

# Expression of Interferon Alpha/Beta Receptor in the Liver of Chronic Hepatitis C Patients

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Interferon (IFN) demonstrates antiviral activity by binding to receptors on the cell surface. Expression of the IFN receptor in hepatocytes may be directly associated with a hepatitis C virus (HCV) infection and the response to IFN therapy. A competitive PCR method was developed to measure IFN alpha/beta ( $\alpha/\beta$ ) receptor mRNA in liver samples obtained by needle biopsy. Thirty-one patients with chronic hepatitis C (21 without cirrhosis, 10 with cirrhosis) and six normal subjects were used. Eighteen of the 21 patients without cirrhosis received the IFN therapy. Competitive PCR was carried out using IFN  $\alpha/\beta$  receptor gene-specific primers and a specific competitor. Expression of the receptor was detected in all liver samples. There was no association between the expression level and serum alanine aminotransferase level, serum (2'–5') oligo (A) synthetase level, amount of serum HCV RNA, or HCV genotype. The expression level in patients with chronic hepatitis was significantly higher than that in normal livers ( $P < 0.05$ ) and in cirrhotic livers ( $P < 0.01$ ). Seven of the 18 patients treated with IFN demonstrated a sustained response to IFN (sustained responders), and the remaining 11 did not (nonsustained responders). The expression level of IFN  $\alpha/\beta$  receptor mRNA in the sustained responders was significantly higher than that in the nonsustained responders ( $P < 0.01$ ). Thus, the expression of IFN  $\alpha/\beta$  receptor mRNA may be one of the host factors influencing the response to IFN therapy. *J. Med. Virol.* 56:217–223, 1998.

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**KEY WORDS:** IFN  $\alpha/\beta$  receptor; IFN therapy; 2–5AS; HCV RNA; HCV genotype

## INTRODUCTION

Interferon (IFN) therapy is the only strategy at present potentially capable of eliminating hepatitis C virus (HCV) from hepatocytes. However, only about one

third of the patients with HCV infection are able to eliminate HCV RNA from their serum with IFN [Kanai et al., 1995; Tanaka et al., 1996]. The efficacy of IFN therapy is of major concern for treating patients with hepatitis C. The determinants of IFN responsiveness may be complicated and it is believed that viral factors may influence this responsiveness. These include the amount of HCV RNA in the serum [Lau et al., 1993], the genotypes of HCV [Tsubota et al., 1994], [Yoshioka et al., 1995], viral heterogeneity in the serum [Kanazawa et al., 1994], and the IFN sensitivity-determining sequence of the HCV genome [Enomoto et al., 1996]. Host factors also influence responsiveness. The liver histology before IFN therapy is reported to be associated with the response [Jouet et al., 1994].

Alpha and beta IFNs are grouped type I IFN, and elicit their antiviral activity by binding to specific receptors on the cell surface [Aguet et al., 1983; Zoon et al., 1984]. The type I IFN receptor has a multichain structure composed of at least three different components [Uze et al., 1995]. One component, the IFN alpha/beta ( $\alpha/\beta$ ) receptor, has been cloned recently and it has been demonstrated that the receptor is involved directly in signal transduction [Novick et al., 1994]. Thus, the IFN  $\alpha/\beta$  receptor on hepatocytes infected with HCV may be involved directly in the antiviral activity of IFN as one of the host factors influencing this response. In the present study, we investigated the expression level of IFN  $\alpha/\beta$  receptor mRNA in the liver of patients with chronic hepatitis C using reverse transcription-PCR, and analyzed the relationships between the expression level before IFN treatment and the effect of therapy.

## MATERIALS AND METHODS

### Cell Culture

Four human hepatoma cell lines—HepG2 [Aden et al., 1979], HuH7 [Nakabayashi et al., 1982], SK-Hep-1 [Shouval et al., 1982], and Hep3B [Aden et al., 1979]—

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Accepted 18 May 1998

were studied as well as two nonhepatoma cell lines: Daudi [Spira et al., 1977] and HeLa [Gey et al., 1952]. The hepatoma cell lines and HeLa cells were maintained in culture in DMEM (Gibco, Grand Island, NY), and Daudi cells in RPMI 1640 medium (Gibco) with 10% fetal bovine serum (Gibco).

### Patients and IFN Therapy

Thirty-one patients with chronic hepatitis C admitted to Kanazawa University Hospital between January 1993 and December 1995 were studied. They included 17 men and 14 women whose mean age was 53.3 years. All patients were positive for both antibodies to HCV (anti-HCV) and HCV RNA in the serum. None of the patients were homosexual, intravenous drug users, or positive for either hepatitis B virus surface antigens (HBsAg) or antinuclear antibodies. A liver sample was surgically removed from one patient and 30 liver samples were obtained by needle liver biopsy. Informed consent was obtained from each patient before the biopsy. The biopsy specimen was divided into two parts, one for histological examination and another for RNA extraction. The histological diagnosis was made by two experts in the field of liver histology. Liver histology was classified according to the classification of Desmet et al. [1994]. This system provided a grade ranging from 0 to 4 according to the degree of liver fibrosis, with a score of 4 corresponding to liver cirrhosis. The degree of inflammation was graded from minimal chronic hepatitis to severe chronic hepatitis. Twenty-one of the patients were diagnosed as having chronic hepatitis without cirrhosis and 10 patients with cirrhosis. Eighteen of the 21 patients received IFN therapy after liver biopsy. Seven patients were treated with IFN- $\alpha$  (total amount: 480–720 million units) and 11 patients with IFN- $\beta$  (336 million units). The response to IFN was categorized by the alanine aminotransferase (ALT) levels after treatment. A complete response was defined as the normalization of ALT within 6 months of the initiation of treatment and its continuation over 6 months after the completion of treatment (sustained responder). Those categorized as having an incomplete response were defined as a nonsustained responder. The activity of (2'–5') oligo (A) synthetase (2–5AS) in the blood was measured by radioimmunoassay using a commercial kit (Eiken Immunochemical Laboratory, Tokyo, Japan). The amount of serum HCV RNA was measured by the multicyclic reverse transcription-PCR technique as described previously [Ishiyama et al., 1993]. HCV genotypes were determined by the method of Okamoto et al. [1992].

We obtained normal liver tissues from seven patients who had operations for colon cancer with liver metastasis. A noncancerous liver tissue of the resected sample was used for Northern blot analysis. Six tissues were obtained by needle biopsy from a noncancerous part of the resected liver. Informed consent was obtained from each patient before the operation. All seven patients were negative for both HBsAg and anti-

HCV in the serum and their serum ALT levels were normal.

### Preparation of IFN $\alpha/\beta$ Receptor cDNA

IFN  $\alpha/\beta$  receptor cDNA was obtained by reverse transcription-PCR using specific primers for the gene. Two primers, 5'-ATTGGATCCTTAATCACTGGGGCA-CAGGGA-3' and 5'-ATCCATGGAATTCATGCTTTT-GAGCCAGAATGCC-3', were designed to comprise the full cDNA of the IFN  $\alpha/\beta$  receptor gene with restriction enzyme sites at the terminal. Total RNA was isolated from Daudi cells, and reverse transcription-PCR was carried out with the primer set. The PCR fragment was cut with *Eco*RI and *Bam*HI, and cloned into the *Eco*RI and *Bam*HI sites of a pSG5 vector (Stratagene, La Jolla, CA). The nucleotide sequence of the insert was confirmed to contain the full sequence of the IFN  $\alpha/\beta$  receptor gene as reported [Novick et al., 1994].

### Northern Blotting

Total RNA was isolated from cultured cells and liver samples, and poly(A)<sup>+</sup> RNA selected with Oligotex-dT30<Super> (JSR and Roche, Tokyo, Japan). Poly(A)<sup>+</sup> RNA was fractionated by electrophoresis through a 1.5% agarose gel. The cloned IFN  $\alpha/\beta$  receptor gene was labeled with (<sup>32</sup>P) dCTP by random priming and used for Northern blot hybridization as previously described [Novick et al., 1994]. The signal was measured using an imaging analyzer (FUJIX BAS 1000, Fuji Film, Tokyo, Japan).

### Measurement of IFN $\alpha/\beta$ Receptor Expression by Competitive PCR

Total RNA was isolated from the liver samples. Twelve  $\mu$ g of total RNA was reverse-transcribed in a 30  $\mu$ l reaction containing 3 mM MgCl<sub>2</sub>, 50 mM Tris-HCl, 75 mM KCl, 0.5 mM of each dNTP, 10 mM DTT, 200 U Moloney Murine Leukemia Virus reverse transcriptase (Gibco, Grand Island, NY), and 100 pmol 3'-specific primer for the IFN  $\alpha/\beta$  receptor gene, 5'-CCCTCTGACTGTTCTTCAATG-3'. The mixture was incubated for 1 hr at 45°C and heated at 95°C for 10 min, followed by a 5 min incubation on ice. The PCR primer sequences were as follows: upstream, 5'-GAAGGTGGTTAAGAACTGTGC-3'; downstream, 5'-CCCTCTGACTGTTCTTCAATG-3'. Competitive PCR fragments that were designed to be amplified by the same PCR primers for the amplification of the IFN  $\alpha/\beta$  receptor gene were constructed with a PCR MIMIC Construction Kit (Clontech, CA). PCR reactions were performed in a total volume of 50  $\mu$ l containing 5  $\mu$ l of  $\times 10$  Ex Taq buffer, 1  $\mu$ l Ex Taq polymerase (Takara, Tokyo, Japan), 0.2 mM of each of dNTP, a competitive fragment, a quarter of the reverse transcription reaction, and 2  $\mu$ M of each of the 3'-specific primer and 5'-specific primer. Amplifications were carried out as above using the following conditions: cycle 1, denaturing at 94°C for 3 min; cycle 2–35, denaturing at 94°C for 1 min, annealing at 62°C for 1.3 min, and extension at 72°C for 1.5 min. The PCR products were electro-

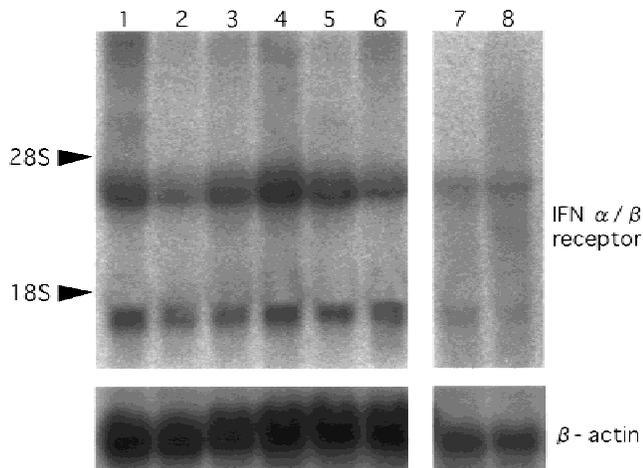


Fig. 1. Northern blot hybridization of poly(A)<sup>+</sup> RNA from six cell lines (lanes 1–6) and two human livers (lanes 7 and 8). Poly(A)<sup>+</sup> RNA (10  $\mu$ g from a cell line and 5  $\mu$ g from a human liver) was electrophoresed through an agarose gel and transferred to a nylon membrane. The membrane was hybridized with an IFN  $\alpha/\beta$  receptor probe (upper lanes), and a  $\beta$ -actin probe (lower lanes). The 28S and 18S ribosomal RNAs are indicated. Lane 1, Daudi; lane 2, HeLa; lane 3, HepG2; lane 4, HuH7; lane 5, SK-Hep-1; Lane 6, Hep3B; lane 7, normal liver; lane 8, liver with chronic hepatitis C.

phoresed on a 1.5% agarose gel containing ethidium bromide, visualized by UV light and photographed with Polaroid film (Polaroid, Cambridge, MA). The intensity of the band signals was measured by a densitometer with an image program (ATTO, Tokyo, Japan). Competitive PCR, as previously described [Kaneko et al., 1992], was used to semiquantify alterations in IFN  $\alpha/\beta$  receptor mRNA.

### Statistical Analysis

Data obtained from the patients were analyzed using the paired t-test and the Mann-Whitney's U test, with  $P < 0.05$  considered to be statistically significant. Data obtained at three degree expression levels of the IFN  $\alpha/\beta$  receptor were analyzed using the Kruskal-Wallis's test, with  $P < 0.05$  considered to be statistically significant.

## RESULTS

### Expression of IFN $\alpha/\beta$ Receptor mRNA

Expression of the IFN  $\alpha/\beta$  receptor gene in hepatoma cell lines and human livers was examined with two nonhepatoma-derived cell lines that are known to express the IFN  $\alpha/\beta$  receptor gene on their cell surface [Novick et al., 1994]. Northern blot hybridization of 10  $\mu$ g of poly(A)<sup>+</sup> RNA from four hepatoma cell lines revealed two transcripts of 1.5 Kb and 4.5 Kb corresponding to the expected sizes of IFN  $\alpha/\beta$  receptor mRNA [Novick et al., 1994] (Fig. 1). The amount of mRNA was similar among the six cell lines. The two transcripts were also found in 5  $\mu$ g of poly(A)<sup>+</sup> RNA from the livers. The expression level in a liver infected with HCV was similar to that in a normal liver without HCV infection. Thus, expression of the IFN  $\alpha/\beta$  receptor gene in hepatoma cell lines and human livers was demonstrated.

### Semiquantitative Analysis of IFN $\alpha/\beta$ Receptor mRNA by Competitive PCR

The amount of liver sample obtained by needle biopsy was not enough to analyze the expression level of the IFN  $\alpha/\beta$  receptor gene by Northern blot hybridization. Therefore, semiquantitation of IFN  $\alpha/\beta$  receptor mRNA was performed using competitive PCR. Twelve  $\mu$ g of total RNA obtained from each liver biopsy sample was reverse-transcribed and coamplified with a standard series of decreasing amounts of the competitor, MIMIC (Fig. 2). Two bands corresponding to the expected size of the products from liver IFN  $\alpha/\beta$  receptor mRNA (291 bp) and the competitor (442 bp) were demonstrated. The intensities of the bands derived from the liver sample were similar while those derived from the competitor decreased. At the point where the amounts of liver product (291 bp) and the competitor (442 bp) were nearly equivalent (i.e., ratio = 1), the starting concentration of liver IFN  $\alpha/\beta$  receptor mRNA for PCR was found to be equal to the known starting concentration of the competitor (Fig. 2A, lane 5; Fig. 2B, lane 4; Fig. 2C, lane 3). Thus, the expression level of IFN  $\alpha/\beta$  receptor mRNA was found to be  $3 \times 10^2$  molecules/ $\mu$ g total RNA and was defined as "1+" (Fig. 2A),  $3 \times 10^3$  molecules/ $\mu$ g as "2+" (Fig. 2B), and  $3 \times 10^4$  molecules/ $\mu$ g as "3+" (Fig. 2C).

### Expression Level of IFN $\alpha/\beta$ Receptor mRNA in Liver

Competitive PCR was subsequently used to quantify the expression level of IFN  $\alpha/\beta$  receptor mRNA in 36 liver samples (Fig. 3). IFN  $\alpha/\beta$  receptor mRNA was detected in every sample and the expression level ranged from 1+ to 3+ as described above (Fig. 2). The expression level in the liver of the patients with chronic hepatitis C without cirrhosis was significantly higher than that in normal livers ( $P < 0.05$ ), and also higher than that in cirrhotic livers ( $P < 0.01$ ). The histological activity of chronic hepatitis did not correlate with the expression level. The serum ALT levels, serum 2–5AS, amount of HCV RNA, HCV genotype, and histological activity did not differ significantly between the patients with chronic hepatitis C and cirrhotic patients (Table I). There was no association between serum ALT level, serum 2–5AS level, amount of HCV RNA, or HCV genotype and the expression levels of IFN  $\alpha/\beta$  receptor mRNA in the liver of patients with chronic hepatitis C or cirrhotic patients (Fig. 4A–D). Thus, the expression level of IFN  $\alpha/\beta$  receptor mRNA in the liver as discerned by competitive PCR correlated only with liver fibrosis.

### Expression of IFN $\alpha/\beta$ Receptor mRNA and Effect of IFN Therapy

Eighteen of the patients received IFN therapy after liver biopsy. All had chronic hepatitis without cirrhosis. Seven of the 18 patients (38%) demonstrated normalization of serum ALT levels within 6 months of the initiation of treatment and its continuation over 6

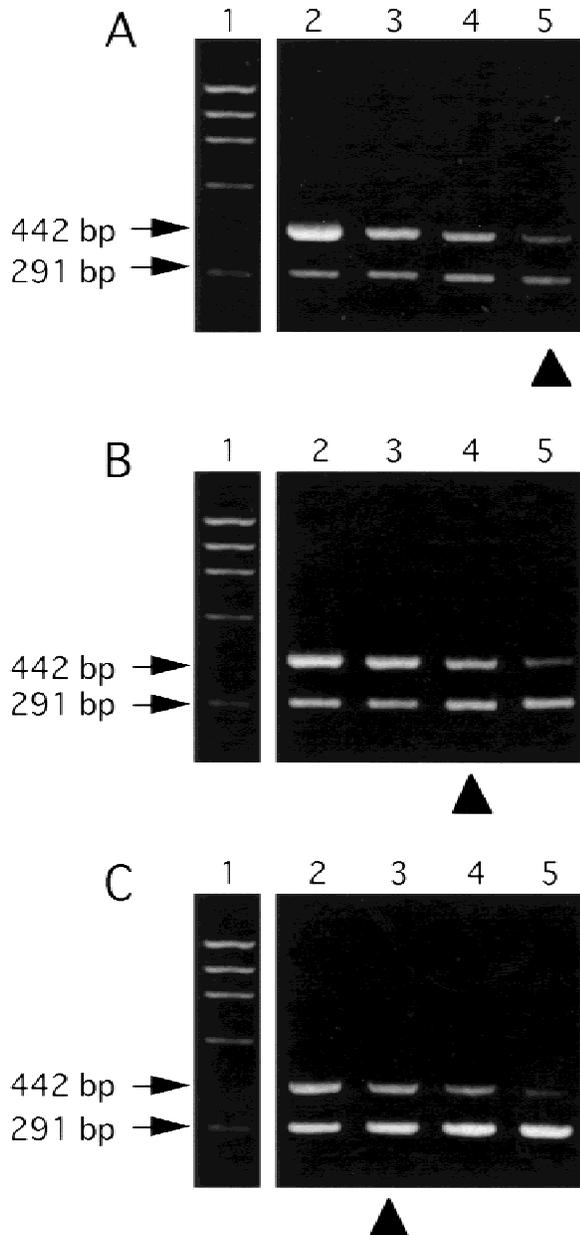


Fig. 2. Competitive PCR of IFN  $\alpha/\beta$  receptor mRNA from three representative liver samples. Twelve  $\mu\text{g}$  of total RNA was reverse-transcribed and one quarter of the resulting mixture was used to semiquantify IFN  $\alpha/\beta$  receptor mRNA by competitive PCR. Tenfold dilutions of a competitor MIMIC (lane 2,  $3 \times 10^5$  molecules of MIMIC; lane 3,  $3 \times 10^4$  molecules; lane 4,  $3 \times 10^3$  molecules; lane 5,  $3 \times 10^2$  molecules) were coamplified. The PCR products were electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light. Thin arrows indicate the PCR products of the competitor, MIMIC (442 bp) and IFN  $\alpha/\beta$  receptor cDNA (291 bp). Thick arrows indicate the dilution at which the competitor and IFN  $\alpha/\beta$  receptor cDNA are approximately equivalent. Lane 1 is the marker that is  $\phi\text{X} 174$  digested by *Hae*III. **A:** The expression level of IFN  $\alpha/\beta$  receptor mRNA in this liver sample is defined as 1+. **B:** The level is defined as 2+. **C:** The level is defined as 3+.

months after the termination of IFN treatment (i.e., sustained responder). The other 11 did not meet the criteria (i.e., nonsustained responder) (Table II). One of the seven sustained responders demonstrated the pres-

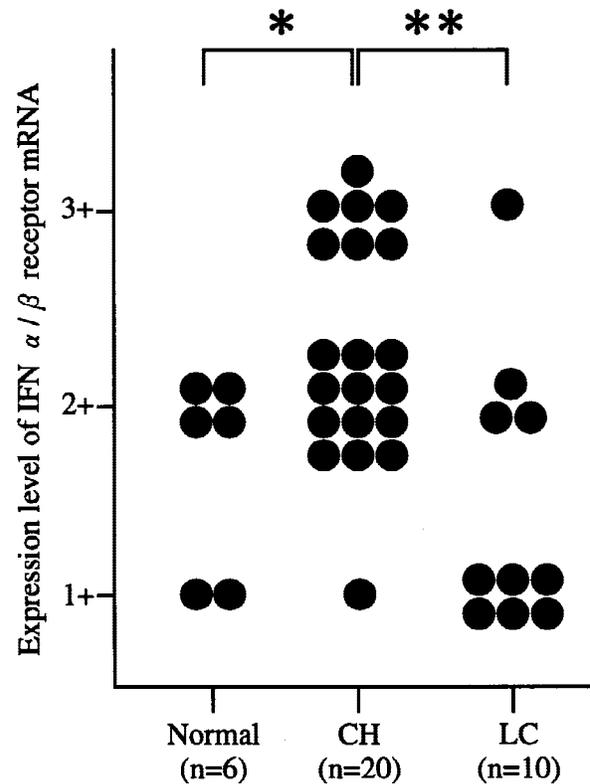


Fig. 3. Expression level of IFN  $\alpha/\beta$  receptor mRNA in the liver and the histology. Thirty-six liver samples obtained by needle biopsy (6 normal livers, normal; 20 chronic hepatitis without cirrhosis, CH; 10 chronic hepatitis with cirrhosis, LC) were used for competitive PCR to semiquantify IFN  $\alpha/\beta$  receptor mRNA. The expression level of the samples was shown as one of three levels (1+ to 3+), as shown in Figure 2. \* denotes  $P < 0.05$ ; \*\*,  $P < 0.01$ .

ence of serum HCV RNA after IFN treatment, while all 11 nonsustained responders remained positive for HCV RNA. The clinical characteristics of the two groups were similar with respect to gender, age, and serum ALT level. The amount of HCV RNA in the responders was  $10^{5.0 \pm 1.8}$  copies/ml, higher than that in the nonresponders ( $10^{4.3 \pm 1.5}$  copies/ml) in this study. The proportion of grading and staging scores of the liver histology in each group was not significant. The IFN regimen in the two groups was similar. The expression level of IFN  $\alpha/\beta$  receptor mRNA was analyzed in the two groups (Fig. 5). Five of seven responders and only 1 of 11 nonresponders demonstrated a 3+ expression level of IFN  $\alpha/\beta$  receptor mRNA. The expression level in the responders was significantly higher than that in the nonresponders ( $P < 0.01$ ). Thus, the expression level of IFN  $\alpha/\beta$  receptor mRNA before treatment with IFN may be one of the host factors that influence IFN therapy efficacy in patients with chronic hepatitis C.

## DISCUSSION

The precise structure of type I IFN receptor is not known. One component of the receptor was cloned by transferring human DNA into mouse cells and selecting for cells responding to human IFN  $\alpha/\beta$  [Uze et al.,

TABLE I. Clinical Characteristics of Patients in Whom the Liver IFN  $\alpha/\beta$  Receptor Expression Was Measured by Competitive PCR

	Normal	CH	LC
Number of patients	6	20	10
Sex (M/F)	3/3	11/9	6/4
Serm ALT level* (IU/l)	16.2 $\pm$ 3.8	56.8 $\pm$ 40.8	78.6 $\pm$ 47.1
Serum 2-5AS* (pmol/dl)	ND	44.3 $\pm$ 26.6	52.7 $\pm$ 41.7
Amount of HCV RNA* (copies/ml)	—	10 <sup>4.7<math>\pm</math>1.5</sup>	10 <sup>4.0<math>\pm</math>1.3</sup>
Genotype of HCV RNA (II/III/IV)	—	16/3/1	8/1/1
Grading and staging of liver histology			
Minimal/mild/moderate/severe		10/9/1/0	1/5/4/0
Staging score 0/1/2/3/4		0/9/5/6/0	0/0/0/0/10

\*denotes expressed as mean  $\pm$  SD; normal, normal livers; CH, chronic hepatitis without cirrhosis; LC, chronic hepatitis with cirrhosis; ND, not determined; —, negative for HCV-RNA.

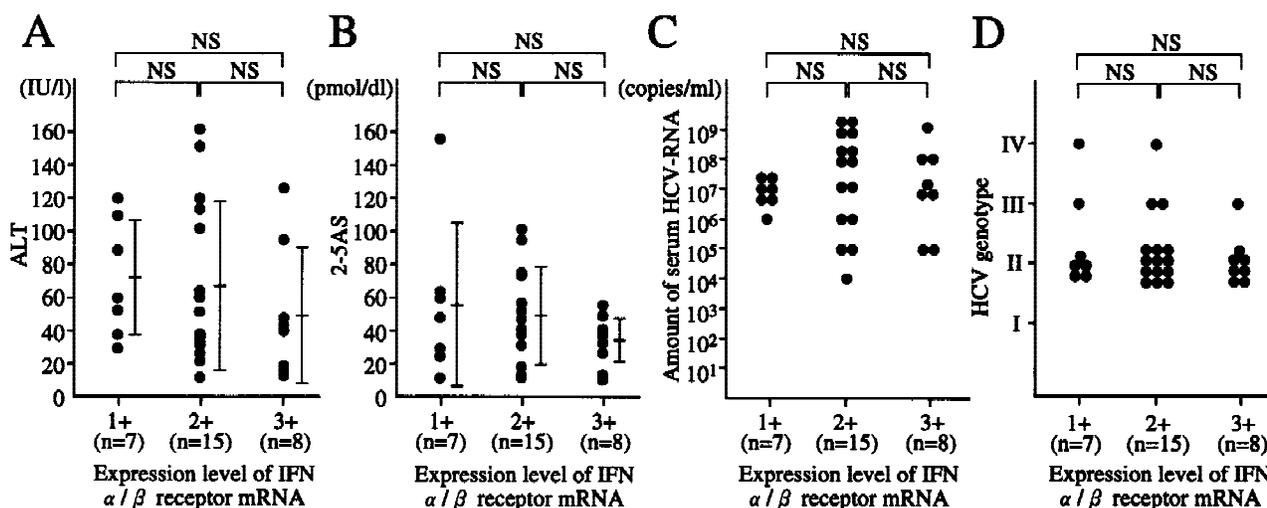


Fig. 4. Expression level of IFN  $\alpha/\beta$  receptor mRNA in the liver and serum markers. The expression level and serum alanine aminotransferase (ALT) level (A); serum (2'-5') oligo (A) synthetase (2-5AS) level (B); the amount of HCV RNA (C); and HCV genotype (D). The expression level of IFN  $\alpha/\beta$  receptor mRNA was shown as one of three levels (1+ to 3+), as shown in Figure 2. NS, There was no statistical significance.

TABLE II. Clinical Characteristics of Patients Who Received Previous IFN Therapy

	Sustained responders	Nonsustained responders
Number of patients	7	11
Sex (M/F)	4/3	7/4
Age*	52.0 $\pm$ 16.4	45.8 $\pm$ 9.0
Serum ALT level* (IU/l)	52.1 $\pm$ 39.7	67.5 $\pm$ 41.2
Amount of HCV RNA* (copies/ml)	10 <sup>5.0<math>\pm</math>1.8</sup>	10 <sup>4.3<math>\pm</math>1.5</sup>
Genotype of HCV RNA (II/III/IV)	6/1/0	9/1/1
Grading and staging of liver histology		
Minimal/mild/moderate/severe	4/3/0/0	4/6/1/0
Staging score 0/1/2/3/4	0/4/2/1/0	0/4/2/5/0
Regimens of IFN therapy (A/B)†	5/2	4/7

\*denotes expressed as mean  $\pm$  SD; †A denotes IFN- $\alpha$  therapy (total amount: more than 480 million units); B, IFN- $\beta$  therapy (total amount: more than 336 million units).

1990]. Recently, Novick et al. [1994] cloned a universal ligand-binding component of the type I IFN receptor (IFN  $\alpha/\beta$  receptor) and demonstrated that the receptor was involved directly in signal transduction. The receptor is present at a low abundance; therefore, several  $\mu$ g of poly(A)+ RNA have been used to detect IFN  $\alpha/\beta$  receptor mRNA by Northern blot hybridization [Novick et al., 1994]. In fact, 5  $\mu$ g of poly(A)+ RNA from the liver samples was necessary to show clear bands by this method (Fig. 1). Since it is unethical to obtain a large amount of liver from patients with chronic hepatitis, we developed a new method using competitive

receptor mRNA by Northern blot hybridization [Novick et al., 1994]. In fact, 5  $\mu$ g of poly(A)+ RNA from the liver samples was necessary to show clear bands by this method (Fig. 1). Since it is unethical to obtain a large amount of liver from patients with chronic hepatitis, we developed a new method using competitive

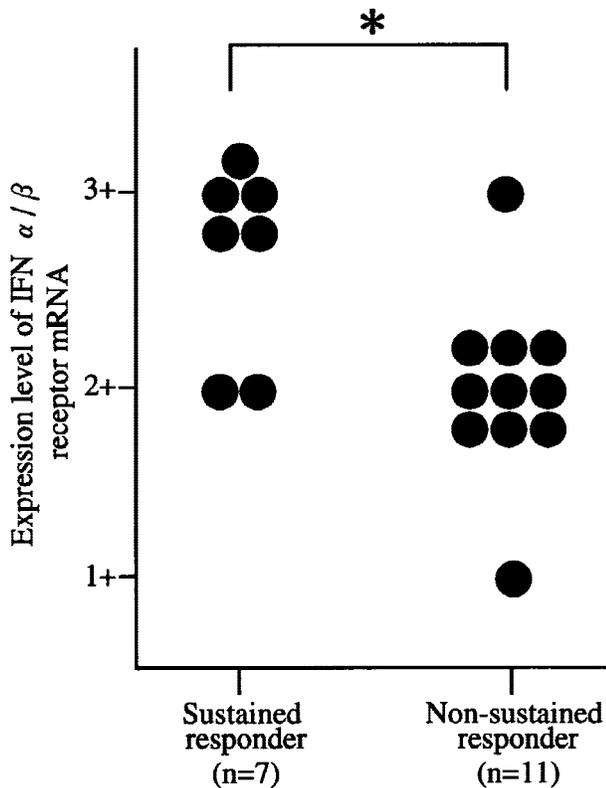


Fig. 5. Expression level of IFN  $\alpha/\beta$  receptor mRNA in the liver and the IFN response. Eighteen patients with chronic hepatitis without cirrhosis received IFN therapy after sampling. Seven patients demonstrated a sustained response to IFN therapy (sustained responder), and the other 11 did not (nonsustained responder). The expression level of IFN  $\alpha/\beta$  receptor mRNA was shown as one of three levels (1+ to 3+), as shown in Figure 2. \* denotes  $P < 0.01$ .

PCR. Competitive PCR has been used successfully for accurate semiquantitative analysis of RNA [Dukas et al., 1993; Li et al., 1996]. The expression level of IFN  $\alpha/\beta$  receptor mRNA in each liver sample was semiquantified at one of three levels (i.e., 1+ to 3+) in this study (Fig. 2). Thus, this method enables semiquantitation of IFN  $\alpha/\beta$  receptor mRNA in liver samples obtained by needle biopsy.

The expression level of IFN  $\alpha/\beta$  receptor mRNA in 20 livers from patients with chronic hepatitis without cirrhosis was significantly higher than that in six normal livers and in 10 cirrhotic livers but the level did not correlate with the amount of serum HCV RNA. Modulation of the IFN system by virus infection has been studied. However, expression of the IFN receptor in a chronic hepatitis virus infection is not fully understood. In a hepatitis B virus infection, inhibition of both IFN production and the action has been reported [Foster et al., 1991; Whitten et al., 1991]. There are two reports regarding IFN receptor expression in peripheral blood mononuclear cells of patients with chronic hepatitis B [Nakajima et al., 1990; Lau et al., 1991]. They described that the expression of IFN receptor was similar between patients with chronic hepatitis B and normal subjects. Similar to our study, Yatsushashi et al. [1997]

reported that the expression of IFN receptor mRNA in the liver of patients with chronic hepatitis C was significantly higher than that of control patients using measurement of cycle differences of the PCR-amplified products. However, contrary to our study, they described that there was a significant negative correlation with the amount of serum HCV RNA, but not with the level of liver fibrosis.

There are at least three explanations for the higher expression of IFN  $\alpha/\beta$  receptor in patients with chronic hepatitis C: (1) HCV may directly increase the expression of IFN  $\alpha/\beta$  receptor in hepatocytes as reported by Yatsushashi et al. [1997]. However, the amount of HCV RNA in serum did not correlate with the expression level (Fig. 4C) in our study. Further study using a large number of patients is clearly necessary to explain the difference. (2) Nonhepatocytes, such as infiltrating lymphocytes, may express abundant IFN  $\alpha/\beta$  receptor mRNA and the total level of gene expression in the liver from hepatitis patients is increased. Although there was no significant difference in the expression of IFN  $\alpha/\beta$  receptor between the two nonhepatoma-derived cell lines and four hepatoma cell lines (Fig. 1), cellular components of the liver, such as hepatocytes, fibroblasts, and infiltrating lymphocytes, may express different amounts of IFN  $\alpha/\beta$  receptor. Thus, the expression level of IFN receptor in the liver may not directly reflect the expression of hepatocytes. This assumption may also explain the low expression of the gene in a cirrhotic liver compared to a noncirrhotic liver (Fig. 3) if fibroblasts in a cirrhotic liver express a lower amount of the receptor compared with the hepatocytes. (3) Cytokines are closely related to expression of IFN receptors. In a previous study, the modulation of human IFN receptor expression by IFN- $\gamma$  and IFN- $\alpha$  was reported [Hannigan et al., 1984; Lau et al., 1986]. Serum cytokines, such as IFN- $\gamma$ , are known to be elevated in chronic hepatitis [Chu et al., 1995; Cacciarelli et al., 1996]. Thus, cytokines in hepatitis may modulate IFN  $\alpha/\beta$  receptor expression in the hepatocytes of patients.

Our results showed that the expression level of IFN  $\alpha/\beta$  receptor in IFN responders was significantly higher than that in nonresponders. IFN receptor expression has been reported to be significantly increased in IFN responders as well [Fukuda et al., 1997; Yatsushashi et al., 1997]. These results indicate that IFN receptor expression in the liver may be one of the host factors influencing IFN efficacy. This assumption is interesting, considering the action of IFN in viral elimination. IFN demonstrates antiviral activity by binding to a specific receptor on the cells and subsequently inducing signal transduction. Thus, the number of IFN receptors may be directly associated with the IFN efficacy in patients with chronic hepatitis C.

The determinants of IFN responsiveness are known to be complicated, and multiple factors are considered to interact with each other. Viral factors (amount of HCV RNA, HCV genotype) and IFN regimen, which were known to be associated closely with the IFN re-

sponse [Lau et al., 1993; Tsubota et al., 1994; Poynard et al., 1995; Yoshioka et al., 1995], were not uniform among the patients in this study, although there was no difference between the two groups. Further study using a large number of patients is clearly necessary to conclude that the expression level of IFN  $\alpha/\beta$  receptor is a determinant of the IFN effect.

## REFERENCES

- Aden DP, Fogel A, Plotkin S, Damjanov I, Knowles BB (1979): Controlled synthesis of HBsAg in a differentiated human liver carcinoma-derived cell line. *Nature* 282:615–616.
- Aguet M, Mogens KE (1983): Interferon receptors. In Gresser I (ed): "Interferon 5." New York: Academic Press, pp 1–22.
- Cacciarelli TV, Martinez OM, Gish RG, Villanueva JC, Krams SM (1996): Immunoregulatory cytokines in chronic hepatitis C virus infection: Pre- and posttreatment with interferon alfa. *Hepatology* 24:6–9.
- Chu CM, Sheen IS, Yeh CT, Hsieh SY, Tsai SL, Liaw YF (1995): Serum levels of interferon-alpha and -gamma in acute and chronic hepatitis B virus infection. *Digestive Diseases and Sciences* 40: 2107–2112.
- Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ (1994): Classification of chronic hepatitis: Diagnosis, grading and staging. *Hepatology* 19:1513–1520.
- Dukas K, Sarfati P, Vaysse N, Pradayrol L (1993): Quantitation of changes in the expression of multiple genes by simultaneous polymerase chain reaction. *Annals of Biochemistry* 215:66–72.
- Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C (1996): Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *New England Journal of Medicine* 334:77–81.
- Foster GR, Ackrill AM, Goldin RD, Kerr IM, Thomas HC, Stark GR (1991): Expression of the terminal protein region of hepatitis B virus inhibits cellular responses to interferons alpha and gamma and double-stranded RNA. *Proceedings of the National Academy of Sciences U.S.A.* 88:2888–2892.
- Fukuda R, Ishimura N, Kushiyama Y, Moriyama N, Ishihara S, Nagasawa S, Miyake T, Niigaki M, Satoh S, Sakai S, Akagi S, Watanabe M, Fukumoto S (1997): Effectiveness of interferon-alpha therapy in chronic hepatitis C is associated with the amount of interferon-alpha receptor mRNA in the liver. *Journal of Hepatology* 26:455–461.
- Gey GO, Coffman WD, Kubicek MT (1952): Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. *Cancer Research* 12:264–265.
- Hannigan GE, Fish EN, Williams BR (1984): Modulation of human interferon-alpha receptor expression by human interferon-gamma. *Journal of Biological Chemistry* 259:8084–8086.
- Ishiyama N, Katayama K, Ishimi N, Takahashi S, Igarashi H, Nakajima H, Saito S, Aoyagi T, Andoh T, Oya A (1993): Quantitative detection of hepatitis C virus RNA by multicyclic RT-PCR. *International Hepatology Communications* 1:72–79.
- Jouet P, Roudot Thoraval F, Dhumeaux D, Metreau JM (1994): Comparative efficacy of interferon alfa in cirrhotic and noncirrhotic patients with non-A, non-B, C hepatitis. *Le Groupe Francais pour l'Etude du Traitement des Hepatites Chroniques NANB/C. Gastroenterology* 106:686–690.
- Kanai K, Kako M, Aikawa T, Kumada T, Kawasaki T, Hatahara T, Oka Y, Mizokami M, Sakai T, Iwata K, Okamoto H, Mayumi M (1995): Clearance of serum hepatitis C virus RNA after interferon therapy in relation to virus genotype. *Liver* 15:185–188.
- Kanazawa Y, Hayashi N, Mita E, Li T, Hagiwara H, Kasahara A, Fusamoto H, Kamada T (1994): Influence of viral quasispecies on effectiveness of interferon therapy in chronic hepatitis C patients. *Hepatology* 20:1121–1130.
- Kaneko S, Murakami S, Unoura M, Kobayashi K (1992): Quantitation of hepatitis C virus RNA by competitive polymerase chain reaction. *Journal of Medical Virology* 37:278–282.
- Lau AS, Hannigan GE, Freedman MH, Williams BR (1986): Regulation of interferon receptor expression in human blood lymphocytes in vitro and during interferon therapy. *Journal of Clinical Investigation* 77:1632–1638.
- Lau JY, Sheron N, Morris AG, Bomford AB, Alexander GJ, Williams R (1991): Interferon-alpha receptor expression and regulation in chronic hepatitis B virus infection. *Hepatology* 13:332–338.
- Lau JY, Davis GL, Kniffen J, Qian KP, Urdea MS, Chan CS, Mizokami M, Neuwald PD, Wilber JC (1993): Significance of serum hepatitis C virus RNA levels in chronic hepatitis C. *Lancet* 341:1501–1504.
- Li J, Tripathi BJ, Chalam KV, Tripathi RC (1996): Transforming growth factor-beta 1 and -beta 2 positively regulate TGF-beta 1 mRNA expression in trabecular cells. *Investigative Ophthalmology and Visual Science* 37:2778–2782.
- Nakabayashi H, Taketa K, Miyano K, Yamane T, Sato J (1982): Growth of human hepatoma cells lines with differentiated functions in chemically defined medium. *Cancer Research* 42:3858–3863.
- Nakajima S, Kuroki T, Shintani M, Kurai O, Takeda T, Nishiguchi S, Shiomi S, Seki S, Kobayashi K (1990): Changes in interferon receptors on peripheral blood mononuclear cells from patients with chronic hepatitis B being treated with interferon. *Hepatology* 12: 1261–1265.
- Novick D, Cohen B, Rubinstein M (1994): The human interferon alpha/beta receptor: Characterization and molecular cloning. *Cell* 77:391–400.
- Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y, Tanaka T, Sato K, Tsuda F, Miyakawa Y, Mayumi M (1992): Typing hepatitis C virus by polymerase chain reaction with type-specific primers: Application to clinical surveys and tracing infectious sources. *Journal of General Virology* 73:673–679.
- Poynard T, Bedossa P, Chevallier M, Mathurin P, Lemonnier C, Trepo C, Couzigou P, Payen JL, Sajus M, Costa JM, Vidaud M, Chaput JC, Multicenter study group (1995): A comparison of three interferon alfa-2b regimens for the long-term treatment of chronic non-A, non-B hepatitis. *New England Journal of Medicine* 332:1457–1462.
- Shouval D, Wands JR, Zurawski VR Jr, Isselbacher KJ, Shafritz DA (1982): Selecting binding and complement-mediated lysis of human hepatoma cells (PLC/PRF/5) in culture by monoclonal antibodies to hepatitis B surface antigen. *Proceedings of the National Academy of Sciences U.S.A.* 79:650–654.
- Spira J, Povey S, Wiener F, Klein G, Andersson Anvret M (1977): Chromosome banding, isoenzyme studies and determination of Epstein-Barr virus DNA content on human Burkitt lymphoma/mouse hybrids. *International Journal of Cancer* 20:849–853.
- Tanaka E, Kiyosawa K, Matsumoto A, Kashiwakuma T, Hasegawa A, Mori H, Yanagihara O, Ohta Y (1996): Serum levels of hepatitis C virus core protein in patients with chronic hepatitis C treated with interferon alfa. *Hepatology* 23:1330–1333.
- Tsubota A, Chayama K, Ikeda K, Yasuji A, Koida I, Saitoh S, Hashimoto M, Iwasaki S, Kobayashi M, Hiromitsu K (1994): Factors predictive of response to interferon-alpha therapy in hepatitis C virus infection. *Hepatology* 19:1088–1094.
- Uze G, Lutfalla G, Gresser I (1990): Genetic transfer of a functional human interferon alpha receptor into mouse cells: Cloning and expression of its cDNA. *Cell* 60:225–234.
- Uze G, Lutfalla G, Mogens KE (1995): Alpha and beta interferons and their receptor and their friends and relations. *Journal of Interferon Cytokine Research* 15:3–26.
- Whitten TM, Quets AT, Schloemer RH (1991): Identification of the hepatitis B virus factor that inhibits expression of the beta interferon gene. *Journal of Virology* 65:4699–4704.
- Yatsuhashi H, Yamasaki K, Aritomi T, Maria P, Carmen D, Inoue O, Koga M, Yano M (1997): Quantitative analysis of interferon  $\alpha/\beta$  receptor mRNA in the liver of patients with chronic hepatitis C: Correlation with serum hepatitis C virus-RNA levels and response to treatment with interferon. *Journal of Gastroenterological Hepatology* 12:460–467.
- Yoshioka K, Higashi Y, Yamada M, Aiyama T, Takayanagi M, Tanaka K, Okumura A, Iwata K, Kakumu S (1995): Predictive factors in the response to interferon therapy in chronic hepatitis C. *Liver* 15:57–62.
- Zoon KC, Arnheiter H (1984): Studies of the interferon receptors. *Pharmacological Therapy* 24:259–278.