29 Perrillo R, Schiff E, Hann HWL, Buti M, Strasser S, Watkins KM, Moorat AE, Woessner MA, Vig P, Brosgart CL, Bourne EC, Atkins MC: The addition of adefovir dipivoxil to lamivudine in decompensated chronic hepatitis B with YMDD variant HBV and reduced response to lamivudine (abstract). Hepatology 2001;34(suppl):349A.
30 Peters M, Hann H, Martin P, Heathcote E, Buggisch P, Moorat AE, Sullivan M, Kleber K, Ebrahimi R, Xiong S, Brosgart C: Adefovir dipivoxil (ADV) alone and in combination with lamivudine (lam) suppresses lam-resistant hepatitis B virus (HBV) replication: 16 week interim analysis (abstract). J Hepatol 2001;36(suppl 31):A13.
31 Rang A, Gunther S, Will H: Effect of interferon alpha on hepatitis B virus replication and gene expression in transiently transfected human hepatoma cells. J Hepatol 1999;31:791–799.
32 Angus P, Vaughan R, Xiong S, Yang H, Delaney W, Gibbs C, Brosgart C, Colledge D, Edwards R, Ayres A, Bartholomeusz A, Locarnini S: Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. Gastroenterology 2003;125:292–297.
33 Yang H, Westland CE, Delaney WE IV, Heathcote EJ, Ho V, Fry J, Brosgart C, Gibbs CS, Miller MD, Xiong S: Resistance surveillance in chronic hepatitis B patients treated with adefovir dipivoxil for up to 60 weeks. Hepatology 2002;36:464–473.
34 Westland C, Delaney WE IV, Yang H, Chen SS, Marcellin P, Hadziyannis S, Gish R, Fry J, Brosgart C, Gibbs C, Miller M, Xiong S: Hepatitis B virus genotypes and virologic response in 694 patients in phase III studies of adefovir dipivoxil. Gastroenterology 2003;125:107–116.

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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

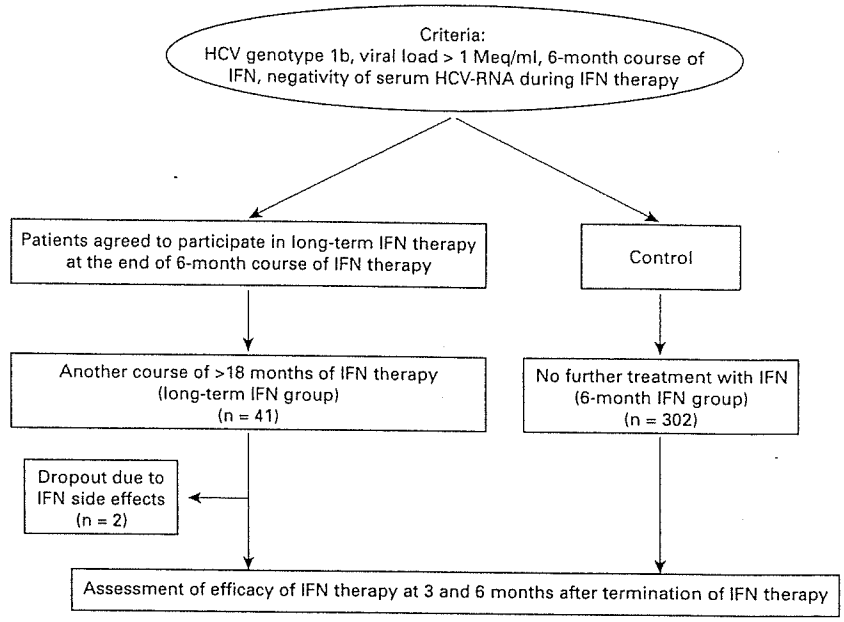

Fig. 1. Study protocol and treatment groups.

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.

References

1 Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, Arase Y, Fukuda M, Chayama K, Murashima N, Kumada H: Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. J Hepatol 1998;28:930–938.

2 Tong MJ, el-Farra NS, Reikes AR, Co RL: Clinical outcome after transfusion-associated hepatitis C virus infection. N Engl J Med 1995; 332:1463–1466.

3 Simonetti RG, Camma C, Fiorello F, Cottone M, Rapicetta M, Marino L, Fiurentino G, Craxi A, Cicaglione A, Giuseppetti R: Hepatitis C virus as a risk factor for hepatocellular carcinoma in patients with cirrhosis: A case control study. Ann Intern Med 1992;116:97–102.

4 Tsubota A, Chayama K, Arase Y, Koida I, Saitoh S, Ikeda K, Iwasaki S, Matsumoto T, Kobayashi M, Kumada H: Factors predictive of response interferon-alpha therapy in hepatitis C virus infection. Hepatology 1994;19:1088–1094.

5 Yoshioka K, Kakumu S, Wakita T, Ishikawa T, Itoh Y, Takayanagi M, Hayashi Y, Shibata M, Morishima T: Detection of hepatitis C virus by polymerase chain reaction and response to interferon-α therapy: Relationship to genotypes of hepatitis C virus. Hepatology 1992;16:293–299.

6 Hagiwara H, Hayashi N, Mita E, Ueda K, Takehara T, Kasahara A, Fusamoto H, Kumada T: Detection of hepatitis C virus RNA in serum of patients with chronic hepatitis C treated with interferon-α. Hepatology 1992;15: 37–41.

7 Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, J Goodman Z, Banks SM, Hoofnagle JH: Recombinant interferon alpha therapy for chronic hepatitis C: A randomized, double blind placebo-controlled trial. N Engl J Med 1989;321: 1506–1510.

8 Causse X, Godinot H, Chevallier M, Chossegros P, Zoulim F, Ouzan D, Heyraud JP, Fontanges T, Albrecht J, Meschievitz C: Comparison of 1 or 3 MU of interferon alfa-2β and placebo in patients with chronic non-A non-B hepatitis. Gastroenterology 1991;101:497–502.

9 Chayama K, Saitoh S, Arase Y, Ikeda K, Matsumoto T, SakaiY, Kobayashi M, Unakami M, Morinaga T, Kumada H: Effect of interferon administration on serum hepatitis C virus RNA in patients with chronic hepatitis C. Hepatology 1991;13:1040–1043.

10 Reichard O, Glaumann H, Fryden A, Norkrans G, Schvarcz R, Sonnerborg A, Yun ZB, Weiland O: Two-year biochemical, virological and histological follow-up in patients with chronic hepatitis C responding in a sustained fashion to interferon alfa-2β treatment. Hepatology 1995: 21:918–922.

11 Yoshioka K, Yano M, Kusakabe A, Hirofuji H, Fuji A, Kuriki J, Arao M, Murase K, Kidokoro R, Kakumu S: Randomized controlled trial of lymphoblastoid interferon α for chronic hepatitis C. Am J Gastroenterol 1999;94:164–168.

12 Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, Iijima A, Urushihara A, Kiyosawa K, Okada M, Hino K, Okita K: Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Hepatology 1998;27:1394–1402.

13 Imai Y, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, Maeda Y, Shirai Y, Fukuzaki T, Kaji I, Ishikawa H, Matsuda Y, Mishikawa M, Seki K, Matsuzawa Y: Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C: Osaka Hepatocellular Carcinoma Prevention Study Group. Ann Intern Med 1998;129:94–99.

14 Okanoue T, Itoh Y, Minami M, Sakamoto S, Yasui K, Sakamoto M, Nishioji K, Murakami Y, Kashima K: Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. Viral Hepatitis Therapy Study Group. J Hepatol 1999;30:653–659.

15 Hoofnagle JH, Di Bisceglie AM: The treatment of chronic viral hepatitis. N Engl J Med 1997; 336:347–356.

16 Lau JY, Davis GL, Kniffen J, Qian KP, Urdea MS, Chan CS, Mizokami M, Neuwald PO, Wilber JC: Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. Lancet 1993;341:1501–1504.

17 Noubaum JB, Pol S, Nalpas B, Landais P, Berthlot P, Brechot C: Hepatitis C virus type 1b infection in France and Italy. Ann Intern Med 1995;122:161–168.

18 Shiratori Y, Kato N, Yokosuka O, Imazeki F, Hashimoto E, Hayashi N, Nakamura A, Asada M, Kurada H, Tanaka N, Arakawa Y, Omata M: Predictors of the efficacy of interferon therapy in chronic hepatitis C virus infection. Gastroenterology 1997;113:558–566.

19 Arase Y, Ikeda K, Chayama K, Murashima N, Tsubota A, Suzuki Y, Saitoh S, Kobayashi M, Suzuki F, Kumada H: Fluctuation patterns of HCV-RNA serum level in patients with chronic hepatitis C. J Gastroenterol 2000;35: 221–225.

20 McHuchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman Z, Ling MH, Cort S, Albrecht JK: Interferon alfa-2β alone or in combination with ribavirin as initial treatment for chronic hepatitis C. N Engl J Med 1998;339:1485–1492.

21 Poynard T, Marcellin P, Lee S, Niederau C, Minuk GS, Ideo G, Bain V,Heathcote J, Zeuzem S, Trepo C, Albrecht J: Randomised trial of interferon alpha 2β plus ribavirin for 48 weeks or 24 weeks versus interferon alpha 2β plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 1998;352:1426–1432.

22 Reichard O, Norkrans G, Fryden A: Randomised, double-blind, placebo controlled trial of interferon alpha 2b with and without ribavirin for chronic hepatitis C. Lancet 1998;351:83–87.

23 Tsubota A, Akuta A, Suzuki F, Suzuki Y, Someya T, Kobayashi M, Arase Y, Saitoh S, Ikeda K, Kumada H: Viral dynamics and pharmacokinetics in combined interferon alfa-2b and ribavirin therapy for patients infected with hepatitis C virus of genotype 1b and high pretreatment viral load. Intervirology. 2002;45: 33–42.

24 Suzuki H, Tango T, Consensus Interferon Group: A multicenter, randomized, controlled clinical trial of interferon alfacon-1 in comparison with lymphoblastoid interferon-alpha in patients with high-titer chronic hepatitis C virus infection. Hepatol R 2002;22:1–12.

25 Giuseppe B, Giorgio B: Consensus interferon for chronic hepatitis C patients with genotype 1 who failed to respond to, or relapsed after, interferon alpha-2b and ribavirin in combination: an Italian pilot study. Eur J Gastroenterol Hepatol 2002;14:477–483.

26 Zeuzem S, Feinman SV, Rasenack J, Heathcote ej, Lai My, Gane E, O'Grady J, Reichen J, Diago M, Lin A, Hoffman J, Brunda MJ: Peginterferonα-2a in patients with chronic hepatitis C. N Engl J Med 2000;343:1666–1672.

27 Lindsay KL, Trepo C, Heintges T, Shiffmann ML, Gordon SC, Hoefs JC, Schiff ER, Goodman ZD, Laughlin M, Yao R, Albrecht JK: A randomized, doubled- blinded trial comparing pegylated interferonα-2β to interferon α-2β as initial treatment for chronic hepatitis C. Hepatology 2001;34:395–403.

28 Kasahara A, Hayashi N, Hiramatsu N, Oshita M, Hagiwara H, Katayama K, Kato M, Matsuzawa M, Yoshihara H, Kishida Y: Ability of prolonged interferon treatment to suppress relapse after cessation of therapy in patients with chronic hepatitis C: A multicenter randomized controlled trial. Hepatology 1995;21:291–297.

29 Poynard T, Bedossa P, Chevallier M, Mathurin P, Lemonnier C, Trepo C, Couzigou P, Payen JL, Sajus M, Costa JM: A comparison of three interferon alfa-2b regimens for the long-term treatment of chronic non-A, non-B hepatitis. N Engl J Med 1995;32:1457–1462.

30 Reichard O, Foberg U, Fryden A, Mattsson L, Norkrans G, Sonnerborg A, Wejstal R, Yun ZB, Weiland O: High sustained response rate and clearance of viremia in chronic hepatitis C after treatment with interferon-α 2β for 60 weeks. Hepatology 1994;19:280–285.

31 Saracco G, Borghesio E, Mesina P, Solinas A, Spezia C, Macor F, Gallo V, Chiandussi L, Donadon V, Spirito F, Mangia A, Andriulli A: Prolonged treatment (2 years) with different doses (3 versus 6 MU) of interferon 2b for chronic hepatitis type C. J Hepatol 1997;27: 56–62.

32 Arase Y, Ikeda K, Tsubota A, Suzuki Y, Saitoh S, Kobayashi M, Akuta N, Someya T, Kobayashi M, Suzuki F, Kumada H: Efficacy of prolonged interferon therapy for patients with chronic hepatitis C with HCV-genotype 1b and high virus load. J Gastroenterol 2003;38:158–163.
33 Garson JA, Tedder RS, Briggs M, Tuke P, Ring C, Machin SJ, Tedder RS: Detection of hepatitis C viral sequences in blood donations by 'nested' polymerase chain reaction and predictor of infectivity. Lancet 1990;335:1419–1422.
34 Detmer J, Lagier R, Flynn J, Zayati C, Kolberg, Collins M, Urdea M, Sauchez-Pescador R: Accurate quantification of hepatitis C virus (HCV) RNA from HCV-genotypes by using branched-DNA technology. J Microbiol 1996; 34:901–907.
35 Desmet V J, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ: Classification of chronic hepatitis: Diagnosis, grading and staging. Hepatology 1994;19:1513–1520.
36 Dusheiko G, Schmilovitzs-Weiss H, Brown D, McOmish F, Yap P-I, Sherlock S, McIntyre N, Simmonds P: Hepatitis C virus genotypes: An investigation of type-specific differences in geographic origin and disease. Hepatology 1994;19:13–18.
37 Mellor J, Holmes EC, Jarivis LM, Yap PL, Simmonds P, International HCV Collaborative Study Group: Investigation of the pattern of hepatitis. C virus sequence diversity in different geographical regions: Implications for virus classification. J Gen Virol 1995;76:2493–2507.

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/

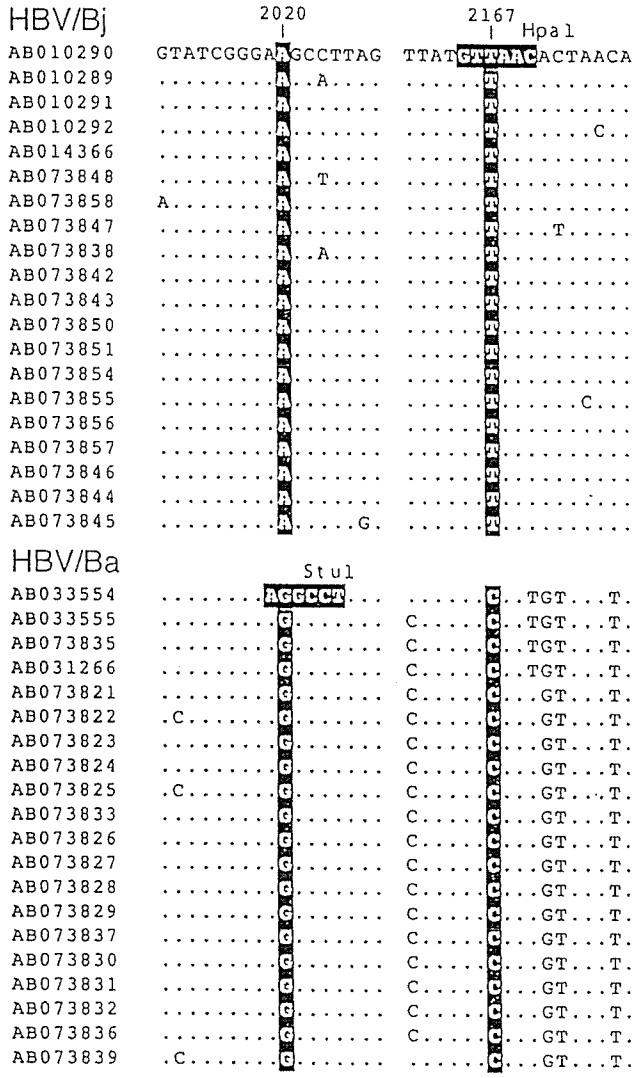

Figure 1. Nucleotide sequences of a part of the core region in 40 hepatitis B virus (HBV) isolates of genotype B. Sequences of 20 HBV isolates of subtype Bj (HBV/Bj) and 20 HBV isolates of subtype Ba (HBV/Ba) are shown, which are representative of 29 HBV/Bj isolates and 41 HBV/Ba isolates reported previously [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

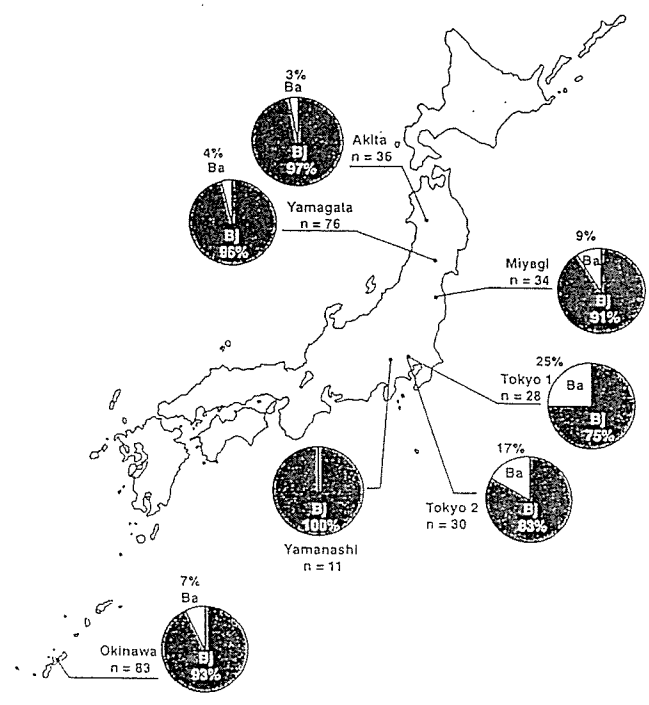

Figure 2. Geographic distribution of 298 patients who had chronic infection with hepatitis B virus (HBV) genotype B subtypes Ba and Bj and who visited 7 different hospitals throughout Japan. Subtypes of HBV genotype B were determined by the PCR restriction fragment–length polymorphism method involving 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		Total
	Ba (n = 38)	Bj (n = 275)	
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.

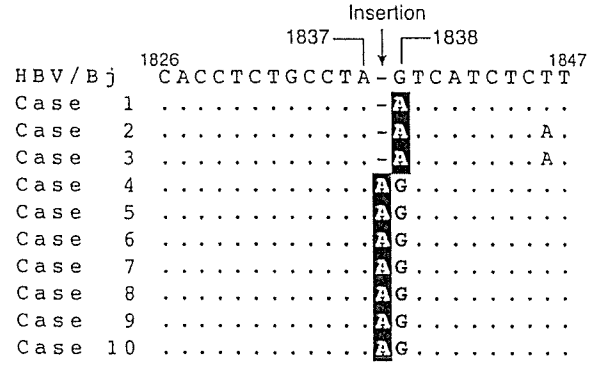

Figure 3. Partial precore sequences of the 10 hepatitis B virus isolates of genotype B subtype Bj (HBV/Bj isolates) that gave false results by the PCR restriction fragment–length polymorphism method involving 1 single nucleotide polymorphism with restriction enzymes *SpeI* and *MseI* [19]. The sequence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

References

1. Okamoto H, Tsuda F, Sakugawa H, et al. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. J Gen Virol 1988; 69:2575–83.
2. Norder H, Courouce AM, Magnius LO. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology 1994; 198:489–503.
3. Stuyver L, De Gendt S, Van Geyt C, et al. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J Gen Virol 2000; 81:67–74.
4. Arauz-Ruiz P, Norder H, Robertson BH, Magnius LO. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. J Gen Virol 2002; 83:2059–73.
5. Lindh M, Hannoun C, Dhillon AP, Norkrans G, Horal P. Core promoter mutations and genotypes in relation to viral replication and liver damage in East Asian hepatitis B virus carriers. J Infect Dis 1999; 179: 775–82.
6. Mayerat C, Mantegani A, Frei C. Does hepatitis B virus (HBV) genotype influence the clinical outcome of HBV infection? J Viral Hepat 1999;6: 299–304.
7. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology 2000; 118:554–9.
8. Orito E, Ichida T, Sakugawa H, et al. Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. Hepatology 2001; 34:590–4.
9. Orito E, Mizokami M, Sakugawa H, et al. A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Japan HBV Genotype Research Group. Hepatology 2001; 33:218–23.
10. Chu CJ, Hussain M, Lok AS. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. Gastroenterology 2002; 122:1756–62.
11. Chu CJ, Lok AS. Clinical significance of hepatitis B virus genotypes. Hepatology 2002; 35:1274–6.
12. Sumi H, Yokosuka O, Seki N, et al. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. Hepatology 2003; 37:19–26.
13. Yuen MF, Sablon E, Yuan HJ, et al. Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications, and hepatocellular carcinoma. Hepatology 2003; 37:562–7.
14. Bollyky PL, Rambaut A, Harvey PH, Holmes EC. Recombination between sequences of hepatitis B virus from different genotypes. J Mol Evol 1996; 42:97–102.
15. Bowyer SM, Sim JG. Relationships within and between genotypes of hepatitis B virus at points across the genome: footprints of recombination in certain isolates. J Gen Virol 2000; 81:379–92.
16. Morozov V, Pisareva M, Groudinin M. Homologous recombination between different genotypes of hepatitis B virus. Gene 2000; 260:55–65.
17. Owiredu WK, Kramvis A, Kew MC. Hepatitis B virus DNA in serum of healthy black African adults positive for hepatitis B surface antibody alone: possible association with recombination between genotypes A and D. J Med Virol 2001; 64:441–54.
18. Sugauchi F, Orito E, Ichida T, et al. Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. J Virol 2002; 76:5985–92.
19. Sugauchi F, Orito E, Ichida T, et al. Epidemiologic and virologic characteristics of hepatitis B virus genotype B having the recombination with genotype C. Gastroenterology 2003; 124:925–32.
20. Usuda S, Okamoto H, Iwanari H, et al. Serological detection of hepatitis B virus genotypes by ELISA with monoclonal antibodies to type-specific epitopes in the preS2-region product. J Virol Methods 1999; 80:97–112.
21. Usuda S, Okamoto H, Tanaka T, et al. Differentiation of hepatitis B virus genotypes D and E by ELISA using monoclonal antibodies to epitopes on the preS2-region product. J Virol Methods 2000; 87:81–9.

22. Mizokami M, Nakano T, Orito E, et al. Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. FEBS Lett 1999; 450:66–71.
23. Sugauchi F, Mizokami M, Orito E, et al. A novel variant genotype C of hepatitis B virus identified in isolates from Australian Aborigines: complete genome sequence and phylogenetic relatedness. J Gen Virol 2001; 82:883–92.
24. Dienes HP, Gerken G, Goergen B, Heermann K, Gerlich W, Meyer zum Buschenfelde KH. Analysis of the precore DNA sequence and detection of precore antigen in liver specimens from patients with anti–hepatitis B e-positive chronic hepatitis. Hepatology 1995; 21:1–7.
25. Kramvis A, Kew MC, Bukofzer S. Hepatitis B virus precore mutants in serum and liver of southern African Blacks with hepatocellular carcinoma. J Hepatol 1998; 28:132–41.
26. Bressac B, Kew M, Wands J, et al. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. Nature 1991; 350:429–31.
27. Hassan MM, Hwang LY, Hatten CJ, et al. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. Hepatology 2002; 36:1206–13.
28. Kao JH, Liu CJ, Chen DS. Hepatitis B viral genotypes and lamivudine resistance. J Hepatol 2002; 36:303–4.
29. Ben-Ari Z, Daudi N, Klein A, et al. Genotypic and phenotypic resistance: longitudinal and sequential analysis of hepatitis B virus polymerase mutations in patients with lamivudine resistance after liver transplantation. Am J Gastroenterol 2003; 98:151–9.
30. Akuta N, Suzuki F, Kobayashi M, et al. The influence of hepatitis B virus genotype on the development of lamivudine resistance during long-term treatment. J Hepatol 2003; 38:315–21.

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Efficacy of 6-month interferon therapy in chronic hepatitis B virus infection in Japan

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Background. In Japan, there are few studies of long-term (more than 1 month) interferon (IFN) therapy for chronic hepatitis B (CHB). In this retrospective study, we investigated the efficacy and predictors of response to 6-month IFN therapy. ***Methods.*** We analyzed 66 Japanese patients with CHB who were treated with IFN for 6 months. They comprised patients who were hepatitis B e antigen (HBeAg)-positive (n = 45) and -negative (n = 21). One (2%), 8 (12%), and 51 (77%) patients were infected with hepatitis B virus (HBV) genotypes A, B, and C, respectively. Responders in patients positive for HBeAg were defined as those who showed normalization of serum alanine aminotransferase (ALT) level, HBeAg loss, and HBV DNA negativity at 6 months after completion of IFN therapy. In patients negative for HBeAg, responders were defined as those patients who showed normalization of ALT level and HBV DNA negativity at the same 6-month time point. ***Results.*** Of the 45 patients with HBeAg at the commencement of IFN therapy, 9 (20%) were responders. Young patients, especially those with a high serum ALT level, were significantly more likely to respond to IFN therapy. Of the 21 patients negative for HBeAg, 13 (62%) were responders. There were no significant differences in clinical characteristics between responders and nonresponders among patients negative for HBeAg. Multivariate analyses identified HBeAg negativity and young age as independent factors associated with a positive response to 6-month IFN therapy. However, long-term follow-up of the treated patients showed a fall in the response rate. ***Conclusions.*** The response rate to 6-month IFN therapy among HBeAg-positive patients was low. However, young patients may require long-term IFN therapy.

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Introduction

Hepatitis B virus (HBV) infection is a common disease that can lead to a chronic carrier state, and it is associated with the risk of development of progressive disease and hepatocellular carcinoma.[1] Interferon (IFN) and lamivudine are two currently approved treatments for chronic hepatitis B (CHB) in most countries.[2] IFN is associated with significant adverse effects, whereas long-term therapy with lamivudine may result in drug resistance. A metaanalysis of IFN therapy published in 1993 reviewed 15 randomized controlled studies involving 837 adult patients who received IFN-α at doses of 5–10 million units (MU), administered at intervals ranging from daily to three times weekly, for 4–6 months.[3] Loss of hepatitis B e antigen (HBeAg) occurred in 33% of the treated patients compared with 12% of controls. Loss of detectable HBV DNA and normalization of alanine aminotransferase (ALT) level were also more common in treated than control patients. The major pretreatment factors that correlated with a response were high ALT levels,[4–6] low HBV DNA,[4,5] female sex, and greater degrees of activity and fibrosis on liver biopsy.[2] However, the optimal duration of IFN therapy for CHB is not well established. Moreover, in the 1990s in Japan, the duration of IFN therapy was mainly 1 month, and the efficacy was limited.[7–9]

Recently, HBV genotypes have been implicated in HBeAg seroconversion as well as response to antiviral treatment. Genotype A was found to be associated with a higher rate of IFN-induced HBeAg seroconversion than genotype D in a study of 64 German patients with HBeAg-positive CHB.[10] Another study, of 58 Taiwanese patients who received IFN treatment for HBeAg-positive CHB, found that patients with genotype B had

a significantly higher rate of HBeAg loss compared with those with genotype C.[11] Moreover, Sugauchi et al.[12] proposed that genotype B could be provisionally classified into a Ba ("a" for Asia) subgroup and a Bj ("j" for Japan) subgroup, and that such virological differences could explain the clinical differences in various Asian countries. Our previous study indicated that, in Japan, the proportions of HBV infection associated with genotypes B and C were 9% and 88%, respectively.[13] Our study also showed that the majority of genotype B patients were HBeAg-negative at the first examination and showed a mild degree of hepatic fibrosis, while genotype C infection was associated with progressive liver fibrosis.[13] Therefore, mainly patients with genotype C of CHB have received antiviral treatment in Japan.

In Japan, there are few studies of long-term (more than 6 months) IFN therapy for CHB. The present study was designed to re-examine retrospectively the efficacy of 6-month IFN therapy and to determine the potential predictors of a positive response to IFN treatment in Japan.

Patients and methods

Patients

We retrospectively studied 66 Japanese adult patients (19 women and 47 men) who commenced IFN treatment between June 1988 and October 2002 at the Department of Gastroenterology of Toranomon Hospital (Table 1). All patients were followed up from the commencement of therapy at our hospital. They all were positive for hepatitis B surface antigen (HBsAg) in the serum for more than 6 months. Causes of hepatitis other than HBV were excluded, such as infection with hepatitis C virus, as well as autoimmune hepatitis. None of the patients had a history of drug abuse or alcoholic hepatitis, and none had received lamivudine therapy before the commencement of IFN.

Interferon therapy and assessment of response to therapy

Patients received 3 to 12 Mega Units (MU) of IFN-α or -β (Sumiferon; Sumitomo Pharmaceutical, Osaka, Japan; Canferon A; Takeda Chemical Industries, Osaka, Japan; Intron A; Schering-Plough, Osaka, Japan; or Feron; Toray, Tokyo, Japan). The regimen in 36 patients was two or three times a week for 6 months, while that applied for the remaining 30 patients was daily for 4 or 8 weeks, followed by three times a week for 20 or 16 weeks. The duration of treatment was 6 months (23–26 weeks) and the median total dose of IFN was 363 MU (Table 1). In patients with HBeAg, responders were defined as those patients who showed normalization of serum ALT level (normal level, 6–50 IU/l), HBeAg loss, and HBV DNA negativity at 6 months after the completion of IFN therapy. On the other hand, in patients negative for HBeAg, responders were defined as those patients who showed normalization of ALT level and HBV DNA negativity at 6 months after the completion of IFN therapy. All patients except for responders were considered nonresponders.

Table 1. Characteristics of patients at commencement of interferon therapy

Demographic data	
Total number of patients	66
Sex (female/male)	19/47
Age (years)[a]	36 (21–61)
Family history of liver disease	37 (56%)
Previous interferon treatment	13 (20%)
Total dose of interferon (Mega Units)[a]	363 (120–1892)
Duration of treatment (weeks)[a]	24 (23–26)
Laboratory data	
Aspartate aminotransferase (IU/l)[a]	87 (30–755)
Alanine aminotransferase (IU/l)[a]	169 (47–802)
Bilirubin (mg/dl)[a]	0.8 (0.3–1.8)
Albumin (g/dl)[a]	3.9 (3.1–4.8)
Staging of liver history (F1/2/3/4/ND)[b]	37/15/6/2/6
Serum HBV DNA[c] (bDNA; Meq/ml)[a]	41.5 (0.5–4000)
HBeAg-positive	45 (68%)
HBV genotype (A/B/C/unknown)	1/8/51/6

[a] Data valves are medians (ranges)
[b] Scores could range from 0 to 4; a score of 4 indicates liver cirrhosis. ND, not done
[c] HBV DNA levels were measured by branched-chain DNA probe assay (bDNA). All HBV DNA values below the lower limit of detection ($<0.7 \times 10^6$ viral genomic equivalents/ml) were set to 0.5 and those over the upper limite of detection ($>3800 \times 10^6$ viral genomic equivalents/ml genomic equivalents) were set to 4000 for calculation purposes

Blood tests and serum viral markers

Routine biochemical tests were performed, using standard procedures, before and at least once every month during therapy. Serial blood samples were taken from some patients before and during therapy and were stored at −80°C until used for measuring HBV DNA. HBsAg, HBeAg, and anti-HBe were determined by radioimmunoassay kits (Abbot Diagnostics, Chicago, IL, USA). HBV DNA was measured by branched-chain DNA probe assay (bDNA; Chiron Laboratory Service, Van Nuys, CA, USA); the lower limit of detection of this assay is 0.7×10^6 viral genomic equivalents/ml (0.7 Meq/ml).

HBV genotype

The six major genotypes of HBV (A, B, C, D, E, and F) were determined by enzyme-linked immunosorbent assay (ELISA; HBV Genotype EIA, Institute of Immu-

nology, Tokyo, Japan) according to the method described by Usuda et al.[14] This method involves the use of monoclonal antibodies directed against five epitopes, which are exposed on the product of the preS2 region of the HBV genome. Because the expression of the five preS2 epitopes is influenced by the HBV genotype, their combination enables determination of the genotypes serologically. Thus, the genotype was determined as A to F. The validity of this ELISA for serological determination of the five HBV genotypes has been verified previously.[14]

Subgroups Ba and Bj of genotype B were determined by a polymerase chain reaction (PCR) method. For this purpose, DNA was extracted from 100 µl of serum. The first PCR for detection of the precore and core region (nucleotide [nt] 1690 to 2600) of HBV DNA was performed using primers BJF3 (5′-CCGACCTTGAGGC ATACTTC-3′; sense) and BJR4 (5′-GGGTCCCACA AATTGCTTAC-3′; antisense) under conditions of initial denaturation for 4 min, 35 cycles of amplification at 94°C for 1 min, 55°C for 2 min, 72°C for 3 min, and 72°C for 7 min. The second PCR reaction was performed under the same reaction conditions, using primers BJF1 (5′-GCTGTGCCTTGGGTGGCTTTG-3′; sense) and BJR2 (5′-GCGACGCGGTGATTGAGACCT-3′; antisense). The amplified PCR products were purified and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems Japan, Tokyo, Japan). Phylogenetic analysis was performed by the following method. The total numbers of synonymous and non-synonymous substitutions among nucleotide sequences were estimated by the method of Gojobori et al.[15] Using this number, a phylogenetic tree was constructed by the neighbor-joining method.[16] Genotype B subgroups (Ba and Bj) were determined by these results.

Statistical analysis

Differences between groups were examined for statistical significance using the χ^2 test or Fisher's exact test and the Mann-Whitney *U*-test where appropriate. The above calculations were performed using StatView software (version 4.5J; Abacus Concepts, Berkeley, CA, USA). Independent predictive factors associated with response to IFN treatment were determined using multivariate multiple logistic regression. The following 12 potential predictors of the response to IFN treatment were assessed in this study: age, sex, family history of an HBV carrier, pretreatment with IFN, IFN total dose, method of IFN administration, HBV genotype, severity of liver disease (mild fibrosis [F1] or not [F2–4]), aspartate aminotransferase (AST), ALT, HBeAg, and HBV DNA level. All factors found to be at least marginally associated with response to IFN therapy ($P < 0.15$) were entered into the multivariate multiple logistic regression analysis. Multivariate multiple logistic regression was performed using the Windows SPSS software package (SPSS, Chicago, IL, USA). The odds ratio (OR) and 95% confidence interval (CI) were calculated to assess the relative risk. A two-tailed *P* value of less than 0.05 was considered statistically significant.

Results

Study population

One (2%), 8 (12%), and 51 (77%) patients were infected with HBV genotypes A, B, and C, respectively. The genotype in the remaining 6 patients could not be determined. The baseline characteristics of the patients are shown in Table 1. Although the numbers of patients with genotypes A and B were small, the distribution of HBV genotype was similar to that in patients with chronic hepatitis B who received care in our hospital, with a follow-up period of more than 2 years.[13] The 1 patient with genotype A, 1 of the 8 with genotype B, 37 of the 51 with genotype C, and all 6 with unknown genotype had HBeAg at the commencement of treatment. Seven of the 8 patients with genotype B were Bj and the other was the Ba type. At the commencement of IFN therapy, the ALT levels of 2 patients (both HBeAg-positive) were normal, and the HBV DNA levels of 9 patients (3 who were HBeAg-positive and 6 who were-negative) were less than 0.7 Meq/ml. However, these parameters in the above patients had been elevated within the 3 months before the treatment started.

Response to interferon therapy

Of the 45 patients with HBeAg at the commencement of IFN therapy, 9 (20%) were responders. Table 2 shows the demographic and clinical characteristics of responders and non-responders in these patients with HBeAg. Patients of younger age or higher ALT level had significantly higher rates of antiviral response to IFN than the others. Other characteristics were not related to the response to therapy. The one patient with HBeAg of genotype B (Bj) responded to IFN therapy.

On the other hand, of the 21 patients negative for HBeAg, 13 (62%) were responders. There were no significant differences in the clinical characteristics of responders and non-responders in this group (Table 3). Among the patients negative for HBeAg, the genotype (B or C) did not correlate with response to IFN therapy. The single patient with genotype Ba was a non-responder. Of the 6 patients with genotype Bj, 4 were responders while the remaining 2 were non-responders.

Table 2. Analysis of predictors of response to interferon therapy in patients positive for HBeAg

	Responders (n = 9)	Non-responder (n = 36)	P value
Sex (female/male)	4/5	11/25	0.45
Age (years)[a]	30 (21–35)	39 (23–59)	0.0048
Family history of liver disease	5 (56%)	22 (61%)	1.0
Previous interferon treatment	1 (11%)	8 (22%)	0.66
Total dose of interferon (MU)[a]	408 (120–1892)	408 (120–774)	0.80
Method of interferon administration (ED + I/I)[b]	5/4	21/15	1.0
Staging of liver history (F1/2/3/4/ND)	3/1/1/1/3	21/9/4/0/2	0.11
ALT (IU/l)[a]	263 (126–500)	149 (47–701)	0.049
Serum HBV DNA (bDNA; Meq/ml)[a]	7.9 (0.5–4000)	303 (0.5–4000)	0.18
HBV genotype (A/B/C/unknown)	0/1/7/1	1/0/30/5	0.12

[a] Data values are medians (ranges)
[b] ED + I, initially every day following intermittent therapy; I, only intermittent therapy

Table 3. Analysis of predictors of response to interferon therapy in patients negative for HBeAg

	Responders (n = 13)	Non-responders (n = 8)	P value
Sex (female/male)	3/10	1/7	1.0
Age (years)[a]	42 (30–60)	37 (28–61)	0.54
Family history of liver disease	6 (46%)	4 (50%)	1.0
Previous interferon treatment	3 (23%)	1 (13%)	1.0
Total dose of interferon (MU)[a]	219 (129–624)	152 (120–533)	0.45
Method of interferon administration (ED + I/I)[b]	3/10	1/7	1.0
Staging of liver histology (F1/2/3/4/ND)	6/4/1/1/1	7/1/0/0/0	0.36
ALT (IU/l)[a]	185 (58–712)	153 (54–802)	0.66
Serum HBV DNA (bDNA; Meq/ml)[a]	7.7 (0.5–770)	1 (0.5–47)	0.32
HBV genotype (A/B/C/unknown)	0/4/9/0	0/3/5/0	1.0

[a] Data values are medians (ranges)
[b] ED + I, initially every day following intermittent therapy; I, only intermittent therapy

Two patients with normal ALT levels at commencement were non-responders. Of six HBeAg-negative patients with undetectable levels of HBV DNA at commencement, three were responders. On the other hand, of three HBeAg-positive patients with undetectable levels of HBV DNA at commencement, one patient was a responder.

Long-term outcome after IFN therapy

In this study, the median follow-up period after IFN therapy was 2 years (range, 0.5–6 years). We analyzed long-term outcome in 31 patients positive for HBeAg and 18 patients negative for HBeAg, in whom the follow-up period was 1 year or more. In the 31 HBeAg-positive patients, the number of responders had decreased from 8 (26%) after 6 months to 5 (16%) at 1–5 years. On the other hand, in the 18 HBeAg-negative patients, the number of responders decreased from 11 (61%) after 6 months to 6 (33%) at 1–6 years.

Evaluation of efficacy of IFN in relation to clinical factors

Data for all patients were subjected to univariate analysis to determine the clinical factors that contributed to the efficacy of IFN treatment. In this analysis, the following two factors significantly influenced the response to IFN: HBeAg negativity (OR, 6.5; 95% CI, 2.1–20.4; P = 0.0013) and low HBV DNA level (<100 vs ≧ 100 Meq/ml; OR, 3.4; 95% CI, 1.0–10.8; P = 0.043). Moreover, ALT level (≧200 vs <200 IU/l) and age (≤35 vs >35 years of age) showed borderline significance with a higher chance of response among all patients (P = 0.056 and P = 0.082, respectively). Next, we investigated the significance of response to IFN therapy by multivariate logistic regression analysis. Both HBeAg and age independently and significantly influenced the outcome of IFN therapy (Table 4).

Discussion

Although IFN is reported to have beneficial effects in the treatment of chronic hepatitis B, the response rate is not high. A metaanalysis published in 1993 reviewed 15 randomized controlled studies involving 837 adult patients who received IFN-α for 4–6 months; loss of HBeAg occurred in 33% of the treated patients.[3] In our study, the response rate to IFN among the HBeAg-positive patients was lower than that in the above studies. The reasons for the difference between studies may

Table 4. Factors associated with response to interferon therapy

Variable	Multivariate odds ratio	95% Confidence interval[a]	*P* value
HBeAg (negative vs positive)	11.1	2.7–46.1	0.0009
Age (≤35 vs >35 years)	5.2	1.3–21.0	0.0209

[a] Values are the odds of having a response to interferon

be differences in ethnic groups and/or in HBV genotype. Kao et al.[11] reported that HBV genotype C, compared to genotype B, was associated with a lower response rate to IFN-α therapy among chronic hepatitis B patients with HBeAg. The response rate among our patients with genotype C was low similar to the results of Kao et al.[11] (response rate; 15%). In our study, in the HBeAg-positive patients, young patients, especially those with a high ALT level at baseline, were significantly more likely to respond to IFN. These prognostic factors were similar to those reported in previous studies,[4–6] although the sample size in our study was small. On the other hand, our previous report[17] showed that 16 of 52 (31%) patients who received IFN-α, given twice per week for 52 weeks, were responders. Therefore, a long-term therapeutic regimen may be necessary to secure a better response.

On the other hand, in the present study the response rate in patients negative for HBeAg was higher than that in those with HBeAg. Previous reports showed that response rates to a 6- to 12-month course of IFN-α in patients with HBeAg-negative CHB ranged from 10% to 47% (average, 24%).[18–21] Moreover, another previous study of ours[22] showed that 9 of 12 (75%) patients who received IFN-β, given twice per week for 24 weeks, responded to the therapy. Considered together, these findings show that the efficacy of IFN in patients negative for HBeAg is high. However, the factors that could predict a sustained response are less well defined in HBeAg-negative patients than in HBeAg–positive patients.[2] The dose of IFN also had little effect, but the duration of therapy (12 vs 5–6 months) was associated with a doubling of sustained response rates.[23]

Our study included two patients with normal ALT levels and 9 patients with undetectable leels of HBV DNA at the commencement of IFN therapy. In these patients, these parameters had decreased by chance at the commencement of IFN therapy, although they had been increased in the 3 months before the treatment. However, the two patients with normal ALT levels at commencement were non-responders. While the response to IFN of patients with normal ALT levels may be poor, that of patients with undetectable levels of HBV DNA at commencement seems similar to other patients. Furthermore, among patients with undatectable levels of HBV DNA at commencement there was no difference in the response rate of those with and without HBeAg.

In our study, no difference in the response to IFN monotherapy was noted between genotypes B and C in patients without HBeAg. Previous reports showed that HBV genotype B was associated with a higher rate of antiviral response to IFN-α treatment in Chinese patients with HBeAg-positive chronic hepatitis B than genotype C.[11,24] It is not clear at present whether this phenomenon applies in patients who are negative for HBeAg.

Recently, Sugauchi et al.[12] proposed that genotype B could be provisionally classified into Ba and Bj subgroups. In Japan, Bj is the major group (93% with genotype B) and most patients with Bj are HBeAg-negative (92%).[25] In our study, six of the seven patients with genotype Bj were HBeAg-negative. Therefore, it is difficult to investigate whether HBV genotype B is associated with a higher rate of antiviral response to IFN treatment than genotype C in Japanese patients with HBeAg-positive chronic hepatitis B.

In our study, HBeAg negativity and younger age were identified as independent and significant determinants of the outcome of IFN therapy. However, an important issue in the treatment of HBeAg-negative chronic hepatitis B is the sustainability of the response to treatment. Our results, which showed a decrease in the response to treatment at long-term follow-up, were similar to those reported by Papatheodoridis et al.[26] In their long-term follow-up of treated patients, it was reported that the sustained response rates decreased from 41% after 6 months to 22% at 2–5 years and thereafter.[26] Lampertico et al.[27] recently reported that 24-month IFN treatment resulted in sustained disease suppression in a significant proportion of patients with HBeAg-negative chronic hepatitis B.[27] Long-term IFN therapy may improve the response to IFN therapy in HBeAg-negative patients. On the other hand, lamivudine, another approved treatment for chronic hepatitis B, requires long-term continuous therapy and could potentially be associated with the development of viral resistance.[28] Considering these aspects of treatment modalities, patients of younger age may require long-term IFN therapy.

In conclusion, we investigated the efficacy of 6-month IFN therapy for Japanese patients. The response rate to