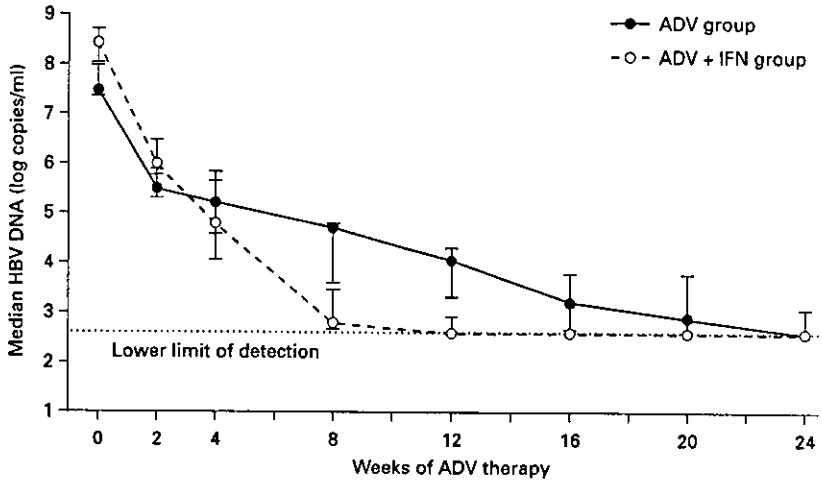

Fig. 1. Median change in serum HBV DNA levels after administration of ADV in the ADV group (continuous line) and ADV + IFN group (dashed line).

Table 1. Clinical and virological profiles of the 14 patients

	ADV group (n = 10)	ADV + IFN group (n = 4)
Time on treatment, weeks	36 (7–56)	38 (24–44)
Age, years	51 (26–57)	39 (36–50)
Gender (M/F)	8/2	4/0
Liver disease (CH/LC)	6/4	4/0
HBV genotype (B/C/unknown)	1/8/1	0/4/0
HBeAg (positive/negative)	8/2	2/2
HBV DNA, log copies/ml	7.4 (6.6–8.3)	8.5 (6.9 to <8.7)
YMDD motif mutant (I/V/I + V)	3/1/6	2/1/1
Baseline AST, × ULN	2.5 (1–7.92)	23.9 (6.52–35.2)
Baseline ALT, × ULN	2.76 (0.46–6.86)	23 (11.5–32.6)
Baseline T-bilirubin, mg/dl	0.7 (0.3–16.9)	2.6 (0.7–4)

Values are shown as the median with the range in parentheses or as the number of patients.

CH = Chronic hepatitis; LC = liver cirrhosis; AST = aspartate aminotransferase. YMDD mutant = codon M552I, M552V, M552I + M552V.

patient in the ADV group had already developed decompensated cirrhosis and had a serum bilirubin concentration of 16.9 mg/dl before adefovir treatment, and died from hepatic failure at week 6.

Virological Response

The median HBV DNA level at baseline was 7.4 log copies/ml in the ADV group and 8.5 log copies/ml in the ADV + IFN group. Serum HBV DNA concentrations diminished in all patients in both the ADV and ADV + IFN groups after ADV therapy. The log change in HBV DNA is shown in figure 1 and table 2. At week 8, serum HBV DNA levels had decreased by a median of 2.75 log copies/ml in the ADV group and by 5.6 log copies/ml in the ADV + IFN group. HBV DNA levels decreased earlier in the ADV + IFN group than in the ADV group until week 8. At week 24, serum HBV DNA levels had significantly decreased from baseline by a median of over 4.8 log copies/ml in the ADV group and by over 5.9 log copies/ml in the ADV + IFN group. By week 24, 55.6% of patients in the ADV group and 100% of those in the ADV + IFN group achieved serum levels of HBV DNA below the limit of detection by PCR assay. Loss of HBeAg was achieved in one patient of the ADV group but in no patients of the ADV + IFN group. However, no patients had undergone HBeAg seroconversion by week 24. There was no difference in the extent of decrease of HBV DNA level in one patient with HBV genotype B compared to the other patients with genotype C.

Biochemical Response

The median ALT levels at baseline were 132 IU/l in the ADV group and 1,152 IU/l in the ADV + IFN group, which were significantly different. The median change in serum ALT levels in the ADV group is shown in figure 2a, excluding one patient treated with corticosteroid. Serum ALT levels decreased immediately until week 4 and normalized until week 24 in 75% (6/8) of patients. Table 2 shows the median change in serum biochemical mark-

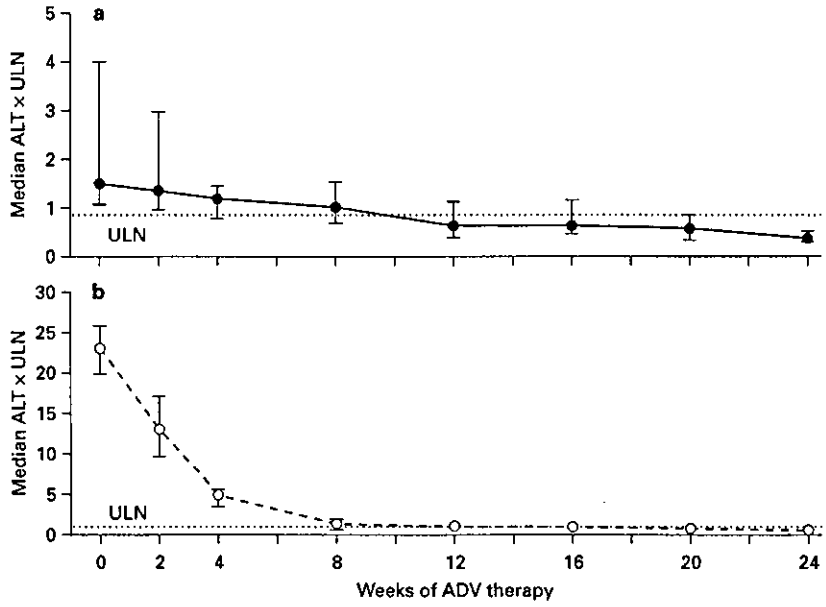

Fig. 2. Median change in serum ALT levels after administration of ADV in the ADV group (**a**) and ADV + IFN group (**b**).

Table 2. Median changes in serum virological and biochemical markers from baseline to week 24

	Baseline	Week 24	Median change from baseline	p value
ADV group (n = 10)				
HBV DNA, log copies/ml	7.4 (6.6–8.3)	<2.6 (<2.6–3.8)	>–4.8	0.008
HBeAg positive, %	80 (8/10)	77.7 (7/9)	–	–
Serum ALT, × ULN	1.47 (0.46–6.86)	0.42 (0.3–2.78)	–1.05	0.012
Serum bilirubin, mg/dl	0.7 (0.3–16.9)	0.75 (0.3–1.4)	0.05	NS
Serum albumin, g/dl	3.5 (2.9–4.0)	4.05 (2.9–4.5)	0.55	NS
ADV + IFN group (n = 4)				
HBV DNA, log copies/ml	8.5 (6.9 to <8.7)	<2.6 (2.6–2.6)	>–5.9	0.068
HBeAg positive, %	50 (2/4)	50 (2/4)	–	–
Serum ALT, × ULN	23 (11.5–32.6)	0.63 (0.38–0.8)	–22.3	0.068
Serum bilirubin, mg/dl	3.1 (0.7–4)	0.4 (0.3–0.6)	–2.7	0.068
Serum albumin, g/dl	3.6 (3.2–3.8)	3.6 (3.5–4.1)	0	NS

Values are shown as the median with the range in parentheses. NS = Not significant.

ers from baseline to week 24, including serum ALT, bilirubin and albumin. The median decrease in ALT levels from baseline to week 24 was –1.05 times the ULN, and was significant at week 24 compared with baseline (p = 0.012). The median changes in serum bilirubin and albumin levels from baseline were not significant at week 24.

The median change in serum ALT levels in the ADV + IFN group is shown in figure 2b. Serum ALT levels decreased immediately and normalized until week 20 in all patients. The median decrease in ALT levels from baseline to week 24 was –22.3 times the ULN, and the median decrease in the serum bilirubin level was –2.7 mg/dl. However, the change was not statistically significant probably because of the small sample size. The median

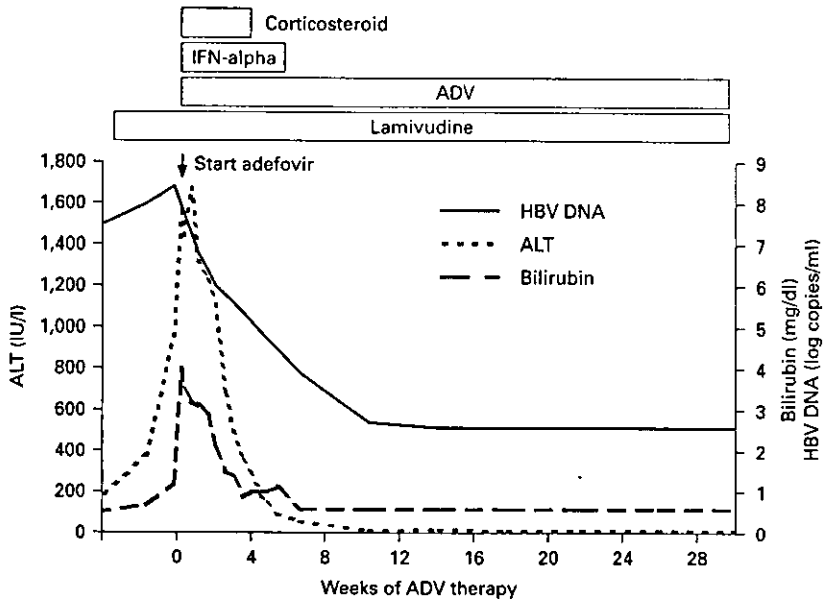

Fig. 3. Clinical course of one patient in the ADV + IFN group who presented with HBeAg-negative chronic hepatitis B and developed severe acute exacerbation due to breakthrough hepatitis following ADV therapy, IFN and corticosteroid combination therapy in addition to lamivudine. HBV DNA (continuous line), ALT (dotted line) and bilirubin (dashed line) responses are plotted at various time points from the commencement of ADV.

change in albumin levels from baseline was not significant. Three of 4 patients in the ADV + IFN group were treated with corticosteroid, and it seemed that not only ADV and IFN but also corticosteroid had a significant impact on the reduction of ALT levels.

Safety

None of the 14 patients complained of drug-associated symptoms, and ADV was generally well tolerated. One patient in the ADV + IFN group had transient increases in ALT levels until week 2, after which they rapidly decreased (fig. 3). This event seemed to be due to the influence of IFN and not ADV. None of the 14 patients had significant changes in any additional biochemical markers of liver function. There were no significant changes in blood cell count, serum creatinine, urea nitrogen or phosphate. All patients have continued on treatment with ADV to the present time, except for one patient in the ADV group who had already developed decompensated cirrhosis and had serum bilirubin levels of 16.9 mg/dl before treatment with ADV, and who died of hepatic failure at week 6.

In the ADV + IFN group, serum bilirubin levels in 3 of 4 patients were elevated in parallel with ALT levels, and the range of bilirubin levels at baseline in these 3 patients was 2.5–4.0 mg/dl over the ULN (ULN = 1.1 mg/dl). Furthermore, their prothrombin times were extended, and they were considered at risk of progressing to hepatic decompensation. In this context, we administered IFN-alpha and corticosteroid at the same time as adefovir therapy for these patients. IFN-alpha and corticosteroid were administered until the virological and biochemical relapses were controlled. ALT and bilirubin levels rapidly decreased, and hepatic decompensation did not occur.

Figure 3 shows the clinical course of a patient in the ADV + IFN group. The patient was a 39-year-old man who presented with HBeAg-negative chronic hepatitis B and who developed severe acute exacerbation due to breakthrough hepatitis following ADV therapy. He was commenced on lamivudine monotherapy, and his HBV DNA became negative by PCR assay. However, he developed breakthrough hepatitis 22 months after commencement of lamivudine therapy, and was treated with IFN for breakthrough hepatitis. The HBV DNA and ALT levels were reelevated, and he developed severe acute exacerbation 35 months after lamivudine therapy, despite showing a transient response to IFN for the breakthrough hepatitis. He was treated with ADV, IFN-alpha and corticosteroid, as shown in figure 3. His HBV DNA and ALT levels normalized at week 12 and week 8, respectively. IFN-alpha and corticosteroid were withdrawn for 4–6 weeks. He remains on a good course on ADV alone added on to lamivudine.

Discussion

Emergence of lamivudine-resistant HBV mutants is a frequent and significant therapeutic problem. Clinical deterioration in patients with ALT flares has been reported in some studies; furthermore, severe acute exacerbations of hepatitis due to lamivudine-resistant mutants have also been reported [3, 9, 12, 22, 23]. These exacerbations were accompanied by hepatic decompensation and resulted in mortality in some cases [23]. This is the reason it is important to manage lamivudine-resistant mutants. ADV has antiviral activity against both wild-type HBV and lamivudine-resistant HBV mutants [17, 20, 21]. Our pilot study showed that ADV add-on to lamivudine decreased HBV DNA levels and ALT levels, as in previous studies [20, 29, 30]. In this study, ALT levels of patients in the ADV group were moderately elevated (median 2.76 times the ULN), and serum ALT levels decreased after administration of ADV. Add-on of ADV alone to lamivudine in patients who had slight or moderate exacerbation and did not take other nucleoside analogues, such as anti-HIV agents, was effective. This therapy was expected to improve the necroinflammatory state in the liver due to lamivudine-resistant mutants, because our study did not detect the elevation of ALT levels after administration of ADV that was reported previously by Benhamou et al. [21].

Marked elevation of serum ALT levels after emergence of lamivudine-resistant mutants has been observed and sometimes leads to complications such as ascites and/or jaundice and hepatic decompensation [22, 23]. In the present study, severe acute exacerbations of hepatitis (ALT >10 times the ULN) were observed in 4 patients of the ADV + IFN group. We administered IFN add-on to adefovir for those 4 patients, and in addition, corticosteroid for 3 of the patients with elevated bilirubin, to prevent hepatic decompensation. IFN has multiple sites of action in the viral life cycle, independent of the reverse transcriptase activity of HBV [31]. IFN has been reported to have antiviral effects against lamivudine-resistant mutants. Suzuki et al. [12] reported that daily IFN therapy was more effective than 3-times-weekly IFN therapy against lamivudine-resistant mutants. We used short-term daily IFN therapy as reported previously, as an add-on to ADV for about 4 weeks. The decrease of HBV DNA levels from baseline occurred earlier in the ADV + IFN group than in the ADV group until week 8 (fig. 1), and ALT levels were rapidly normalized (fig. 3). Consequently, all patients in the ADV + IFN group were rescued from severe acute exacerbation caused by lamivudine-resistant mutants. Because this study was not controlled and the background of patients in the ADV + IFN group differed from that of the ADV group, we could not definitively document that ADV and short-term IFN-alpha combination therapy had additive effects against lamivudine-resistant mutants. However, we consider that adefovir and short-term IFN-alpha combination therapy may become a therapeutic option for severe acute exacerbation of hepatitis caused by lamivudine-resistant mutants.

ADV is effective against lamivudine-resistant mutant HBV polymerases in vitro to an extent comparable to its effectiveness against wild-type polymerases [17]. Several clinical trials have shown that adefovir had antiviral effects against both wild-type HBV and lamivudine-resistant mutants [18–21, 29, 30]. Consistent with previous studies, we found that ADV administration in addition to lamivudine decreased HBV DNA levels and improved biochemical markers; however, we did not evaluate the effects of switching from lamivudine to ADV. Peters et al. [30] reported preliminary data showing that there was no difference in the decrease of HBV DNA between lamivudine plus ADV and ADV alone. Recently, Angus et al. [32] detected the emergence of a mutant resistant to ADV in domain D of the HBV polymerase at week 80 after ADV treatment. Moreover, they reported that lamivudine therapy was effective against this ADV-resistant mutant. However, it appears that, at least in the first year of treatment, resistance to ADV is less common than resistance to lamivudine [10, 33]. It is important to prevent emergence of resistant mutants to antiviral drugs and disease progression in the treatment of chronic hepatitis B. Based on these findings, and because ADV and lamivudine combination therapy can make up for a weak point, this combination therapy is potentially useful for treatment of patients who need long-term antiviral therapy. It will be necessary to assess current ongoing clinical trials of antiviral drug combination therapy.

Westland et al. [34] showed that ADV therapy resulted in potent reductions in serum HBV DNA with no significant differences due to genotype or race in worldwide phase III studies. Almost all patients treated with ADV had HBV genotype C. In contrast to previous studies, there was no difference in the efficacy of ADV based on HBV genotype.

In conclusion, administration of ADV add-on to lamivudine for patients with breakthrough hepatitis caused by lamivudine-resistant mutants reduced HBV DNA and ALT levels. For patients with severe acute exacerbation of hepatitis due to lamivudine-resistant mutants, early use of ADV and IFN (and corticosteroid) add-on to lami-

vudine could prevent a fatal course. This was a pilot trial, and the number of patients was small. It is necessary to undertake large-scale, long-term and/or controlled trials to confirm that treatment with adefovir and IFN add-on to lamivudine is effective. Moreover, clinical trials will be necessary to evaluate the long-term prognosis of patients with breakthrough hepatitis.

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Sustained Negativity for HCV-RNA over 24 or More Months by Long-Term Interferon Therapy Correlates with Eradication of HCV in Patients with Hepatitis C Virus Genotype 1b and High Viral Load

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Key Words
Chronic hepatitis C · Long-term interferon therapy · HCV-genotype 1b

Abstract
Objective: We assessed whether sustained negativity for HCV-RNA over 24 or more months by long-term interferon (IFN) therapy correlates with eradication of HCV in patients with hepatitis C virus genotype 1b and high viral load or not. ***Methods:*** The number of patients with HCV-genotype 1b and high viral load exceeding 1 Meq/ml who received 6 MU of natural IFN-α daily for 2–8 weeks, followed by three times/week for 16–22 weeks and negativity for HCV-RNA during IFN administration was 403. Forty-one of 403 patients received 6 MU of natural IFN-α three times/week for more than 18 months after the initial IFN therapy (long-term-IFN-group). Three hundred and two patients did not receive any IFN treatment for 6 months after the termination of the 6-month course (6-month-IFN-group). Sustained virological response (SVR) was defined as negative HCV-RNA at both 3 and 6 months after the completion of IFN therapy. ***Results:*** SVR was noted in 73.2% (30/41) of long-term-IFN-group and 18.2% (55/302) of 6-month-IFN-group. Multivariate analysis showed that long-term IFN therapy was the most significant contributor to SVR ($p < 0.0001$). ***Conclusion:*** Sustained negativity of HCV-RNA for 24 or more months by long-term IFN therapy correlated with SVR in patients with genotype 1b and high viral load.

Introduction

Chronic hepatitis C is a slowly progressive liver disease that could evolve into liver cirrhosis or hepatocellular carcinoma (HCC) [1–3]. It has been reported that clearance of hepatitis C virus (HCV) or normalization of serum alanine aminotransferase (ALT) after interferon (IFN) therapy contribute to the notably suppressed incidence of HCC caused by chronic HCV infection [4–14]. Previous studies have identified various factors that could predict the response to IFN, including a high response (e.g. low HCV RNA level, HCV genotype 2a, short duration of the disease, and absence of cirrhosis) and low response (e.g., high

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HCV RNA level and genotype 1b) [15–18]. In Japan, approximately 50–60% of patients with chronic HCV infection exhibit genotype 1b and have a high level of serum HCV-RNA (>1 mega equivalents/ml, Meq/ml) [19].

Recent studies demonstrated the positive effects of new treatments for HCV infection, such as the combination of IFN-ribavirin, IFN-alfacon-1 (consensus IFN), and pegylated-IFN [20–27]. In Japan, however, the HCV-RNA clearance rates by these new treatments were at most about 20–50% in patients with HCV-genotype 1b and a high virus load. Despite the low HCV clearance rate by IFN therapy in patients with genotype 1b and high level of serum HCV-RNA, the serum level of HCV-RNA is often negative when determined by reverse transcription nested polymerase chain reaction (RT-nested PCR) during IFN administration. However, many patients relapse after termination of IFN therapy. Recently, several centers reported an increase in the frequency of responders among patients on prolonged IFN therapy [28–32].

The present study was designed to further clarify this point, focusing specifically on the efficacy of prolonged IFN therapy in patients with genotype 1b and a high viral load. That is, we conducted this clinical trial to determine the significance of an additional long-term course of IFN in patients who were HCV-RNA negative during a 6-month course of IFN therapy. A retrospective study was used to examine the efficacy of prolonged IFN therapy in the present trial.

Methods

Patients and Treatment Protocol

A total of 403 patients with chronic hepatitis C satisfied the following conditions in our hospital from 1993 to 2000: (1) Who had HCV-genotype 1b and serum HCV-RNA levels greater than the ≥1 Meq/ml as determined by the branched DNA probe assay (version I or II) before IFN therapy. (2) Who had average ALT greater than the upper normal limit (ALT normal range, 12–50 IU) for more than 3 months before the initial course of IFN treatment. (3) Who had histological evidence of chronic hepatitis within 1 year before the IFN administration. (4) Who hadn't been given corticosteroids, immunosuppressants, or antiviral agents used within 6 months before IFN therapy. (5) Who received 6 MU of natural IFN-α (human lymphoblastoid IFN; Sumitomo pharmaceuticals, Tokyo, Japan) intramuscularly daily for 2–8 weeks, followed by three times/week for 16–22 weeks and had negative serum HCV-RNA level by RT-nested PCR [33] during the 6-month course of IFN therapy. (6) Who were negative for hepatitis B surface antigen (HBsAg) or hepatitis virus DNA (HBV-DNA) in the serum, as determined by radioimmunoassay and spot hybridization. (7) Who were negative for antinuclear antibodies (ANA) or antimitochondria antibodies (AMA) in the serum as determined by immunofluorescence on rat liver and kidney.

Patients with the following conditions were excluded from the study: (1) HCC or severe liver failure; (2) pregnant women; (3) febrile patients with leukocyte counts $<3 \times 10^3$ cells/μl and/or platelet count $<1 \times 10^5$/μl; (4) patients with renal disorders; (5) patients with past history of hypersensitivity reactions to biological preparations such as vaccines.

Forty-one of these 403 patients received continuous treatment of 6 MU of natural IFN-α three times weekly for more than another 18 months after the initial 6-month IFN therapy (long-term IFN group). In long-term IFN group, the initial course of IFN treatment consisted of 6 MU of natural IFN-α given according to one of two schedules. In 31 patients, the daily natural IFN-α was administered for 8 weeks, followed by IFN three times a week for 16 weeks. In another ten patients, the daily natural IFN-α was administered for 2 weeks, followed by IFN three times a week for 22 weeks. Three hundred and two of 403 patients did not receive any IFN treatment for 6 months after the termination of the 6-month course (6-month IFN group). In 6-month IFN group, the initial course of IFN treatment consisted of 6 MU of natural IFN-α given according to one of three schedules. In 245 patients, the daily natural IFN-α was administered for 8 weeks, followed by IFN three times a week for 16 weeks. In another 6 patients, the daily natural IFN-α was administered for 4 weeks, followed by IFN three times a week for 20 weeks. In the third group of 51 patients, the daily natural IFN-α was administered for 2 weeks, followed by IFN three times a week for 22 weeks. The remaining 60 of 403 patients were continuously treated with IFN after the initial 6-month course of IFN treatment. However these patients did not receive continuous treatment of 6 MU of natural IFN-α three times weekly for more than another 18 months after the initial 6-month IFN therapy. The study protocol of this clinical trial is shown in figure 1. The physicians in charge explained the purpose and method of the clinical trial, as well as potential adverse reactions, to each patient and informed consent for participation was obtained from all patients. The clinical trial commenced in December 1993 and ended in May 2000. All patients were followed-up monthly for at least 6 months after cessation of IFN therapy, and blood samples were taken during each visit. The criterion of termination of long-term IFN therapy was defined as the attainment of constantly negative HCV-RNA for the period of more than 24 months during IFN therapy. Termination of long-term IFN therapy in the former group was decided by conference between the physician in charge and each patient.

Definition of Response to IFN Therapy

The presence or absence of HCV-RNA and improvement of serum ALT concentrations were evaluated both at 3 and 6 months after cessation of long-term IFN treatment using the following three grades: Sustained virological response (SVR) was defined as negative HCV-RNA by RT-nested PCR at both 3 and 6 months after the completion of long-term IFN therapy. Biochemical response (BR) was defined as normalization of serum ALT in the presence of positive HCV-RNA by RT-nested PCR at both 3 and 6 months after cessation of long-term IFN therapy. Non-response (NR) was applied to patients who did not show SVR or BR.

Blood Testing

Blood samples were obtained just before therapy and stored at −80°C until assayed. Serum ALT concentrations were measured at least once per month for 3 months prior to the initiation of long-term

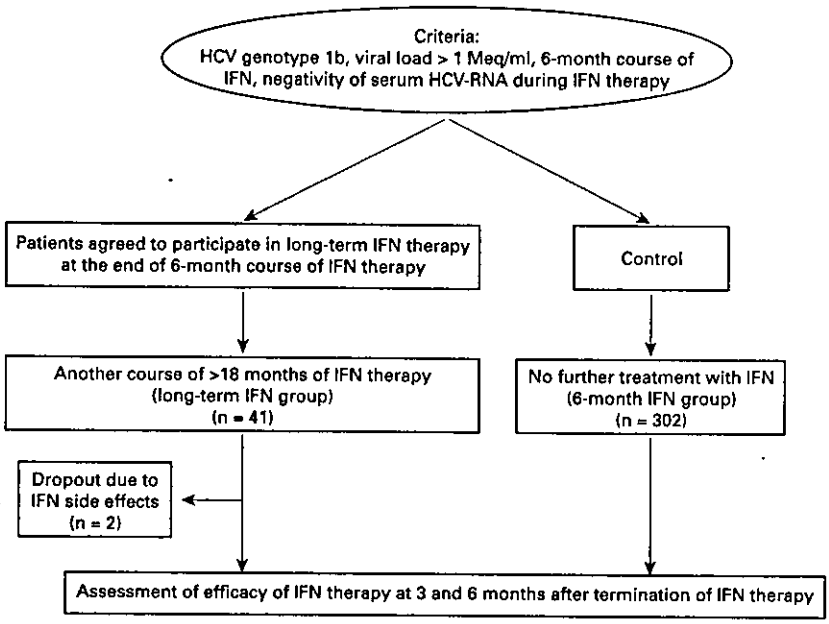

Fig. 1. Study protocol and treatment groups.

IFN therapy, one to four times per month during long-term IFN therapy, and once or twice per month thereafter. HCV-RNA levels before IFN therapy were analyzed at the same time by a branched DNA probe assay (b DNA probe assay, version 2.0, Chiron, Dai-ichi Kagaku, Tokyo) and the results were expressed in Meq/ml [34]. Blood samples obtained during and after long-term IFN therapy were also tested by the RT-nested PCR.

Histopathological Examination of Liver Biopsy

Liver biopsy specimens were obtained percutaneously or at laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo). The histopathological state was determined using the criteria of Desmet et al. [35]. Baseline liver histology of chronic hepatitis prior to IFN therapy was also classified into three stages based on the extent of fibrosis: mild (periportal expansion), moderate (portoportal septa), and severe (portocentral linkage or bridging fibrosis).

Statistical Analysis

The Fisher's exact test or Mann-Whitney U test was used for comparison of group frequencies as appropriate. The efficacy of IFN treatment was assessed by the intention to treat (ITT) analysis. As for Mann-Whitney U test A, a $p < 0.05$ was considered statistically significant. In the Fisher's exact test, a $p < 0.05$ by the two-tailed test was considered statistically significant. The associated factors for attainment of SVR after IFN therapy were examined by logistic regression analysis. Statistical analyses were performed using the SPSS software package (SPSS Inc., Chicago, Ill., USA).

Results

Baseline Clinicopathological Features of Both Groups

Table 1 summarizes the profiles and laboratory data of patients on long-term IFN therapy and those on 6-month IFN therapy. Both groups had HCV-genotype 1b and serum HCV-RNA level exceeding 1 Meq/ml at entry into the study. There were no significant differences between the two groups with regard to age, sex, liver histology, serum HCV-RNA level, AST, and ALT levels. The rate of IFN retreatment on long-term IFN group was significantly higher ($p < 0.001$) compared with those of 6-month IFN therapy group.

Safety and Tolerability of Prolonged IFN Therapy

Of the 41 patients on prolonged IFN therapy, two (4.9%) stopped the treatment at 9 and 13 months after its commencement due to the appearance of natural IFN-α-related side effects. These included anorexia and general fatigue. These IFN-α-related adverse effects disappeared one month after cessation of long-term IFN therapy. The remaining 39 patients continued to be treated without IFN-related side effects. Four of these 39 patients showed reactivation of HCV-RNA at 6, 7, 10 and 13 months, respectively, on prolonged IFN therapy.

Table 1. Characteristics of patients at study entry

	Long-term IFN group	6-Month IFN group	p value
n	41	302	
Male/female	30/11	196/106	0.162
Age, years*	46 (24–64)	48 (21–67)	0.828
Liver histology staging (F1/F2/F3)	25/12/4	197/99/6	0.378
HCV-RNA, Meq/ml*	9.6 (1.1–54.6)	5.7 (1–64)	0.258
AST, IU/l*	58 (19–366)	59 (16–230)	0.705
ALT, IU/l*	86 (14–699)	96 (16–594)	0.280
History of IFN therapy naïve/retreatment	19/22	261/41	<0.0001
Period of IFN therapy in this study, months	26 (26–68)	6	<0.0001

* Data are expressed as median (range).

Table 2. Effects of long-term IFN therapy examined by the intention to treat analysis

	Outcome of IFN therapy		
	SVR	BR	NR
Long-term IFN group	30/41 (73.2%)	3/41 (7.3%)	8/41 (19.5%)
6-Month IFN group	55/302 (18.2%)	68/302 (22.5%)	179/302 (59.3%)

SVR = Sustained virological response; BR = biochemical response; NR = non-response.

Table 3. Analysis of predictors of sustained virological response (SVR) after IFN therapy

	SVR (n = 85)	Non-SVR (n = 258)	p value
Sex (male/female)	61/24	161/97	0.065
Age, years*	45 (27–64)	47 (21–67)	0.111
Liver histology staging (F1/F2/F3)	60/21/4	162/90/6	0.169
HCV-RNA, Meq/ml*	3.8 (1–29)	6.3 (1–64)	0.009
AST, IU/l*	65 (19–366)	60 (16–144)	0.109
ALT, IU/l*	105 (14–807)	90 (16–594)	0.060
Duration of IFN therapy (>24 months/6 months)	30/55	11/247	<0.0001

* Data are expressed as median (range).

Efficacy of Prolonged IFN Therapy

Table 2 compares the efficacy of IFN in long-term IFN therapy group to that of a 6-month course. The efficacy of IFN therapy was estimated based on ITT analysis. SVR was noted in 30 of the 41 (73.2%) patients on long-term IFN therapy and in 55 of the 302 (18.2%) of the 6-month course of IFN therapy.

Predictive Factors for Virological Response

A total of 85 patients were confirmed to show SVR at 6 months after the completion of IFN therapy. In the next step, we determined the predictive factors for SVR. The following factors were evaluated: age, sex, liver histology, viral load, transaminase, and type of protocol of IFN treatment (table 3). Univariate analysis showed that long-

Table 4. Factors associated with SVR after IFN therapy by multivariate analysis

Factors	Category	Odds ratio	95% CI	p value
Duration of IFN	long term 6 months	11.61	4.50–29.94	<0.0001
HCV-RNA	<5 Meq/ml ≥5 Meq/ml	2.88	1.32–5.87	0.0051

CI = Confidence interval.

term IFN therapy and low level of HCV-RNA were significant factors that contributed to SVR Because the variables were mutually correlated; multivariate logistic regression analysis was performed with two statistically significant variables in the univariate analysis. As a result, the multivariate analysis showed that the period of IFN administration was the most important factor for attaining of SVR. That is, the risk ratio for SVR appearance in patients treated with more than 24 months (long-term treatment group) was 11.61 compared with patients treated with IFN for 6 months (table 4).

Discussion

Many investigators have reported that IFN therapy is effective in reducing serum levels of ALT, reducing/eliminating HCV-RNA level, improving liver histology and reducing the incidence of HCC in patients with chronic hepatitis C [4–14]. However, clearance of HCV-RNA was achieved in only 30–40% of patients who received a 6-month course of IFN therapy. Moreover, in patients with HCV-genotype 1b and a high viral load exceeding 1 Meq/ml by the DNA probe assay, the clearance of HCV-RNA was even lower (achieved in only 10%) [11, 18, 32]. However, genotype 1b is the predominant genotype in Japan [19], similar to many European [36] and Western countries [37]. Therefore, there is a pressing need to develop an effective strategy for the treatment of patients with genotype 1b and a high HCV-RNA viral load.

Recent reports indicated that increases in the total dosage and duration of IFN therapy enhance the therapeutic efficacy of such treatment [28–32]. However, due to the lack of data on the effects of long-term monotherapy, the optimal duration of IFN in patients who initially fail to respond to such treatment is yet to be determined. We examined here the efficacy of long-term IFN therapy in patients who showed HCV-RNA negativity during the cycle of IFN therapy within a 6-month course. Our results showed that attainment of consistently negative serum HCV-RNA for a period of more than 24 months by long-term IFN therapy correlates significantly with SVR. In general, patients with genotype 1b and a high viral load are often negative for HCV-RNA in the serum during IFN therapy. However, the relapse rate of HCV-RNA after a 6-month IFN course is high [11, 18]. In the present study, only a few patients showed relapse of HCV-RNA among those who remained negative for HCV-RNA over more than 24 months during long-term IFN therapy. The above results indicated that patients with a high viral titer of HCV-genotype 1b who become HCV-RNA negative after initiation of IFN therapy are highly likely to show SVR following sustained negativity for HCV-RNA in response to long-term IFN therapy. However, since long-term IFN therapy can be associated with increased chance of development of adverse effects and is costly, selection of patients for long-term IFN therapy is extremely important.

The two-drug regimen of IFN and ribavirin enhances sustained viral response rates. Despite the increased efficacy, such combination therapy is also associated with serious adverse effects, particularly those associated with ribavirin, e.g. anemia, teratogenesis. Therefore, although the combination therapy of IFN and ribavirin is the first choice therapy in patients with genotype 1 and high viral load, viral eradication could be achieved at least in some patients with long-term IFN therapy.

In conclusion, we have demonstrated in the present study that attainment of persistent negativity for HCV-RNA for a period of more than 24 months during long-term IFN therapy correlates significantly with SVR.

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MAJOR ARTICLE

Two Subtypes of Genotype B (Ba and Bj) of Hepatitis B Virus in Japan

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We have previously reported 2 subtypes of hepatitis B virus (HBV) genotype B, one of which has the recombination with genotype C over the precore region plus core gene (Ba) and the other of which does not (Bj). A restriction fragment–length polymorphism method with 2 endonucleases was newly developed for distinguishing between subtypes Ba and Bj and was applied to 313 carriers of HBV genotype B in Japan. Subtype Ba was detected in 38 (12%) and subtype Bj in 275 (88%) of the carriers of HBV genotype B. Hepatitis B e antigen in serum was found more frequently in patients with chronic infection with subtype Ba than in those with chronic infection with subtype Bj (8 [32%] of 25 vs. 25 [9%] of 273; $P < .01$). The new method for distinguishing between Ba and Bj by restriction fragment–length polymorphism would be useful in examining the distribution of these 2 subtypes in situations in which HBV genotype B is prevalent.

Hepatitis B virus (HBV) has been classified into 7 genotypes, designated A to G, by a divergence of >8% in the entire genomic sequence, and these 7 genotypes have characteristic geographic distributions [1–3]. Very recently, an eighth genotype with a provisional designation of 'H' was proposed [4], but its classification as a new genotype or as a subtype of genotype F needs further phylogenetic analyses. There have been increasing lines of evidence for the influence of HBV genotypes in the manifestation of clinical liver diseases in hosts [5–11]. It has been reported that HBV genotype B, compared with genotype C, is associated with earlier seroconversion from hepatitis B e antigen (HBeAg) to the corresponding antibody (anti-HBe) and with lower histological activity scores, and that genotype B is less prevalent than genotype C among patients with cirrhosis. These data indicate that HBV genotype B induces less-active and less-advanced liver disease than does genotype C, although recent reports [12, 13] indicate that there is no difference in long-term outcome between patients infected with genotype B and those infected with genotype C. In Taiwan, however, HBV genotype B is reported to enhance the development of hepatocellular carcinoma (HCC) in individuals younger than 50 years of age [7]; this is not the case in patients of the same age in Japan, however [8]. There is a possibility that such remarkable clinical differences among carriers of HBV genotype B are attributable to virological differences in the HBV strains that are infecting the hosts (including differences even in strains of the same genotype). In addition, host differences and the presence or absence of cofactors may make a difference.

The recombination between HBV genomes of distinct genotypes has been reported [14–17]. It is not known,

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however, how they influence the outcome of HBV infections in hosts. Recently, we reported 2 subtypes of genotype B, one of which possesses the recombination with genotype C over the precore region plus core gene (Ba) and the other of which does not (Bj) [18]. By means of PCR-based restriction fragment–length polymorphism (RFLP) in the precore region, HBV of subtype Bj (HBV/Bj) was found to be endemic in Japan, and HBV of subtype Ba (HBV/Ba) is ubiquitous in the other countries in Asia [19]. Furthermore, in a case control study, HBeAg and core promoter mutation (T1762/A1764) are found to be more frequent in the carriers of HBV/Ba than in carriers of HBV/Bj [19].

Recently, 2 HBV/Bj isolates that produced discordant test results with the previously reported PCR-RFLP [19] were recovered from Japanese HBV carriers and sequenced (GenBank accession nos. AB106884 and AB106885). They possessed adenine as nucleotide (nt) 1838 or an insertion of adenine between nt 1837 and nt 1838 in the *MseI* restriction site used in the PCR-RFLP [19], which may give a false result for subtype Ba in a few HBV isolates of subtype Bj. To distinguish between HBV/Ba and HBV/Bj precisely, therefore, a novel PCR-RFLP method was developed involving 2 single nucleotide polymorphisms (SNPs) in the core region. The method was applied to isolates from 313 Japanese carriers of HBV genotype B to examine geographical and clinical differences between HBV/Ba and HBV/Bj infections in Japan.

MATERIALS AND METHODS

Patients. A total 313 serum samples containing HBV genotype B were obtained from chronic carriers of HBV who visited 7 hospitals that were scattered from the north of the mainland of Japan to its southern islands, where HBV genotype B is prevalent [8]. The hospitals included: Yamagata University Hospital, Yamagata; Tohoku University Hospital, Sendai; Akita City Hospital, Akita; Toranomon Hospital, Tokyo; Tokyo National Hospital, Tokyo; Yamanashi Medical University Hospital, Yamanashi; and Ryukyu University Hospital, Okinawa. Serum samples from each of the hospitals were tested to determine alanine aminotransferase (ALT) and asparate aminotransferase (AST) levels and to determine the presence of HBeAg and anti-HBe using commercial kits (EIA, Dinabot; Tokyo, Japan). The presence of HBV genotype B in the serum samples was determined by ELISA on preS2-region products [20, 21], and the results were confirmed by PCR-RFLP of the S gene [22].

Chronic carriers were classified into 3 groups after they had been followed up for ≥12 months, as follows: (1) the asymptomatic carrier group, defined as noncirrhotic carriers who had no subjective symptoms and who maintained normal serum ALT levels throughout the follow-up period; (2) the chronic hepatitis group, defined as noncirrhotic carriers with ALT levels exceeding the upper limit of normal (defined as 35 U/L); and (3) the liver cirrhosis group, defined as patients with clinical evidence of cirrhosis revealed by ultrasonography (e.g., coarse liver architecture, nodular liver surface, and blunt liver edge) and evidence for hypersplenism (e.g., splenomegaly revealed by ultrasound and a platelet count of <100,000 platelets/mm^3). The diagnosis of acute hepatitis was established by the loss of hepatitis B surface antigen from serum within 6 months after beginning the follow-up period. Patients who were coinfected with hepatitis C virus were excluded, and none had received antiviral treatments during the follow-up period. The study protocol conformed to the 1975 Declaration of Helsinki and was approved by the ethics committees of the institutions, and informed consent was obtained from each HBV carrier.

PCR-RFLP for distinguishing between HBV/Ba and HBV/Bj. Nucleic acids were extracted from 100 μL of serum that had been stored at −40°C using a DNA extractor kit (Genome Science Laboratory). HBV/Ba and HBV/Bj were determined using 2 kinds of PCR-RFLP. They were essentially the same method, but they used different enzymes. One of the methods was described previously and has a target in the precore region [18]. The other method was newly developed and targeted at the core region. In the new method, the first-round PCR was carried out with sense primer (PC1-HBV: 5′-CAT GCA ACT TTT TCA CCT CTG CCT-3′ [nt 1813–1836]) and antisense primer (COR-HBV: 5′-GAG TGC GAA TCC ACA CTC CA-3′ [nt 2285–2266]). The second-round PCR was performed with another sense primer (PC2-HBV: 5′-TGT TCA AGC CTC CAA GCT GTG-3′ [nt 1861–1881]) and COR-HBV. A portion (5 μg) of the amplification product of 425 base pairs (bp) in size was digested with 5 U of *HpaI* and *StuI* at 37°C for 3 h. Digests with *HpaI* and *StuI* were run on electrophoresis in 3.0% (weight/volume) agarose gel, stained with ethidium bromide, and examined for their sizes under the ultraviolet light.

For serum samples producing discrepant results by 2 different PCR-RFLP methods, the precore region plus core gene in the HBV DNA obtained from the samples was sequenced with primers reported previously for confirmation of HBV/Ba or HBV/Bj [23]. The standard precautions for avoiding contamination during PCR were exercised carefully, and a negative control serum sample was included in each run of tests to ensure the specificity.

Statistical analyses. Statistical differences were evaluated using the Mann-Whitney nonparametric test, Fisher's exact probability test, and Student's *t* test, when appropriate. Differences were considered significant for *P* values of <.05.

RESULTS

PCR-RFLP for distinguishing between HBV/Ba and HBV/Bj. When the 70 HBV genotype B isolates retrieved from the DDBJ/

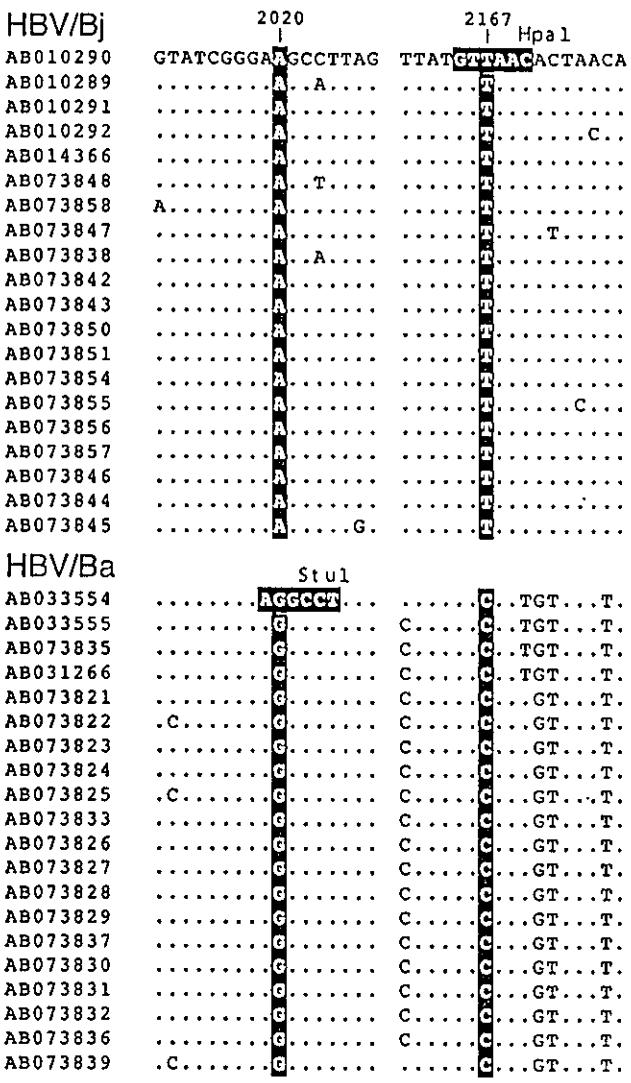

Figure 1. Nucleotide sequences of a part of the core region in 40 hepatitis B virus (HBV) isolates of genotype B. Sequences of 20 HBV isolates of subtype Bj (HBV/Bj) and 20 HBV isolates of subtype Ba (HBV/Ba) are shown, which are representative of 29 HBV/Bj isolates and 41 HBV/Ba isolates reported previously [18]. All of the HBV/Bj isolates possessed nt 2020 of A and nt 2167 of T, which made a part of an *Hpa*I site (GTTAAC); in remarkable contrast, all HBV/Ba isolates possessed nt 2020 of G, which made a part of a *Stu*I site (AGGCCT), and nt 2167 of C.

EMBL/GenBank database were compared over the entire genome, it was found that all of the 29 HBV/Bj isolates possessed nt 2020 of A (A2020) and nt 2167 of T (T2167), creating an *Hpa*I site (GTTAAC [nt 2165–2170]). This was in remarkable contrast to all of the remaining 41 HBV/Ba isolates, which possessed G2020, giving rise to an *Stu*I site (AGGCCT [nt 2019–2024]) in combination with C2167. Sequences of 20 HBV/Bj and 20 HBV/Ba isolates are shown in figure 1. Taking advantage of these 2 SNPs of A or G at nt 2020 and T or C at nt 2167, an RFLP method with 2 endonucleases was developed for distinguishing between subtypes Bj and Ba. PCR products of 425 bp (nt 1861–2285), amplified on HBV/Bj isolates, were split by *Hpa*I digestion into 2 fragments of 306 bp and 119 bp, respectively, and those on HBV/Ba isolates were not. Conversely, the PCR products of 425 bp, amplified on HBV/Ba isolates, were broken down by *Stu*I digestion into 2 fragments of 265 bp and 160 bp, respectively, and those on HBV/Bj isolates were not.

When the PCR-RFLP method was applied to 313 serum samples obtained from Japanese carriers of HBV genotype B, HBV/Ba was found in 35 (11%) of the samples and HBV/Bj was found in 253 (81%) of the samples; subtypes were indistinguishable in the remaining 25 samples (8%). To confirm the reliability of this PCR-EFLP method, the precore region plus core gene was sequenced directly on all 35 of the 35 HBV/Ba isolates, 44 of the 253 HBV/Bj isolates, and 25 of the 25 isolates of indistinguishable subtypes. All of the 44 HBV/Bj isolates determined by PCR-RFLP were confirmed for the Bj subtype by sequencing. One of the 35 (3%) isolates of HBV/Ba determined by PCR-RFLP, however, turned out to be of subtype Bj by sequencing. Of the 25 isolates for which subtypes were undistinguished by PCR-RFLP, 4 (16%) of the isolates were classified into HBV/Ba and 21 (84%) of the isolates were classified into HBV/Bj by sequencing.

Distribution of HBV/Ba and HBV/Bj in Japan by acute or

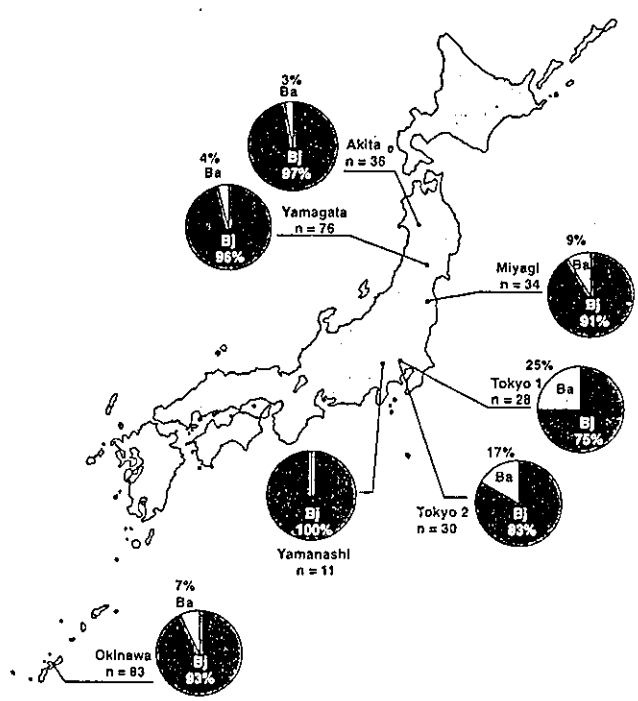

Figure 2. Geographic distribution of 298 patients who had chronic infection with hepatitis B virus (HBV) genotype B subtypes Ba and Bj and who visited 7 different hospitals throughout Japan. Subtypes of HBV genotype B were determined by the PCR restriction fragment–length polymorphism method involving 2 single nucleotide polymorphisms with restriction enzymes *Stu*I and *Hepa*I (see Materials and Methods).

Table 1. Demographic, clinical, and virological characteristics of patients in Japan who were persistently infected with hepatittis B virus (HBV) genotype B, subtype Ba or Bj, by subtype.

Characteristic	HBV genotype B subtype		
	Ba (n = 25)	Bj (n = 273)	P
Age, mean years ± SD	42.0 ± 15.0	48.7 ± 14.4	<.05
No. of male subjects/no. of female subjects	15/10	193/80	NS
Liver disease			
Asymptomatic carrier state	40	38	NS
Chronic hepatitis	52	54	NS
Liver cirrhosis	8	8	NS
ALT level, mean U/L ± SD	75.8 ± 112.0	72.1 ± 154.3	NS
AST level, mean U/L ± SD	54.9 ± 63.9	54.5 ± 107.7	NS
Positive for HBeAg			
All, no. positive/no. of patients (%)	8/25 (32)	25/273 (9)	<.01
Age >30 years, no. positive/no. of patients (%)	6/19 (32)	9/236 (4)	<.01
Postive for anti-HBe			
All, no. positive/no. of patients (%)	17/25 (68)	241/273 (88)	<.01
Age >30 years, no. positive/no of patients (%)	14/19 (74)	222/236 (94)	<.01

NOTE. ALT, alanine aminotransferase; anti-Hbe, antibody to hepatitis B e antigen; AST, asparate aminotransferase; HBeAg, hepatitis B e antigen; NS, not significant.

chronic hepatitis and by geographic region. There were 15 patients with acute hepatitis B and 298 patients with chronic hepatitis B who were infected with HBV genotype B and for whom subtypes Ba or Bj were determined by PCR-RFLP. Subtype Ba was detected in 13 (87%) of the 15 patients with acute hepatitis, which is significantly more frequently than it was detected among patients with chronic hepatitis (25 [8%] of 298 patients) (P < .01).

Figure 2 illustrates the geographic distribution of subtypes Ba and Bj on the basis of data reported from 7 Japanese hospitals for 298 patients with chronic hepatitis. There were regional differences in the distribution of Ba and Bj subtypes (P < .01). Subtype Ba was detected in 12 (21%) of the 58 patients in the Tokyo metropolitan area (combining data on patients from 2 hospitals), and it was detected less often in the other districts (in a total of 13 [5%] of 240 patients, with rates of detection in individual hospitals ranging from 0% to 9%).

Comparison of HBV/Ba carriers with HBV/Bj carriers. Table 1 compares the demographic, virological, and clinical characteristics of 25 carriers of HBV/Ba with those of 273 carriers of HBV/Bj. The mean age ±SD was significantly higher in carriers of HBV/Bj than in carriers of HBV/Ba (48.7 ± 14.4 years vs. 42.0 ± 15.0 years; P < .01). No differences were observed in clinical manifestations (in terms of transaminase levels and the distribution of chronic liver diseases) between carriers of subtype Ba and carriers of subtype Bj.

There were marked differences in HBeAg/anti-HBe status between the patients infected with the Ba subtype and those infected with the Bj subtype. The prevalence of HBeAg was significantly higher in patients infected with HBV/Ba than in those infected with HBV/Bj (32% vs. 9%; P < .01), and the difference was even more prominent among patients >30 years old (32% vs. 4%; P < .01). Conversely, anti-HBe was significantly less frequent in patients infected with HBV/Ba than in patients infected with HBV/Bj, both overall (68% vs. 88%; P < .01) and in patients >30 years old (74% vs. 94%; P < .01).

Comparison of 2 PCR-RFLP methods for distinguishing HBV/Ba from HBV/Bj. The previous PCR-RFLP method with restriction endonucleases *Spe*I and *Mse*I [18] involved only 1 SNP of G or A at nt 1838. It was applied to the 313 isolates of HBV genotype B for which subtypes had been determined

Table 2. Classification of 313 hepatitis B virus (HBV) genotype B isolates as subtype Ba or subtype Bj, as determined by PCR restriction fragment–length polymorphism (PCR-RFLP) methods involving either 1 single nucleotide polymorphism (previous method) or 2 single nucleotide polymorphisms (new method).

Subtype classification by previous method	Subtype classification by new method		
	Ba (n = 38)	Bj (n = 275)	Total
Ba	34 (89)	10 (4)	44
Bj	0 (0)	238 (86)	238
Unclassified	4 (11)	27 (10)	31

NOTE. Data are no. (%) of isolates.

in this study (table 2). Of 38 HBV/Ba isolates, 34 (89%) were classified as HBV/Ba and 4 (11%) were unclassifiable using the previous PCR-RFLP method. Of the 275 HBV/Bj isolates, 238 (86%) were classified as HBV/Bj and 27 (10%) were unclassifiable using the previous PCR-RFLP method.

There were, however, 10 HBV/Bj isolates that were classified as HBV/Ba using the previous PCR-RFLP method. Sequences of a part of the precore region in the 10 HBV/Bj isolates classified as HBV/Ba by the previous PCR-RFLP are shown in figure 3. A point mutation from G to A at nt 1838 was detected in 3 of the isolates. An insertion of A between nt 1837 and 1838 in the remaining 7 isolates induced a frame-shift in the product of precore region and resulted in an HBeAg-negative phenotype. Because these mutations created a restriction site for *MseI* enzyme (TTAA), they gave a false result for HBV/Bj in HBV/Ba isolates by the previous PCR-RFLP method, which involved the *MseI* restriction site [19]. None of the 7 carriers of HBV/Ba in whom HBV DNA sequences with an insertion of A were detected had serum samples that tested positive for HBeAg; a serum sample obtained from the remaining carrier was not available for testing. Thus, the role of this single nucleotide insertion in inducing an HBeAg-negative phenotype was confirmed.

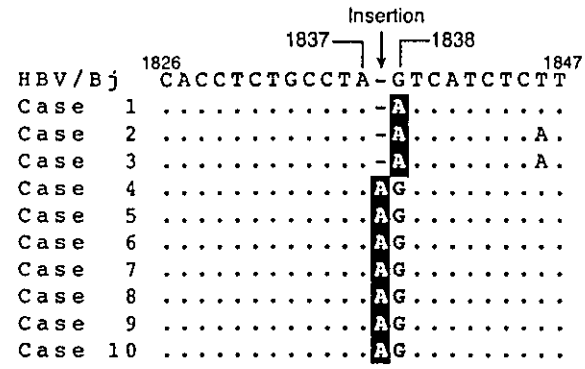

Figure 3. Partial precore sequences of the 10 hepatitis B virus isolates of genotype B subtype Bj (HBV/Bj isolates) that gave false results by the PCR restriction fragment–length polymorphism method involving 1 single nucleotide polymorphism with restriction enzymes *SpeI* and *MseI* [19]. The sequence of the reference HBV/Bj isolate (AB010290) is shown at the top of the figure.

DISCUSSION

In this study, a novel PCR-RFLP method involving 2 SNPs, A or G at nt 2020 and T or C at nt 2167 in the core gene, was developed for distinguishing between HBV/Bj and HBV/Ba isolates. With use of this method, 288 (92%) of the 313 HBV isolates of genotype B were classified as subtype Bj or subtype Ba. The validity of this new PCR-RFLP method was confirmed by sequencing 104 HBV DNA samples, including those from all 35 HBV/Ba isolates and those from 44 of the 253 HBV/Bj isolates. Only a single HBV/Bj isolate possessed G2020 and C2167 and produced a false result for HBV/Ba by the new PCR-RFLP method. In comparison with the previous PCR-RFLP method, which involved 1 SNP [19] and which, on rare occasions, misidentified HBV/Bj isolates as HBV/Ba, the new PCR-RFP was more specific in distinguishing between HBV/Ba and HBV/Bj isolates (table 2).

Precore sequences of 7 HBV/Bj isolates that had discordant results when analyzed using previous and new PCR-RFLP methods revealed a unique frame-shift insertion between nt 1837 and nt 1838 for aborting the expression of HBeAg (figure 3). Of the 7 individuals from whom these HBV/Bj isolates were recovered, 2 were asymptomatic carriers, and 5 were found to have chronic hepatitis; all 7 had negative results when tested for HBeAg. A similar insertion at this position in the precore region has been described in patients with HBeAg-negative HCC or chronic hepatitis [24, 25].

In the present study, HBV/Bj was detected in 275 (88%) of the 313 serum samples in Japan that contained HBV genotype B; these included 273 (92%) of the 298 serum samples obtained from individuals who were persistently infected with HBV. Patients with HBV/Ba infection were found to have clinical outcomes that were distinctly different from those of patients with HBV/Bj infection. HBV/Ba was detected significantly more frequently in the patients with acute, resolving (rather than persistent) HBV infection (13 [87%] of 15 vs. 25 [8%] of 298; $P < .01$). The association between recombinant genotypes of HBV and clinical manifestations has thus far not been looked into, although several recent studies indicate that HBV genotypes have clinical consequences [5–11]. The carriers of HBV genotype C have more cases of advanced liver disease than do carriers of HBV genotype B [7, 9]. Of possible relevance to this difference, the presence of the double mutation in the core promoter (T1762/A1764) is more frequent and the point mutation in the precore region (A1896) is less frequent in patients infected with HBV genotype C than in those infected with HBV genotype B [9, 10]; these mutations decrease and abolish, respectively, the expression of HBeAg. There are differences even among infections with HBV genotype B; such differences are associated with different subtypes. Thus, patients infected with HBV/Bj have a significantly lower prevalence of HBeAg and a higher prevalence of anti-HBe than do those patients with HBV/Ba infection, indicating that seroconversion takes place earlier in patients with HBV/Bj infection [19]. These results were corroborated by the findings of the present study (table 1).

The prevalence of HBeAg in carriers of HBV genotype B varies widely and depends on the geographical region from which it is reported. In Hong Kong, where HBV/Ba infection seems to account for almost all HBV genotype B infections

[19], Chu et al. [10] found HBeAg in ~40% of patients infected with HBV genotype B who were >30 years old. By contrast, in Japan, where HBV/Bj infection accounts for most HBV genotype B infections, HBeAg was detected in only 10% of patients infected with HBV genotype B who were >30 years old [8]. These differences in the prevalence of HBeAg may be explained by different distributions of HBV/Ba and HBV/Bj subtypes between the 2 countries.

No significant differences were observed in the severity of liver disease between patients with HBV/Ba infection and those with HBV/Bj infection (table 1). Only a limited number of patients with HBV/Ba infection were investigated, however, none of whom had HCC. Evidence has been accumulating that indicates the influence of HBV genotypes on the development of severe chronic liver disease, including HCC [6–11], although there are some arguments against this [12, 13]. In Taiwan, HBV genotype B infection may be responsible for the development of HCC in carriers of HBV genotype B aged <50 years [7]. By outstanding contrast, in Japan, HBV genotype B is not found in patients with HCC aged <60 years [8]. Because HBV genotype B isolates from Taiwan are HBV/Ba [19], there is a possibility that the recombination between genotypes B and C in Taiwanese HBV/Ba isolates might have enhanced an early development of HCC there. It does need to be pointed out, however, that the development of HCC is probably multifactorial, given the possible presence of chronic inflammatory changes in the liver that would enhance hepatocarcinogenesis. Furthermore, specific environmental hepatotoxins, such as aflatoxin [26] and alcohol [27], probably contribute to high incidence rates of HCC in some areas of the world. HBV genotypes influence the response to lamivudine in the treatment of patients with chronic hepatitis B [28, 29]. The response may differ even among patients infected with HBV of the same genotype. Recently, Akuta et al. [30] reported that the response to lamivudine was less frequent in patients infected with HBV/Ba than in those infected with HBV/Bj. Taken altogether, a large-scale study, in collaboration with many countries in which HBV/Ba is prevalent, is required to evaluate any clinical differences between HBV/Ba and HBV/Bj infections.

In conclusion, a new PCR-RFLP method involving 2 SNPs was developed specifically for distinguishing between HBV/Ba and HBV/Bj isolates. Of these 2 subtypes of HBV genotype B, HBV/Bj was the predominant subtype throughout Japan and was associated with the development of acute liver disease less frequently than was HBV/Ba. The recombination with HBV genotype C in HBV/Ba would contribute to a delayed seroconversion of HBeAg in individuals who are infected with it. The new PCR-RFLP would be useful in evaluating clinical, epidemiological, and virological differences between HBV/Ba and HBV/Bj infections in countries in which HBV genotype B is prevalent.

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