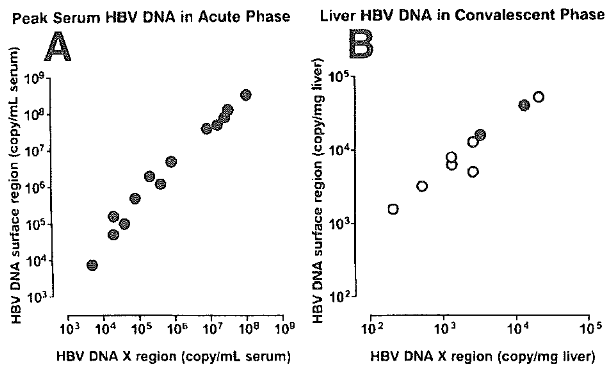

Fig. 2. HBV DNA levels measured by real-time detection PCR using primers derived from the surface region were correlated with those measured by PCR with primers from the X region. (A) A significant correlation was observed for peak serum HBV DNA in the early phase of acute self-limited hepatitis B ($r = 0.989$; $P = .001$). (B) The correlation was also significant for liver HBV DNA, which was persistently detected in the convalescent phase of the disease ($r = 0.946$; $P = .008$). ●, Positive for serum HBV DNA; ○, negative for serum HBV DNA.

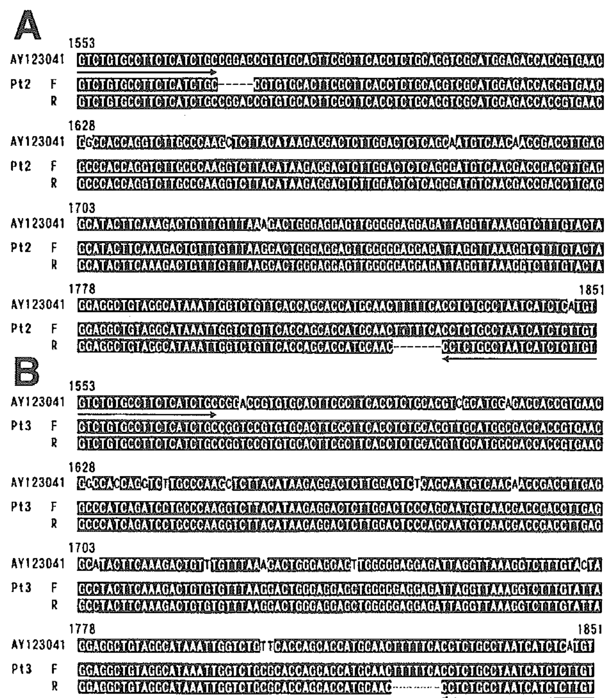

Fig. 4. The direct repeat PCR products recovered from liver tissue samples from patients (A) 2 and (B) 3 were sequenced in both directions. The forward (F) and reverse (R) nucleotide sequences obtained were aligned with the reference sequence of an HBV isolate (GenBank accession no. AY123041). Nucleotide positions are indicated at the **top**. Identical nucleotides are shown by white letters on a black background. **Dashes** denote undetermined nucleotides. Primer sequences used (DRF1/DRR2) are shown by **arrows**.

Histopathologic examination showed that a low grade of liver inflammation had persisted for a decade. Mild portal inflammation was observed in 7 patients (78%), one of which had mild focal necrosis. None of the patients had piecemeal necrosis or confluent necrosis. Liver fibrosis was frequently observed in the late convalescent phase. The fibrosis score ranged from 0 to 3, and 8 patients (89%) retained liver fibrosis. Six patients showed fibrous expansion of most portal areas accompanied by occasional portal-to-portal bridging, and their fibrosis stage was scored 3. Examples of these histologic alterations encountered are presented in Fig. 5. Overall, only 1 of the 9 patients examined had normal liver histology. The histologic outcomes were unlikely to be affected by an alcoholic factor. The 9 patients with liver biopsies had low levels of alcohol intake (Table 1). Moreover, similar histologic findings were observed for the 5 patients with very low levels of alcohol intake (nondrinker or <5 g/d, if any). Mild portal inflammation persisted in 4 patients, accompanied by mild focal necrosis in 1 patient. Four patients retained liver fibrosis, and the stage was scored 3 in 2 patients. Mild steatosis was seen in patients 1 and 9, but there was no evidence of pericellular/perisinusoidal fibrosis suggestive of alcoholic or nonalcoholic steatohepatitis.

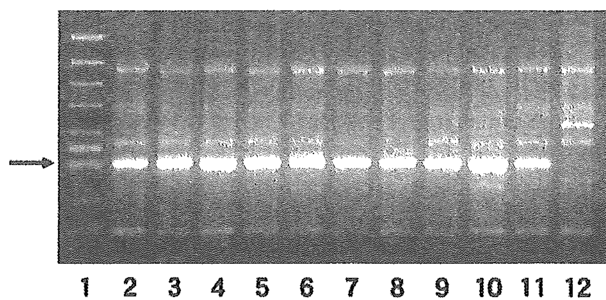

Fig. 3. Detection of a 299-base pair intact direct repeat sequence in liver tissue from patients with a remote history of acute self-limited hepatitis B. Lane 1, molecular weight marker (px174 DNA/Hae III [Toyobo]); lanes 2, 3, 4, 5, 6, 7, 8, 9, and 10, liver tissue from patients 1, 2, 3, 4, 9, 11, 12, 14, and 6, respectively; lane 11, positive control (liver tissue from a chronic HBsAg carrier); lane 12, negative control (liver tissue from a patient without HBV infection).

Liver viral loads and fibrosis stage in the late convalescent phase were further correlated with patient clinical courses. Liver viral loads estimated from the levels of the HBV DNA surface and X regions had no relation to the peak levels of serum alanine aminotransferase activity, bilirubin, and HBV DNA in the acute phase. The relationship was not evident between the liver viral loads and time from the onset of acute hepatitis B. Advanced liver fibrosis stage years after clinical and serologic recovery was associated with high levels of peak serum HBV DNA at the onset ($P = .046$) but not with the liver viral loads in the

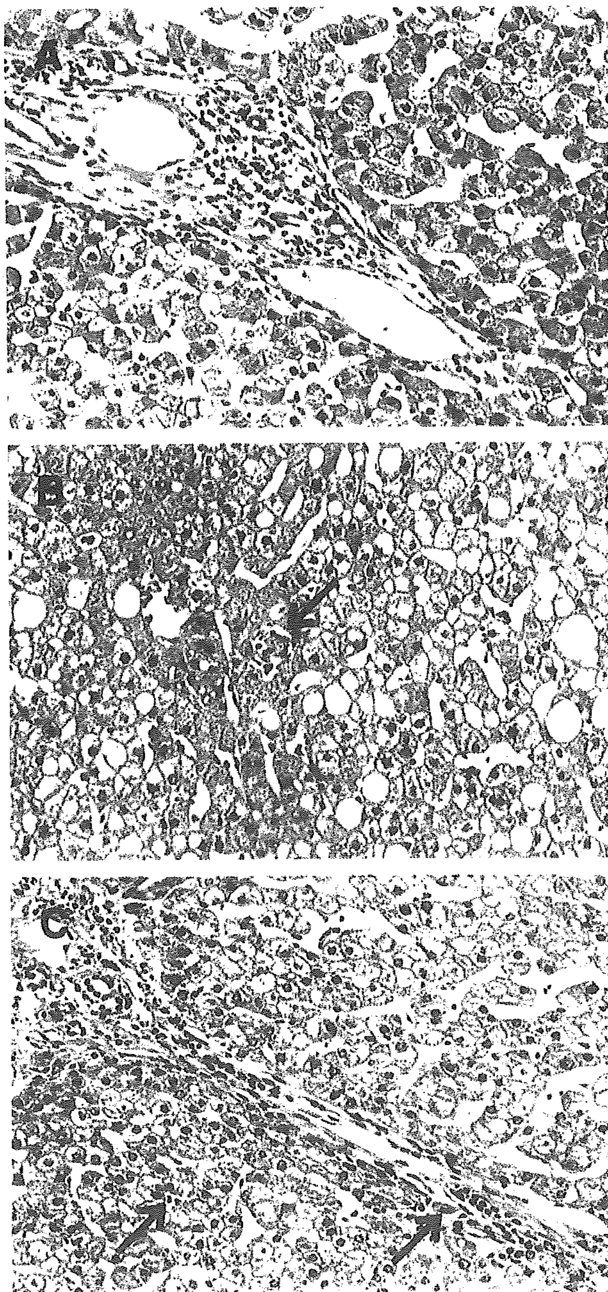

Fig. 5. Representative liver histologic alterations in patients with a remote history of acute self-limited hepatitis B. (A) Mild infiltration of inflammatory cells and fibrous expansion of portal areas were observed in most patients (patient 3). (B) Mild focal necrosis (**arrow**) and mild steatosis were further found in 1 patient (patient 9). (C) Six patients had occasional portal-to-portal bridging of fibrosis. **Arrows** indicate apoptotic cell and apoptotic body seen in this case (patient 11). (Original magnification: A and B, ×400; C, ×200.)

late convalescent phase. No relationship was found between liver fibrosis stage and time from the onset of the disease. The influence of alcohol intake on fibrosis stage was also not evident.

Discussion

At present, the long-term impact of acute self-limited hepatitis B on the liver is unknown in humans. The main aims of the present study were to explore the long-term histologic and virologic outcomes of acute self-limited hepatitis B. The duration and pathologic implications of serologically undetectable HBV persistence in the liver were investigated. Enhanced HBV-specific immune responses are induced following acute exposure to the virus,[4-7] which presents a striking contrast to chronic HBV infection and is believed to lead to termination of the disease. However, there is a report of a chimpanzee that continued to harbor a nonreplicating episomal form of HBV DNA in the liver after resolution of acute hepatitis B by PCR criteria.[20] Similar findings of the persistence of woodchuck hepatitis virus (WHV) DNA in the liver have been described for apparently healthy animals after recovery from acute WHV hepatitis.[21]

All patients studied had demonstrated clinical and serologic recovery from acute hepatitis B. Most (80%) of the patients had developed anti-HBs and were clear of HBV from the serum by PCR criteria. Nevertheless, occult HBV persisted in the livers of all 9 patients studied up to a decade after resolution. One report in the literature showed that HBV DNA was detected in the livers of 2 of 4 patients who had had acute self-limited hepatitis B 30 years previously.[10] Collectively, complete HBV eradication seems to be a very rare event, if it occurs at all, after resolution of acute hepatitis B. In the current study, both of the HBV DNA surface and X sequences derived from 2 distinct virus genomic regions were detected in all liver samples tested. A close correlation was further shown between the levels of the HBV DNA surface sequences and those of the X sequences in the liver, which ensured reliable identification of HBV traces. Moreover, all liver samples tested positive by the PCR methodology to detect an intact direct repeat region, which will amplify the ccc HBV DNA but is unlikely to amplify the incomplete virion HBV DNA and integrated HBV DNA. Although we must stress a possibility that some forms of integrated HBV DNA and other HBV replicative intermediates are amplified, the procedure has been used as a fairly specific assay to detect ongoing occult HBV infection. Collectively, these observations suggest that a replication-competent episomal form of HBV DNA persists in the liver of all patients with a remote history of acute self-limited hepatitis B.

The present study provides direct evidence that liver tissue is a site of virus propagation during convalescence. A previous study showed that HBV infection persisted in the liver but not in peripheral blood mononuclear cells

from patients with acute self-limited hepatitis B 30 years earlier, thus indicating that liver tissue can be the main site of virus propagation in such cases.[10] Unfortunately, occult HBV infections in multiple organs were not compared in the present study, which aimed to explore liver histologic and virologic outcomes. We must stress that further studies are obviously necessary to address this issue. The liver HBV DNA levels were not affected by the severity of liver injury and the viral loads in the acute phase. Interestingly, the levels did not decline with the time elapsed after resolution, and low-grade liver inflammation persisted. Taken together, the presence of ccc HBV DNA may imply low levels of ongoing replication. After infected cells have been massively eliminated by virus-specific immune responses, the HBV replication may be balanced against the host immune pressure for years. Vigorous HBV-specific CTL reactivity has been shown in patients years after clinical and serologic recovery from acute hepatitis B.[7,8] Ample evidence now exists that cytokines (*e.g.*, tumor necrosis factor α and interferon gamma) produced by the CTLs play a major role in the posttranscriptional down-regulation of HBV genome expression without significant liver injury.[22,23] The virus may establish a dynamic equilibrium with the host immune system, where it stimulates the immune response, which in turn keeps HBV replication at an extremely low level. Further quantitative analysis of the liver ccc HBV DNA will shed more light on this issue.

The unresolved problem in the occult HBV infection issue is whether it has any clinical impact. A persistent replication-competent state of HBV infection in the liver raises clinically important problems in the context of liver transplantation and immunosuppressive treatment. In contrast, in an immune-competent state, there is no clear proof that strongly suppressed HBV infection can be involved in the development of advanced chronic liver disease. In the present study, the liver persistence of the replication-competent virus was accompanied by low-grade liver inflammation. A recent study on woodchucks convalescent from acute WHV hepatitis also showed the lifelong persistence of occult infection in the liver, which induces a very mild liver inflammation continuing for life.[21] We must stress that its clinical relevance remains uncertain. Most of our patients retained mild liver fibrosis irrespective of the time elapsed between recovery and liver biopsies. The sample size is not large enough to draw a solid conclusion on factor(s) contributive to liver fibrosis. However, the fibrosis stage was related to the viral loads in the acute phase and seemed to be determined by the nature of the disease at the onset, although the possibility remains that persistent inflammation exerted some influence on the regression of fibrosis. We cannot completely exclude a possibility that low-level alcohol intake in some patients may have affected their fibrosis stage.

The oncogenic potential of occult HBV after resolution of acute hepatitis B is further doubtful. Many studies indicate that occult HBV infection might play a critical role in the development of hepatocellular carcinoma in patients with serologically unidentified pathogenesis of hepatocellular carcinoma.[24,25] Woodchucks that have been infected by the corresponding hepadnavirus (WHV) are at high risk of developing hepatocellular carcinoma after complete resolution of acute WHV hepatitis.[21] In humans, however, long-standing active HBV replication accompanied by significant liver injury is likely to be a very important factor that causes the development of HBV-related hepatocellular carcinoma. Patients with hepatocellular carcinoma with occult HBV may possibly have had chronic HBV infection and lost HBsAg. It is conceivable that hepatocellular carcinoma is unlikely to occur in individuals who recovered from acute hepatitis B.

References

1. Kuhns M, McNamara A, Mason A, Campbell C, Perrillo R. Serum and liver hepatitis B virus DNA in chronic hepatitis B after sustained loss of surface antigen. Gastroenterology 1992;103:1649-1656.
2. Fong T-L, Di Bisceglie AM, Gerber MA, Waggoner JG, Hoofnagle JH. Persistence of hepatitis B virus DNA in the liver after loss of HBsAg in chronic hepatitis B. HEPATOLOGY 1993;18:1313-1318.
3. Komori M, Yuki N, Nagaoka T, Yamashiro M, Mochizuki K, Kaneko A, Yamamoto K, et al. Long-term clinical impact of occult hepatitis B virus infection in chronic hepatitis B patients. J Hepatol 2001;35:798-804.
4. Penna A, Chisari FV, Bertoletti A, Missale G, Fowler P, Giuberti T, Fiaccadori F, et al. Cytotoxic T lymphocytes recognize an HLA-A2-restricted epitope within the hepatitis B virus nucleocapsid antigen. J Exp Med 1991;174:1565-1570.
5. Bertoletti A, Ferrari C, Fiaccadori F, Penna A, Margolskee R, Schlicht HJ, Fowler P, et al. HLA class I-restricted human cytotoxic T cells recognize endogenously synthesized hepatitis B virus nucleocapsid antigen. Proc Natl Acad Sci U S A 1991;88:10445-10449.
6. Missale G, Redeker A, Person J, Fowler P, Guilhot S, Schlicht HJ, Ferrari C, et al. HLA-A31- and HLA-Aw68-restricted cytotoxic T cell responses to a single hepatitis B virus nucleocapsid epitope during acute viral hepatitis. J Exp Med 1993;177:751-762.
7. Rehermann B, Fowler P, Sidney J, Person J, Redeker A, Brown M, Moss B, et al. The cytotoxic T lymphocyte response to multiple hepatitis B virus polymerase epitopes during and after acute viral hepatitis. J Exp Med 1995;181:1047-1058.
8. Penna A, Artini M, Cavalli A, Levrero M, Bertoletti A, Pilli M, Chisari FV, et al. Long-lasting memory T cell responses following self-limited acute hepatitis B. J Clin Invest 1996;98:1185-1194.
9. Quint WGV, De Bruijn I, Kruining H, Heijtink RA. HBV-DNA detection by gene amplification in acute hepatitis B. HEPATOLOGY 1990;12:653-656.
10. Bläckberg J, Kidd-Ljunggren K. Occult hepatitis B virus after acute self-limited infection persisting for 30 years without sequence variation. J Hepatol 2000;33:992-997.
11. Michalak TI, Pasquinelli C, Guilhot S, Chisari FV. Hepatitis B virus persistence after recovery from acute viral hepatitis. J Clin Invest 1994;93:230-239.

12. Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. Nat Med 1996; 2:1104-1108.
13. Cabrerizo M, Bartolomé J, Caramelo C, Barril G, Carreño V. Molecular analysis of hepatitis B virus DNA in serum and peripheral blood mononuclear cells from hepatitis B surface antigen-negative cases. HEPATOLOGY 2000;32:116-123.
14. Hagiwara H, Hayashi N, Mita E, Takehara T, Kasahara A, Fusamoto H, Kamada T. Quantitative analysis of hepatitis C virus RNA in serum during interferon alfa therapy. Gastroenterology 1993;104:877-883.
15. Abe A, Inoue K, Tanaka T, Kato J, Kajiyama N, Kawaguchi R, Tanaka S, et al. Quantitation of hepatitis B virus genomic DNA by real-time detection PCR. J Clin Microbiol 1999;37:2899-2903.
16. Mizokami M, Nakano T, Orito E, Tanaka Y, Sakugawa H, Mukaide M, Robertson BH. Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. FEBS Lett 1999;450:66-71.
17. Mason AL, Xu L, Guo L, Kuhns M, Perrillo RP. Molecular basis for persistent hepatitis B virus infection in the liver after clearance of serum hepatitis B surface antigen. HEPATOLOGY 1998;27:1736-1742.
18. Kwok S, Higuchi R. Avoiding false positives with PCR. Nature 1989;339: 237-238.
19. Ishak K, Baptista A, Bianchi L, Callea F, Groote JD, Gudat F, Denk H, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995; 22:696-699.
20. Liang TJ, Blum HE, Wands JR. Characterization and biological properties of a hepatitis B virus isolated from a patient without hepatitis B virus serologic markers. HEPATOLOGY 1990;12:204-212.
21. Michalak TI, Pardoe IU, Coffin CS, Churchill ND, Freake DS, Smith P, Trelegan CL. Occult lifelong persistence of infectious hepadnavirus and residual liver inflammation in woodchucks convalescent from acute viral hepatitis. HEPATOLOGY 1999;29:928-938.
22. Guidotti LG, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. Science 1999;284:825-829.
23. Chisari FV. Viruses, immunity, and cancer: lessons from hepatitis B. Am J Pathol 2000;156:1117-1132.
24. Paterlini P, Gerken G, Nakajima E, Terre S, D'Errico A, Grigioni W, Nalpas B, et al. Polymerase chain reaction to detect hepatitis B virus DNA and RNA sequences in primary liver cancers from patients negative for hepatitis B surface antigen. N Engl J Med 1990;323:80-85.
25. Kamito H, Nakata K, Hamasaki K, Daikoku M, Mawatari F, Ueki T, Hayashida Y, et al. Detection of hepatitis B virus genome in hepatocellular carcinoma from both hepatitis B surface antigen- and antibody to hepatitis C virus-negative patients: a study using polymerase chain reaction. Oncol Reports 1996;3:619-623.

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Changes in virus loads and precore mutations in chronic hepatitis B patients treated with 4 weeks of daily interferon alfa-2a therapy

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Abstract

Interferon (IFN) alfa-2a was administered to 23 patients with chronic hepatitis B daily for 4 weeks and the relation between the efficacy of the treatment and changes in total hepatitis B virus (HBV) DNA and precore mutant levels was investigated. At 6 and 12 months after the completion of IFN therapy, 39.1% (9/23) and 36.8% (7/19) of patients, respectively, showed alanine transaminase (ALT) normalization; 31.3% (5/16) and 50.0% (7/14), respectively, became negative for HBe-antigen (HBeAg); and 42.1% (8/19) and 41.2% (7/17), respectively, became undetectable for HBV DNA. All 18 of the patients who were positive for HBeAg at baseline nevertheless had the precore mutation. The level of precore mutant as a proportion of the total HBV DNA level was constant at baseline, and 3 and 6 months after the completion of therapy. Thus, the investigation showed that in chronic hepatitis B, the precore mutation occurs at a constant proportion beginning in the HBeAg-positive phase, and IFN therapy inhibits the growth of the wild-type and precore mutant viruses equally.
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Keywords: Chronic hepatitis B; rIFNα-2a; HBV DNA; Precore mutant

1. Introduction

Drugs that have been used to treat chronic hepatitis B include interferon (IFN), propagermanium, and steroids. Recently, lamivudine has been introduced and its use in combination with IFN has attracted interest. IFN therapy was first reported by Greenberg et al. [1] in 1976, whose work shows that IFN inhibits viral growth. In the US and Europe, IFN monotherapy generally consists of long-term administration of 5–10MU per day three times per week for 4–6 months [2,3]. In Japan, in 1986, the National Health Insurance coverage established 4 weeks as the standard treatment period. Consequently, in the present study, rIFN alfa-2a was administered daily for 4 weeks at a dose of 9MU per day for the first 3 days and 18MU per day thereafter.

The efficacy of IFN therapy is estimated by seroconversion from HBe-antigen (HBeAg) to HBe-antibody (HBeAb), undetectable response for hepatitis B virus (HBV) DNA, and normalization of alanine transaminase (ALT). Factors reported to be associated with response to IFN therapy are the baseline levels of HBV DNA and ALT [2,3]. Moreover, the clinical significance of infection with the precore mutant virus, which does not produce HBeAg, has recently drawn attention. We therefore, quantitatively analyzed precore mutant levels and examined the changes in these levels with IFN monotherapy.

2. Materials and methods

The subjects were 23 patients with chronic hepatitis B, 16 males and 7 females, with a mean age of 36.3 ± 9.8 years. Eighteen of the patients were positive for HBeAg and five were negative. Although all 23 patients were positive for HBV DNA in the polymerase chain reaction (PCR) assay, two patients were below detection limits by the bDNA probe assay. The precore mutant level was not less than 10^7 copies/ml in 12 patients and less than 10^7 copies/ml in 11

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Table 1
Baseline characteristics of patients

Patients number	Sex	Age	Grading[a]	Staging[a]	Interval from prior IFN (Month)	ALT (IU/l)	HBeAg (index)	HBeAb (%)	HBVDNA bDNA-p (Meq/ml)	HBVDNA PCR (copy/ml)	Precore mutant (copy/ml)
1	Female	35	–	–	38	287	0.8	96.4	96	8×10^7	8×10^7
2	Male	46	A1	F1	–	67	0.8	95.1	<0.7	5×10^3	<100
3	Female	52	A2	F1	10	310	179.8	0	1900	3×10^8	3×10^8
4	Female	29	–	–	24	304	3.6	57.8	3.4	3×10^6	3×10^6
5	Male	34	A1	F0	–	74	53.5	0	44	3×10^7	8×10^6
6	Female	22	A1	F1	–	259	407.7	0	78	3×10^7	3×10^7
7	Female	34	A1	F1	7	116	385.4	0	190	7×10^7	4×10^7
8	Male	43	A2	F2	14	206	113.3	0	1800	4×10^8	7×10^8
9	Male	56	A3	F2	–	148	7.2	58.0	1.6	9×10^5	9×10^5
10	Male	33	A1	F0	–	142	271.9	0	350	1×10^8	1×10^5
11	Male	33	–	–	41	87	292.2	0	150	3×10^7	3×10^7
12	Male	40	A2	F0	–	995	451.8	0	2100	4×10^8	4×10^8
13	Male	28	A1	F1	–	42	0.8	82.1	710	8×10^8	8×10^8
14	Male	44	A2	F2	49	57	1.8	17.2	2.8	5×10^6	8×10^5
15	Female	30	A2	F1	–	211	183.4	0	3800<	8×10^8	8×10^8
16	Female	27	A2	F2	–	87	9.2	23.3	<0.7	9×10^4	5×10^3
17	Male	36	–	–	10	448	231.8	0	25	1×10^7	1×10^6
18	Male	33	A3	F3	7	44	5.2	54.7	1	3×10^6	1×10^6
19	Male	27	A1	F2	5	184	53.9	0	400	3×10^7	3×10^7
20	Male	58	A3	F3	13	367	0.8	87.6	74	3×10^7	3×10^7
21	Male	26	A1	F3	8	115	172.8	0	1000	2×10^8	1×10^8
22	Male	28	A2	F0	10	338	174.7	0	490	1×10^7	7×10^6
23	Male	42	–	–	96	191	2.2	78.6	2.1	1×10^5	5×10^3

[a] Five cases were not measured.

patients, 1 of whom had a precore mutant level of less than 10^2 copies/ml. Fourteen patients had previously received IFN therapy for intervals from 5 to 96 months, during which they had been administered 477MU daily for 4 weeks. Baseline ALT was 34 to 66 IU/l in three patients and not less than 67 IU/l in 20 patients. Eighteen patients underwent liver biopsy, of whom four had a fibrosis score of F0, six a score of F1, five a score of F2, and three a score of F3 (Table 1).

rIFN alfa-2a was initially administered at a dose of 9MU per day for three consecutive days and 18MU per day for the subsequent 25 days (total dose, 477MU). Excluded were patients who had received an antiviral agent or immunomodulator within 3 months before the study; those who had received an injectable agent containing glycyrrhizin/cysteine/glycine or shosaiko-to (Chinese herbal medicine) within 1 month before the study; and those with a white blood cell (WBC) count of less than 3000/mm^3 or a platelet count of less than 100,000/mm^3.

The virological tests performed were the total amount of HBV DNA, using a bDNA probe assay (Quantiplex, Chiron) and competitive polymerase chain reaction assay (nested-PCR, Otsuka Assay), and the HBV precore mutant levels, using a quantitative mutation-site specific polymerase chain reaction assay (PCR-MSSA, Otsuka Assay). Using PCR-MSSA assay, precore point mutation (G–A, 83rd base of precore region) was examined using a mutation-trapped oligonucleotide primer, which yields a polymerase chain reaction amplification product only with precore mutantsand within the detection limits of 10^2 to 10^9 copies/ml [4]. Each measurement was performed immediately before treatment initiation, at treatment completion, and 6 months after treatment completion. HBeAg and HBeAb levels were measured immediately before treatment initiation, at treatment completion, and 3, 6, and 12 months after treatment completion. They were measured by radioimmunoassay (RIA), and a cutoff index higher than 2.1 for HBeAg was judged to be positive, and an inhibition percent higher than 50 for HBeAb was judged to be positive. Liver histology findings were assessed according to the Knodell histologic activity index [5] and the Desment scoring system [6].

The efficacy of the treatment was evaluated at its completion and at 6 and 12 months after completion according to ALT normalization and loss of HBeAg and HBV DNA.

The statistical analysis was performed using Fisher's exact test and the Wilcoxon 2-sample test.

3. Results

3.1. Efficacy

The rate of patients with normalized ALT levels was 4.3% (1/23) at treatment completion, 39.1% (9/23) at 6 months after completion, and 36.8% (7/19) at 12 months after completion. Although all measurement rates changed during the follow-up, there were many cases with normalized ALT levels after the treatment completion. Of the patients who were positive for HBeAg at baseline, the rate of patients who

Table 2
The rate of biochemical and virological response

	At treatment completion	3 months after treatment completion	6 months after treatment completion	12 months after treatment completion
ALT normalized	4.3% 1/23	34.8% 8/23	39.1% 9/23	36.8% 7/19
HBeAg lost[a]	38.9% 7/18	29.4% 5/17	31.3% 5/16	50.0% 7/14
HBV DNA cleared[b]	47.6% 10/21	40.0% 8/20	42.1% 8/19	41.2% 7/17

Reduction of the number of patients during the follow-up is caused by without patient's consent.
[a] Five patients were excluded because negative at study initiation.
[b] Two patients were excluded because undetectable at study initiation.

became negative for HBeAg was 38.9% (7/18) at treatment completion, 31.3% (5/16) at 6 months after completion, and 50.0% (7/14) at 12 months after completion. Thus, the highest negative rate was at 12 months after the treatment completion. The rate of patients who became undetectable for HBV DNA (bDNA probe assay) was 47.6% (10/21) at treatment completion, 42.1% (8/19) at 6 months after completion, and 41.2% (7/17) at 12 months after completion. A rate of more than 40% undetectable was maintained after the treatment completion. The inability to obtain consent resulted in a reduction in the number of patients followed (Table 2).

3.2. *Efficacy according to patient baseline characteristics*

Examination of baseline patient characteristics, ALT normalization, and loss of HBeAg and HBV DNA at 6 months after treatment completion revealed a trend toward greater efficacy with respect to the rate of ALT normalization and

Table 3
Efficacy according to baseline characteristics of patients

Features		*n*	6 Months after treatment completion: ALT normalized ($n = 23$)	HBeAg lost[a] ($n = 16$)	HBV DNA cleared[b] ($n = 19$)
Sex	Male	16	25.0% (4/16)	20.0% (2/10)	38.5% (5/13)
	Female	7	71.4% (5/7)	50.0% (3/6)	50.0% (3/6)
Age	<40	15	40.0% (6/15)	25.0% (3/12)	38.5% (5/13)
	40≦	8	37.5% (3/8)	50.0% (2/4)	50.0% (3/6)
Prior IFN therapy	Yes	14	35.7% (5/14)	40.0% (4/10)	53.8% (7/13)
	No	9	44.4% (4/9)	16.7% (1/6)	16.7% (1/6)
Staging	F0,F1	10	50.0% (5/10)	28.6% (2/7)	25.0% (2/8)
	F2,F3	8	37.5% (3/8)	20.0% (1/5)	66.7% (4/6)
	Non-perform	5	20.0% (1/5)	50.0% (2/4)	40.0% (2/5)
ALT (IU/ml)	67≦	20	35.0% (7/20)	33.3% (5/15)	37.5% (6/16)
	34–66	3	66.7% (2/3)	0% (0/1)	66.7% (2/3)
HbeAg (index)	100–1000	11	27.3% (3/11)	22.2% (2/9)	33.3% (3/9)
	2.1–100	7	42.9% (3/7)	42.9% (3/7)	50.0% (3/6)
	<2.1	5	60.0% (3/5)	–	50.0% (2/4)
HBeAb (%)	50–100	8	62.5% (5/8)	50.0% (2/4)	42.9% (3/7)
	0–50	15	26.7% (4/15)	25.0% (3/12)	41.7% (5/12)
HBV DNA (Meq./ml)	100≦	11	27.3% (3/11)	25.0% (2/8)	33.3% (3/9)
	0.7–100	10	40.0% (4/10)	42.9% (3/7)	50.0% (5/10)
	<0.7	2	100% (2/2)	0% (0/1)	–
HBV DNA (copies/ml)	10^7–10^9	16	31.3% (5/16)	27.3% (3/11)	35.7% (5/14)
	10^2–10^7	7	57.1% (4/7)	40.0% (2/5)	60.0% (3/5)
	$<10^2$	0	–	–	–
Precore mutant (copies/ml)	10^7–10^9	12	41.7% (5/12)	37.5% (3/8)	45.5% (5/11)
	10^2–10^7	10	30.0% (3/10)	25.0% (2/8)	37.5% (3/8)
	$<10^2$	1	100% (1/1)	–	–

[a] Five patients were excluded because negative at study initiation.
[b] Two patients were excluded because undetectable at study initiation.

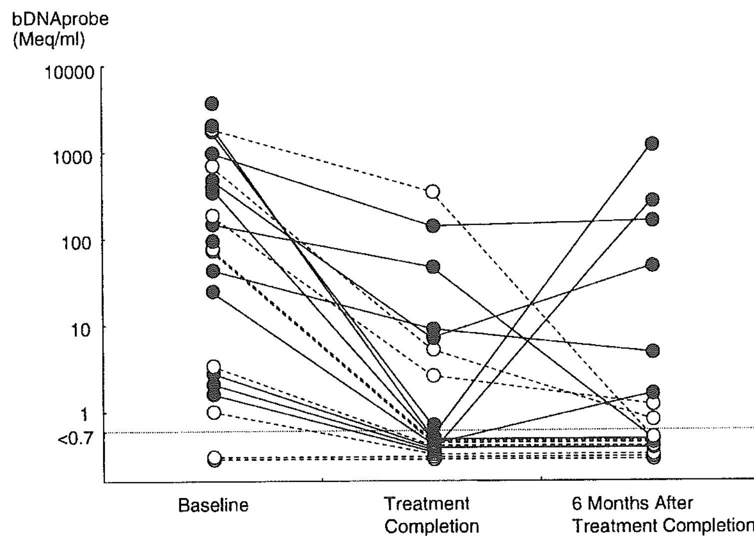

Fig. 1. Changes in Serum HBV DNA levels on interferon (IFN) therapy. And normalization of ALT in each patients at 6 months after the treatment completion. HBV DNA levels were significantly lower in patients with normalized ALT than with abnormal ALT at 6 months after the treatment completion. Open circles with dotted lines showed the changes in serum HBV DNA levels in normalized alanine transaminase (ALT) at 6 months after treatment completion, and closed circles with solid lines showed the abnormal alanine transaminase (ALT) at the same time. It was performed by the bDNA probe assay, since the changes in total hepatitis B virus (HBV) DNA levels were clearer than by the polymerase chain reaction (PCR) assay.

loss of HBeAg and HBV DNA in female patients than in male patients. Moreover, patients who had undergone previous IFN therapy showed greater efficacy, as indicated by the negative rate for HBeAg and HBV DNA, than patients who had not undergone previous therapy. In addition, the lower the baseline viral load, the greater was the efficacy with respect to the rate of ALT normalization and loss of HBeAg and HBV DNA. However, there were no significant differences between baseline characteristics and efficacy (Table 3).

3.3. *Efficacy based on changes in viral markers*

Regardless of its level at baseline, HBV DNA tended to decrease from the initiation of IFN therapy to its completion. After treatment completion, this decrease continued in five patients, while HBV DNA levels increased in the remaining patients. Two of the patients who exhibited more than 100 Meq/ml of the virus at treatment completion had ALT normalization at 6 months after treatment completion; conversely, seven patients who had less than 100 Meq/ml of the virus or were undetectable for the virus at treatment completion did not show ALT normalization at 6 months after treatment completion. Thus, there was no relation between the virus level at treatment completion and efficacy at 6 months after completion. However, HBV DNA levels at 6 months after treatment completion were significantly lower in patients with normalized ALT than with abnormal ALT at the same time (the ALT normalization rate of positive HBV

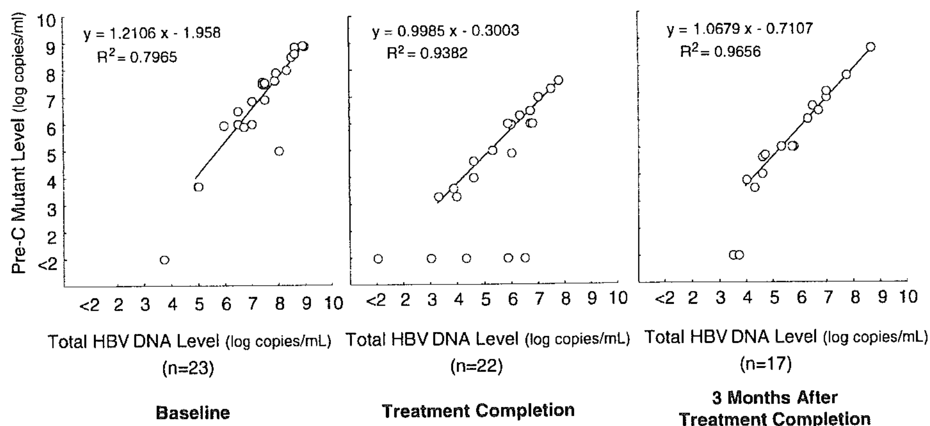

Fig. 2. Total HBV DNA Levels and Precore Mutant Levels on interferon (IFN) Therapy. Total hepatitis B virus (HBV) DNA and precore mutant were measured in: 23 patients at baseline, 22 patients at the treatment completion and 17 patients at 3 months after the treatment completion. Correlation coefficients are shown at each point. From the point of sensitivity of detection limit during the treatment, hepatitis B virus (HBV) DNA was performed by the polymerase chain reaction (PCR) assay.

DNA patients was 25%, and 70% for negative HBV DNA patients; $P = 0.0359$). It performed by the bDNA probe assay, since the changes in total HBV DNA levels were clearer than by the PCR assay (Fig. 1).

At baseline, only one patient had a precore mutant level of less than 10^2 copies/ml and was thus considered to have the wild-type virus. A correlation was seen between the total HBV DNA level and the precore mutant level at baseline, with no consistent trend seen between the proportion of precore mutant in relation to total HBV DNA. A correlation between total HBV DNA and precore mutant level was also seen at treatment completion and 3 months after completion, with no trend seen between the proportion of precore mutant at these timepoints. At all timepoints, the slope of the curve was equal, and no change was seen in the mutant proportion. However, there were exceptions in four patients: only the precore mutant level decreased to the limit of detection at treatment completion, indicating that inhibition of precore mutant growth exceeded inhibition of wild-type growth. From the point of sensitivity of detection limit during the treatment, HBV DNA was performed by the PCR assay (Fig. 2).

4. Discussion

Although no drugs have shown adequate efficacy in chronic hepatitis B, lamivudine has recently been introduced. Lamivudine has potent anti-HBV activity, but long-term lamivudine treatment frequently produces the YMDD mutation and increases HBV DNA and ALT levels [7]. As treatment discontinuation in this case may produce a rebound effect and result in acute exacerbation of the hepatitis, strategies such as continued lamivudine therapy with concurrent IFN use have been adopted [8]. Combined therapy with lamivudine and IFN reportedly increases the negative rate for HBeAg [9].

The first results of 4 weeks of IFN monotherapy in Japan were reported in 1983 by Matsumura et al. [10]. Since then, there have been occasional reports on topics such as the virological and immunological changes seen during IFN monotherapy, but there have been few reports on the efficacy of IFN therapy. The criteria for evaluating the efficacy of IFN therapy are not clearly defined. Generally, negative response for HBeAg, seroconversion to HBeAb, and normalization of ALT are assessed. Recently, however, the results of clinical studies using liver carcinogenesis or survival as the endpoint have been reported. Factors such as IFN therapy, age, histological progression in the liver, and post-treatment negative for HBeAg, HBV DNA, and HBsAg and normalization of ALT, play significant roles in efficacy after treatment completion [11–13].

In the present study, treatment with rIFN alfa-2a for 4 weeks resulted in, at 6 months and 12 months after treatment completion, normalization of ALT and almost the same undetectable HBV DNA rate, however the negative HBeAg rate rose from about 30 to 50%. Thus, in this study, treatment with rIFN alfa-2a did not show its effectiveness at each timepoint. It will be necessary to perform long-term follow-up observations of these patients, using liver carcinogenesis or survival as the endpoint.

The close relation between HBV mutation and disease type has been shown, and there have been numerous studies on this topic. Mutation at nucleotide 1896 of the precore region is considered particularly important, because it is thought to be related to the pathophysiology of fulminant and other hepatitis. In this study, we evaluated the frequency of the occurrence of precore mutants using MSSA for the detection of point mutations at the 83rd base in the precore region for the mutant HBV genome. MSSA can be used for the detection of 10^2 copies/ml of precore mutants in the presence of 10^7 copies/ml of wild-types, its sensitivity is considered to be at least 0.001%. With the use of this MSSA, even if only wild-type genomes are present, precore mutant-type can be identified on electrophoresis [4]. There have been numerous reports in the US and Europe indicating that this precore mutant is resistant to IFN [14,15]. In Japan, however, IFN efficacy in patients positive for HBeAb was first reported by Muraoka et al. [16], and since then other groups have also reported inhibition of HBV growth and ALT normalization with IFN therapy [17,18]. Possible factors in this discrepancy include the fact that, in the US and Europe, negative responses, not only for HBeAg and HBV DNA, but also for HBsAg, are the objectives of treatment and baseline characteristics such as the period of infection and HBV genotype differ greatly. Therefore, an examination of the response to IFN in which there is uniformity with respect to these factors is needed. In the present study, the total HBV DNA level and precore mutant level were correlated before and after IFN treatment. Among the 18 patients who were positive for HBeAg at baseline, none was negative for the precore mutant. Thus, in chronic hepatitis B, the precore mutation occurred at a constant proportion beginning from the HBeAg-positive phase, and IFN therapy inhibited growth of the wild-type virus and the precore mutant virus equally in most of the patients. In some patients, inhibition of the precore mutant growth exceeded inhibition of the wild-type growth; there were no cases in which wild-type growth was inhibited. Shindo et al. [19] reported that the precore wild-type and mutant have similar sensitivities to IFN. However, because they used the restriction fragment length polymorphism (RFLP) assay [20] to determine mutant virus levels and did not perform a quantitative examination, and because the IFN was administered intermittently (three times per week for 17 weeks) and the efficacy rate was 26.1% (6/23 patients; defined as showing seroconversion to HBeAb, loss of HBV DNA and normalization of ALT), the results of their study cannot be adequately compared with those of our investigation.

In an investigation in patients with type B cirrhosis, Ikeda et al. [21] reported high rates of carcinogenesis when the precore mutant is present at high concentrations. They

further report that high precore mutant concentrations correlate with a high total HBV DNA level in such patients, indicating that the inflammation associated with the hepatitis is severe, and acts to indirectly promote carcinogenesis. In addition, it is reported that a primary infection by HBV with a gene mutation contributes to the infection becoming fulminant and severe [22]. However, it is also reported that mutation of the precore region during the natural course of chronic hepatitis B is related to quiescence of the hepatitis and a decrease in the virus level [23].

The time required to reach the true endpoint of the present study, carcinogenesis or survival, makes it difficult to provide conclusions regarding whether the hepatic lesions in patients will progress or whether the hepatitis will become quiescent and the patients will become asymptomatic carriers. However, it is already evident that IFN treatment inhibits the growth of both the wild-type and precore mutant viruses seen in chronic hepatitis B and that it is also effective in patients who are positive for HBeAg and have a predominance of the wild-type virus.

References

[1] Greenberg HG, Pollard RB, Lutwick LI, Gregory PB, Robinson WS, Merigan TC. Effect of human leukocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis. N Engl Med 1976;295:517–22.

[2] Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337:1733–45.

[3] Hoofnagle JH. Therapy of viral hepatitis. Digestion 1998;59:563–78.

[4] Kinoshita M, Seno T, Fukui T, Shin S, Tsubota A, Kumada H. A detection method for point mutation in the precore region of human hepatitis B virus (HBV)-DNA using mutation-site-specific assay. Clin Chim Acta 1994;228:83–90.

[5] Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1:431–5.

[6] Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19:1513–20.

[7] Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papaioannou C. Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B. Hepatology 2000;32:847–51.

[8] Someya T, Suzuki Y, Arase Y, et al. Interferon therapy for flare-up of hepatitis B virus infection after emergence of lamivudine-induced YMDD motif mutant. J Gastroenterol 2001;36:139–41.

[9] Mutimer D, Dowling D, Cane P, Ratcliffe D, Tang H, O'Donnell K, et al. Additive antiviral effects of lamivudine and alpha-int0erferon in chronic hepatitis B infection. Antivir Ther 2000;5:273–7.

[10] Matsumura N, Yoshikawa O, Kondo M, Kawakami H, Kishida T. Therapeutic effect of a low dosage of human leukocyte interferon on chronic hepatitis B virus interferon. Digestion 1983;26:205–12.

[11] Niederau C, Heintges T, Lange S, Goldmann G, Niederau CM, Mohr L, et al. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. N Engl J Med 1996;334:1422–7.

[12] Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999;29:971–5.

[13] Perrillo R, Tamburro C, Regenstein F, Balart L, Bodenheimer H, Silva M, et al. Low-dose, titratable interferon alfa in decompensated liver disease caused by chronic infection with hepatitis B virus. Gastroenterology 1995;109:908–16.

[14] Brunetto MR, Giarin M, Saracco G, Oliveri F, Calvo P, Capra G, et al. Hepatitis B virus unable to secrete e antigen and response to interferon in chronic hepatitis B. Gastroenterology 1993;105:845–50.

[15] Fattovich G, McIntyre G, Thursz M, Colman K, Giuliano G, Alberti A, et al. Hepatitis B virus precore/core variation and interferon therapy. Hepatology 1995;22:1355–62.

[16] Muraoka H, Sanefuji T, Keida R, Tsuji R, Abe H, Uchimura Y, et al. A case of acute exacerbation of chronic hepatitis B accompanied by antibody to HBeAg with remission of liver damage after long-term treatment with interferon. Kurume Med J 1995;42:307–11.

[17] Aikawa T, Kanai K, Kako M, Kawasaki T, Hino K, Iwabuchi S, et al. Interferon-alpha 2a for chronic hepatitis B with e antigen or antibody: comparable antiviral effects on wild-type virus and precore mutant. J Viral Hepat 1995;2:243–50.

[18] Kako M, Kanai K, Aikawa T, Iwabuchi S, Takehira Y, Kawasaki T, et al. Response to interferon-alpha 2a in patients with e antigen-negative chronic hepatitis B. J Clin Gastroenterol 1997;25:440–5.

[19] Shindo M, Okuno T. Genomic variations in precore and cytotoxic T lymphocyte regions in chronic hepatitis B in relationship to interferon responsiveness. Liver 2000;20:136–42.

[20] Okamoto H, Yotsumoto S, Akahane Y, Yamanaka T, Miyazaki Y, Sugai Y, et al. Hepatitis B viruses with precore region defects prevail in persistently infected hosts along with seroconversion to the antibody against e antigen. J Virol 1990;64:1298–303.

[21] Murashima N, Arase Y, Chayama K, Ikeda K, Kumada H, Saitoh S, et al. Relationship of hepatocellular carcinogenesis with precore mutant virus and serum hepatitis B virus DNA concentration. A longitudinal analysis of patients with cirrhosis. Hepatol Res 1998;10:142–55.

[22] Aritomi T, Yatsuhashi H, Fujino T, Yamasaki K, Inoue O, Koga M, et al. Association of mutations in the core promoter and precore region of hepatitis virus with fulminant and severe acute hepatitis in Japan. J Gastroenterol Hepatol 1998;13:1125–32.

[23] Karino Y, Toyota J, Sato T, Ohmura T, Yamazaki K, Suga T, et al. Early mutation of precore (A1896) region prior to core promoter region mutation leads to decrease of HBV replication and remission of hepatic inflammation. Dig Dis Sci 2000;45:2207–13.

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Circulating soluble Fas levels in patients with hepatitis C virus infection and interferon therapy

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Background. The clinical relevance of the circulating soluble form of the Fas-Receptor (sFas) was investigated in patients with hepatitis C receiving type 1 interferon (IFN) therapy. ***Methods.*** sFas was quantified by enzyme-linked immunosorbent assay in 66 hepatitis C virus (HCV) carriers and 30 HCV-naive or previously infected controls. The levels were then monitored during enhanced treatment with type 1 IFNs in 15 chronic hepatitis C patients. ***Results.*** The HCV carriers had high levels of sFas compared with controls (3.8 ± 1.3 vs 2.7 ± 0.8 ng/ml; $P < 0.001$). sFas levels in patients with chronic HCV infection were directly related to serum alanine aminotransferase levels ($r = 0.440$; $P < 0.001$) and the histological grade ($r = 0.403$; $P = 0.019$). Among necroinflammatory reactions, only piecemeal necrosis showed a correlation with sFas levels ($r = 0.556$; $P = 0.001$). Pretreatment sFas levels, however, were not predictive of therapeutic outcomes. A sustained virological response to enhanced IFN therapy showed a relation to only the pretreatment HCV load. Interestingly, circulating sFas was upregulated when IFN-β was administered at short intervals (3 MU/every 12 h). This upregulation was accompanied by parallel aminotransferase elevation, which was observed regardless of a virological response. ***Conclusions.*** sFas elevation, in parallel with the severity of liver injury, suggests the possible upregulation of hepatic Fas expression and the Fas-mediated pathway in both HCV- and type 1 IFN-induced liver injury. The essential function of sFas to protect hepatocytes against Fas-mediated liver injury was not evident in these clinical settings.

Key words: hepatitis C, type 1 interferon, apoptosis, soluble Fas

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Introduction

Fas-Receptor/Fas ligand-mediated apoptosis of liver cells plays a significant role in the pathogenesis of hepatitis C. Hepatic upregulation of Fas was found to be correlated with more severe inflammation caused by ongoing hepatitis C virus (HCV) infection.[1,2] Parallel activation of T lymphocytes expressing Fas ligand was detected in liver-infiltrating mononuclear cells, allowing transduction of the apoptotic death signal to Fas-bearing hepatocytes.[3] Interferon (IFN)-α and IFN-β, members of the type 1 IFN family, are currently used for the treatment of chronic hepatitis C. Hepatic injury in patients treated with systemic administration of type 1 IFNs remains an unresolved problem. A recent study showed that apoptosis may also be involved in type 1 IFN-induced hepatotoxicity.[4]

Fas-Receptor (APO-1/CD95) has a single membrane-spanning domain and is expressed in hepatocytes. An alternatively spliced soluble form of the Fas-Receptor (sFas) without the transmembrane-spanning domain is produced by Fas-expressing hepatocytes,[5,6] and upregulation of Fas expression in hepatocytes contributes to the increase in serum sFas levels. It is of interest that sFas may serve to bind Fas ligand on cytotoxic T lymphocytes, thereby minimizing Fas-mediated liver injury.[7–9] However, the clinical relevance of circulating sFas is not yet fully understood in relation to chronic hepatitis C and type 1 IFN therapy. sFas elevation has been reported in chronic HCV infection,[10] but controversy remains. Possible upregulation of sFas during IFN therapy has also been shown, based on sFas levels within 24 h after IFN administration,[11] but the data are limited. To further our understanding of sFas, we studied serum sFas levels in patients with HCV infection as compared with HCV-naive subjects or previously infected controls. sFas levels were then monitored in the course of treatment with type 1 IFNs, and the results were examined in relation to liver injury.

Patients and methods

Patients

The subjects were 66 patients with chronic HCV infection (39 men and 27 women; age, 27–80 years [median, 59 years]) and 30 controls without ongoing HCV infection (21 men and 9 women, age 26–73 years [median, 56 years]). No significant difference was seen in sex (P = 0.426) or age (P = 0.761) distribution between the two groups. The 66 HCV carriers had been persistently positive for serum HCV RNA for more than 6 months. During the entire follow-up, none of the patients displayed any confounding etiology of liver disease, such as hepatitis B virus, autoimmune markers, or alcohol abuse (>25 g/day). Sera were obtained from all patients, and liver biopsy specimens could be obtained from 35 patients at the time of serum collection. After enrollment, 15 of the chronic hepatitis C patients (10 men and 5 women, age 33–63 years [median, 45 years]) were assigned to two different regimens of 4-week enhanced IFN induction therapy. Eight patients (group A) were treated with a high dose of IFN-α2a (Roferon-A; Nippon Roche, Tokyo, Japan; 9 MU/daily for 4 weeks, followed by 9 MU/thrice weekly; total dose, 720–1206 MU [median, 941 MU]). The remaining 7 patients (group B) received a high dose of IFN-β (Feron; Toray Industries, Tokyo, Japan; 3 MU/twice a day for 4 weeks, followed by 6 MU/thrice weekly; total dose, 276–636 MU [median, 384 MU]). Using stored serum samples obtained at 0, 1, 2, 4, and 8 weeks after initiation of the induction therapy, we investigated changes in serum alanine aminotransferase (ALT) activity, HCV RNA, and sFas levels.

Sera were also obtained from the 30 control subjects. The controls consisted of 8 healthy individuals without serum HCV RNA and HCV antibody, 8 serum HCV RNA-negative but HCV antibody-positive healthy individuals with no history of IFN therapy, and 14 nonviremic HCV antibody-positive patients who had been successfully treated with IFN therapy for chronic hepatitis C. Patients in the last group had remained negative for serum HCV RNA for 0.7–8.0 years (median, 4.0 years) after the end of previous IFN therapy. All serum samples were stored at −80°C without thawing until use. The study was approved by the local Research Ethics Committee, in accordance with the 1975 Declaration of Helsinki, and all the patients provided written informed consent.

Laboratory testing

HCV antibody was tested with a third-generation enzyme-linked immunosorbent assay (ELISA) (Ortho Diagnostic Systems, Tokyo, Japan). Serum HCV RNA was detected by a polymerase chain reaction assay (Amplicor HCV Monitor; Roche Diagnostics, Tokyo, Japan) and quantified using a branched DNA (bDNA) assay (Quantiplex HCV-RNA; Chiron, Emeryville, CA, USA). HCV RNA-positive serum samples were subjected to HCV genotyping with the genotype-specific NS4 antibody assay (Immucheck-HCV Gr; International Reagent, Kobe, Japan), which is based on a comparison between antibody responses to the two genotype-specific NS4 antigens (C14-1 and C14-2).

Detection of sFas

Serum sFas levels were measured by ELISA (Medical and Biological Laboratories [MBL], Nagoya, Japan).[12,13] Serum sFas was first captured by polyclonal antibody to an intracellular sequence of human Fas (KDITSDSENSNFRNEIQSLV), and then detected by monoclonal antibody to an extracellular epitope (KCRCKPNFFC). Thus, the assay is theoretically expected to detect a complete sFas molecule composed of the extracellular and intracellular domains of Fas-Receptor, but is unlikely to detect incomplete cleaved forms of Fas-Receptor, which can be released following the destruction of Fas-expressing cells. In order to quantify the sFas levels, human Fas (GenBank accession no. CAA45250) was expressed in WR19L-12a cells, and standard Fas protein was purified from the membrane fraction. The assay was performed according to the manufacturer's instructions. In brief, a 96-well plate coated with the polyclonal antibody was incubated with 100 μl of fivefold diluted serum samples and standard Fas protein (0–2 ng/ml) at room temperature for 1 h. After thorough washing, the plate was further incubated with the peroxidase-conjugated monoclonal antibody at room temperature for 1 h. The plate was then washed, and a solution containing tetramethyl benzidine was added. After 30-min incubation, the reaction was stopped, and the optical density at 450 nm was measured. All assays for sFas were done in duplicate, and the concentration of sFas was determined from the standard curve. High levels of sFas were quantified using diluted serum samples.

Histological evaluation

Liver biopsy specimens were fixed in formalin and embedded in paraffin for routine staining with hematoxylin-eosin. All specimens were examined by the same experienced pathologist, who was unaware of the biochemical, serological, and virological data. Biopsy specimens were semiquantitatively evaluated by the histological activity index described by Knodell et al.[14]

Statistical analysis

All data values are expressed as means ± SD. For numeric variables, group comparisons were performed by the Wilcoxon nonparametric test. The χ^2 test or Fisher's exact test was used for categorical variables. Correlations between the variables were calculated using Spearman rank order correlations. A value of $P < 0.05$ (two-tailed) was considered to indicate significance. For multivariate analysis, multiple logistic regression analysis, with a stepwise procedure, was performed with the computer program, SAS 8.02 (SAS Institute, Cary, NC, USA).

Results

Figure 1 shows serum sFas levels in relation to the presence of ongoing HCV infection. The 66 chronic HCV carriers had significantly higher levels of sFas (3.8 ± 1.3 ng/ml) than the 30 controls without ongoing HCV infection (2.7 ± 0.8 ng/ml; $P < 0.001$). In the control group, remote history of HCV infection exerted no impact on sFas levels. Serum sFas levels were 2.5 ± 0.3 ng/ml for the serum HCV RNA-negative but HCV antibody-positive healthy individuals who were spontaneously cleared of HCV. The levels were compatible with those for healthy individuals with no HCV markers (3.1 ± 0.9 ng/ml). sFas levels were equally low in chronic hepatitis C patients who had been clear of serum HCV RNA for 0.7–8.0 years (median, 4.0) after the end of IFN therapy (2.5 ± 0.9 ng/ml). No relation of sFas levels with time after IFN therapy was evident in these patients. No correlation was seen between sFas levels and age in the chronic HCV carriers ($r = 0.007$; $P = 0.954$) or the control subjects ($r = 0.103$; $P = 0.579$).

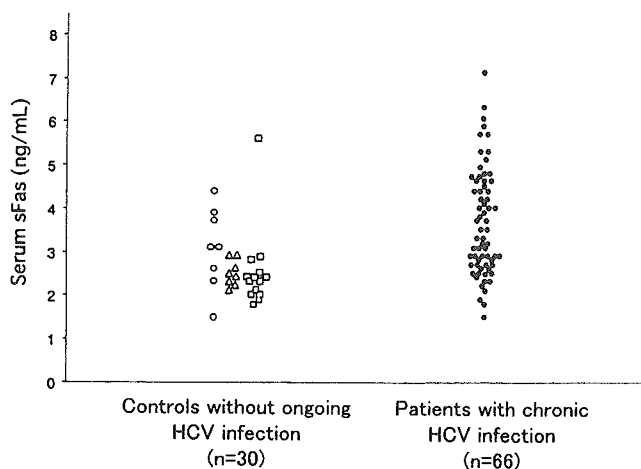

Fig. 1. Serum sFas levels in 66 patients with chronic hepatitis C virus (HCV) infection were compared with those in 30 controls without ongoing HCV infection (3.8 ± 1.3 vs 2.7 ± 0.8 ng/ml; $P < 0.001$). The controls consisted of 8 healthy individuals without serum HCV RNA and HCV antibody (*open circles*), 8 serum HCV RNA-negative but HCV antibody-positive healthy individuals without interferon (IFN) therapy (*open triangles*), and 14 patients who had remained negative for serum HCV RNA for 0.7–8.0 years (median, 4.0 years) after the end of IFN therapy for chronic hepatitis C (*open squares*). The levels of serum soluble Fas-Receptor (sFas) were 3.1 ± 0.9, 2.5 ± 0.3, and 2.5 ± 0.9 ng/ml for these three control groups, respectively, with no significant difference

The elevation of circulating sFas levels in chronic HCV infection was investigated in relation to virological, biochemical, and histological characteristics. In the 66 chronic HCV carriers, sFas levels had no relation to HCV replicative levels, assessed by serum HCV RNA titers (Fig. 2). This was also the case when the correlation was analyzed for each HCV genotype. sFas levels were 3.6 ± 1.2 ng/ml for the 19 patients infected with genotype 1, 3.6 ± 1.1 ng/ml for the 22 patients with genotype 2, and 3.8 ± 1.4 ng/ml for the remaining 25 patients with undetermined genotype(s), with no significant differences among the values. On the other hand, a significant correlation was found between serum sFas levels and ALT activity ($r = 0.440$; $P < 0.001$; Fig. 3). In 35 chronic HCV carriers, the circulating sFas levels were also correlated with liver histological findings (Fig. 4). A significant correlation was revealed between sFas levels and the total grading score ($r = 0.403$; $P = 0.019$), whereas no correlation was evident between sFas levels and the staging score ($r = 0.321$; $P = 0.057$). As for correlations with necroinflammatory scores, sFas levels showed a correlation with piecemeal necrosis ($r = 0.556$; $P = 0.001$), but not with lobular necrosis and inflammation ($r = 0.224$; $P = 0.192$) or portal inflammation ($r = 0.316$; $P = 0.066$).

Changes in serum sFas levels during 8 weeks of enhanced induction treatment with type 1 IFNs were

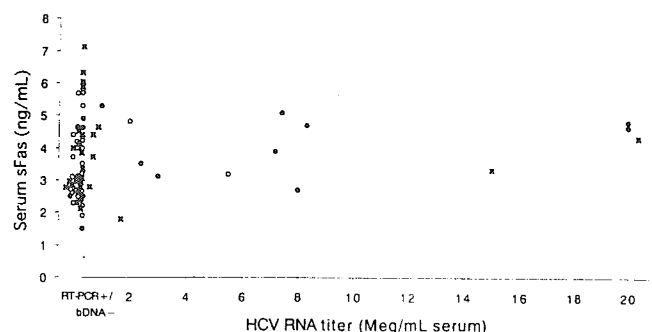

Fig. 2. Relation of serum sFas levels with serum HCV RNA titers and HCV genotypes in the 66 patients with chronic HCV infection. Serum sFas levels had no relation to serum HCV RNA titers and HCV genotypes. *Solid circles*, patients with genotype 1; *open circles*, patients with genotype 2; *crosses*, patients with undetermined genotype

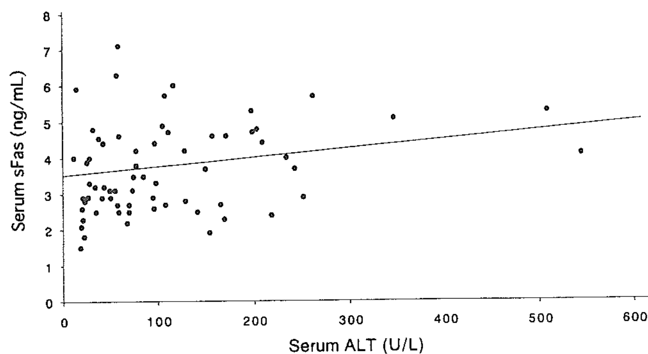

Fig. 3. Relationship between serum sFas levels and serum alanine aminotransferase (*ALT*) levels in the 66 patients with chronic HCV infection ($r = 0.440$; $P < 0.001$)

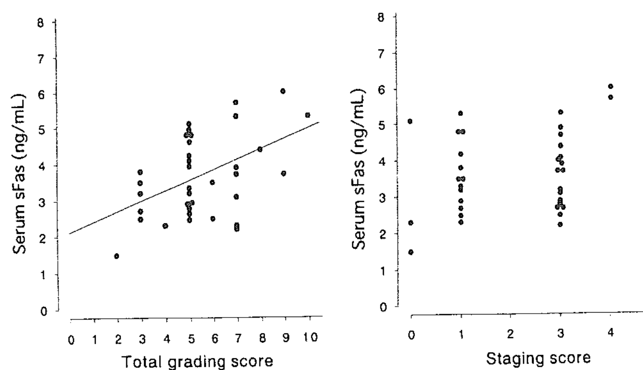

Fig. 4. Relationship between serum sFas levels and histological findings in 35 patients with chronic HCV infection. Serum sFas levels showed a correlation with the total grading score ($r = 0.403$; $P = 0.019$), but not with the staging score ($r = 0.321$; $P = 0.057$)

investigated in 15 chronic hepatitis C patients (Fig. 5). The pretreatment viral load ranged from less than 0.5 to 20.0 Meq/ml (median, 3.0 Meq/ml). Genotypes 1 and 2 were found in 9 and 6 patients, respectively. Of the 8 patients treated with IFN-α2a (group A), serum HCV RNA was cleared during the treatment in 7 patients. In these virological responders, no significant change was seen in serum sFas levels at weeks 1, 2, 4, and 8 as compared with the baseline level (3.7 ± 0.9 ng/ml). Thus, HCV clearance was not accompanied by a decrease in serum sFas levels. Serum ALT activity was normalized in 4 virological responders, but remained elevated in the other 3. In group B, the 7 patients received enhanced induction treatment with IFN-β. A virological response was achieved during the treatment by 6 patients. Despite HCV clearance, the virological responders showed an increase in sFas levels after the initial intensive treatment (3 MU/twice a day for 4 weeks; 4.5 ± 0.9 at baseline vs 7.1 ± 1.3 ng/ml at week 4; $P = 0.018$). The levels returned to the baseline after the subsequent treatment (6 MU/thrice weekly for 4 weeks). In parallel with the elevation of sFas levels, serum ALT activity was also elevated in the absence of HCV viremia in 5 of the 6 virological responders.

A sustained virological response, defined as serum HCV RNA clearance at 6 months posttreatment, was achieved by five patients (four patients in group A and one patient in group B). Table 1 shows the clinical and virological characteristics of the sustained virological responders and those of the patients showing no sustained virological response. A significant difference was observed only for the pretreatment viral load. All sustained virological responders had a low pretreatment viral load of less than 0.5 Meq/ml, whereas the levels ranged from less than 0.5 to 20.0 Meq/ml (median, 7.3 Meq/ml) in patients with no sustained virological response ($P = 0.014$). Pretreatment serum sFas levels were the same for patients with a sustained virological response and those without (4.5 ± 0.6 vs 3.8 ± 1.0 ng/ml; $P = 0.089$). Multivariate analysis also showed only the pretreatment viral load to be predictive of a sustained virological response ($P = 0.038$).

Discussion

Currently available information suggests that apoptosis-inducing death ligands and corresponding receptors mediate cell death in human liver diseases. Thus, knowledge regarding how this system functions may help in developing rational therapeutic strategies to ameliorate clinical liver diseases. Apoptosis-inducing death receptors belong to the tumor necrosis factor/nerve growth factor receptor superfamily. Fas-Receptor (APO-1/CD95), one of the death receptors, has a single membrane-spanning domain and is expressed in hepatocytes. The particular importance of the Fas-Receptor in chronic hepatitis C has been amply documented.[1,2] Hepatic Fas-Receptor expression has been shown to be upregulated in HCV infection, with a close correlation to the necroinflammatory grade. The Fas-Receptor-mediated apoptotic pathway may also play a significant role in the pathogenesis of other forms of liver injury. An alternatively spliced soluble form of the Fas-Receptor without the transmembrane-spanning domain is produced by Fas-expressing hepatocytes and is considered to serve to bind Fas ligand on cytotoxic T cells, thereby minimizing liver injury.[7–9] However, its clinical relevance has not been well explored.

The present study demonstrated that patients with chronic HCV infection had higher levels of circulating sFas than healthy individuals without previous HCV exposure and patients who had a remote history of HCV infection but had been cleared of HCV spontaneously or following IFN therapy. sFas levels in chronic HCV infection had no relation to HCV replication

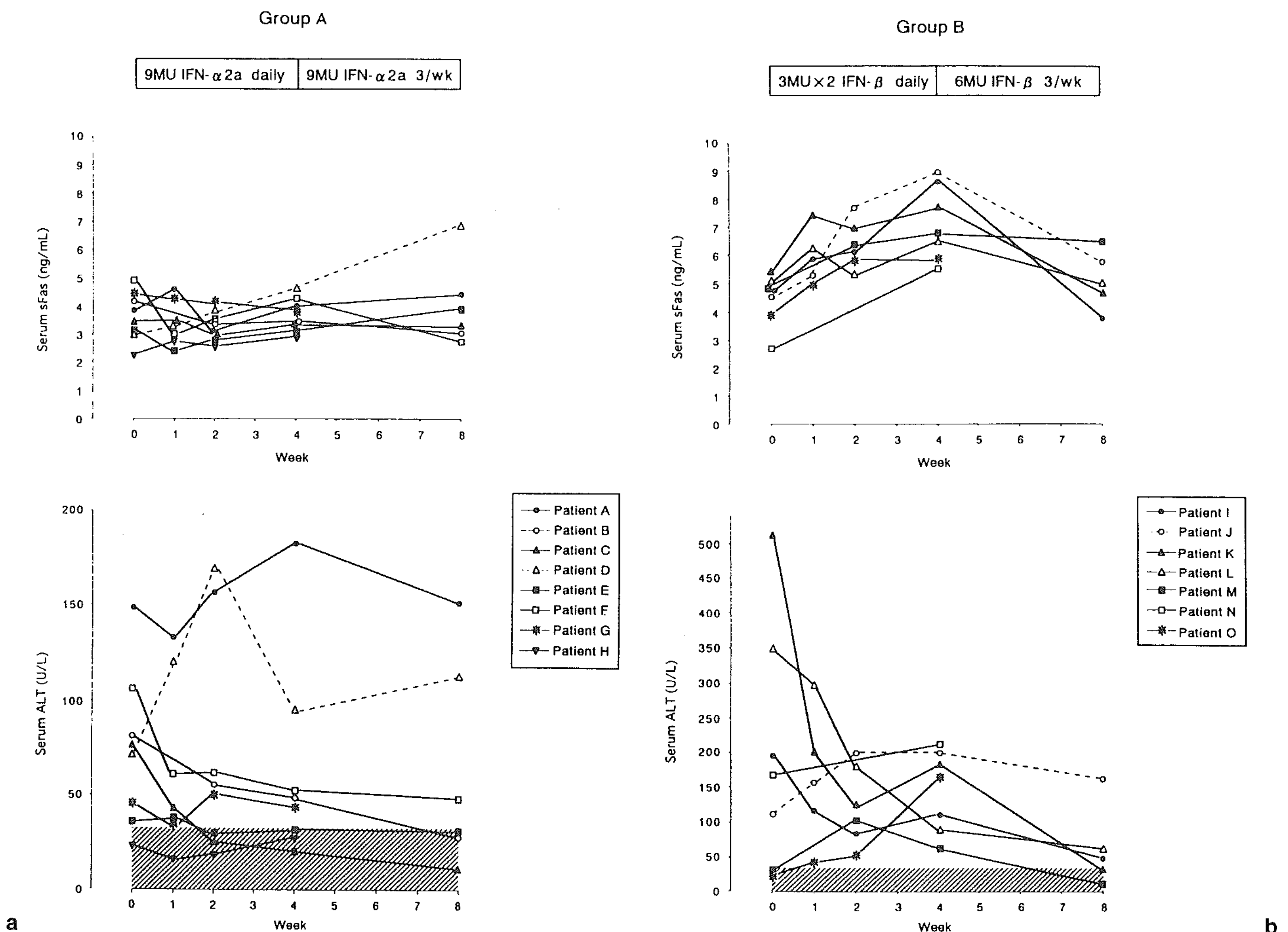

Fig. 5a,b. Changes in serum sFas and ALT levels during 8 weeks of enhanced IFN therapy for chronic hepatitis C. **a** Eight patients (patients A–H) received 9 megaunits (MU) of IFN-α2a daily for 4 weeks, followed by the same dose thrice a week for the subsequent 4 weeks (*group A*). **b** The other patients (patients I–O) received 3MU of IFN-β twice a day continuously for 4 weeks, and then 6MU of IFN-β thrice a week for 4 weeks (*group B*). The *Solid* and *dashed lines* in **a** and **b** indicate patients who were negative and positive for serum HCV RNA, respectively, at the end of treatment. In the end-of-treatment virological responders, serum sFas levels showed no significant change during IFN-α2a therapy in group A, while the levels increased following the initial 4-week intensive IFN-β therapy in group B (4.5 ± 0.9 before treatment vs 7.1 ± 1.3 ng/ml at 4 weeks; $P = 0.018$) and returned to the baseline levels at 8 weeks. Serum sFas elevation at 4 weeks was associated with serum ALT elevation observed during IFN-β therapy. *Shaded areas* indicate the normal range of serum ALT activity

and genotypes, but were correlated with biochemical and histological disease activity. Serum sFas levels measured by the assay we used have been shown to correlate with hepatic Fas expression.[10] Hepatic Fas expression in chronic hepatitis C is particularly prominent at the advancing edge of piecemeal necrosis.[1] sFas levels were also correlated with the severity of necroinflammatory reactions, especially that of piecemeal necrosis. Collectively, the data obtained suggest the upregulation of hepatic Fas expression and the involvement of the Fas-mediated pathway in HCV-related liver injury. In contrast with experimental studies, sFas in our study failed to protect hepatocytes against Fas-mediated liver injury. This discrepancy may be attributable to the amount of circulating sFas protein. The inhibition of Fas-Receptor-mediated liver injury by sFas has been reported in mice which were given very large amounts of sFas.[8] Serum sFas levels in mice could have been much higher than those observed in humans, and the levels in chronic hepatitis C patients may not be enough to inhibit the apoptosis caused by Fas ligand on cytotoxic T lymphocytes and its soluble form. Further studies are necessary to clarify the impact of sFas on the Fas-mediated pathway in humans. Thus far, elevation of sFas levels in chronic HCV infection has also been reported,[10] but controversy remains.[11] The

Table 1. Factors predictive of a sustained virological response to IFN therapy with 4-week enhanced induction treatment for chronic hepatitis C

Characteristics	Sustained response (*n* = 5)	No sustained response (*n* = 10)	*P*
Age (years)	50 ± 12 52 (37–63)	47 ± 9 45 (33–58)	0.540
Sex (M/F)	4/1	6/4	0.600
Serum sFas (ng/ml)	4.5 ± 0.6 4.4 (3.7–5.3)	3.8 ± 1.0 3.7 (2.3–5.1)	0.178
HCV genotype (1/2)	1/4	8/2	0.089
Serum HCV RNA titer (Meq/ml)	<0.5 (<0.5)	7.3 (<0.5 to 20.0)	0.014
Serum ALT (U/l)	177 ± 189 105 (43–508)	108 ± 103 75 (21–345)	0.327
Histological grading score	7.4 ± 2.3 8 (5–10)	5.0 ± 1.5 5 (3–7)	0.072
Histological staging score	2.2 ± 1.1 3 (1–3)	1.7 ± 1.3 1 (0–3)	0.463
Total IFN dose (MU)	863 ± 302 891 (384–1206)	611 ± 271 597 (276–990)	0.111

Values are given as means ± SD and medians (ranges)

different findings may be attributable to differences in the necroinflammatory grade of the study populations and the different sFas assays used.

In the present study, changes in sFas levels following type 1 IFN administration were investigated. IFN-α and IFN-β, members of the type 1 IFN family, are currently used to treat a number of infectious diseases and cancers. A growing number of chronic hepatitis C patients have been treated with type 1 IFNs, but the effectiveness of these agents is often limited due to adverse side effects. The pathophysiological mechanisms have not been well defined. Type 1 IFN-induced hepatotoxicity is one of such adverse effects and causes serum ALT elevation in patients with a virological response. Recently, several studies showed that the antiviral effects were more pronounced in patients treated twice daily with IFN-β compared with once-daily dosing, but that IFN-induced liver injury was more common with the twice-daily treatment protocol.[15,16] The present study showed that virological response to high-dose type 1 IFN therapy was not accompanied by a reduction of sFas levels and that serum ALT levels were likely to remain elevated, despite the clearance of circulating HCV RNA. Interestingly, sFas levels in virological responders increased during intravenous twice-daily treatment with IFN-β, and the increase was accompanied by an exacerbation of biochemical disease activity.

Acute hepatopathy with elevated serum aminotransferase levels has also been reported in sheep treated with twice-daily administration of IFN tau, a type 1 IFN, and apoptosis was shown to play a critical role in the IFN tau-induced hepatotoxicity.[4] Type 1 IFNs can affect the expression of various genes and induce apoptosis, but the mechanism of the type 1 IFN-induced apoptosis remains to be explored. Thus far, regulatable expression of the IFN-induced double-stranded RNA-dependent protein kinase, PKR, has been shown to trigger apoptosis, possibly through upregulation of the Fas-Receptor.[17,18] The data obtained in our study support this hypothesis. Elevation of sFas levels and serum ALT activity in virological responders to IFN indicates IFN-induced upregulation of hepatic Fas-Receptor expression and its involvement in type 1 IFN-induced hepatotoxicity. Again, we found that sFas exerted no apparent inhibition of Fas-mediated liver injury during IFN therapy, thus raising further questions as to whether the Fas-mediated pathway is controlled by sFas in humans. Recently, it has been reported that upregulation of sFas can be induced in the very early phase of IFN therapy.[11] Further studies are necessary to work out sFas dynamics in more detail during various treatment regimens.

At present, it remains to be worked out whether sFas levels in chronic hepatitis C are relevant to the therapeutic efficacy of IFN. In the present study, the majority of the patients achieved an end-of-treatment virological response after the enhanced IFN treatment, irrespective of the pretreatment patient characteristics. However, a sustained virological response was related to only the pretreatment viral load. Thus, it was shown that pretreatment circulating sFas levels have little impact on the final therapeutic outcome when patients are subjected to enhanced IFN therapy. Finally, it is likely that upregulation of the Fas-mediated pathway also occurs in other types of liver injury and is accompanied by changes in serum sFas levels. More studies are necessary to address this issue and to further our understanding of sFas.

References

1. Hiramatsu N, Hayashi N, Katayama K, Mochizuki K, Kawanishi Y, Kasahara A, et al. Immunohistochemical detection of Fas antigen in liver tissue of patients with chronic hepatitis C. Hepatology 1994;19:1354–9.
2. Okazaki M, Hino K, Fujii K, Kobayashi N, Okita K. Hepatic Fas antigen expression before and after interferon therapy in patients with chronic hepatitis C. Dig Dis Sci 1996;41:2453–8.
3. Mita E, Hayashi N, Iio S, Takehara T, Hijioka T, Kasahara A, et al. Role of Fas ligand in apoptosis induced by hepatitis C virus infection. Biochem Biophys Res Commun 1994;204:468–74.
4. Kim HT, Stoica G, Bazer FW, Ott TL. Interferon tau-induced hepatocyte apoptosis in sheep. Hepatology 2000;31:1275–84.
5. Cascino I, Fiucci G, Papoff G, Ruberti G. Three functional soluble forms of the human apoptosis-inducing Fas molecule are produced by alternative splicing. J Immunol 1995;154:2706–13.
6. Papoff G, Cascino I, Eramo A, Starace G, Lynch DH, Ruberti G. An N-terminal domain shared by Fas/Apo-1 (CD95) soluble variants prevents cell death in vitro. J Immunol 1996;156:4622–30.
7. Cheng J, Zhou T, Liu C, Shapiro JP, Brauer MJ, Kiefer MC, et al. Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. Science 1994;263:1759–62.
8. Kondo T, Suda T, Fukuyama H, Adachi M, Nagata S. Essential roles of the Fas ligand in the development of hepatitis. Nat Med 1997;3:409–13.
9. Krams SM, Fox CK, Beatty PR, Cao S, Villanueva JC, Esquivel CO, et al. Human hepatocytes produce an isoform of Fas that inhibits apoptosis. Transplantation 1998;65:713–21.
10. Iio S, Hayashi N, Mita E, Ueda K, Mochizuki K, Hiramatsu N, et al. Serum levels of soluble Fas antigen in chronic hepatitis C patients. J Hepatol 1998;29:517–23.
11. Yoneyama K, Goto T, Miura K, Mikami K, Ohshima S, Nakane K, et al. The expression of Fas and Fas ligand, and the effects of interferon in chronic liver diseases with hepatitis C virus. Hepatol Res 2002;24:327–37.
12. Seishima M, Takemura M, Saito K, Sano H, Minatoguchi S, Fujiwara H, et al. Highly sensitive ELISA for soluble Fas in serum: increased soluble Fas in the elderly. Clin Chem 1996;42: 1911–4.
13. Endo S, Inada K, Takakuwa T, Kasai T, Yamada Y, Wakabayashi G, et al. Nitrite/nitrate (NOx) and sFas antigen levels in patients with multiple organ failure. Res Commun Mol Pathol Pharmacol 1996;92:253–6.
14. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1:431–5.
15. Shiratori Y, Perelson AS, Weinberger L, Imazeki F, Yokosuka O, Nakata R, et al. Different turnover rate of hepatitis C virus clearance by different treatment regimen using interferon-beta. J Hepatol 2000;33:313–22.
16. Asahina Y, Izumi N, Uchihara M, Noguchi O, Tsuchiya K, Hamano K, et al. A potent antiviral effect on hepatitis C viral dynamics in serum and peripheral blood mononuclear cells during combination therapy with high-dose daily interferon alfa plus ribavirin and intravenous twice-daily treatment with interferon beta. Hepatology 2001;34:377–84.
17. Balachandran S, Kim CN, Yeh WC, Mak TW, Bhalla K, Barber GN. Activation of the dsRNA-dependent protein kinase, PKR, induces apoptosis through FADD-mediated death signaling. EMBO J 1998;17:6888–902.
18. Donze O, Dostie J, Sonenberg N. Regulatable expression of the interferon-induced double-stranded RNA dependent protein kinase PKR induces apoptosis and Fas receptor expression. Virology 1999;256:322–9.

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C型慢性肝炎に対するインターフェロンα-2bとリバビリン併用療法におけるヘモグロビン減少に関する検討

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Study on Decreased Hemoglobin Levels in Combination Therapy with Interferon α-2b and Ribavirin for Chronic Hepatitis C

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The approval of ribavirin for reimbursement under the health insurance scheme in Japan in 2001 started a new era in interferon (IFN) therapy against chronic hepatitis C, enabling a change from IFN mono therapy to combination therapy with IFN and ribavirin. As the major safety problems of ribavirin, teratogenicity, hemolytic anemia and skin symptoms may be given. Also, there is known to be a greater incidence of decreased hemoglobin (Hb) in combination therapy with IFN α-2b and ribavirin than in the case of IFN mono therapy. Despite this, however, it has been considered that combination therapy may be accomplished if the criteria for reducing the dosage of ribavirin are strictly followed.

In this study, patients receiving combination therapy with IFN α-2b and ribavirin in Osaka National Hospital were followed up to evaluate the decrease in Hb in cases in which the ribavirin was reduced in dosage or discontinued (reduced/discontinued group). Hb levels in this group decreased by 3.0 ± 1.6 g/dL on average after 4 weeks, significantly lower than pretreatment levels. In these subjects, it seemed that the decreased Hb levels after 4 weeks of the combination therapy aggravated anemia symptoms and subjective symptoms such as general fatigue, with the result that combination therapy could not be accomplished.

Key words —— chronic hepatitis C, interferon therapy, ribavirin, decreased hemoglobin level

緒　言

C型慢性肝炎に対するインターフェロン(IFN)療法が，1992年に導入されてから10年以上が経過した．日本人の場合，C型肝炎ウイルス(HCV)の遺伝子型がgenotype1bでHCV-RNA量が分岐鎖DNAプローブ法で1 Meq/mL以上，またはアンプリコア定量法で100KIU/mL以上あるIFN難治性症例が多く，これまで林ら[1)]や加藤ら[2)]，奥新らなど[3,4)]によりIFN療法における投与方法の工夫が行われてきた．1998年にMcHutchisonら[5)]やPoynardらなど[6,7)]が，C型慢性肝炎患者に対してリバビリンとIFN α-2bの併用療法とIFN α-2b単独療法での臨床比較試験を行い，併用療法の高い治療効果と安全性について報告した．現在，欧州肝臓学会(EASL)やアジア太平洋肝臓学会(APASL)などにおいて併用療法を標準的治療法としている．わが国でも1998年から2000年にかけて行われた臨床比較試験の結果[8,9)]，2001年にリバビリンが保険適応になり，C型慢性肝炎に対するIFN療法は，IFN単独療法からIFNとリバビリンの併用療法という新しい時代になった．リバビリンの安全性上の問題点として，催奇形性と溶血性貧血，皮膚症状などが挙げられる．

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療センター)で行われた併用療法施行症例において追跡調査を行い，リバビリンの減量，または併用療法中止症例(減量・中止群)における要因を明らかにすることを目的に，ヘモグロビン(Hb)値の推移と減少量などについての検討を行ったので報告する．

対　　象

平成14年2月より平成15年3月までに大阪医療センター消化器科において，C型慢性肝炎患者で高ウイルス量(HCV-RNA 量が分岐鎖 DNA プローブ法で 1 Meq/mL 以上)の IFN 初回治療症例，または IFN 治療後再燃症例に対してリバビリンと IFN α-2b の併用療法を行った全症例を対象とした．投与方法は，IFN α-2b 6～10MIU/day を2週間連日投与後，週3回22週間歇投与に，リバビリン600mg(体重60kg 未満)～800mg(体重60kg 以上)/day を連日併用投与とした．

方　　法

併用療法施行患者を6カ月間の継続終了群と減量・中止群の2群に分類した．両群において，投与開始2週後，4週後の Hb 値の推移について投与前値との比較を行った．両群間では投与開始4週後の Hb 値，および Hb 減少量について比較検討を行った．投与前 Hb 値による(投与前 Hb 値<12g/dL，12g/dL≦Hb 値<14g/dL，14g/dL≦Hb 値の3群に分類)リバビリンの減量，併用療法中止の割合，投与開始4週後の Hb 減少量による(Hb 減少量≧3.0g/dL，Hb 減少量<3.0g/dL の2群に分類)リバビリンの減量，併用療法中止の割合について調査した．合併症および既往歴については，診断記録より調査した．リバビリンの減量および投与中止基準として，Hb 値10g/dL 未満で200mg 減量とし，8.5g/dL 未満で IFN とともに投与中止とした．Hb 減少以外の有害事象が発現し，投与量の減量または併用療法中止を行う必要がある場合は，主治医の判断でそれを行うこととした．Hb 値の推移における投与前値との比較および群間比較には分散分析を使用し，Hb 減少量の群間比較には対応のない t 検定を使用した．有意差の判定基準は $p<0.05$ を有意差とした．

結　　果

併用療法施行症例は44例であった．うち1例は，抗ウイルス効果が認められないため，主治医の判断により併用療法が中止となったため除外した．解析対象症例43例のうち，IFN 初回治療症例が6例，再燃症例が37例であり，継続終了群は23例，減量・中止群は20例(リバビリンの減量症例は7例，併用療法の中止症例は13例)であった．減量・中止群における平均年齢は，62.1±8.4歳と継続終了群よりも有意に高く，併用療法開始前の血小板数(PLt)は，平均11.6±3.2×10^4/μL と有意に低値であった．患者背景を **Table 1** に示す．リバビリンの減量理由の内訳は，Hb 値が減量基準に達した症例が6例，併用療法による全身倦怠感症状のため主治医が判断した症例が1例であった．併用療法中止理由の内訳は，Hb 値が

Table 1. 患者背景

背景因子	継続終了群	減量・中止群	検定
症例(M/F)	23(18/5)	20(11/9)	
年齢(歳)	56. 3±10. 9	62. 1±8. 4	p<0.05
セロタイプ(1/2)	19/2 不明 2	16/4	
IFN投与量(6MIU/10MIU)	16/7	11/9	
Rib投与量/day(600mg/800mg)	12/11	11/9	
体重当たりのRib投与量(mg/kg/day)	11. 3±2. 4 (n=21)	11. 8±1. 1 (n=14)	N. S
AST(IU/L)	79. 6±39. 8	75. 0±26. 5	N. S
ALT(IU/L)	102. 2±77. 2	95. 0±37. 7	N. S
T−bil値(mg/dL)	0. 9±0. 3	0. 8±0. 3	N. S
RBC(×10^4/μL)	4. 4±0. 5	4. 3±0. 6	N. S
WBC(×10^3/μL)	4. 9±1. 6	4. 3±1. 1	N. S
PLt(×10^4/μL)	15. 7±6. 4	11. 6±3. 2	p<0.05
Hb値(g/dL)	13. 9±1. 8	13. 7±1. 5	N. S
Scr(mg/dL)	0. 8±0. 2	0. 7±0. 1	p<0.05
BUN(mg/dL)	15. 5±4. 5	15. 6±2. 7 (n=19)	N. S

mean±S. D
対応のないt検定
P<0. 05　N. S.　:not significant

中止基準に達した症例が2例，Hb値が減量基準に達しリバビリンを減量後，併用療法による嘔吐症状または眼底出血のため，主治医が併用療法中止と判断した症例が2例であった．全身倦怠感または貧血症状のため主治医が併用療法中止と判断した症例が，それぞれ7例と2例であった(**Table 2**)．

合併症に関しては，両群において高血圧症，糖尿病，心疾患，鉄欠乏性貧血，甲状腺機能低下症の合併またはその既往歴を有する患者を認めたが，偏りは認められなかった(**Table 3**)．中止症例のうち2症例は，心不全(症例7)または甲状腺機能低下症(症例11)に対する投薬を有する症例であった．症例7は，ジゴキシン，ワルファリン，ゾピクロンを継続服用していた．投与開始19週後にHb値は13.3g/dLであったが，全身倦怠感のため主治医の判断により併用療法が中止になった．症例11は，ベシル酸アムロジピン，テプレノン，エチゾラム，メコバラミン，レボチロキシンナトリウムを継続服用していた．投与開始1週後にPLtが$2.4\times10^4/\mu L$に減少し，IFNが連日投与から間歇投与に変更となり，リバビリンが200mg/day減量になった．投与開始5週後にPLtが$2.7\times10^4/\mu L$に回復しHb値は11.6g/dLであったが，全身倦怠感のため主治医の判断により併用療法が中止になった．投与開始前の甲状腺刺激ホルモン(TSH)値は2.09μU/mL，遊離トリヨードサイロニン(F-T3)値は2.38pg/mL，遊離サイロキシン(F-T4)値は0.92ng/dLであり，投与開始5週後においては，それぞれ0.53μU/mL，1.98pg/mL，1.08ng/dLに変動しいずれも低値であった．心疾患，甲状腺機能低下症の合併またはその既往歴を有する他の症例において，その疾患に対する投薬治療は認められなかった．

両群におけるHb値の推移は，投与前値と比べ投与開始2週後，4週後は有意に低下した(**Fig. 1**)．投与開始

Table 2．併用療法中止症例

症例	性別	投与前Hb値 (g/dL)	中止時期	中止時Hb値 (g/dL)	リバビリン投与量の経緯	主な中止理由
1	M	15.0	6週	10.5	800mg/day継続	全身倦怠感
2	M	12.1	10週	9.3	600mg/dayから6週後400mg/dayに減量	貧血症状
3	M	13.0	6週	7.3	800mg/day継続	中止基準により
4	M	17.5	12週	12.2	800mg/dayから4週後600mg/dayに減量	全身倦怠感
5	M	14.9	12週	9.9	800mg/day継続	貧血症状
6	M	14.6	12週	11.3	600mg/day継続	全身倦怠感
7	M	15.5	19週	13.3	800mg/day継続	全身倦怠感
8	F	12.8	21週	9.2	600mg/day継続	全身倦怠感
9	F	11.5	6週	8.2	600mg/dayから4週後400mg/dayに減量	中止基準により
10	F	13.9	6週	10.4	600mg/day継続	全身倦怠感
11	F	11.5	5週	11.6	600mg/dayから1週後400mg/dayに減量	全身倦怠感
12	F	11.8	16週	9.7	600mg/dayから8週後400mg/dayに減量	嘔吐
13	F	13.5	16週	10.5	600mg/dayから5週後400mg/dayに減量	眼底出血

Table 3．合併症および既往歴の比較

疾患名	継続終了群(n=23)	減量・中止群(n=20)
高血圧症	7例	4例
胃・十二指腸潰瘍	5例	5例
不眠症	5例	5例
2型糖尿病	5例	4例
鉄欠乏性貧血	3例	2例
甲状腺機能低下症	3例	4例
肥大型心筋症	2例	なし
めまい症	なし	4例
心不全	なし	2例
神経症	なし	2例
その他	11例	13例

(重複あり)

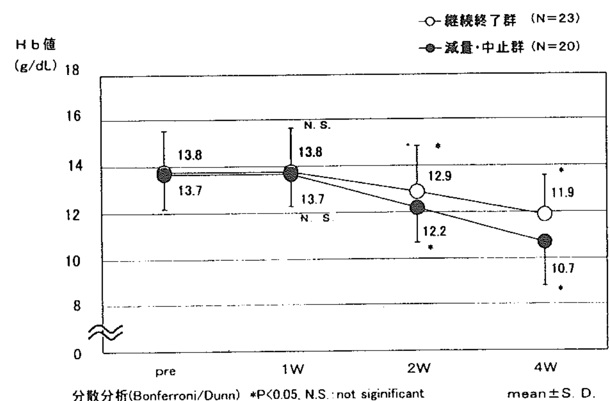

Fig. 1．Hb値の推移についての比較