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Ribavirin Enhances Interferon-γ Levels in Patients with Chronic Hepatitis C Treated with Interferon-α

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Key Words
Ribavirin · Hepatitis C · Combination therapy · Interferon-γ

Abstract
Some patients with chronic hepatitis C respond to interferon (IFN)-α treatment, and the efficiency can be improved by combining it with ribavirin. The mechanism of this improvement is unknown. To investigate the effects of these two regimens on the immune responses in 51 patients with chronic hepatitis C, we examined the hepatitis C core antigen-specific proliferative response and cytokine production profiles, natural killer (NK) cell cytotoxicity and cytotoxic T cell function during treatment. The results are as follows: (1) both viral clearance and biochemical normalization occurred more frequently in patients receiving combination therapy; (2) the function of NK cells increased after treatment in the responders of both groups ($p < 0.05$); (3) the level of IFN-γ produced by hepatitis C core antigen-stimulated peripheral blood mononuclear cells was higher in patients receiving combination therapy, especially in responders; (4) the core antigen-specific proliferative response decreased after treatment, and (5) in addition, the core-specific cytotoxic T cell activities of five responder patients also increased significantly after therapy. In conclusion, enhancement of immune responses, especially those related to type-1 T helper cell activity, may contribute to better efficacy in combining ribavirin with IFN-α for treatment of chronic hepatitis C.

Introduction

Hepatitis C virus (HCV) is the major agent causing non-A, non-B hepatitis worldwide [1], and is responsible for 60–90% of posttransfusion hepatitis cases [2, 5, 20, 26]. The infection is characterized by an unusually high frequency of persistent infection after the acute stage in adults, and about 20% of these patients develop cirrhosis and eventually hepatocellular carcinoma several decades later [20]. Therefore, effective treatment is desperately needed. Interferon (IFN)-α has been shown to be effective [13]. Unfortunately, less than 20–25% of treated patients can achieve a sustained response after the recommended regimen of 3×10^6 units (3 MU) 3 times weekly for 24 weeks [4, 23]. More than half of the responders relapse after IFN-α is discontinued [27]. To overcome this, a con-

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sensus of extending the treatment to 12 months has been reached, but the sustained response after discontinuation of the treatment is still far from satisfactory. Thus, combination with other drugs is actively being investigated. Ribavirin is a guanosine analogue with broad-spectrum antiviral activities. In patients with chronic hepatitis C, ribavirin monotherapy has been shown to induce a gradual decrease in serum alanine aminotransferase (ALT) activities [21], but there are no sustained biochemical or virological responses [7, 22]. Nevertheless, combining ribavirin with IFN-α has been shown to be more effective than IFN alone for the treatment of chronic hepatitis C [3, 14]. After long-term follow-up, we were able to show that this combination therapy can cure nearly half of patients with chronic hepatitis C [14].

Despite this progress, the mechanisms of action of this combination treatment for chronic hepatitis C remain unclear, although it has been suggested that IFN-α may enhance the antiviral response by increasing the number of cytotoxic CD8+ T cells, and that ribavirin may modify the cytokine production profiles of T cells both in vivo and in vitro [18, 25]. We therefore investigated the immunological changes in patients with chronic hepatitis C receiving IFN-α monotherapy or combination therapy with ribavirin.

Patients and Methods

Patients and Treatment Schedule

Fifty-one patients (29 men, 22 women) with a mean age of 49 years (range 26–65 years) were studied. They all had chronic HCV infection documented by positive viremia and elevated serum ALT levels for at least 6 months, and were randomized into two treatment groups: groups A and B.

Group A received IFN-α alone and was composed of 15 men and 10 women with a mean age of 46 years. The HCV genotypes of group A were genotype 1b (13 patients), genotype 2a (7 patients) and genotype 2b (3 patients). Two of these 25 patients were infected with more than one strain of HCV: 1 patient had genotypes 1b and 2a and another patient had genotypes 1a, 1b and 2b. Group B was composed of 14 men and 12 women with a mean age of 49 years who received a combination of IFN-α and ribavirin. The HCV genotypes of group B were genotype 1b (15 patients), genotype 2a (7 patients) and genotype 2b (4 patients). In all patients, recombinant IFN-α2a (Roferon-A®, provided by Hoffmann-La Roche, Basel, Switzerland) was given subcutaneously at a dosage of 6 MU thrice weekly from the 1st to the 12th week and then 3 MU from the 13th to the 24th week. In group B patients, a daily dose of 1,200 mg of oral ribavirin (Virazole®, ICN Pharmaceuticals, Costa Mesa, Calif., USA) was added for the entire treatment course of 24 weeks. Blood samples were collected regularly to monitor the immune responses at week 0 (pretreatment), week 12 (during treatment) and week 26 (2 weeks after treatment).

Meanwhile, some of the patients with HLA-A2 (+) were examined for cytotoxic T cell activity during treatment. Among them, patient 1 was a responder of group A, while patients 2 and 3 were responders of group B. Another 2 patients (patients 4 and 5) completed the follow-up monitoring of cytotoxic T cell activity during the entire treatment course. Patient 4 was a nonresponder of group A who was then retreated with 5 MU of IFN-α combined with ribavirin for 24 weeks. Patient 5 was a patient from group A with relapsed disease, who was retreated with 5 MU of IFN-α combined with ribavirin for 24 weeks and 3 MU of IFN for another 24 weeks. Patients 6 and 7 were nonresponders of group A. For patients 4 and 5, blood samples were collected regularly to monitor the cytotoxic T cell responses at weeks 0, 12, 24, 36, 48 and 72. The other patients were examined at weeks 0 and 24. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque (Pharmacia) density gradient centrifugation. Cells were washed twice with Hanks' balanced salt solution and resuspended in RPMI-1640 complete medium with 2% human AB serum prior to immunological studies.

Detection of Serum HCV RNA

Serum HCV RNA was detected by using a reverse transcription polymerase chain assay as described before [12]. Briefly, RNA was extracted from 100 μl of serum by the single-step acid guanidinium thiocyanate-phenol-chloroform method and converted into cDNA with a random hexamer and Moloney murine leukemia virus reverse transcriptase in a volume of 20 μl. The cDNA was amplified with *Taq* DNA polymerase and type-specific primers.

Phenotypic Analysis

Surface staining was performed by incubating 1×10^6 PBMCs with phycoerythrin (PE)-conjugated anti-CD8 and fluorescein isothiocyanate (FITC)-conjugated anti-CD3, PE-conjugated anti-CD4 and FITC-conjugated anti-CD45RA, PE-conjugated anti-CD14 and FITC-conjugated anti-CD45, PE-conjugated anti-CD19 and FITC-conjugated anti-CD3, or PE-conjugated anti-CD16 plus CD56 and FITC-conjugated anti-CD3 (PharMingen, San Diego, Calif., USA). All antibody incubations were performed at 4°C for 30 min. Cells were washed and resuspended in 0.5 ml of PBS and 0.1% sodium azide and subjected to FACscan analysis. A total of 5,000 cells were counted, and the frequency of each cell surface marker was determined on a FACSCalibur® using Cell Quest software (Becton Dickinson, Mountain View, Calif., USA). Controls were cells suspended in medium only. Flow cytometry was regularly calibrated with CaliBRITE beads (Becton Dickinson).

Serum Levels of Soluble CD4 and CD8 Molecules

Levels of soluble CD4 (sCD4) and CD8 (sCD8) were determined with a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Quantikine, R&D, Minneapolis, Minn., USA). The minimum detectable level of sCD4 was typically less than 60 pg/ml and that of sCD8 was lower than 0.1 ng/ml.

Expression and Purification of HCV Core Protein

To clone the core gene, primers C190-N (5′-AAGAATTCATGAGCACGAATCCTAA-3′) and C190-C (5′-TTACTCGAGATAAGCGGAAGCTGG-3′) were used to perform polymerase chain reaction. The resulting DNA fragment was digested with *Eco*R I and *Xho* I and cloned in a pET-21a vector. The open reading frame of the core gene was followed by six histidine codons. The recombinant plasmid was then transformed into BL 21 cells.

Expression of core protein was induced by the addition of 1 m*M* IPTG when the BL 21 cells had grown to $OD_{600} = 0.6$. After a 2-hour

induction, cells were harvested, resuspended in binding buffer (5 m*M* imidazole, 0.5 *M* NaCl, 20 m*M* Tris, pH 7.9) and sonicated. After centrifugation, an equal volume of 6 *M* guanidine-HCl was added to the supernatant. The soluble fraction of the cell lysate was then used to purify the core protein using a denaturing nickel column. All reagents used in the course of purification contained 3 *M* guanidine-HCl. The core protein was eluted by 300 m*M* imidazole and then dialyzed with TNE buffer (10 m*M* Tris, pH 7.5, 100 m*M* NaCl, 1 m*M* EDTA).

To perform Western blot, total lysate protein or the purified core protein was run by sodium dodecyl sulfate-12.5% polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane and then visualized by mouse anti-core antibody and by an ECL (enzymatic chemiluminescence) detection method (NEN™ Life Science Products, Mass., USA).

HCV Core-Specific Proliferative Response

To further study the HCV core-specific proliferative response in patients, 1×10^4 PBMCs were incubated with or without 1 μg/ml HCV core protein (C190) in 96-well round-bottomed microtiter plates (200 μl/well). After 6 days, cultures were pulsed with 1 μCi of [^{3}H]-thymidine and harvested 18 h later. The result of the core-specific proliferative response was expressed as a stimulation index. A value above 3 was defined as positive.

Natural Killer Cell Cytotoxicity Assay

PBMCs were incubated with 1×10^4 ^{51}Cr-labeled K562 cells in 96-well round-bottomed microtiter plates at an effector/target ratio of 10, 50 and 100, respectively. Cytotoxic activity was evaluated with the standard 4-hour ^{51}Cr release assay. Specific lysis was calculated as follows:

$$^{51}\text{Cr released (\%)} = [(E - S)/(T - S)] \times 100,$$

where E is the amount of chromium released in the presence of effector cells, S is the spontaneous release of the label in media alone and T is the total amount of chromium released in the presence of 50% HCl.

IFN-γ Assay

To determine the concentration of IFN-γ, 1×10^4 PBMCs were incubated with or without 1 mg/ml C190 antigen in 24-well flat-bottomed plates for 3 days, and the culture supernatant was collected and analyzed using ELISA. The lower limit of this assay was 40 pg/ml and the upper limit was 25,000 pg/ml. Standards were prepared from recombinant human IFN-γ (PharMingen).

Preparation of Core-Expressed Target Cells

HLA typing of the HepG2 cell line or PBMCs from HCV patients by complement-dependent microcytotoxicity was performed with Terasaki HLA typing trays (One Lambda, Canoga Park, Calif., USA) according to the manufacturer's instructions. The HLA type of HepG2 cells consists of A2, A24, B13, B52 and Cw6. Patients 1–7 were HLA-A2 (+). The cDNA fragment coding for the HCV core gene was inserted at the cloning sites of the S2 vector [11], denoted as C190/S2. The viral medium from C190/S2 DNA-transfected GP+env Am12 packaging cells [15] was used to infect HepG2 cells, and then they were selected with 1.2 μg/ml G418. Resistant colonies were further checked before expression of the core protein (data not shown) and used as the HLA-A2 (+) allogeneic target cells.

Table 1. Serum ALT levels and hepatitis C viremia before and after treatment

	Group A (n = 25)	Group B (n = 26)	p value
Serum ALT, IU/ml			
week 0	125 ± 16	148 ± 22	
week 24	74 ± 5*	24 ± 4*	
Cleared HCV RNA[1], n	8 (32)	17 (66)	<0.05

Group A received IFN-α alone. Group B received a combination of IFN-α and ribavirin. Figures in parentheses represent percentages. * $p < 0.05$ compared to the value at week 0.

[1] HCV RNA detected by a nested polymerase chain reaction assay with a sensitivity of 100 copies/ml.

Table 2. The cytotoxic activity of NK cells at different effector/target (E/T) ratios before and after treatment

E/T ratio	Group	Treatment response	Cytotoxicity, %	
			week 0	week 26
10	A	R	0.3 ± 0.3	4.7 ± 1.2*
		NR	1.8 ± 0.5	5.3 ± 1.0*
	B	R	0.7 ± 0.3	3.6 ± 1.0*
		NR	1.8 ± 0.8	5.0 ± 1.3*
50	A	R	2.1 ± 1.1	15.1 ± 2.8*
		NR	10.2 ± 2.0	16.2 ± 2.7
	B	R	4.4 ± 1.1	11.5 ± 2.5*
		NR	10.7 ± 4.5	14.9 ± 5.7
100	A	R	4.4 ± 1.8	23.1 ± 4.3*
		NR	16.8 ± 2.7	23.0 ± 3.4
	B	R	8.7 ± 1.9	17.0 ± 3.1*
		NR	16.8 ± 6.4	24.2 ± 7.5

Data are represented as the mean ± SEM. R = Responders; NR = nonresponders. * $p < 0.05$ compared to the value at week 0.

Core-Specific Cytotoxic T Cell Activity Assay

In brief, PBMCs were isolated as described above and stimulated with irradiated C190/HepG2 cells. On day 7, cultures were restimulated with irradiated C190/HepG2 cells. The PBMCs were fed with fresh RPMI-1640 medium containing IL-2 on days 3, 5, 10 and 12 and then tested for core-specific cytolytic activity on day 14. In vitro stimulated effector cells from PBMCs were incubated with 1×10^4 ^{51}Cr-labeled S2/HepG2 or C190/HepG2 cells in 96-well round-bottomed microtiter plates at effector/target ratios of 10, 20 and 40. The cytotoxic activity was evaluated with the standard 4-hour ^{51}Cr release assay as described above.

Statistical Analysis

Data are presented as the mean ± SEM. Statistical significance ($p < 0.05$) was analyzed by paired Student's t test.

Results

Virological and Biochemical Responses of Patients

Results of viremia and serum ALT levels of patients before and after treatment are summarized in table 1. Both viral clearance and ALT responses were better in patients receiving IFN-α plus ribavirin compared to those receiving IFN-α only ($p < 0.05$). The HCV genotypes of the responders among those who received IFN-α treatment only (group A) were genotype 1b (1 patient), genotype 2a (4 patients), genotype 2b (2 patients), genotypes 1b and 2a (1 patient) and genotypes 1a, 1b and 2b (1 patient). On the other hand, the HCV genotypes of the responders among those who received IFN-α plus ribavirin treatment (group B) were genotype 1b (4 patients), genotype 2a (7 patients) and genotype 2b (4 patients). This suggests that genotype 1b may be more sensitive to combination therapy ($p < 0.05$).

Phenotypic Analysis of PBMCs and Serum Levels of sCD4 and sCD8 Molecules

To analyze the effects of the two different treatments on the percentage of PBMCs, appropriate PE- or FITC-conjugated antibodies were used against cell surface markers for staining. In both group A and group B patients, the percentages of CD8+ T cell, memory CD4+ T cell, total T cell and B cell populations in PBMCs showed no significant changes before, during or after treatment (data not shown). In addition, there was no difference in the percentage of natural killer (NK) cells between responders and nonresponders of these two groups. However, the activation status of effector cells tended to decline after treatment, since both sCD4 and sCD8 molecules decreased significantly after treatment (fig. 1). There was no difference in this respect between the two treatment groups, nor between responders and nonresponders.

Cytolytic Activity of NK Cells

Furthermore, in these two groups, the cytotoxicity of NK cells increased dramatically after 24 weeks of treatment (table 2). The data suggest that the function of NK cells increases after treatment with IFN-α or IFN-α plus ribavirin ($p < 0.05$). The extent of the increase in cytolytic activity of NK cells in group A and B patients appeared to be similar. Furthermore, it was interesting to discover that patients with low NK cell cytolytic activity responded better to either IFN-α or IFN-α plus ribavirin treatment.

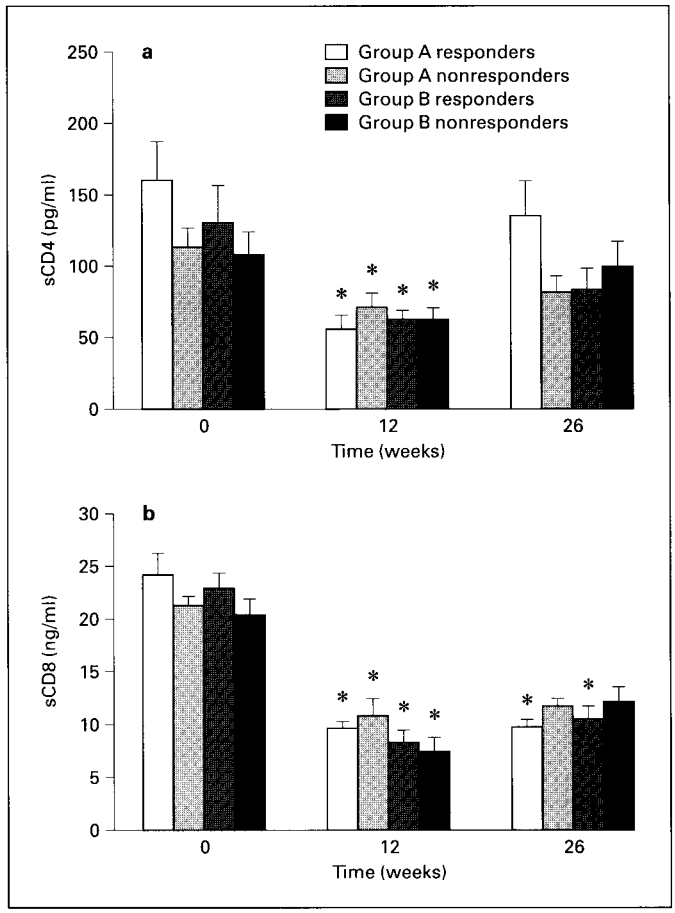

Fig. 1. Serum levels of sCD4 (**a**) and sCD8 (**b**) in both groups of patients before (week 0), during (week 12) and 2 weeks after (week 26) treatment. Group A patients received IFN-α only; group B patients received IFN-α combined with ribavirin. sCD4 and sCD8 levels decreased significantly after treatment in both groups (* $p < 0.05$ compared to the value at time 0 in the same group). Data are presented as the mean ± SEM of three experiments.

HCV Core Protein-Specific Proliferative Response

To further understand the effects of antiviral therapy on HCV core antigen-specific immune responses, we purified the HCV core protein (fig. 2) and performed a core protein-specific proliferation assay. The positive rates (stimulation index value over 3) of HCV core-specific proliferation response in the 0, 12th and 26th weeks were 8/25, 5/25 and 6/25, respectively, in group A and 11/26, 1/26 and 5/26, respectively, in group B. Group B showed a reduced response in the 12th and 26th weeks compared to that of week 0 ($p < 0.05$); however, there was no significant difference between the responders and nonresponders in each group (fig. 3).

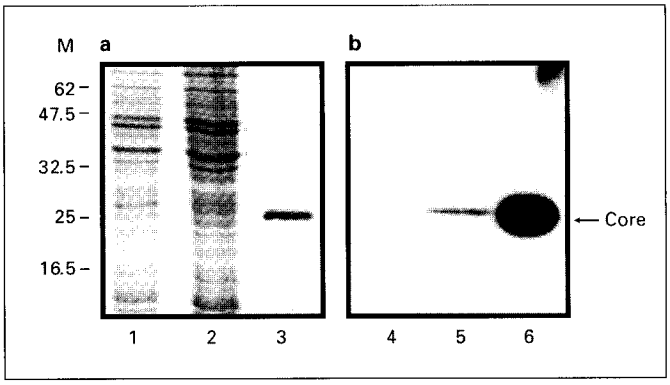

Fig. 2. Sodium dodecyl sulfate-12.5% polyacrylamide gel electrophoresis (**a**) and immunoblotting analysis (**b**) of HCV core protein. Total protein at 10 μg from BL 21 cells containing the core-expressing plasmid was analyzed before induction (lanes 1 and 4) and after induction (lanes 2 and 5). Lanes 3 and 6 contain 1 μg of purified protein. M = Molecular mass markers (in kilodaltons).

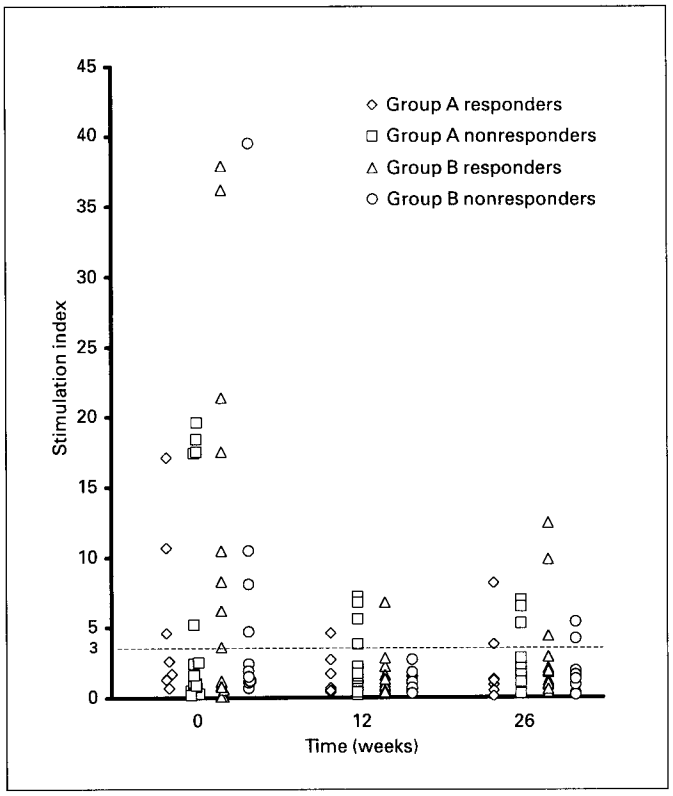

Fig. 3. Hepatitis C viral core protein-specific proliferative response in patients treated with IFN-α only (group A) or IFN-α combined with ribavirin (group B). Results of the core-specific proliferative response are expressed as a stimulation index. A value above 3 (indicated by a dashed line) is defined as positive. The results are representative of three experiments.

IFN-γ Produced by Core-Stimulated PBMCs

Spontaneous secretion of IFN-γ increased after treatment in both groups of patients (fig. 4a). Additionally, we also investigated IFN-γ produced by PBMCs after incubation with the HCV core protein during the course of treatment. The data show that core-induced IFN-γ production also increased after either monotherapy or combination therapy, but the increase was more dramatic in responders who received combination therapy (fig. 4b). Responders and nonresponders of the monotherapy group showed significantly different IFN-γ production in the 12th week, i.e. 18.7 ± 19.4 pg/ml and an undetectable level ($p < 0.01$), respectively. On the other hand, responders and nonresponders of the combination therapy group also showed markedly different levels of IFN-γ, i.e. 258.3 ± 53.9 and 112.2 ± 31.2 pg/ml ($p < 0.01$), respectively, in the 26th week.

Core-Specific Cytotoxic T Cell Activities of Retreated Patients

Core-specific cytotoxic T cell activities of five responders (patient 1, who received monotherapy, and patients 2–5, who received combination therapy) were measured at weeks 0 and 26 (fig. 5a). Among them, a nonresponder of group A (patient 4) and a patient with relapsed disease from group A (patient 5) were monitored completely during the retreatment course and after treatment (fig. 5b, c). Cytotoxic T cell activities of the other two nonresponders (patients 6 and 7) were nondetectable before and after treatment (data not shown). The results show that core-specific cytotoxic activities increased significantly when the viremia and serum ALT levels of these patients reached normal levels.

Discussion

Previous studies have shown that using either IFN or ribavirin as a single agent in treating chronic hepatitis C is apparently inadequate. Combining ribavirin with IFN has recently been shown to increase sustained virological, biochemical and histological responses in patients chronically infected with HCV [14, 16]. IFN and ribavirin seem to act synergistically, and the responses are sustained in 30–50% of cases. Despite this progress, the mechanism underlying the greatly improved efficacy achieved by this combination therapy is largely unknown. Because ribavirin monotherapy has been shown to have little effect on hepatitis C viremia but can decrease serum aminotransferase levels, changes in host responses after ribavirin

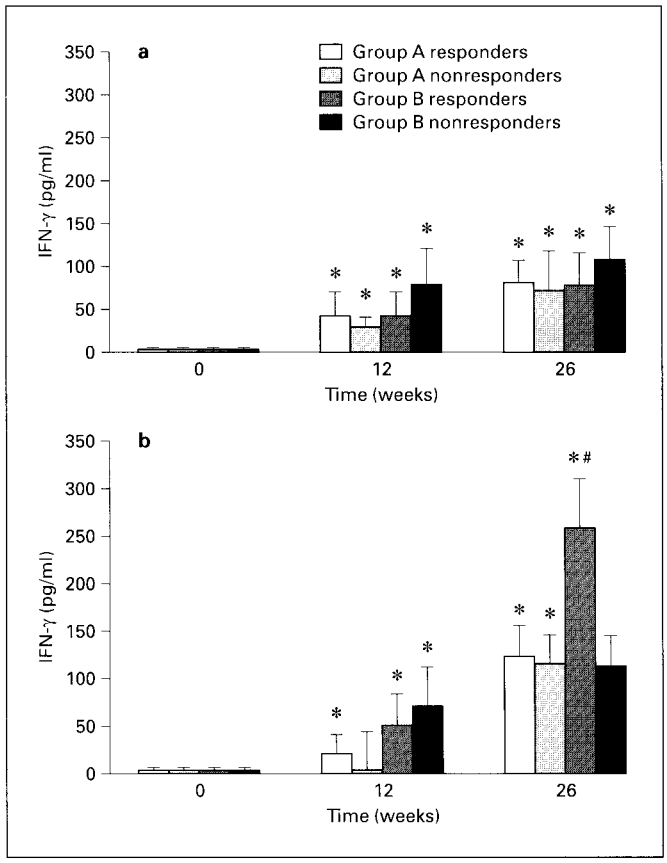

Fig. 4. IFN-γ produced by PBMCs without (**a**) and with (**b**) HCV core antigen (C190) incubation as determined by ELISA. Spontaneous and antigen-stimulated secretions represented the concentration of the culture supernatant from 1 × 10^4 PBMCs from patients treated with IFN-α only (group A) or IFN-α combined with ribavirin (group B) and incubated without or with 1 μg/ml C190 antigen for 3 days, respectively. * $p < 0.05$ compared to the value at time 0 in the same group; # $p < 0.05$ compared to the value for the nonresponders at the same time point. Data are presented as the mean ± SEM of three experiments.

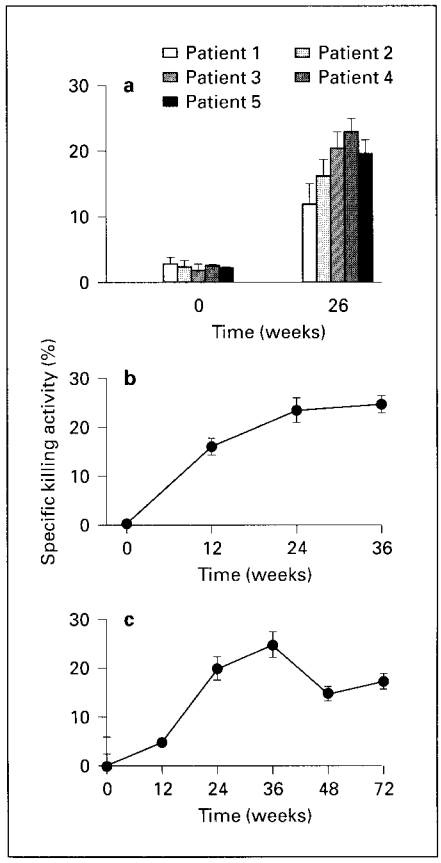

Fig. 5. Core-specific cytotoxic T cell activities of patients during the course of treatment. **a** PBMCs of patients 1–5 collected in weeks 0 and 26 were stimulated twice with irradiated C190/HepG2 cells, and then cytotoxicities were measured by the standard 4-hour ^{51}Cr release assay as described in Patients and Methods. Effector/target ratios were set at 40:1, 20:1 and 10:1 in triplicate. Lysis is shown with an effector/target ratio of 40. Specific killing activity is defined as the cytotoxicities against C190/HepG2 cells minus the cytotoxicities against S2/HepG2 cells. PBMCs of patients 4 (**b**) and 5 (**c**) were collected at different time points as indicated, and then cytotoxicities were measured as described above. The results suggest that core-specific cytotoxic activity gradually increased during the treatment in patients 4 and 5.

treatment may be more importantthan its antiviral activities. We therefore studied several immune parameters in our patients with chronic hepatitis C, comparing those receiving IFN-α alone and those receiving a combination with ribavirin.

At the end of treatment, normal serum ALT levels were observed in 12 of 25 patients in the group treated with IFN-α alone (48%) and in 20 of 26 patients in the combination group (77%). Furthermore, the histopathological examination also demonstrated improvement for these patients [unpubl. data]. In the meantime, immune responses during the course of treatment were serially monitored. Results show no significant changes in percentages of B cells, CD8+ T cells, memory CD4+ T cells, total T cells or NK cells during the course of either combination therapy or monotherapy. However, levels of both sCD4 and sCD8 decreased after treatment, suggesting reduced activity of immune effector T cells even as the cell number remained unchanged. In addition, the HCV core protein has been defined as the most potent T cell immunogen in chronic HCV patients [9]. In our studies, HCV core-specific proliferative responses were reduced in the 12th and 26th weeks compared to those of week 0 in

patients receiving combination therapy ($p < 0.05$). However, there was no significant difference between responders and nonresponders. It is likely that the decreased proliferative response of PBMCs in these patients might have resulted from either decreased functional activity of T cells or clearance of the viral load in vivo. It has long been known that NK cells are important in viral clearance. We found that activities of NK cells of responders in these two groups increased significantly after IFN treatment ($p < 0.05$). However, the increment was similar between group A and B patients. This implies that the effect may be caused by IFN-α alone. An interesting finding was that patients with initially low NK cell activities had better responses to both IFN-α and combined treatments. It is possible that defective NK cell function is partly accountable for persistent infection of HCV and can be reversed by IFN-α or combined treatment.

Importantly, the concentration of IFN-γ increased after IFN therapy among responders and nonresponders of groups A and B. Thus, the effect was probably caused by IFN-α alone. Therefore, these immunological changes are probably not the major factor leading to improvement in clinical manifestations. In particular, the HCV core-specific release of IFN-γ increased dramatically after combination therapy with IFN-α and ribavirin (fig. 4). IFN-γ might be produced by either T helper cells, activated NK cells or cytotoxic T cells. It has been reported that IFN-α favors the differentiation of CD4+ T cells into type-1 T helper (Th1) cells [19] and directly upregulates production of IFN-γ by CD4+ T cells [24]. A previous study suggested that IL-12, one of the cytokines produced early in many infections by macrophages, can also elicit production of a large amount of IFN-γ by NK cells [10]. Meanwhile, we also found that HCV-specific cytotoxic T cell activity increases in patients given the combination treatment (fig. 5) and correlates with normalized levels of ALT and viremia. HCV-specific cytotoxic T cell and CD4+ Th1 cell activities have been found to correlate better with the resolution of HCV infection [6, 17]. An animal study also demonstrated that ribavirin can enhance HCV core-specific Th1 and CTL responses with the increased IL-12 levels [8]. Treatment with ribavirin may increase the Th1-related cellular immune response and subsequently enhance cytotoxic T cell activity to clear the virus.

Our observations suggest that adding ribavirin to IFN-α greatly enhances the IFN-γ level of HCV-stimulated mononuclear cells and HCV-specific cytotoxic T cell activity. This may be the mechanism underlying the increased efficacy of this combination therapy for chronic hepatitis C.

Acknowledgements

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References

1 Alter HJ. The hepatitis C virus and its relationship to the clinical spectrum of NANB hepatitis. J Gastroenterol Hepatol 5(suppl 1):78–94; 1990.
2 Alter HJ, Holland PV, Morrow AG, Purcell RH, Feinstone SM, Moritsugu Y. Clinical and serological analysis of transfusion-associated hepatitis. Lancet ii:838–841;1975.
3 Brillanti S, Garson J, Foli M, Whitby K, Deaville R, Masci C, Miglioli M, Barbara L. A pilot study of combination therapy with ribavirin plus interferon alfa for interferon alfa-resistant chronic hepatitis C. Gastroenterology 107: 812–817;1994.
4 Castillo I, Bartolome J, Navas S, Gonzales S, Herrero M, Carreno V. Virological and biochemical long-term follow-up of patients with non-A, non-B hepatitis treated with interferon. Hepatology 19:1342–1346;1994.
5 Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B hepatitis genome. Science 244:359–362;1989.
6 Cooper S, Erickson A, Adams EJ, Kansopon J, Weiner AJ, Chien DY, Houghton M, Parham P, Walker CM. Analysis of a successful immune response against hepatitis C virus. Immunity 10:439–449;1999.
7 Di Bisceglie AM, Shindo M, Fong TL, Fried MW, Swain MG, Bergasa NV, Axiotis CA, Waggoner JG, Park Y, Hoofnagle JH. A pilot study of ribavirin therapy for chronic hepatitis C. Hepatology 16:649–654;1992.
8 Fang S-H, Hwang L-H, Chen D-S, Chaing B-L. Ribavirin enhancement of hepatitis C virus core antigen-specific type 1 T helper cell response correlates with the increased IL-12 level. J Hepatol 33:791–798;2000.
9 Ferrari C, Valli A, Galati L, Penna A, Scaccaglia P, Giuberti T, Schianchi C, Missale G, Marin MG, Fiaccadori F. T-cell response to structural and nonstructural hepatitis C virus antigens in persistent and self-limited hepatitis C virus infection. Hepatology 19:286–295; 1994.
10 Gumperez JE, Parham P. The enigma of the natural killer cells. Nature 378:245–248;1995.
11 Hsieh CL, Chen BF, Wang CC, Liu HH, Chen DS, Hwang LH. Improved gene expression by a modified bicistronic retroviral vector. Biochem Biophys Res Commun 214:910–917; 1995.
12 Kao JH, Chen PJ, Lai MY, Yang PM, Sheu JC, Wang TH, Chen DS. Mixed infections of hepatitis C virus as a factor in acute exacerbations of chronic type C hepatitis. J Infect Dis 170: 1128–1133;1994.
13 Keeffe EB, Hollinger FB. Therapy of hepatitis C: Consensus interferon trials. Consensus Interferon Study Group. Hepatology 26(3 suppl 1):101S–107S;1997.
14 Lai MY, Kao JH, Yang PM, Wang JT, Chan KW, Chu JS, Chan KW, Chu JS, Chen DS. Long-term efficacy of ribavirin plus interferon alpha in the treatment of chronic hepatitis C. Gastroenterology 111:1307–1312;1996.

15 Markowitz D, Goff S, Bank A. Construction and use of a safe and efficient amphotropic packaging cell line. Virology 167:400–406; 1988.
16 McHutchison JG, Gordon GC, Schiff ER, Schiffman ML, Lee WM, Rustgi VK, Goodman ZD, Ling MH, Cort S, Albrecht JK. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. N Engl J Med 339:1485–1492;1998.
17 Missale G, Bertoni R, Lamonaca V, Valli A, Massari M, Mori C, Rumi MG, Houghton M, Fiaccadori F, Ferrari C. Different clinical behaviors of acute hepatitis C virus infection are associated with different vigor of the anti-viral cell-mediated immune response. J Clin Invest 98:706–714;1996.
18 Ning Q, Brown D, Parodo J, Cattral M, Gorczynski R, Cole E, Fung L, Ding JW, Liu MF, Rotstein O, Phillips MJ, Levy G. Ribavirin inhibits viral-induced macrophage production of TNF, IL-1, the procoagulant fgl2 prothrombinase and preserves Th1 cytokine production but inhibits Th2 cytokine response. J Immunol 160:3487–3493;1998.
19 Parronchi P, De Carli M, Manetti R, Simonelli C, Sampognaro S, Piccinni MP, Macchia D, Maggi E, Del Prete G, Romagnani S. IL-4 and IFN (alpha and gamma) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones. J Immunol 149:2977–2983;1992.
20 Purcell RH. Hepatitis viruses: Changing patterns of human disease. Proc Natl Acad Sci USA 91:2401–2406;1994.
21 Reichard O, Anderson J, Schvarcz R, Weiland O. Ribavirin treatment for chronic hepatitis C. Lancet 337:1058–1061;1991.
22 Reichard O, Yun ZB, Sonnerborg A, Weiland O. Hepatitis C viral RNA titers in serum prior to, during, and after oral treatment with ribavirin for chronic hepatitis C. J Med Virol 41: 99–102;1993.
23 Saracco G, Rosina F, Abate ML, Chiandussi L, Gallo V, Cerutti E, Di Napoli A, Solinas A, Deplano A, Tocco A, et al. Long-term follow-up of patients with chronic hepatitis C treated with different doses of interferon-alpha 2b. Hepatology 18:1300–1305;1993.
24 Schandene L, del Prete GF, Cogan E, Stordeur P, Crusiaux A, Kennes B, Romagnani S, Goldman M. Recombinant interferon-alpha selectively inhibits the production of interleukin-5 by human CD4+ T cells. J Clin Invest 97:309–315;1996.
25 Tabor E, Gerety RJ, Drucker JA, Seeff LB, Hoofnagle JH, Jackson DR, April M, Barker LF, Pineda-Tamondong G. Transmission of non-A, non-B hepatitis from man to chimpanzee. Lancet i:463–466;1978.
26 Tam RC, Pai B, Bard J, Lim C, Averett DR, Phan UT, Milovanovic T. Ribavirin polarizes human T cell responses towards a type I cytokine profile. J Hepatol 30:376–382;1999.
27 Tine F, Magrin S, Craxi A, Pagliaro L. Interferon for non-A, non-B hepatitis. A meta-analysis of randomised chemical trials. J Hepatol 13: 192–199;1991.