CLINICAL IMPLICATION OF HBV GENOTYPES

DISTINCT CLINICAL AND/OR virological characteristics of the HBV infection have been reported in different geographical parts of the world and are increasingly associated with host factors, environmental factors and the genetic diversity of the infecting virus.[47] HBV is classified into at least eight genotypes (A–H) based on an intergroup divergence of 8% or more in the complete nucleotide sequence and a number of subgenotypes (Aa/A1, Ae/A2, Bj/B1, Ba/B2, Cs/C1, Ce/C2, D1, D2, and so forth) that are currently known to have distinctive association with ethnic and/or geographical distribution.[48]

Association between HBV genotype and clinical manifestation

Acute hepatitis

The universal vaccination program against HBV has significantly reduced the number of new infection cases in most countries with levels of endemicity estimated from intermediate to high.[49] However, efficiency of universal vaccination in countries with a low level of endemicity still remains controversial. Japan is one of the countries with a low level of endemicity and mainly vertical (mother to baby) transmission route.[50] In Japan, HBV vaccination in combination with HBV immunoglobulin treatment is the only recommended measure for infants born to HBsAg positive mothers. Studies in Japan indicated genotype C (subgenotype Ce/C2) to be the major type in the country and genotype B (subgenotype Bj/B1) is the second distributed. Surveillance studies have shown a recent trend toward increase in number of acute hepatitis B infection among young adults mainly through sexual contacts.[51,52] Although most cases are associated with genotype C infection, there is a continuous trend toward increase in prevalence of genotype A among acute hepatitis cases.[51,53–56] Patients infected with genotype C have been known to be rarely associated with development of chronic persistence after acute infection in immune competent adults in Japan (1%) in contrast to the higher rates of those infected with genotype A (6–23%).[53,54] A recent multicenter study in Japan indicated a trend among chronic hepatitis B patients toward increase in prevalence of genotype A (from 1.7% in 2002 to 3.5% in 2006), whereas other genotypes remained stable at their prevalence during the same period.[57] The shift in genotype prevalence with the increase of genotype A among chronically infected carriers can be explained by higher risk of genotype A to develop persistence. This is consistent with higher rates of chronic persistence after acute infection in adults in European countries where genotype A is prevalent (10%).[48,58] This is also consistent with results of *in vitro* and *in vivo* comparisons of different genotype strains showing different dynamics of replication: slow for genotype A and rapid by genotype C.[59,60] The surveillance study indicated that all patients treated with lamivudine (LVD) recovered from acute hepatitis, whereas none of the three patients who developed a chronic outcome had received antiviral treatment during their acute phase of infection, indicating that LVD might be able to prevent the chronic outcome.[54] Cumulatively, these data indicate the clinical importance of routine genotyping for acute hepatitis B patients.

Fulminant hepatitis

One of the most serious complications of acute HBV infection is fulminant hepatitis. In Japan, the annual number of fulminant hepatitis reported was approximately 400 cases, with approximately half of these caused by HBV infection. Despite its rather low incidence, fulminant hepatitis is a national problem because the mortality rate is extremely high.[61] It is important to understand factors predisposing for development of fulminant hepatitis. Viral factors associated with the development of fulminant hepatitis are mutations in the core promoter (T1762/A1764)[62] and the pre-core region (A1896).[54,63,64] However, these findings were not consistent with studies in Europe and the USA.[65–67] A large-scale cross-sectional study in Japan revealed association between genotype B (subgenotype Bj/B1) infection and development of fulminant hepatitis; on the other hand, no cases of fulminant hepatitis were registered among those infected with genotype A (subgenotype Ae/A2).[54] Differences in genotypes circulating in Asia and Europe/USA may indicate that distinct viral factors are playing roles in manifestation of infection by different genotype.

Chronic hepatitis

Chronic HBV infection is the most common cause of HCC in Asia.[68] Efficient surveillance and early diagnosis of development of this life complication requires risk stratification of chronic hepatitis B patients. Older age, male sex and liver cirrhosis are well recognized factors associated with increased risk of HCC.[69,70] In addition, recent large-scale population-based and clinical case–control studies carried out in Asia, have shown that infecting virus factors associated with a high risk of HCC, include HBV DNA levels,[71,72] HBV basal core promoter mutations,[35] genotype C (vs B),[22,36,73,74] and sub-

genotype Ce/C2.[71,75] There are data indicating that genotype C infection associated with a higher viral load than genotype B.[76] Association of genotype F with HCC was found to be higher than that of genotype C in Alaskan natives.[77,78] Unfortunately, there are few prospective studies examining other HBV genotypes for association with adverse outcomes. Genotype A (subgenotype Aa/A1) was found in association with development of HCC in young adults in South Africa.[79,80] However, very high rates of detection of subgenotype Aa/A1 among asymptomatic carriers suggest contribution of environmental factors (aflatoxin contained in food) for the development of HCC. In comparison with Aa/A1, HCC associated with Ae/A2 is found primarily in older individuals. In addition, the rate of complications, including HCC, for those infected with subgenotype Ae/A2 appears to be less than that found in those infected with genotype D, C or F1.[77,81] A prospective study in Spain showed that genotype A (presumably Ae/A2) infection was associated with a significantly higher cumulative rate of sustained biochemical remission, HBV DNA and HBsAg clearance in patients with chronic HBV infection than genotype D infection.[81]

Consensus 4

4-1 Recently, there is an increase of HBV genotype A proportion among acute hepatitis B infection cases in Japan. (Level 3.)

4-2 HBV genotype A acute infection has a tendency to evolve in chronic hepatitis compared to genotype B/C. (Level 3.)

4-3 Antiviral therapy of acute infection might be efficient in prevention of chronic carrier stage. (Level 3.)

4-4 Genotype C compared with genotype B is associated with higher risk of outcome in HCC in chronic carriers. (Level 2a, grade B.)

4-5 Genotype A compared with genotype D and F in chronic carriers is associated with better prognosis in terms of spontaneous ALT normalization and DNA clearance. (Level 2a, grade B.)

HBV MUTATIONS AND THEIR POTENTIAL IMPACT ON PATHOGENESIS OF HBV INFECTION

THE HBV GENOME consists of double-stranded DNA, 3200 bp in length. HBV replicates through reverse transcription of a RNA intermediate, the pregenome RNA, different from all known mammalian DNA viruses. HBV infection is characterized by high levels of virus production, however, the HBV reverse transcriptase is an error-prone enzyme lacking proof-reading capacity, resulting in a large number of nucleotide substitutions during replication. The misincorporation rate has been estimated to be of the order of 10^{10} incorrect nucleotide incorporations per day. As a result, HBV has a quasispecies distribution in infected patients.

Naturally occurring mutations identified in the HBV genome are more prevalent in patients with chronic hepatitis than in HBeAg positive asymptomatic carriers. Among them, several specific mutations have been shown to be associated with the pathogenesis of HBV infection.

HBeAg seroconversion

A HBV strain harboring stop codon mutation in the precore region was first reported in anti-HBe positive patients with chronic hepatitis.[25] The precore region located upstream of the core region is involved in the production and secretion of HBeAg protein. HBeAg is secreted into blood after removal of N-terminal 19 amino acids (a.a.) and C-terminal 34 a.a. from HBeAg precursor protein composed of precore and core regions. Nucleotide substitution of G to A at nt 1896 confers stop codon (TAG) mutation from tryptophan (TGG) at codon 28 in the precore region, resulting in a failure to produce HBeAg protein.[82–84] Although controversial, 10 genotypes have been identified tentatively so far[85] and genotypes affect the occurrence of stop codon mutation in the precore region. The stop codon mutation in the precore region (G1896A) is rarely encountered in HBV genomes of genotype A, some of genotype C and F, because they possess C at position 1858 that makes a pair with G at position 1896 in the stem-loop structure of the *cis*-encapsidation signal.[86]

The HBV core promoter regions located upstream of core region are involved in the transcription of precore mRNA and pregenomic RNA. Nucleotide substitution of A to T at nt 1762 combined with substitution of G to A at nt 1764 in the core promoter region give rise to a reduced transcription of precore mRNA and increased level of viral DNA, resulting in a decreased production of HBeAg protein and enhanced viral replication.[87–89]

Consensus 5

Nucleotide substitution G1896A confers stop codon mutation in the precore region. Nucleotide substitution A1762T combined with substitution G1764A in

the core promoter region give rise to a reduced transcription of precore mRNA. These nucleotide changes in combination with a reduction of HBeAg caused by suppressed replication of HBV are closely associated with HBeAg seroconversion. (Level 2b, grade B.)

Association between HBV mutations and clinical manifestation

Fulminant hepatitis

Precore and core promoter mutations are very frequent in patients with fulminant hepatitis from Asia[62,63,90] and the Middle East.[64] However, these mutations were not detected in those from Western countries.[65,67,91,92] This difference could be attributable to the difference of genotype prevalence, frequent genotype Ae and rare Bj in Western countries.[86] The patients infected with the former genotype rarely have precore mutant virus, while the latter frequently have the mutant virus. Stop codon mutation in the precore region is inhibited in genotype A because of C at position 1858 that makes a pair with G at position 1896 in the stem-loop structure of the *cis*-encapsidation signal.[93]

Ozasa *et al.* analyzed the difference of host and viral factors between 40 patients with fulminant hepatitis B and 256 with acute self-limited hepatitis B in a multicenter cross-sectional study,[54] and showed that precore stop codon mutation of G1896A and genotype Bj are associated with fulminant hepatitis in Japan. They also reported the marked enhancement of viral replication by introducing either G1896A or A1762T/G1764A mutation into the Bj clone in *in vitro* transfection study. Because this type of HBV mutant is found not only in patients with fulminant hepatitis but also in asymptomatic HBV carriers,[94] the interaction between the virus and the host's immune response might influence the outcome of HBV infection.

In addition to the mutants mentioned above, pre-S2 defective virus or HBV defective in secretion because of surface gene mutations are reported in patients with fulminant hepatitis. These mutant viruses showed a characteristic feature of virus retention in hepatocytes and misassembly with high replication capacity.[95–97]

HCC development

Evidence has been accumulating over the past decade that the risk of developing cirrhosis and HCC is influenced by the patient's viral status, such as genotype, viral load and genomic mutations. Naturally occurring mutations have been identified in the structural and non-structural genes as well as the regulatory elements of the virus, and these mutations are more prevalent in patients with chronic hepatitis than in HBeAg positive asymptomatic carriers.[98]

A double mutation, A1762T/G1764A in the basal core promoter region has been found in patients with advanced liver disease and HCC. Several case–control studies,[30,35,99–102] retrospective cohort studies[103,104] and one prospective cohort study[105] confirmed this finding, while some conflicting results were also reported in the case–control studies[106,107] and one prospective study.[108]

The role of deletions in the pre-S region of the HBV genome has been shown to be associated with the development of progressive liver diseases including HCC. Several case–control studies confirmed this finding.[27,107–110] A further mapping study of the pre-S region showed that all the deletion regions encompassed T- and B-cell epitopes and most of them lost one or more functional sites including the polymerized human serum albumin-binding site.[109] Deletion of these functional sites may cause intracellular retention of HBV envelope proteins and viral particles and contribute to more progressive liver damage and HCC development.

In addition to these common mutations, several other mutations, C1653T in the enhancer II region, T1753C/A/G in the core promoter region, and G1317A/T1341C/A/G in enhancer I region, have been reported to be associated with the development of HCC in some case–control studies.[30,107,111]

Consensus 6
There is some evidence that emergence of HBV genomic mutations arising during the course of chronic infection influence the outcome of chronic liver disease. Among them, core promoter mutations A1762T/G1764A might have a potential for developing progressive liver disease and HCC. (Level 2a, grade B.)

HBsAg escape mutant

The HBsAg mutant was first described in a child born to a HBsAg positive mother who developed acute hepatitis B in spite of vaccination and passive immunization against HBV.[112] This viral strain contained a substitution of glycine to arginine at position 145 (sG145R) and was able to escape the immune surveillance, resulting in an infection despite the presence of anti-HBs antibodies, vaccine escape mutant. Similar mutants have been detected all over the world.[113–115]

Patients after liver transplantation for HBV-related chronic liver disease who had received anti-HBs antibodies to prevent re-infection of the graft showed an "immune escape mutant".[116–118] Furthermore, "diagnosis escape mutants" have also been described because HBsAg detection assays are based on anti-HBs antibodies.[119] The emergence of these variants may contribute to occult HBsAg negative HBV infection.[120]

The HBV genome is organized in such a way that the envelope gene is overlapped by the polymerase gene; therefore, HBV with changes in the polymerase gene associated with resistance to the nucleos(t)ide analog which are described in detail in section 5 may have consequent changes in the envelope gene. A triple mutant causing LVD resistance (rtV173L + rtL180M + rtM204V), which have an enhanced replication capacity compared with rtL180M + rtM204V alone, causes two amino acid changes in the overlapping surface gene (sE164D + sI195M). This mutant reduces anti-HBs binding to levels seen only with the vaccine escape mutant sG145R.[121] Some patients treated with LVD showed seroclearance of HBsAg with detectable circulating HBV DNA. An sP120A mutation was associated with HBsAg seroconversion in these patients and this mutation produces a reduced anti-HBs binding which causes the failure to detect HBsAg.[122]

Consensus 7
Amino acid substitutions, deletions or insertions across the "a" determinant of HBsAg, such as a substitution sG145R, give rise to vaccine and immunoglobulin escape mutant. (Level 4, grade C.)

INDICATIONS FOR ANTIVIRAL TREATMENT OF CHRONIC HEPATITIS B

ONCE THE LIVER is persistently infected with HBV, it is difficult to eradicate the virus. It is reported that the natural clearance rate of HBsAg in asymptomatic HBsAg carriers is approximately 1–2% per year.[123] Therefore, the first goal in treating chronic hepatitis B is to prevent patients from progression to cirrhosis and occurrence of HCC.

When the initiation of antiviral therapy for chronic hepatitis B is considered, it is very important to estimate the fibrosis stage of each patient. If possible, a liver biopsy should be performed in order to obtain sufficient information to determine the extent of hepatic fibrosis. When the fibrosis stage of patients with chronic hepatitis B is moderate to severe, or when the patients have cirrhotic liver, the administration of antiviral therapy should be considered. When inflammatory activity is high and the fibrosis seems to be progressive, the introduction of antiviral therapy should also be considered.

In order to prevent the occurrence of hepatic fibrosis and HCC, virological factors as well as biochemical factors are important. A long-term follow-up study of untreated HBsAg positive individuals in Taiwan in which the cumulative incidence of HCC and cirrhosis were studied for 13 years revealed that high baseline HBV DNA was associated with increased risk of HCC and cirrhosis. Incidence rate of HCC in patients whose viral load of HBV DNA was less than 300 copies/mL was 1.3%, whereas in patients whose viral load was more than 1 000 000 copies/mL the incidence rate was 14.9%.[33] Moreover, incidence of cirrhosis in patients whose viral load was less than 300 copies/mL was 4.5%, whereas it was 36.2% in patients whose viral load was more than 1 000 000 copies/mL.[124] Therefore, the introduction of antiviral therapy should be considered based on biochemical and virological findings.

As mentioned above, although high viral load of HBV DNA is one of the strong risk factors in predicting poor prognosis of HBV carriers, low HBV DNA level does not rule out risk in Asian patients. Among HBeAg positive patients, HBV DNA levels of less than 10^5 copies/mL predicted better histological outcome; however, 14.3% of patients still had established fibrosis.[125] The liver biopsy is also very useful for such cases.

Recommendation 4
- 4-1 Introduction of antiviral therapy should be considered on the biochemical and virological findings. (Level 2a, grade B.)
- 4-2 Antiviral therapy should be considered for patients with low virus load but progressed hepatic fibrosis. (Level 2a, grade B.)
- 4-3 Liver biopsy finding (if available) should be useful to determine the introduction of antiviral therapy. (Level 2a, grade B.)

On the other hand, when patients with HBV have obscure or mild fibrosis, a close observation without any medication could be considered for them. Once antiviral therapy with a nucleos(t)ide analogue is started, it is very difficult to stop. Therefore, for patients who are in an inactive carrier state and whose fibrosis stage is relatively mild, a coarse observation without any treatment could be a useful choice to treat the patients.

Young patients with chronic hepatitis B, especially those who are HBeAg positive, often face the flare-up of hepatitis. Because such patients are likely to achieve spontaneous HBe seroconversion and go into an inactive carrier state, unnecessary antiviral therapy should be avoided for them. A coarse observation without any medications should be considered for young patients or those with mild fibrosis.

Recommendation 5
Indication of antiviral therapy for chronic hepatitis B: Observation without therapy should be considered for young patients or those with mild fibrosis. (Level 3, grade B.)

NUCLEOS(T)IDE ANALOGUES FOR CHRONIC HEPATITIS B

AS STATED ABOVE, the goal of antiviral therapy in patients with chronic hepatitis B is to prevent cirrhosis and HCC. Maintaining persistent suppression of HBV replication reduces the development of cirrhosis and HCC. In the last decade, there has been a major advance in the treatment of chronic hepatitis B with nucleos(t)ide analogues such as LVD, adefovir (ADV), entecavir (ETV), telbivudine and tenofovir.[126–132] In treatment by nucleos(t)ide analogues for chronic hepatitis B in Japan, LVD, ADV and ETV are mainly used at present. Nucleos(t)ide analogues are potent inhibitors of the polymerase/reverse transcriptase and are easy to administrate p.o. to chronic hepatitis B patients because of low adverse effects and strong efficacy to suppress HBV replication. Thus, nucleotide analogue therapy could rescue liner decompensation, reduce fibrosis progression and prevent the development of HCC.[133–136] On the other hand, there are major disadvantages including requirement of prolonged or even indefinite therapy for most patients and the high incidence of antiviral resistance. Disadvantages of nucleos(t)ide analogues include the development of antiviral resistance.[137–140] Drug-resistant viruses emerge during the treatment and could be associated with flare-up of hepatitis. Due to no proof of reading activity of HBV polymerase, the spontaneous substitution rate of HBV genome is high in the natural course of the disease. Through the selection of pre-existing resistant variants and gradual accumulation of new a.a. substitutions, the mutations exhibiting the best replication capacity in the presence of the drug are selected under the circumstance of antiviral pressure. The level of intrinsic resistance and the replicative fitness determine the mutant spread and hence the annual incidence of drug resistance.

LVD

Lamivudine was the first nucleoside analogue licensed for the treatment of chronic HBV infection in Japan in 1999. LVD was given at a dose of 100 mg daily and has excellent safety and tolerability.[141–143]

Liaw *et al.* reported that continuous treatment with LVD delays the clinical progression of chronic hepatitis B with advanced fibrosis or cirrhosis by significantly reducing the incidence of hepatic decompensation and risk of HCC (level 1b).[134] Matsumoto *et al.* also showed that LVD therapy effectively reduces the incidence of HCC in Japanese patients with chronic hepatitis B.[144] Thus, it is generally considered that control of viral load using nucleos(t)ide analogues is effective to prevent complicating HCC in patients with active chronic hepatitis B.

Consensus 8
The control of viral load using nucleos(t)ide analogues reduces the risk of complicating HCC in patients with chronic hepatitis B. (Level 1b, grade B.)

Lamivudine resistance is characterized by the mutation of the highly conserved tyrosine, methionine, aspartate, aspartate (YMDD) nucleotide-binding motif in the catalytic domain of the enzyme. YMDD to YIDD (rtM204I) or YVDD (rtM204V) mutations are associated with LVD resistance.[142,145,146] These resistant mutants appear to replicate less efficiently than the wild-type virus *in vitro*, however, additional mutations such as rtV173L and rtL180M can restore partially the replication capacity *in vitro*.[147,148] LVD resistance occurred in approximately 20% of patients after 1 year, which increased to approximately 70% after 5 years (Fig. 1).

A meta-analysis, which included Asian patients and North American/European patients, indicated that HBV subtype ayw (genotype D) appears to respond significantly better to LVD treatment than does HBV subtype adw (genotype A). Insufficient suppression of the adw subtype during the early phase of treatment may lead to the high incidence of LVD resistance in HBV subtype adw.[149] In a study comparing the virological outcome among infections with HBV genotypes A, B and C, patients infected with genotype A had the lowest rate of HBV DNA clearance than those with genotype B or C, and had the highest incidence of resistant mutations.[150]

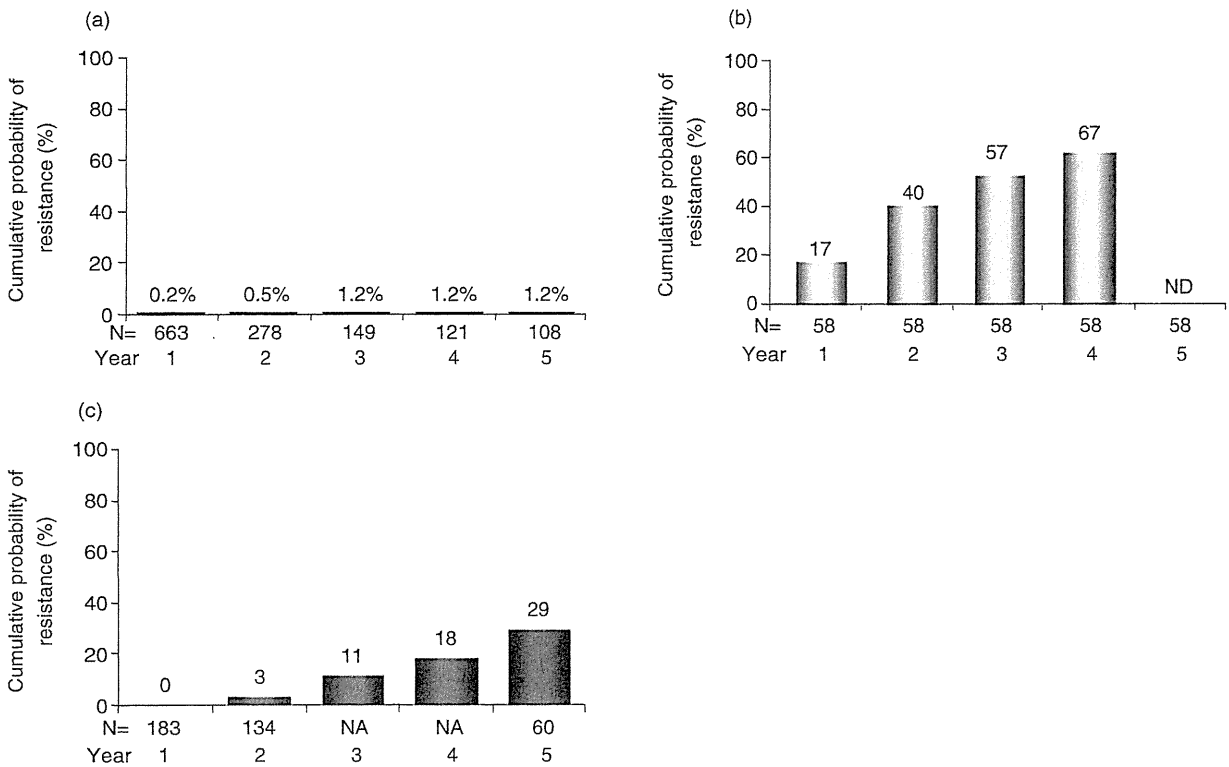

Figure 1 Cumulative probability of resistance after the initiation of entecavir (ETV), lamivudine (LVD) and adefovir (ADV) for patients with hepatitis B e-antigen. (a) Cumulative probability of resistance after the initiation of ETV.[159] (b) Cumulative probability of resistance after the initiation of LVD.[138] (c) Cumulative probability of resistance after the initiation of ADV.[153]

Lamivudine or hepatitis B immunoglobulin (HBIg) treatment induced vaccine/HBIg-escape mutations sP120T and sG145R in combination with LVD-resistance mutations. These mutations are associated with rtT128N and rtW153Q in the polymerase protein and have been found to partially restore the *in vitro* replicative capacity of LVD-resistant HBV.[121]

Another LVD resistant mutation, rtA181T, concomitantly generates a stop codon in the surface antigen (sW172stop), resulting in impaired secretion of HBsAg.[151] Neither the adefovir associated resistance mutation rtN236T nor the tenofovir associated resistance mutation rtA194T causes changes in the envelop protein.

ADV

Adefovir dipivoxil is a prodrug of ADV and has structural similarity to the natural substrate, dATP. Several studies have also been conducted using ADV.[128,152–154] In HBeAg positive patients, treatment with ADV for 1 year resulted in HBeAg seroconversion in 12%, serum HBV DNA in less than 10^3 copies/mL in 21% and normalization of ALT in approximately 48% of patients.[127] The rate of HBeAg seroconversion increased to 29% after 2 years and 43% after 3 years of treatment. In HBeAg negative patients, serum HBV DNA of less than 10^3 copies/mL and normalization of ALT were observed in 51% and 72%, respectively, after 1 year of ADV.[154] After 5 years of therapy, the serum HBV DNA were less than 10^3 copies/mL in 67% of patients, and ALT level normalized in 69%. The reported incidence of ADV resistance is 0% after 1 year, 3% after 2 years and 29% after 5 years of antiviral therapy (Fig. 1).[154] The primary mutations associated with ADV resistance are rtN236T and rtI233V in the D domain and rtA181V in the B domain of HBV polymerase. In comparison with more than 100-fold decrease in sensitivity to LVD associated with the two primary mutations, the rtN236T mutation confers only a 5–10-fold decrease in sensitivity to ADV *in vitro*,[155] which may explain the delayed emergence of this mutant.

In LVD-resistant patients treated with ADV monotherapy, the rate of antiviral resistance was 6–18% after

1 year and 21–38% after 2 years.[156,157] Switching therapy from LVD to ADV may enhance the acquisition of another mutation and induce replication of HBV DNA.[158–160] On the other hand, combination therapy of LVD and ADV effectively suppressed viral replication and maintained high efficacy in LVD-resistant patients with chronic HBV infection.

ETV

Entecavir is a guanine analogue and Chang *et al.* have reported that ETV is effective in reducing the serum level of HBV DNA compared with LVD in HBeAg positive patients (Table 2).[159] The cumulative proportion of patients with undetectable HBV DNA (<300 copies/mL) increased to 81% after 1 year of therapy and 93% after 5 years of therapy.[160] After 1 year of treatment with ETV, the serum ALT level was normalized in approximately 70% of patients, and increased to 90% of patients after 5 years. Lai *et al.* have reported that ETV is more efficacious in HBeAg negative patients compared with LVD (Table 2).[161] ETV is the most potent of the currently available anti-HBV drugs because it affects multiple functions of the polymerase, including priming, reverse transcription and DNA elongation.[162]

Entecavir was licensed for the treatment of chronic hepatitis B in Japan in 2006. In nucleos(t)ide-naive patients, ETV is given at dose of 0.5 mg/day.

The rate of ETV resistance was extremely low in nucleoside-naive patients.[160,163,164] The incidence of ETV resistance in nucleos(t)ide analogue-naive patients was reported to be 1.2% at 3 years (Fig. 1).[160,163,164] HBeAg loss was observed in 8% of these patients. The response to ETV was lower in LVD-resistant patients than in nucleos(t)ide analogue-naive patients. In LVD-resistant patients, 20% of patients had undetectable HBV DNA levels after 48 weeks of ETV therapy, and the resistance rate to ETV was 26% at 3 years. Patients with HBeAg at the initiation of ETV had a resistance rate to ETV of 36% at 3 years. On the other hand, patients without HBeAg at the initiation of ETV did not have resistance to ETV at 3 years (Fig. 2).[160,165] In LVD-resistant patients, the risk of the development of resistance to ETV is much higher than those without LVD resistance.[160,165]

The resistance to ETV is principally associated with the mutations rtM250V, rtI169T or rtS202I in addition to the primary LVD resistance mutations rtM204V + rtL180M. The need for multiple mutations to induce ETV resistance suggests a higher genetic barrier to resistance and explains the low rate of resistance to ETV in nucleos(t)ide analogue-naive patients.

Table 2 Efficacy of nucleoside analogues for chronic hepatitis B

	Subject: HBeAg positive patients[159]						
	n	Change of HBV DNA (log copies/mL)		Negativity of HBV DNA of <300 copies/mL		Normalization of ALT	SC
ETV 0.5 mg	354	−6.9	$P < 0.001$	67%	$P < 0.001$	68%	$P < 0.05$	21%	$P = 0.33$
LVD 100 mg	355	−5.4		36%		60%		18%	
	Subject: HBeAg negative patients[161]						
	n	Change of HBV DNA (log copies/mL)		Negativity of HBV DNA of <300 copies/mL		Normalization of ALT	
ETV 0.5 mg	325	−5.0	$P < 0.001$	90%	$P = 0.001$	78%	$P < 0.05$		
LVD 100 mg	323	−4.5		72%		71%			

ALT, alanine aminotransferase; ETV, entecavir; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus; LVD, lamivudine; SC, seroconversion; VR, virological response.

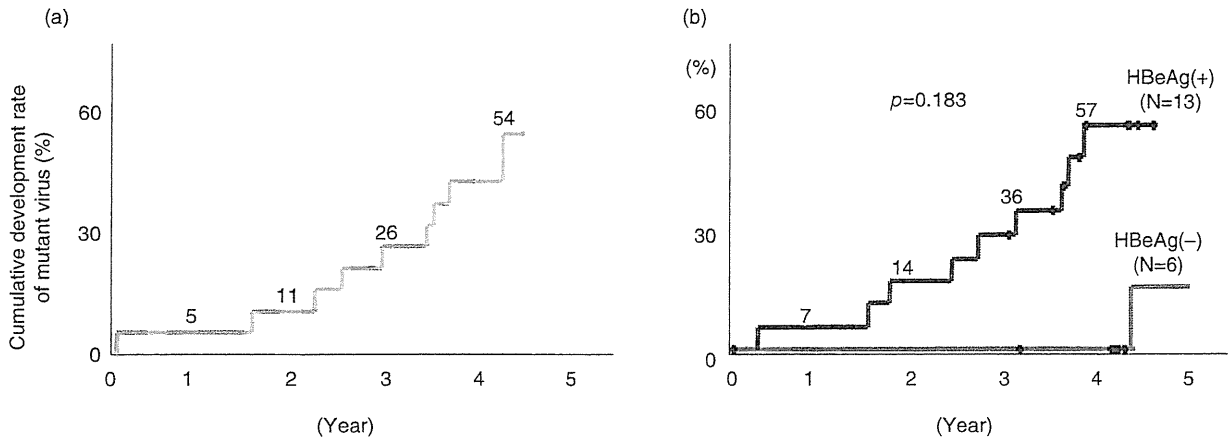

Figure 2 Cumulative development rate of mutant virus after the initiation of entecavir monotherapy in hepatitis B patients with resistance after the administration of lamivudine monotherapy.[164] (a) Cumulative development rate of mutant virus in all patients. (b) Cumulative development rate of mutant virus based on the difference of hepatitis B patients with positive hepatitis B e-antigen (HBeAg) and hepatitis B patients with negative HBeAg.

Consensus 9
Drug-resistant virus with specific mutations in the polymerase/reverse transcriptase gene emerges during nucleos(t)ide analogue therapy in chronic hepatitis B patients. The rtM204V/I and rtL180M mutations are associated with LVD resistance, the rtN236T and rtI233V or rtA181V with ADV resistance, and the rtM250V or rtT184G or rtS202I combined with rtM204V + rtL180M with ETV resistance. (Level 4, grade C.)

Recommendation 6
When patients with chronic hepatitis B are treated with nucleos(t)ide analogues, ETV should be given as the first-line drug because of its high efficacy and low emergence of viral resistant mutant. (Level 1b, grade A.)

Recommendation 7
The combination therapy of LVD and ADV is an effective treatment for LVD-resistant patients. (Level 1b, grade B.)

INTERFERON THERAPY FOR CHRONIC HEPATITIS B

INTERFERON (IFN) WAS the first antiviral treatment approved for chronic HBV infection. IFN-α and -β have a predominantly antiviral effect but also have an immunomodulatory effect and antiproliferative effect which is in contrast to direct antiviral agents such as nucleos(t)ide analogues. The duration of treatment is defined (usually 24–48 weeks) in IFN therapy. This finite duration of therapy is an advantage over direct antiviral agents which are usually given indefinitely. The long-term outcome of therapy is more precisely described in IFN compared to LVD due to its longer history of clinical usage.

Selection of patients

Factors associated with favorable response to IFN therapy are vigorously studied (Table 3). For HBeAg positive patients, high pretreatment ALT levels,[166] high grade of necroinflammation on liver histology and low serum HBV DNA level have consistently been shown to be predictive of favorable response.[167] Other predictive factors include female sex,[166] younger age,[168,169] and HBV genotype A versus D or B versus C.[169,170] Patients fulfilling these predictors are the best candidates for IFN treatment. For HBeAg negative patients, there is no consistent predictor of response. Adverse events such as severe infection or exacerbations of liver disease were common when IFN was given for decompensated cirrhosis. Thus, patients with decompensated cirrhosis should not be treated with IFN due to a risk of precipitating hepatic failure and fatal complications.[171,172]

Table 3 Predictive factors for response to interferon therapy

Predictive factors	HBeAg positive	HBeAg negative
Race	No correlation	No correlation
Age	No correlation or Younger	No correlation or Younger
Sex	No correlation or Female	No correlation or Female
ALT	Higher level	No correlation or Higher level
Activity	Higher grade	No correlation
Fibrosis	Conflicting	No correlation
HBV DNA titer	Lower titer	No correlation or lower titer
Genotype	A > D, B > C	A > D, B > C
Precore	Conflicting	No correlation
Core promoter	mutant	

ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus.

Recommendation 8
Younger age, high ALT levels, low HBV load, genotype A or B and high inflammatory activity in liver biopsy are predictive of good response to IFN. IFN therapy should be considered in patients fulfilling these predictors. (Level 2a, 2b, grade B.)

Recommendation 9
Interferon should be avoided for patients with decompensated cirrhosis. (Level 4, grade D.)

Standard IFN therapy in HBeAg positive chronic hepatitis B

A meta-analysis of 16 randomized controlled studies have shown that treatment with IFN-α for 16–24 weeks versus an untreated control is associated with higher rate of HBeAg loss (33% vs 12%), HBeAg seroconversion (difference of 18%), undetectable HBV DNA by hybridization or branched chain assay (37% vs 17%), HBsAg loss (7.8% vs 1.8%) and ALT normalization (difference of 23%) (Table 4).[173] A controlled trial has shown that extending therapy for up to 32 weeks in patients who remained HBeAg positive at the end of 16 weeks of therapy improved the rate of HBeAg seroconversion.[174] The durability of HBeAg seroconversion is more than 80%, and even delayed seroconversion could occur in 10–15% of patients 1–2 years after completion of therapy.[175–177] The loss of HBsAg is reported to occur in 12–65% of patients who cleared HBeAg.[175,178] However, this is a rare event in Asian patients.[176,177]

Consensus statement 10
10-1 In HBeAg positive patients, treatment with IFN versus untreated control is associated with higher rate of HBeAg loss, HBeAg seroconversion, undetectable HBV DNA, HBsAg loss and ALT normalization. Extension of therapy improves the rate of HBeAg seroconversion. (Level 1a,1b.)
10-2 Durability of HBeAg seroconversion is more than 80%. The loss of HBsAg is rare in Asian patients. (Level 1b.)

Standard IFN therapy in HBeAg negative chronic hepatitis B

Although the rate of response at the end of therapy is 60–90%, the durability of long-term response is less

Table 4 Standard interferon therapy for HBeAg positive chronic hepatitis B. Meta-analysis of 16 randomized controlled trials

	Interferon		Control	*P*-value
Loss of HBV DNA	37%		17%	0.0001
Loss of HBeAg	33%		12%	0.0001
Loss of HBsAg	7.8%		1.8%	0.001
Seroconversion		Difference of 18%		0.002
ALT normalization		Difference of 23%		0.0001

ALT, alanine aminotransferase; HBeAg, hepatitis B e-antigen; HBV, hepatitis B virus.

than 50%.[179,180] Longer duration of therapy is associated with improved durability of response: 10–15% with 4–6 months of therapy, 22–30% with 6–12 months of therapy and 30% with 24 months of therapy.[181–184]

Consensus statement 11

11-1 Durability of response is less than 50% in HBeAg negative patients. (Level 1b.)

11-2 Longer duration of therapy (>48 weeks) is associated with improved durability of response. (Level 2b.)

Pegylated IFN (PEG IFN)

Twenty four weeks of PEG IFN-α-2a monotherapy had higher rate of combined response (loss of HBeAg, suppression of HBV DNA <500 000 copies/mL and ALT normalization) compared to standard IFN-α-2a.[185] Another study with 24 weeks of PEG IFN-α-2b monotherapy also showed a higher rate of HBeAg loss and HBV DNA suppression compared to standard IFN-α-2b.[169]

Controlled studies comparing the 48 weeks of PEG IFN-α-2a and LVD in HBeAg positive and negative patients revealed that PEG IFN had a higher rate of sustained response.[170,171] Seroconversion of HBeAg (32% vs 19%), ALT normalization (41% vs 28% in HBeAg positives and 59% vs 44% in HBeAg negatives), HBV DNA suppression (HBV DNA <10 000 copies/mL, 32% vs 22% in HBeAg positives; HBV DNA <20 000 copies/mL, 43% vs 29% in HBeAg negatives) and negative HBV DNA (14% vs 5% in HBeAg positives and 19% vs 7% in HBeAg negatives) were more frequent in PEG IFN treated patients.

Differences were reported in outcome of the antiviral treatment of patients infected with different genotypes; genotype B is associated with a higher rate of antiviral response to IFN treatment than HBV genotype C among Asian patients with HBeAg positive chronic hepatitis B.[169,186,187] In multicenter trials comparing combination therapy of PEG IFN-α-2b and LVD versus PEG IFN-α-2b alone, it was shown that treatment with PEG IFN-α-2b is the best therapy to achieve HBsAg clearance in patients with genotype A compared with D.[188,189]

Combination or sequential therapy

Combination of two antiviral agents with different mechanisms of action seems a logical approach to improve efficacy. In fact, simultaneous combination of LVD and PEG IFN has a higher rate of HBV suppression, ALT normalization and less frequent emergence of LVD-resistant mutant virus compared to LVD alone. However, there is no difference in treatment response between the simultaneous combination of LVD and IFN or PEG IFN compared to IFN or PEG IFN alone (Table 5).[132,133,170]

There are several clinical trials of sequential therapy with LVD followed by IFN.[190–194] Common to all studies is that the sequential therapy had no advantage over IFN alone. Some studies have shown the suggestive evidence that sequential therapy had a higher rate of HBV suppression, ALT normalization and less frequent emergence of LVD-resistant mutant virus compared to LVD alone (Table 5).[190–194] However, because the study protocols and their results are variable, a conclusive result could not be drawn.

Table 5 Sequential therapy of lamivudine and interferon

		BR	SC	VR	LVD-R
Manesis *et al.* 2006	Sequential	39%	NA	28%	
($n = 36$)[190]	IFN	22%	NA	19%	
Shi *et al.* 2006	Sequential	53%	NA	14%	0%
($n = 162$)[191]	LVD	36%	NA	18%	23%
Yurdaydin *et al.* 2005	Sequential	51%	NA	54%	24%
($n = 78$)[193]	LVD	41%	NA	59%	53%
Sarin *et al.* 2005	Sequential	40%	40%	40%	15%
($n = 75$)[194]	LVD	14%	11%	16%	8%
Schalm *et al.* 2000	Sequential	50%	36%	55%	0%
($n = 226$)[192]	IFN	50%	22%	49%	0%
	LVD	63%	19%	63%	31%

BR, biochemical response; IFN, interferon; LVD, lamivudine; LVD-R, lamivudine resistant mutation; NA, not applicable because hepatitis B e-antigen patients are studied; SC, seroconversion; VR, virological response.

Long-term outcome

The end-point of antiviral therapy is to prevent liver cirrhosis and HCC. Meta-analysis of five studies including 935 patients revealed that IFN treatment significantly decreased the incidence of cirrhosis with the combined risk ratio of 0.65 (95% confidence interval [CI] = 0.47–0.91).[195] Meta-analysis of 11 studies including 2082 patients revealed that IFN treatment significantly decreased the incidence of HCC with the combined risk ratio of 0.59 (95% CI = 0.43–0.81).[195] These results suggest that IFN prevents progression of liver disease to liver cirrhosis or delays the development of HCC, as long as it is within 4–7 years of follow up which is the length of follow up in these studies. Sustained response to IFN therapy was associated with increased survival.[175,181,196,197] To further elucidate the impact of IFN on the natural course of chronic hepatitis B, studies with larger populations followed for longer periods may be needed.

Consensus statement 12

12-1 IFN therapy prevents progression to cirrhosis or the development of HCC. (Level 1a.)

12-2 IFN therapy is associated with improved survival. (Level 1b.)

Adverse effects

The most frequent adverse effects are flu-like symptoms, fatigue, myelosuppression and dermal reaction at the injection site. Others include alopecia, depression and thyroid dysfunction. Less frequent but severe adverse events include interstitial pneumonitis, exacerbation of underlying autoimmune disorders, cerebral vascular events and flare of hepatitis.

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Original Article

Serum chemokine levels are associated with the outcome of pegylated interferon and ribavirin therapy in patients with chronic hepatitis C

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Aim: Serum chemokine levels and amino acid substitutions in the interferon-sensitivity determining region (ISDR) and core region have been associated with treatment outcome of pegylated interferon and ribavirin therapy in genotype 1 hepatitis C virus (HCV)-infected patients. The present study was conducted to clarify the association between serum chemokines and treatment outcome in patients with chronic HCV-1 infection in a Japanese cohort.

Methods: A total of six serum chemokines were quantified before, during and after pegylated interferon and ribavirin treatment in 79 genotype 1 chronic HCV patients using a multiple bead array system. Viral ISDR and core region variants were determined by direct sequencing.

Results: The baseline serum levels of eotaxin, IP-10 and RANTES were significantly higher in chronic HCV patients than in controls. High levels of eotaxin and macrophage inflammatory protein (MIP)-1β before therapy and more than two mutations in the ISDR were associated with a sustained virological response, and patients with more than two mutations in the ISDR also had significantly higher MIP-1β levels. Receiver–operator curve analysis showed a 77% sensitivity and 73% specificity for predicting an SVR using MIP-1β values.

Conclusion: Serum MIP-1β levels may predict the response to HCV treatment with pegylated interferon and ribavirin and are associated with amino acid substitutions in the ISDR.

Key words: chemokines, core, interferon sensitivity determining region, MIP-1β, pegylated interferon, ribavirin

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is a major cause of chronic liver disease that leads to liver cirrhosis and/or hepatocellular carcinoma (HCC).[1] HCC is ranked fourth in men and fifth in women as a cause of death from malignant neoplasms in Japan.[2,3] Interferon (IFN)-based therapy can achieve HCV eradication and decrease the risk of HCC to improve prognosis; with pegylated (PEG) IFN and ribavirin therapy, approximately 50% of patients with genotype 1 HCV infection achieve a sustained virological response (SVR).[4,5]

Chemokines and their receptors play an important role in the pathogenesis of HCV infection.[6,7] Despite the growing amount of published research supporting the complex interactions of these inflammatory biomarkers in the outcome of antiviral therapy, the majority of recent studies have nearly exclusively concentrated on only one or a few markers. Thus, it is possible that a test evaluating several biomarkers may prove to be of greater value in predicting responses to therapy.

In the present study, we sought to determine the levels of six chemokines in patients with chronic HCV-1b

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infection who underwent treatment with PEG IFN and ribavirin using a broad-spectrum bead-based multiplex immunoassay.

METHODS

Subjects

A TOTAL OF 79 treatment-naïve patients with chronic hepatitis C (40 men and 39 women; median age 60 years [range: 17–74]) were seen at Shinshu University Hospital or its affiliated hospitals in the Nagano Interferon Treatment Research Group. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (γ-GTP) were tested using standard methods.[8] All patients, who were infected with genotype 1b HCV, received PEG IFN-α-2b (PegIntron; Schering-Plough, Tokyo, Japan; 1.5 μg/kg of bodyweight) and ribavirin (Rebetol; Schering-Plough; 600–1000 mg/day) adjusted to bodyweight for 48 weeks as described previously.[9] The pretreatment median value for ALT was 54 IU/L (range: 22–389), AST was 44 IU/L (range: 20–288) and HCV RNA was 1700 KIU/mL (range: 11–5100), as measured by COBAS AMPLICOR assays (Roche Diagnostic Systems, Tokyo, Japan). A group of 26 healthy individuals (13 men and 13 women; mean age 54 years [range: 28–60]) with hepatitis B and C negative serologies and normal transaminases were used as the control. All patients and controls were negative for the antibody to HIV. The protocol of this study was approved by the ethics committee of Shinshu University School of Medicine and all patients provided written informed consent. All serum samples were immediately stored at −70°C and remained in storage until testing.

Definition of treatment outcome

An SVR was concluded in those whose serum HCV RNA was undetectable 24 weeks after completing therapy. Post-treatment relapse was defined as the reappearance of HCV RNA in the serum after treatment in patients whose HCV RNA was undetectable during or at the completion of therapy. A non-response was defined as a decrease in HCV RNA to less than 2 log copies/mL at week 12 and detectable HCV RNA during the treatment course.

Detection of amino acid substitutions in core and interferon-sensitivity determining regions (ISDR)

The sequences of 1–191 amino acids (a.a.) in the core protein and 2209–2248 a.a. in the NS5A region of genotype 1b HCV were determined by direct sequencing using stored serum samples obtained before therapy, as reported previously. Nucleotide and a.a. sequences were compared with the nucleotide sequences of genotype 1b HCV-J.[10] Substitutions of a.a.70 arginine (Arg70) and glutamine (Gln70) or a.a.91 leucine (Leu91) and methionine (Met91)[11] and the number of a.a. substitutions in the ISDR were defined as wild-type (0), intermediate-type (1) and mutant-type (≥2).[12] Of the 79 patients, 75 were determined to have substitutions at a.a.70 and a.a.91, and 76 could be sequenced for their ISDR.

Detection of chemokines

Six chemokines (macrophage inflammatory protein [MIP]-1α, MIP-1β, eotaxin, IP-10, RANTES and interleukin [IL]-8) were quantified using Luminex Multiplex Cytokine Kits (Procarta Cytokine assay kit; Panomics, Fremont, CA, USA) from serum samples obtained before the start of treatment, 4 weeks after the start of treatment and 24 weeks after the completion of treatment, according to the manufacturer's instructions.[13]

Statistical analysis

The Mann–Whitney *U*-test and Kruskal–Wallis test were used to analyze continuous variables as appropriate. The Wilcoxon rank sum test and the Friedman test were used to evaluate changes in serum chemokine levels over time. Spearman's rank correlation coefficients were used to evaluate the relationship between each pair of markers. The χ^2-test with Yate's correction was used for the analysis of categorical data. In cases where the number of subjects was less than 5, Fisher's exact test was used. $P \leq 0.05$ was considered significant. To predict treatment outcome, each cut-off point for continuous variables was determined by receiver–operator curve (ROC) analysis. Statistical analyses were performed using SPSS ver. 18.0J.

RESULTS

OF THE 79 patients receiving PEG IFN and ribavirin therapy, 31 (39%) achieved an SVR, 23 (29%) relapsed, and 25 (32%) did not respond to treatment and were termed null viral responders (NVR). When stratified into three groups based on treatment outcome, patients with an NVR had a higher female ratio ($P = 0.030$) (Table 1). Before treatment, the median AST and γ-GTP levels in the SVR group were significantly lower than those in the relapsed and NVR groups. Substitutions of a.a.70 in the core region