REVIEW ARTICLE

MECHANISMS OF DISEASE

Hepatitis B Virus Infection — Natural History and Clinical Consequences

Don Ganem, M.D., and Alfred M. Prince, M.D.

From the Departments of Microbiology and Immunology and Medicine and the Howard Hughes Medical Institute, University of California, San Francisco (D.G.); and the Laboratory of Virology, Lindsley F. Kimball Research Institute, New York Blood Center, and the Department of Pathology, New York University School of Medicine — both in New York (A.M.P.). Address reprint requests to Dr. Prince at the Laboratory of Virology, Lindsley F. Kimball Research Institute, New York Blood Center, 310 E. 67th St., New York, NY 10021, or at aprince@nybloodcenter.org.

I N THE PAST 10 YEARS, REMARKABLE STRIDES HAVE BEEN MADE IN THE understanding of the natural history and pathogenesis of hepatitis B virus (HBV) infection. In this article we will review these advances, with particular reference to the implications for antiviral therapy.

HISTORY

Clinical and epidemiologic studies began to differentiate among various types of acute hepatitis in the decades after World War II. The groundbreaking studies of Krugman and colleagues in 1967 firmly established the existence of at least two types of hepatitis,[1] one of which (then called serum hepatitis, and now called hepatitis B) was parenterally transmitted. Links to the virus responsible for this form of hepatitis were derived by serologic studies conducted independently by Prince and colleagues[2-4] and by Blumberg and colleagues.[5] Blumberg and colleagues, searching for serum protein polymorphisms linked to diseases, identified an antigen (termed Au) in serum from patients with leukemia, leprosy, and hepatitis, though the relationship of this antigen to hepatitis was initially unclear. By systematically studying patients with transfusion-associated hepatitis, Prince and coworkers independently identified an antigen, termed SH, that appeared in the blood of these patients during the incubation period of the disease, and further work established that Au and SH were identical.[6,7] The antigen represented the hepatitis B surface antigen (HBsAg).[8,9] These seminal studies made possible the serologic diagnosis of hepatitis B and opened up the field to rigorous epidemiologic and virologic investigation.
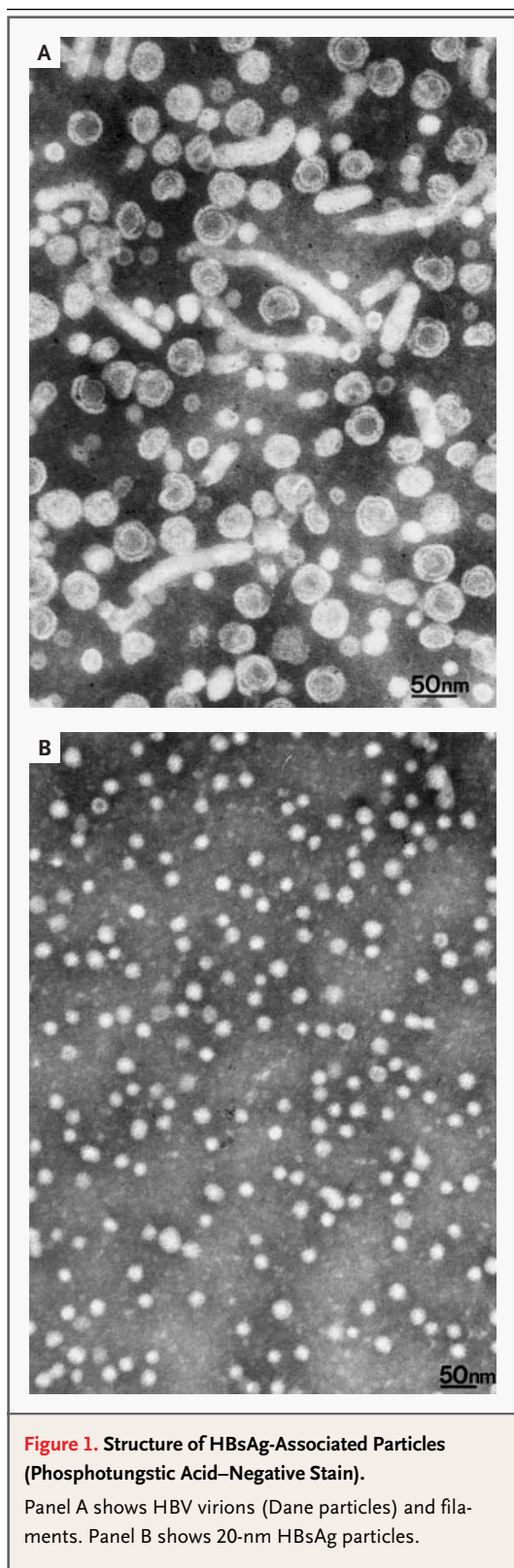
VIROLOGIC FEATURES

CLASSIFICATION AND STRUCTURE

Hepatitis B virus (HBV) is the prototype member of the Hepadnaviridae (hepatotropic DNA virus) family. Hepadnaviruses have a strong preference for infecting liver cells, but small amounts of hepadnaviral DNA can be found in kidney, pancreas, and mononuclear cells. However, infection at these sites is not linked to extrahepatic disease.[10-13]

HBV virions are double-shelled particles, 40 to 42 nm in diameter (Fig. 1A),[14] with an outer lipoprotein envelope that contains three related envelope glycoproteins (or surface antigens).[15] Within the envelope is the viral nucleocapsid, or core.[16] The core contains the viral genome, a relaxed-circular, partially duplex DNA of 3.2 kb, and a polymerase that is responsible for the synthesis of viral DNA in infected cells.[17] DNA sequencing of many isolates of HBV has confirmed the existence of multiple viral genotypes, each with a characteristic geographic distribution.[18]

In addition to virions, HBV-infected cells produce two distinct subviral lipoprotein particles: 20-nm spheres (Fig. 1B) and filamentous forms of similar diameter

Figure 1. Structure of HBsAg-Associated Particles (Phosphotungstic Acid–Negative Stain).

Panel A shows HBV virions (Dane particles) and filaments. Panel B shows 20-nm HBsAg particles.

(Fig. 1A).[16] These HBsAg particles contain only envelope glycoproteins and host-derived lipids and typically outnumber virions by 1000:1 to 10,000:1.

VIRAL GENES AND PROTEINS

The HBV genome has only four long open reading frames. The preS–S (presurface–surface) region of the genome encodes the three viral surface antigens by differential initiation of translation at each of three in-frame initiation codons.[15,16,19] The most abundant protein is the 24-kD S protein (which is known as HBsAg). Initiation at the most upstream start codon generates the M (or preS2) protein, the function of which is unknown. Initiation at the most upstream start codon yields the L (or preS1) protein, which is thought to play key roles in the binding of the virus to host-cell receptors[20] and in the assembly of the virion and its release from the cell.[21]

The preC–C (precore–core) region encodes hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg). These two proteins are also derived by alternative initiation of translation at two in-frame AUG codons.[15,19] The internal AUG encodes the 21-kD C protein, the structural polypeptide of the viral capsid, whereas the upstream AUG directs production of the 24-kD preC protein. The preC region encodes a signal sequence, which directs the chain into the secretory pathway. As the chains traverse the Golgi complex, cleavage by cellular proteases generates HBeAg, a 16-kD fragment that is secreted into the blood.[22] HBeAg plays no role in viral assembly, and its function is not clear. It is not required for viral replication; mutants bearing chain-terminating lesions within the preC region replicate well in culture and, in fact, arise frequently during natural infection.[23]

The P coding region is specific for the viral polymerase, a multifunctional enzyme involved in DNA synthesis and RNA encapsidation. The X open reading frame encodes the viral X protein (HBx), which modulates host-cell signal transduction and can directly and indirectly affect host and viral gene expression.[19] X-protein activity is absolutely required for the in vivo replication and spread of the virus.[24]

VIRAL REPLICATION CYCLE

Figure 2 shows the main features of the hepadnavirus replication cycle, the cardinal feature of which is the replication of the DNA genome by reverse transcription of an RNA intermediate.[25] Incoming HBV virions are bound by cell-surface receptors, the
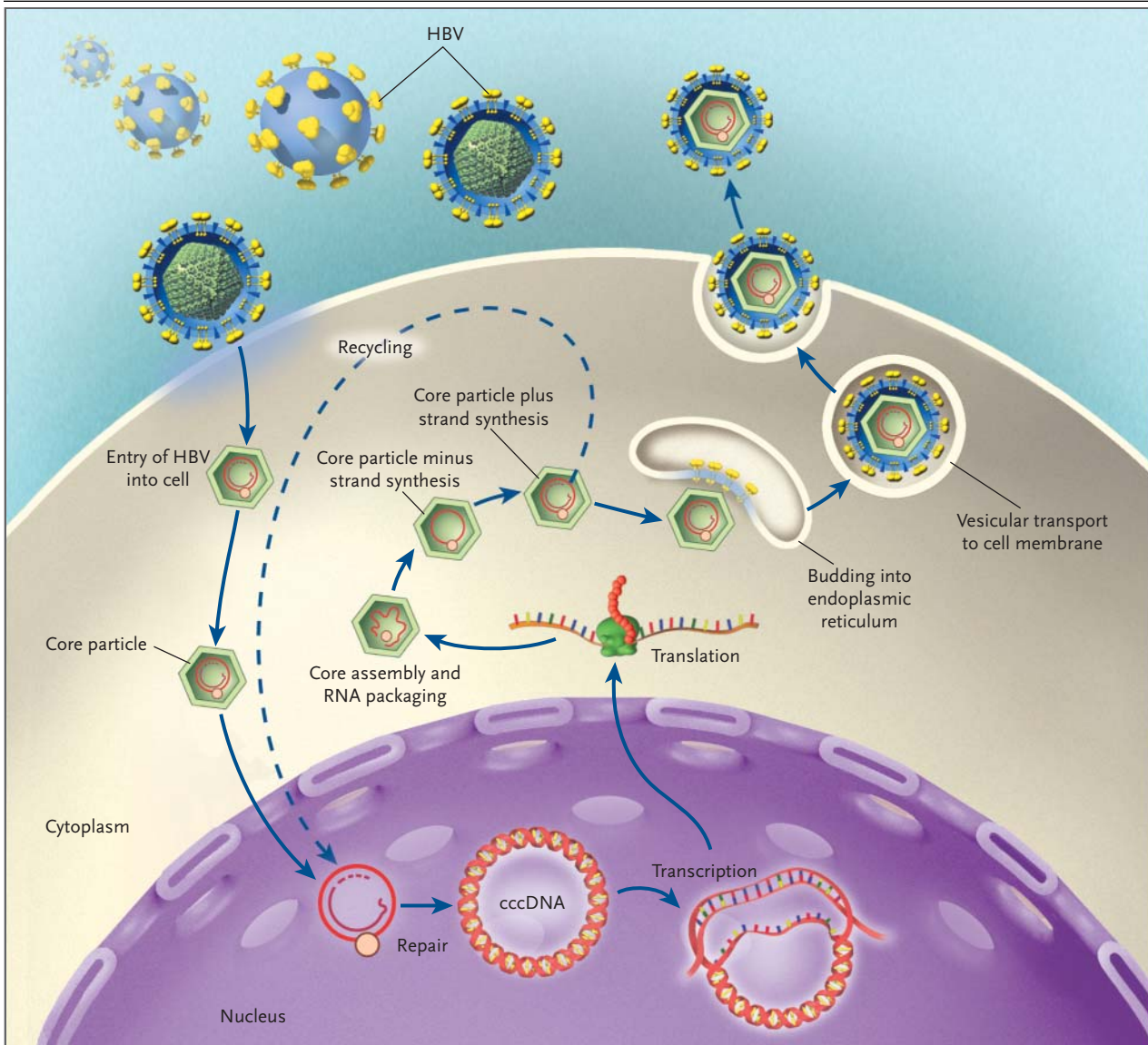
Figure 2. The Replication Cycle of HBV.

HBV virions bind to surface receptors and are internalized. Viral core particles migrate to the hepatocyte nucleus, where their genomes are repaired to form a covalently closed circular DNA (cccDNA) that is the template for viral messenger RNA (mRNA) transcription. The viral mRNA that results is translated in the cytoplasm to produce the viral surface, core, polymerase, and X proteins. There, progeny viral capsids assemble, incorporating genomic viral RNA (RNA packaging). This RNA is reverse-transcribed into viral DNA. The resulting cores can either bud into the endoplasmic reticulum to be enveloped and exported from the cell or recycle their genomes into the nucleus for conversion to cccDNA. The small, peach-colored sphere inside the core particle is the viral DNA polymerase.

identity of which remains unknown. After membrane fusion, cores are presented to the cytosol and transported to the nucleus. There, their DNA genomes are converted to a covalently closed circular (ccc) form,[26] which serves as the transcriptional template for host RNA polymerase II. This enzyme generates a series of genomic and subgenomic transcripts.[27]

All viral RNA is transported to the cytoplasm, where its translation yields the viral envelope, core, and polymerase proteins, as well as the X and preC polypeptides. Next, nucleocapsids are assembled in the cytosol, and during this process a single molecule of genomic RNA is incorporated into the assembling viral core.[28] Once the viral RNA is encapsidated, reverse transcription begins.[28] The

synthesis of the two viral DNA strands is sequential. The first DNA strand is made from the encapsidated RNA template; during or after the synthesis of this strand, the RNA template is degraded and the synthesis of the second DNA strand proceeds, with the use of the newly made first DNA strand as a template.[25,27,29] Some cores bearing the mature genome are transported back to the nucleus, where their newly minted DNA genomes can be converted to cccDNA to maintain a stable intranuclear pool of transcriptional templates.[26] Most cores, however, bud into regions of intracellular membranes bearing the viral envelope proteins. In so doing, they acquire lipoprotein envelopes containing the viral L, M, and S surface antigens and are then exported from the cell.

PATHOGENESIS OF HEPATITIS B

The HBV replication cycle is not directly cytotoxic to cells. This fact accords well with the observation that many HBV carriers are asymptomatic and have minimal liver injury, despite extensive and ongoing intrahepatic replication of the virus.[30] It is now thought that host immune responses to viral antigens displayed on infected hepatocytes are the principal determinants of hepatocellular injury. This notion is consistent with the clinical observation that patients with immune defects who are infected with HBV often have mild acute liver injury but high rates of chronic carriage.[31]

The immune responses to HBV and their role in the pathogenesis of hepatitis B are incompletely understood. Correlative clinical studies show that in acute, self-limited hepatitis B, strong T-cell responses to many HBV antigens are readily demonstrable in the peripheral blood.[32] These responses involve both major-histocompatibility-complex (MHC) class II–restricted, CD4+ helper T cells and MHC class I–restricted, CD8+ cytotoxic T lymphocytes. The antiviral cytotoxic T-lymphocyte response is directed against multiple epitopes within the HBV core, polymerase, and envelope proteins; strong helper T-cell responses to C and P proteins have also been demonstrated in acute infection. By contrast, in chronic carriers of HBV, such virus-specific T-cell responses are greatly attenuated, at least as assayed in cells from the peripheral blood. However, antibody responses are vigorous and sustained in both situations (although free antibodies against HBsAg [anti-HBs antibodies] are not detectable in carriers because of the excess of circulating HBsAg). This pattern strongly suggests that T-cell responses, es-

pecially the responses of cytotoxic T lymphocytes, play a central role in viral clearance. Figure 3 summarizes the major types of cellular immune response to HBV.

The mechanisms by which cytotoxic T lymphocytes kill liver cells and cause viral clearance have been incisively investigated in transgenic mice that express viral antigens or contain replication-competent viral genomes in the liver.[32,33] Because these mice harbor HBV genes in their germ-line DNA, they are largely tolerant to HBV proteins, and accordingly, clinically significant liver injury does not develop. However, if antiviral cytotoxic T lymphocytes of syngeneic animals are transferred into such mice, acute liver injury with many of the features of clinical hepatitis B develops.[34] It is striking that, in this model, the number of hepatocytes killed by direct engagement between cytotoxic T lymphocytes and their targets is very small and clearly insufficient to account for most of the liver damage. This suggests that much of the injury is due to secondary antigen-nonspecific inflammatory responses that are set in motion by the response of the cytotoxic T lymphocytes. Presumably, much of the damage occurring in this context is due to cytotoxic by-products of the inflammatory response, such as tumor necrosis factor (TNF), free radicals, and proteases. Other immune-cell populations, notably natural killer T cells,[35] probably also contribute to liver injury.

Recent experiments suggest that some of the inflammatory by-products, notably interferon-γ (IFN-γ) and TNF-α, can have antiviral effects that do not involve killing the target cells. When cytotoxic T lymphocytes are transferred to mice that bear replicating HBV, viral DNA and RNA throughout the liver rapidly disappear, even from viable, uninjured hepatocytes — an effect that can be blocked by the administration of antibodies to TNF-α and IFN-γ.[34] Such noncytocidal antiviral effects may be important for viral clearance in natural infection. In fact, cytokine release triggered by unrelated hepatic infections in HBV-transgenic mice can have the same effect.[36] This phenomenon may explain the suppression and occasional clearance of chronic HBV infection in patients with superimposed acute hepatitis caused by unrelated viruses.

NATURAL HISTORY

Primary HBV infection in susceptible (nonimmune) hosts can be either symptomatic or asymptomatic. The latter is more common than the former, espe-
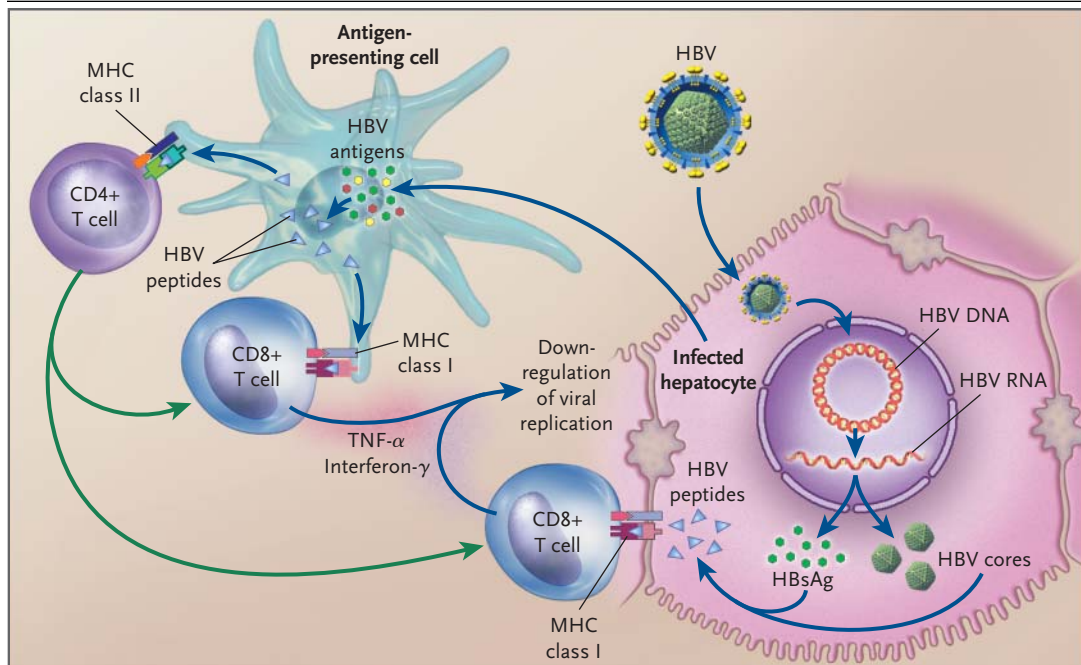
Figure 3. Cellular Immune Responses to HBV.

HBV replicates in hepatocytes to produce HBsAg particles and virions. Both types of particle can be taken up by antigen-presenting cells, which degrade the viral proteins to peptides that are then presented on the cell surface bound to MHC class I or II molecules. (Antigen-presenting cells can also process and display viral antigens taken up by phagocytosis of killed infected hepatocytes.) These peptide antigens can be recognized by CD8+ or CD4+ T cells, respectively, which are thereby sensitized. Virus-specific CD8+ cytotoxic T cells (with help from CD4+ T cells, green arrow) can recognize viral antigens presented on MHC class I chains on infected hepatocytes. This recognition reaction can lead to either direct lysis of the infected hepatocyte or the release of interferon-γ and TNF-α, which can down-regulate viral replication in surrounding hepatocytes without direct cell killing.

cially in young children. Most primary infections in adults, whether symptomatic or not, are self-limited, with clearance of virus from blood and liver and the development of lasting immunity to reinfection.[37,38] However, some primary infections in healthy adults (generally less than 5 percent) do not resolve but develop into persistent infections. In such cases, viral replication continues in the liver and there is continual viremia, although the titers of virus in the liver and blood are variable. Persistent HBV infection may be symptomatic or asymptomatic. People with subclinical persistent infection, normal serum aminotransferase levels, and normal or nearly normal findings on liver biopsy are termed asymptomatic chronic HBV carriers; those with abnormal liver function and histologic features are classified as having chronic hepatitis B. Cirrhosis, a condition in which regenerative nodules and fibrosis coexist with severe liver injury, develops in about 20 percent of people with chron-

ic hepatitis B. The resulting hepatic insufficiency and portal hypertension make this process one of the most feared consequences of chronic HBV infection.

Primary Infection

In primary infection, HBsAg becomes detectable in the blood after an incubation period of 4 to 10 weeks, followed shortly by antibodies against the HBV core antigen (anti-HBc antibodies), which early in infection are mainly of the IgM isotype.[38] Viremia is well established by the time HBsAg is detected, and titers of virus in acute infection are very high — frequently 10^9 to 10^{10} virions per milliliter.[39] Circulating HBeAg becomes detectable in most cases, and studies of chimpanzees and other animals with primary hepadnaviral infection show that 75 to 100 percent of hepatocytes are infected when this antigen is evident.[40] Thus, it is not surprising that epidemiologic studies consistently show high rates of

both vertical and horizontal transmissibility during acute HBV infection.[41]

When liver injury does occur in primary infection, alanine aminotransferase levels do not increase until after viral infection is well established, reflecting the time required to generate the T-cell–mediated immune response that triggers liver injury. Once this response is under way, titers of virus in blood and liver begin to drop. The fact that infection can be cleared from virtually all hepatocytes without massive hepatic destruction (in most cases) is a testament to the extraordinary power of the noncytolytic clearance mechanisms described above. With clearance of the infection, the viral antigens HBsAg and HBeAg disappear from the circulation, and free anti-HBs antibodies become detectable.

Surprisingly, in self-limited infection, as defined by the disappearance of the viral antigens and the appearance of anti-HBs antibodies, low levels of HBV DNA in the blood may persist for many years, if not for life.[42] It is not known whether this DNA contains the entire HBV genome, or even whether it is contained in virions. However, inoculation of serum from three subjects with persistent HBV DNA into chimpanzees has not led to documented infectivity.[42]

Persistent Infection

In persistent HBV infection, the early events unfold as in self-limited infection, but HBsAg remains in the blood and virus production continues, often for life. However, levels of viremia in chronic infection are generally substantially lower than during primary infection, although they can vary considerably from person to person. High titers of HBV in the blood are often indicated by the continued presence of HBeAg. Typically, there are 10^7 to 10^9 virions per milliliter in the blood[43] in such cases, which are highly infectious. But most people with persistent infection, especially those with anti-HBe antibodies, have somewhat lower levels of viremia.

One feature of chronic HBV infection that is not widely appreciated is its dynamic natural history. Even though, in most cases, HBsAg remains detectable for life, titers of viral DNA tend to decline over time. With the passage of time, there is also a tendency for HBeAg to disappear from the blood, along with seroconversion to positivity for anti-HBe antibodies — a progression that occurs at a rate of 5 to 10 percent per year in persistently infected people.[39] Often, the disappearance of HBeAg is preceded or accompanied by a transient rise in alanine aminotransferase levels, known as a flare, which suggests that the process reflects immune-mediated destruction of infected hepatocytes. Reductions in the level of viremia as great as five orders of magnitude may accompany seroconversion to anti-HBe antibodies.[44] Thus, the natural history of HBV persistence suggests that there is an ongoing immune attack on infected cells in the liver — an attack that is usually inadequate to eradicate infection altogether, but that does reduce the number of infected cells and thereby lowers the circulating viral load. Figure 4 shows typical patterns of serologic and molecular markers in both acute self-limited and chronic HBV infection.

The widely held view that circulating viral DNA disappears when anti-HBe antibodies appear is incorrect; this idea reflects the fact that, for many years, HBV DNA was measured by relatively insensitive hybridization methods with a detection limit of 10^5 to 10^6 virions per milliliter. Thanks to the advent of the polymerase-chain-reaction (PCR) method, we now know that at least 70 to 85 percent of people with anti-HBe antibodies have detectable viral DNA in the circulation, typically in the range of 10^3 to 10^5 molecules per milliliter, and sometimes higher.[44] Although these levels of HBV DNA are relatively low, they are hardly negligible. (For reference, they are similar to levels of human immunodeficiency virus [HIV] and HCV DNA in many patients with symptomatic acquired immunodeficiency syndrome or hepatitis C.) Given the short half-life of HBV virions (approximately one day),[39] such levels can be sustained only by ongoing viral replication; therefore, the claim that HBV enters a so-called nonreplicative phase later in its course is not correct. For this reason, anyone who has a positive test for HBsAg should be presumed to have some level of ongoing viremia. For example, when a decision must be made about immunoprophylaxis after a needle stick involving blood from an HBsAg-positive patient, prophylaxis should be offered irrespective of that patient's HBeAg status.

HBeAg-negative carriers are a heterogeneous group. Most such carriers have low levels of viral DNA, relatively normal levels of alanine aminotransferase, and a good prognosis.[30] However, particularly in southern Europe and in Asia, at least 15 to 20 percent of such carriers have elevated levels of alanine aminotransferase and viral DNA in the blood.[45] The virus in many such carriers harbors mutations in the preC region that prevent the production of HBeAg.[46] It has been suggested that per-
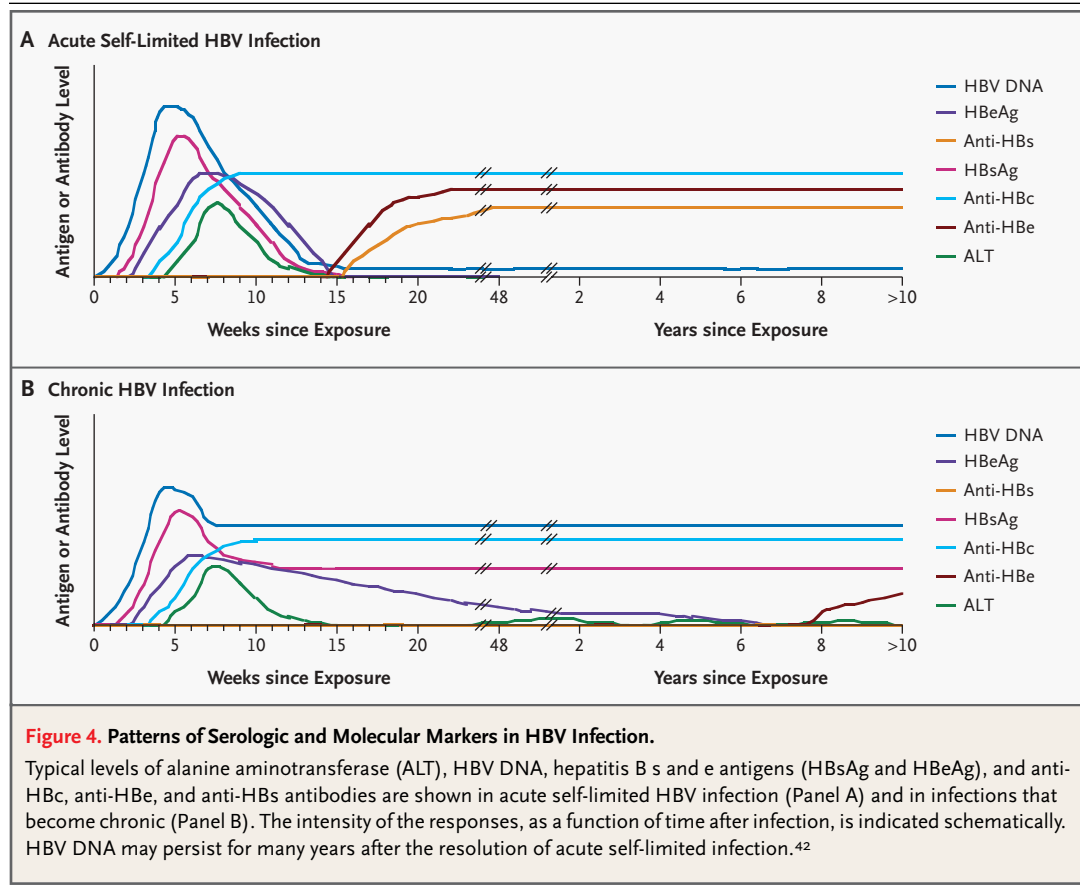
Figure 4. Patterns of Serologic and Molecular Markers in HBV Infection.
Typical levels of alanine aminotransferase (ALT), HBV DNA, hepatitis B s and e antigens (HBsAg and HBeAg), and anti-HBc, anti-HBe, and anti-HBs antibodies are shown in acute self-limited HBV infection (Panel A) and in infections that become chronic (Panel B). The intensity of the responses, as a function of time after infection, is indicated schematically. HBV DNA may persist for many years after the resolution of acute self-limited infection.[42]

sistently abnormal levels of alanine aminotransferase and elevated levels of viral DNA may denote a subgroup of HBeAg-negative carriers who should receive active antiviral therapy.[47]

Hepatocellular Carcinoma
Another feature of the natural history of HBV infection is its link to primary hepatocellular carcinoma. Chronically infected subjects have a risk of hepatocellular carcinoma that is 100 times as high as that for noncarriers[48]; within the HBsAg-positive group, HBeAg-positive carriers have the highest risk of hepatocellular carcinoma, but even carriers with anti-HBe antibodies have a substantial risk of cancer.[49] (Although the role of HBV in provoking hepatocellular carcinoma is undisputed, its cellular and molecular mechanisms remain incompletely understood.[50-54]) Given these facts, twice-a-year screening of chronically infected patients with measurements of serum alpha fetoprotein or hepatic ultrasonography, or both, is warranted.[47] However, there is debate as to when such screening should begin. Furthermore, screening is imperfect — alpha fetoprotein screening, for example, has an excellent negative predictive value, but its positive predictive value ranges from 9 to 30 percent.[47]

IMPLICATIONS FOR THERAPY

The goals of therapy in patients with HBV infection are a reduction in the level of viremia and amelioration of hepatic dysfunction. Most clinical studies have focused on chronically infected patients with elevated aminotransferase levels and circulating HBeAg, in whom viral loads can be readily measured even with first-generation DNA tests. There are clear indications for therapy in HBeAg-positive patients. They have an increased risk of early progression to chronic active hepatitis and cirrhosis,[55] and they have a risk of hepatocellular carcinoma that is substantially higher than that for other carriers.[49] By contrast, asymptomatic HBeAg-negative chronic carriers with viral loads below 10^5 genomes per milliliter and normal alanine aminotransferase

values tend to have a relatively stable course, with low rates of clinical or pathological progression.[30] At present, therapy is usually not offered to such persons. As noted above, some HBeAg-negative patients have liver dysfunction and substantial viremia ($>10^5$ molecules per milliliter). Discussions of the treatment of such patients are rare in the published literature, but results of a recent trial suggest that many of these patients would also benefit from effective antiviral therapy.[56] A recent clinical practice guideline[47] includes this group in its discussion of treatment; clearly, future clinical investigations should focus more attention on this group.

The usual markers of successful therapy are the loss of HBeAg, seroconversion to anti-HBe antibodies, and reduction of the circulating viral load. These are useful indicators, since patients with stable seroconversion to anti-HBe–positive status typically have improved histologic findings in the liver, and this improvement tends to be maintained over the long term.[57] True cure of infection (loss of HBsAg and complete disappearance of viremia, as measured by stringent PCR assays) is achieved only infrequently (in 1 to 5 percent of patients) with current regimens, although the increasing numbers of active antiviral drugs might lead to an upward revision of this figure in the future. In the case of patients with HBeAg-negative chronic hepatitis, there is no information about which markers best measure the response to therapy. Quantitation of viremia by PCR assays would seem a logical starting place, but there have been no systematic studies to guide the clinical interpretation of results.

INTERFERON

For many years, administration of interferon alfa (5 million to 10 million units subcutaneously three times per week, for at least three months) was the mainstay of therapy. About 30 percent of patients who tolerated this regimen had a successful response, defined as a loss of HBeAg, the development of anti-HBe antibodies, and a decline in serum alanine aminotransferase levels.[58] With HBe seroconversion and normalization of alanine aminotransferase levels, improvement is usually sustained well after therapy has been discontinued.[58] Interferon alfa treatment of chronic HBV infections in patients with cirrhosis has even been reported to reduce the risk of hepatocellular carcinoma.[59]

However, the side effects of therapy with interferon alfa (fever, myalgias, thrombocytopenia, and depression) make it a difficult treatment for many patients. Moreover, in many patients a flare of liver injury occurs during administration of interferon alfa, often just before or during clearance of HBeAg. This phenomenon may reflect the immunomodulatory activity of interferon alfa, which, in addition to impairing HBV replication, can also cause up-regulation of MHC class I antigens on hepatocytes and thereby augment the recognition of infected cells by cytotoxic T lymphocytes. Although sometimes disquieting to patients and physicians alike, these flares are intrinsic to the therapy and, as markers of enhanced antiviral immune responsiveness, often presage a successful outcome. However, treatment with interferon alfa is generally contraindicated in very advanced liver disease, since in such cases the flares may precipitate overt liver failure. Moreover, patients with advanced cirrhosis and splenomegaly usually have base-line leukopenia and thrombocytopenia, which can be exacerbated by the drug.

ANTIVIRAL DRUGS

Lamivudine

In the past decade, therapy for HBV has been revolutionized by the advent of drugs that directly block replication of the HBV genome. All these drugs (to date) are nucleoside or nucleotide analogues that selectively target the viral reverse transcriptase. The first successful drug, lamivudine, emerged from screening for inhibitors of the HIV reverse transcriptase and was introduced into clinical practice for the management of HIV infection. Carriers of HIV who are also infected with HBV had substantial declines in HBV viremia when treated with lamivudine,[60] and such declines were also observed in patients with chronic hepatitis B who did not have HIV infection.[61] In general, treatment with lamivudine results in a reduction of 3 to 4 log in circulating levels of HBV DNA in the first three months of therapy; this decline is associated with more rapid loss of HBeAg, seroconversion to anti-HBe–positive status, and improvement in serum aminotransferase levels. The drug is usually well tolerated, a factor that has led to the rapid displacement of interferon alfa from the roster of first-line therapies for HBV. Lamivudine is not immunomodulatory and can be used in patients with decompensated cirrhosis.[62] Even polyarteritis nodosa associated with HBV has been shown to respond dramatically to treatment with lamivudine plus plasma exchange.[63]

Although lamivudine is not an immunomodulator, there is strong evidence that successful treatment with lamivudine relies to some extent on an

adequate host immune response. This evidence emerged from a retrospective examination of subgroups of patients with optimal responses to therapy, which revealed a strong correlation between HBeAg clearance and elevated pretreatment values for alanine aminotransferase.[64] HBeAg seroconversion occurred in 65 percent of cases in which pretreatment values for alanine aminotransferase were more than five times the upper limit of the normal range, as compared with only 26 percent in patients with elevations in alanine aminotransferase values that were two to five times the upper limit of the normal range. Only 5 percent of patients with pretreatment alanine aminotransferase values that were less than twice the upper limit of the normal range had clearance of HBeAg — a rate similar to that for the spontaneous loss of this marker. This finding suggests that by reducing the viral load, lamivudine allows the immune and inflammatory responses to deal more effectively with the remaining infected hepatocytes in the host.

The principal limitation of lamivudine monotherapy is the development of drug resistance, which is mediated largely by point mutations at the YMDD motif at the catalytic center of the viral reverse transcriptase. The resulting mutants are slightly less fit than wild-type HBV in the absence of the drug, but they are strongly selected for in its presence.[65] Viral resistance emerges much more slowly in HBV infection than in HIV infection, for complex reasons beyond the scope of this review. By the end of one year of therapy, 15 to 20 percent of patients have resistant variants in the circulation; the figure rises to 40 percent by two years, and to 67 percent by the fourth year.[66]

The clinical significance of the development of resistance is still being debated. Clearly, in many patients, resistance presages a return of higher-level viremia, and in some of these patients further liver injury develops. However, although the level of viremia rises, in many patients it may still remain below pretreatment levels — perhaps as a result of the reduced fitness of the variants. In addition, some patients continue to undergo conversion from HBeAg-positive status to HBeAg-negative status, even after the appearance of lamivudine-resistant mutants in the circulation; by the end of four years of therapy, 40 to 50 percent of patients treated with lamivudine have undergone such conversion. Therefore, some experts favor the continuation of lamivudine therapy in patients with resistant variants

but no evidence of overt clinical failure, especially since transient exacerbations of liver injury develop in some patients when antiviral therapy is withdrawn.[67] (Such postwithdrawal flares are not limited to lamivudine but have also been observed with other anti-HBV regimens.[56,68]) Now that newer anti-HBV drugs are available, additional options exist for patients with resistant strains of HBV.

Other Nucleotide Analogues

Recently, the Food and Drug Administration (FDA) approved a second antiviral drug, adefovir, to treat HBV infection. Adefovir, a nucleotide (adenosine monophosphate) analogue, is a prodrug that undergoes two intracellular phosphorylations to yield the active drug, an inhibitor of the viral polymerase. Initially developed as an inhibitor of HIV reverse transcriptase, it proved nephrotoxic in the doses that were required for effective inhibition of HIV replication. However, in lower doses (10 mg per day) it has shown little nephrotoxicity and retains good efficacy against HBV in HBeAg-positive patients, with a reduction of 3 to 4 log in viremia; the frequency of HBeAg seroconversion is enhanced, and there is histologic improvement in the liver.[68] Similar efficacy was documented in HBeAg-negative patients with abnormal liver function and elevated levels of viral DNA.[56] Moreover, the drug effectively inhibits the replication of lamivudine-resistant HBV mutants, both in vitro and in vivo.[69,70] Clear evidence of the emergence of adefovir-resistant HBV mutants has not been found in the clinical trials performed to date,[56,68,71] although this issue remains open now that large numbers of patients will be using the drug.

Tenofovir, another adenine nucleotide analogue that was approved by the FDA for the treatment of HIV, also has activity against the HBV polymerase. In recent trials in HIV carriers who were positive for HBeAg, treatment with standard doses of tenofovir led to a reduction of 4 log in circulating HBV DNA levels, even in patients who had evidence of lamivudine-resistant virus.[72,73] However, the FDA has not yet approved tenofovir for use in patients with HBV infection.

Several investigational drugs are now in advanced stages of clinical trials. Entecavir is a guanosine analogue that, unlike the drugs discussed above, is highly selective for the HBV polymerase and has no activity against HIV. It is extremely potent on a molar basis, and doses as low as 0.1 mg per

day can cause reductions of 4 log in levels of HBV DNA; a dose of 0.5 mg per day caused a reduction of nearly 5 log in 24 weeks of monotherapy.[74] The drug is also active against lamivudine-resistant HBV. L-deoxythymidine is a thymidine analogue that also selectively inhibits the HBV polymerase and is advancing through clinical trials; it, too, appears to be active against lamivudine-resistant virus.[75] Two other nucleoside analogues (emtricitabine and clevudine) have also shown promise in vitro and in vivo, although neither is reliably active against all lamivudine-resistant variants.[76]

Thus, the past few years have witnessed the development of a plethora of new drugs for the treatment of HBV infection. It seems likely that combination therapy will become the wave of the future in HBV therapy, but many questions remain to be resolved by clinical investigation. Is combination therapy truly superior to monotherapy, clinically as well as virologically? If, as most expect, it proves to be superior, which combination of drugs should be used for initial treatment? Will there be an evolution of cross-resistance among drugs that are effective against lamivudine-resistant viruses? What should be the duration of therapy? (One recent practice guideline recommends stopping antiviral therapy at one year in HBeAg-positive patients who have seroconversion to anti-HBe antibodies but continuing therapy in those who do not have seroconversion in the first year or in whom breakthrough viremia develops.[47]) More speculatively, could more potent combination therapy reduce HBV levels to a point where the remaining virus could be cleared by the host's own immune system, either spontaneously or after therapeutic vaccination with HBV antigens?

LIVER TRANSPLANTATION

Liver transplantation for HBV disease is attended by recurrent viral infection in more than 80 percent of patients if no interventions are undertaken to prevent reinfection.[77] Recurrent HBV infection in the context of the level of immunosuppression needed to prevent graft rejection is typically associated with high viral loads and a poor outcome, especially for patients with chronic HBeAg-positive hepatitis. Hepatitis B immune globulin, administered over long periods, cuts the reinfection rate in half and extends the two-year survival rate from 50 percent to approximately 80 percent.[77,78] Use of lamivudine after transplantation has resulted in similar improvement,[79] but without question, the best results have been obtained with post-transplantation prophylaxis consisting of both hepatitis B immune globulin and lamivudine. This regimen reduces the rate of reinfection to about 10 percent and has boosted the five-year rate of HBV-free survival to approximately 80 percent.[80] However, many problems remain. Hepatitis B immune globulin is very expensive and contributes substantially to the cost of transplantation. It seems likely that, in the near future, a more limited course of prophylaxis with hepatitis B immune globulin (6 to 12 months), in concert with long-term drug therapy, perhaps with more than one agent, will become popular. Whether the use of hepatitis B immune globulin can be avoided altogether by the use of multidrug regimens will require careful study.

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CORRECTION

Hepatitis B Virus Infection — Natural History and Clinical Consequences

Hepatitis B Virus Infection — Natural History and Clinical Conse-quences . On page 1119, lines 7 through 9 of the first full paragraph should have read, ``Initiation at the next upstream start codon,´´ rather than ``Initiation at the most upstream start codon,´´ as printed. We re-gret the error.