The American Journal of Medicine (2005) 118, 1413.e15-1413.e22

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THE AMERICAN JOURNAL *of* MEDICINE®

REVIEW

Hepatitis B virus infection: Current status

Ponsiano Ocama, MD,[a] Christopher K. Opio, MD,[a] William M. Lee, MD[b]

[a]Mulago Hospital, Kampala, Uganda, and [b]University of Texas Southwestern Medical School, Division of Digestive and Liver Diseases, Dallas, Tex.

KEYWORDS:
Hepatitis B;
Nucleoside analogues;
Hepadna viruses;
Viral hepatitis

ABSTRACT: Hepatitis B virus currently infects more than 400 million people worldwide. Despite the availability of hepatitis B vaccine, the overall prevalence of hepatitis B virus infection has declined little in recent years. Hepatitis B virus causes liver injury by an immune response against the virus-infected liver cells and is not directly cytopathic, although immunosuppression appears to enhance replication and lead to direct cytotoxicity. The interplay of the host immune response and the virus's ability to replicate is a prime determinant of the likelihood of liver injury, its intensity, and progression to cirrhosis. A series of stages evolve in the life cycle of each patient's infection, with associated decreases in viral load at each successive stage. Viral mutations in the polymerase or the core gene affect replication and may enhance liver injury. Recently, genotypes have been identified that are linked to clinical outcomes, drug responses, and mutations. Four drugs (interferon alpha, lamivudine, adefovir, and entecavir) have been approved by the US Food and Drug Administration for treatment of hepatitis B virus; they effectively decrease replication and reduce inflammation and fibrosis. Treatment of hepatitis B virus in complex situations such as co-infection with human immunodeficiency virus or immunosuppressive therapy remains challenging. The use of hepatitis B vaccine has been shown to reduce the incidence of new infection in many regions. A decline in the prevalence of hepatitis B infection worldwide will require changes in high-risk behavior and the wider use of vaccination.

Since the discovery of hepatitis B virus in 1966, our understanding of its intricacies has continued to unfold. A major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma, hepatitis B virus ranks as an important pathogen throughout the world. Although potent antiviral agents have now emerged, the virus itself and the diseases it causes continue to evolve. The availability of new treatments is currently resulting in new interest in and understanding of the importance of treatment. Coupled with effective vaccines and risk avoidance, these treatments should begin to diminish the burden of chronic hepatitis B in the near future. This review will highlight our current understanding of hepatitis B virus, including both its virology and clinical features.

Epidemiology

Two billion people worldwide have evidence of hepatitis B virus exposure, and an estimated 400 million are actively infected.[1] Worldwide, the prevalence of hepatitis B virus varies greatly. In hyperendemic areas, such as China, Southeast Asia, Western Pacific, and sub-Saharan Africa, the carrier rate exceeds 8% and transmission occurs mainly from mother to child at time of parturition, as well as by horizontal transmission among children less than 5 years of

William M. Lee, MD, receives research support from Schering, Roche, Bristol Myers Squibb, Gilead, and Glaxo SmithKline.

Requests for reprints should be addressed to William M. Lee, MD, Division of Digestive and Liver Diseases, University of Texas Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas, TX 75390-9151.

Manuscript submitted May 24, 2005, and accepted in revised form June 15, 2005.

E-mail address: William.Lee@UTSouthwestern.edu.

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doi:10.1016/j.amjmed.2005.06.021

age, and to a lesser extent between sexually active adults. In North America and Europe less than 1% are chronically infected, the result of injection drug use, sexual transmission, nosocomial infection, or emigration from endemic areas. In 30%, no clear mode of transmission is found. In the United States, 1.25 million have chronic hepatitis B virus infection, approximately half of whom are Asian-Americans.[2] The large quantities of hepatitis B virus in serum and other body fluids ($\sim 10^8$ copies/mL) allow spread by mucosal and percutaneous routes with greater efficiency than is observed with hepatitis C virus ($\sim 10^6$ copies/mL) or human immunodeficiency virus (HIV; $\sim 10^4$ copies/mL).

Virology

Hepatitis B virus is a doubled-stranded DNA virus with 4 partially overlapping open reading frames: S (surface or envelope, hepatitis B surface antigen [HBsAg]) gene, C (core, hepatitis B core antigen [HBcAg]) gene, X gene, and the P (polymerase) gene.[3] Once hepatitis B virus enters the hepatocyte, it is uncoated and enters the nucleus as covalently closed circular DNA, a template from which RNA intermediates are made. These are subsequently encapsulated and transcribed in the cytoplasm, first as negative then positive DNA strands that acquire the envelope (HBsAg) and leave the cell. In addition, hepatitis B 'e' antigen (HBeAg), HBcAg peptides, and excess HBsAg in the form of spheres and rods appear on the cell surface or are secreted.

Viral mutations

Because hepatitis B virus DNA polymerase replicates through RNA intermediates, it is prone to mutations similar to HIV or hepatitis C virus. Under pressure from external (drugs) or internal (immunologic) stimuli, the polymerase and core genes are most affected. Mutations in the P gene are observed in patients treated with antiviral nucleoside analogues such as lamivudine.[4] Spread of lamivudine resistance beyond patients treated with the drug has been documented. Most mutations occur in the YMDD binding motif, a 4-amino acid sequence comprising the catalytic site for the replicating RNA strand. The presence of lamivudine in the chain interrupts replication. A single base pair substitution replacing methionine with isoleucine or leucine in the YMDD motif impairs binding of lamivudine, allowing renewed replication capacity. The C gene and its pre-core region encode the nucleocapsid (HBcAg) and HBeAg, a soluble secreted peptide associated with high levels of replication. Seroconversion of HBeAg with its loss and the appearance of anti-HBe are associated with greatly diminished replication and disease quiescence.[5] However, patients with mutations in the pre-core or core region continue to replicate actively, failing to secrete HBeAg and show progressive liver disease. In Asia and southern Europe, 30% to 90% of patients demonstrate these HBeAg-negative mutations, compared with 10% to 40% in the United States.[6]

Antibodies to HBcAg appear early in infection; they are not protective but signify previous exposure. The immunoglobulin-M component of anti-HBc indicates acute infection or reactivation. Antibodies produced in response to the surface antigen (anti-HBsAg) are protective but evolve more slowly in acute infection and are the hallmark of resolved infection and immunity to hepatitis B virus. Vaccine preparations containing HBsAg result in formation of anti-HBs without concomitant anti-HBc.

Genotypes of hepatitis B

Hepatitis B virus has been classified into 8 genotypes, A to H, based on genetic sequence variability between genotypes of more than 8%.[7] Certain genotypes predominate within different geographic, regional, and racial groups. Different genotypes are associated with somewhat disparate clinical outcomes, treatment responses, and mutations. The role of genotypes in the clinical management of chronic hepatitis B virus is still under evolution.[8]

Immunopathogenesis

Hepatitis B virus does not cause direct cell injury except in unusual circumstances. The immune response of the host, both cellular and humoral, determines the course of infection and the degree of liver injury in targeted virus-infected hepatocytes.[5] Recognition of hepatitis B virus determinants by cytotoxic T lymphocytes (CD8+ cells) leads to destruction specific to infected hepatocytes, with augmentation by CD4+ cellular responses. If there is abrogation of the immune response, such as in patients receiving immunosuppressive therapy, control of infection may be lost, hepatitis B virus replication is exaggerated, and a direct cytopathic effect is produced, leading to fibrosing cholestatic hepatitis (Figure 1).[9,10] On the other hand, severe hepatitis is sometimes observed on withdrawal of immunosuppression, presumably the result of an enhanced immune response.

Natural history of hepatitis B virus

Because the host immune status governs whether hepatitis B virus infection is resolved or sustained, perinatal infection, which is associated with a partial immune tolerance, leads to chronic ongoing infection in 95% compared with 30% in children 1 to 5 years and less than 5% in adult cases. Neonatal transmission of infection is prevented by treatment of the newborn with hepatitis B immunoglobulin and/or vaccination, although nearly equal efficacy is obtainable with vaccination alone.

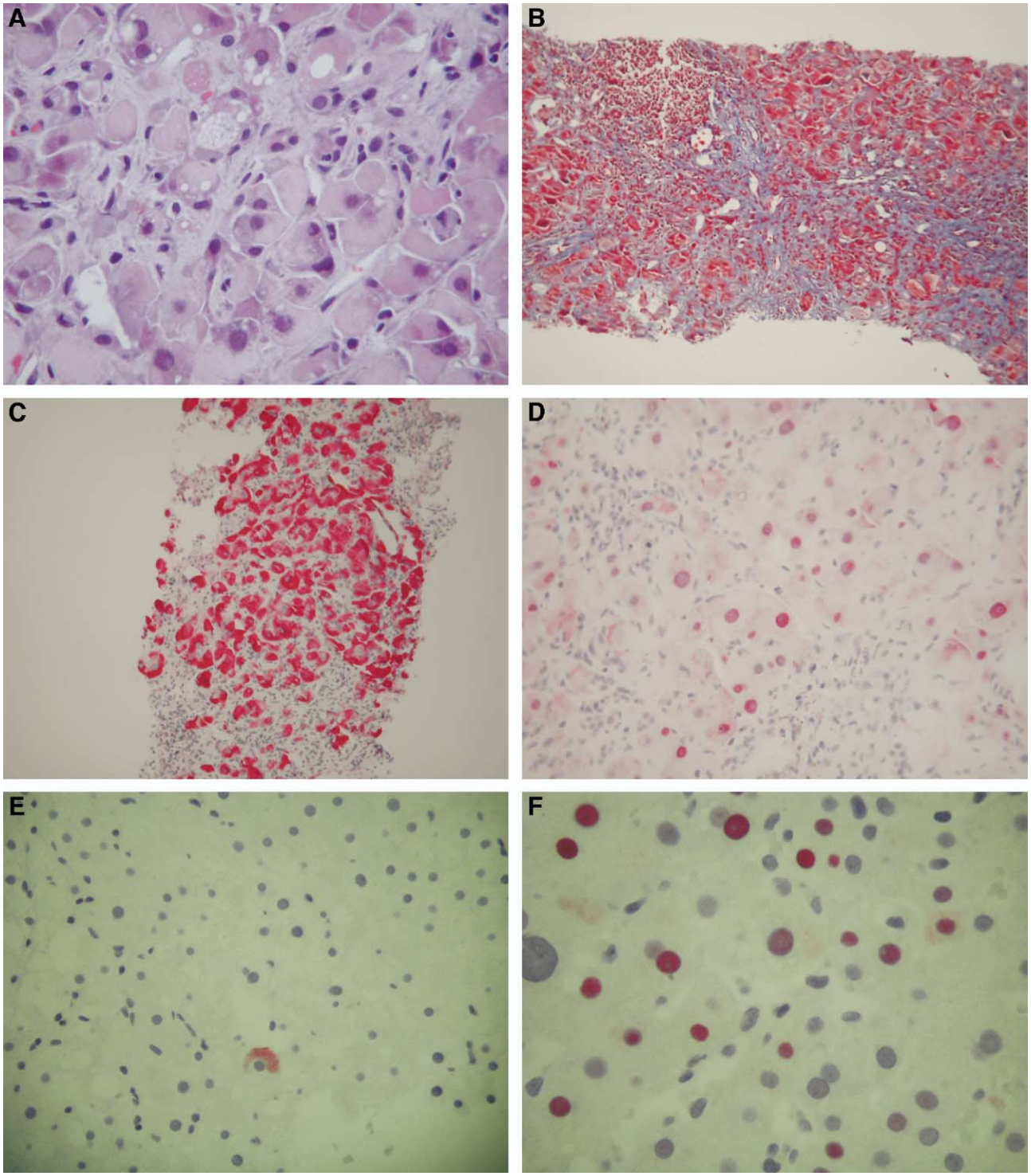

Figure 1 Overwhelming hepatitis B infection with immunosuppression. Photomicrographs providing evidence that immunosuppression leads to overwhelming infection. A–D: Liver biopsy from a patient who developed severe liver failure after chemotherapy for non-Hodgkin lymphoma, which was ultimately fatal. A: Extensive hepatocyte injury and inflammation involving the portal tract and hepatic lobule. Prominent ground glass cytoplasmic inclusions representing excess HBsAg are present (100× hematoxylin-eosin). B: Severe architectural disruption with intercellular fibrosis and hepatocyte necrosis (fibrosing cholestatic hepatitis) (100× Masson trichrome stain). C: Immunohistochemical stain for HBsAg confirming the overwhelming burden of HBsAg giving diffuse cytoplasmic reactivity in virtually all cells (alkaline phosphatase stain 100×). D: Overwhelming infection is also evident with HBcAg-positive staining in virtually every nucleus and in cytoplasm as well (alkaline phosphatase stain 200×). E, F: In contrast, the same immunohistochemical stains observed above applied to the liver biopsy of a patient with chronic hepatitis B. E: Only a single cell demonstrates immunoreactivity to HBsAg (alkaline phosphatase stain 400×). F: Scattered nuclei are reactive to staining for HBcAg (alkaline phosphatase stain 200×).

Table 1 Natural history of hepatitis B virus infection

	I	IIa	IIb	IIc	IIIa	IIIb	IV
HBsAg	+	+	+	+	+	+	−−
Anti-HBs	−−	−−	−−	−−	−−	−−	+
Anti-HBc	+	+	+	+	+	+	+
HBeAg	+	+	+	+	−−	−−	−−
Anti-HBe	−−	−−	−−	−−	+	+	+
HBV DNA	10^9	10^7	$<10^5$	10^7	$<10^5$	10^6	$<10^2$
ALT	−−	+++	−−	+++	−−	+++	−−
Key	tolerant	active	lam or adv	lam mutant	e neg	e neg mutant	immune

Four stages have traditionally been recognized, from immune tolerant with high viral titers to resolution of infection and the development of immunity to the virus. The new categories represent common patterns observed by the clinician in patients who are on treatment (stage IIb) or display important mutations (stages IIc or IIIb) that are associated with relapse with return of hepatitis B virus DNA and active liver disease.

ALT = alanine aminotransferase; lam = lamivudine; adv = adefovir dipivoxil; e neg = HBeAg negative.

The natural course of hepatitis B virus infection has been described as occurring in 4 stages leading from immune tolerance to full immunity. This construct now requires revisions to include the therapies and mutants described (Table 1). In the early stages, HBeAg is present as the replication marker, signifying at least 10^5 copies/mL present in serum. Patients who seroconvert to become HBeAg negative and develop anti-HBe will typically have DNA below this level, the threshold of positivity for earlier hepatitis B virus detection methods.[11]

Current hepatitis B virus DNA testing using polymerase chain reaction assays allows accurate quantification of viral particles over a wide dynamic range than previously (10^2-10^9 copies/mL). HBeAg seroconversion is associated with persistent low-level viral titers (10^3-10^5) but with improved overall outcomes. HBeAg testing, formerly used to mark viral clearance, is a less reliable marker of disease resolution because of the increasing frequency of HBeAg-negative mutations. Hepatitis B virus DNA quantitation must be used to evaluate and follow disease evolution.

Up to 20% of patients who develop HBeAg seroconversion may reactivate, becoming HBeAg positive again. Thus, regular follow-up with quantitative measurement of hepatitis B virus DNA and aminotransferase levels is required after seroconversion to ensure its durability. In persistently HBeAg-negative individuals, the presence of more than 10^5 copies/mL indicates a mutation (Table 1).[12] Similarly, resistance to lamivudine or other nucleos(t)ide analogues must be considered when hepatitis B virus DNA levels return toward baseline values despite continued treatment. Specific tests to confirm the presence of HBeAg and lamivudine-resistant mutations are commercially available.

Diagnosis and treatment

Treatment for hepatitis B virus infection is targeted at diminishing viral replication, because lessening viral burden appears to decrease hepatic inflammation and even the degree of fibrosis. In addition, 10% to 20% of patients under treatment lose HBeAg from serum annually, and 1% to 5% completely resolve infection with clearance of HBsAg. Every HBsAg-positive patient deserves evaluation. It is useful to determine the presence of HBeAg and the level of hepatitis B virus DNA in serum and to measure aminotransferase levels. Imaging the liver with ultrasonography, computed tomography scan, or magnetic resonance imaging is needed to identify cirrhosis or liver mass lesions such as hepatocellular carcinoma. Liver biopsy is usually required to determine whether treatment is indicated. Bridging fibrosis or cirrhosis on biopsy indicates progressive disease usually mandating treatment. Suppression of hepatitis B virus replication follows virtually all treatments, but serum hepatitis B virus DNA levels often return to baseline with cessation of treatment unless there is seroconversion, that is, loss of HBeAg, and development of anti-HBe antibodies. Favorable features for treatment include high aminotransferase and low hepatitis B virus DNA levels.[13] Four drugs have been approved by the US Food and Drug Administration (FDA) for the treatment of chronic hepatitis B virus infection: interferon alfa-2b, lamivudine, adefovir dipivoxil, and entecavir. A simple algorithm for treatment is shown in Figure 2.

Interferon α-2b

Interferon alfa-2b, approved by the FDA in 1992, has immunomodulatory and antiviral effects. In patients with HBeAg-positive hepatitis B, interferon alfa given subcutaneously at a dose of 5 MU daily or 10 MU thrice weekly induced loss of hepatitis B virus DNA and HBeAg in 37% and 33%, respectively, compared with 17% and 12%, respectively, in the placebo group after 12 to 24 weeks of therapy. Interferon is effective only in selected patients with favorable features as outlined above.[13] Other factors that increase the likelihood of response include absence of immunosuppression, female sex, HBeAg positive, short duration of illness, horizontal acquisition of virus, and history of acute icteric hepatitis.

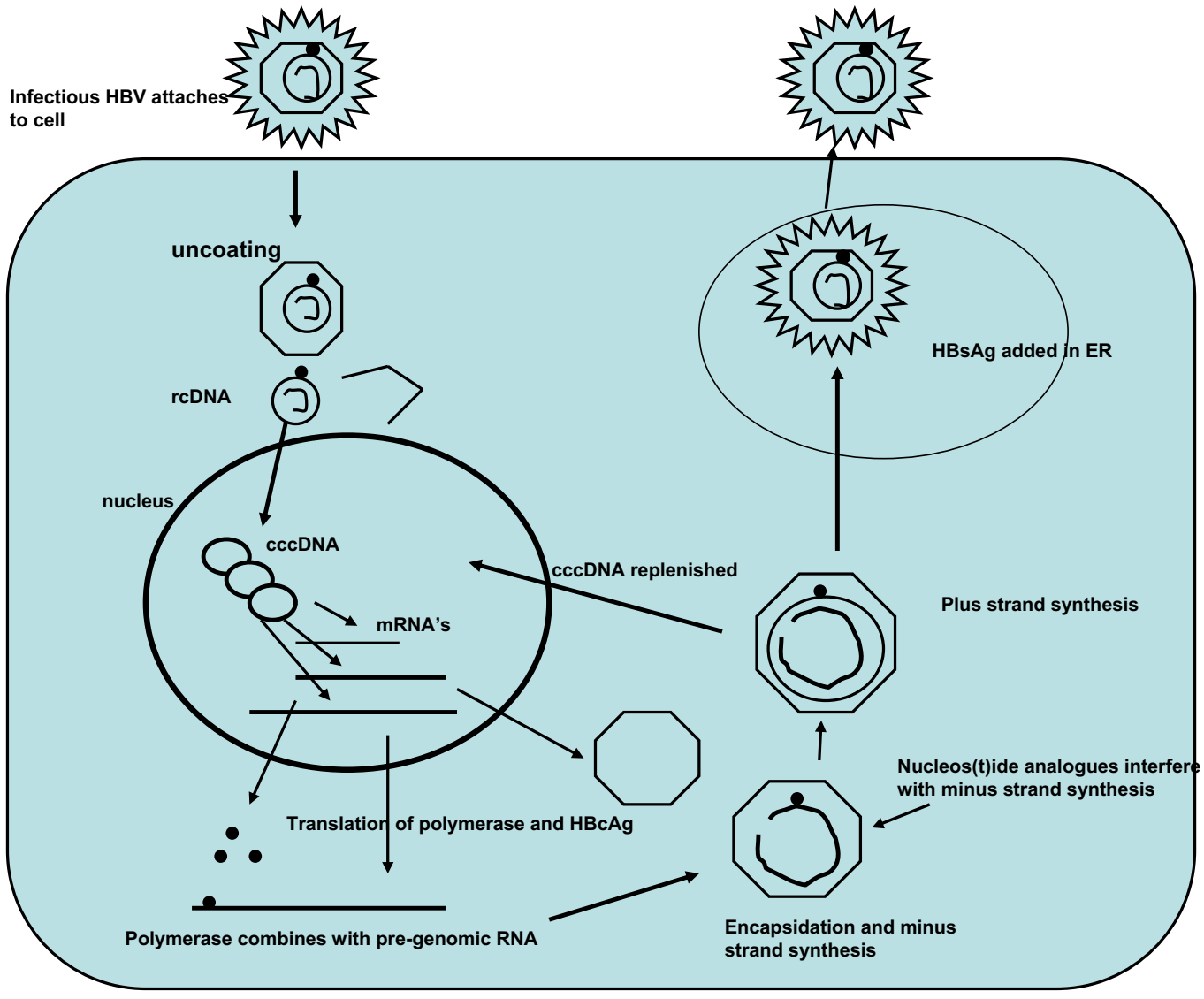

Figure 2 An algorithm for the management of chronic hepatitis B.[19-22] HBV = hepatitis B virus; HBcAg = hepatitis B core antigen; HBsAg = hepatitis B surface antigen; ER = endoplasmic reticulum; cccDNA = covalently closed circular DNA.

Side effects of interferon are well known and may require dose adjustment or discontinuation. In patients with cirrhosis, interferon may cause worsening of liver function. At present, interferon therapy is infrequently used because oral agents are easier to administer and have fewer side effects. Pegylated interferons will probably replace conventional interferons in the treatment of hepatitis B virus, because current evidence from phase III clinical trails suggest pegylated interferons are more efficacious than lamivudine in the treatment of chronic hepatitis B virus.[14]

Nucleos(t)ide analogues

Nucleos(t)ide analogues decrease hepatitis B virus replication but have no immunomodulatory effect. Currently available nucleos(t)ide analogues decrease DNA levels in plasma by approximately $3x\log_{10}$ up to $7X\log_{10}$ but seldom eradicate the virus and usually must be given long term to maintain viral suppression.

Lamivudine

Lamivudine (3TC) is a nucleoside analogue effective in both HBeAg-positive and HBeAg-negative patients as well as interferon failures. After 1 year of treatment of HBeAg-positive patients, lamivudine induced HBeAg seroconversion in 16% to 17% compared with 4% to 6% in those on placebo.[15] If seroconversion occurs, lamivudine may be withdrawn with a low risk of relapse provided treatment is continued for at least 3 to 12 months after seroconversion. The optimal interval is unclear. Long-term benefit in terms of improved histology, seroconversion, and fewer liver can-

cers has been observed with nucleos(t)ide therapy. Patients have been removed from liver transplantation waiting lists in some instances. In patients with HBeAg-negative chronic hepatitis B receiving lamivudine therapy, two thirds will demonstrate a reduction in serum hepatitis B virus DNA to less than 10^5 copies/mL and will normalize aminotransferases, compared with 6% of placebo recipients after 24 weeks. Lamivudine is effective in patients with cirrhosis, including those with hepatic decompensation, and can prevent recurrent hepatitis B virus after liver transplant.[16] An oral drug with an excellent safety profile, lamivudine's major drawback is the development of resistance (YMDD mutation) that occurs in approximately 24% at 1 year and 67% by 4 years.

Development of the lamivudine-resistant YMDD mutations leads to rebound in hepatitis B virus DNA, followed by an increase of alanine aminotransferase and worsening inflammation on biopsy typically over several months. Acute liver failure has been reported in this setting. Seroconversion with long-term benefit has been demonstrated in some patients despite lamivudine-resistant mutations.

Adefovir dipivoxil

Adefovir dipivoxil, a nucleotide analog of adenosine, was approved by the FDA in 2002. In patients who are HBeAg positive or HBeAg negative, adefovir decreased hepatitis B virus DNA by at least $3x\log_{10}$. Aminotransferase levels became normal at 48 weeks in 72% compared with 29% on placebo; hepatitis B virus DNA became undetectable by a sensitive polymerase chain reaction assay in 51% compared with 0% on placebo. HBeAg seroconversion occurred in 12% compared with 6% of those on placebo.[17] Significant histologic improvement occurred in both treated groups. Adefovir is equally effective against wild-type and lamivudine-resistant virus in studies of naïve patients, as well as post-liver transplant recipients. Adefovir is mainly excreted unchanged by the kidneys. Nephrotoxicity has been observed at higher doses, but the drug appears to be safe at the current recommended dose. Frequent monitoring of renal function is therefore important. Dose adjustments are necessary for patients with creatinine clearances below 50 mL/min and for those on hemodialysis. Resistance against adefovir in HBeAg-positive patients has been reported but is much less frequent than that observed with lamivudine (1.6% at 2 years).

Entecavir

Entecavir recently received FDA approval in the United States for use in naïve patients and those with evidence of lamivudine resistance. Diminution of viral titers may reach $7X\log_{10}$ in naïve patients.[18] Long-term studies are not yet available.

Beginning therapy and choice of agent

The decision to treat must be individualized and is made on the basis of several data: age and clinical condition of the patient, viral load, aminotransferase levels, coexisting liver disease, family history of hepatocellular cancer, and, where available, biopsy findings. A full discussion of treatment is found in several consensus guideline publications.[19-22] In general, those with active liver disease as evidenced by elevated aminotransferases and viral titers exceeding 1 million copies/mL are considered in need of treatment (Figure 2).

Newer agents

Several additional drugs are under clinical evaluation to improve treatment responses. A number of nucleoside analogues used in the treatment of HIV have activity for hepatitis B virus. Tenofovir, emtricitabine, and the combination of emtricitabine and tenofovir (Truvada; Gilead Sciences, Foster City, Calif.) all show promise but have not been fully studied. As observed with HIV, the use of combinations of drugs acting at different replication stages might further enhance replication suppression; however, no synergistic combination has been defined. The pegylated interferons have promise but have not been fully evaluated. Surprisingly, there appears to be no additional benefit from use of interferons with nucleoside analogues: Pegylated interferon alfa-2a alone or in combination with lamivudine was found to result in a significantly higher (but similar) rate of sustained response than use of lamivudine alone in HBeAg-negative patients.

Special populations: Human immunodeficiency virus co-infection

Co-infection of hepatitis B virus and HIV is common because of shared modes of transmission. In the presence of HIV infection, hepatitis B virus is more likely to persist with more rapid evolution of liver damage and progression to cirrhosis than is observed in mono-infection. Lamivudine-based highly active retroviral therapy combinations may lead to improvement in liver disease in patients with co-infection because lamivudine is effective against both viruses; however, resistance to lamivudine develops more rapidly than in mono-infected patients. In contrast, adefovir dipivoxil, is effective against lamivudine-resistant mutants, but has little effect on HIV replication in doses used for hepatitis B virus. Agents such as Truvada may be the drug

of choice in patients with co-infection, but this has not been studied systematically.

Hepatocellular carcinoma

Among other causative factors, chronic hepatitis B virus appears responsible for a large number of hepatocellular carcinoma cases worldwide. Resolution of chronic hepatitis B significantly diminishes the risk of subsequent hepatocellular carcinoma, as does seroconversion to HBeAg negativity. Vaccination against hepatitis B virus has decreased viral carriage, resulting in a lower incidence of hepatocellular carcinoma in high-density regions, and greater future gains are expected. The use of routine screening with ultrasonography on an annual basis is often recommended but may not be cost-effective.

Liver transplantation

More than one third of patients with chronic hepatitis B infection can expect to develop cirrhosis or liver cancer. Liver transplantation is effective therapy for patients with hepatitis B virus-induced end-stage liver disease but was associated with a high reinfection rate leading to graft loss and poor survival before the use of suppressive therapy. Hepatitis B virus recurrence after liver transplantation has been significantly reduced using a combination of hepatitis B immunoglobulin and lamivudine. Adefovir dipivoxil is relatively safe and effective in lamivudine-resistant hepatitis B virus pre- and posttransplantation.

Cancer chemotherapy/immunosuppressive therapy

Patients with active or inactive chronic hepatitis B virus infection on cancer chemotherapy or after bone marrow transplantation may undergo reactivation leading to severe hepatitis during and/or after the chemotherapy (Figure 1). It is important to screen for hepatitis B virus infection in all patients before cancer chemotherapy or other immunosuppressive therapy. Lamivudine appears effective as prophylaxis or treatment of hepatitis B virus reactivation in these patients.

Acute liver failure

Approximately 1% of patients with acute hepatitis B virus infection develop hepatic failure, but the incidence of acute liver failure caused by hepatitis B virus is decreasing in the United States. The findings of coagulopathy and encephalopathy confirm the diagnosis that is fatal in approximately 75% without liver transplantation. Although there is no proven treatment for acute liver failure caused by hepatitis B infection, nucleoside analogues may be useful in this setting.

Hepatitis B vaccine

Until recently, the cost of hepatitis B vaccines has precluded its widespread use; where available, its effect in reducing the prevalence of hepatitis B virus infection is dramatic. To prevent chronic hepatitis B virus infection and related consequences, Taiwan reduced the prevalence of their chronic HBsAg carrier rate in children from 9.8% to 1.3% after 10 years of neonatal vaccinations. Such strategies have become the standard in the United States. Vaccination programs have been initiated through the World Health Organization in much of Africa in the last 2 to 3 years.

The future

Given the huge burden of hepatitis B virus infection worldwide and the number of advances made in the past several decades, it is surprising that more progress in limiting infection has not been realized. The number of acute hepatitis B cases has decreased by 76% in the United States between 1987 and 1998, in large part because of changes in high-risk behavior. However, hepatitis B continues to spread in endemic areas where universal vaccination has not yet reached. The availability of vaccination and its use to prevent neonatal transmission, as well as the increasing use of suppressive therapies, should yield greater gains toward eradication of hepatitis B in another generation.

References

1. Mast EE, Mahoney FJ, Alter MJ, Margolis HS. Progress toward elimination of Hepatitis B transmission in the United States. *Vaccine*. 1998;16(Suppl):S48-S51.
2. McQuillan GM, Coleman PJ, Kruszon-Moran D, Moyer LA, Lambert SB, Margolis HS. Prevalence of hepatitis B virus infection in the United States: the National Health and Nutrition Examination Surveys, 1976 through 1994. *Am J Public Health*. 1999;89:14-18.
3. Seeger C, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev*. 2000;64:51-68.
4. Hunt CM, McGill JM, Allen MI, Condreay LD. Clinical relevance of hepatitis B virus mutations. *Hepatology*. 2000;31:1037-1044.
5. Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. *Ann Rev Immunol*. 1995;13:29-60.
6. Hadziyannis SJ, Vassilopoulos D. Hepatitis B e antigen-negative chronic hepatitis B. *Hepatology*. 2001;34:617-624.
7. Ljunggren KK, Miyakawa Y, Kidd AH. Genetic variability in hepatitis B viruses. *J Gen Virol*. 2002;83;1267-1280.
8. Fung SK, Lok AS. Hepatitis B genotypes: do they play a role in the outcome of HBV infection? *Hepatology*. 2004;40:790-792.
9. Davies SE, Portmann BC, O'Grady JG, Aldis PM, Chaggar K, Alexander GJ, et al. Hepatic histological findings after transplantation for

chronic hepatitis B virus infection, including a unique pattern of fibrosing cholestatic hepatitis. *Hepatology*. 1991;13:150-157.
10. Yeo W, Chan PKS, Zhong S, Ho WM, Steinberg JL, Tam JS, et al. Frequency of hepatitis B virus reactivation in cancer patients undergoing cytotoxic chemotherapy: a prospective study of 626 patients with identification of risk factors. *J Med Virol*. 2000;62:299-307.
11. Chu CJ, Hussain M, Lok ASF. Quantitative serum HBV DNA levels during different stages of chronic hepatitis B infection. *Hepatology*. 2002;36:1408-1415.
12. Hadziyannis SJ, Vassilopoulos D. Hepatitis B e antigen-negative chronic hepatitis B. *Hepatology*. 2001;34:617-624.
13. Brook MG, Karayiannis P, Thomas HC. Which patients with chronic hepatitis B virus infection will respond to alfa-interferon therapy? A statistical analysis of predictive factors. *Hepatology*. 1989;10:761-763.
14. Marcellin P, Lau GK, Bonino F, Farci P, Hadziyannis S, Jin R, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination inpatients with HBeAg-negative chronic hepatitis B. *N Engl J Med*. 2004;351:1206-1217.
15. Lai CL, Chien RN, Leung NWY, Chang TT, Guan R, Tai DI, et al. A one year trial of lamivudine for chronic hepatitis B. *N Engl J Med*. 1998;339:61-68.
16. Perrillo RP, Wright T, Rakela J, Levy G, Schiff E, Gish R, et al. A multi-center United States-Canadian trial to assess lamivudine monotherapy before and after liver transplantation for chronic hepatitis B. *Hepatology*. 2001;33:424-443.
17. Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, et al. Adefovir dipivoxil for the treatment of patients with hepatitis B e antigen-positive chronic hepatitis B. *N Engl J Med*. 2003;348:808-816.
18. Lai CL, Rosmawati M, Lao J, Vlierberghe HV, Anderson FH, Thomas N, et al. Entecavir is superior to lamivudine in reducing hepatitis B virus DNA in patients with chronic hepatitis B infection. *Gastroenterology*. 2002;123:1831-1838.
19. Liaw YF, Leung N, Guan R, Lau GK, Merican I; Asian-Pacific Consensus Working Parties on Hepatitis B. Asian-Pacific consensus statement on the management of chronic hepatitis B: an update. *J Gastroenterol Hepatol*. 2003;18:239-245.
20. Franchis R, Hadengue A, Lau GK, Lavanchy D, Lok A, McIntyre N, et al; EASL Jury. EASL International Consensus Conference on Hepatitis B. *J Hepatol*. 2003:39(Supp1):S3-S25.
21. Lok AS, McMahon BJ. Chronic hepatitis B: Update of recommendations. *Hepatology*. 2004;39:857-861.
22. Keeffe EB, Dieterich DT, Schiff ER, Tobias H, Han SH, Wright TL, et al. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States. *Clin Gastroenterol Hepatol*. 2004;2: 87-106.