Acta Medica et Biologica
Vol. 40, No. 2, 59-67, 1992

Titers of Antibodies Associated with Hepatitis C Virus during and after Interferon Therapy: Evaluation of anti-C100 and anti-core antibodies

Tadashi KOBAYASHI

Department of Internal Medicine III, Niigata University School of Medicine, Asahimachi 1, Niigata 951, Japan

Received March 17, 1992

Summary. **To clarify useful indicators during treatment with interferon-α (IFN-α) against chronic hepatitis C, 25 patients were surveilled. Several viral markers for the evaluation of the effects of IFN therapy were examined. The antibody to C100 (anti-C100), antibody to the protein encoded in the core region of HCV (anti-core), and hepatitis C virus RNA (HCV-RNA) by polymerase chain reaction (PCR) were assayed before and after IFN treatment in each patient.**

In 6 out of 25 patients, alanine aminotransferase (ALT) levels decreased to normal values within 6 months after IFN therapy and remained within the normal range thereafter. In 8 patients, ALT levels showed transient normal levels within one month after the therapy, but in the other 11 patients, ALT levels did not change during the course of IFN therapy.

In 6 patients with good responses and 8 patients with transient responses, histological findings in the liver biopsy specimens before treatment were more moderately changed than those in patients with no response to IFN. Titers of anti-C100 before treatment in patients with good responses to IFN were higher than those in patients with no response.

Changes in the titers of anti-C100 and anti-core and changes of HCV-RNA in each patient correlated well with the changes in serum aminotransferase levels. Therefore, HCV antibody titers and HCV-RNA are considered to be useful indicators in predicting the prognoses of patients who receive IFN therapy.

INTRODUCTION

Chronic hepatitis and liver cirrhosis are believed to be induced mainly by the non-A, non-B hepatitis virus.[1] Drugs such as acyclovir and corticosteroids have been administered to patients with non-A, non-B hepatitis without effect.[2,3] In 1986, Hoofnagle et al. reported that interferon-α (IFN-α) was effective in 8 out of 10 patients with chronic non-A, non-B hepatitis.[4] Since then, many studies have indicated the usefulness of IFN therapy.[5-11]

Recently, the genome of the etiological agent of non-A, non-B hepatitis was cloned, and named hepatitis C virus (HCV).[12] A serum antibody to C100 antigen expressed from a part of the non-structual region of the HCV genome was used for the diagnosis of hepatitis C, and an enzyme-linked immunosorbent assay (ELISA) kit for the measurement of this antibody (anti-C100) has been established.[13]

As anti-C100 does not necessarily reflect the amount of HCV directly, the measurement of HCV-RNA being required for the evaluation of IFN therapy. The polymerase chain reaction (PCR) is a very sensitive method for detecting very small amounts of DNA.[14] HCV-RNA has been detected by this method used in combination with reverse transcription (RT-PCR). HCV-RNA was quite frequently detected through this method in the sera of patients with non-A, non-B hepatitis who were positive for anti-C100.[15-18] It was also detected by using primers derived from the 5' non-coding region, the most conserved part of the HCV genome,[18] in patients who were negative for anti-C100. This assay has also been used for the evaluation of IFN therapy urder various conditions.[19,20]

To evaluate the effects of IFN therapy in patients with chronic hepatitis C, the anti-C100 and HCV-RNA as well as the antibody to the putative core protein of the HCV genome (anti-core) were measured.[21] The factors that influence the effectiveness of IFN therapy for hepatitis C patients were also discussed.

PATIENTS AND METHODS

Twenty-five patients (18 males and 7 females) with chronic hepatitis C were surveilled in this study, their

ages ranging from 28 to 68 years (mean 51.3). They were treated with IFN-α at the 3rd Department of Internal Medicine of Niigata University between May, 1987 to December, 1990 (Table 1). All cases showed elevated serum aminotransferase (alanine aminotransferase: ALT) levels for at least 6 months before the treatment. Twenty-two cases were diagnosed histologically by liver biopsies within 6 months before IFN therapy. Chronic active hepatitis was diagnosed when piecemeal necrosis and lymphocyte infiltration in the portal area were observed. Thirteen patients had histories of blood transfusions (these episodes had occurred between 9 months and 34 years before the treatment had commenced).

The IFNs were administered as follows (Table 1): natural IFN-α (lymphoblastoid IFN, provided by Otsuka Pharmaceutical Company Tokyo, Japan and Sumitomo Pharmaceutical Company Osaka, Japan), recombinant α-2a (provided by Takeda Pharmaceutical Company Osaka, Japan) IFNs were administered in 21, 2, and 2 cases, respectively. Schedules for IFN administration were different for each patient. Natural IFN-α was administered intramusculary at a dosage of 1-10 Mega units (MU) daily for 4-8 weeks in 8 cases. In 7 cases, 3 MU natural IFN-α was administered daily for 2 or 4 weeks, followed by three times weekly at the same dosage for 5-24 weeks. Only one case was given 10 MU natural IFN-α daily for 2 weeks, followed by three times weekly at the same dosage for 14 weeks. In 5 cases, 1-10 MU natural IFN-α was administered three times weekly for 12 weeks.

Recombinant IFN α-2a was given intramusculary at dosage of 3 or 9 MU three times weekly for 12 weeks in 2 cases. Recombinant IFN α-2b was administered at the dosage of 10 MU daily for 8 weeks in one case, and the same dosage of the IFN was given intramusculary daily for 2 weeks followed by three times weekly for 12 weeks in one case.

Total doses of administered natural IFN-α, recombinantα-2a, and α-2b IFNs were 204.6±170.8 MU, 216.0± 152.7 MU, and 520.0±56.6 MU, (mean±SD) respectively.

Follow-up periods after therapy ranged from 0 to 48 months.

To investigate the factors that affected the effectiveness of IFN therapy, we analyzed the clinical background of each patient (Table 2). The factors examined were age, sex, history of blood transfusions and serum ALT levels just before treatment, and histological findings of liver biopsy specimens obtained within 6 months before IFN treatment.

Anti-C100 was measured with an ELISA kit (Abbott Laboratories Tokyo, Japan). In most patients, the optical density (OD) value was over 2.0 before IFN treatment. To perform semiquantitative analysis of the antibody to C100, the sera were diluted with phosphate buffered saline (PBS) until the OD value ranged betweem 1.0 and 2.0. In the present study, this dilution value in each patient was regarded as the titer of C100 during the course of IFN treatment.

To determine and compare the titers of anti-C100 in the sera patients before treatment, the sera were diluted with PBS; the maximal dilution value in which anti-C100 showed positive was considered to be a titer of anti-C100.

Anti-core was measured with an ELISA kit using core protein expressed in Escherichia coli encoded in the core region of the HCV genome.[21] All sera were diluted 50-fold with PBS before being assayed.

Two oligonucleotide primers used for RT-PCR were synthesized on a DNA synthesizer (Applied Biosystems). The primers used were HCVC (5'-GTGCTCATGGTGCACGGTCTA-3') and HCVCR (5'-AGAGCCATAGTGGTCTGCGGA-3') which have the sequences derived from nucleotides 117 to 137 and 312 to 332 of the published sequence of HCV.[22] They correspond mainly to the 5'-non coding region of the HCV genome, which is considered to be highly conserved among HCV strains.[19,23]

RNA was prepared from serum by an SDS-proteinase K method. Briefly, 200 μl of serum was diluted with a buffer to a final concentration of 10 mM Tris (pH 8.0), 5 mM EDTA, and 0.5% sodium dodecyl sulfate (SDS) in a total volume of 500 μl. This was incubated for 30 min at 37°C after the addition of 10 μg of proteinase K. An RNA pellet was obtained after phenol-chloroform extraction and ethanol precipitation. It was dissolved in 10 μl of water, and then 5 μl of this solution was used for the RT-PCR assay.[17]

The PCR assay was carried out as follows: RNA was annealed with 1 ng of antisense primer (HCVCR) in a total volume of 10 μl. Reverse transcription was then performed with 200 units of Molony murine leukemia virus reverse transcriptase for 1 h at 37°C. The PCR was then carried out by a DNA thermal cycler (Perkin Elmer Cetus, USA) for 35 cycles (each cycle being 55°C for 45 sec, 72°C for 2 min, and 90°C for 1 min). After the reaction, the products were electrophoresed in 3% agarose gel and stained with ethidium bromide. The PCR products were further analyzed by Southern blot hybridization, and probed with a 32P-labeled cloned fragment of HCV. The filter was washed twice with 1× SSC/0.5% (W/V) SDS for 30 min at 55°C (1×SSC was 0.15 M NaCl/15 mM sodium citrate, pH 7.0), and exposed to Kodak X-AR film for 24 h at −80°C.

We performed tentative semiquantification of HCV-RNA as follows (Fig. 1). When a specific band was

Table 1. Patients and Their Laboratory Data before Treatment

Case	Age	Sex	BTF*a	Liver Histology	IFN	Dose*b (MU)	Methods of Administration	Total Dose (MU)	GPT (IU/L)	Anti C100	Anti Core	HCV RNA	Effect*c
1	54	F	−	CAH*d	Nat-α*f	1	Daily for 4W	28	130	+	+	++	3
2	52	M	−	CAH	Nat-α	3	Daily for 4W	84	194	+	+	++	1
3	30	M	−	−	Nat-α	3	Daily for 4W	84	372	+	+	−	3
4	38	M	−	CAH	Nat-α	3	Daily for 4W	84	84	−	+	−	3
5	56	F	26 Y	CAH	Nat-α	3	Daily for 4W	84	157	−	+	++	2
6	61	F	−	CAH	Nat-α	9	Daily for 4W	252	218	−	+	++	3
7	48	M	−	CPH*e	Nat-α	10	Daily for 8W	560	175	+	+	++	2
8	57	M	27 Y	CPH	Nat-α	10	Daily for 8W	560	58	+	+	++	1
9	28	M	−	CPH	Nat-α	3	Daily for 2W→3 times/w for 5W	87	372	+	+	++	2
10	63	M	4 Y	CAH	Nat-α	3	Daily for 4W→3 times/w for 7W	147	98	+	+	++	2
11	68	M	23 Y	CAH	Nat-α	3	Daily for 4W→3 times/w for 7W	147	105	+	+	++	3
12	55	F	3 Y	CAH	Nat-α	3	Daily for 4W→3 times/w for 8W	156	317	+	+	++	2
13	53	F	34 Y	CAH	Nat-α	3	Daily for 4W→3 times/w for 8W	156	181	+	+	++	3
14	35	M	25 Y	CAH	Nat-α	3	Daily for 2W→3 times/w for 24W	258	400	+	+	++	1
15	30	M	−	CPH	Nat-α	3	Daily for 2W→3 times/w for 24W	258	153	+	+	++	2
16	34	M	−	CPH	Nat-α	10	Daily for 2 W→3 times/w for 14W	560	504	+	+	++	1
17	60	M	−	CAH	Nat-α	1	3 times/w for 12W	36	261	+	+	++	3
18	66	F	26 Y	CAH	Nat-α	1	3 times/w for 12W	36	234	-	+	++	3
19	66	M	−	−	Nat-α	5	3 times/w for 12W	180	162	-	+	++	3
20	46	F	2 Y	−	Nat-α	5	3 times/w for 12W	180	204	+	+	++	2
21	54	M	34 Y	CPH	Nat-α	10	3 times/w for 12W	360	307	+	+	++	1
22	64	M	27 Y	CAH	α-2a*g	3	3 times/w for 12W	108	206	+	+	++	2
23	64	M	−	CAH	α-2a	9	3 times/w for 12W	324	139	+	+	++	3
24	59	M	9 M	CAH	α-2b*h	10	Daily for 8W	560	126	+	+	++	3
25	39	M	2 Y	CPH	α-2b	10	Daily for 2W→3 times/w for 12W	480	290	+	+	++	1

*a: history of blood transfusions and tive since blood trasfusion. *b: administered IFN doses per day. *c: effect of IFN therapy. *d: chronic active hepatitis. *e: chronic persistent hepatitis. *f: natural-α interferon. *g: recombinant α-2a interferon. *h: recombinant α-2b interferon.

Table 2. Characteristics of the Three Groups of Patients before IFN Treatment

Group	1 (n=6)	2 (n=8)	3 (n=11)	Statistical analysis
Age	45.2±10.3	48.8±13.7	56.3±12.1	N. S
Sex (M : F)	6 : 0	5 : 3	7 : 4	N. S
BTF*[a]	4	5	4	N. S
ALT (IU/L)	292.2±155.7	210.3±90.7	182.9±83.6	N. S
Histology*[b] (CAH : CPH)	2 : 4	4 : 3	9 : 0	*1, *2 $p<0.05$
Anti-C100*[c]	7.83±2.04 (6/6)	8.14±2.67 (7/8)	5.57±1.62 (7/11)	*3 $p<0.05$
Anti-Core(×50 OD)*[d]	1.018±0.227 (6/6)	0.798±0.354 (8/8)	0.835±0.278 (11/11)	N.S
HCV-RNA (++ or +:−)	6 : 0	8 : 0	9 : 2	N.S
Total dose*[e] (MU)	364.4±204.0 (n=5)	210.3±165.1 (n=7)	111.4±77.1 (n=9)	*4 $p<0.05$

*a: History of blood transfusions.
*b: Liver biopsies were not performed in one patient in Group 1 and in 2 patients in Group 3. CAH: chronic active hepatitis. CPH: chronic persistent hepatitis.
*c: Titer of anti-C 100. One patient in Group 2 and 4 in Group 3 were negative for anti-C100.
*d: Mean OD value of anti-core after sera were diluted 50-fold.
*e: Patients who received recombinant IFN-α were excluded. (one in Group 1, one in Group 2 and two in Group 3)

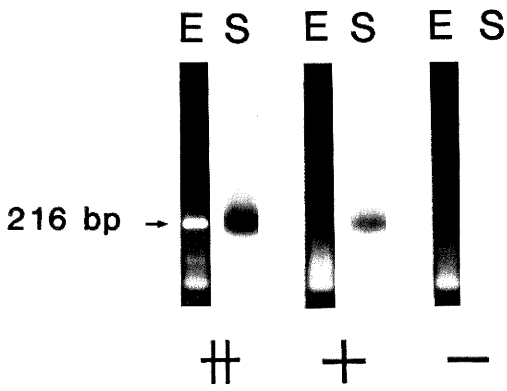

Fig. 1. Grading of the amount of HCV-RNA in the serum. When a specific band was visible in agarose gel by ethidium bromide staining, it was graded as ++, and when the signal was detected only by Southern blot analysis, it was graded as +. When both system failed to detect the specific HCV sequence, HCV-RNA was judged to be negative (−). E: ethidium bromide staining. S: Southern blot analysis.

visible in the agarose gel by ethidium bromide staining, we graded the amount of HCV-RNA as ++, and when the signal was detected only by Southern blot analysis, the amount was graded as +. When both systems failed to detect the specific HCV sequence, HCV-RNA was judged to be negative (−).

RESULTS

Patients were divided into 3 groups according to changes in ALT levels after treatment. Group 1: ALT was normalized within 6 months after the end of IFN therapy and remained within normal limits thereafter. Group 2: ALT was normalized transiently during IFN therapy or within 1 month after therapy. Group 3: ALT did not change during the course of IFN therapy. The effects of therapy were evaluated according to this classification: six patients were placed in Group 1, 8 in Group 2, and 11 in Group 3.

The results of IFN therapy in our series of 25 patients with chronic hepatitis C, together with the factors which

may influence the effectiveness of IFN therapy, are summarized in Table 1.

The clinical, biochemical, histological, and virological characteristics of the three groups before IFN treatment are shown in Table 2. Statistical analysis showed that there were no significant differences among the three groups with regard to sex differences, history of blood transfusion, or serum ALT levels. With regard to histological findings, however, the numgber of patients with chronic active hepatitis against chronic persistent hepatitis in Group 3 was significantly more than that in Groups 1 and 2. As to viral markers, all patients in Group 1, 7 of 8 (88%) in Group 2, and 7 of 11 (64%) in Group 3 were positive for anti-C100. The titers of anti-C100 in each group (mean±SD) were 7.83±2.04, 8.14±2.67, and 5.57±1.62, respectively. A significant difference in the titer of anti-C100 was observed between Groups 1 and 3 ($p<0.05$). Although all 25 patients were positive for anti-core before treatment, no differences in the titer of anticore were observed among 3 groups. Twenty-three were positive for HCV-RNA before treatment. The two patients who were negative for HCV-RNA (cases 3 and 4) belonged to Group 3 (Table 1).

The average of total IFN doses given was more in Group 1 than in Group 3.

The changes of viral markers in each patient before and after IFN treatment were as follows. Although anti-C100 did not become seronegative in any of the 20 patients during the observation periods, changes in the titer of anti-C100 appeared to be different in each group (Fig. 2-a, b, c). In Group 1, anti-C100 tended to decrease at the end of treatment and continued to be in lower titers during the follow-up period. In most patients in Group 2, the titer of anti-C100 decreased just after treatment but increased soon after cessation of the therapy. In most Group 3 patients, the titer of anti-C100 was not changed by IFN therapy.

Anti-core remained positive in all 25 patients during and after IFN treatment. Changes in the titer of anti-core in each group were similar to the changes in anti-C100 (Fig. 3-a, b, c).

Changes in the amount of HCV-RNA are shown in Fig. 4-a, b, c. In Groups 1 and 2, HCV-RNA decreased immediately after treatment, but increased within 3 months in 3 out of 6 patients in Group 1 and in 5 of 6

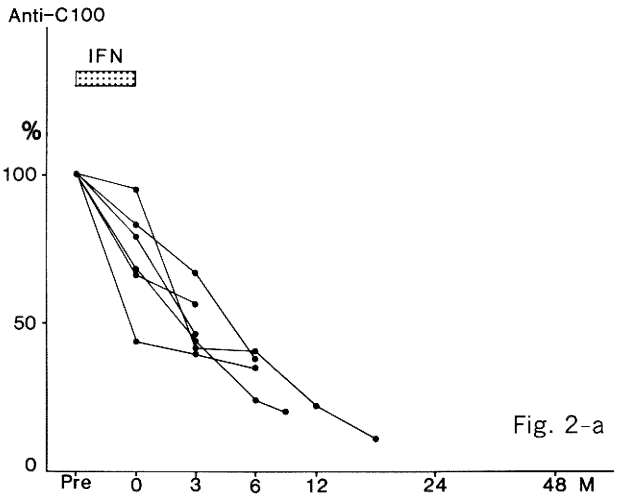

Fig. 2-a

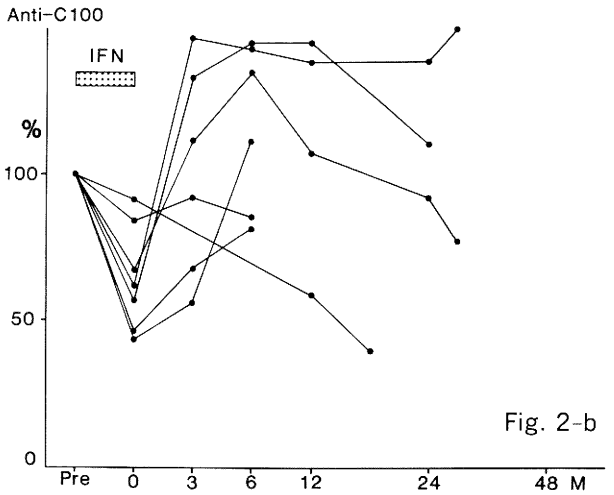

Fig. 2-b

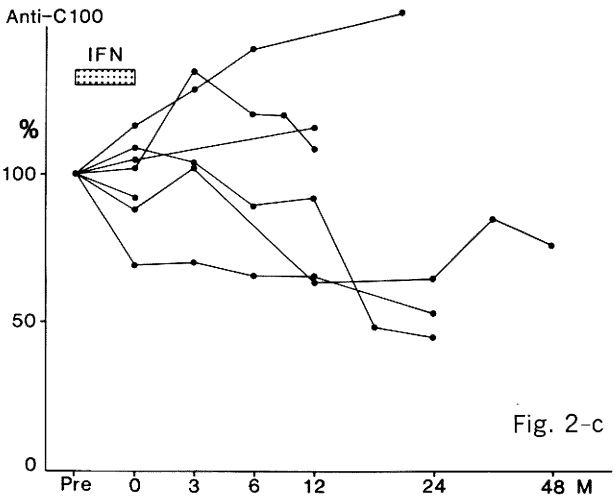

Fig. 2-c

Fig. 2. Changes in anti-C100 titers after diluting with PBS. (a) In Group 1, anti-C100 tended to decrease at the end of treatment, and continued to decrease during and the follow-up period. (b) In most patients in Group 2, the titer of anti-C100 decreased just after treatment but increased soon after cessation of therapy. (c) In most patients in Group 3, the titer of anti-C100 did not change.

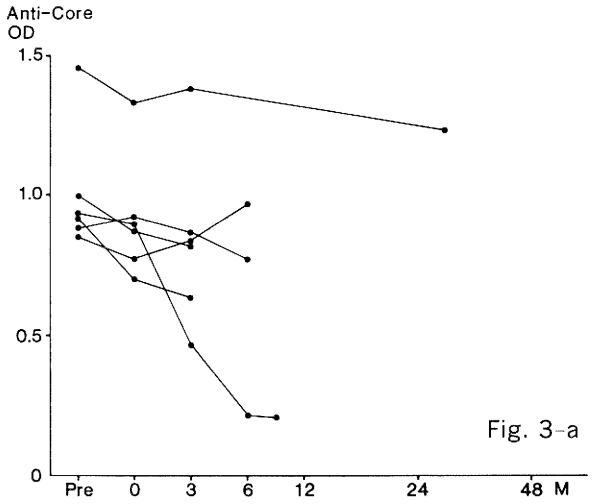

Fig. 3-a

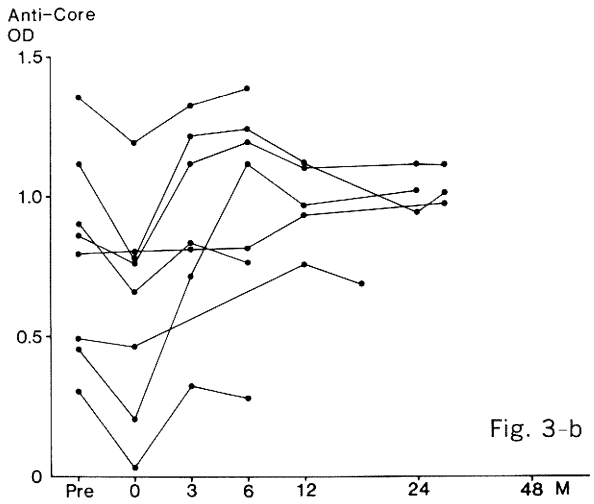

Fig. 3-b

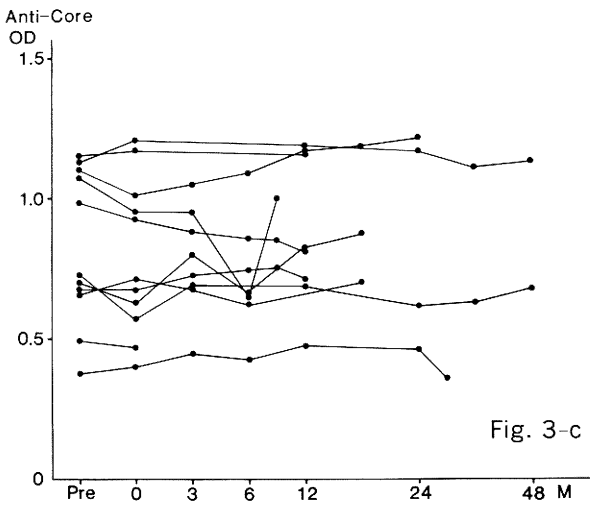

Fig. 3-c

patients in Group 2. In Group 3, 4 of 9 patients positive for HCV-RNA before treatment showed no change in HCV-RNA. In 2 patients who were negative for HCV-RNA before treatment, HCV-RNA continued to be negative both during and after treatment.

DISCUSSION

Interferon therapy for chronic hepatitis C is reported to be beneficial in controlling the disease, even though its effects are often transinet and relapse occurs after the cessation of treatment.[4,9,10] Studies have clarified the changes in serum aminotransferase levels and histological findings as indicators of the efficacy of IFN therapy, whereas changes in HCV markers such as anti-C100, anti-core, and HCV-RNA have not been tested. The reduction of HCV replication levels may be also an important factor in the evaluation of IFN therapy for chronic hepatitis C, and in the present study, we investigated the significance of HCV markers for this evaluation.

In this study, the factors affecting the IFN therapy were differences in histological findings, titers of anti-C100, and the total dosage of IFN administered. IFN therapy was more effective for patients with moderate histological changes in the liver. Schvarsz also reported that patients with moderate histological changes in liver biopsy specimens showed a good response to IFN-α.[8] In chronic hepatitis B, ALT levels before IFN treatment were higher in responders than in non-responders, but histological findings of the liver before treatment did not significantly differ in each group.[24]

In this study, patients receiving high total doses of IFN tended to have a better prognosis than patients who received low doses of IFN. This was compatible with the results of other studies.[9,26]

We performed serial titration of serum and quantitated titers of anti-C100 antibody. The anti-C100 titer was lower in Group 3 for whom IFN therapy was not effective-than in Group 1, for whom the therapy was judged effective. Moreover, four of the 11 patients in Group 3 were negative for anti-C100.

These results were different from those in previous studies, which showed that the response of ALT to IFN administration did not differ between anti-C100-postitive and anti-C100-negative patients.[20,25,26] The reason why IFN therapy was not so effective in patients with negative anti-C100 has not yet been determined, but we

Fig. 3. Changes in the anti-core titers after diluting 50-fold with PBS were similar to those in the anti-C100 titers. (a) Group 1, (b) Group 2, and (c) Group 3.

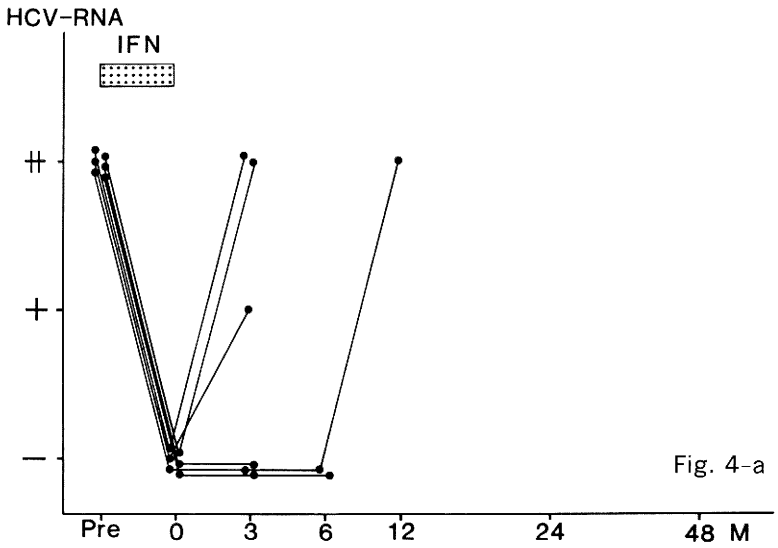

Fig. 4-a

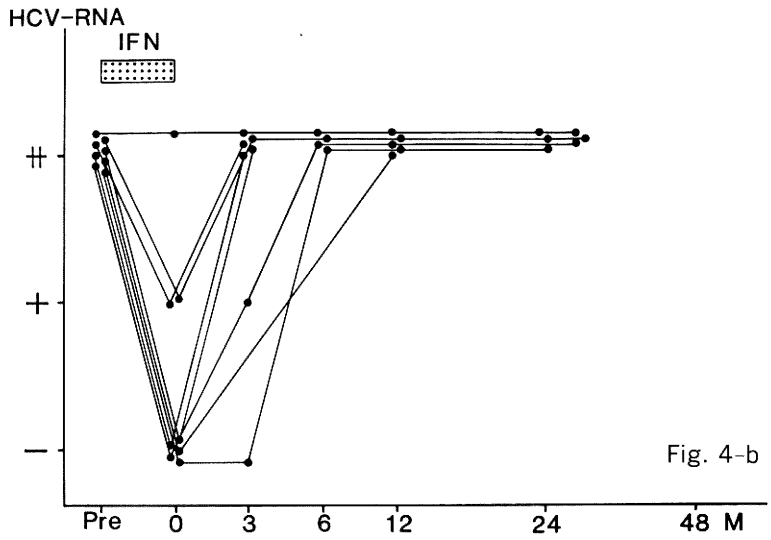

Fig. 4-b

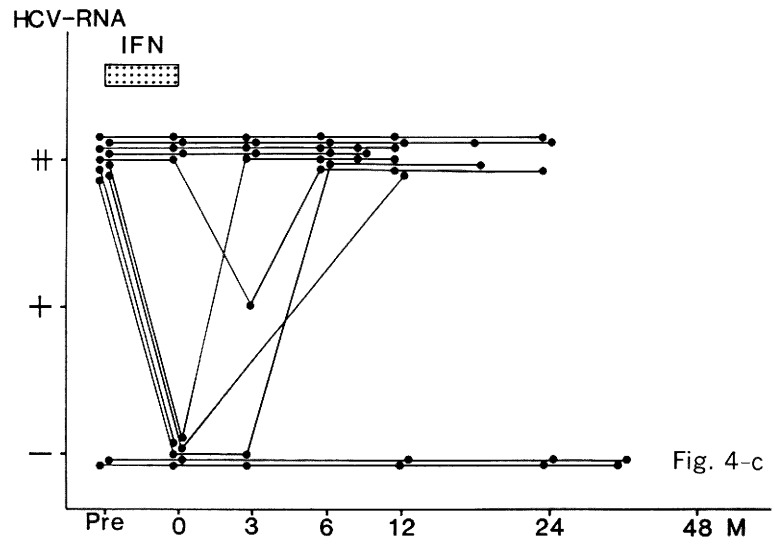

Fig. 4-c

Fig. 4. Changes in HCV-RNA. In Groups 1 (a) and 2 (b), HCV-RNA decreased immediately after treatment, but increased within 3 months in 3 out of 6 patients in Group 1 and in 5 out of 6 patients in Group 2. (c) In Group 3, 4 out of 9 patients positive for HCV-RNA before treatment showed no change in HCV-RNA. In 2 patients who were negative for HCV-RNA before treatment, HCV-RNA continued to be negative both during and after treatment.

may be able to predict the effects of IFN therapy for patients with chronic hepatitis C based on their anti-C100 titers before treatment.

Changes in the titer of anti-C100 during and after IFN therapy seemed to correlate well with liver function tests and HCV-RNA. Changes in the anti-core titer in diluted serum were similar to the changes in the titer of anti-C100. These results suggest that changes in anti-C100 and anti-core titers are related to changes in hepatitis C virus replication in chronic hepatitis C, and that these markers are useful for the evaluation of IFN therapy.

In many patients, the HCV genome was decreased or depleted in the serum when responding to IFN. Since the response of HCV-RNA to IFN administration was immediate, HCV was suggested to be very sensitive to IFN, as has been described previously.[19,20] In many patients, however, the reappearance of HCV-RNA was observed, followed by an elevation of serum aminotransferase. This finding suggests that HCV continues to exist in the hepatocytes and that IFN is not able to eliminate HCV completely. It remains to be seen whether any regimen, and if so which regimen, of IFN administration can obtain complete efficacy.

Acknowledgments. We wish to thank Prof. Hitoshi Asakura, Assist. Prof. Tomoteru Kamimura, and Dr. Showgo Ohkoshi (Department of Internal Medicine III, Niigata University School of Medicine) for their advice during this research. Also thanks go to Dr. Kunitada Shimotono (Virology Division, National Cancer Center Research Institute) for providing the ELISA kit of anti-core antibody, and special thanks to Naomi Chou for her assistance in the experiment.

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