; Phe, Phenylalanine; Ser, Serine; Thr, Threonine; Val, Valine.

An additional concern is that there is significant overlap between conserved regions in the HBV polymerase gene and the "a" determinant of the HBsAg gene (Figure 2). Thus, mutations within the shared DNA sequence will induce amino acid changes in both protein products, leading to a virus with a variant HBsAg and antiviral resistance. Amino acid substitutions in the HBsAg, including Glu164Asp, Trp196Ser, and Ile195Met, are associated with reduced binding to anti-HBs antibody without affecting the function of the protein. These genetic changes correspond to the HBV polymerase gene with changes in the protein of Val173Leu, Met204Ile, and Leu180Met, which renders the virus resistant to lamivudine treatment.[13,63] Equally likely is the possibility that the HBV polymerase substitutions, Thr128Asn and Trp153Gln, may lead to the selection of an antibody escape mutant. These changes in the polymerase gene lead to substitutions at Pro120Thr and Gly145Arg in the HBsAg gene. The importance of this possibility is that HBsAg mutations selected during antiviral therapy can lead to a virus escaping HBV vaccination.[13] It is clear that mutations emerge, leading to changes in both proteins. This appears to be a unique feature of HBV due to the overlapping nature of the genes.

Summary

Chronic HBV infection is a major cause of morbidity and mortality worldwide. An accurate diagnosis of infected patients is essential to disease detection and effective treatment the infection. The introduction of the HBV vaccine has reduced the rate of new infections by effectively preventing the transmission of this virus.

However, in the last 20 years a number of HBV mutants have emerged. The fluid nature of HBV lends itself to rapid change due to the imprecise RNA polymerase allowing random heterogeneity. This permits selection of mutated HBV by host immunity and antiviral therapy. A number of well-characterized HBV mutations have been recognized, leading to vaccine failure, loss of HBV detection by diagnostic assays, increased viral replication leading to hepatic damage, and resistance to antiviral agents.

This issue requires constant surveillance of infected patients and should include vaccine reformulation including known HBsAg mutations. To determine the significance of HBV variants, active surveillance is needed along with continued evaluation of diagnostic assays and therapeutic agents. This issue requires constant surveillance of infected patients and should include vaccine reformulation including known HBsAg mutations. To determine the significance of HBV variants, active surveillance is needed along with continued evaluation of diagnostic assays and therapeutic agents.

References

1. Sorrell MF, Belongia EA, Costa J, et al. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis B. *Ann Intern Med*. 2009;150:104–110.

2. Shepard CW, Simard EP, Finelli L, et al. Hepatitis B virus infection: Epidemiology and vaccination. *Epidemiol Rev*. 2006;28:112–125.

3. Degertekin B, Lok AS. Update on viral hepatitis: 2008. *Curr Opin Gastroenterol*. 2009;25:180–185.

4. Mahoney FJ. Update on diagnosis, management, and prevention of hepatitis B virus infection. *Clin Microbiol Rev*. 1999;12:351–366.

5. Lin CL, Kao JH, Chen BF, et al. Application of hepatitis B virus genotyping and phylogenetic analysis in intrafamilial transmission of hepatitis B virus. *Clin Infect Dis*. 2005;41:1576–1581.

6. Coleman PF. Detecting hepatitis B surface antigen mutants. *Emerg Infect Dis*. 2006;12:198–203.

7. Gerlich WH, Bremer C, Saniewski M, et al. Occult hepatitis B virus infection: Detection and significance. *Dig Dis*. 2010;28:116–125.

8. Alter HJ, Blumberg BS. Further studies on a "new" human isoprecipitin system (Australia antigen). *Blood*. 1966;27:297–309.

9. Blumberg BS, Alter HJ, Visnich S. A "new" antigen in leukemia sera. *JAMA*. 1965;191:541–546.

10. Chisari FV. Hepatitis B virus biology and pathogenesis. *Mol Genet Med*. 1992;2:67–104.

11. Liang TJ. Hepatitis B: The virus and disease. *Hepatology*. 2009;49(5 suppl):S13–S21.

12. Günther S. Genetic variation in HBV infection: Genotypes and mutants. *J Clin Virol*. 2006;36(suppl 1):S3–S11.

13. Harrison TJ. Hepatitis B virus: Molecular virology and common mutants. *Semin Liver Dis*. 2006;26:87–96.

14. Yim HJ, Lok AS. Natural history of chronic hepatitis B virus infection: What we knew in 1981 and what we know in 2005. *Hepatology*. 2006;43(2 suppl 1):S173–S181.

15. Bertoletti A, Gehring AJ. The immune response during hepatitis B virus infection. *J Gen Virol*. 2006;87:1439–1449.

16. Krajden M, McNabb G, Petric M. The laboratory diagnosis of hepatitis B virus. *Can J Infect Dis Med Microbiol*. 2005;16:65–72.

17. Lok AS, McMahon BJ. Chronic hepatitis B: Update 2009. *Hepatology*. 2009;50:661–662.

18. McMahon BJ. The natural history of chronic hepatitis B virus infection. *Hepatology*. 2009;49(5 suppl):S45–S55.

19. Carman WF, Thursz M, Hadziyannis S, et al. Hepatitis B e antigen negative chronic active hepatitis: Hepatitis B virus core mutations occur predominantly in known antigenic determinants. *J Viral Hepat*. 1995;2:77–84.

20. Bowden S. Serological and molecular diagnosis. *Semin Liver Dis*. 2006;26:97–103.

21. Centers for Disease Control and Prevention. Recommendations for preventing transmission of human immunodeficiency virus and hepatitis B virus to patients during exposure-prone invasive procedures. *MMWR Recomm Rep*. 1991;40:1–9.

22. Valsamakis A. Molecular testing in the diagnosis and management of chronic hepatitis B. *Clin Microbiol Rev*. 2007;20:426–439.

23. Andersson KL, Chung RT. Monitoring during and after antiviral therapy for hepatitis B. *Hepatology*. 2009;49(5 suppl):S166–S173.

24. Wursthorn K, Manns MP, Wedemeyer H. Natural history: The importance of viral load, liver damage and HCC. *Best Pract Res Clin Gastroenterol*. 2008;22:1063–1079.

25. Krajden M, Minor J, Cork L, et al. Multi-measurement method comparison of three commercial hepatitis B virus DNA quantification assays. *J Viral Hepat*. 1998;5:415–422.

26. Heermann KH, Gerlich WH, Chudy M, et al. Quantitative detection of hepatitis B virus DNA in two international reference plasma preparations. Eurohep Pathobiology Group. *J Clin Microbiol*. 1999;37:68–73.

27. Doherty R, Garland S, Wright M, et al. Effectiveness of a bivalent Haemophilus influenzae type B-hepatitis B vaccine in preventing hepatitis B virus infection among children born to hepatitis B e antigen-positive carrier mothers. *Pediatr Infect Dis J*. 2009;28:777–781.

28. Lavanchy D. Viral hepatitis: Global goals for vaccination. *J Clin Virol*. 2009 Jul 3. [Epub ahead of print].

29. Daniels D, Grytdal S, Wasley A, et al. Surveillance for acute viral hepatitis—United States, 2007. *MMWR Surveill Summ*. 2009;58:1–27.

30. Dentinger CM, McMahon BJ, Butler JC, et al. Persistence of antibody to hepatitis B and protection from disease among Alaska natives immunized at birth. *Pediatr Infect Dis J*. 2005;24:786–792.

31. McMahon BJ, Bruden DL, Petersen KM, et al. Antibody levels and protection after hepatitis B vaccination: Results of a 15-year follow-up. *Ann Intern Med*. 2005;142:333–341.

32. Carman WF, Zanetti AR, Karayiannis P, et al. Vaccine-induced escape mutant of hepatitis B virus. *Lancet*. 1990;336:325–329.

33. Gerlich WH. Breakthrough of hepatitis B virus escape mutants after vaccination and virus reactivation. *J Clin Virol*. 2006;36 (suppl 1):S18–S22.

34. Kajiwara E, Tanaka Y, Ohashi T, et al. Hepatitis B caused by a hepatitis B surface antigen escape mutant. *J Gastroenterol*. 2008;43:243–247.

35. Zheng X, Weinberger KM, Gehrke R, et al. Mutant hepatitis B virus surface antigens (HBsAg) are immunogenic but may have a changed specificity. *Virology*. 2004;329:454–464.

36. Gerlich WH, Glebe D, Schüttler CG. Deficiencies in the standardization and sensitivity of diagnostic tests for hepatitis B virus. *J Viral Hepat*. 2007;14(suppl 1):16–21.

37. Mizuochi T, Okada Y, Umemori K, et al. Evaluation of 10 commercial diagnostic kits for *in vitro* expressed hepatitis B virus (HBV) surface antigens encoded by HBV of genotypes A to H. *J Virol Methods*. 2006;136:254–256.

38. Osiowy C. Detection of HBsAg mutants. *J Med Virol*. 2006;78(suppl 1):S48–S51.

39. Hollinger FB. Hepatitis B virus genetic diversity and its impact on diagnostic assays. *J Viral Hepat*. 2007;14(suppl 1):11–15.

40. Petit MA, Maillard P, Capel F, et al. Immunochemical structure of the hepatitis B surface antigen vaccine—II. Analysis of antibody responses in human sera against the envelope proteins. *Mol Immunol*. 1986;23:511–523.

41. Echevarría JM, Avellón A. Improved detection of natural hepatitis B virus surface antigen (HBsAg) mutants by a new version of the VITROS HBsAg assay. *J Med Virol*. 2008;80:598–602.

42. Thakur V, Kazim SN, Guptan RC, et al. Transmission of G145R mutant of HBV to an unrelated contact. *J Med Virol*. 2005;76:40–46.

43. Song BC, Kim SH, Kim H, et al. Prevalence of naturally occurring surface antigen variants of hepatitis B virus in Korean patients infected chronically. *J Med Virol*. 2005;76:194–202.

44. Hsu CW, Yeh CT, Chang ML, et al. Identification of a hepatitis B virus S gene mutant in lamivudine-treated patients experiencing HBsAg seroclearance. *Gastroenterology*. 2007;132:543–550.

45. Tabor E. Infections by hepatitis B surface antigen gene mutants in Europe and North America. *J Med Virol*. 2006;78 (suppl 1):S43–S47.

46. Cao GW. Clinical relevance and public health significance of hepatitis B virus genomic variations. *World J Gastroenterol*. 2009;15:5761–5769.

47. Lada O, Benhamou Y, Poynard T, et al. Coexistence of hepatitis B surface antigen (HBs Ag) and anti-HBs antibodies in chronic hepatitis B virus carriers: Influence of "a" determinant variants. *J Virol*. 2006;80:2968–2975.

48. Roth WK, Weber M, Petersen D, et al. NAT for HBV and anti-HBc testing increase blood safety. *Transfusion*. 2002;42:869–875.

49. Seitz R, Heiden M. Quality and safety in blood supply in 2010. *Transfus Med Hemother*. 2010;37:112–117.

50. Alter HJ, Klein HG. The hazards of blood transfusion in historical perspective. *Blood*. 2008;112:2617–2626.

51. Mu SC, Lin YM, Jow GM, et al. Occult hepatitis B virus infection in hepatitis B vaccinated children in Taiwan. *J Hepatol*. 2009;50:264–272.

52. Kusumoto K, Yatsuhashi H, Nakao R, et al. Detection of HBV core promoter and precore mutations helps distinguish flares of chronic hepatitis from acute hepatitis B. *J Gastroenterol Hepatol*. 2008;23:790–793.

53. Tong S, Kim KH, Chante C, et al. Hepatitis B virus e antigen variants. *Int J Med Sci*. 2005;2:2–7.

54. Chotiyaputta W, Lok AS. Hepatitis B virus variants. *Nat Rev Gastroenterol Hepatol*. 2009;6:453–462.

55. Liu S, Zhang H, Gu C, et al. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: A meta-analysis. *J Natl Cancer Inst*. 2009;101:1066–1082.

56. Nguyen MH, Keeffe EB. Chronic hepatitis B: Early viral suppression and long-term outcomes of therapy with oral nucleos(t)ides. *J Viral Hepat*. 2009;16:149–155.

57. Pawlotsky JM, Dusheiko G, Hatzakis A, et al. Virologic monitoring of hepatitis B virus therapy in clinical trials and practice: Recommendations for a standardized approach. *Gastroenterology*. 2008;134:405–415.

58. Rotman Y, Brown TA, Hoofnagle JH. Evaluation of the patient with hepatitis B. *Hepatology*. 2009;49(5 suppl):S22–S27.

59. Zoulim F, Buti M, Lok AS. Antiviral-resistant hepatitis B virus: Can we prevent this monster from growing? *J Viral Hepat*. 2007;14(suppl 1):29–36.

60. Pastor R, Habersetzer F, Fafi-Kremer S, et al. Hepatitis B virus mutations potentially conferring adefovir/tenofovir resistance in treatment-naive patients. *World J Gastroenterol*. 2009;15:753–755.

61. Akarsu M, Sengonul A, Tankurt E, et al. YMDD motif variants in inactive hepatitis B carriers detected by Inno-Lipa HBV DR assay. *J Gastroenterol Hepatol*. 2006;21:1783–1788.

62. Han Y, Huang LH, Liu CM, et al. Characterization of hepatitis B virus reverse transcriptase sequences in Chinese treatment naive patients. *J Gastroenterol Hepatol*. 2009;24:1417–1423.

63. Torresi J. The virological and clinical significance of mutations in the overlapping envelope and polymerase genes of hepatitis B virus. *J Clin Virol*. 2002;25:97–106.

Abbreviations

HBV, hepatitis B virus; WHO, World Health Organization; ORFs, open reading frames; cccDNA, covalently closed, circular DNA; HBsAg, HB surface antigen; anti-HBs, hepatitis B surface antibody; HBeAg, hepatitis B e antigen; anti-HBc, hepatitis B core antigen; IgM, immunoglobulin M; HBIG, HBV immune globulin; anti-HBe, antibody to the hepatitis B e antigen; DTaP, diphtheria, tetanus, and acellular pertussis; IPV, inactivated poliovirus; BCP, basal core promoter; PC, precore; HCC, hepatocellular carcinoma; RT, reverse transcriptase; YMDD, Tyr-Met-Asp-Asp; ADV, Adefovir Dipivoxil