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Review

Update of research and management of hepatitis B

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Introduction

Approximately 350 million people in the world are chronically infected with hepatitis B virus (HBV), the main cause of hepatocellular carcinoma (HCC) especially in many Asian countries. Recent advances in molecular biology have expanded our knowledge of the biology of HBV, the mechanisms of liver disease, and the development of HCC associated with HBV infection. Eight genotypes have been discovered[1–4] that have an uneven geographical distribution.[5,6] It has also been clarified that intertypic recombination was noted in genotypes A, B, and others.[7]

Recently, new nucleos(t)ide analogues and long-acting interferon (pegylated interferon) were introduced to treat chronic hepatitis B, but there is no consensus on the treatment of chronic hepatitis B. The main aim when treating chronic hepatitis B is to suppress persistent virus replication. Interferon (IFN) was first introduced as an antiviral agent; and recently nucleos(t)ide analogues such as lamivudine,[8–10] adefovir dipivoxil,[11–13] and entecavir[14,15] as well as the long-acting IFN peginterferon[16,17] have become available in many countries, but they show low rates of sustained response and are associated with various adverse events. There is a possibility that combination therapy has additive or synergistic antiviral effects and decreases the rate at which resistant viruses develop.[18–20] However, the data for these combination therapies are still short term.

Recent advances in molecular biology have also clarified the clinical significance of the HBV genotype[21,22] and the mutation of precore and core promoter regions.[7,23] Most HBV carriers in Asian countries have resulted from maternal transmission of the infection during early childhood, and around 80% of the carriers show natural seroconversion from a hepatitis B e antigen (HBeAg)-positive state to an HBe antibody (HBeAb)-positive state before 25 years of age. Furthermore, HBeAg to HBeAb seroconversion frequently occurs in chronic hepatitis patients with a high serum alanine aminotransferase (ALT) level. Thus, it is important to clarify the natural course of HBV carriers before antiviral treatment. This article focuses on the recent advances in basic research of HBV and suggests a strategy of antiviral therapy for chronic hepatitis B patients.

HBV genotype

There are currently eight HBV subgroups based on genetic differences. HBV genotypes A, B, C, and D were first classified by an intergroup divergence of more than 8%.[1] HBV genotypes E and F were then identified,[2] followed by recent reports of genotypes G and H.[3,4] One cannot discriminate these genotypes by four serological subtypes (adw, adr, ayw, ayr) of HBV, which are classified by antigenic determinants of the hepatitis B surface antigen, but a there is a partial correlation between genotypes and serotypes (Table 1). There are also a few reports on a serological method for determining HBV genotypes using several monoclonal antibodies to preS2 and S proteins.[24,25] These HBV genotypes show a close relation to ethnicity (Table 1); more importantly, recent investigations have revealed associations between HBV genotypes and clinical features of the infection.

Two major genotypes, HBV/B and HBV/C, prevail in East Asia including Japan. HBV genotype C was more prevalent than genotype B in cirrhotic patients in Japan,[5,26] China,[27] and Taiwan.[28] Another study from

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Table 1. Correlation between HBV subtypes and genotypes and their geographic distribution

HBV genotype	HBV subtypes	Geographic distribution
A	adw2, ayw1	Europe, North and South America, Central Africa, Philippines
B	adw2, ayw1	East Asia
C	adr, adw2, ayr	East Asia
D	ayw2, 3	Mediterranean area, Middle East, South Africa
E	ayw4	West Africa
F	adw4	Native Americans, Central and South America
G	adw2	USA, France
H	adw4	Central America

Japan found that the risk of progression to cirrhosis and HCC was similar in patients with genotypes B and C, but those with genotype B showed slower progression of liver disease.[22] A study in the United States also demonstrated a low frequency of decompensated cirrhosis among those with genotype B.[29] These studies have been corroborated by several observations that showed a lower HBeAg-positive rate[22,23,29–31] and higher prevalence of HBeAg seroconversion[22,31–34] with genotype B than with genotype C.

A cross-sectional study from Taiwan reported an association of genotype B with the development of HCC in young people (<35 years old).[28] However, a recent cohort study from the same group failed to confirm this association in HBsAg-positive children. Further studies are needed to clarify the relation between HBV genotypes and HCC.[34]

Regarding HBV genotypes A and D, there was a report from India that genotype D is associated with more severe liver disease than genotype A.[35] A study from Spain demonstrated that HBsAg clearance occurred more often in patients chronically infected with genotype A than in those with genotype D. A study from Switzerland reported that acute infection is more likely to develop into a chronic infection in patients with genotype A than in those with genotype D.[36] Several reports from Japan also support this tendency toward chronicity from acute horizontal infection with genotype A.[37–40] Taken together, these reports suggest that HBV genotype A causes mild but persistent liver disease that shows a good response to antiviral therapy. These characteristics contrast sharply with those of an HBV genotype C infection.

Clinical significance of HBV gene mutation

Mutations that affect HBeAg production

The precore/core region of HBV encodes for hepatitis B core antigen (HBcAg) and HBeAg. One point mutation at the precore region (G1896A; eW28X) that aborts HBeAg production has been identified particularly in anti-HBe-positive HBV carriers.[41] Later studies revealed that this G1896A mutation occurs in a genotype-dependent manner.[42,43]

The precore region of the HBV pregenomic RNA forms a stem loop structure where nucleotides at 1896 and 1858 couple. In genotypes A and F, a G1896A mutation rarely occurs because a C residue at 1858 in these genotypes favors a G at 1896. In contrast, in genotypes B, D, and most of C, a T (U in RNA) residue at 1858 can pair more covalently after a G to A mutation. In the usual HBV infection course, loss of HBeAg means low viral replication and an inactive inflammation state. In some HBV carriers, however, chronic active hepatitis occurs after HBeAg seroconversion, with a G1896A mutation often observed in these patients. This type of HBeAg-negative hepatitis is frequently seen in Asia and the Mediterranean area, where HBV genotypes C and D are prevalent.

Core promoter variants are other naturally occurring mutations that can affect HBeAg production. The most common one involves a dual mutation at A1762T and G1764A. Several in vitro studies demonstrated that this double mutation appears to reduce HBeAg expression and enhance viral replication.[44–46] It is suggested that alterations in transcription factors bound to the mutated core promoter region mediate a decrease in precore mRNA and an increase in pregenomic RNA.[47,48]

HBeAg is not essential for replication and, based on several clinical and virological studies, is thought to be an immunological tolerogen. Investigations of vertical transmission cases have demonstrated that neonates born to HBeAg-negative mothers frequently developed a transient acute (sometimes severe) hepatitis, whereas neonates born to HBeAg-positive mothers became chronic virus carriers.[49–51] Precore and core promoter variants have been found in association with fulminant hepatitis.[52–58] These facts suggest an aggressive immune response in individuals who do not have circulating HBeAg. Furthermore, a recent in vitro study demonstrated that HBeAg, but not HBcAg, could elicit an

immune tolerance in double- and triple-transgenic mice expressing an HBV-specific T cell receptor and HBcAg with or without HBeAg.[59] It is somewhat intriguing that HBeAg-defective mutants are selected during chronic infection even though HBeAg is a tolerogen. As a possible explanation, Milich and Liang proposed that HBeAg has dual roles: It acts as a tolerogen when secreted, whereas cytosolic HBeAg may be a target for the host's immune system.[60]

Mutations in X gene

Mutations at basal core promoters (A1762T and G1764A) simultaneously affect codon 130 and 131 of the X gene (xK130M and xV131I). Several studies have demonstrated that these mutations were found more frequently in patients with HCC than in those with chronic hepatitis B.[61–64] Because these core promoter variants may result from a long-lasting immune response and may be associated with more severe liver disease, it is unclear if the core promoter mutations or X protein alterations are directly involved in hepatocarcinogenesis. However, a recent report suggested that they could at least be viewed as a predictor of the development of HCC.[65]

Yeh et al. reported that the mutation at codon 31 (xS31A) was frequently found in association with HCC in Taiwan.[66] However, as there are no other reports at present, studies in other countries and genotypes are needed.

Mutations in S gene

The S region encodes for the major B cell epitopes ("a" determinant) of HBsAg. Mutations in this "a" determinant are known as vaccine escape mutants.[67]

The preS region contains cytotoxic T-lymphocyte epitopes, and mutations in this region are often selected as a result of host immune pressure. Deletion mutants of the preS region are detected in around 10% of individuals with chronic hepatitis B infection, particularly along with acute exacerbation of inflammation.[68–72] These preS defective mutants tend to be retained in the cytoplasm and possibly modify virion formation probably to escape the host's immune response.[69,72,73] They usually coexist with wild-type viruses to be encapsidated into the virion and secreted.[72,73] The association of preS deletion mutants with HCC has recently been reported[74] and is discussed later.

Recently Hass et al. reported a novel mutation in the S region that decreased HBsAg production via a unique mechanism.[75] The study reported a point mutation at a splicing donor site in the S region in an immunodeficient patient who showed reactivation of chronic hepatitis B. It proved that this splicing donor site is necessary for persistence and cytoplasmic transport of the preS2/S mRNA, and a point mutation at this donor site could abolish HBsAg production.

Molecular mechanism of the development of hepatocellular carcinoma

Epidemiological evidence has revealed a close relation between HBV infection and the development of HCC.[76–78] Like other cancers, HBV-related carcinogenesis is thought to involve a multistep process, but a precise molecular mechanism remains to be elucidated. However, several viral mechanisms may correspond to each carcinogenic step, including *cis* and *trans* activation of cellular genes by viral proteins, antiapoptotic action, induction of genomic instability, and insertional mutagenesis.[78–81] In addition, indirect hepatocarcinogenesis by HBV-induced chronic necroinflammation appears to play an important role.[79,80]

In this review we focus on direct carcinogenesis related to HBx protein, HBs protein, and HBV integration into the host genome.

HBx and hepatocarcinogenesis

HBx is the smallest protein encoded by HBV. It is not indispensable for viral replication, but it may enhance viral transcription in cultured cells.[80] Whereas mammalian hepadnaviruses such as HBV and woodchuck hepatitis virus (WHV) encode X protein and cause HCC in affected animals, avian hepadnaviruses, which do not cause HCC, lack X protein. Furthermore, development of HCC was observed in transgenic mice that express X protein.[82] These facts prompted an interest in HBx in relation to hepatocarcinogenesis.

X protein does not act by directly binding DNA, but it associates with several components of the transcriptional apparatus, such as TFIIB, TFIIH, and RPB5, through protein–protein interaction. Other studies suggest that X protein can stimulate several cytoplasmic signal transduction pathways, such as the Ras–Raf–MAP kinase and JAK–STAT pathways, in an Src kinase-dependent manner[78–81] (Table 2). Although these results were first demonstrated by in vitro overexpression of X protein, a recent report proved that Wnt/β-catenin signal is activated only in hepatoma cell lines with HBV integration. This Wnt signal activation was also Src kinase-dependent and was observed in hepatoma cell lines without HBV infection by overexpression of HBx.[83] When overexpressed, HBx can interact with many other proteins, including p53, UVDDB (a DNA repair protein), and proteasomes.[80,81]

The 3′ terminal of the X region is frequently deleted when HBV is integrated into the host genome. Some

Table 2. Interaction between HBx and host factors

HBx can activate transcription from DNA binding domain of NF-κB, AP-1, AP-2, c-EBP, ATF/CREB, NF-AT, RNA pol I, RNA pol III (HBx does not directly bind to DNA but acts through protein–protein interaction.)	
HBx can activate the transcription factors CREB, TFIIB, TFIIH, RPB5, c-EBPα, NF-κB	
HBx can activate the signal transduction pathway Ras-Raf-MAP kinase, JAK-STAT	
Other cellular molecules that interact with HBx:	
p53	controversial reports exist
UVDDB	related to DNA repair
HVDAC3	related to cation channel of mitochondria
Proteasome	related to degradation of transcription factor (?)

Table 3. Cancer and clonal proliferation in association with insertional mutagenesis by integration of oncogenic viruses

Species	Viruses	Notes	References
Mouse	Murine leukemia virus	Common integration sites in leukemia and malignant lymphoma	90, 91
Woodchuck	Woodchuck hepatitis virus (WHV)	Common integration into N-*myc* in hepatocellular carcinoma (HCC)	Reviewed in 79, 81
Mouse	Type B leukemogenic virus	Common integration into Rorgamma region in malignant lymphoma	92
Sheep	Jaagsiekte sheep retrovirus	Common integration sites in lung cancer	93
Mouse	Murine leukemia virus	Insertional activation of clonal proliferation of hematopoietic stem cell	94
Mouse	Murine stem cell virus	Common integration sites in soft tissue tumor and osteosarcoma	95
Human	Retrovirus vector	Insertional activation of clonal proliferation of T lymphocytes	96
Human	Hepatitis B virus (HBV)	Common integration sites in HCC	98–103
Human	Human papillomavirus (HPV)	Common integration sites in uterine cervical cancer	101

studies have demonstrated that carboxyl terminal-truncated X protein could inhibit cell cycle arrest and apoptosis in vitro.[84,85] One or several of these properties of X protein may play some role in hepatocarcinogenesis, but further investigation is needed.

HBs and hepatocarcinogenesis

Transgenic mice expressing HBs protein have been found to develop HCC.[86] It has been reported that C-terminally truncated preS2 protein in an HBV-related hepatoma cell line activated protein kinase C pathway. More recently, naturally occurring preS2 deletion protein was reported to up-regulate cyclin A expression in vivo and in vitro.[74] Thus, it is suggested that HBs protein or its modified form is a transactivator and is potentially related to hepatocarcinogenesis in some cases.

HBV integration and hepatocarcinogenesis

Hepatitis B virus shares with oncogenic retroviruses a unique replication strategy through reverse transcription and a characteristic life cycle that includes integration into the host genome. Studies during the 1980s and 1990s demonstrated a few cases where HBV integration occurred near genes closely related to cell proliferation, such as retinoic acid receptor beta[87] and cyclin A.[88] However, in many other cases, HBV integration seemed to occur randomly, and one could not find any preferred sites or genes.[89]

With recent information on the human genome and progress in strategies to identify viral–host junctions, growing evidence demonstrates that viral insertional mutagenesis is an important oncogenic mechanism for mammalian tumor viruses, such as retroviruses, human papillomavirus (HPV), and hepadna virus (Table 3). Analyses of retrovirus integration revealed many common integration sites near genes related to carcinogenesis and stem cell renewal.[90–96] In woodchucks, WHV-related HCCs frequently show WHV insertion into the N-*myc* gene.[81]

For HBV integration, Brechot and colleagues developed a polymerase chain reaction (PCR)-based approach using a human *Alu* repeat, allowing a large number of rapid analyses on HBV flanking host sequences.[97] With HBV-*Alu* PCR, they demonstrated that HBV insertion into cellular genes occurred in around

70% of HCCs and that genes related to telomere synthesis, the Ras signaling pathway, and calcium signaling were recurrently affected.[98,99] In particular, HBV integration into the *hTERT* gene was the first one found that was common to different HCCs. Two independent groups other than Brechot and colleagues have reported HBV integration into *hTERT* in HCCs and hepatoma cell lines,[100,101] and one study demonstrated that expression of *hTERT* gene was *cis*-activated by HBV integration in vitro.[100] It is of note that viral integrations into *hTERT* were recurrently found in uterine cervical cancers with HPV infection,[101] underlining the importance of viral integration and its insertional mutagenesis regardless of viral species or organ.

Recent reports revealed the second common integration site for HCC. Murakami et al. found HBV integration into the *MLL* gene in 1 of 68 cases,[102] and Tamori et al. independently found it in 3 of 15 HCCs.[103] Taken together, HBV integration and the resulting insertional mutagenesis are not rare events, and they play a role in hepatocarcinogenesis possibly by producing fusion protein,[104,105] by *cis*-activation of cellular genes, or by disrupting gene function.

Integration of HBV is not a late event during a course of chronic infection. One can identify HBV integration in chronic hepatitis tissues and even in tissues after acute self-limiting hepatitis.[106] We have analyzed host genes affected by HBV integration in chronic hepatitis tissues and identified candidate cellular genes related to cell growth and survival.[107]

Pathogenesis of hepatitis B and natural history

Hepatitis B virus is not directly hepatotoxic. Many HBV carriers are asymptomatic and have no or minimal liver injury even with high viral replication. It has been demonstrated that host immune responses to viral antigens result in hepatocellular injury, and it is clear clarified that covalently closed circular DNA (cccDNA) plays an important role in maintaining chronic HBV infection.[108]

Antiviral therapy

Recent studies have demonstrated that sustained viral suppression ($<10^5$ copies/ml in serum HBV DNA) results in normal serum ALT and prevents progression to cirrhosis. Thus, the treatment goal is sustained viral suppression with antiviral therapy including interferon, lamivudine, adefovir dipivoxil, entecavir, and various combination therapies. We describe here antiviral treatment, including combination therapy, mainly focusing on the treatments available in Japan.

Interferon

Conventional interferon-α (IFNα), IFNβ, and pegylated IFNα (PEG-IFNα) are available for treating chronic hepatitis B. IFN has many actions including antiviral and immunomodulatory effects.

Initially, IFN had been used for only 4 weeks in chronic hepatitis B patients in Japan, and its effects were limited, whereas 4–6 months of therapy was popular in many Western and Asian countries. With the latter regimen, HBeAg loss was achieved in approximately 33% of HBeAg-positive patients (three times that in controls),[109] and loss of HBsAg was noted in 7.8% (controls 1.8%) after IFN therapy.[110] More than 12 months of therapy was more effective in HBeAg-positive patients with low serum HBV DNA levels.[111] The daily dosage of IFN was 3–10 MU thrice weekly.

A high seroconversion rate was noted in patients with high serum ALT levels, low serum HBV DNA levels, and moderate to severe hepatitis, whereas a lower response rate was observed in patients with lower baseline serum ALT levels (≦1.3–3.0 times the upper limit of normal),[112] high serum HBV DNA levels, and minimal inflammatory changes. Corticosteroid withdrawal therapy induced long-term clinical remission in chronic hepatitis B patients,[113] and priming with a corticosteroid before IFN therapy resulted in a higher seroconversion rate.[114] A long-term follow-up study demonstrated that the sustained virological response was 10%–15% within 4–6 months of treatment, 22% within 12 months, and 30% within 24 months.[115–118] Furthermore, IFN-induced HBeAg seroconversion is durable and results in good overall survival and survival free of hepatic decompensation.[119–121] It was reported that IFN therapy for cirrhotic patients significantly decreased the rate of HCC development, especially in patients with a high level of HBV DNA.[122] HBsAg loss was seen in up to 10% of patients in Western countries but was rare in Asian patients.

Most HBV infections in Asian patients are acquired perinatally or during early childhood, and the distribution of their HBV genotype is quite different from that in Caucasian patients. More than 80% of HBV carriers in Asian countries including Japan naturally seroconvert by the age of 25–30 years. Thus, we must take into consideration the age and HBV genotype of those who are given antiviral therapy.

A pilot study suggested that IFNβ is effective and safe for re-treating patients with chronic hepatitis B who had not responded to a previous IFNα cycle.[123]

Recently, PEG-IFNα2a[19,124] and PEG-IFNα2b[125] (long-acting forms of IFNα) have been used in both Asian and Caucasian patients but are still not available in Japan. Both 24-week and 52-week courses were found to be tolerable and produced a higher

seroconversion rate in Asian and Caucasian patients.[19,124,125]

Lamivudine

Lamivudine, an oral nucleoside analogue, inhibits HBV replication. A daily dose of 100 mg lamivudine markedly reduces the serum HBV DNA level. However, when short-term treatment is stopped, the serum HBV DNA levels generally return to pretreatment levels.[8,126,127]

Lamivudine has usually been administered for 1 year. The first-year HBeAg seroconversion rate in 100 mg lamivudine-treated patients with a pretreatment ALT level more than five times the upper limit of normal was 80%; there was no further increase in HBeAg seroconversion during a second year of therapy.[128] However, reappearance of HBeAg and hepatitis flares occurred at a high rate after seroconversion with lamivudine therapy for HBeAg-positive chronic hepatitis patients with high serum HBV DNA levels.[129,130]

It is thought that prolonged therapy is needed in patients with low ALT levels or a long endogenous antiviral immune response.[131] Three years of lamivudine therapy reduced necroinflammatory activity and reversed fibrosis formation in most patients, but the emergence of YMDD variants blunted the histological response.[132] The response to lamivudine therapy in HBeAg-negative patients is similar to that of HBeAg-positive patients.[133,134]

A lamivudine-resistant strain with altered YMDD motif of the polymerase gene (rtM204I and rtM204V with or without rtL180M) developed in 10%–20% after 1 year, 30%–40% after 3 years, and 50%–70% after 5 years, resulting in flare-up hepatitis due to resistant virus.[129,135,136] A flare-up due to a YMDD mutant results in hepatic failure in some cirrhotic patients, who then require liver transplantation[137]; however, most patients with flare-up hepatitis have a serum ALT level of <80 IU/l.[136] It was reported that lamivudine initially selected wild-type virus from precore/core promoter mutants, but precore mutation reappeared during prolonged therapy.[138]

Lamivudine resistance in HBV does not seem to depend on the HBV genotype, although it was significantly higher in the Ba ("a" means Asia) subgroup of HBV than in the Bj ("j" means Japan) subgroup.[139]

A highly sensitive method to detect the YMDD motif mutant demonstrated that the mutant was noted in a few patients with HBeAb-positive chronic hepatitis B without previous administration of lamivudine.[140] YMDD motif mutants may be selected during continuing lamivudine therapy and elicit another hepatitis flare-up.[19,140,141] Flare-up hepatitis develops when the serum YMDD motif mutant level is $>10^{2.7}$ copies/ml.[142]

Adefovir dipivoxil

Adefovir dipivoxil (Adefovir) is an acyclic nucleotide analogue, and it has been proven that adefovir is effective for both wild-type and lamivudine-resistant HBV strains. A daily dose of 10 mg was recently approved for treatment of both HBeAg-positive and HBeAg-negative chronic hepatitis B patients.[11,12,143,144] Histological improvement, HBV DNA suppression, ALT normalization, and HBsAg loss (1.6%–2.0% vs. 0%) was seen in HBeAg-positive and HBeAg-negative chronic hepatitis B patients.[11,12] HBeAg loss and HBeAg seroconversion also increased compared to that in controls (12% vs. 6%).[11] There was no significant difference in the antiviral effect of adefovir among the various HBV genotypes.[13] Combination therapy with lamivudine led to a stronger antiviral effect and achieved HBV DNA levels of <200 copies/ml, as seen by PCR. HBeAg seroconversion was 6%–8% after 1 year of combination therapy compared with 0%–2% with lamivudine monotherapy and 11% with adefovir monotherapy; at week 104, HBeAg seroconversion increased 12% on combination therapy.[144] A few patients showed a relapse after seroconversion when they stopped adefovir administration. Long-term adefovir therapy decreased the replicative form of HBV DNA (termed cccDNA) levels by a noncytolytic mechanism.[145]

It has been reported that the rate of adefovir-resistant mutant appearance is low compared with that after lamivudine therapy. Recently, it was clarified that resistance mutations rtN236T and rtA181V were identified in 5.9% of HBeAg-negative chronic hepatitis B patients after 144 weeks.[146] Adefovir dipivoxil-resistant rtN236T mutant is susceptible to lamivudine and other nucleoside analogues, such as entecavir, emtricitabine, and telbivudine.[147,148]

Disturbed renal function was reported with a daily dose of adefovir of 30 mg but not with 10 mg. Increased serum creatinine was reported in 2.5% when therapy was extended to 3 years, but it was reversible upon stopping therapy.[149]

Entecavir

Entecavir became available for chronic hepatitis B patients in the United States in 2005 and might become available in Japan in 2006. A Phase III clinical trial demonstrated that entecavir was superior to lamivudine for reducing HBV DNA in both HBeAg-positive and HBeAg-negative patients.[150] Entecavir at daily doses of 1.0 and 0.5 mg resulted in significantly greater reductions in the HBV DNA level and normalization of serum ALT levels than lamivudine 100 mg daily after as little as 24 weeks of treatment.[151] At 48 weeks, the

mean reductions in HBV DNA levels were 5.06, 4.46, and 2.86 $\log_{10}$ copies/ml after entecavir 1.0, 0.5, and 0.1 mg, respectively, which is significantly higher than the 1.37 $\log_{10}$ copies/ml achieved with lamivudine,[151] and these amounts of entecavir were well tolerated. Entecavir 1 mg might be used for lamivudine-resistant mutants, and 0.5 mg may be suitable for the wild strain. In HBeAg-positive chronic hepatitis B patients, 96 weeks of treatment with entecavir 0.5 mg results in continued clinical benefit as measured by the reduction in serum HBV DNA (<300 copies/ml by PCR; 80% vs. 39%; $P < 0.0001$) and ALT levels and continued HBeAg seroconversion compared with lamivudine (31% vs. 25%, P = NS).[152] No resistant mutants were noted during the 96 weeks of treatment. Entecavir demonstrated an overall safety profile comparable to that of lamivudine throughout the 96 weeks.[152]

Combination therapy

Interferon and lamivudine

There have been many reports concerning the combination or sequential therapies with lamivudine and IFN;[18,153–157] however, its efficacy is not certain and might be limited. We tried combination and sequential therapy with lamivudine and natural IFNα in genotype C patients according to the method by Serfaty et al.,[18] but HBV DNA suppression, HBeAg negativity, and ALT normalization in our study were not comparable to their results (unpublished data). This discrepancy might be due to the differences in the distribution of HBV genotype (genotype A was prevalent in the study of Serfaty et al.) and in the mode of HBV transmission. Controlled studies demonstrated the efficacy of combination therapy for HBeAg-positive patients with high serum ALT levels[153,154] but not for HBeAg-negative patients.[156,158]

Pegylated interferon and lamivudine

As mentioned already, PEG-IFN might be more effective than conventional IFNα.[19,124,125] Patients with HBeAg-negative chronic hepatitis B given PEG-IFNα2a had significantly higher response rates (which were sustained 24 weeks after the cessation of therapy) than did patients given lamivudine. Addition of lamivudine to PEG-IFNα2a did not improve the posttherapeutic response rate.[19,159] In patients with HBeAg-positive chronic hepatitis, combination treatment of PEG-IFNα2b (32 weeks) and lamivudine (52 weeks) produced a higher sustained virological response than did lamivudine monotherapy (52 weeks) after up to 3 years after treatment.[160] At the end of treatment, HBeAg loss occurred in 63% of patients in the combination group and in 28% of patients in the lamivudine group (P = 0.0001). The probabilities of sustained response for combination treatment and lamivudine monotherapy were, respectively, 33% and 13% at week 24, 31% and 11% at week 52, and 29% and 9% at week 76 (log-rank test, $P = 0.0015$).

Other antiviral agents

Many promising nucleoside and nucleotide analogues for chronic hepatitis B are being evaluated in Phase I, II, and III studies. Unfortunately, these studies are not ongoing in Japan. Telbivudine suppresses wild-type HBV by 5–8 $\log_{10}$ and was more potent than lamivudine in a Phase II study.[161] Clevudine 30 mg/day for 24 weeks resulted in an HBV DNA reduction of 4.46 $\log_{10}$ undetectable by PCR in 59%, HBeAg loss in 24%, and ALT normalization in 76%.[162] Tenofovir disoproxil fumarate show strong suppression of HBV with YMDD motif mutants and has a good safety record.[163]

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