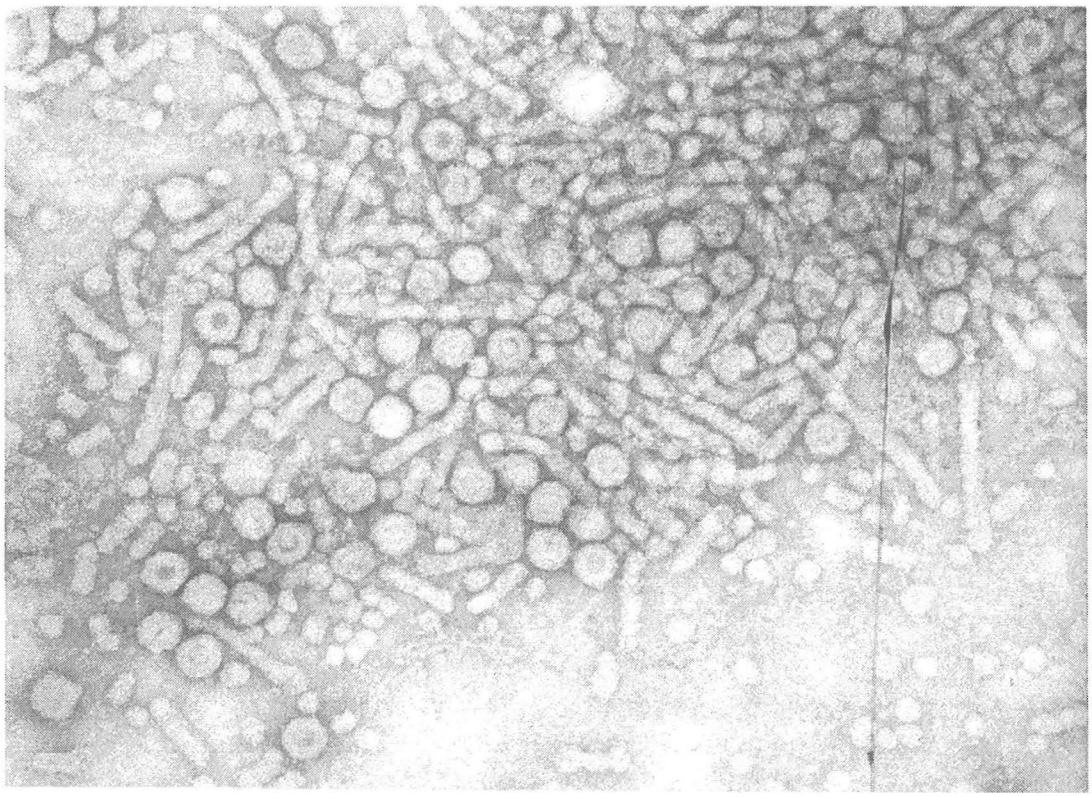
Hepatitis B Virus Infection: New Mutations, New Drugs



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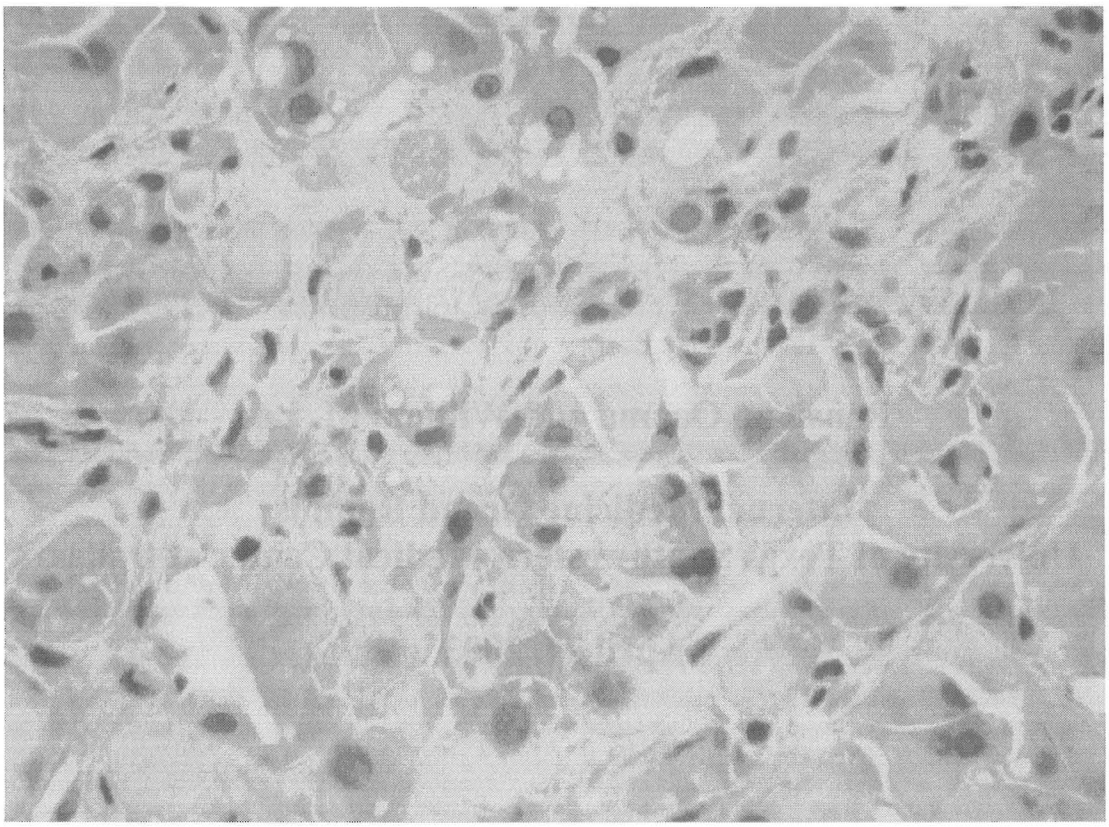
This is to acknowledge that Drs. Ocama and Lee have disclosed financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Lee will be discussing off-label uses in his presentation.

Dr. Ponsiano Ocama has spent the academic year at UT Southwestern as the William and Eleanor Crook Fellow in Liver Disease for 2002-03. He is a specialist medical officer at the Makerere University Teaching Hospital in Kampala, Uganda. Fifteen percent of the Ugandan population has hepatitis B, compared to 5% with human immunodeficiency virus (HIV) infection. He wishes to thank Mrs. Eleanor Crook for making this year possible.

Dr. William M. Lee is the Meredith Mosle Distinguished Professor in Liver Disease. His research interests include viral hepatitis, acute liver failure and drug hepatotoxicity. UT Southwestern is the central site for the NIH-sponsored Acute Liver Failure Study Group and is one of ten US sites engaged in the HALT C Study, both under Dr. Lee's direction. Dr. Lee will give this Grand Round but wishes to acknowledge the major contribution of Dr. Ocama to this presentation. He also wishes to acknowledge the contributions of useful visual material from Drs. Anna S.F. Lok, University of Michigan, W. Ray Kim, Mayo Clinic, Mark Kane, CDC, and the Gilead Corporation.



Introduction

Since the discovery of hepatitis B virus (HBV) in 1966,[1] our understanding of its intricacies has continued to unfold. A major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma, hepatitis B virus ranks as a primary pathogen throughout the world but continues to change. While potent anti-viral agents have now emerged, the virus itself and the diseases it causes continue to evolve. New treatments, available effective vaccines and changing behaviors are beginning to diminish the burden of chronic hepatitis B. This review will highlight our current understanding of hepatitis B virus as well as the therapies now available.

Epidemiology

Two billion people worldwide have evidence of HBV exposure and an estimated 400 million are actively infected.[2,3] Widely distributed, hepatitis B's endemicity varies greatly. In hyperendemic areas, such as China, Southeast Asia, Western Pacific and sub-Saharan Africa, the carrier rate exceeds 8 percent and transmission occurs mainly from mother to child at time of delivery and to a lesser extent by horizontal transmission primarily among children less than five years of age, or between sexually active adults.[4-7] In North America and Europe less than three percent are chronically infected, primarily due to injection drug use, sexual transmission or emigration from endemic areas. Transmission also occurs via nosocomial infection.[2,5] In 30 percent, no clear mode of transmission is found.[8] In the U.S., 1.25 million have chronic HBV infection, approximately half of whom are Asian-Americans.[8-10] The large quantities of HBV in serum and other body fluids (~10^8 copies/mL) allow spread via mucosal and percutaneous routes with greater efficiency than is observed with hepatitis C virus (HCV;~10^6 copies/mL) or human immunodeficiency virus (HIV;~10^4 copies/mL).

Virology

Hepatitis B virus is a small DNA virus belonging to the Hepadna virus family that includes the ground squirrel hepatitis virus, woodchuck hepatitis virus and duck hepatitis B virus with similar infection characteristics.[11] The HBV genome is double-stranded, with four partially overlapping open reading frames: S (surface or envelope, HBsAg) gene, C (core, HBcAg) gene, X gene and the P (polymerase) gene.[11,12] HBsAg has several unique antigenic determinants (a, d, w, y and r), the combination of which

determines the different serotypes of hepatitis B virus. Serum of infected patients contains excess HBsAg, as small spheres or rods in quantities exceeding the number of whole virions by 10 to 100-fold.[11,12]

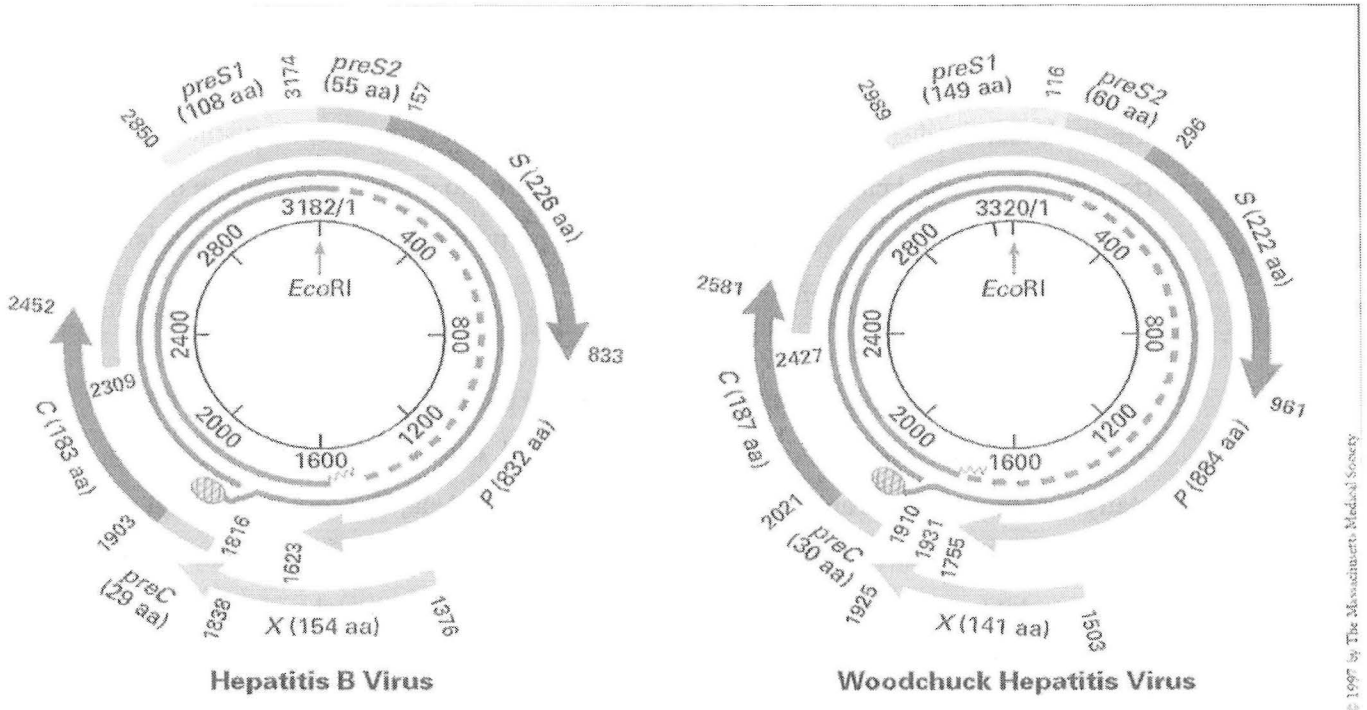


Figure 1. HBV genome compared to WHV genome.

Viral mutations

The HBV DNA polymerase replicates by reverse transcription using RNA intermediates, leaving it prone to mutations similar to HIV or HCV.[12] Under pressure from external (drugs) and internal (immunologic) stimuli, the surface and core genes are most subject to mutations. Mutations in the P gene are observed in patients treated with antiviral nucleoside analogues such as lamivudine.[13] Use of these agents has resulted in spread of lamivudine resistance beyond those patients treated with the drug.[14] Most mutations occur in the YMDD binding motif, a 4-amino acid sequence which secures the nucleosides as the replicating RNA strand is formed.[15,16] The presence of lamivudine in the chain arrests replication. A single base pair substitution which replaces methionine with isoleucine or leucine in the YMDD motif impairs binding of lamivudine and other nucleosides so that the mutant virus replicates less efficiently than wild type virus.[17,18]

The C gene and its pre-core region encode the nucleocapsid and the hepatitis B 'e' antigen (HBeAg), a soluble secreted peptide that correlates with active replication, high circulating serum DNA levels and infectivity. HBeAg may also convey immune tolerance and promote chronic infection.[11,19] Absence of HBeAg has been associated with greatly diminished replication and disease quiescence.[20] However, patients with mutations in the pre-core or core region fail to secrete HBeAg but continue active replication and progressive liver disease.[20-22] In Asia and southern Europe, 30 to 90 percent of patients demonstrate HBeAg negative mutations, compared to 10 to 40 percent in the U.S.[19] Core peptides displayed on the cell surface are important for immune recognition, subsequent liver cell injury, and, presumably, clearance of virus-infected cells.[9]

Antibodies to HBcAg that appear early in infection are not protective but provide lifelong evidence of infection. Antibodies to HBeAg appear in patients who have greatly diminished replication or have developed an HBeAg-negative mutation. Antibodies produced in response to the surface antigen (anti-HBs) are protective but evolve more slowly in acute infection and are the hallmark of resolved infection and immunity. Vaccine preparations containing HBsAg result in formation of anti-HBs without concomitant anti-HBc.[23] The IgM component of anti-HBc appears both in acute infection and in flares of disease activity during chronic hepatitis B infection.[24]

Serotypes of Hepatitis B

Variations in the antigenic determinants of HBsAg result in four commonly recognized serotypes (adr, adw, ayr and ayw).[25] Antibody to the 'a' determinant, which is common to all leads to protective immunity. Mutations have been described in the 'a' determinant of the S gene in association with 'vaccine escape',[26] where re-infection occurs despite vaccination or administration of hepatitis B immunoglobulin (HBIG).[27,28]

HBV genotypes

Hepatitis B virus has been classified into 7 genotypes, A-G, based on genetic sequence variability between genotypes of > 8 percent. These genotypes conform to a great extent to serotype patterns.[29]

Genotype	Serotype	Distribution	Comments
A	adw2, ayw1	NW Europe, N America, Central America	Rare HBeAg neg mutations
B	adw2, ayw1	SE Asia, China, Japan	Earlier HBeAg sero-conversion, better IFN response than C
C	ayr, ardq+, adrq--, adw2	SE Asia, China, Japan	Faster disease progression than B
D	ayw2, ayw3	S Europe, Middle East, India	Poorer response to IFN than genotype A
E	ayw4	Africa	Unknown significance
F	adw4q--	American natives, Polynesia, Central and South America	Unknown significance
G	adw2	United States, France	

Table 1. Geographic distribution of genotypes and their clinical significance.[30-33]

Specific genotypes are found in different geographic regions, and the genotypes (B and C) that predominate in Asian patients are also found in Asian-Americans. Genotypes are associated with various clinical outcomes, treatment responses and mutations.[30,31] For example, genotype C shows lower rates of spontaneous HBeAg seroconversion, higher rates of cirrhosis and hepatocellular carcinoma and a poorer response to interferon than genotype B.[31-33] Pre-core mutation is common in genotypes B, C and D and rare in genotype A,[34] accounting for the lower incidence of HBeAg-negative mutations in U.S. patients, who are commonly genotype A. The absence of pre-core mutations in genotype A can be explained by the differences in nucleotides in the stem loop structure of the core gene. To create the commonly observed stop codon (number 28) associated with HBeAg negative mutations, genotype A would require a double mutation to maintain base-pairing across the stem loop, whereas the same mutation in genotypes B-D requires only one base pair change.[34]

Immunopathogenesis

Hepatitis B virus does not cause direct cell injury except in unusual circumstances. The immune system of the host, both cellular and humoral, directs the course of infection resulting in liver injury by targeting virus-infected hepatocytes.[35] Hepatocytes process and present epitopes on the cell surface, mainly specific HBcAg peptides from amino acid 8 to 27, via HLA class I molecules. Recognition by cytotoxic

T-lymphocytes (CD8+ cells) leads to destruction of the infected hepatocyte by apoptosis (programmed cell death). Antigen-presenting cells, mainly macrophages, process other HBV peptides, presenting them via HLA class II molecules, leading to recognition by T-helper (CD4+) cells yielding cytokine and antibody production.

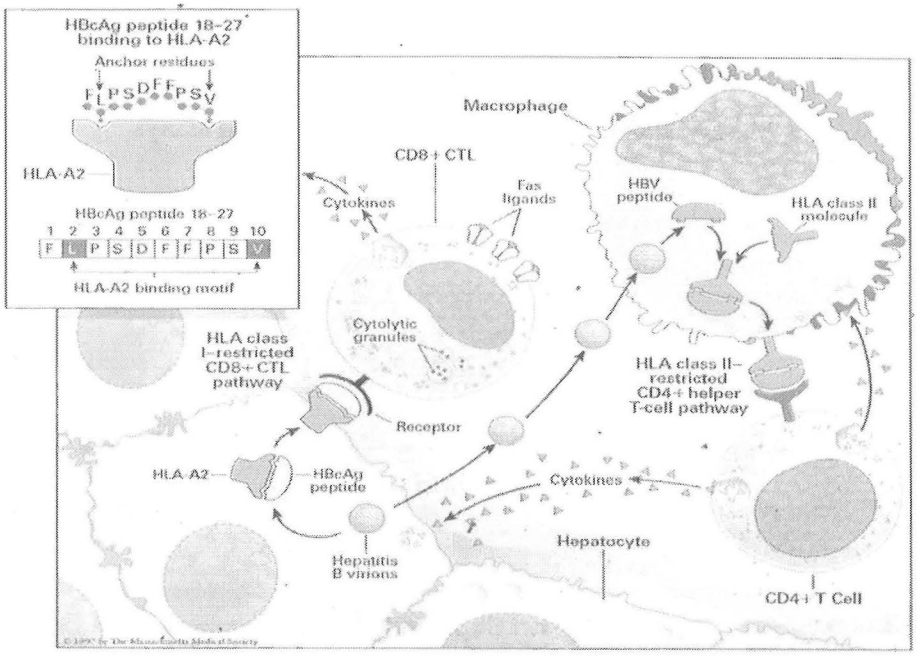


Figure 2. Immunopathogenesis of HBV infection.

With abrogation of the immune response, such as in patients receiving immunosuppressive therapy, the natural control of infection is lost, HBV replication is exaggerated and a direct cytopathic effect is produced, leading to fibrosing cholestatic hepatitis. This pattern of apparent direct injury is observed in liver grafts and in patients with intensive chemotherapy or following renal or bone marrow transplants.[36-39] This may be the result of withdrawal of immunosuppression or a direct toxic effect of HBsAg proteins on cellular excretion mechanisms.[39]

Natural history of HBV

Since the host immunological status governs whether HBV infection is resolved or sustained, infection acquired perinatally evolves to chronic disease in 95 percent compared to 30 percent for infection in children 1 to 5 years and less than 5 percent in adult cases.[24] High neonatal transmission of infection has conventionally been prevented

by treatment of the newborn with HBIG and/or vaccination, although efficacy is equally good with vaccination alone.[40-42]

The natural course of HBV infection has been described as occurring in 4 stages leading from immune tolerance to full immunity.[9] This construct requires revisions to incorporate new information regarding important mutation variants (Table 2). If HBeAg is positive, active replication affords at least 10^5 copies/mL of serum, but titers may vary considerably.[43] All patients who seroconvert to become HBeAg negative and develop anti-HBe will have undetectable DNA using earlier hybridization assays

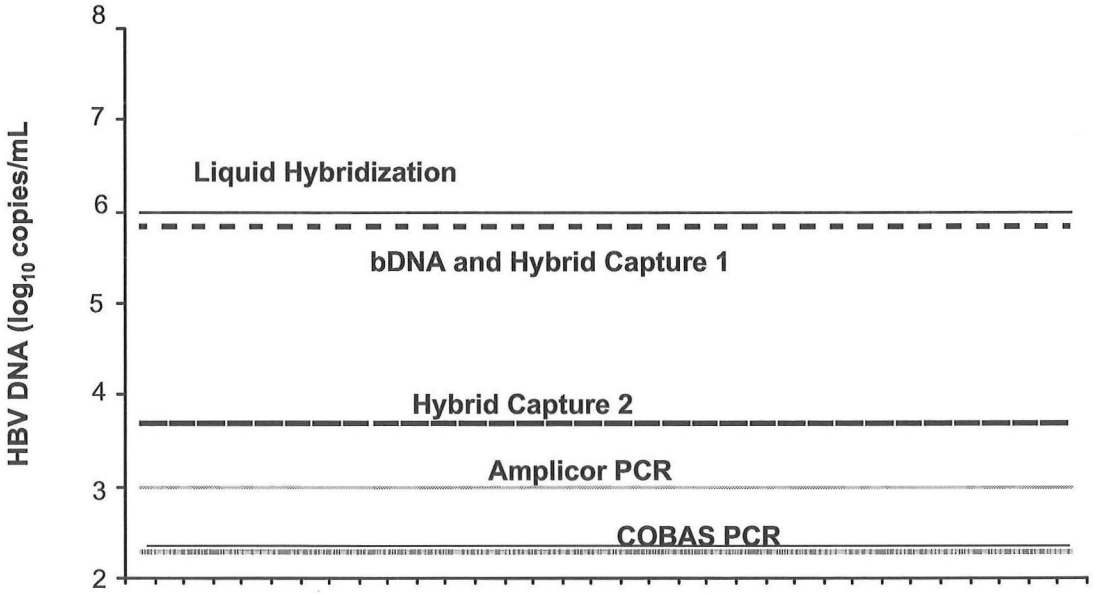


Figure 3. Sensitivity of various current HBV DNA assays.

Current HBV DNA testing, using the more sensitive polymerase chain reaction (PCR) assays, allows accurate quantification of viral particles over a wider dynamic range than previously possible (10^9 to 10^2 copies/mL). The current high prevalence of HBeAg-negative patients with active replication (>10^5 copies/mL) makes HBeAg testing less helpful. At the same time, the more sensitive HBV DNA assays now available render the previous threshold of quantitation, which coincided well with HBeAg seroconversion, no longer applicable.[44] However, the significance of small quantities of viral DNA persisting in serum is unclear, since HBeAg negativity without mutations is associated with improved outcomes, despite low but repeatedly positive DNA titers.[13,43]

Up to 20 percent of patients who develop HBeAg seroconversion may subsequently reactivate, becoming HBeAg positive again.[45] Thus, regular follow-up with quantitative measurement of HBV DNA as well as aminotransferase levels is required after seroconversion to ensure its durability. In HBeAg-negative individuals, the presence of $>10^5$ copies/mL indicates a mutation.[43] Similarly, lamivudine resistance must be considered if low HBV DNA levels revert to previous values despite continued treatment. Specific tests to confirm the presence of HBeAg and lamivudine resistant mutations are commercially available.[44,46]

	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	--
ALT	--	+++	--	+++	--	+++	--
Key	tolerant	active	on lam or adv	lamiv mutant	e neg	e neg mutant	immune

Table 2. Four stages of HBV infection (Updated 2003)

Diagnosis and Treatment

Treatment for HBV infection is targeted at viral replication. While therapy may not eradicate the virus, a decrease in viral burden decreases hepatic inflammation. In addition, 10-20 percent each year lose HBeAg from serum and 1-5 percent have complete resolution of infection with clearance of HBsAg. In evaluating every HBsAg positive patient, it is necessary to determine the presence of HBeAg, the level of HBV DNA in serum and measure aminotransferase levels. Ultrasonography will help identify cirrhosis or liver mass lesions such as hepatocellular carcinoma. A simple algorithm for treatment is shown in Figure 4. Liver biopsy is usually required to determine whether treatment is

indicated.[47] Bridging fibrosis or cirrhosis on biopsy indicates past and potentially future active disease mandating treatment.

The goal of therapy is to improve liver inflammation and, if possible, to decrease hepatic fibrosis. Suppression of DNA replication follows virtually all treatments but serum DNA levels return to baseline with cessation of treatment unless there is loss of HBeAg, with or without seroconversion to detectable anti-HBe antibodies.[45] Early discontinuation of treatment following seroconversion leads to relapse, although the optimal treatment interval following sero-conversion is unclear.[48] Three drugs have been approved for the treatment of chronic hepatitis B infection: interferon-alfa, lamivudine and adefovir dipivoxil.

Interferon α-2b

Interferon alfa 2b, approved by the Food and Drug Administration (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 HBV DNA and HBeAg in 37 percent and 33 percent respectively compared to 17 percent and 12 percent in the placebo group after 12-24 weeks of therapy.[49] Interferon is effective only in a selected group of patients: those with low pretreatment HBV DNA (<200 pg/ml), high levels of serum aminotransferases (>100 IU/L), and hepatic necroinflammation. Other factors that increase likelihood of response include absence of immunosuppression, female sex, HBeAg-positive, short duration of illness, horizontal acquisition of virus and a history of acute icteric hepatitis.[50]

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. However, low doses of interferon have been used with some success in patients with cirrhotic decompensation.[51] Interferon therapy is infrequently used at present because of the ease of administration and infrequent side effects of oral agents. The availability of pegylated interferons should lead to further testing of these agents in combination with nucleoside analogues.[52]

Nucleos(t)ide analogues

Nucleos(t)ide analogues decrease HBV replication, but have no immunomodulatory role. HBV persists in the nucleus in the form of covalently closed

circular (ccc) DNA. Clearance of cccDNA results only from the clearance of infected hepatocytes.[53,54] Most nucleos(t)ide analogues lower DNA levels in plasma by approximately $3x\log_{10}$, accompanied by improved inflammation although this is not universal. Replication is not eradicated and thus drugs have to be given long-term,[55] and do not necessarily result in viral eradication even after long periods of treatment.

Lamivudine

Lamivudine, a (-) enantiomer of 2'-deoxy-3'-thiacytidine (3TC), is a nucleoside analogue effective in both HBeAg-positive and HBeAg-negative patients as well as interferon failures. After one year of treatment of HBeAg-positive patients, lamivudine induces HBeAg seroconversion in 16-17 percent compared to 4-6 percent in those on placebo.[56,57] Seroconversion is permanent in most patients, associated with improvement in fibrosis, so that lamivudine may be withdrawn with low risk of relapse.[48,58] In patients with HBeAg-negative chronic hepatitis B, complete response (loss of detectable HBV DNA using older assays, plus normalization of alanine aminotransaminase) was seen in 63 percent of patients on lamivudine therapy compared to 6 percent in those on placebo at 24 weeks of treatment.[59] It is effective in patients with cirrhosis, including those with hepatic decompensation,[60] as well as in preventing recurrent hepatitis B virus after liver transplant.[61,62] Lamivudine is an oral drug with an excellent safety profile. Its major drawback is the development of resistance which occurs at approximately 14 percent at one year and 67 percent at 4 years.[56,63,64]

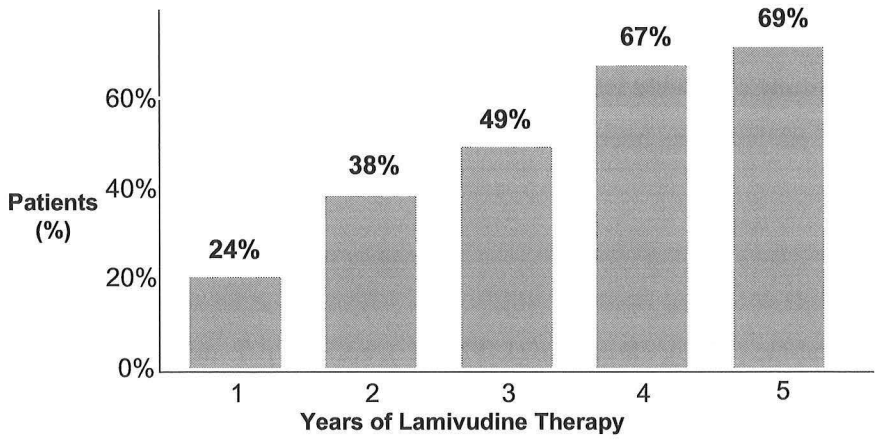


Figure 5. Progression of lamivudine resistance

Development of a YMDD mutation leads to rebound in HBV DNA, followed by an increase of ALT and deterioration in inflammation on biopsy.[58,65] That HBV DNA and ALT remain lower than pre-treatment values, at least initially, suggests less robust replication by the mutant virus.[17,18] Should lamivudine be withdrawn, wild type virus reappears; if the drug is continued disease progression occurs because of the presence of the mutant virus.[58] Seroconversion has been demonstrated in some patients with lamivudine-resistant mutation.[64,65] Long-term benefit is seen where viral suppression occurs.[58,59]

Adefovir dipivoxil

This nucleotide analog of adenosine is a diester pro-drug of adefovir monophosphate, approved for chronic hepatitis B virus infection by Food and Drug Administration in 2002. In patients with HBeAg-positive chronic HBV, adefovir at 10 mg or 30 mg daily induced HBeAg seroconversion in 12 percent and 14 percent respectively compared to 6 percent in those on placebo at 48 weeks of treatment.[66] There were significant decreases in inflammation and loss of HBV DNA in the adefovir group with improvement of alanine aminotransferase levels. In HBeAg-negative patients taking 10 mg adefovir daily, ALT levels became normal at 48 weeks in 72 percent compared to 29 percent on placebo; HBV DNA became undetectable by PCR assay in 51 percent compared to 0 percent on placebo. There was significant histologic improvement in both treated groups.[66,67] Adefovir is effective against both wild-type and lamivudine-resistant virus.[68,69] Adefovir is excreted largely unchanged by the kidneys and appears to be safe, although nephrotoxicity occurs at higher doses. Dose adjustments are necessary in patients with creatinine clearance less than 50mL/min and in those on hemodialysis.[70] Resistance against adefovir has been reported but is less frequent than that observed with lamivudine.[71]

Newer Agents

Several drugs are being tested to improve treatment responses, including entecavir, emtricitabine (FTC), clevudine (L-FMAU), and the β-L-nucleosides (L-dA, L-dT, val-LdC). In a phase II clinical trial, entecavir was found to be superior to lamivudine in viral load reduction and normalization of alanine aminotransferase after 22 weeks of treatment.[72] In a phase II trial, emtricitabine was shown to have significant effect on loss

of HBeAg in HBeAg-positive patients and loss of HBV DNA and normalization of ALT in HBeAg-negative patients at 48 weeks. However, resistance including cross-resistance with lamivudine may occur due to the structural similarity between the two drugs.[73] Clevudine, in phase I/II trial caused a >2 log drop after 28 days of treatment and this was sustained for at least 6 months without further treatment.[74] The β-L-nucleosides have a profound effect on HBV replication, with a dose-dependent reduction of 4.0 log10 in DNA at 4 weeks. The safety profile is similar to placebo.[75] As has been the case with HIV, the use of combinations of drugs acting at different levels in the HBV DNA replication process may produce greater suppression but no synergistic combination has yet been defined.

Special Conditions:

HBV and HIV co-infection

Co-infection of hepatitis B virus and HIV is common due to shared modes of transmission.[76,77] In the presence of HIV infection, HBV is more likely to persist with rapid evolution of liver damage and progression to cirrhosis.[78] Co-infected individuals have higher risk of liver-related deaths especially when the CD4+ count is low.[79] The introduction of highly active antiretroviral therapy (HAART) has also been associated with poorer liver outcomes for dual infections.[80] Lamivudine-based HAART combinations may lead to improvement in liver disease in patients with co-infection since lamivudine is effective against both viruses; however, resistance to one or both viruses may develop.[80,81] By contrast, adefovir dipivoxil, is effective against lamivudine escape mutants , but has little effect on HIV replication in doses used for HBV.[82] A related nucleotide analog, tenofovir, recently approved for HIV, is active against HBV including lamivudine-resistant strains but has not been extensively studied.[83]

HBV and HDV

Hepatitis delta virus (HDV) is a defective RNA virus that requires HBsAg for its assembly and replication.[84] HDV is the smallest animal virus and the only RNA virus with a circular genome, otherwise observed only in plant viruses. It can be acquired together with HBV (co-infection) or as a super-infection in a patient with established HBV. As a co-infection, delta is associated with more severe and sometimes fulminant liver injury, but also with more likelihood of viral clearance. Only rarely does the co-

infected patient develop chronic delta infection. On the other hand, super-infection tends to become chronic and in most cases progression to cirrhosis occurs more rapidly than with HBV alone.[85]

There is no satisfactory therapy for HDV infection. Interferon-alpha at 9MU TIW given for a year induced a biochemical response (normalization of ALT in 71 percent at end of treatment and in 50 percent of patients after 6 months of follow-up) associated with histological improvement despite no loss of HDV RNA.[85] End-stage liver disease caused by HDV can be managed by orthotopic liver transplant.[86]

HBV and HCV Treatment

Hepatitis B and C viruses share modes of transmission and occur together, especially in intravenous drug users.[87,88] In dual infection, disease progression may be more severe than with either agent,[89] and a higher dose of alpha-interferon may be required to treat the co-infection.[90]

HBV and Hepatocellular Carcinoma

Among other etiological factors, chronic hepatitis B virus appears responsible for the largest number of hepatocellular carcinoma cases worldwide.[91] Although the oncogenic mechanism is not fully understood, the X gene of Hepatitis B cause trans-activation of many cellular genes associated with cell proliferation.[92] Hepatocellular carcinoma typically but not invariably occurs in the setting of cirrhosis, since 30 percent to 50 percent of HBV-associated hepatocellular carcinoma occurs in the absence of cirrhosis.[45] Resolution of chronic hepatitis B significantly diminishes the risk of developing hepatocellular carcinoma, and resolution of active replication (HBeAg-positive state) also greatly diminishes the likelihood of HCC.[93]

Family history of HCC, male gender, age >45 years, cirrhosis and co-infection with hepatitis C virus are risk factors for development of hepatocellular carcinoma.[45,94] Periodic screening of chronic HBV infected patients using alpha-fetoprotein and ultrasound scanning every 6 months may be used to detect hepatocellular carcinoma in patients with chronic HBV infection,[45] but has not been shown to be cost-effective. Vaccination against hepatitis B virus has led to decreased viral carriage and a lower incidence of hepatocellular carcinoma in high density regions, and greater future gains are expected.[95]

HBV and liver transplantation

Liver transplantation is effective therapy for patients with HBV-induced end-stage liver disease, but has been associated with a very high re-infection rate leading to graft loss and poor survival.[96] HBV recurrence after liver transplant has been significantly reduced using a combination of hepatitis B immunoglobulin (HBIG) and lamivudine.[97,98] Adefovir dipivoxil is safe and effective in lamivudine-resistant HBV post-transplantation.[99]

HBV and cancer chemotherapy

Patients with active or inactive chronic hepatitis B virus infection on cancer chemotherapy or after bone marrow transplantation may undergo reactivation of their disease.[37,100,101]This often leads to severe hepatitis during and/or after the chemotherapy; corticosteroid-containing regimens have been particularly implicated. It is important to screen for HBV infection in all patients prior to cancer chemotherapy or other immunosuppressive therapy. Lamivudine appears effective either as prophylaxis or in treatment of HBV reactivation in these patients.[37,102,103]

Acute liver failure

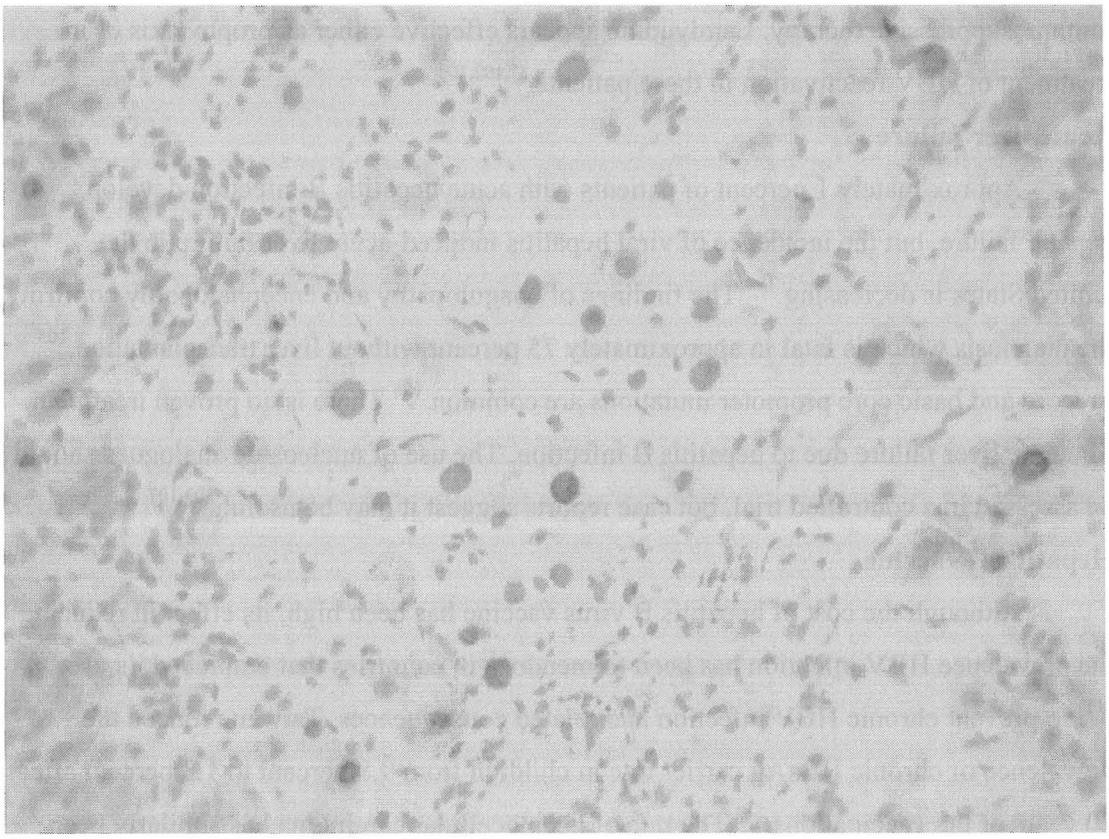
Approximately 1 percent of patients with acute hepatitis B infection develop hepatic failure, but the incidence of viral hepatitis induced-acute liver failure in the United States is decreasing.[104] The findings of coagulopathy and encephalopathy confirm the diagnosis which is fatal in approximately 75 percent without liver transplantation.[105] Precore and basic core promoter mutations are common.[106] There is no proven treatment for acute liver failure due to hepatitis B infection. The use of nucleoside analogues should be assessed in a controlled trial, but case reports suggest it may be useful.[103,107]

Hepatitis B vaccine

Although the cost of hepatitis B virus vaccine has been high, its effect in reducing the prevalence HBV infection has been tremendous in countries that embraced its use.[108-111] To prevent chronic HBV infection and related consequences, Taiwan reduced the prevalence of chronic HBsAg carrier rate in children from 9.8 percent to 1.3 percent after 10 years of the vaccinations.[112] The rate of hepatocellular carcinoma has similarly been reduced in these children.[95]

The Future

Given the huge burden of HBV infection worldwide, and the number of advances made in the past several decades, it is surprising that more progress in limiting the spread of infection has not yet been realized. The number of acute hepatitis B cases has dropped by 76 percent in the United States between 1987 and 1998.[7] However, hepatitis B continues to be spread in endemic areas where universal vaccination has not yet reached. The availability of vaccination, the use of HBIG to prevent neonatal transmission, and the availability of suppressive therapies is likely to result in greater gains toward the limitation and eradication of hepatitis B in another generation.



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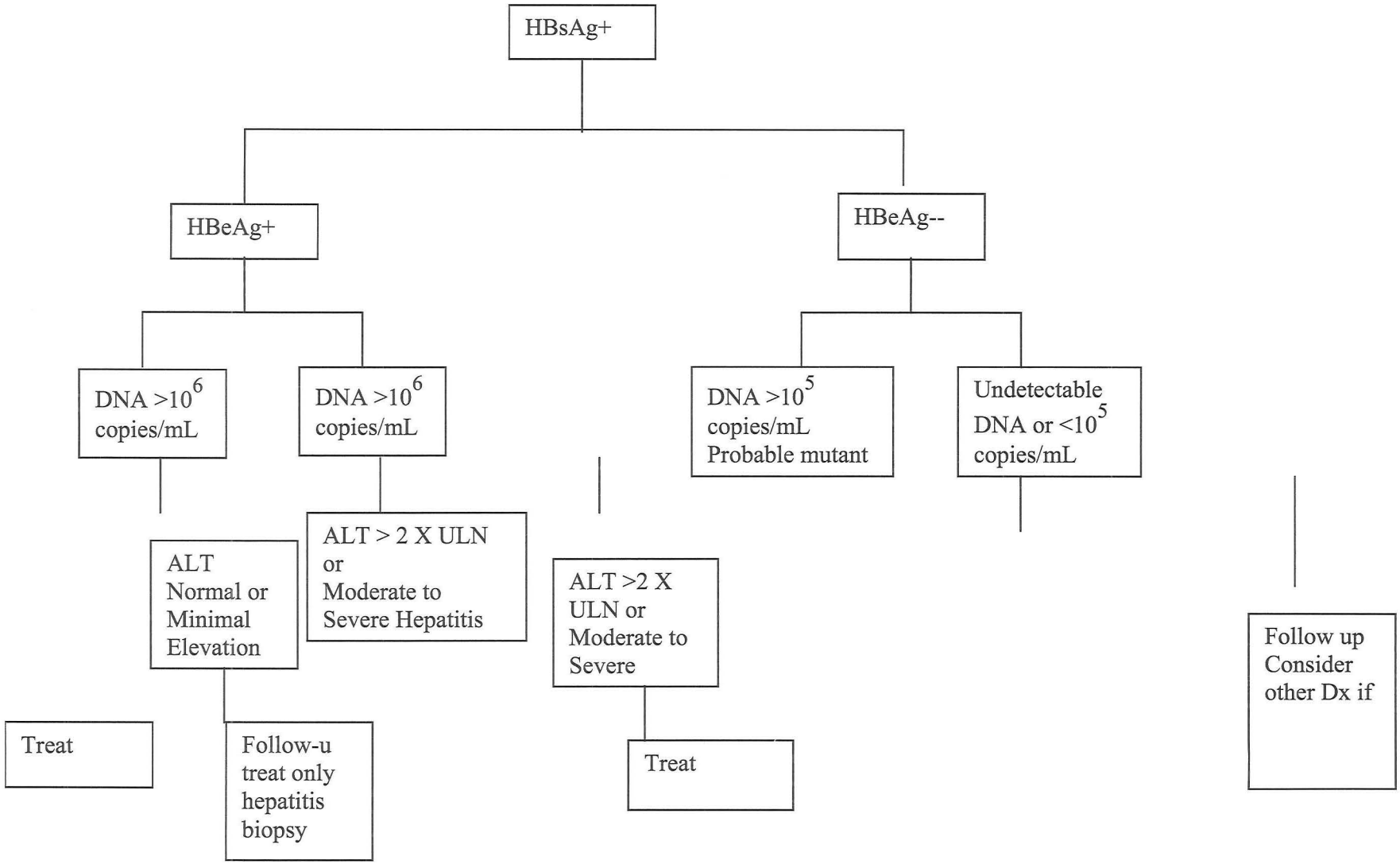

Figure 4. An approach to the treatment of most patients with chronic hepatitis B.