Jikeikai Med J 2004 ; 51 : 1-11

Cytokine Profiles of Hepatitis C Virus Carriers who Show Persistently Normal Aminotransferase Levels

Tomofumi Atarashi, Mikio Zeniya, Hiroki Takahashi, Kazuhiko Koike, George Okuda, and Masami Sakaguchi

Division of Gastroenterology and Hepatology, Department of Internal Medicine, The Jikei University School of Medicine

ABSTRACT

Some patients whose serum shows hepatitis C virus (HCV) RNA have persistently normal alanine aminotransferase (ALT) levels and are termed HCV asymptomatic carriers (ASC). In contrast, patients who achieve this state only after being treated with interferon are termed biochemical responders (BR). However, the immune mechanisms of these states are unclear. We sought to clarify the relationship between the balance of T helper (Th)1 and Th2 cytokines and HCV-ASC. We studied 53 patients with HCV chronic hepatitis and elevated ALT levels and 20 HCV-ASC patients who were serum HCV-RNA positive. We investigated cytokine levels in peripheral blood with flow cytometry, to detect intracellular cytokines, and with whole blood assays, to detect cytokine titers. Levels of Th1 cytokines were higher in HCV-infected patients than in healthy controls ($p < 0.001$). With both measurement methods the Th1/Th2 ratio was higher in the HCV-ASC patients than in patients with elevated levels of ALT and chronic hepatitis ($p < 0.01$). No correlation was found between the Th1/Th2 ratio and levels of HCV-RNA in serum. These findings indicate that HCV infection increases levels of Th1 cytokines regardless of ALT levels and that the Th1/Th2 ratio reflects the activity of hepatitis. We suggest that the predominance of Th1 in HCV infection is related to the ASC state. (Jikeikai Med J 2004 ; 51 : 1-11)

Key words : hepatitis C virus, asymptomatic carrier, Th1/Th2 ratio, cytokine

Introduction

Hepatitis C virus (HCV) is most important cause of chronic hepatitis in Japan. Chronic liver disease develops in an estimated 80% of patients infected with HCV, and at least 40% of these cases result in liver cirrhosis[1-3]. Moreover, hepatocellular carcinoma develops annually in approximately 7% of patients with liver cirrhosis[4,5]. To prevent the development of liver cirrhosis in patients with HCV-related chronic hepatitis, several antiviral therapies have been established in Japan. One such treatment, interferon (IFN) monotherapy, can eradicate HCV infection in at least 30% of cases. However, its antiviral effects are incomplete, even in patients with a low serum viral load or a genotype other than 1b. Recently, the combination therapy of IFN and ribavirin has achieved markedly improved rates of HCV eradication, perhaps through ribavirin's modification of the infected host's immune condition. These results strongly suggest that host-related immune responses are involved in both the chronicity of HCV infection and its progression to liver cirrhosis. The precise mechanism responsible for immune-mediated liver cell injury in chronic HCV infection remains unclear. However, many reports have shown the importance of the host

Received for publication, November 26, 2003
新　智文，銭谷　幹男，高橋　宏樹，小池　和彦，奥田　丈二，坂口　正巳
Mailing address : Mikio Zeniya, Division of Gastroenterology and Hepatology, Department of Internal Medicine, The Jikei University School of Medicine, 3-25-8, Nishi-Shimbashi, Minato-ku, Tokyo, 105-8461, Japan.
E-mail : zeniya@jikei.ac.jp

immune response in mediating liver cell injury[6–8]. In liver tissue, most infiltrating lymphocytes express T-cell markers[9,10] and CD8, suggesting that cytotoxic T lymphocytes (CTLs) are involved in the host immune response. Furthermore, an HCV-specific CTL response has been reported in patients with HCV infection[11–13].

Patients with HCV infection who persistently show normal aminotransferase levels and are termed asymptomatic carriers (ASC). The outcome of IFN therapy in such ASC patients is worse than that in patients with chronic hepatitis whose serum aminotransferase levels fluctuate[14,15]. These findings strongly suggest that the immune status of an infected host might relate to the pathogenesis of HCV-ASC. Recently it has reported that T helper (Th)1/Th2 cytokine balance might play a crucial role in the immunopathogenesis of HCV infection[16]. However, whether the actual Th1/Th2 ratio is related to the immunologic condition in HCV infection is unknown. To clarify immunologic conditions in HCV-ASC, we examined cytokine profiles by measuring intralymphocyte cytokine levels and cytokine production levels in whole peripheral blood.

Methods

Subjects

The subjects were 53 patients in whom HCV infection had been diagnosed at The Jikei University Hospital from 1995 through 2000. Informed consent was obtained from all subjects. The initial diagnoses were based on the presence in serum of HCV RNA as determined with the reverse transcription polymerase chain reaction (Amplicore, Dynabot, Tokyo, Japan) and histologic findings consistent with chronic hepatitis using international criteria[17]. Specimens of whole peripheral blood were collected from each patient by venipuncture to quantitatively measure HCV RNA and levels of ALT in serum. Samples of heparinized peripheral blood obtained on the same day were used for both the intracellular cytokine assay and the whole blood culture.

The HCV-ASC group consisted of 20 patients (9 men and 11 women; average age, 55 years; 12 with HCV genotype 1b, 4 with HCV genotype 2a, and 4 with HCV genotype 2b) who had shown normal serum levels of ALT on monthly blood tests for at least 19 months (average, 42 months; range, 19–75 months). Of these 20 patients, 9 had been treated with IFN at least 2 years before enrollment without HCV RNA being eradicated. Seven patients had normal ALT levels before and after IFN treatment. Two patients maintained normal ALT levels after IFN treatment despite being serum-positive for HCV RNA (biological responders; BR). The remaining patients received no specific antiviral treatment. In 12 of 20 patients, an HCV-ASC liver biopsy was performed and showed both mild fibrosis and inflammation.

Thirty-three subjects (25 men and 8 women; average age, 53 years) showed high serum levels of ALT (average, 95 IU/L) during the observation period (chronic hepatitis C active; CHC-A group). Tables 1 and 2 show the characteristics of the patients. In addition, 23 healthy volunteers (15 men and 8 women; average age, 51 years) with no liver damage and with normal serum levels of ALT served as healthy control subjects.

Whole blood culture

Whole blood was cultured with the method of De Groote et al.[18]. Briefly, heparinized whole blood was diluted twice with RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) and cultured under the stimulation of 1 μg/ml of lipopolysaccharide (from *Escheri-*

Table 1. Clinical background of the subjects

	HCV-ASC	CHC-A
Number of patients	20	33
Average of age (years)	55±13	53±9
Gender (M : F)	9 : 11	25 : 8
ALT (IU/l)	26±6	95±57
Platelets ($\times 10^4$/mm^3)	17.2±5.1	18.1±4.4
γ globulin (g/dl)	1.61±0.35	1.58±0.41
HCV-RNA (kcopy/ml)	666±463	800±685
Range (median)	28–1,700 (570)	3.2–2,100 (550)
Genotype 1b : 2a : 2b	12 : 4 : 4	21 : 7 : 5
IFN treatment	9	18
Period of ASC (months) (mean)	19～75 (42)	

Table 2. Clinical and histologic background of HCV-ASC patients

	Age	Sex	IFN	Period of ASC (Month)	ALT (IU/l)	HCV-RNA (kcopy/ml)	HCV Genotype	Histologic grade
	65	F	−	75	31	330	1b	NT
	59	F	−	49	24	320	1b	F0A1
	68	F	−	48	18	64	1b	NT
	50	F	−	48	18	28	2a	NT
	63	M	−	38	30	860	1b	NT
	62	M	−	37	34	1,700	2b	NT
	33	F	−	36	35	360	1b	F0A1
	70	M	−	35	30	1,200	1b	F0A1
	70	F	−	35	27	440	1b	NT
	70	F	−	25	18	670	1b	NT
	27	F	−	23	25	99	1b	F0A1
	63	M	+	66	21	770	1b	F1A2
	49	M	+	65	33	1,400	2b	F0A1
	49	M	+	49	22	1,100	2b	F1A1
	53	F	+	47	22	830	2a	F1A1
	46	M	+	43	32	1,200	1b	NT
	61	M	+	38	16	420	2b	F0A1
	62	F	+	36	24	570	2a	F1A1
	31	M	BR	32	26	570	1b	F1A1
	44	F	BR	19	24	380	2a	F1A1
average	55			42	26	666		

Histologic grading was based on the Desmet criteria.

chia coli O55 : B5 ; Sigma Chemical Co., St. Louis, MO, USA). Blood was incubated in upright sealed tubes (Falcon, Oxnard, CA, USA) in a CO_2 incubator containing 95% CO_2 for 24 hours at 37°C. After incubation, the culture supernatant was separated by centrifugation and used for assays of interleukin (IL)-10 and IFN-γ.

Cytokine assays

Levels of both IL-10 and IFN-γ in whole blood culture supernatant were measured with ELISA kits (Amersham International plc, Little Chalfont, Buckinghamshire, England, UK).

Intracellular cytokine production assessed by flow cytometry

Intracellular cytokines were studied with the flow cytometry technique described by Kohsaka et al.[19]. The procedure is summarized below.

Cell preparation and immunostaining

Peripheral whole blood, diluted 2 times with RPMI 1640 containing 2 mM L-glutamine (Wako Pure Chemical Co., Ltd., Osaka, Japan), was stimulated with phorbol 12-myristate 13 acetate (10 ng/ml, Sigma) and ionomycine (1 μg/ml, Sigma) for 4 hours in the presence of 10 μg/ml of brefeldin A (Sigma) at 37°C in 7% CO_2. Next, 20 μl of 4-fold diluted R-phycoerythrin covalently linked to cyanin 5.1 (PC5)-conjugated anti-CD4 mouse IgG1 monoclonal antibody (Beckman Coulter, Fullerton, CA, USA) was added to 500 μl of diluted whole blood in a dark room and incubated for 15 minutes at room temperature. The erythrocytes were then lysed with 4 ml of FACS Lysing Solution (Becton Dickinson) and the samples were centrifuged at 500 g for 5 minutes. The supernatant was removed, and 1.5 ml of FACS Permeabilizing Solution (Becton Dickinson) was added for 10 minutes at room temperature. The cells were washed with 3 ml of phosphate-buffered saline (Wako Pure Chemical) and centrifuged at 500 g for 5 minutes.

For the immunostaining of cytokines, both 20 μl of fluorescein isothiocyanate (FITC)-conjugated mouse IgG2b monoclonal antibody against human IFN-γ (Becton Dickinson) and phycoerythrin (PE)-conjugated mouse IgG1 monoclonal antibody against human IL-4 (Becton Dickinson) were added to the cells for 30 minutes in a darkened room at room temperature. As a negative control, cells were stained with FITC- or PE-conjugated anti-keyhole limpet hemocyanin (KLH) mouse IgG2a or IgG1 monoclonal antibody (Becton Dickinson). KLH is the antigen that is not expressed on human cells. After incubation the cells were resuspended and fixed in 500 μl of 1% paraformaldehyde (Wako Pure Chemical).

Flow cytometry

A FACScan flow cytometer (Beckton Dickinson) equipped with a 15 mW argon ion laser at a filter setting for FITC (530 nm), PE (585 nm), and PC5 emitting in deep red (670 nm) was used. We acquired 5×10^5 to 10^6 cells in the list mode and analyzed them with CellQuest software (Beckton Dickinson). Analysis gates were set for lymphocytes according to the forward and side-scatter properties. The results are expressed as the percentage of cytokine-producing cells in the CD4-positive cell population.

Statistical analysis

Statistical analysis of the tested values was performed using the nonparametric Mann-Whitney test to compare the three different groups. Data are presented as means$\pm$SD. Statistical significance was set at $p<0.05$.

Results

Whole blood culture

Figure 1 shows the cytokine profiles obtained by culture of whole blood from HCV-ASC patients. The level of IFN-γ in the culture supernatant in the HCV-ASC group (2,389$\pm$1,081 pg/ml) was significantly higher than that in the CHC-A group (1,501$\pm$1,347 pg/ml ; $p<0.05$). However, the level of IL-10 in the HCV-ASC group (181$\pm$147 pg/ml) was lower than that in the CHC-A group (327$\pm$211.6 pg/ml ; $p<0.05$), such that the IFN-γ/IL-10 ratio was higher in the HCV-ASC group (23.20$\pm$20.75) than in the CHC-A group (4.91$\pm$4.27 ; $p<0.01$; Fig. 1).

Intracellular cytokine assay

The percentage of IFN-γ-positive cells in the HCV-ASC group (28.35$\pm$7.92%) was significantly higher than that in the control group (19.31$\pm$5.49% ; $p<0.001$) but did not differ significantly from that in the CHC-A group (26.43$\pm$7.55% ; Fig. 2). The per-

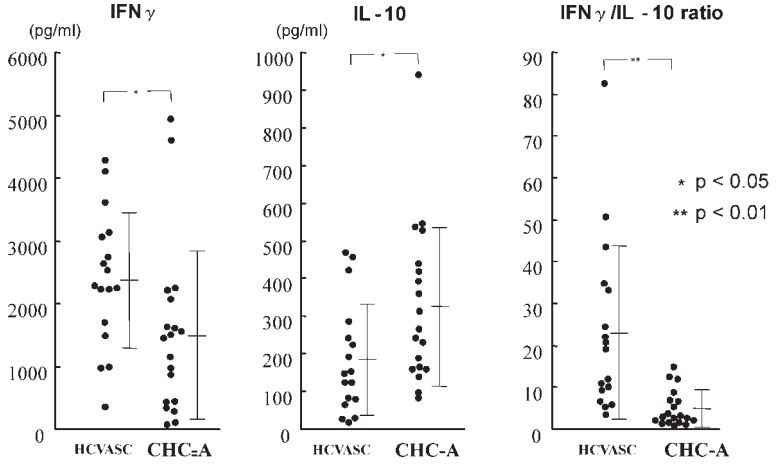

Fig. 1. Cytokine profiles determined with whole blood culture

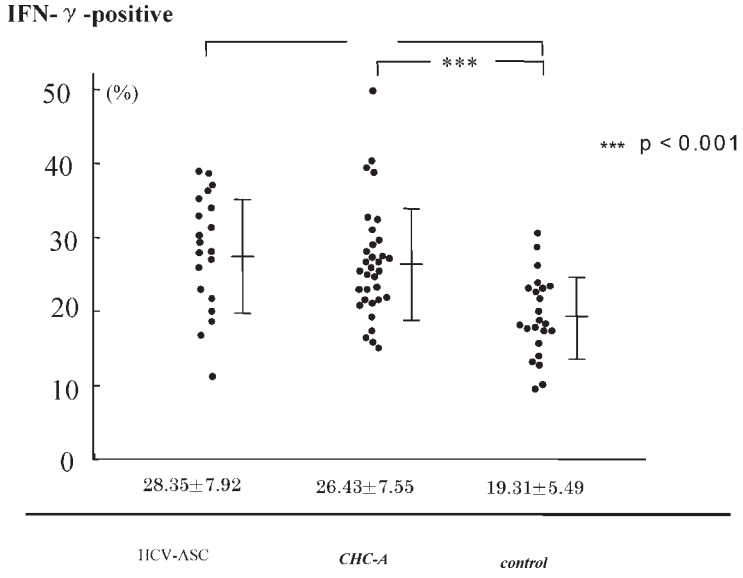

Fig. 2. Percentage of IFNγ-positive cells determined with intracellular cytokine assay using flow cytometry

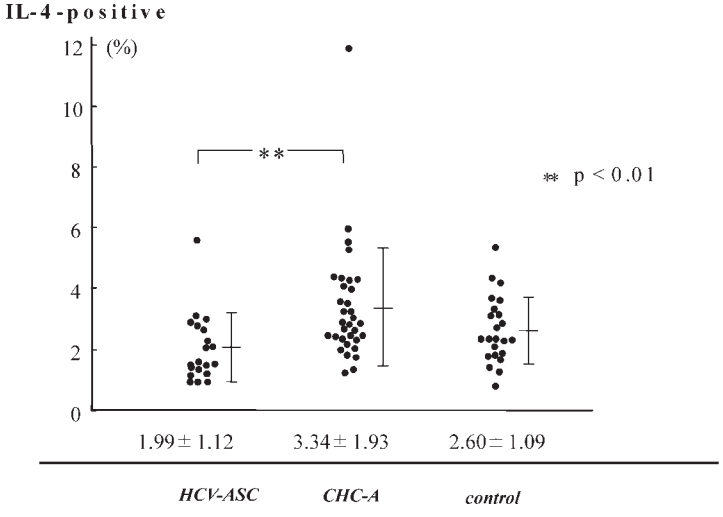

Fig. 3. Percentage of IL-4-positive cells determined with intracellular cytokine assay using flow cytometry

centage of IL-4-positive cells in the HCV-ASC group (1.99±1.12%) was lower than that in CHC-A (3.34±1.93%; $p<0.01$). The IFN-γ/IL-4 ratio in the HCV-ASC group (18.27±10.63) was significantly higher than the ratios in either the CHC-A group (9.81±5.29; $p<0.001$) or the control group (8.33±3.18; $p<0.001$). Similar to the results of whole blood culture, there were no differences between IFN-treated HCV-ASC and innate HCV-ASC. Furthermore, there were no differences between naive HCV-ASC and post-IFN treatment HCV-ASC (BR) in any category (data not shown).

The serum level of HCV RNA did not correlate with the percentage of IFN-γ-positive cells, the percentage of IL-4-positive cells, or the IFN-γ/IL-4 ratio (Fig. 5). The serum level of ALT did not correlate with the percentage of IFN-γ positive cells (Fig. 6) but did correlate weakly with the percentage of IL-4-positive cells ($r=0.3368$; $p<0.05$; Fig. 7) and the IFN-γ/IL-4 ratio ($r=0.3818$; $p<0.01$; Fig. 8).

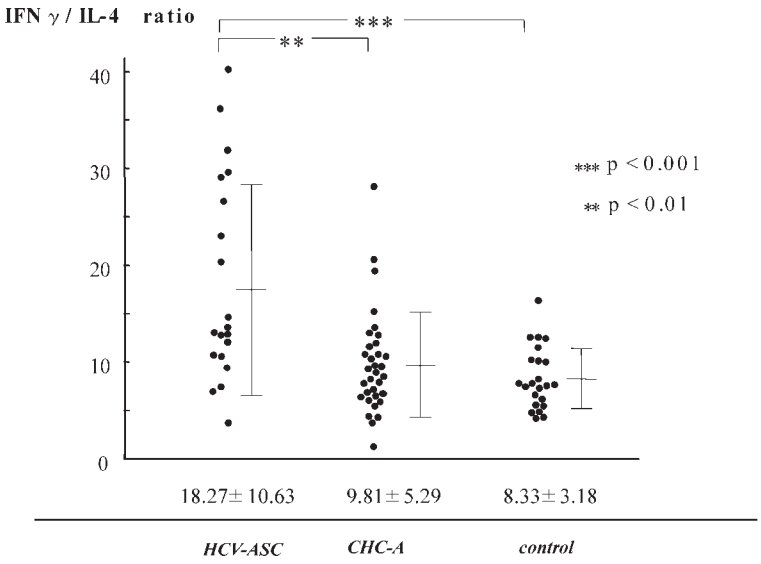

Fig. 4. IFN-γ/IL-4 ratio determined with intracellular cytokine assay using flow cytometry

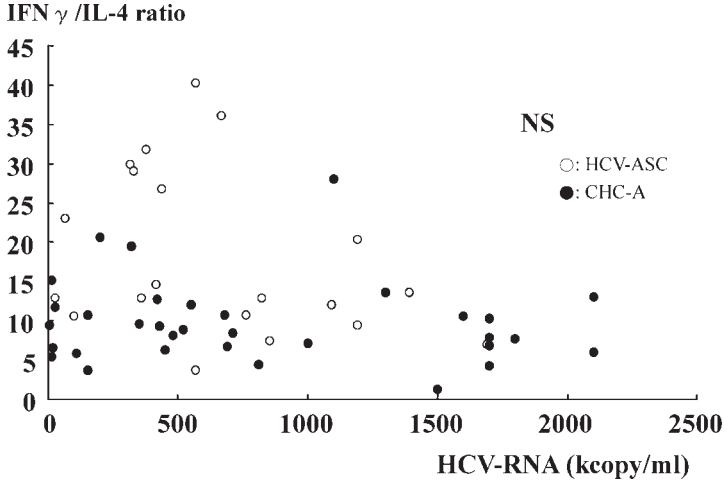

Fig. 5. Correlation between IFN-γ/IL-4 ratio and serum level of HCV-RNA

Follow-up of HCV-ASC

Ten patients who were categorized as being in the HCV-ASC group at the start of the study were followed up for at least 15 months and underwent re-evaluation of cytokine profiles. The serum HCV load remained high in these patients (average: 577±302 kcopy/ml). The cytokine profiles at follow-up also remained similar to those initially (Fig. 9). In 3 patients, follow-up biopsy showed no histologic progression.

Discussion

Patients infected with HCV often have no clinical signs of liver disease and have serum aminotransferase levels within the normal range; these patients may be defined as HCV-ASCs. Morisco et al.[20] have reported that these patients have mild histological change as we reported in our subjects (Table 2). Prati et al.[21] and Persico et al.[22] have also reported similar observations. Furthermore, a large cohort study by Marthurin et al.[23] has found that HCV-ASC

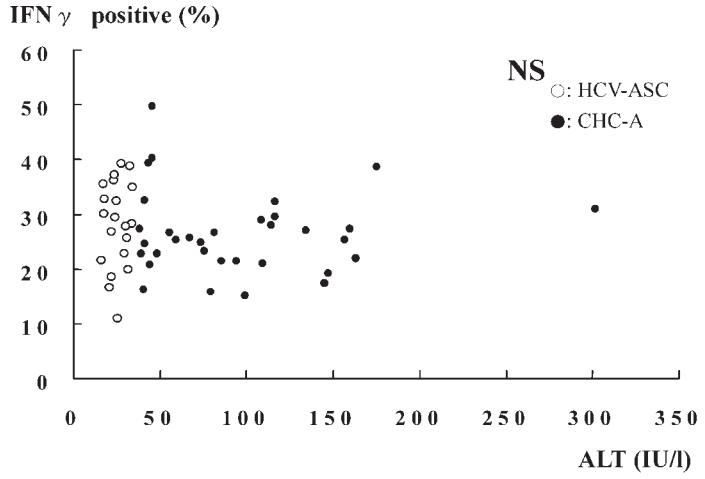

Fig. 6. Correlation between IFN-γ and serum level of ALT

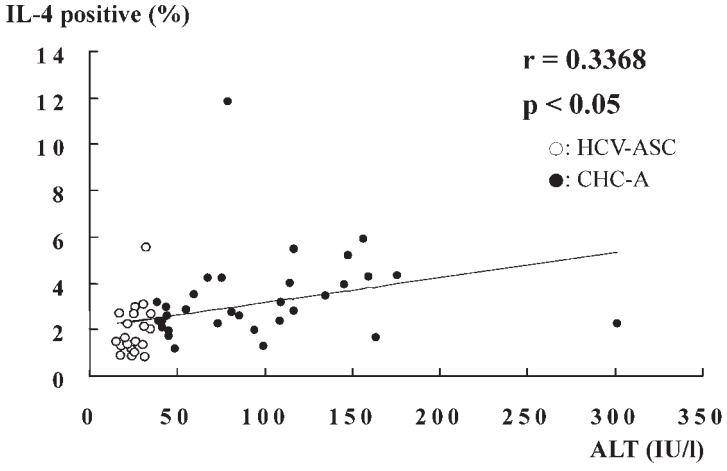

Fig. 7. Correlation between IL-4 and serum level of ALT

patients have less severe fibrosis than do patients with elevated levels of ALT. Pouti et al.[24] have estimated that as many as 25% of patients with HCV infection have persistently normal aminotransferase levels. Interestingly, viral factors involving viral load and HCV genotype do not relate to this condition. However, the immunologic characteristics of HCV-ASC are not well defined. The two-assay system used in the present clearly showed that HCV-ASC patients have a Th1-dominant immune condition.

The predominance of Th1 cytokine responses plays an important role in viral clearance of patients with either acute and chronic hepatitis B[25,26]. Cacciarelli et al.[27] have reported that serum levels of Th1 cytokines, including IFN-γ and IL-2, are elevated in patients with HCV infection. Furthermore, Napoli et al.[28] have shown that levels of IFN-γ and IL-2 mRNA in the liver are also elevated in patients with chronic HCV infection. These data strongly suggest that the Th1 cytokines IFN-γ and IL-2 are produced in the liver and are involved in inducing chronic inflammation.

These Th1-dominant cytokine profiles possibly play a role in inducing HCV-specific CTLs in the liver, resulting in liver damage manifested as elevated serum aminotransferase levels. An interesting finding is that HCV-ASC patients showed Th1-dominant cytokine profiles. However, in HCV-ASC

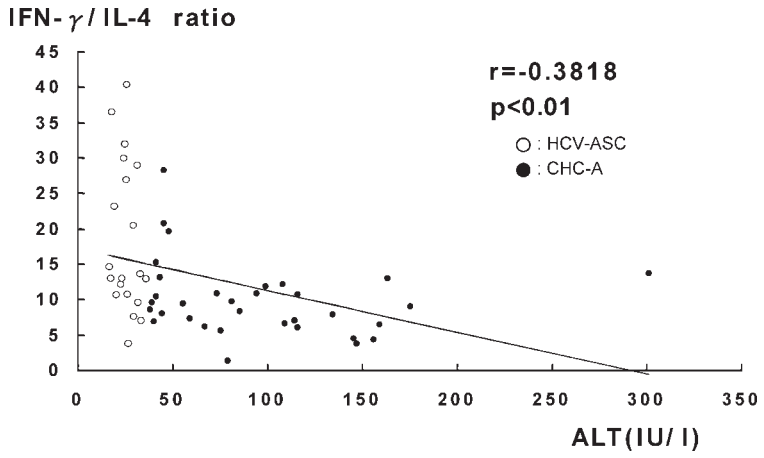

Fig. 8. Correlation between IFN-γ and serum level of ALT

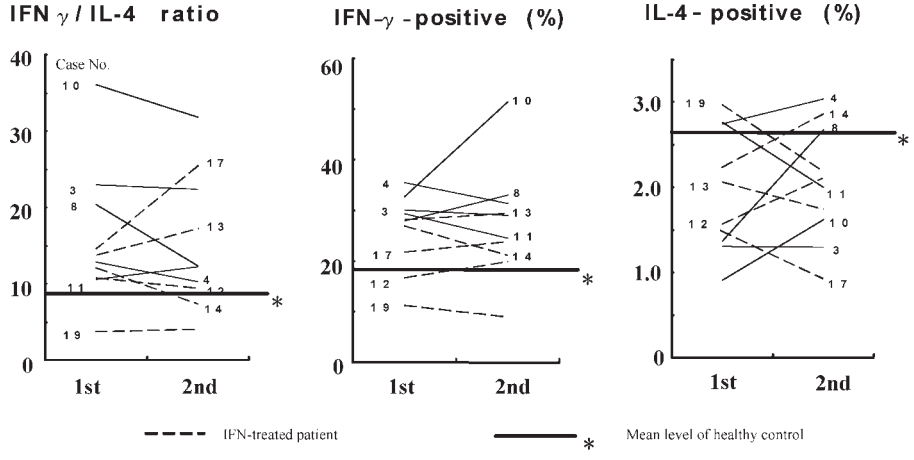

Fig. 9. Changes in intracellular cytokine profiles in HCV-ASC

patients serum aminotransferase levels stay within the normal range. The Th1-dominant cytokine profiles may enhance CTLs, resulting in hepatocyte injury and elevation of serum ALT levels. This apparent contradiction might be partly explained by the following two factors: 1) Th1-dominant cytokine profiles may in turn bolster infected-host defenses against HCV-infection and 2) although Th1-related cytokines are enhanced, Th2-type cytokines are also enhanced, such that the Th1/Th2 pattern ultimately shifts to Th1.

In the present study, CHC-A patients showed a positive correlation between IL-4 and serum ALT levels, a finding that suggests augmentation of Th2 cytokines might be involved in the Th1/Th2 pattern that induced immunological liver injury. However, the serum HCV-RNA level was not correlated with cytokine levels.

In hepatitis B, the ASC condition is induced by immunological tolerance[29] due to high levels of HBV-related antigens in serum. However, in CHC no evidence for the existence of immune tolerance has been reported. In the present study, HCV-ASC patients showed a high serum level of HCV RNA, as measured with competitive reverse transcription polymerase chain reaction. Even so, the amount of antigen in HCV infection was smaller than that in HBV infection, indicating that a high serum level of HCV-RNA cannot induce immunologic tolerance in HCV infection.

Clerici and Shearer[30] have reported that the Th1/Th2 pattern is closely related to the progression of the disease, especially with acquired immunodeficiency syndrome (AIDS). They have shown that a Th1-dominant immune condition is critical for the progression of AIDS, suggesting that Th1-type responses might be immunoprotective and can prevent the progression of viral infection. This finding suggests that Th1 dominance is involved in the pathogenesis of HCV-ASC, because HIV infects lymphocytes whereas HCV infects hepatocytes.

The function of the dendritic cell, a potent professional antigen-presenting cell, is reduced by HCV infection[31], indicating that HCV infection itself can also induce immune dysfunction of an HCV-infected host. This immune dysfunction might affect the Th1/Th2 pattern to induce the HCV-ASC condition. In the present study, we did not analyze the significance of dendritic cells, although doing so might may help clarify the pathogenesis of HCV-ASC.

The recent findings of Sobue et al.[32] differ from those of the present study. They also studied the Th1/Th2 balance in both peripheral blood and intrahepatic lymphocytes with flow cytometry and ELISA for cytokines. They found that Th1-dominant response was correlated with disease activity and progression. They showed that the IFN-γ/IL-10 ratio in HCV-ASC is smaller than that in either chronic hepatitis C or liver cirrhosis, indicating that a Th1-dominant immune response is not observed in HCV-ASC. There were several technical differences between their study and ours: they used intrahepatic lymphocytes and did not focus on IL-4. Our study used only peripheral blood, which suggests a possible difference between peripheral and intrahepatic lymphocytes. Even so, they showed a similar result in their study of peripheral blood, although we noted several problems in their analysis: 1) they did not examine histologic changes in HCV-ASC patients and 2) the average age of HCV-ASC patients might be significantly higher than that of CHC-A patients. This suggests that their HCV-ASC patients might include patients with liver cirrhosis. Furthermore, if, as they have reported, HCV-ASC patients have no Th1 response, how these patients show mild or slight inflammation in the liver is difficult to understand. In our study, we examined similar factors and consistently found Th1 dominance in peripheral lymphocytes as a feature of HCV-ASC involving BR after IFN therapy.

Bozdayi et al.[33] have shown that HCV-infected patients having 1 dominant strain in the circulation may show a relatively weaker immune response, resulting in lower ALT levels. We did not analyze HCV sequences in the present study. However, our patients showing normal serum ALT levels tended to have a high serum viral load, a finding that suggests the absence of marked HCV polymorphism and a lower likelihood of having a certain strain causing immunopathogenesis of HCV-ASC.

Mochida et al.[34] have reported that a HCV core peptide can inhibit Fas-mediated hepatocyte apoptosis in transgenic mice induced to express HCV proteins. They have reported that apoptotic cell death in the liver of mice expressing HCV protein is significantly reduced and that HCV proteins suppress Fas-mediated apoptotic cell death and inhibit activities of caspase-9 and -3/7. These findings might explain the Th1 dominance in HCV-ASC. In the present study, most HCV-ASC patients showed high serum levels of HCV RNA, indicating a high HCV core protein level that could suppress CTL attacks on hepatocytes. A Th1-dominant immune condition that can augment CTLs and does not cause liver inflammation results in the HCV-ASC state with relatively mild histologic changes in the liver.

Interestingly, the HCV-ASC state can be induced with IFN treatment. Some patients (BRs) had a normal ALT level in serum after IFN treatment despite the presence in serum of HCV RNA. We found that naive HCV-ASC patients and BRs had similar cytokine profiles. This finding suggests that IFN treatment might induce an immunologic condition similar to that observed in HCV-ASC.

In cases of HCV-ASC, especially those in which serum levels of HCV-RNA are high, Th1-dominant cytokines appear to be induced with augmentation of CTLs. Simultaneously, the apoptosis of CTL was reduced, resulting in a normal serum level of ALT and mild inflammation in the liver. Furthermore, we

found that the augmentation of Th2 cytokines by IL-4 was also involved in this process.

Our results strongly suggest the presence of a Th1-dominant immune response in HCV-ASC. However this immune response does not cause severe inflammation and the reason for this lack of inflammation remains to be established in future studies.

Acknowledgement: The authors thank Professor Gotaro Toda, chairman of the Division of Gastroenterology and Hepatology, Department of Internal Medicine, The Jikei University School of Medicine, for his valuable guidance and encouragement. The authors also thank all members of the Second Laboratory of the Division of Gastroenterology and Hepatology for their help on this work. This work was presented in part at Digestive Disease Week (D D W) Japan 1999, Tokyo.

References

1. Gretch DR, dela Rosa C, Carithers RL, Willson RA, Williams B, Corey L. Assessment of hepatitis C viremia using molecular amplification technologies: correlations and clinical implications. Ann Intern Med 1995; 123: 321-9.
2. Gross JB, Persing DH. Hepatitis C: advances in diagnosis. Mayo Clin Proc 1995; 70: 296-7.
3. Davis GL, Lau JYN, Lim HL. Therapy for chronic hepatitis C. Gastroenterol Clin North Am 1994; 23: 603-13.
4. Di Bisceglie AM. Hepatitis C and hepatocellular carcinoma. Hepatology 1997; 26: 34S-8S.
5. Caselmann WH, Alt M. Hepatitis C virus infection as a major risk factor for hepatocellular carcinoma. J Hepatol 1996; 24: 61-6.
6. Nelson DR: The immunopathogenesis of hepatitis C virus infection. Clin Liver Dis 2001; 5: 931-53.
7. Fan XG, Tang FQ, Yi H, Liu WE, Houghton M, Hu GL. Effect of IL-12 on T-cell immune responses in patients with chronic HCV infection. APMIS 2000; 108: 531-8.
8. Sun J, Bodola F, Fan X, Irshad H, Soong L, Lemon SM, Chan TS. Hepatitis C virus core and envelope proteins do not suppress the host's ability to clear a hepatic viral infection. J Virol 2001; 75: 11992-8.
9. Nelson DR, Marousis CG, Davis GL, Rice CM, Wong J, Houghton M, et al. The role of hepatitis C virus-specific cytotoxic T lymphocytes in chronic hepatitis C. J Immunol 1997; 158: 1473-81.
10. Wong DK, Dudley DD, Afdhal NH, Dienstag J, Rice CM, Wang L, et al. Liver-derived CTL in hepatitis C virus infection: breadth and specificity of responses in a cohort of persons with chronic infection. J Immunol 1998; 160: 1479-88.
11. Diepolder HM, Zachoval R, Hoffmann RM, Wierenga EA, Santantonio T, Jung MC, et al. Possible mechanisms involving T-lymphocyte response to non-structural protein in viral clearance in acute hepatitis C virus infection. Lancet 1995; 346: 1006-7.
12. Rehermann B, Chang KM, McHutchison JG, Kokka R, Houghton M, Chisari FV. Quantitative analysis of the peripheral blood cytotoxic T lymphocyte response in patients with chronic hepatitis C virus infection. J Clin Invest 1996; 98: 1432-40.
13. Lechner F, Wong DK, Dunbar PR, Chapman R, Chung RT, Dohrenwend P, et al. Analysis of successful immune responses in persons infected with hepatitis C virus. J Exp Med 2000; 191: 1499-512.
14. Sangiovanni A, Morales R, Spinzi GC, Rumi MG, Casiraghi A, Ceriani R, et al. Interferon alpha treatment of HCV RNA carriers with persistently normal transaminase levels: A pilot randomized controlled study. Hepatology 1998; 27: 853-6.
15. Yagura M, Murai S, Kojima H, Tokita H, Kamitsukasa H, Harada H. Interferon treatment in patients with chronic hepatitis C with normal alanine-aminotransferase activity. Hepatogastroenterology 1999; 46: 1094-9.
16. Fan XG, Liu WE, Li CZ, Wang ZC, Luo LX, Tan DM, et al. Circulating Th1 and Th2 cytokines in patients with hepatitis C virus infection. Mediators Inflamm 1998; 7: 295-7.
17. Ichida F, Tsuji T, Omata M. New Inuyama classificatio: new criteria for histological assessment of chronic hepatitis. International Hepatology Communication 1996; 6: 112-9.
18. De Groote D, Gevaret Y, Lopez Y, Garhy R, Fauchet F, Dehart I, et al. Novel method for measurement of cytokine production by one-stage procedure. J Immunol Method 1993; 163: 259-67.
19. Kohsaka T, Miyazaki T, Noma Y, Kobayashi Y, Higuchi N, Kudoh Y, et al. Examination for detection of intracellular cytokines by flow cytometry(in Japanese). Igaku to Yakugaku 1997; 38: 875-82.
20. Morisco F, Leone D, Tuccillo C, Iasevoli P, Sessa G, De Luise G, et al. Subjects positive for hepatitis C virus RNA with normal aminotransferase levels, a "trompe l'oeil" clinical picture. Dig Liver Dis 2000; 32: 598-602.
21. Prati D, Capelli C, Zanella A, Mozzi F, Bosoni P, Pappalettera M, et al. Influence of different hepatitis C virus-positive subjects with normal liver biochemical values. Hepatology 1995; 22: 418-25.
22. Perisco M, Perisco E, Suozzo R, Conte S, De Seta M, Coppola L, et al. Natural history of hepatitis C virus carriers with persistently normal aminotransferase levels. Gastroenterology 2000; 118: 760-4.

23. Mathurin P, Moussalli J, Cadranel JF, Thibault V, Charlotte F, Dumouchel P, et al. Slow progression rate of fibrosis in hepatitis C virus patients with persistently normal alanine aminotransferase activity. Hepatology 1998 ; 27 : 868–72.
24. Pouti C, Castellacci R, Montagnese F. Hepatitis C virus carriers with persistently normal aminotransferase levels : healthy people or true patients ? Dig Liver Dis 2000 ; 32 : 634–43.
25. Romagnani S. Lymphokine production by human T cells in disease states. Annu Rev Immunol 1994 ; 12 : 227–57.
26. Barnaba V, Franco A, Paroli M, Benvenuto R, De Petrillo G, Burgio VL, et al. Selective expansion of cytotoxic T lymphocytes with a CD4+ CD56+ surface phenotype and a T helper type 1 profile of cytokine secretion in the liver of patients chronically infected with hepatitis B virus. J Immunol 1994 ; 152 : 3074–87.
27. Cacciarelli TV, Martinez OM, Villaneuva JC, Gish RG, Krams SM. Immunoregulatory cytokines in hepatitis C virus (HCV) infection pre and post treatment with interferon-α (Abstract). Gastroenterology 1995 ; 108 : A1042.
28. Napoli J, Bishop GA, McGuiness P, Painter DM, McGaughan GW. Increased intrahepatic TH1 cytokine mRNA levels correlate with tissue damage in chronic hepatitis C (Abstract). Hepatology 1995 ; 22 : 222A.
29. Milich DR, Schodel F, Peterson DL, Jones JE, Hughes JL. Characterization of self-reactive T cells that evade tolerance in hepatitis B antigen transgenic mice. Eur J Immunol 1995 ; 25 : 1663–72.
30. Clerici M, Shearer GM. A TH1→TH2 switch is a critical step in the etiology of HIV infection. Immunol Today 1993 ; 14 : 107–11.
31. Akbar SMF, Horiike N, Onji M, Hino O. Dendritic cells and chronic hepatitis virus carriers. Intervirology 2001 ; 44 : 199–208.
32. Sobue S, Nomura T, Ishikawa T, Ito S, Saso K, Ohara H, et al. Th1/Th2 cytokine profiles and their relationship to clinical features in patients with chronic hepatitis C virus infection. J Gastroenterol 2001 ; 36 : 544–51.
33. Bozdayi AM, Uzunalimoglu O, Aslan N, Bozkaya H, Turkyilmaz AR, Kayhan B, et al. Influence of viral load and alanine aminotransferase on viral genetic heterogeneity in patients with chronic hepatitis C infection. Intervirology 2000 ; 43 : 61–6.
34. Machida K, Tsukiyama-Kohara K, Seike E, Tone S, Shibasaki F, Shimizu M, et al. Inhibition of cytochrome c release in Fas-mediated signaling pathway in transgenic mice induced to express hepatitis C virus proteins. J Biol Chem 2001 ; 276 : 12140–6.