Natural History of Chronic Hepatitis B Virus Infection: What We Knew in 1981 and What We Know in 2005

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Remarkable progress has been made in our understanding of the natural history of chronic hepatitis B virus (HBV) infection in the past 25 years. Availability of sensitive HBV DNA assays and application of sophisticated immunological techniques led to the recognition that HBV replication persists throughout the course of chronic HBV infection, and host immune response plays a pivotal role in HBV-related liver disease. Knowledge of the HBV genome organization and replication cycle led to the unraveling of HBV genotypes and molecular variants, which contribute to the heterogeneity in outcome of chronic HBV infection. The natural course of chronic HBV infection is now perceived as consisting of 4 phases: immune tolerance, immune clearance [hepatitis B e antigen (HBeAg)–positive chronic hepatitis], inactive carrier state, and reactivation (HBeAg-negative chronic hepatitis B). Understanding the dynamic nature of chronic HBV infection is crucial in the management of HBV carriers and underscores the need for long-term monitoring. Accumulating evidence indicates that antiviral therapy can prevent progression of HBV-related liver disease, particularly among patients with sustained response. Newer antiviral therapies with improved efficacy and decreased risk of resistance may lead to a complete revision of the chapter on the natural history of chronic HBV infection on the occasion of the golden jubilee of HEPATOLOGY. (HEPATOLOGY 2006;43:S173-S181.)

The first issue of HEPATOLOGY appeared around the same time the corresponding author decided to pursue hepatology training. Back then, hepatitis B was a major focus in hepatology research. Hepatitis B vaccine and treatment were still experimental; hepatitis C, nonalcoholic fatty liver disease, and liver transplantation were not part of the hepatology vocabulary. The availability of molecular virology tools fueled rapid growth in hepatitis B research. In this review article, I invited a fellow to accompany me in recounting what I knew about the natural history of chronic hepatitis B at the beginning of my fellowship and what we know at the beginning of his fellowship.

Natural History of Chronic Hepatitis B Virus Infection: What We Knew in 1981

Progression From Acute to Chronic Infection. Epidemiological and clinical studies estimated that approximately 10% of patients with acute hepatitis B virus (HBV) infection progress to chronic infection.[1] Recovery from acute HBV infection with hepatitis B surface antigen (HBsAg) to antibody (anti-HBs) seroconversion was thought to indicate virus clearance.

Markers of HBV Replication and Significance of Hepatitis B e Antigen. In 1981, evaluation of hepatitis B patients relied on serology assays.[2,3] It was recognized that infectivity of HBV carriers varied and hepatitis B e antigen (HBeAg) was a reliable marker of infectivity.[3] HBV was known to be a DNA virus, and many investigators were developing assays to measure HBV DNA polymerase activity and later HBV DNA level.[4-6] These assays relied on hybridization techniques with a lower limit of detection of approximately 10^6 copies/mL, and most assays were semi-quantitative.[5,6]

HBeAg was observed to be present during the initial phase of chronic HBV infection, and its presence correlated with the detection of HBV DNA polymerase activity and
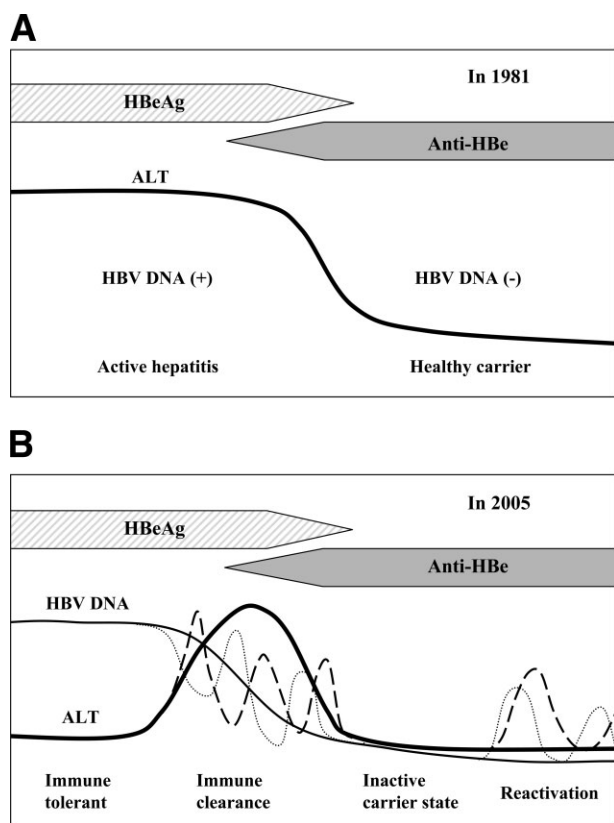
A

B

Fig. 1. Phases of chronic HBV infection known in 1981 (A) and in 2005 (B). In 1981, it was thought that chronic HBV infection consists of 2 phases: replicative and non-replicative. HBV no longer replicates and ALT normalizes after HBeAg seroconversion. It is now known that HBV replication persists throughout the course of chronic HBV infection, and exacerbations of ALT and fluctuations in serum HBV DNA level (dot line) may occur even after HBeAg seroconversion. The course of chronic HBV infection is now considered to consist of 4 phases: immune tolerance, immune clearance (HBeAg-positive chronic hepatitis), inactive carrier, and reactivation (HBeAg-negative chronic hepatitis) although not all patients go through every phase. HBeAg, hepatitis B e antigen; anti-HBe, antibody to HBeAg; HBV, hepatitis B virus; ALT, alanine aminotransferase.

HBV DNA in serum.[6,7] In some patients, spontaneous HBeAg to HBe antibody (anti-HBe) seroconversion occurred during the course of chronic HBV infection.[8,9] This event was usually accompanied by disappearance of HBV DNA in serum and normalization of aminotransferases.[8,9] A few astute investigators noticed that HBV DNA could be detected in some HBeAg-negative patients, but the significance of this finding was not clear.[5,6]

Spontaneous HBsAg clearance with or without anti-HBs seroconversion also was observed in rare cases.[10]

Phases of Chronic HBV Infection. The course of chronic HBV infection was thought to consist of two phases: an initial phase characterized by presence of HBeAg, detection of serum HBV DNA, and active liver disease, and a later phase characterized by absence of HBeAg, undetectable serum HBV DNA, and inactive liver disease (Fig. 1A).[8,9] HBV, it was thought, no longer

replicates after HBeAg seroconversion, so patients in the latter phase were referred to as "healthy" carriers with nonreplicative infection.

Chronic HBV Infection and Cirrhosis. Epidemiological studies found that chronic HBV infection was a common cause of cirrhosis and hepatocellular carcinoma (HCC), particularly in endemic countries.[11,12] Factors associated with increased risk of cirrhosis included older age, male sex, and superinfection with a newly discovered virus—delta virus (now known as hepatitis D virus, HDV).[13]

Chronic HBV Infection and Hepatocellular Carcinoma. HBV has been suspected as a cause of HCC based on epidemiologic and molecular virological studies.[12,14-18]

In a landmark paper published in 1981, Beasley et al. provided the best evidence for an etiological relationship between chronic HBV infection and HCC. They followed 22,707 Taiwanese men (3,454 HBsAg positive and 19,253 HBsAg negative) for a mean of 3.3 years; the relative risk of HCC in HBsAg-positive men was found to be 223:1.[15] Risk factors for HCC included older age, male sex, and presence of cirrhosis.[11,16] Exposure to aflatoxin was also suggested to be a risk factor.[19]

The role of HBV infection in HCC development was further strengthened by the demonstration of integration of HBV DNA into the cellular genome in HCC tissue.[17,18] Despite the use of relatively insensitive techniques such as Southern blot hybridization, several investigators also found integrated HBV DNA in HCC tissues from HBsAg-negative patients suggesting a bigger role of HBV in HCC development.[18]

Natural History of Chronic HBV Infection: What We Know in 2005

Availability of sensitive HBV DNA assays, knowledge of the HBV genome organization and replication cycle, and understanding of the host immune response to HBV infection have changed our concept of the natural history of chronic HBV infection.[20-22]

Using currently available HBV DNA assays, which can detect <50 IU/mL (<250 copies/mL), we now recognize that most patients who were previously considered to have nonreplicative infection have detectable serum HBV DNA. Knowledge that HBV replicates via reverse transcription of pregenomic RNA led to the recognition of a high mutation rate during HBV replication and existence of HBV genotypes and molecular variants.[20,23,24] Application of sophisticated immunological techniques demonstrated that HBV-related liver disease is immune-mediated, and patients with chronic HBV infection have impaired immune response to HBV.[25,26]

Progression From Acute to Chronic Infection. In the 1980s, exacerbations of hepatitis were recognized as

being common during the course of chronic HBV infection.[27-29] Careful analyses of patients presenting with "acute hepatitis B" found that the risk of progression to chronic HBV infection among immunocompetent adults was <1% after exclusion of patients with acute exacerbations of chronic HBV infection.[30] The overall risk of chronicity was 5% to 10%, being higher in those infected perinatally (90%) or during childhood (20%).[31,32]

Using sensitive assays, HBV DNA can be detected in liver as well as serum up to 10 years after "recovery" from an acute HBV infection.[33,34] It is now recognized that HBV persists, albeit at low levels being held in check by the host immune response.[34] This accounts for reports of chemotherapy-induced reactivation of HBV replication in persons with serological markers of recovered HBV infection.[35,36] Several investigators have reported evidence of chronic hepatitis and fibrosis on liver biopsy specimens in patients who have no recognized cause of liver disease other than prior acute hepatitis B.[37] Because these patients were not serially monitored and the number of patients studied is small, additional studies are needed to determine the clinical consequences of a self-limiting acute hepatitis B.

Markers of HBV Replication, Significance of HBeAg, Precore and Core Promoter Variants. In the late 1980s, it became clear that the presence and absence of HBeAg is not an indicator of replicative and nonreplicative infection, but rather high and low levels of HBV replication. Most HBeAg-negative patients, including those with normal aminotransferases, have detectable HBV DNA in serum, although the levels are usually lower than in HBeAg-positive patients and can at times fall below the limit of detection. Some HBeAg-negative patients have HBV DNA levels up to 8 to 9 log10 IU/mL, persistent or intermittent elevations in aminotransferases, and continued hepatic necroinflammation. In 1989, Brunetto et al.[38] and Carman et al.[39] simultaneously demystified the incongruous finding of high serum HBV DNA levels in HBeAg-negative patients. They found a single nucleotide change in the precore region ($G_{1896}A$), which leads to a premature stop codon that abolishes HBeAg production. Shortly after, a core promoter variant with 2 nucleotide changes ($A_{1762}T$ and $G_{1764}A$) that down-regulates precore/core mRNA and HBeAg production was described.[40] Understanding the molecular basis by which HBV replication can occur in patients who are HBeAg negative is a major milestone in hepatitis B research.

Although testing for HBeAg is still important in determining the phase of chronic HBV infection, assessment of HBV replication in 2005 must rely on quantification of serum HBV DNA using sensitive assays that have detection limit <50 IU/mL.

Phases of Chronic HBV Infection. Our understanding of the natural course of chronic HBV infection in 2005 has been radically changed as a result of the recognition that HBV replication persists throughout the course of chronic HBV infection, host immune response plays a pivotal role in HBV-related liver injury, and the balance between host immune response and HBV replication is dynamic.

The natural course of chronic HBV infection evolved from 2 to 3 phases in the mid 1980s with the recognition of an immune tolerance phase,[41] and to 4 phases in the early 1990s with the understanding and acceptance of "HBeAg-negative chronic hepatitis" (Fig. 1B), although not all patients go through every phase. In patients with perinatally acquired HBV infection, the first phase (immune tolerance) is characterized by the presence of HBeAg, high levels of serum HBV DNA, normal serum aminotransferases, and minimal or no inflammation on liver biopsy. During this phase, which may last 1 to 4 decades, spontaneous and treatment-induced HBeAg seroconversion is infrequent (<5% per year). A study from Taiwan followed 240 patients (54% men, mean age 27.6 years) who presented in this phase and found that only 5% progressed to cirrhosis and none to HCC during a follow-up period of 10.5 years.[42] These findings indicate that prognosis is generally favorable for patients who are in the immune tolerant phase. In patients with childhood- or adult-acquired HBV infection, the "immune tolerant" phase is short-lived or absent.

The second phase (immune clearance/HBeAg-positive chronic hepatitis) is characterized by the presence of HBeAg, high or fluctuating serum HBV DNA levels, persistent or intermittent elevation in serum aminotransferases, and active inflammation on liver biopsy. A hallmark of this phase is flares of aminotransferases, which are believed to be manifestations of immune-mediated lysis of infected hepatocytes secondary to increased T cell responses to hepatitis B core antigen (HBcAg) and HBeAg.[43,44] In the early 1980s, Liaw et al.[45,46] demonstrated that these flares may precede HBeAg seroconversion, but many flares only result in transient decreases in serum HBV DNA levels without loss of HBeAg, and some flares may lead to hepatic decompensation.[45,46] It is now recognized that the duration of the "immune clearance" phase, and the frequency and severity of the flares, correlate with the risk of cirrhosis and HCC.[47,48] Recurrent flares occur more commonly in men and may explain why HBV-related cirrhosis and HCC are more common in men than in women.[29]

An important outcome of the "immune clearance" phase is HBeAg to anti-HBe seroconversion. Factors associated with higher rates of spontaneous HBeAg sero-

**Table 1. Factors Asociated With Higher Rates of
Spontaneous HBeAg Seroconversion**

Older age*
Higher ALT levels at presentation*
Acute exacerbations*
HBV genotype (B > C)
Ethnicity (other than Asian)

*Supported by strong evidence

conversion include older age,[47] higher aminotransferase levels,[49,50] and more recently HBV genotypes (Table 1).[51,52] High aminotransferase level is believed to be a surrogate marker for vigorous host immune response, accounting for its strong correlation with spontaneous as well as treatment-related HBeAg seroconversion. Studies from Asian countries, where genotypes B and C predominate, showed that genotype B is associated with a lower prevalence of HBeAg, HBeAg seroconversion at an earlier age, and more sustained virological and biochemical remission after HBeAg seroconversion.[51,52]

The third phase (inactive HBsAg carrier state) is characterized by absence of HBeAg, presence of anti-HBe, persistently normal aminotransferase levels, and low or undetectable serum HBV DNA ($<10^5$ IU/mL). Liver biopsy usually shows mild hepatitis and minimal fibrosis, but inactive cirrhosis may be observed in patients who had accrued severe liver injury during the preceding "immune clearance" phase. The inactive HBsAg carrier state may persist indefinitely, in which case the prognosis is generally favorable, especially if this state is reached early. This is supported by a long-term follow-up study of HBsAg-positive healthy blood donors in northern Italy.[53] No difference in survival was found between 296 HBsAg-positive blood donors and 157 uninfected controls over a 30-year period, and no episodes of hepatic decompensation were reported.

Unfortunately, some inactive carriers have reactivation of HBV replication. Reactivation may occur spontaneously or as a result of immunosuppression.[36,54] In one study of 283 Taiwanese patients followed for a median of 8.6 years after spontaneous HBeAg seroconversion, 67% had sustained remission, 4% had HBeAg reversion, and 24% had HBeAg-negative chronic hepatitis B. Cirrhosis developed in 8% and HCC in 2%, the risk being higher in those who had active hepatitis after HBeAg seroconversion.[55]

The fourth phase (reactivation of HBV replication/ HBeAg-negative chronic hepatitis B) is characterized by negative HBeAg, positive anti-HBe, detectable HBV DNA, elevated aminotransferases, and continued necro-inflammation.[56] Whereas most patients reach this phase after a variable duration of inactive carrier state, some

progress directly from HBeAg-positive chronic hepatitis to HBeAg-negative chronic hepatitis.[55] Patients in this phase are usually older and have more advanced liver disease because this represents a later phase in the course of chronic HBV infection. Serum HBV DNA levels are lower than in HBeAg-positive patients but may reach 10^{8-9} IU/mL. The hallmark of this phase is its fluctuating course. In a study of 164 anti-HBe–positive patients who were monitored at monthly intervals for a median period of 21 months, 64% had fluctuating alanine aminotransferase (ALT) levels, including 44% whose ALT levels were intermittently normal.[57] Several investigators have attempted to define cutoff HBV DNA levels that would differentiate patients with HBeAg-negative chronic hepatitis from inactive carriers, but in view of the fluctuating course, serial testing is more reliable than a single test.[58]

HBeAg-negative chronic hepatitis B was originally reported in Mediterranean countries.[38,39] It has now been reported in all parts of the world.[59] The geographic variations in prevalence of HBeAg-negative chronic hepatitis B are related to the predominant HBV genotype in that region. Recent studies in Europe, Asia, and the United States have all reported an increased prevalence of HBeAg-negative and a decreased prevalence of HBeAg-positive chronic hepatitis[59,60]; this may be related to increased awareness, decrease in new HBV infections, and aging of existing carriers. Regardless, this shift has an important impact on treatment strategies.

Spontaneous HBsAg seroclearance has been reported to occur at the rate of 0.5% to 1% per year in patients with chronic HBV infection.[47,53] HBsAg seroclearance is generally accompanied by undetectable serum HBV DNA, normalization of liver biochemistries, and improved liver histology.[61] However, HCC has been reported in a small percent of patients, the risk being higher in those with cirrhosis, HCV coinfection, or older age at the time of HBsAg seroclearance.[61,62]

Chronic HBV Infection and Cirrhosis. The annual incidence of cirrhosis has been estimated to be 2% to 6% for HBeAg-positive and 8% to 10% for HBeAg-negative patients (Fig. 2).[48,63-65] The higher rate of cirrhosis among HBeAg-negative patients is related to older age and more advanced liver disease at presentation.[64,65] Among HBeAg-positive patients, the rate of cirrhosis development is higher in those who remained HBeAg positive during follow-up.[48] In the last 25 years, additional factors have been identified to be associated with progression to cirrhosis: habitual alcohol intake,[66] concurrent infection with hepatitis C virus (HCV) or human immunodeficiency virus (HIV),[67,68] high levels of HBV replication, and HBV genotype (C>B) (Table 2).[51,69-72] In a recent study from Taiwan, the 10-year cumulative probability of
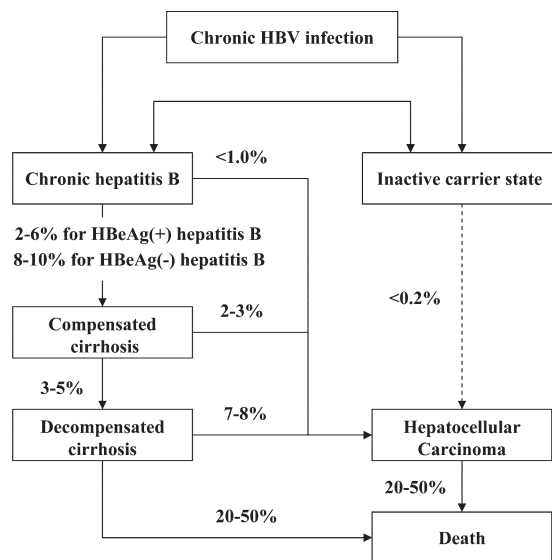
Fig. 2.   Estimated annual rates (%) of progression through different clinical states during the course of chronic HBV infection. Adapted from Fattovich et al.[78]

cirrhosis among chronic hepatitis B patients with HCV superinfection, HDV superinfection, and no superinfection was 48%, 21%, and 9% respectively.[67] Co-infection of HIV and HBV has also been shown to increase the risk of cirrhosis and liver-related mortality compared with HBV monoinfection.[68] Several studies showed that patients who had HBeAg reversion had increased risk of cirrhosis compared with those who had sustained HBeAg seroconversion.[47,54,55] In one study of 3774 HBsAg carriers aged 30 to 65 years, the adjusted relative risk of cirrhosis for patients with baseline serum HBV DNA $>10^4$ and $>10^6$ copies/mL was 2.3 (95% CI, 1.6-3.5) and 9.3 (95% CI, 6.5-13.1), respectively.[69] Collectively, these data suggest that persistent high levels of HBV replication (with accompanying hepatitis) increase the risk of cirrhosis, but the prognostic significance of a high serum HBV DNA level at a single time point in a young carrier (<30 years old) is unclear. As discussed earlier, studies in Asia showed that genotype C is associated with HBeAg seroconversion at a later age and more active hepatitis than genotype B; it is therefore not surprising that these studies also found that genotype C is associated with a more rapid rate of progression to cirrhosis than genotype B.[51,52,70,71]

In patients who have progressed to cirrhosis, persistent high levels of HBV replication as indicated by presence of HBeAg and serum HBV DNA detectable by hybridization assays have also been found to be associated with increased risk of hepatic decompensation and mortality.[73,74]

Chronic HBV Infection and HCC.   More than 2 decades after Beasley's landmark paper, HBV was offi-

cially recognized as a carcinogen. The annual incidence of HCC has been estimated to be <1% for non-cirrhotic carriers and 2% to 3% for patients with cirrhosis (Fig. 2).[47,55,74-78] Additional risk factors for HCC identified in the last 25 years include co-infection with HCV,[77] a family history of HCC,[79] habitual alcohol intake,[66,80] high levels of HBV replication,[81-83] HBV genotype (C$>$B),[75,82] and core promoter mutations (Table 3).[84,85] Recent studies found that obesity, diabetes, and smoking also may contribute to the risk of HCC.[86]

Several lines of evidence support an association between HBV replication and the risk of HCC.[81-83] In a prospective study of 11,893 Taiwanese men aged 30 to 65 years, followed for a mean of 8.5 years, the adjusted relative risk of HCC was 6- to 7-fold higher among HBsAg men who were HBeAg positive at entry than those who were HBsAg positive, HBeAg negative.[81] Another study from Taiwan found that the risk of HCC increased with increasing baseline serum HBV DNA level. The adjusted odds ratio for patients with the highest quintile of HBV DNA level versus those with the lowest was 7.26 (95% CI, 3.54-14.89).[82] Studies in Senegal and mainland China also confirmed an increased risk of HCC among carriers with high baseline serum HBV DNA levels.[83] Unfortunately, none of these studies monitored serum HBV DNA and aminotransferase levels over time. The duration of high levels of HBV replication as well as the intensity and frequency of hepatitis activity may be more important than a high HBV DNA level on a random occasion in predicting the risk of HCC in individual carriers.

Several studies from Asia demonstrated that genotype C is associated with increased risk of HCC compared with genotype B.[70,82] This may be related to a longer duration of high levels of HBV replication and active hepatitis and a higher frequency of core promoter mutations. Core promoter mutations have been shown in many studies to be associated with increased risk of HCC and to precede HCC diagnosis.[84,85] Core promoter mutations also have been found to be associated with more active hepatitis and

Table 2. Factors Associated With Increased Risks of Progression to Cirrhosis

Host Factors	Virus Factors	Environmental Factors
Older age* (longer duration)	High levels of HBV replication*	Concurrent infection (HCV*, HDV, HIV)
Male*	Genotype (C $>$ B)*	Alcohol consumption*
Immune status	HBV variant (core promoter)	Diabetes mellitus†
		Obesity†

*Supported by strong evidence.
†Further studies needed.

Table 3. Factors Associated With Increased Risks of HCC Development

Host Factors	Virus Factors	Environmental Factors
Older age (longer duration)*	High levels of HBV replication*	Concurrent infection (HCV*, HDV)
Male*	Genotype (C>B)	Alcohol consumption*
Presence of cirrhosis*	HBV variant (core promoter)	Aflatoxin
		Smoking†
Family history of HCC*	X gene transactivation	Diabetes mellitus†
Race (Asian, African)		Obesity†

*Supported by strong evidence.
†Further studies needed.

are more frequently associated with genotype C than B.[60,85] In addition, the most common core promoter mutations ($A_{1762}T$, $G_{1764}A$) result in corresponding changes in the overlapping X gene.[87] The HBx protein is a potent transactivator and may activate host genes including oncogenes.[88]

Interventions to Modify the Natural History of Chronic HBV Infection. Accumulating evidence in the past 25 years showed that the risk of cirrhosis and HCC are higher in HBV carriers who undergo HBeAg seroconversion later in life, have persistent high levels of HBV replication, and long durations of active hepatitis. Thus, treatment that is effective in inducing sustained suppression of HBV replication may reduce the risk of cirrhosis and HCC.

Long-term follow-up studies found that interferon therapy has a minimal effect on reducing the risk of cirrhosis, HCC, and liver-related mortality.[89,90] However, a significant benefit was observed among responders.[91] The overall lack of benefit may be related to the low rate of sustained response after a single course of interferon therapy.

The availability of oral antiviral therapy with minimal side effects has changed the paradigm of hepatitis B treatment. A study of patients who have received 3 years of lamivudine therapy found that necroinflammation as well as fibrosis was decreased, although maintenance of histologic benefit was mainly seen in patients who did not have evidence of lamivudine resistance.[92] Several uncontrolled studies reported that lamivudine treatment was associated with decreased risk of cirrhosis and major complications, and improved survival.[93,94] The most convincing evidence that antiviral therapy can prevent progression of chronic hepatitis B was provided by a prospective, double-blind, randomized, controlled trial of lamivudine reported in 2004.[95] In this trial, 651 Asian patients with compensated liver disease, who were positive for HBeAg or had serum HBV DNA >700,000 genome equivalents/mL, with bridging fibrosis or cirrhosis on liver biopsy were randomized to receive lamivudine or placebo. After a median follow-up of 32 months, a significant difference

in disease progression as well as HCC was observed between the 2 groups despite a very high rate (49%) of lamivudine resistance.

These data indicate that antiviral therapy can modify the natural history of chronic HBV infection. The results are likely to be better with newer antiviral agents such as adefovir and entecavir that have lower rates of drug resistance.[96,97] Given the propensity for HBV to persist and the low efficacy of currently available antiviral agents to induce sustained virus suppression, long-term treatment will be required. Thus, issues regarding long-term safety, drug resistance, and costs must be considered. Because liver damage occurs at a slower pace during the "immune tolerance phase," spontaneous HBeAg seroconversion can occur uneventfully in some patients, and many patients remain in the inactive carrier state after HBeAg seroconversion for years if not for life; careful consideration is needed before initiating treatment with medications with limited safety record and unknown risk of drug resistance beyond the first few years. For patients with high serum HBV DNA levels and advanced liver disease, antiviral treatment has been demonstrated to prevent disease progression. For other patients, the benefits of long-term antiviral therapy remain to be determined.

Concluding Remarks

This article summarized the progress in our understanding of the natural history of chronic HBV infection in the past 25 years. The availability of sensitive quantitative serum HBV DNA assays has made the biggest impact on our understanding of the interplay between the virus and the host and the role of HBV replication in the outcome of chronic HBV infection. During the next 25 years, we anticipate that advances in virology, immunology, genomics, and proteomics will improve our understanding of the host immune response to HBV; the role of host genetics, HBV genotypes, and viral variants in HBV-related liver disease will be better defined; and it is possible that the success of antiviral therapy will lead to a complete

revision of the chapter on the natural history of chronic HBV infection.

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