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Helper T cell cytokine response to ribavirin priming before combined treatment with interferon alpha and ribavirin for patients with chronic hepatitis C

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Abstract

The viral genotype and serum viral level influence the response to interferon (IFN) treatment in patients with chronic hepatitis C virus (HCV) viremia. The aim of this study was to investigate a possible relationship between early virological response and helper T (Th) cell cytokine expansion by 4 weeks of ribavirin (RIB) alone followed by IFN and RIB combined in patients with genotype 1b and a high HCV RNA level, patients reported not to respond well to IFN treatment. Eighty-one patients with genotype 1b and a high HCV RNA level, over 100 international unit per milliliter (KIU/mL) (by Amplicor HCV Monitor), were assigned to two groups: Group A ($N = 40$) with a 4-week RIB administration followed by a 24-week combination treatment, and Group B ($N = 41$) with a 24-week combination treatment only. Blood was obtained from each patient on the following schedule: at Baseline (4 weeks before day 0), on day 0 (initiation day of the RIB and IFN combination treatment), weeks 4 (4 weeks after the start of the combination treatment), and at the end of the combination treatment. Flow cytometry was used to investigate sequential changes of IFN-γ producing (Th1) and interleukin-4 producing (Th2) cells from whole blood samples after stimulation with PMA and ionomycin. Serum HCV RNA clearances were 32.5% at week 4, 43.2% at week 8, 85.7% at the end of the combination treatment, and 22.9% within the 24-week follow-up in Group A; and 17.1%, 27.0%, 66.7% and 19.4% in Group B, respectively. The mean Th1/Th2 ratio significantly increased from 15.9 at baseline to 17.6 at day 0 with a decrease of Th2 cells, and then significantly decreased from 17.6 at day 0 to 15.5 at week 4 in Group A, while there was no significant change in Group B between baseline and day 0. In Group A, 13 patients with HCV RNA clearance within 4 weeks had a significantly increased Th1/Th2 ratio, from 14.0 at baseline to 22.1 at day 0, and then a significantly decreased ratio, from 22.1 at day 0 to 15.0 at week 4, while the others had no significant change in the ratio. RIB administration preceding combined treatment of RIB with IFN was more effective in Th2 cell expansion than the usual combined treatment of IFN with RIB and led to a relatively early virological clearance in chronic hepatitis C patients with genotype 1b and a high HCV RNA level.

Keywords: Interferon-α (IFN-α); Ribavirin (RIB); Hepatitis C virus (HCV); Interferon-γ (IFN-γ); Interleukin-4 (Il-4)

1. Introduction

Chronic hepatitis C virus (HCV) infection is alarmingly prevalent, 2–15%, throughout the world. Of Japanese patients with HCV, 30% have a consistently abnormal alanine aminotransferase (ALT) level and develop hepatocellular carcinoma (HCC) (Hayashi et al., 1997, 2000), with the incidence of HCC rising in recent years (Taylor-Robinson et al., 1997; El-Serag and Mason, 1999). Sustained virological response (SVR) rate is 20–30% in chronic hepatitis C patients following interferon (IFN) monotherapy, but only 5% in patients with both HCV genotype 1b and a high HCV RNA level (Di Bisceglie et al., 1989; Furusyo et al., 1997, 2002; Hayashi

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et al., 1998). Unfortunately, HCV genotype 1b is the most common genotype (80%) in Japan. Therefore, there are many chronic hepatitis C patients who do not respond well to IFN monotherapy in Japan.

Ribavirin (RIB) (1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a synthetic guanosine analogue that possesses a broad spectrum of action against DNA and RNA viruses (Sidwell et al., 1972), including the flaviviruses (Patterson and Fernandez-Larsson, 1990). It is the only available drug that has demonstrated in many studies some beneficial effect in the treatment of chronic hepatitis C (Reichard et al., 1991; Di Bisceglie et al., 1992). However, RIB monotherapy had only a transient effect, with no significant impact on HCV replication, but reduced the ALT level in 60% of the patients and had an effect on necroinflammatory activity (Di Bisceglie et al., 1992, 1995). These findings suggest that RIB administration stimulates the activity of cytotoxic T-cells against infected hepatocytes, not directly against HCV replication. Moreover, the combination treatment with IFN and RIB leads to higher effectiveness in terms of virological response (Khakoo et al., 1998), which would enhance the activity of the immune system against HCV. Therefore, we hypothesized that RIB priming before the combination treatment would be effective for pre-setting the immune system.

An SVR rate of 40% for chronic hepatitis C patients upon combination therapy of IFN-α with RIB has been reported (Kakumu et al., 1993; Schvarcz et al., 1995), but the exact mechanism responsible for the success is unclear. IFN-α has a direct antiviral effect and induces a number of immunomodulatory activities that can enhance antiviral immune response (Peters, 1996; Hayashi et al., 1995; Furusyo et al., 1999). HCV-specific T helper (Th)-cell response has been shown to be important in the resolution of acute HCV infection, with strong T-cell reactivity found in patients who clear HCV spontaneously and a weak response in those with progressive chronic infection (Diepolder et al., 1995; Missale et al., 1996). Moreover, several studies have reported such T-cell reactivity in association with IFN treatment (Missale et al., 1997; Kawakami et al., 2000; Murata et al., 2002). These findings raise the possibility that enhancement of T-cell reactivity may be a mechanism involved in the successful antiviral effect seen with the combination IFN-α and RIB therapies.

We here report the findings of a controlled pilot study designed to assess the relationship between the virological efficacy of and immunological response to IFN-α and RIB combined after 4 weeks of RIB pre-treatment of Japanese chronic hepatitis C patients.

2. Patients and methods

2.1. Patients

This controlled pilot study was designed to assess the relationship between the virological efficacy of and immunological response to IFN-α and RIB combined after 4 weeks of RIB pre-treatment of Japanese patients with chronic HCV viremia. All 81 patients with chronic hepatitis C (49 men and 32 women, mean age 56.7 years, age range 27–66 years) were randomly allocated by sealed envelope to the following treatment groups: Group A, RIB pre-treatment followed by a combination therapy of IFN-α and RIB, and Group B, combination therapy of IFN-α and RIB without RIB pre-treatment. Data on patient characteristics at entry are given in Table 1. All patients were positive for antibody to HCV and HCV RNA. No patient positive for hepatitis B virus surface antigen or antibody to human immunodeficiency virus or having other possible causes of hepatocellular injury such as autoimmunity or drug-induced liver disease were included. No patient had received antiviral or corticosteriod therapy within the 12 months prior to inclusion. All patients were infected with HCV of genotype 1b and had a high HCV RNA level, over 100 KIU/mL, determined by Amplicor HCV Monitor. The mean ALT level at entry was 88.8 IU/L (range, 41–333 IU/L). Needle biopsy of the liver was done for each patient within 2 months of the start of therapy, and two pathologists examined the biopsy specimens independently without prior knowledge of the patients. All patients were diagnosed with chronic active hepatitis with piecemeal necrosis or fibrosis formation of portal–portal bridging. Cirrhosis and non-cirrhosis were histologically diagnosed by biopsy. No significant differences were observed between Group A and B patients at entry. Serum ALT and HCV RNA level changes were measured during the observation. All patients gave their informed consent, and the study was approved by the ethics committee of Kyushu University Hospital. All the patients were followed for 6 months after cessation of IFN treatment.

Table 1
Patient characteristics at entry

Characteristics	Group A ($N = 40$)	Group B ($N = 41$)
Male:female (N)	24:16	25:16
Age (years)	57.5 ± 8.5	55.9 ± 12.6
Weight > 60 kg N (%)	21 (48.8)	22 (52.4)
Previous IFN N (%)	22 (51.2)	16 (38.1)
HCV RNA level (kilocopies/mL)	733.7 ± 228.1	719.0 ± 241.0
ALT (IU/L)	89.2 ± 39.2	89.1 ± 40.1
Hb (g/dL)	14.4 ± 1.2	13.9 ± 1.4
Non-cirrhosis:cirrhosis N (%)	40:0	40:1

IFN, interferon; HCV, hepatitis C virus; ALT, alanine aminotransferase; Hb, hemoglobin. No significant differences in each characteristic were observed between Groups A and B patients. All patients were chronically infected with HCV genotype 1b.

2.2. Treatment protocol

Both groups of patients were given IFN-α 2b (Intron-A, Schering-Plough, Kenilworth, NJ) intramuscularly at a dose of 6 million units (MU) daily for 2 weeks, then 6 MU thrice weekly for 22 weeks (total dose 480 MU). Patients received RIB (Rebetol, Schering-Plough) 600–800 mg orally twice daily according to weight: 600 mg for patients less than 60 kg and 800 mg for those 60 kg or over. Fig. 1 shows the intervention and study timeline of the IFN plus RIB com-

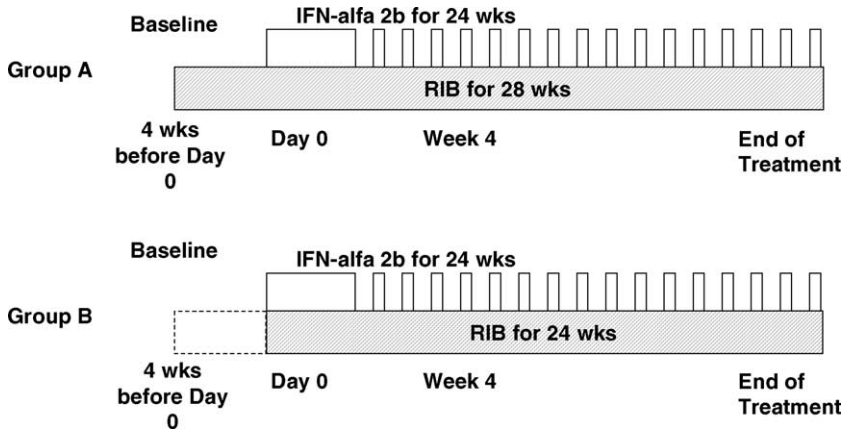

Fig. 1. Intervention protocol and study timeline of the IFN plus RIB combination treatment. IFN, interferon-α; RIB, ribavirin. Blood samples were taken at baseline (4 weeks before day 0), on day 0 (initiation day of RIB and IFN combination treatment), at week 4 (4 weeks after the start of the combination treatment), and at the end of the combination treatment.

bination treatment. The Group A patients were treated with RIB for 4 weeks, after which they received the combination therapy of IFN plus RIB for 24 weeks. The Group B patients received the combination therapy of IFN plus RIB for 24 weeks. Whole blood was obtained from each patient, to investigate sequential changes of IFN-γ producing (Th1) and interleukin-4 producing (Th2) cells on the following schedule: at baseline (4 weeks before day 0), on day 0 (IFN plus RIB combination therapy, initiation day), week 4 (4 weeks after the start of combination therapy), and at the end of the combination therapy.

The above duration and dose of IFN and RIB were approved by the Japanese Minister of Health, Labour and Welfare. The 48 weeks of IFN and RIB and the RIB dosage of 1000–1200 mg recommended by the international guideline were not permitted under the rules of the Japanese national health insurance system during the period of this study.

2.3. Definition of virological response during treatment

In this study, an early HCV RNA clearance response (ER) was defined as a undetectable HCV RNA test at 4 weeks after the initiation of the combination therapy of IFN plus RIB and Non-ER as a positive HCV RNA test at this time point. Sustained virological response (SVR) was defined as undetectable HCV RNA and a normal ALT level (under 36 IU/L) at 6 months after the cessation of treatment.

2.4. Serum assay methods

Serum samples were also drawn at monthly intervals while on treatment and at 4, 12, and 24 weeks after completion of treatment, stored at −20 °C, and frozen and thawed only once before doing the HCV RNA analysis.

2.5. HCV RNA determination by PCR

RNA was extracted from 50 μL of serum by Sep Gene RV (Sanko Junyaku, Tokyo, Japan). Complementary DNA was synthesized by use of random primers and reverse transcriptase (Super Script II; Life Technologies, Gaithersburg, MD). HCV RNA was detected by 2-stage PCR with primers from the 5′NC of the HCV genome (Choo et al., 1989): 5′-CTGTGAGGAACTACTGTCTT-3′ (sense) and 5′-AACACTACTCGGCTAGCAGT-3′ (antisense) in the first stage and 5′-TTCACGCAGAAAGCGTCTGT-3′ (sense) and 5′-GTTGATCCAAGAAAGGACCC-3′ (antisense) in the second stage.

2.6. HCV RNA genotyping

The HCV RNA genotype of each patient with HCV viremia was determined by 2-stage PCR using universal and type-specific primers from the putative core gene of the HCV genome by a modification of the method of Okamoto et al. (1992) and our previous report (Hayashi et al., 2000). The genotype nomenclature was based on the system proposed by Simmonds et al. (1994).

2.7. Quantity of serum HCV RNA

Serum HCV RNA levels were determined by the second-generation Cobas Amplicor HCV Monitor assay (COBAS v2.0, Roche Diagnostics Systems, Meylan, France) (Amplicor monitor). The range of the linear relationship provided was 0.5×10^3 KIU/mL to 850 KIU/mL for Amplicor monitor (Doglio et al., 1999). Samples over 850 KIU/mL by Amplicor monitor were re-measured after 10 and 100 times dilution to determine the accurate HCV RNA level.

2.8. IFN-γ and interleukin-4 producing peripheral CD4+ T cells by flow cytometric analysis

Identification of the capacity for cytokine production by CD4+ T cells for all the studied patients was done by three-color flow cytometry. The following analysis, as previously described elsewhere (Kawakami et al., 2000; Murata et al.,

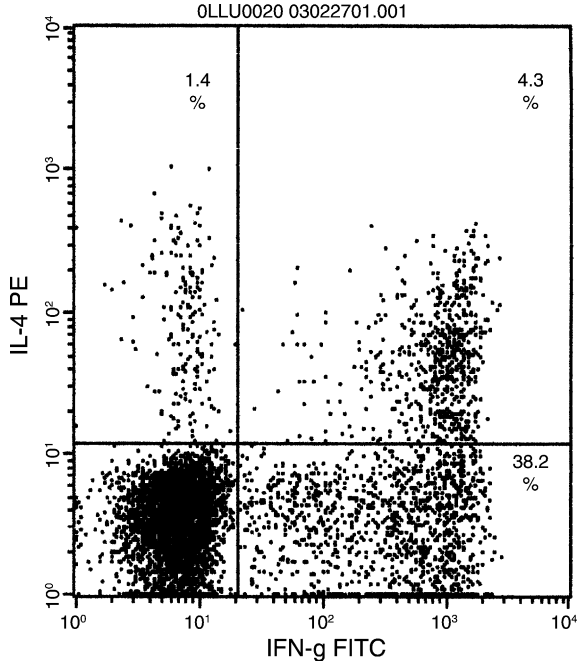

Fig. 2. A representative three-color flow cytometric pattern of the peripheral CD4+ T cells of one patient. Intracellular cytokines in CD4+ T cells after stimulation with mitogen were stained with anti-IFN-γ and IL-4 mAbs. The percentage of cytokine-positive cells to CD4+ T cells is shown in each quadrant.

2002), was immediately done after sampling whole blood from the patients. Based on these primary experiments, the system using IFN-γ and interleukin (IL)-4 (IL-4) proved to be the most stable and representative markers of Th1 and Th2 cell cytokines, respectively. Aliquots of diluted whole blood (1 mL) were cultured in a 24-well culture plate (Becton Dickinson, NJ) with 50 ng/mL PMA and 1 mg/mL of ionomycin in the presence of a protein-secretion inhibitor, GolgiPlug™, containing brefeldin A, and then incubated at 37 °C in 5% CO_2 for 4 h. Fixation, permeabilization, and intracellular cytokine staining of cultured cells were done with CytoStain™ Kits according to the Manufacturer's instructions (whole blood method). Cells were stained with both anti-IFN-γ and anti-IL-4 monoclonal antibodies (mAbs), and then incubated at 4 °C for 60 min. Stained cells were analyzed by flow cytometry, CYTORON ABSOLUTE with ImmunoCount 2 software (Ortho Diagnostic Systems, Raritan, NJ). Live-gating of lymphocytes was done, and up to 30,000 events were acquired for each analysis.

Fig. 2 shows a representative three-color flow cytometric pattern of the peripheral CD4+ T cells of one of the patients studied. Intracellular cytokines in CD4+ T cells after stimulation with mitogen were stained with anti-IFN-γ and IL-4 mAbs. The percentage of cytokine-positive cells to CD4+ T cells is shown in each quadrant. The percentages of IFN-γ and IL-4 producing cells in CD4+ lymphocytes were 38.2% and 1.4%, respectively. We used this method to investigate sequential changes of IFN-γ producing (Th1) and IL-4 producing (Th2) cells from whole blood samples of each patient.

2.9. Statistical analysis

Continuous data were expressed as mean values ± standard deviation (S.D.) of the mean. Statistical differences in the continuous data were determined by paired *t*-test, unpaired *t*-test, or Kruskal–Wallis test, and categorical data were compared by chi-square test and Fisher's exact test. Statistical analysis of the IFN-γ and IL-4 producing cell percentages and the ratio classified by response to treatment was done by analysis of variance with repeated measures. For each level of IFN-γ and IL-4 producing cell, percentage and ratio, a model that involves the fixed effects of the response-group to treatment (early HCV viremia clearance response or no response), time and a random effect of patients was assumed as follows:

$$Y_{ijk} = M + A_i + B_j + G_{ik} + E_{ijk}$$

where Y_{ijk} is the jth value of each level of IFN-γ and IL-4 producing cell percentages and the ratio for the kth patient in the ith response-group; M the mean of all observations; A_i the fixed effect of the response-group; B_j the fixed effect of time; G_{ik} a random effect of the kth patient in the ith response-group; E_{ijk} a random effect corresponding to error.

Stepwise logistic regression analysis was done using a commercially available software package (BMDP Statistical Software Inc., Los Angeles, CA) for the IBM (Yorktown Heights, NY) 3090 computer system. The BMPD program LR was used to evaluate the complicated relationship between the clinical features and the rates of ER and SVR to IFN among patients.

A P-value less than 0.05 was regarded as being statistically significant.

3. Results

3.1. HCV RNA level change during the 4-week RIB administration

In Group A, the mean serum HCV RNA level significantly decreased, from 733.7 ± 228.1 KIU/mL at baseline to 510.3 ± 224.0 KIU/mL at day 0, despite no patient having cleared HCV RNA, but the mean ALT did not significantly change from baseline (89.2 ± 39.2 IU/L) to day 0 (82.0 ± 47.7 IU/L) The mean Hb level did not significantly change in Group A (14.4 ± 1.2 g/dL to 13.2 ± 1.6 g/dL). In Group B, no significant difference in HCV RNA, ALT, or Hb level was observed during the period, and no patient had HCV RNA clearance (HCV RNA, 719.0 ± 241.0 KIU/mL to 705.2 ± 259.5 KIU/mL: ALT, 89.1 ± 40.1 IU/L to 95.8 ± 46.2 IU/L: Hb, 13.9 ± 1.4 g/dL to 13.6 ± 1.6 g/dL).

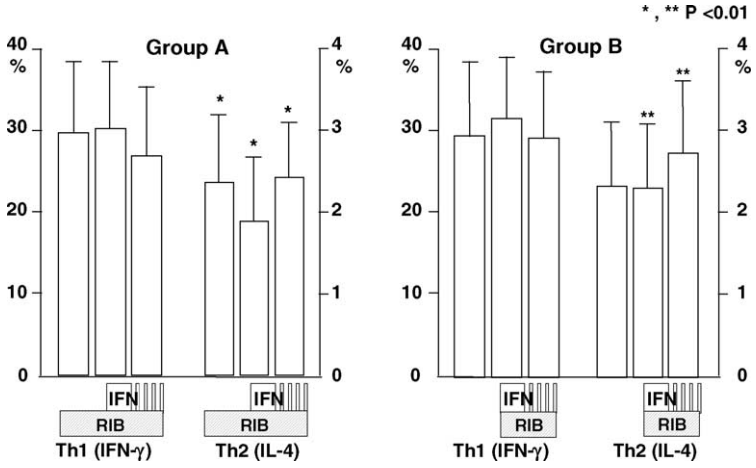

Fig. 3. Changes in the percentages of IFN-γ (Th1) and IL-4 (Th2) producing CD4+ T cells of 40 Group A and 41 B patients during the 4-week RIB administration and the combination treatment with IFN-α (at baseline, day 0, and week 4). IFN, interferon; Th, helper T; IL, interleukin; RIB, ribavirin.

3.2. IFN-γ and IL-4 producing peripheral CD4+ T cell percentage changes

Fig. 3 shows the changes in the percentages of IFN-γ (Th1) and IL-4 (Th2) producing CD4+ T cells of Group A and B patients during the 4-week RIB alone administration and the combination therapy of IFN plus RIB. In Group A patients during the 4-week RIB administration, the mean percentage of Th1 cells did not significantly change from baseline, but the mean percentage of Th2 cells significantly decreased, from 2.3% at baseline to 1.9% at day 0. In Group B patients, neither Th1 nor Th2 cells changed during the study period. On the other hand, in both Group A and B patients the mean percentage of Th1 cells did not significantly change between day 0 and week 4 of the combination therapy, but the mean percentage of Th2 cells significantly increased in Group A and B patients, from 1.9% and 2.4% at day 0 to 2.3% and 2.7% at week 4, respectively.

3.3. Th1/Th2 ratio change

Fig. 4 shows the changes in the ratio of IFN-γ (Th1) to IL-4 (Th2) producing CD4+ T cells of Group A and B patients at baseline, day 0 (after 4 weeks of RIB alone administration) and at week 4 of the combination therapy of IFN plus RIB. In Group A patients, the mean Th1/Th2 ratio significantly increased, from 15.4 at baseline to 18.6 at day 0, and significantly decreased between day 0 till week 4, from 18.6 to 14.6. In Group B patients, the Th1/Th2 ratio did not change during the study period (14.9 at baseline, 15.0 at day 0, and 13.3 at week 4).

Fig. 5 shows the relationship between early HCV RNA clearance and changes in the ratio of IFN-γ (Th1) to IL-4 (Th2) producing CD4+ T cells at baseline, day 0, and week 4. Interestingly, 13 of the Group A patients with an ER had a significantly increased Th1/Th2 ratio, from 14.0 at baseline to 22.1 at day 0, and then a significantly decreased ratio, from

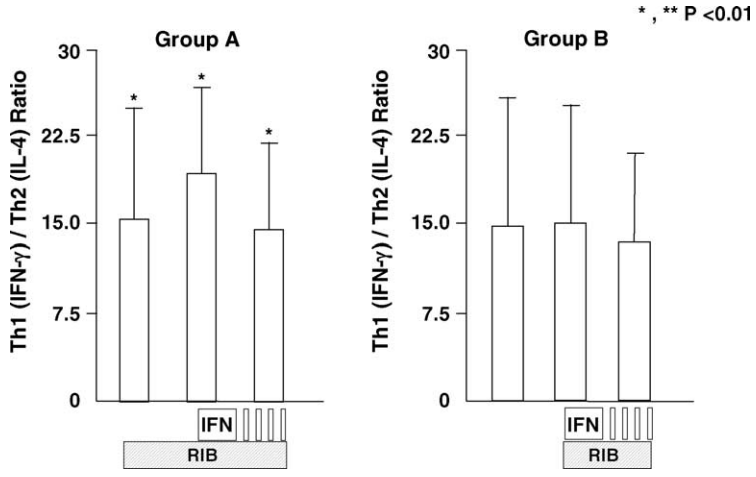

Fig. 4. Changes in the ratio of IFN-γ (Th1) to IL-4 (Th2) producing CD4+ T cells of 40 Group A and 41 B patients during the 4-week RIB administration and the combination treatment with IFN-α (at baseline, day 0, and week 4). IFN, interferon; Th, helper T; IL, interleukin; RIB, ribavirin.

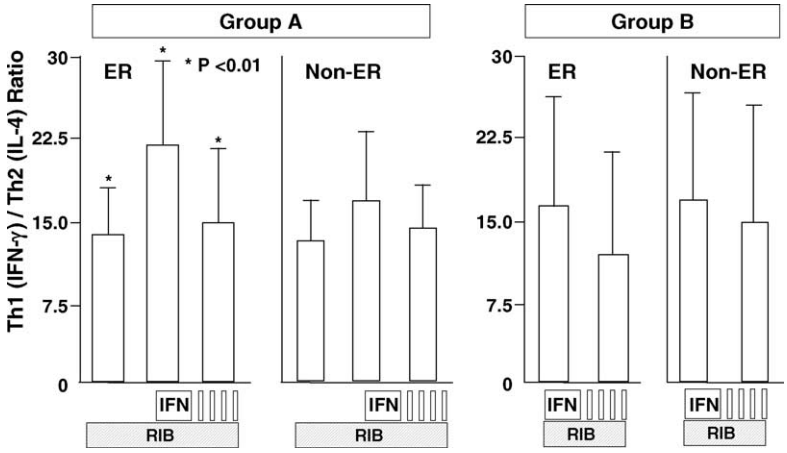

Fig. 5. Relationship between early HCV viremia clearance and changes in the ratio of IFN-γ (Th1) to IL-4 (Th2) producing CD4+ T cells during the 4-week RIB administration and the combination treatment with IFN-α (at baseline, day 0, and week 4). IFN, interferon; Th, helper T; IL, interleukin; RIB, ribavirin.

22.1 at day 0 to 15.0 at week 4. The other patients, without ER, had no significant change in the Th1/Th2 ratio during the observation period.

In order to determine the factors contributing to the virological response, a stepwise logistic regression analysis was done. However, no significant predictive factors of SVR or EVR were found.

3.4. Clinical course

No group A patient discontinued treatment during the 4-week RIB alone administration. Within 4 weeks of the start of the combination therapy, no Group A or B patients discontinued treatment, however, several patients discontinued after 4-weeks of combination therapy: 3 from Group A and 4 from Group B within 8 weeks of the start of the combination therapy, then 2 more Group A patients and one more in Group B patient between week 8 and the end of the treatment. In total, 12.5% of the Group A patients (5 of 40) and 12.2% of the Group B patients (5 of 41) discontinued treatment. No significant difference in the rate of discontinuation was found between Groups A and B. The reasons for discontinuation were mainly general fatigue and hematological disorders.

Fig. 6 shows the HCV RNA clearance rates of Group A and B patients during the combination therapy of IFN plus RIB and the 24 weeks of follow-up. Virological response (negative HCV RNA by PCR) was relatively high in Group A during the combination therapy and the 24 weeks of follow-up, although no statistical difference in virological response between Groups A and B was found.

Fig. 7 shows the changes in the mean ALT level of Group A and B patients after the start of therapy. The mean ALT level of Group A was lower than Group B, even though there was no significant difference in virological response between the two groups.

With regard to hematological disorders during treatment, no significant difference in hemoglobin (g/dL) change was

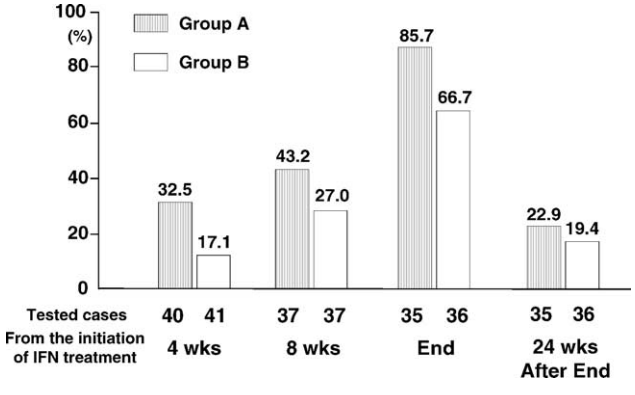

Fig. 6. Clearance rate of HCV viremia in Group A and B patients during the combination RIB and IFN treatment and at 24 weeks after the end of treatment. HCV, hepatitis C virus; RIB, ribavirin; IFN, interferon. Of the patients in each group, five did not complete treatment.

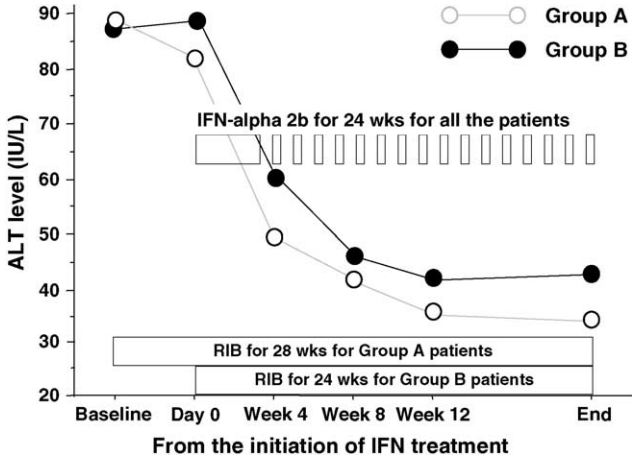

Fig. 7. Changes in the mean ALT level of Group A and B patients during the treatment. ALT, alanine aminotransferase.

found between Groups A and B. Group A patients were 14.4 ± 1.2 at baseline, 13.2 ± 1.6 on day 0, 12.3 ± 2.0 at week 4, 11.5 ± 2.2 at week 8, 11.9 ± 2.0 at week 12, and 12.0 ± 2.0 at the end. Group B patients were 13.9 ± 1.4 at baseline, 14.1 ± 1.4 on day 0, 12.8 ± 2.0 at week 4, 12.1 ± 2.3 at week 8, 12.4 ± 2.0 at week 12, and 12.6 ± 2.1 at the end. No significant difference in the changes of leukocyte or platelet counts was observed (data not shown).

4. Discussion

In the present study, a shift to a higher Th1 cytokine profile was found during the 4-week RIB alone administration, followed by a shift to a higher Th2 cytokine profile during the combination therapy of IFN plus RIB, which was significantly correlated with ER in these chronic hepatitis C patients. We previously reported that ER to IFN monotherapy was found in all patients with SVR, suggesting that monitoring serum HCV RNA during IFN administration is useful in evaluating the antiviral effect (Furusyo et al., 1999, 2002; Yamaji et al., 1998). Patients with both HCV genotype 1b and high HCV RNA levels do not respond well to IFN treatment. Therefore, the present study focused on ER among chronic hepatitis C patients with both genotype 1b and a high viral load.

The study would have been strengthened if some of the classic predictive factors of good viral response, for example, age, stage, and viral load, could have been explored using Th1 and Th2 monitoring. In order to find the factors contributing to virological response, stepwise logistic regression analysis was done. Because our patients had a high viral level and were all chronically infected with genotype 1b, which is known to be highly IFN-resistant, and also the groups were small, no significant predictive factors of SVR or EVR were found.

A pharmacokinetic study showed that the range of mean half-lives following multiple dosing of 600 mg RIB orally twice daily was 274–298 h, and that the steady state assessment from the trough concentrations in 3, 4, and 5 weeks of multiple dose administration indicated no significance between RIB alone treatment and a combination treatment with IFN (Khakoo et al., 1998). Therefore, a 4-week RIB pretreatment was chosen so that the plasma RIB concentration would be at steady state in the present study.

The two Th cell subsets, Th1 and Th2, are generally characterized by distinct and mutually exclusive patterns of cytokine production with different functions. Th1 cells produce IFN-γ and IL-2, as well as other cytokines, and promote cellular immune reaction, while Th2 cells produce IL-4, IL-6, IL-10, and other cytokines, and enhance humoral immune response. The Th1 and Th2 subsets have been proposed to play a pivotal role in the development of chronic viral infections and autoimmune diseases (Mosmann and Sad, 1996). With respect to chronic HCV infection, the data are still controversial because different methodologies have been used for evaluating the cytokine levels (Masaki et al., 2002). Recently, identification of Th subsets at the single cell level has become practical with the development of an intracellular cytokine assay using flow cytometry, as we already reported elsewhere (Kawakami et al., 2000; Murata et al., 2002). The stability of IFN-γ and the IL-4 cytokine system were shown in our primary studies. Therefore, this methodology was used in the present study to investigate the possible relationship between ER and expansion of Th cells by 4 weeks of RIB alone followed by a combination therapy of IFN plus RIB.

Immune response by a shift from the Th1 to the Th2 subset has been described in patients with chronic HCV infection during an IFN and RIB combination treatment (Lee et al., 2002). However, to our knowledge, no study has been done on the effect of a 4-week RIB administration followed by a combination therapy of IFN plus RIB. RIB in vitro was reported to promote or preserve Th1 cytokine-immune response, but to inhibit Th2 cytokines (Ning et al., 1998; Tam et al., 1999). We documented that a shift to a Th1 cytokine profile occurred during the 4-week RIB administration, followed by a shift to a Th2 cytokine profile during the IFN and RIB combination treatment, which was significantly correlated with early virological clearance. Other researchers, using the same methodology as ours, reported that a lower Th1/Th2 ratio before IFN monotherapy was a significant factor for long-term virological response in Japanese patients with chronic hepatitis C (Masaki et al., 2002). Moreover, an increase in Th1 profile and a decrease in Th2 after IFN-α therapy were observed in SVR patients, whereas the opposite result was obtained in non-SVR patients (Piazzolla et al., 2001). These findings may reflect our data that Th1/Th2 changes occurred and were related to viral clearance after preceding RIB pre-treatment followed by the combined treatment.

Crotty and colleagues proposed that RIB may act as an RNA mutagen, an effect that mutates the virus and reduces its infectivity, thus inducing the production of defective HCV particles (Crotty et al., 2000). Indeed, the mean HCV RNA level significantly decreased in our patients after a 4-week RIB alone administration, although no significant impact on HCV replication was reported (Di Bisceglie et al., 1992, 1995). This discrepancy can be explained by differences in the study population. Our patients had both high a HCV RNA level and genotype 1b.

The efficacy of a pegylated formulation of IFN-α (Peg-IFN) plus RIB combination treatment was reported to be superior to IFN-α plus RIB combination treatment and Peg-IFN monotherapy (Manns et al., 2001). A 48-week combination treatment of Peg-IFN and RIB has replaced the 24-week IFN monotherapy. Although no statistically significant difference in virological clearance was found between the two treatment schedules, the 4-week RIB administration followed by a 24-week IFN-α and RIB combination treatment (Group A), showed a higher frequency of virological clearance during treatment than the standard 24-week combination treatment (Group B) which is the most common in Japan and which has been considered the most effective. Peg-IFN plus RIB com-

bination treatment has not yet been approved for clinical use in patients with chronic HCV viremia by the Japanese Ministry of Health, Labour and Welfare at the time of the present study. In December 2004, the Peg-IFN plus RIB combination treatment received the official approval. Moreover, RIB administration for over 6 months has also not yet been approved for use with these patients, although IFN treatment for over 6 months was allowed. So far, the most effective and available treatment is the 6-month IFN-α plus RIB combination. The 4-week RIB pre-treatment did not increase the rate of discontinuation or the incidence of hematological disorders. Modifying this RIB pre-treatment regimen to take advantage of the potent antiviral effect seems necessary to obtain the highest possible rate of sustained response to IFN treatment. We, therefore, feel that RIB priming combined with peg-IFN-α plus RIB treatment may be beneficial for obtaining the most potent antiviral effects to improve the sustained response rate.

In conclusion, RIB administration followed by a combined therapy with IFN resulted in greater Th2 cell expansion than the usual combined treatment of IFN with RIB and led to relatively early virological clearance in chronic hepatitis C patients with genotype 1b and a high HCV RNA level.

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