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

IFN among HBeAg-positive patients was low. In this group, patients of young age with high ALT levels were significantly more likely to respond to IFN monotherapy than other patients. On the other hand, the response rate to IFN among HBeAg-negative patients was high. Multivariate analysis identified HBeAg negativity and young age as independent determinants of the outcome of 6-month IFN therapy. Further studies, such as longer-term therapy (over 1 year) may be necessary in order to confirm these findings and establish the true response to IFN therapy.

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Peripheral CD8+/CD25+ lymphocytes may be implicated in hepatocellular injuries in patients with acute-onset autoimmune hepatitis

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Background. The mechanism of liver injuries in autoimmune hepatitis (AIH) is not fully understood, especially because the onset is insidious and the clinical courses fluctuate with spontaneous exacerbation and improvement even without immunosuppressive therapies. ***Methods.*** Eleven patients with acute-onset AIH, some of whom were without hypergammaglobulinemia or anti-nuclear antibodies, and 41 patients with chronic AIH were compared serologically, biochemically, and histologically to determine differences in liver injuries between patients with acute-onset and chronic AIH. All patients fulfilled the diagnostic criteria according to a scoring system proposed in 1999. ***Results.*** Lymphocytes with CD8+/CD25+ markers in pretreatment blood were significantly more prevalent in acute-onset than chronic AIH patients (24% vs 14%; $P < 0.05$). After treatment, however, CD8+/CD25+ lymphocytes were fewer in patients with acute-onset than in those with chronic AIH at 1 and 2 weeks ($P = 0.0001$). No other differences were noted in clinical characteristics or immunological parameters between patients with acute-onset and those with chronic AIH. In a patient with typical acute-onset AIH, CD8+/CD25+ lymphocytes increased and decreased in parallel with the activity of liver disease. ***Conclusions.*** Activated CD8+ T lymphocytes with CD25 markers may be implicated in the development of acute-onset AIH.

Key words: autoimmune hepatitis, acute-onset, cytotoxic T lymphocytes, flow-cytometry, immunosuppressive therapies

Introduction

Autoimmune hepatitis (AIH) is an organ-specific disease caused by immune responses in the genetic background.[1] The mechanism of AIH is not clear, although CD4+ T-cell responses are implicated in hepatocyte injuries, unlike viral hepatitides, in which CD8+ cells play a major role in inducing inflammation of the liver.[2] Most patients with AIH have an insidious disease onset and rarely, if ever, visit hospitals before they develop full-blown disease. Moreover, hepatitis in AIH patients waxes and wanes spontaneously, even without immunosuppressive therapies. These background makes it extremely difficult to determine the mechanism underlying the initiation of hepatic injuries in patients with this disease.

There are patients with AIH who do not fulfil the criteria for a definite diagnosis during the acute phase, and in whom delayed diagnosis can prevent the timely commencement of corticosteroids. These drawbacks for patients are caused principally by the lack of autoantibodies specific for AIH, and failure to diagnose all cases by the present criteria for the diagnosis of AIH.[3]

In the present study, to examine ways of achieving an early diagnosis of AIH, the disease in patients with acute onset was confirmed by histological analysis, which is the gold standard for this disease, and the patients were compared with patients with chronic AIH or nonviral acute hepatitis in terms of various clinical parameters and host factors. This was done with the aim of elucidating the pathogenetic mechanism underlying the initiation of AIH, and determining factors that lead to differences among AIH patients. Peripheral lymphocytes with T cell-markers were also examined, with reference to the in response to immunosuppressive therapies.

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Patients and methods

Patients

The patient population examined consisted of 52 patients with AIH and 12 with nonviral acute hepatitis who were treated at the Department of Gastroenterology, Toranomon Hospital, Tokyo, Japan from 1979 to March 2001. The diagnosis in all of them was confirmed by histological findings and the present diagnostic criteria.[3] Patients with alanine aminotransferase (ALT) levels at presentation exceeding 250 IU/l were categorized as having acute-onset AIH (n = 11) and the others, as having chronic AIH (n = 41). The 12 patients with nonviral acute hepatitis, confirmed by findings on liver biopsies, served as controls. The three groups of patients were compared for hepatobiliary enzymes, autoantibodies, serum levels of IgG, and parameters of histological analysis.

Serological testing

Anti-nuclear antibodies (ANA) were determined by an indirect immunofluorescence method using Hep-2 cells; anti-smooth muscle antibodies were determined by indirect immunofluorescence, anti-liver kidney microsome (LKM1) antibodies were determined by immunofluorescence, hepatitis B surface antigen was determined by radioimmunoassay (Abbott Laboratories, North Chicago, IL, USA); antibody to hepatitis C virus was determined by a second-generation enzyme-linked immunosorbent assay (Dainabot, Tokyo, Japan); and hepatitis C virus (HCV) RNA was determined by reverse-transcription polymerase chain reaction (PCR).

Surface markers on lymphocytes

Peripheral blood mononuclear cells (PBMCs) were separated by centrifugation on a density gradient with Ficoll-Hypaque (Pharmacia, NJ, USA). They were washed twice, counted, and suspended at 1×10^6 cells in RPMI 1640 containing 10% (vol/vol) fetal calf serum. The PBMCs were then cultured at 37°C for 24 h in the wells of a microtiter plate on which anti-CD3 antibodies were immobilized. Then, PBMCs captured by anti-CD3 were analyzed by flow-cytometry with monoclonal antibodies to CD8 and CD25.

Statistical analysis

The χ^2 test was used for evaluating differences in categorical variables and the paired t-test was used to evaluate differences in continuous variables.

Results

Comparison of patients with acute-onset and chronic AIH

Among the 52 patients with AIH, only 11 (21%) were diagnosed with the acute-onset type. Of the 11 patients with acute-onset AIH, the diagnosis in only 4 (36%) was confirmed at presentation by histological findings on the first liver biopsy. Table 1 compares the biochemical, immunologic, and histological features of the patients with acute-onset AIH with these features in patients with chronic AIH and the patients with nonviral acute hepatitis without serological markers of hepatitis viruses. The serum level of IgG in the patients with acute-onset AIH tended to be lower than that in chronic AIH patients. A definite diagnosis based on histological examinations was possible in most cases of acute-onset and chronic AIH (73% and 93%, respectively). There were two cases of possible AIH in the 12 (17%) patients who were diagnosed with nonviral acute hepatitis.

The reason for not being able to diagnose acute-onset AIH in 3 of the 11 (27%) patients was the in failure to achieve a score required in the diagnosis system,[3] with low IgG levels and titers of ANA not high enough. There were patients with acute-onset AIH in whom the diagnosis was made at the second acute exacerbation when their disease had progressed considerably.

Flow-cytometry analysis at pretreatment revealed differences in the decrease of CD8+/CD25+ lymphocytes between patients with acute-onset AIH (4 cases)

Table 1. Comparison of acute-onset AIH with chronic AIH and nonviral acute hepatitis

	Chronic AIH	Acute-onset AIH	Nonviral AH
n	41	11	12
ALT (IU/l)[a]	92 (22–232)	387 (256–2640)	186 (88–452)
IgG (mg/dl)[a]	2650 (2310–4632)	2220 (1854–2940)	1274 (870–2520)
Histological confirmation	38 (93%)	8 (73%)	2 (17%)

AIH, autoimmune hepatitis; AH, acute hepatitis; ALT, alanine aminotransferase
[a]The median value is shown with the range in parentheses

Table 2. Behavior of CD4+/CD25+ lymphocytes in patients with acute-onset and chronic AIH before and during treatment

Patients	CD4+/CD25+ lymphocytes (%)		
	Pretreatment	1 week	2 weeks
Acute-onset (n = 11)	12.3 ± 4.8	18.2 ± 3.2	22.4 ± 7.6
Chronic (n = 41)	15.9 ± 3.3	23.3 ± 6.3	24.2 ± 5.1

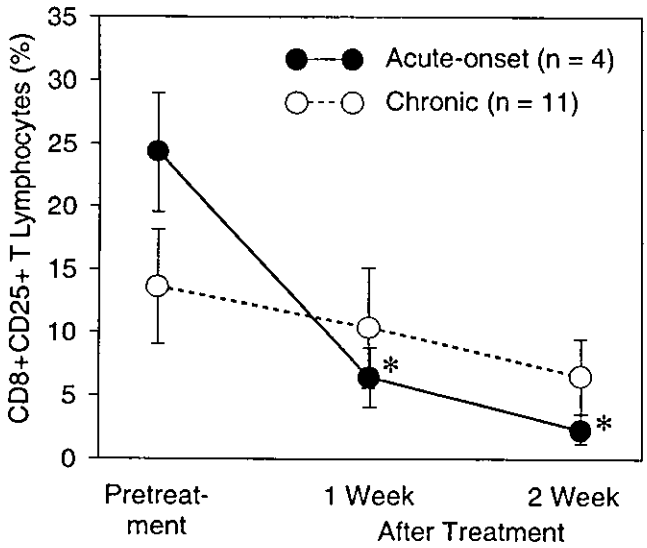

Fig. 1. Decrease in CD8+/CD25+ lymphocytes after treatment in patients with acute-onset and chronic autoimmune hepatitis (AIH). CD8+/CD25+ lymphocytes decreased significantly in patients with acute-onset AIH both 1 week and 2 weeks after treatment, as indicated by *asterisks* (P = 0.0001)

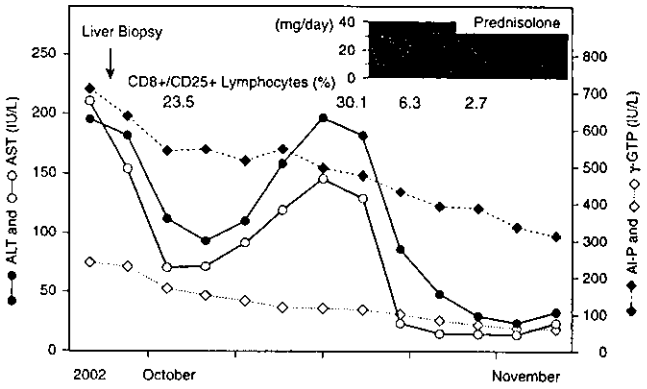

Fig. 2. The clinical course of a patient with acute-onset AIH. Levels of alanine aminotransferase (*ALT*), aspartate aminotransferase (*AST*), alkaline phosphatase (*Al-P*), and γ-glutamyl transpeptidase (γ-*GTP*) are shown, along with the percentages of CD8+/CD25+ lymphocytes. Prednisolone therapy given to the patient is shown *at top of Fig*

and chronic AIH (11 cases) (Fig. 1). Pretreatment numbers of CD8+/CD25+ lymphocytes were significantly higher in acute-onset than chronic AIH (24% vs 14%; $P < 0.05$). After treatment, however, numbers of CD8+/CD25+ lymphocytes were fewer in patients with acute-onset than in those with chronic AIH, at both 1 and 2 weeks (P = 0.0001). In contrast, CD4+/CD25+ lymphocytes at pretreatment, and at 1 and 2 weeks after treatment were not much different between patients with acute-onset AIH and those with chronic AIH (Table 2).

A case of typical acute-onset AIH

A 34-year-old woman without previous liver disease or pertinent family history, or alcohol intake, had a baby by normal delivery in January 2002. No liver function abnormalities were recognized at the delivery. On a regular health check-up in May 2002, however, an ALT level of more than 100 IU/l was detected in her serum, and the attending physician recommended hospitalization. She was diagnosed with non-A, non-B hepatitis, in the absence of autoantibodies or intake of any hepatotoxic drugs. Because therapies had no effect, she was referred to the Department of Gastroenterology, Toranomon Hospital, Tokyo.

At presentation, no anemia or jaundice was detected in examinations of her ocular conjunctivae. She had no hepatomegaly. Biochemical examination of her sera revealed: total protein, 6.3 g/dl; total bilirubin, 1.8 mg/dl; alkaline phosphatase, 634 IU/l; γ-glutamyl transpeptidase, 71 IU/l. Aminotransferases were elevated, with ALT at 154 IU/l and aspartate aminotransferase at 181 IU/l. C-reactive protein (CRP) was negative and γ-globulin was not elevated, at 1.14 g/dl. She did not have evidence of infection with hepatitis viruses, being without hepatitis B surface antigen or antibody to hepatitis C virus in serum.

Immunological examinations disclosed normal levels of immunoglobulins: IgG, 1060 mg/dl; IgA, 286 mg/dl; IgM, 104 mg/dl. Results of tests for autoantibodies were negative. A test for lupus erythematosus (LE) cells produced negative results.

Her clinical course is illustrated in Fig. 2. Specimens from a liver biopsy done 3 days after admission revealed massive fibrosis and severe inflammation in portal areas, with extensive infiltration of lymphocytes (Fig. 3A,B). Her aminotransferase levels decreased sponta-

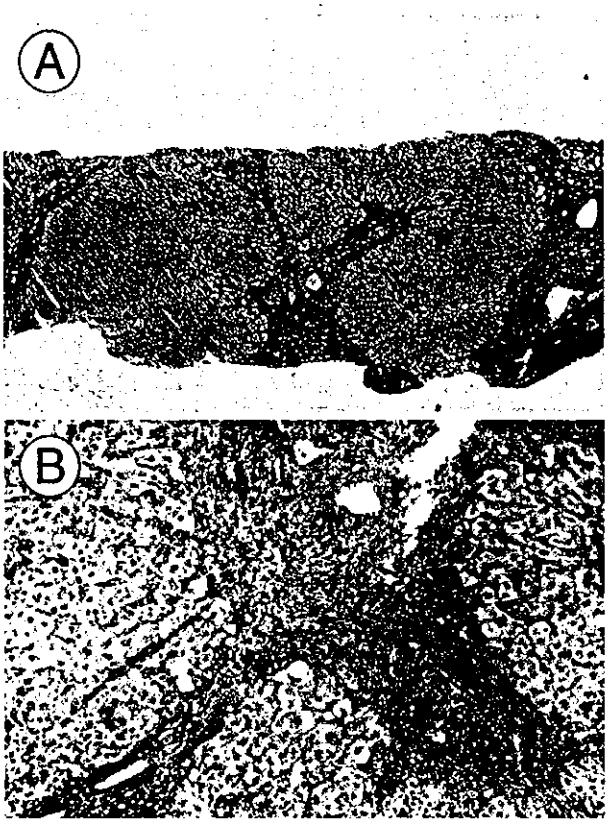

Fig. 3A,B. Liver histology in the patient with acute-onset AIH whose clinical course is shown in Fig. 2. **A** Low-power (×20) field stained with silver; **B** high-power field (×200) stained with periodic acid Schiff

neously without any treatment, and they increased again 3 weeks after admission, whereupon oral prednisolone, at 40 mg/day, was initiated. Aminotransferase levels decreased immediately after the initiation of prednisolone, and the dose was reduced to 30 mg/day. Levels of alkaline phosphatase and γ-glutamyl transpeptidase gradually decreased through her clinical course.

Peripheral lymphocytes were recovered from her blood four times, and they were examined for surface markers. The CD8+/CD25+ lymphocytes reflected her AIH activity. Initially they were detected in 23.5% of total lymphocytes and increased to 30.1% at the time of exacerbation. The CD8+/CD25+ lymphocytes decreased in parallel with lowered aminotransferase levels.

Discussion

A number of theories have been proposed to explain the pathogenesis of hepatocyte injuries in patients with AIH; for example, antibody-dependent cell cytotoxicity (ADCC) and antibody-mediated cytotoxicity (ACC). It has been shown that CD4+ lymphocytes, rather than CD8+ lymphocytes, are heavily implicated in the pathogenesis of AIH.[2,4,5] The involvement of CD4+ lymphocytes is implicated, also, in type 2 AIH—which is characterized by antibodies to liver and kidney mitochondriae (anti-LKM)[6]—and experimental AIH.[7,8] The results obtained in this study, in which acute-onset and chronic cases of AIH were compared in details, suggest that CD8+ cytotoxic T lymphocytes (CTL) may play a significant role in the acute phase of AIH. Specifically, activated CD8+ cells with the CD25 marker were more frequent in patients with acute-onset AIH than in those with chronic AIH, and these cells increased and decreased in parallel with disease activity in a patient with a typical case of acute-onset AIH without elevated IgG or ANA (Fig. 2). As the disease advances into chronicity, a shift would occur from CTL to ADCC in this patient. This view is supported by the lack of hypergammaglobulinemia and high titers of ANA in some patients with acute-onset AIH.[1] The involvement of CD8+ lymphocytes in the pathogenesis of AIH is increasingly be coming obvious.[9,10]

It appears that swift and extensive damage to hepatocytes in the initial stage of AIH would be mediated by CTL through the major histocompatibility (MHC) class 1 pathway. As AIH becomes full-blown, hepatitis would tend to be mediated by helper T cells through the MHC class 2 pathway. Hence the pathology would depend on the balance between Th1 and Th2 subsets of helper T lymphocytes during the course of AIH. This may reflect a crucial involvement of HLA DR antigens in the pathogenesis and expression of AIH, which differs widely from patient to patient, and this involvement deserves further extensive analyses. To pursue this possibility, HLA types were examined in the present series of patients with acute-onset and chronic AIH.

The influence of HLA-DR antigens on the pathogenesis of AIH is obvious, being reflected in characteristic patterns of DR antigens in patients with AIH who are positive for HCV RNA,[11] as well as in patients with other autoimmune diseases.[12,13] The prevalence of DR4 is high in patients with AIH in Japan, while that of DR3 is high in AIH patients in western countries. Because DR4 prevails in patients without DR3 in western countries, these two DR phenotypes have been regarded as independent contributing factors to the genetic susceptibility to AIH. This has been confirmed in studies of peripheral blood mononuclear cells and lymphocytes infiltrating the liver. In lymphocytes infiltrating portal areas, CD4+ cells are more frequent in patients with AIH than in those without it. This may reflect the involvement of class II-mediated immune responses in the pathogenesis of AIH, and the involvement of DR4 and DR15 antigens in AIH.[6]

The predominance of lymphocytes of the Th1 lineage is confirmed by reports of the production of cytokines in a cell line specific for LKM-1 in patients with AIH positive for LKM-1 antibody.[4] Furthermore, CD4+ cells sensitized with liver-derived antigens prevail in liver tissues from patients with AIH, and this feature is not seen in patients with viral hepatitides. These lines of evidence would indicate an important role of DC4 cells in the pathogenesis of AIH mediated by MHC class 2 pathways.

In the present study, only changes in CD8+/CD25+ cells were evaluated to determine their role in the induction of hepatitis in the acute-phase of AIH. The activation of T lymphocytes could be responsible for severe and extensive hepatitis in the acute phase of AIH. Clearly, further analyses along this avenue are required in larger series of patients and with respect to MHC-class 2 antigens to further define the pathology and predict response to treatment in patients with acute-onset AIH. It is hoped that such attempts would characterize more precisely the operation of DR antigens in the pathogenesis of this disease.

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High-dose interferon alpha-2b induction therapy in combination with ribavirin for Japanese patients infected with hepatitis C virus genotype 1b with a high baseline viral load

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Background. Although pegylated interferon (IFN) is now used in many countries as a standard therapy for chronic hepatitis C, the efficacy and safety of combination therapy of high-dose interferon alpha-2b induction with ribavirin are not fully evaluated, especially in Japanese patients infected with hepatitis C virus (HCV) genotype 1b with a high viral load. ***Methods.*** Patients (n = 83) received daily, high-dose induction therapy of interferon alpha-2b (6 million units [MU] once daily for 2 weeks), followed by 6MU three times weekly for 22 weeks. Oral ribavirin (800 or 600mg/day) was given daily for 24 weeks, and then the patients were followed up for 24 weeks. ***Results.*** Of the 83 patients, 67 (81%) had a biochemical response (BR), and 37 (45%) achieved a sustained BR (SBR). Virologic response (VR; undetectable serum HCV RNA level by polymerase chain reaction [PCR]) was noted in 55 (66%) patients, and sustained VR (SVR) in 16 (19%) patients. Baseline viral load did not influence treatment outcome. There was no significant difference in treatment outcome among treatment-naïve patients, relapsers, and nonresponders to previous IFN monotherapy. Multivariate analyses identified serum ribavirin concentrations at week 8 of therapy (odds ratio [OR], 23.7; 95% confidence interval [CI], 1.84–61.1; P = 0.015) and negativity for serum HCV RNA at week 8 (OR, 22.5; CI, 1.76–57.5; P = 0.017, respectively) as two significant and independent predictors of SVR. ***Conclusions.*** The efficacy of 24-week combination therapy of high-dose IFN alpha-2b induction and ribavirin deserves attention in HCV genotype 1b patients with a high viral load, especially in nonresponders to previous IFN monotherapy and patients with a very high viral load.

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Introduction

Combination therapy with interferon (IFN) alpha-2b plus ribavirin is the first-line therapy worldwide for IFN-naïve patients with chronic hepatitis C and even for relapsers and nonresponders to previous IFN monotherapy.[1–5] The standard treatment regimen is subcutaneous three-times-weekly (tiw) administration of IFN alpha-2b, at a dose of 3 or 5 million units (MU) for 24 or 48 weeks, together with the daily oral administration of ribavirin at a dose of 1000–1200mg. This combination therapy has significantly improved the sustained viral clearance rate, compared to the classical Western regimen of IFN monotherapy (3MU tiw without induction therapy). However, the treatment outcome is not always satisfactory, because hepatitis C virus (HCV) genotype 1 (1a/1b) infection correlates strongly with an unfavorable likelihood of response to both combination therapy and IFN monotherapy.[2,3]

In Japan, high-dose, 2- to 8-week daily induction therapy, followed by tiw maintenance administration has been the standard IFN monotherapy regimen. Higher IFN doses have been administered in Japan than in Western countries. The IFN regimen is one of the factors that significantly influences treatment outcome.[6] There are several differences in genotype distributions, patient ages, body weights, and other factors (e.g., ethnic groups) between Westerners and Japanese. Thus, it is unclear whether, in Japanese, the efficacy and safety of combination therapy with high-dose, induction therapy plus ribavirin would be similar to those with the Western regimen. Specifically, Japanese patients infected with HCV genotype 1b with a high viral load comprise about half of the total number of patients with

chronic hepatitis C. This patient subgroup shows a markedly poor response even to high-dose IFN monotherapy, resulting in less than 10% sustained viral clearance rate.[6-10] Strangely, few studies have focused on the outcome of combination therapy for this "IFN-resistant" patient subgroup, all of whom were infected with genotype 1b alone, but not genotype 1 (1a + 1b).

In the present study, we evaluated the efficacy and safety of combination therapy with ribavirin plus high-dose IFN alpha-2b daily induction therapy, followed by intermittent maintenance therapy, for Japanese patients chronically infected with HCV of genotype 1b with a high viral load. In addition, we investigated the factors that could be significantly associated with sustained viral clearance after combination therapy for the "IFN-resistant" patients.

Patients and methods

Study population

Eighty-three Japanese patients chronically infected with HCV genotype 1b and with a high viral load were consecutively enrolled in this study, and received daily, high-dose IFN alpha-2b induction therapy in combination with ribavirin at the Department of Gastroenterology, Toranomon Hospital, between December 2001 and April 2002. Written informed consent was obtained from all patients before they received the combination therapy. Inclusion criteria were: a positive test for anti-HCV antibody; HCV genotype 1b, confirmed by a polymerase chain reaction (PCR)-based method;[11] serum HCV RNA levels more than 200 kilo international units (KIU)/ml on quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0; Roche Molecular Systems, Pleasanton, CA, USA) within the preceding 12 weeks (defined as "high" viral load); persistently high serum alanine transaminase (ALT) concentrations during the preceding 12 weeks; a diagnosis of chronic hepatitis on liver biopsy specimen obtained within the preceding 48 weeks, as assessed by one pathologist, using the ranking system for grading of necroinflammation activity and staging of fibrosis;[12] hemoglobin concentration of 12.0 g/dl or more; platelet count of $100 \times 10^3/\mu l$ or more during the preceding 12 weeks; and age 20 years or more. Patients with the following conditions were excluded from the study: liver cancer or severe liver failure, as described previously;[7] other forms of liver disease, including primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease; coexisting serious psychiatric or medical illness; treatment with any other antiviral or immunomodulatory agent administered within the preceding 24 weeks; history of ribavirin therapy; patients with hepatitis B surface antigen or hepatitis B core antibody, as determined by commercially available radioimmunoassay; patients with hypersensitivity to IFN or ribavirin; and women who were pregnant or lactating.

Study protocol

Treatment was provided for 24 weeks, with a subsequent 24-week follow-up period. Intramuscular IFN alpha-2b (Intron-A; Schering-Plough International, Kenilworth, NJ, USA), at a dose of 6 million units (MU), was administered daily for the initial 2 weeks. Following the 2-week induction therapy, IFN, at a dose of 6 MU, was administered tiw for 22 weeks. Oral ribavirin (Rebetol; Schering-Plough International), at a total dose of 800 mg per day (for patients who weighed more than 60 kg), was administered twice daily for 24 weeks; the dose was 600 mg daily for those who weighed 60 kg or less. The dose of ribavirin was adjusted based on hemoglobin concentrations: ribavirin was reduced to 600 or 400 mg/day when the hemoglobin concentration fell below 10 g/dl, and it was discontinued when the concentration dropped below 8.5 g/dl.

Biochemical and virologic responses to treatment were assessed during the treatment period (weeks 1 through 24), and during the subsequent follow-up period (weeks 25 through 48). A biochemical response (BR) was defined as normalization of serum ALT activity by the end of treatment, and a virologic response (VR) was defined as undetectable serum HCV RNA by the end of treatment, using a qualitative PCR assay (Amplicor HCV version 2.0; Roche Molecular Systems) with a lower detection limit of 100 copies/ml. A sustained biochemical or sustained virologic response (SBR or SVR, respectively) was defined as normalization of serum ALT or as absence of serum HCV RNA, respectively, after the completion of treatment until the end of the follow-up period.

Clinical and laboratory data were assessed twice weekly during the first 2 weeks, at least once weekly during the next 2 weeks, and at least every 4 weeks during the remaining treatment period and the 24-week follow-up period. Virologic assessment and measurement of serum ribavirin concentrations were performed once weekly during the first 2 weeks, and at least every 4 weeks during the remaining treatment and follow-up periods. Serum ribavirin concentrations were determined by a validated high-performance liquid chromatography/tandem mass spectrometric assay, using ^{13}C-ribavirin as an internal standard.[13] The lower detection limit of quantitation for this assay was 50 ng/ml. Adverse effects were monitored clinically by careful interview and medical examination throughout the study. Patient compliance with treatment was evaluated by a questionnaire and medical records and by counting the number of returned capsules.

Statistical analysis

The χ^2 test, Fisher's exact two-tailed test, or the Mann-Whitney test was used for statistical comparisons between group frequencies, where appropriate. Treatment outcomes were analyzed on an intention-to-treat basis. Cumulative probability rates of SVR were calculated using the Kaplan and Meier method. Multivariate analysis (multivariate logistic regression) was used to identify factors independently associated with SVR. Included were 27 demographic, laboratory, histological, virologic, and therapeutic variables (Table 1). In addition, early dynamic variables after therapy, including disappearance of HCV RNA from serum and serum ribavirin concentrations, were also analyzed using univariate and multivariate analyses. Each continuous variable was transformed into two categories, based on the value with the largest capacity to discriminate between patients for univariate and multivariate analyses. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) in univariate analysis were subsequently subjected to multivariate analysis. All P values for statistical tests were two-tailed, and values of less than 0.05 were considered statistically significant. All calculations were performed with the SPSS statistical package (version 7.5; SPSS, Chicago, IL, USA).

Table 1. Baseline characteristics of chronic hepatitis C patients with hepatitis C virus genotype 1b and high viral load treated with 24-week combination therapy of high-dose interferon (IFN) alpha-2b induction plus ribavirin

Sex (M/F)	60/23
Age (years)	51 ± 10[b]
Body weight (kg)	66.1 ± 8.1[b]
History of transfusion (yes/no)	28/55
Bilirubin (mg/dl)	0.8 (0.3–1.4)[a]
AST (IU/l)	60 (24–194)[a]
ALT (IU/l)	100 (50–312)[a]
LDH (IU/l)	153 (107–307)[a]
Alkaline phosphatase (IU/l)	211 (105–437)[a]
GGT (IU/l)	50 (14–244)[a]
Total protein (g/dl)	7.6 (6.3–9.1)[a]
Albumin (g/dl)	3.8 (3.2–4.7)[a]
Cholinesterase (ΔpH)	1.1 (0.5–1.8)[a]
Creatinine	0.8 (0.5–1.1)[a]
Clearance of creatinine	89.7 (42.6–129.8)[a]
Iron (μg/dl)	149 (35–291)[a]
Ferritin (ng/ml)	164 (<10–1611)[a]
Hyaluronate (μg/l)	55.7 (14.3–356)[a]
Platelet count ($\times 10^3/\mu l$)	167 (100–325)[a]
Hemoglobin (g/dl)	15.1 (12.0–17.4)[a]
Prothrombin time (%)	92 (69–117)[a]
ICG_{R15} (%)	19 (4–49)[a]
Liver histology	
Stage (1/2/3/4)	45/21/16/1
Grade (mild/moderate/severe)	53/29/1
Viral load (KIU/ml)	720 (230 to <850)[a]
Naïve/retreatment	24/59
Ribavirin dose/kg weight (mg/kg)	11.4 (8.8–14.3)[a]; 11.4 ± 1.1[b]

AST, aspartate transaminase; ALT, alanine transaminase; LDH, lactic acid dehydrogenase; ICG_{R15}, indocyanine green retention rate at 15 min

[a] Data values are expressed as medians (range)

[b] Data values are expressed as means ± SD

Results

Patient characteristics at baseline are summarized in Table 1. Eighty-three patients (60 men and 23 women) between the ages of 24 and 70 years (median, 52 years) and weighing between 47.2 and 89.8 kg (median, 66.5 kg) participated in the study. Fifty-three (64%) patients fully completed the treatment and were followed-up closely as scheduled. Although patient compliance with ribavirin was satisfactory, the dose of ribavirin was reduced to 600 or 400 mg/day in 17 (20%) patients because of falls in hemoglobin concentrations to less than 10.0 g/dl. Of these 17 patients, 14 received treatment for 24 weeks as scheduled. Sixteen (19%) patients developed severe treatment-related adverse events that led to discontinuation of therapy.

Of the 83 patients, 67 (81%) had a BR, and 37 (45%) achieved an SBR. ALT activities in 30 relapsers returned to the baseline level. Fifty-five (66%) patients showed a VR, and 16 (19%) patients attained an SVR. Serum HCV RNA levels returned to baseline values in 39 virologic responders, who became positive for serum HCV RNA after cessation of treatment. The cumulative probability rates of SVR were 5% at week 4; 22% at week 8; 44% at week 12; 53% at week 16; 68% at week 20; and 68% at week 24 (Fig. 1). In SVR patients, HCV RNA disappeared from serum at times ranging from week 2 to week 14.7 (median, 8 weeks).

VR was noted in 24 of 38 (63%) patients with a pretreatment viral load of more than 200 to less than 700 KIU/ml and in 31 of 45 (69%) patients with a viral load of 700 KIU/ml or more. Furthermore, an SVR was achieved by 9 of 38 (24%) patients with a viral load of more than 200 to less than 700 KIU/ml and by 7 of 45 (16%) patients with a viral load of 700 KIU/ml or more. Thus, viral load did not significantly influence VR and SVR, although patients with very high viral loads were less likely to show SVR.

Of the 24 IFN-naïve patients, 15 (63%) had a VR, and 4 (17%) attained an SVR. Of the 59 patients who had previously received one or two courses of IFN monotherapy, 40 (68%) had a VR, and 12 (20%) attained SVR. Of the 40 virologic responders to combination therapy, 12 had a VR to previous IFN monotherapy, 16 had no VR, and 12 had no available data. Of the 12 sustained virologic responders to combination therapy, 5 showed a VR to previous IFN therapy, 4 had

no VR, and no data were available for the other 3. In all retreatment patients except 1, the total dosage and treatment period of previous IFN monotherapy were equal or equivalent to, or more than, those in combination therapy. There was no significant difference in VR and SVR rates between IFN-naïve patients and those who had previously received IFN monotherapy. Of the 24 patients who showed no VR to previous IFN therapy, 16 (67%), including 4 (17%) sustained virologic responders, exhibited VR to combination therapy.

Despite ribavirin dose reduction because of falls in hemoglobin concentrations, 14 patients received the 24-week treatment as scheduled. Of these 14 patients, 11 (79%) had a VR, and 3 (21%) achieved an SVR. Of 53 patients who fully completed the treatment schedule, 37 (70%) had a VR, and 13 (25%) attained an SVR. There was no statistically significant difference in VR and SVR rates between the subgroups of patients.

In the univariate analysis, four baseline and dynamic variables were significantly or marginally linked to SVR: negativity for HCV RNA in serum at week 8 of therapy ($P = 0.011$), serum ribavirin concentration of 2500 ng/ml or more at week 4 of therapy ($P = 0.046$), serum ribavirin concentration of 3000 ng/ml or more at week 8 of therapy ($P = 0.049$), and γ-glutamyl transpeptidase level of less than 50 IU/l ($P = 0.059$). Because these variables were mutually correlated, multivariate analysis was subsequently performed. In the final step, the following two variables entered the model and could not be removed: serum ribavirin concentration at week 8 of therapy (odds ratio [OR], 23.7; 95% confidence interval [CI], 1.84–61.1; $P = 0.015$) and negativity for HCV RNA in serum at week 8 of therapy (OR, 22.5; 95% CI, 1.76–57.5; $P = 0.017$) (Table 2). These two factors were significantly and independently associated with SVR.

All patients experienced adverse effects during treatment, and showed profiles similar to previously reported symptoms.[1–5] The majority of adverse effects were of mild to moderate severity. Thirteen (16%) patients discontinued treatment because of severe adverse effects (Table 3). After cessation of treatment, all but 1 patient, who suddenly died, recovered completely from the adverse effects without any longterm effects. One patient who developed cerebral hemorrhage had cirrhosis, 1 patient who developed cerebral infarction received antihypertensive therapy, and 1 patient who died suddenly had been operated upon for cardiovascular disease (unspecified) in infancy, but the cause of his death was unknown. For the whole group, hemoglobin concentrations fell from 12.0–17.4 g/dl (median, 15.1 g/dl) to 7.8–14.9 g/dl (median, 10.4 g/dl), representing a fall of 0.8 to 7.6 g/dl (median, 4.2 g/dl). The nadir values were observed between weeks 2 and 24 (median, week 10). In 21 (25%) patients, the dose of ribavirin was reduced to 600 or 400 mg/day due to anemia (hemoglobin concentrations below 10.0 g/dl) between weeks 1 and 17 (median, 5 weeks). No patient required dose adjustment in response to other hematological changes during treatment.

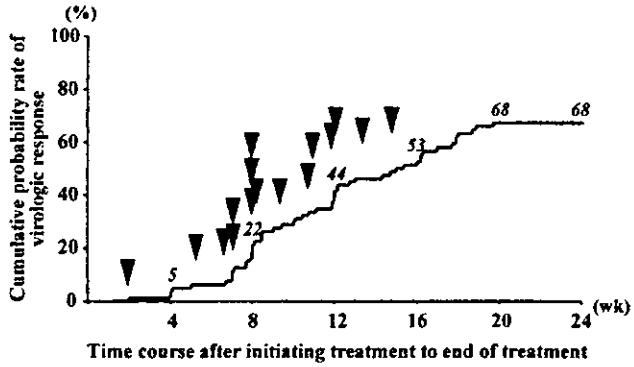

Fig. 1. Cumulative probability rate of virologic response to combination therapy with high-dose interferon alpha-2b induction plus ribavirin for chronic hepatitis C patients infected with hepatitis C virus genotype 1b and a high baseline viral load. *Arrowheads*, time-points at which virologic response was observed in patients with sustained virologic response (SVR). *Numbers in italics* on graph are the cumulative probability rates at the weeks indicated

Table 2. Variables associated with sustained virologic response to combination therapy of IFN alpha-2b plus ribavirin in patients chronically infected with hepatitis C virus genotype 1b with a high viral load

Variables	Category	Odds ratio (95% CI)	*P* value
Serum ribavirin concentration at week 8 after start of therapy	1: <3000 ng/ml	1	0.015
	2: ≥3000 ng/ml	23.7 (1.84–61.1)	
Serum HCV RNA at week 8 after start of therapy	1: Positive	1	0.017
	2: Negative	22.5 (1.76–57.5)	

95% CI, 95% confidence interval

Table 3. Adverse effects of therapy necessitating discontinuation of treatment

Adverse effects	Age (years)	Sex	Time at which therapy was terminated (weeks)[a]
General malaise (n = 5)	70	F	2
	62	F	2
	61	M	9
	48	M	15
	58	M	23
Depression (n = 2)	44	F	3
	64	M	16
Irritability (n = 1)	50	M	14
Severe hemolytic anemia (n = 1)	62	F	11
Bleeding from internal hemorrhoids (n = 1)	67	M	4
Cerebral hemorrhage (n = 1)	44	M	6
Cerebral infarction (n = 1)	47	M	23
Sudden death (n = 1)	24	M	22

[a]Time at which therapy was terminated after commencement of treatment (weeks)

Discussion

In December 2001, the Japanese Government instituted 24-week combination therapy of high-dose IFN alpha-2b induction plus ribavirin for chronic hepatitis C. Our results confirmed that approximately 20% of genotype 1b patients with a high viral load attained an SVR with this regimen. Interestingly, the VR and SVR rates were similar among IFN-naïve patients, relapsers, and nonresponders to previous IFN monotherapy. Of note is that 67% and 17% of the nonresponders achieved VR and SVR, respectively. A recent metaanalysis of controlled trials indicated that the overall SVR rate with the 24-week Western regimen was 13.2% in nonresponders.[14] When confining the analysis to genotype 1 patients, the SVR rate would decrease to approximately 10%. Considering that our nonresponders had previously received higher doses of IFN than those used in the West, the regimen we used may specifically benefit nonresponders. The VR rate of 67% in our nonresponders suggests that longer treatment duration could increase the high SVR rate, even in nonresponders. Recently, one study showed that treatment with IFN (5MU tiw) plus ribavirin (1000mg/day) for 12 months increased the SVR rate to 18% in genotype 1 or and genotype 4 nonresponders.[5] These clinical data support the synergistic antiviral activity of ribavirin with IFN, which is induced by as yet unconfirmed mechanisms.[15,16]

Careful attention should be paid to the interpretation of results with the Western regimen, because there are various differences in treatment-, host-, and virus-related characteristics between Western and Japanese patients. As for virus-related factors, more than half of genotype 1 Western patients are infected with genotype 1a,[1,17] which is very rare in Japan. Strangely, only few studies have focused on the treatment outcome in genotype 1b patients alone, and addressed whether the response to combination therapy may differ between genotype 1a and 1b patients. One study showed that genotype 1a patients were more likely to respond to combination therapy than genotype 1b patients.[1] The possibility that the ratio of genotype 1a to 1b may modify treatment outcome cannot be completely excluded.

When genotype 1b patients with a high viral load were stratified according to baseline viral load, our study showed that the VR and SVR rates were independent of the viral load. These findings are in agreement with those observed previously in patients who were infected with different genotypes and received different treatment regimens.[1,5] Combination therapy could be valuable in such patients with unfavorable virus-related factors, because baseline viral load influences the response to IFN monotherapy.[1,8-10] Specifically, 16% of our patients with a very high viral load (≥700KIU/ml) achieved an SVR when treated with our combination regimen, whereas few patients with such conditions generally had an SVR to IFN monotherapy.

With respect to treatment-related factors, it is not clear whether IFN induction in combination therapy could increase the SVR rate in genotype 1b patients with a high viral load. Certainly, induction therapy induces a more rapid viral decline than intermittent therapy, resulting in a high initial rate of HCV clearance. However, the VR and final SVR rates are unlikely to depend on IFN induction.[17-19] Thus, the beneficial effect of IFN induction in the combination therapy remains controversial pending further studies.

Early loss or persistence of serum HCV RNA could positively or negatively predict SVR to IFN-based

therapy.[4,20,21] The time-point for efficiently discriminating treatment outcome is week 4 after the initiation of IFN monotherapy, and week 12 in combination therapy.[2-4] Among genotype 1b patients with a high viral load who were treated with our combination regimen, the most appropriate time-point was week 8, which was an independent dynamic predictor of SVR in our multivariate analyses. Unlike findings in IFN monotherapy, late clearance of HCV RNA in combination therapy can still be associated with SVR (Fig. 1).[2,3] Our multivariate analyses also identified serum ribavirin concentrations at week 8 as an independent predictor of SVR. Ribavirin concentrations need to reach a critical and stable level to induce SVR,[22-24] and require at least 4–8 weeks to reach a steady level.[13,23,24] Accordingly, our multivariate analysis identified no baseline factor as an independent predictor of SVR to our combination regimen in genotype 1b patients with a high viral load. Predictors described in previous studies include non-genotype 1, low baseline viral load, younger age, female sex, white race, and absence of bridging fibrosis or cirrhosis.[2,3,25]

Previous studies reported that ribavirin dose reductions, caused by hemolytic anemia, were observed in 4%–9% of patients who were treated with 24- or 48-week combination therapy.[1,2,4,17] In these studies, discontinuation of treatment due to adverse events was necessary in 5%–6% of the patients. Compared with these data, our rates of reduction and treatment cessation appear to be higher. The mean or median maximal decrease from baseline hemoglobin level was 0.9–3.1 g/dl in previous studies,[1,2,4] whereas the value in our study was 4.2 g/dl. The difference may, perhaps, have been caused by differences in patient age (mean, 51 years in our study vs 39–44 years in previous studies), body weight (66.1 kg vs 75.6–83.7 kg, respectively), and IFN treatment regimen (with high-dose induction vs with low-dose induction and without induction).[1,2,4,17] Alterations of our combination regimen should be made after careful consideration, because our results reconfirmed that ribavirin dose reductions and subsequent continuation of treatment did not significantly influence the SVR rate.[2] Although the beneficial effect of extending treatment duration from 6 to 12 months was limited to genotype 1 patients with a high viral load,[1,2,3] an increase in the drop-out rate with prolonged treatment duration should be kept in mind.[2]

In conclusion, the effect of 24-week combination therapy of high-dose IFN alpha-2b induction and ribavirin deserves attention in genotype 1b patients with a high viral load, specifically in nonresponders to previous IFN monotherapy and patients with a very high viral load. In the future, a new combination regimen should be established to improve the SVR rate and to reduce adverse effects in these patients.

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Influence of hepatitis B virus genotypes on the response to antiviral therapies

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Hepatitis B virus (HBV) has been classified into eight genotypes (A–H) based on genome sequence divergence. Genotypes of HBV have distinct geographical distributions, and two genotypes account for most HBV worldwide. Hepatitis B e antigen expression lasts longer and liver disease is more severe with graver outcomes in carriers of genotype C than B in Asia. Accumulating lines of evidence indicate a better response to interferon and lamivudine in patients with chronic hepatitis B who are infected with genotype B rather than C. The therapeutic response may differ, however, in patients infected with HBV of the same genotype. For example, the response to lamivudine is poorer in patients infected with subtype Ba, which contains a recombination with genotype C, than in those with subtype Bj without such a recombination. Influence of genotypes on therapeutic response needs to be examined in patients infected with the other genotypes, particularly in those with genotype A or D infection.

Keywords: chronic hepatitis, genotypes, hepatitis B e antigen, hepatitis B virus, interferon, lamivudine

Introduction

Worldwide, 350 million people are estimated to be persistently infected with hepatitis B virus (HBV),[1] and three-quarters of these people reside in Asia. The morbidity and mortality of persistent HBV infection are a major public health concern. More than one million deaths every year are due to end-stage HBV liver disease, such as decompensated liver cirrhosis and hepatocellular carcinoma (HCC). The carrier state of HBV is established mainly through mother-to-baby infection in Japan, where the prevalence of hepatitis B surface antigen carriage (HBsAg) in the general population used to be less than 2%, while horizontal transmission during infancy plays an additional role in the other countries in Asia and accounts for most persistent infections in Africa where HBsAg prevailed in >8% of the general population during the past. Although much less efficient, horizontal transmission can lead to persistent HBV infection in Western countries through sexual contact and illicit intravenous drug use. Individuals with persistent HBV infection need to be identified early and receive efficient antiviral therapy in order to prevent the development of serious liver disease.

Genotypes of HBV

The response to antiviral therapy in HBV infection is influenced by many host and viral factors. Recently, HBV genotypes have attracted increasing attention since they influence the activity and outcome of HBV-associated chronic liver disease, as well as the response to antiviral therapies. In 1988, four genotypes of HBV (A–D) were proposed based on sequence divergence of >8% in the entire HBV genome, which consists of approximately 3200 base pairs.[2] Later, an additional four genotypes (E–H) were identified by the same criteria.[3–5] HBV genotypes have distinct geographical distributions,[6] and the full picture awaits further epidemiological surveys in many as yet unexamined countries. Overall, genotype A prevails in Northwest Europe, sub-Saharan Africa, India and the United States, B and C are frequent in Southeast Asia, Japan and Oceania, and D is common in the Mediterranean countries. Genotype E is restricted to Africa, and F is found mainly in Central and South America. The distribution of genotypes G and H is yet to be determined. Since persistent HBV infection is frequent in Asia, genotypes B and C prevailing there have been studied most extensively with their clinical and therapeutic differences unfolding rapidly. Differences between genotypes A and D prevailing in Western countries, India and the United States, also, are increasingly coming to the fore.

Clinical manifestations of persistent HBV infection with distinct genotypes

Prospective, case-controlled and cross-sectional studies predominantly but not entirely indicate that the severity and outcome of chronic hepatitis B are more serious in patients infected with

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genotype C compared with B.[7-10] Liver cirrhosis and HCC are more frequent in carriers of genotype C than B.[7,11-13] Very recently, chronic liver disease was detected more frequently in Japanese individuals infected with genotype C than D [221/350 (63%) compared with 6/38 (16%), $P<0.001$].[14]

The synthesis of hepatitis B e antigen (HBeAg) is regulated at both translational and transcriptional levels.[15] Mutations to create stop codons in the precore region, typified by the G-to-A mutation at nucleotide (nt) 1896 (G1896A), shut down the translation of HBeAg completely. The double mutation in the core promoter, A-to-T at nt 1762 and G-to-A at nt 1764 (A1762T/G1764A), interferes with the proper transcription of HBeAg precursor, thereby downregulating the synthesis of HBeAg. The precore stop codon mutation (G1896A) is detected more frequently in persons infected with genotype B than C; it is inhibited in those with genotype A, because it destabilizes the e encapsidation signal.[16] In remarkable contrast, the core promoter double mutation (A1762T/G1764A) is more common in those with genotype C than B. Overall, the ramifications of this are that the seroconversion to the loss of HBeAg takes longer in individuals infected with genotype C than B, and is accompanied by the development of severe liver disease.

Sugauchi *et al.*[17] reported two subtypes of genotype B, one of which possesses a recombination with genotype C over the precore region plus core gene (subtype Ba) while the other does not (subtype Bj). The distribution of subtype Bj is restricted to Japan (hence the 'j' for Japan), in contrast to subtype Ba found in all Asian countries other than Japan ('a' for Asia, therefore). Since HBeAg and the double mutation in the core promoter (A1762T/G1764A) are significantly more frequent in carriers of subtype Ba than Bj,[18] subtypes of genotype B may influence the clinical outcome and the response to antiviral therapies for chronic hepatitis B.

To a lesser extent, clinical differences between genotype A and D infections have been reported from Europe, where these genotypes are frequent. HBV infection is contracted in adulthood in these countries, principally through sexual contacts and illicit drug use, and HBV infection is more likely to persist in persons infected with genotype A rather than D or the other genotypes.[19] These findings stand at variance with those of Sanchez-Tapias *et al.*[20] who found sustained biochemical remission and clearance of HBV DNA to be more frequent in infection with genotype A than genotype D (log-rank, 14.2, $P=0.002$) or genotype F (log-rank, 4.2, $P=0.03$); the rate of HBsAg clearance was also found to be higher in genotype A compared with D infection (log-rank, 4.06, $P=0.03$). Likewise in a comparison between 60 and 63 patients in India infected with HBV genotype A or D, respectively, genotype D was significantly associated with severe liver disease (61% compared with 30%, $P<0.05$) and tended to be more frequent in those with HCC below 40 years of age (63% compared with 44%, $P=0.06$).[21] Clinical differences amongst HBV genotypes manifest themselves in the distribution of acute and chronic liver disease in those who visit hospitals. In our Toranomon Hospital in metropolitan Tokyo, 57 adult patients with acute hepatitis B and 1077 with chronic hepatitis B were admitted during the same period.[22] The distribution of genotypes were: genotype A (acute, 22.8% versus chronic, 1.9%; $P<0.00001$); B (14.0% versus 9.4%); C (43.9% versus 87.7%, $P=0.004$); D (1.8% versus 0.2%); F (1.8% versus 0.2%); and untypeable (15.8% versus 0.6%, $P=0.001$).

Influence of HBV genotype on the response to antiviral therapy

Until lamivudine was developed for clinical use, interferon had remained the sole practical antiviral for chronic hepatitis B, ever since initial clinical trials by Hoofnagle and colleagues[23,24] in the mid-1980s. The response to interferon, judged by the loss of HBeAg from serum, is achieved in at most 20% of treated patients.[25] Moreover, Asian patients who have acquired the HBV carrier state at birth or in early infancy respond to interferon more poorly than Caucasian patients who contracted it in adulthood.[26] To make matters even worse, patients with chronic hepatitis B positive for anti-HBe antibodies are much less responsive to interferon than those with serum HBeAg.[27] Limited experience indicates that HBV genotypes make a difference in the response to interferon in patients with chronic hepatitis B.

Zhang *et al.* compared the response between 10 patients with genotype A infection and 21 patients with genotype D or E infection.[28] Since all patients they studied were positive for anti-HBe antibodies, the negative influence of genotype A on seroconversion to anti-HBe was excluded. They found the response to interferon was higher in patients infected with genotype A compared with D or E (70% versus 40%, $P=0.001$).[28] Likewise, Kao *et al.*[29] reported the response to interferon to be higher in patients infected with genotype B rather than C [13/32 (41%) versus 4/26 (15%), $P=0.045$]. More recently, Wai *et al.*[30] compared the response between patients randomized to interferon or placebo. They found the response was better in patients with genotype B than C infection who were allocated to interferon treatment [12/31 (39%) versus 7/42 (17%), $P=0.034$]; the response rate did not differ in those who received placebo.

Lamivudine [(−)-β-L-2′,3′-dideoxy-3′-thiacytidine] is a nucleoside analogue with a potent antiviral activity. Since its approval in 1998, lamivudine has gained wide popularity for the treatment of chronic hepatitis B due to high a efficacy with minimal untoward effects.[31-35] We believe in continued lamivudine treatment for patients with or without serum HBeAg,[36-39] and have accumulated experience with 286 patients including seven who have received lamivudine for 7 years or longer.[40] HBV genotype also makes a difference in the response to lamivudine in patients with chronic hepatitis B. Among the 16 patients who had received lamivudine for 3 years or longer, the virological response with the loss of HBV DNA detectable by non-amplified method was achieved in two of the three (67%) patients infected with genotype B and in seven of the 13 (54%) patients infected with genotype C.

Kao *et al.*[41] reported on the response to lamivudine in patients treated for 6–30 months infected with genotype B compared with C [3/13 (23%) versus 2/18 (11%), no significant differences]. They found resistance to lamivudine in two (15%) patients with genotype B and in four (22%) with genotype C. Chien *et al.*[42] reported that the sustained response to lamivudine was much higher in patients infected with genotype B compared with genotype C [38/62 (61%) versus 5/20 (20%), $P=0.009$]. We monitored 213 patients on continued lamivudine treatment for drug-resistant HBV variants for mutations in the tyrosine-methionine-aspartate-aspartate (YMDD) motif in the viral DNA polymerase/reverse transcriptase.[43] The emergence of YMDD mutants was no different amongst patients infected with genotype A, B or C. However, YMDD mutants developed significantly more frequently in patients infected with subtype Ba

than Bj during 2 years on lamivudine [3/4 (75%) versus 1/14 (7%), $P<0.05$]. Severe acute exacerbation of hepatitis occurred in four of the 185 (2%) patients with genotype C along with the emergence of YMDD mutants, but in none of the 28 patients with the other genotypes. In patients with chronic hepatitis B in Germany, risk of lamivudine resistance was significantly higher in carriers of HBsAg of serotype adw than ayw [7/13 (54%) versus 1/13 (8%), $P=0.03$];[44] serotype adw corresponded to genotype A and ayw to D.[45]

Conclusion

HBV genotypes influence the severity of liver disease and response to interferon and lamivudine. They are also expected to influence the response to adefovir dipivoxil, which has recently been approved for treatment of chronic hepatitis B,[46] as well as the emergence of resistant mutants;[47] although as yet no differences have been observed in the response to adefovir dipivoxil in relation to HBV genotypes.[48] Should poor responses to a given antiviral be predicted in patients infected with HBV of certain genotypes, they can be directed to the other therapeutic options to spare the cost and burden of treatment. In evaluating the association of HBV genotypes with the response to antiviral therapies, however, it needs to be taken into account that patients visiting hospitals are biased for severe liver disease. Moreover, once full-blown disease develops, it would become refractory to any antiviral treatments. Thus, genotype differences may be attenuated in patients with severe liver disease seen in hospitals,[49,50] probably due to exclusion of patients with less severe disease who can still benefit from treatments. This view would be supported by different distributions of genotypes B (12% and 54%, respectively) and C (84% and 47%) between patients visiting hospitals and individuals found with HBV infection at routine check-ups in the same district of Japan.[51,52] Therefore, in evaluating the influence of HBV genotypes on response to antiviral therapies, one has to keep in mind not only patients with liver disease who visit hospitals, but also those who have not and who may benefit from early treatment.

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