Hepatitis B virus (HBV) infects more than 300 million people worldwide and is a common cause of liver disease and liver cancer. HBV, a member of the *Hepadnaviridae* family, is a small DNA virus with unusual features similar to retroviruses. HBV replicates through an RNA intermediate and can integrate into the host genome. The unique features of the HBV replication cycle confer a distinct ability of the virus to persist in infected cells. Virological and serological assays have been developed for diagnosis of various forms of HBV-associated disease and for treatment of chronic hepatitis B infection. HBV infection leads to a wide spectrum of liver disease ranging from acute (including fulminant hepatic failure) to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Acute HBV infection can be either asymptomatic or present with symptomatic acute hepatitis. Most adults infected with the virus recover, but 5%-10% are unable to clear the virus and become chronically infected. Many chronically infected persons have mild liver disease with little or no long-term morbidity or mortality. Other individuals with chronic HBV infection develop active disease, which can progress to cirrhosis and liver cancer. These patients require careful monitoring and warrant therapeutic intervention. Extrahepatic manifestations of HBV infection are rare but can be difficult to diagnose and manage. The challenges in the area of HBV-associated disease are the lack of knowledge in predicting outcome and progression of HBV infection and an unmet need to understand the molecular, cellular, immunological, and genetic basis of various disease manifestations associated with HBV infection. (HEPATOLOGY 2009;49:S13-S21.)

### Virology

The hepatitis B virus (HBV) is a small DNA virus with unusual features similar to retroviruses. It is a prototype virus of the *Hepadnaviridae* family. Related viruses are found in woodchucks, ground squirrels, tree squirrels, Peking ducks, and herons. Based on sequence comparison, HBV is classified into eight genotypes, A to H. Each genotype has a distinct geographic distribution. Three types of viral particles are visualized in infectious serum by electron microscopy. Two of the viral particles are smaller spherical structures with a diameter of 20 nm and filaments of variable lengths with a width of 22 nm (Fig. 1). The spheres and filaments are composed of hepatitis B surface antigen (HBsAg) and host-derived lipids without viral nucleic acids and are therefore noninfectious. The infectious HBV virion (Dane particle) has a spherical, double-shelled structure 42 nm in diameter, consisting of a lipid envelope containing HBsAg that surrounds an inner nucleocapsid composed of hepatitis B core antigen (HBcAg) complexed with virally encoded polymerase and the viral DNA genome. The genome of HBV is a partially double-stranded circular DNA of about 3.2 kilobase (kb) pairs. The viral polymerase is covalently attached to the 5' end of the minus strand.

The viral genome encodes four overlapping open reading frames (ORFs: S, C, P, and X) (Fig. 2A). The S ORF encodes the viral surface envelope proteins, the HBsAg, and can be structurally and functionally divided into the pre-S1, pre-S2, and S regions. The core or C gene has the precore and core regions. Multiple in-frame translation initiation codons are a feature of the S and C genes, which give rise to related but functionally distinct proteins. The C ORF encodes either the viral nucleocapsid HBcAg or hepatitis B e antigen (HBeAg) depending on whether translation is initiated from the core or precore regions, respectively (Fig. 2B).

The core protein has the intrinsic property to self-assemble into a capsid-like structure and contains a highly basic cluster of amino acids at its C-
terminus with RNA-binding activity.\textsuperscript{5} The precore ORF codes for a signal peptide that directs the translation product to the endoplasmic reticulum, where the protein is further processed to form the secreted HBeAg. The function of HBeAg remains largely undefined, although it has been implicated as an immune tolerogen, whose function is to promote persistent infection.\textsuperscript{6} The polymerase (pol) is a large protein (about 800 amino acids) encoded by the P ORF and is functionally divided into three domains: the terminal protein domain, which is involved in encapsidation and initiation of minus-strand synthesis; the reverse transcriptase (RT) domain, which catalyzes genome synthesis; and the ribonuclease H domain, which degrades pregenomic RNA and facilitates replication. The HBV X ORF encodes a 16.5-kd protein (HBxAg) with multiple functions, including signal transduction, transcriptional activation, DNA repair, and inhibition of protein degradation.\textsuperscript{7-10} The mechanism of this activity and the biological function of HBxAg in the viral life-cycle remain largely unknown. However, it is well established that HBxAg is necessary for productive HBV infection in vivo and may contribute to the oncogenic potential of HBV.

Other functionally important elements within the HBV genome include two direct repeats (DR1 and DR2) in the 5′ ends of the plus strand, which are required for strand-specific DNA synthesis during replication.\textsuperscript{11} Two enhancer elements, designated as En1 and En2, confer liver-specific expression of viral gene products.\textsuperscript{12} A glucocorticoid-responsive element (GRE) sequence within the S domain,\textsuperscript{13} a polyadenylation signal within the core gene, and a posttranscriptional regulatory element overlapping En1 and part of HBxAg ORF have also been described.\textsuperscript{14}

The HBV replication pathway has been studied in great detail and is summarized in Fig. 3. The initial phase of HBV infection involves the attachment of mature virions to host cell membranes, likely involving the pre-S domain of the surface protein.\textsuperscript{15} Various cellular factors

Fig. 1. Electron micrograph of circulating forms of HBV particles in the blood is shown at the top and a schematic drawing of Dane particle, the infectious HBV particle, is shown at the bottom with various structural features.

Fig. 2. The HBV genome. (A) The genomic organization, RNA transcripts and gene products are shown with several key regulatory elements. (B) The transcription start sites of various HBV transcripts and the proteins they encode (see text for details).
have been proposed to be the viral receptors, but only carboxypeptidase D has been shown to play an essential role in viral entry for the duck HBV.\textsuperscript{16} Mechanisms of viral disassembly and intracellular transport of the viral genome into the nucleus are not well understood and probably involve modification of the nucleocapsid core protein.\textsuperscript{17} After entry of the viral genome into the nucleus, the single-stranded gap region in the viral genome is repaired by the viral pol protein, and the viral DNA is circularized to the covalently closed circular (cccDNA) form.\textsuperscript{18} This form of HBV DNA serves as the template for transcription of several species of genomic and subgenomic RNAs and is the stable component of the replication cycle that is relatively resistant to antiviral action and immune clearance (Fig. 2B).

The transcripts from the cccDNA are unspliced, polyadenylated, and possess a 5′ cap structure. The 3.5-kb genomic transcripts consist of two species with different 5′ ends: the pregenomic and the precore RNAs. The pregenomic RNA (pgRNA) serves as the template for reverse transcription and the messenger RNA for core and polymerase; the precore RNA directs the translation of the precore gene product. The polymerase translation is initiated at the pol start codon of the pgRNA, probably as a result of a ribosomal scanning mechanism.\textsuperscript{19} The large HBsAg (L-HBsAg) protein is translated from the 2.4-kb subgenomic RNA, the middle (M-HBsAg) and small HBsAg (S-HBsAg) proteins from the various forms of 2.1-kb RNAs, and the HBxAg protein from the 0.7-kb RNA.

The S-HBsAg is the major S gene product and the L and M proteins are the minor species. Each surface protein has a glycosylation site in the S domain. Additional modifications of the L and M proteins occur at the pre-S2 domain with an N-linked oligosaccharide and a myristic acid at the amino-terminal glycine residue of the pre-S1 domain.\textsuperscript{20} The distribution of the three envelope glycoproteins varies among the types of viral particles, with little or no L and M protein in the 20-nm particles but relatively more L protein in the Dane particles.

Replication of HBV begins with encapsidation of the genome. The packaging signal is a cis-acting element referred to as epsilon, which contains a stem-loop structure.\textsuperscript{21} The terminal protein of the pol interacts with the epsilon and in concert with the core protein forms the nucleocapsid. After encapsidation, the pol mediates the reverse transcription of the pgRNA to minus-strand DNA and subsequent positive-strand synthesis. The circular form of the DNA is completed through several complicated steps of strand transfer.\textsuperscript{22} The nucleocapsid then interacts with the envelope proteins in the endoplasmic reticulum to assemble into
mature virions, which are then secreted into the extracellular milieu.

**Diagnosis and Serology**

HBV infection leads to a wide spectrum of liver disease ranging from acute hepatitis (including fulminant hepatic failure) to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). The diagnosis of HBV infection and its associated disease is based on a constellation of clinical, biochemical, histological, and serologic findings. A number of viral antigens and their respective antibodies can be detected in serum after infection with HBV, and proper interpretation of the results is essential for the correct diagnosis of the various clinical forms of HBV infection (Table 1).

The typical course of acute hepatitis B is shown in Fig. 4A. HBV DNA followed shortly afterward by HBsAg and HBeAg are the first viral markers detected in serum. HBsAg may be detected as early as 1-2 weeks or as late as 11-12 weeks after exposure, and its persistence is a marker of chronicity. HBeAg correlates with the presence of high levels of HBV replication and infectivity. Within a few weeks of appearance of viral markers, serum alanine and aspartate aminotransferase (ALT, AST) levels begin to rise and jaundice may appear. HBeAg is usually cleared early, at the peak of clinical illness, whereas HBsAg and HBV DNA usually persist in the serum for the duration of clinical symptoms and are cleared with recovery. Antibodies to the HBV proteins arise in different patterns during acute hepatitis B. Antibody to HBCAg (anti-HBc) generally appears shortly before onset of clinical illness, the initial antibody being mostly immunoglobulin M (IgM) class, which then declines in titer as levels of IgG anti-HBc arise. Antibody to HBeAg (anti-HBe) usually appears shortly after clearance of HBeAg, often at the peak of clinical illness. Thus, loss of HBeAg and appearance of anti-HBe is a favorable serological marker during acute hepatitis B, indicating the initiation of recovery. Antibody to HBsAg arises late during infection, usually during recovery or convalescence after clearance of HBsAg. Anti-HBs persists after recovery, being the antibody associated with immunity against HBV. However, between 10% and 15% of patients who recover from hepatitis B do not develop detectable anti-HBs and have anti-HBc alone as a marker of previous infection. For this reason, anti-HBc testing is the most reliable means of

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**Table 1. Hepatitis B Virus Serological and Virological Markers**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>HBV infection, both acute and chronic</td>
</tr>
<tr>
<td>HBeAg</td>
<td>High-level HBV replication and infectivity; marker for treatment response</td>
</tr>
<tr>
<td>HBV DNA</td>
<td>Level of HBV replication; primary virologic marker for treatment response</td>
</tr>
<tr>
<td>Anti-HBc (IgM)</td>
<td>Acute HBV infection; could be seen in flare of chronic hepatitis B</td>
</tr>
<tr>
<td>Anti-HBc (IgG)</td>
<td>Recovered or chronic HBV infection</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>Recovered HBV infection or marker of HBV vaccination; immunity to HBV infection (titer can be measured to assess vaccine efficacy)</td>
</tr>
<tr>
<td>Anti-HBe</td>
<td>Low-level HBV replication and infectivity; marker for treatment response</td>
</tr>
<tr>
<td>Anti-HBc (IgG) and anti-HBs</td>
<td>Past HBV infection; could lose anti-HBs</td>
</tr>
<tr>
<td>Anti-HBc (IgG) and HBsAg</td>
<td>Chronic HBV infection</td>
</tr>
<tr>
<td>Anti-HBc (IgG) and/or anti-HBs and HBV DNA (PCR)</td>
<td>Latent or occult HBV infection</td>
</tr>
</tbody>
</table>

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Fig. 4. The clinical course and serologic profiles of (A) acute and (B) chronic hepatitis B.
assessing previous infection with HBV, whereas anti-HBs testing is used to assess immunity and response to HBV vaccine.25

Patients who develop chronic hepatitis B (Fig. 4B) have a similar initial pattern of serological markers with appearance of HBV DNA, HBsAg, HBeAg, and anti-HBc. In these persons, however, viral replication persists and HBsAg, HBeAg, and HBV DNA continue to be detectable in serum, often in high titers. The subsequent course of chronic hepatitis B is quite variable. Most persons remain HBsAg-positive for years if not for life and have some degree of chronic liver injury (chronic hepatitis) that can lead to significant fibrosis and cirrhosis. Persons with chronic HBV infection are also at high risk to ultimately develop HCC.

The diagnosis of acute hepatitis B is reliably made by the finding of IgM anti-HBc in serum, particularly in a patient with HBsAg and signs, symptoms, or laboratory features of acute hepatitis. Nevertheless, in some instances, HBsAg is cleared rapidly from the serum, and IgM anti-HBc is the only marker detectable when the patient presents with hepatitis. Testing for anti-HBc (total) and anti-HBs are not useful in diagnosis, and testing for HBeAg and anti-HBe should be reserved for persons who test positive for HBsAg. The finding of HBsAg without IgM anti-HBc suggests the presence of chronic hepatitis B, but this diagnosis generally also rests upon finding of persistence of HBsAg for at least 6 months.23,26 HBV DNA testing can also be helpful in the assessment of level of viral replication and possibly helpful in assessing prognosis and need for antiviral therapy. Assays for HBV DNA level have improved substantially over the years.27

The current real-time polymerase chain reaction–based assay (TaqMan) has a lower limit of detection of 5-10 HBV DNA copies/mL and can accurately quantify a wide range of levels (Fig. 5). With this degree of sensitivity, HBV DNA can be detected early during the course of infection, arising before the appearance of other serological markers, such as HBsAg or anti-HBc. As a consequence, testing for HBV DNA has emerged as a primary approach in the diagnosis and management of HBV infection. HBV DNA testing has now become routinely used in blood product screening (nucleic acid testing)28 and monitoring of patients with HBV during treatment.29 Persistently high levels of HBV DNA following resolution of hepatitis may be indicative of a failure to control the infection and an evolution into chronic infection.

Acute Hepatitis B

About two-thirds of patients with acute HBV infection have a mild, asymptomatic and subclinical illness that usually goes undetected.30 Approximately one-third of adults with acute HBV infection develop clinical symptoms and signs of hepatitis, which range from mild constitutional symptoms of fatigue and nausea, to more marked symptoms and jaundice, and rarely to acute liver failure. The clinical incubation period of acute hepatitis B averages 2-3 months and can range from 1-6 months after exposure, the length of the incubation period correlating, to some extent, with the level of virus exposure.31 The incubation period is followed by a short preicteric or prodromal period of constitutional symptoms such as fever, fatigue, anorexia, nausea, and body aches. During this phase, serum ALT levels rise and high levels of HBsAg and HBV DNA are detectable. The preicteric phase lasts a few days to as long as a week and is followed by onset of
jaundice or dark urine. The icteric phase of hepatitis B lasts for a variable period averaging 1–2 weeks, during which viral levels decrease. In convalescence, jaundice resolves but constitutional symptoms may last for weeks or even months. During this phase, HBsAg is cleared followed by the disappearance of detectable HBV DNA from serum.

Acute liver failure occurs in approximately 1% of patients with acute hepatitis B and jaundice. The onset of fulminant hepatitis is typically marked by the sudden appearance of fever, abdominal pain, vomiting, and jaundice, followed by disorientation, confusion, and coma. HBsAg and HBV DNA levels generally fall rapidly as liver failure develops, and some patients are HBsAg-negative by the time of onset of hepatic coma. Patients with acute liver failure due to hepatitis B require careful management and monitoring and should be referred rapidly to a tertiary medical center with the availability of liver transplantation.

Chronic Hepatitis B

Chronic hepatitis B has a variable and dynamic course. Early during infection, HBeAg, HBsAg, and HBV DNA are usually present in high titers, and there are mild to moderate elevations in serum aminotransferase levels (Fig. 4B). With time, however, the disease activity can resolve either with persistence of high levels of HBeAg and HBV DNA (the “immune tolerance phase”) or with loss of HBeAg and fall of HBV DNA to low or undetectable levels (“inactive carrier state”). Other patients continue to have chronic hepatitis B, although some lose HBeAg and develop anti-HBe (HBeAg-negative chronic hepatitis B). The course and natural history of hepatitis B are discussed in detail elsewhere in these proceedings.

The overall prognosis of patients with chronic hepatitis is directly related to the severity of disease. For those with severe chronic hepatitis and cirrhosis, the 5-year survival rate is about 50%. Among patients with evidence of chronic hepatitis (elevated ALT and inflammation and/or fibrosis on liver biopsy), many are asymptomatic or have nonspecific symptoms, such as fatigue and mild right upper quadrant discomfort. Patients with more severe disease or cirrhosis may have significant constitutional symptoms, jaundice, and peripheral stigmata of end-stage liver disease including spider angiomas, palmar erythema, splenomegaly, gynecomastia, and fetor hepaticus. Ascites, peripheral edema, encephalopathy, and gastrointestinal bleeding are seen in patients with more advanced cirrhosis. ALT and AST are often elevated but may not correlate well with severity of liver disease. Bilirubin, prothrombin time, and albumin often become abnormal with progressive disease. Decreasing platelet count is often a poor prognostic sign.

Patients with chronic hepatitis may develop acute exacerbations with markedly elevated serum ALT. This scenario is more frequently described in those with HBeAg-negative chronic hepatitis B. To distinguish between acute hepatitis B and chronic hepatitis B with a flare, anti-HBc IgM is a useful marker, as described in the previous section. However anti-HBc of the IgM class can be detected occasionally in patients with chronic hepatitis B with exacerbation. Alpha-fetoprotein (AFP), used as a marker for HCC, is often elevated in parallel with ALT during acute exacerbation. However, it is unlikely to exceed 400 ng/mL. In patients with AFP much greater than this level, development of HCC should be suspected.

An estimated one-third of persons with chronic HBV infection will ultimately develop a long-term consequence of the disease, such as cirrhosis, end-stage liver disease, or HCC. The determinants of outcome of chronic hepatitis B appear to be both viral (HBV DNA levels, HBV genotype, some HBV mutation patterns) and host-specific (age, gender, genetic background, immune status).

Extrahepatic Manifestations of Hepatitis B

Extrahepatic manifestations of hepatitis B are present in 1–10% of HBV-infected patients and include serum-sickness–like syndrome, acute necrotizing vasculitis (polyarteritis nodosa), membranous glomerulonephritis, and papular acrodermatitis of childhood (Gianotti-Crosti syndrome). Although the pathogenesis of these disorders is unclear, immune complex–mediated injury related to high level of HBV antigenemia is thought to be the cause.

The serum-sickness–like syndrome occurs in the setting of acute hepatitis B, often preceding the onset of jaundice. The clinical features are fever, skin rash, and polyarteritis. The symptoms often subside shortly after the onset of jaundice, but can persist throughout the duration of acute hepatitis B. The course of this syndrome often parallels the duration and level of HBV viremia: rapid clearance of the virus leads to rapid resolution of the illness. This disorder resembles experimental serum sickness, in which immune complexes activate the complement pathways leading to complement-mediated injury. Patients with this syndrome have low complement levels and high-level circulating immune complexes containing HBV antigens and complement components.

About 30%-50% of patients with acute necrotizing vasculitis (polyarteritis nodosa) are HBV carriers. This entity is more commonly seen in patients with recent
exposure to HBV. Immune-mediated vascular injury can involve large, medium, and small vessels. Early clinical features are marked constitutional symptoms, high fever, anemia, and leukocytosis. Multisystem involvement is common, including arthritis, renal disease (proteinuria and hematuria), heart disease (pericarditis and congestive heart failure), hypertension, gastrointestinal disease (acute abdominal pain and bleeding), skin involvement (vasculitic lesions), and neurological disorders (mononeuropathy multiplex and central nervous system abnormalities). The disease is highly variable and has a mortality rate of 30% within 5 years if not treated.

HBV-associated nephropathy has been described in adults but is more common in children. Membranous glomerulonephritis is the most common form. Liver disease may be mild or absent in many of these patients. This disorder is frequently observed in countries with high prevalence of HBV infection. About 30%-60% of children with this disorder experience spontaneous remission, especially with HBeAg seroconversion. However, about 30% of adults with this condition can progress to renal failure with as many as 10% requiring dialysis or renal transplant.

Papular acrodermatitis (Gianotti-Crosti syndrome) is a distinct skin manifestation of acute HBV infection in childhood. Skin lesions are maculopapular, erythematous, and nonpruritic, and involve the face and extremities. The syndrome lasts about 15-20 days and can either precede or follow the onset of jaundice in acute hepatitis B. Generalized lymphadenopathy and hepatomegaly have been described.

Other immune-mediated hematological disorders, such as essential mixed cryoglobulinemia and aplastic anemia have been described as part of the extrahepatic manifestations of HBV infection, but their association is not as well-defined; therefore, they probably should not be considered etiologically linked to HBV.

**Occult or Latent HBV Infection**

Other atypical HBV infections include seronegative occult or latent HBV infections. This heterogeneous group consists of patients who are HBsAg-negative who are either seronegative for all HBV markers or positive for anti-HBc and/or anti-HBs. Many of these patients are positive for HBV DNA by polymerase chain reaction either in the liver or serum or both. Some of these patients have underlying liver disease, suggestive of ongoing hepatocellular injury from persistent HBV infection. Studies in animal models have demonstrated long-term persistence of viral genomes in the serum and/or liver of animals that have biochemical and serologic evidence of viral clearance and recovery from infection. The important question is whether this observation represents ongoing viral replication and therefore clinically significant infection in terms of liver disease and transmission. Existing evidence supports the notion that it indeed indicates low-level viral replication, capable of transmission. Studies in liver transplantation revealed transmission of HBV infection to recipients if the donors carried the anti-HBc marker. In addition, reactivation of HBV infection in patients with serologic evidence of recovery undergoing immunosuppression or chemotherapy has been reported. These observations, together with the immunologic studies described above, provide compelling evidence that one may not be able to completely eliminate HBV infection. Patients with serologic evidence of recovery probably have low-level viral replication that is effectively controlled by an active immune response. The possibility that these occult infections are caused by HBV mutants has been proposed. Although mutations have been reported in various regions of the viral genome, definitive evidence in support of a pathogenic role of these mutants is lacking. Furthermore, whether liver disease can indeed result from these occult HBV infections is controversial. At present, there are no convincing studies in support of a causal relationship. Therefore, these occult HBV infections, other than the special situations described above, may not be clinically important.

**Important Questions and Needs for Future Research**

1. How does HBV establish productive infection in vivo and what is the host response early during the infection? Despite well-described information on the clinical manifestations and natural history of acute HBV infection, detailed knowledge of the virus-host interaction during this stage remains poorly defined. Advances in this area would offer a better understanding of the pathogenesis of HBV infection and its associated disease.

2. What is the immunologic basis of chronic infection and hepatocellular injury? There have been great strides in understanding the virology and immune response of HBV infection, but the molecular mechanisms whereby the host fails to clear the virus and develops chronic infection remain largely unknown. In addition, the adaptive evolution of virus under host immune pressure remains to be elucidated. Finally, the pathogenesis of various extrahepatic manifestations associated with HBV infection is poorly understood. Further research in these areas is crucial not only in better understanding the natural history and disease progression but also in improving treatment for chronic hepatitis B.
3. What is the genetic basis of the diverse clinical manifestations and disease outcomes of HBV infection? With the recent advances in genetic and genomic medicine, there are increasing opportunities to elucidate the genetic basis for variations in expression and susceptibility to HBV-associated diseases. Genome-wide association studies and other genomic technological advances would provide crucial information to identify useful genetic markers for disease outcome, clinical manifestations, and treatment response of HBV-associated disease.

References


