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Hepatology Research xxx (2004) xxx–xxx
Hepatology Research
www.elsevier.com/locate/ihepcom

Production of IFN-γ and IL-12 by peripheral whole blood is maintained in hepatitis C virus patients with persistently normal alanine transferase activity
A preliminary report

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Received 29 July 2003; received in revised form 10 February 2004; accepted 27 February 2004

Abstract

The current study was designed to investigate the immune status in hepatitis C virus (HCV) patients with persistently normal alanine transferase activity (ALT) (patients with normal alanine transferase). For this purpose, serum levels and lipopolysaccharide (LPS)-induced IFN-γ, IL12 p70, IL12 p40 and IL-10 as well as NK cell activity were assayed in six patients with normal ALT, 22 HCV-infected individuals with chronic hepatitis (CH), 13 cases of liver cirrhosis (LC) and 26 age-matched controls. Cytokine production was assayed with the whole blood induction method. IFN-γ levels were significantly lower in patients with HCV-infected chronic hepatitis and liver cirrhosis than in controls (883.1 ± 1167.3, 777.2 ± 891.2 and 2066.5 ± 2094.8 pg/ml, respectively, $P < 0.05$). However, IFN-γ production in those individuals with normal ALT was not reduced (2627.8 ± 2538.5 pg/ml). Although variation was observed, four of the six patients showed moderate to strong IFN-γ production. No intergroup differences were observed for IL12 p70, IL12 p40 and IL-10 production and NK cell activity. Our results suggest that preserved IFN-γ production in patients with normal ALT, in contrast to the reduction in chronic hepatitis and liver cirrhosis, may be related to a slow rate of disease progression.

Keywords: Lipopolysaccharide; IL12 p70; IL12 p40; NK cell; Carrier; Whole blood induction method

1. Introduction

The hepatitis C virus (HCV), a 9.4 kb single-stranded, positive sense RNA species, is the major etiologic agent of non-A, non-B hepatitis [1]. The most striking feature of HCV infection is its tendency toward chronicity. Most patients with HCV infection develop chronic hepatitis (70–85%) and this progresses to liver cirrhosis (LC), and often to hepatocellular carcinoma [2,3]. However, some HCV-infected patients display normal serum alanine transferase (ALT) levels with no symptoms and signs of liver disease for a long period, despite having a high viral load [4,5]. HCV-infected patients with persistently normal ALT show a slower fibrosis progression rate than those with elevated ALT [5]. This variation is not understood.

Although the mechanisms accounting for hepatocellular damage have yet to be clarified in detail, a pathogenetic role for host immune reactions has been outlined [6,7]. With reference to this point, several immunoregulatory cytokines are believed to be involved in the modulation of the complex virus–host interaction [8]. Cytokines are produced by multiple cell types such as NK cells, macrophages, $CD4^+$ T cells and $CD8^+$ T cells. Responses are referred to as Th1-like and Th2-like after the original description of the cytokine profiles produced by subsets of $C4^+$ T cells [9]. Th1-like responses include IL-2, TNF-α, and IFN-γ secretion and are

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1386-6346/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.hepres.2004.02.013

required for generation of cytolytic T lymphocytes and NK cell activation during the host antiviral immune response. Th2-like responses produce IL-4 and IL-10, which help augment antibody production and inhibit development of the Th1 response. It appears that the Th1 response is activated in the liver in response to HCV infection from earlier data [8,10] and an imbalance of Th1 and Th2 responses may play a role in the development of chronicity [11]. It has been reported that NK cells play an important role not only in the early innate host defense against HCV [12,13] but also in the associated hepatocyte injury [14,15]. A contribution of host genetic influences in HCV infection has also been proposed in relation to disease progression [16,17].

The current study was designed to investigate the immune status in hepatitis C virus patients with persistently normal alanine transferase levels (patients with normal ALT). Serum levels and lipopolysaccharide (LPS)-induced IFN-γ, IL12 p70, IL12 p40 and IL-10 by peripheral whole blood, and NK cell activity, were assayed in six patients with normal ALT, 22 cases of HCV-infected patients with chronic hepatitis, 13 with liver cirrhosis, and 26 age-matched controls.

2. Materials and methods

2.1. Patients

Forty-one patients with HCV infection positive for anti-HCV antibodies and HCV-RNA (18 males, 23 females, age: 68.3 ± 8.4 years; range 52–85 years) and 26 controls (11 males, 15 females, age: 64.4 ± 11.1 years; range 50–86 years) were studied. (a) Six of the patients had persistently normal ALT for more than 3 years with levels checked every 2–6 months, despite being HCV-RNA positive. As it is reported that the peak ALT distribution among normal subjects is in the range 11–20 IU/l [18], ALT levels under 29 IU/l were considered normal in this study. Many HCV-infected patients with normal ALT demonstrate elevation between 12 and 32 months of follow-up [19], and the mean observation period was as long as 5.5 ± 1.2 years (range, 3–10) in this study. Needle biopsy of the liver was performed in one patient and tissue proved to be normal. (b) Twenty-two were patients with chronic hepatitis (CH). Nine of the 22 were diagnosed with needle biopsies of the liver and 13 with liver function tests. (c) Thirteen patients were with liver cirrhosis, four diagnosed with laparoscopic biopsy of the liver and nine with liver function tests and non-invasive imaging of the liver. Three had decompensated and 10 had compensated LC. One compensated LC case was complicated with hepatocellular carcinoma. (d) The 26 controls without liver diseases were age-matched and otherwise comparable to the patients with HCV infection.

Demographic data are summarised in Table 1. Although the sex ratio did not differ between patients with HCV infection and age-matched controls, five of six patients with normal ALT were female. Thirteen cases (patients with normal ALT 3; CH 8; LC 2 had a past history of blood transfusion and periods after blood transfusion were long as 36 ± 1, 38.5 ± 12.7 and 43.5 ± 2.1 years, respectively. Titers of HCV-RNA did not differ between patients with normal ALT and those with chronic hepatitis or liver cirrhosis. However, patients with normal ALT were less frequently infected with genotype 1b HCV-RNA compared to the chronic hepatitis cases. Two patients with liver cirrhosis

Table 1
Characteristics of subjects with HCV infection and age-matched controls

Item	Control ($n = 26$)	Patients with normal ALT ($n = 6$)	CH ($n = 22$)	LC ($n = 13$)
Age (year)	64.4 ± 11.1	67.8 ± 3.9	67.0 ± 8.7	71.1 ± 8.0
Sex (male/female)	(11/15)	(1/5)	(12/10)	(5/8)
Blood transfusion (+/−)		(3/3)	(8/14)	(2/11)
Years after blood transfusion		36.0 ± 1	38.5 ± 12.7	43.5 ± 2.1
Type of HCV-RNA (1b/2a/2b)		(2/3/1)	(18/2/2)[a]	(10/2/1)
Titer of HCV-RNA (k > 850/850-100/100 > KIU/ml)		(2/3/1)	(11/8/3)	(5/7/1)
Total bilirubin (mg/dl)	0.7 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	1.3 ± 0.4[b,**]
Albumin (g/dl)	4.7 ± 0.3	4.6 ± 0.4	4.6 ± 0.3	3.8 ± 0.5[b,**]
ALT (IU/l)	23.3 ± 11.4	19.3 ± 4.9	57.5 ± 43.5[b,**]	72.9 ± 52.8[b,**]
WBCs (mm^{-3})	5565 ± 1424	4717 ± 874	4110 ± 847[**]	3435 ± 1478[b,**]
Lymphocytes (mm^{-3})	2130 ± 679	1485 ± 469[*]	1582 ± 341[*]	1221 ± 626[**]
RBCs ($\times 10^4/mm^3$)	447 ± 52	400 ± 36	441 ± 48	387 ± 72[*]
Hb (g/dl)	13.7 ± 1.6	12.5 ± 1.5	13.6 ± 1.7	12.6 ± 2.7
Platelets ($\times 10^4/mm^3$)	22.5 ± 4.5	19.5 ± 4.7	16.3 ± 4.5[**]	8.6 ± 4.4[b,**]

Variable data are mean ± S.D. values. Parenthesis denotes the number of subjects. Patients with normal ALT: patients with persistently normal serum alanine transferase levels, CH: chronic hepatitis and LC: liver cirrhosis.

[a] $P < 0.05$, statistically significant as compared with the number for patients with normal ALT by Fisher's exact probability test.

[b] $P < 0.01$, statistically significant as compared with the value for patients with normal ALT.

[*] $P < 0.05$, statistically significant by the Mann–Whitney test as compared with the value of controls.

[**] $P < 0.01$, statistically significant by the Mann–Whitney test as compared with the value of controls.

had a past history of high consumption of alcohol. Fifteen patients (patients with normal ALT 1; CH 11; LC 3 had a past history of interferon therapy. Nutrient mixtures for hepatic failure (Aminoleban EN®, Otsuka Co. Tokushima, Japan), which are reported to increase NK cell activity [20], were administered to six patients with liver cirrhosis. All those with HCV infection and controls were seronegative for HBsAg (enzyme-linked immunosorbent assay (ELISA)). Informed consent was obtained from each individual, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Venous blood samples from individuals were obtained in the morning in a fasting state.

2.2. Assay for HCV markers

Anti-HCV was assayed with a CobasR Core anti-HCV EIA kit (Roche Diagnostics GmbH, Mannheim, Germany). The presence and titer of HCV-RNA was assessed by reverse transcription-polymerase chain reaction (RT-PCR) using an AMPLICOR GT HCV monitor version 2.0 (Roche Diagnostics GmbH, Mannheim, Germany). The analytical sensitivity of the assay was found to be 0.5 KIU/ml, and linearity was indicated from a lowest titer of 0.5 to a highest of 850 KIU/ml. The HCV-RNA genotype was assessed by direct sequencing using RT-PCR products of AMPLICOR GT HCV monitor version 2.0 [21]. According to differences in nucleotide sequences for the HCV 5′ non-coding region, HCV-RNA division was made into 1a, 1b, 2a, 2b, 3a and others.

2.3. Whole blood induction method

One millilitre of peripheral blood was drawn and placed in a heparinised tube. Within 1 h of sampling, 0.5 ml heparinised blood was cultured at 37 °C for 24 h in 5 ml RPMI-1640 medium (Gibco, Grand Island, NY) with lipopolysaccharide (LPS; 1 μg/ml, *Escherichia coli* O55: B55, Difco) as previously described [22]. The culture supernatants were stored at −80 °C until IFN-γ, IL-12 and IL-10 were assayed.

2.4. Cytokine assays

The culture supernatants and sera were assayed for their IFN-γ, IL-12 p70, Il-12 p40 and IL-10 contents with enzyme-linked immunoassay, following the manufacturer's protocols. IFN-γ, IL-12 p70, IL-12 p40 and IL-10 were assayed with a HUMAN IFN-γ ASSAY Kit (Japan Antibody Lab., Takasaki, Japan), QuantikineR HS (R&D Systems, MN, USA), Quantikine (R&D Systems, MN, USA) and Human IL-10 US UltraSensitive (BioSource International, Inc., CA, US), respectively. The minimum detectable levels of IFN-γ, IL-12 p70, IL-12 p40 and IL-10 have been established as 7.8, 0.5, 15 and 0.2 pg/ml, respectively.

2.5. Assay of NK cell activity

Preparation of peripheral lymphocytes and target cells, and the assays of NK cell activity were performed as previously described [20]. Briefly, peripheral blood mononuclear cells (PBMCs) were separated from heparinised blood by gradient centrifugation and adjusted to $1 \times 10^6\,ml^{-1}$. The K562 cell line, an erythroblastic cell line established from a chronic myelogenous leukemia, was used as the target and adjusted to $1 \times 10^7\,ml^{-1}$.

NK cell activity was measured by chromium release assay. Two hundred 1 μl of PBMCs and 10 μl of K562 cells were added to plastic microplates (Falcon, Oxnard, CA, USA) and then cultured in 5% CO_2 at 37 °C for 4 h. The effector–target cell (E–T) ratio was 20:1. Maximal release (MR) was estimated by culturing the K562 cells in 2% Triton X-100 (E. Merk, Darmstadt, Germany), and spontaneous release (SR) was measured by culturing the K562 cells without PBMCs. All assays were performed in triplicate. After incubation, the microplates were centrifuged, the supernatant was removed and assayed using a gamma counter. Experimental release (ER) was calculated as the mean of results for triplicate cultures. NK cell activity was expressed as ER−SR/MR−SR × 100%.

2.6. Statistics

Results are given as mean ± standard deviation. Differences among the groups were assessed for significance with the Mann–Whitney test. Differences in number of subjects among the groups were compared with the Fisher's exact probability test. Correlations were calculated by Spearman's correlation coefficients. $P < 0.05$ was regarded as indicating statistical significance.

3. Results

3.1. Cytokine serum levels

Data for serum levels of IFN-γ, IL-12 p70, IL-12 p40 and IL-10 in patients with normal ALT, patients with chronic hepatitis and liver cirrhosis and age-matched controls are summarised in Table 2. Serum IFN-γ was detected in only six patients with HCV infection and four controls. While IL-12 p70 was more frequently detected in patients with HCV infection than in controls (patients with normal ALT 5; CH 10; LC 7; controls 2; $P < 0.05$). IL-12 p40 was detected in all patients and controls, and serum levels were increased in HCV-infected patients with chronic hepatitis and liver cirrhosis (CH 168.4 ± 75.4; LC 176.3 ± 95.2; controls 80.8 ± 49.5 pg/ml; $P < 0.01$). No significant difference was observed for serum levels of IFN-γ, IL-12 p70 and IL-10, and proportions positive for IL-10 between patients with HCV infection and controls.

Table 2
Cytokine serum levels in patients with HCV infection and age-matched controls

	Control ($n = 26$)	Patients with normal ALT ($n = 6$)	CH ($n = 22$)	LC ($n = 13$)
IFN-γ detected (+/−)	(4/22)	(0/6)	(4/18)	(2/11)
IFN-γ (pg/ml)	141.7 ± 232.1	–	12.1 ± 4.2	13.8 ± 2.9
IL-12 p70 detected (+/−)	(4/22)	(5/1)**	(10/12)*	(7/6)*
IL-12 p70 (pg/ml)	1.3 ± 1.1	1.2 ± 1.2	0.9 ± 0.4	1.6 ± 1.0
IL-12 p40 (pg/ml)	80.8 ± 49.5	138.4 ± 94.7	168.4 ± 75.4[a]	176.3 ± 95.2[a]
IL-10 detected (+/−)	(17/9)	(5/1)	(17/5)	(11/2)
IL-10 (pg/ml)	1.9 ± 1.7	2 ± 0.2	2.1 ± 1.5	2.1 ± 1.3

Variable data mean ± S.D. values. Parenthesis denotes the number of subjects. Patients with normal ALT: patients with persistently normal serum alanine transferase levels, CH: chronic hepatitis and LC: liver cirrhosis.
[a] $P < 0.01$, statistically significant by the Mann–Whitney test as compared with the value of controls.
* $P < 0.05$, statistically significant as compared with the number of controls by Fisher's exact probability test.
** $P < 0.01$, statistically significant as compared with the number of controls by Fisher's exact probability test.

3.2. LPS-induced cytokine production by peripheral whole blood

Data for LPS-induced IFN-γ, IL-12 p70, IL-12 p40 and IL-10 production by peripheral whole blood in patients with normal ALT, patients with chronic hepatitis and liver cirrhosis, and age-matched controls are summarised in Figs. 1–3. IFN-γ production was significantly lower in patients with chronic hepatitis and liver cirrhosis than in controls (883.1 ± 1167.3, 777.2 ± 891.2, and 2066.5 ± 2094.8 pg/ml, respectively, $P < 0.05$) (Fig. 1). However, IFN-γ production in patients with normal ALT was not reduced (2627.8 ± 2538.5 pg/ml) (Fig. 1). Although variation between individuals in IFN-γ production of patients with normal ALT was observed, four of the six patients showed moderate to strong IFN-γ production. No significant difference was observed for IL-12 p70, IL-12 p40 and IL-10 production between patients with HCV infection and controls (Figs. 2 and 3), or for the ratios of Th-1 associated (IFN-γ)/Th-2 associated (IL-10) parameters (Fig. 3).

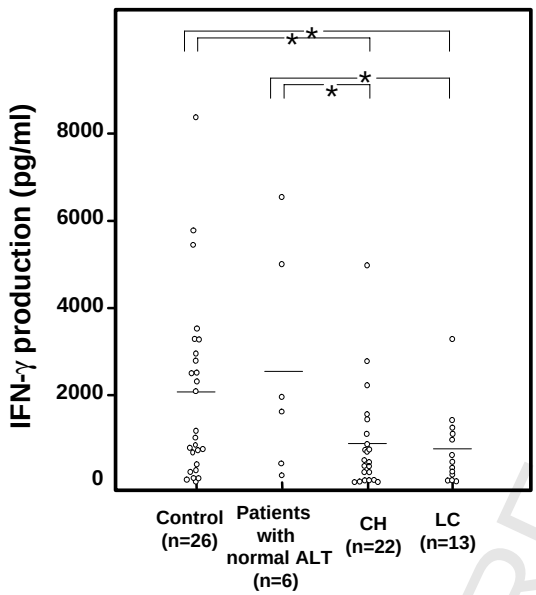

Fig. 1. Production of IFN-γ by lipopolysaccharide-stimulated peripheral whole blood in patients with normal ALT, HCV-infected individuals with chronic hepatitis, liver cirrhosis cases and age-matched controls. Cytokine production was assayed by the whole blood induction method. Small circles represents a single subject. Mean cytokine concentrations are illustrated by horizontal bars. Patients with normal ALT: patients with persistently normal serum alanine transferase levels. $P < 0.05$, statistically significant by the Mann–Whitney test. IFN-γ production was significantly lower in patients with HCV-infected chronic hepatitis and liver cirrhosis, but not patients with normal ALT, than in controls.

3.3. NK cell activity

No significant difference was observed for NK cell activity between patients with HCV infection and controls (Fig. 3).

3.4. Correlation coefficients between IFN-γ and other variables in controls and patients with HCV infection

Correlations of IFN-γ production and other variables (age, sex, genotype and titer of HCV-RNA, peripheral blood analysis, liver function test, IL-12 p70, IL-12 p40 and IL-10 production, serum levels of IL-12 p40 and NK cell activity) were assessed in 26 controls and 41 patients with HCV infection (Table 3). Among controls, IFN-γ production was positively correlated with WBC, lymphocyte count, RBC, IL-12 p70, IL-12 p40 and IL-10 production, and negatively correlated with serum IL-12 p40 levels by Spearman's correlation coefficients. However, among patients with HCV infection, the IFN-γ production was correlated not only with RBC, IL-12 p70, IL-12 p40 and IL-10 production but also with genotype of HCV-RNA. IFN-γ production was significantly lower in patients infected with genotype 1b (30 cases) than those with 2a and 2b (11 cases) (692.9 ± 1011.8 and 2184.4 ± 1997.7 pg/ml, respectively, $P < 0.01$). With respect to age, sex, titer of HCV-RNA and liver function test, there were no significant correlations with IFN-γ production (Table 3).

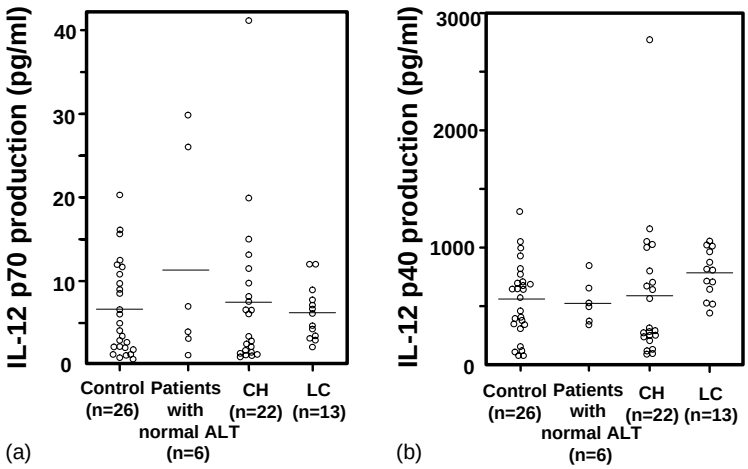

Fig. 2. (a) Production of IL-12 p70 and (b) IL-12 p40 by lipopolysaccharide-stimulated peripheral whole blood in patients with normal ALT, HCV-infected individuals with chronic hepatitis, liver cirrhosis cases and age-matched controls. Cytokine production was assayed by the whole blood induction method. Small circles represents a single subject. Mean cytokine concentrations are illustrated by horizontal bars. Patients with normal ALT: patients with persistently normal serum alanine transferase levels. No significant difference was observed for IL-12 p70, IL-12 p40 production between patients with HCV infection and controls.

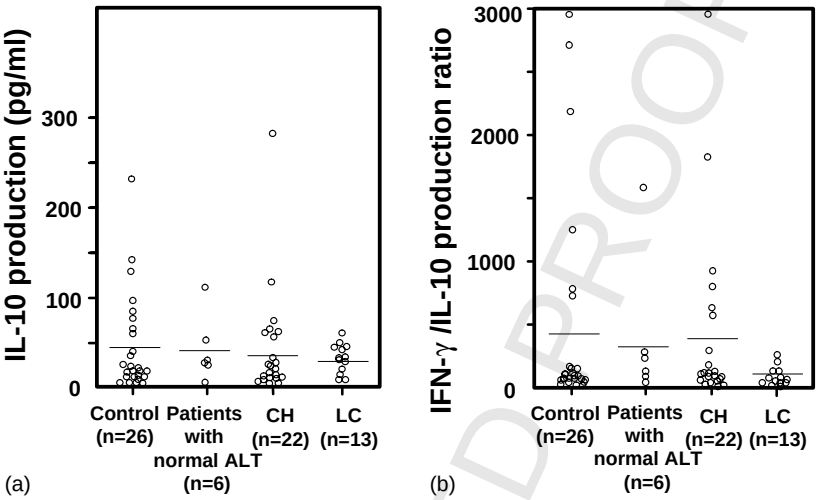

Fig. 3. (a) Production of IL-10 by lipopolysaccharide-stimulated peripheral whole blood and (b) the ratios of Th-1 associated (IFN-γ)/Th-2 associated (IL-10) parameters in patients with normal ALT, HCV-infected individuals with chronic hepatitis, liver cirrhosis cases and age-matched controls. Cytokine production was assayed by the whole blood induction method. Small circles represents a single subject. Mean cytokine concentrations are illustrated by horizontal bars. Patients with normal ALT: patients with persistently normal serum alanine transferase levels. No significant difference was observed for IL-10 production and the ratios of TH-1 associated/Th-2 associated parameters between patients with HCV infection and controls.

4. Discussion

It is reported that there are several factors that could have an impact on progression of chronic hepatitis C [23]. In this study, age at infection was suspected as being almost the same between patients with normal ALT and patients with chronic hepatitis judging from years after blood transfusion. Regarding sex, there is moderate evidence to indicate that the rate of progression of liver disease is lower among females than males [23,24] and in this study, although the sex ratio did not differ between patients with HCV infection and age-matched controls, five of six patients with normal ALT were females. Although there is no evidence that the viral concentration has any effect on disease progression [23], there is a report that viral genotype exerts an influence, as a higher rate of progression was found among persons infected with genotype 1b [25]. In this study, only two of six patients with normal ALT, in comparison with 80% (28/35) of patients with chronic hepatitis and liver cirrhosis, were infected with genotype 1b (Table 1).

The immune status has been investigated with reference to variation between individuals in the rate of progression of HCV-infected chronic liver diseases by a number of investigators [15,23,26–29]. It is reported that percentages of $CD8^+$ T cells [15] and $\delta\gamma$ TCR^+ T cells [15,26], NK cell activity [15], and serum IL-10 level [29] are higher in car-

Table 3
Spearman's correlation coefficients between IFN-γ and other variables in controls and patients with HCV infection

Items	IFN-γ production	
	Controls ($n = 26$)	Patients with HCV infection ($n = 41$)
Age	NS	NS
Sex	NS	NS
Type of HCV-RNA (1b/others)	–	0.512**
Titer of HCV-RNA (k > 850/850 > KIU/ml)	–	NS
Total bilirubin	NS	NS
Albumin	NS	NS
ALT	NS	NS
WBC	0.415*	NS
Lymphocyte	0.434*	NS
RBC	0.490*	0.310*
Hb	NS	NS
Platelet	NS	NS
IL-12 p70 production	0.510**	0.716**
IL-12 p40 production	0.474*	0.408**
Serum IL-12 p40 level	−0.420*	NS
IL-10 production	0.493*	0.351*
NK cell activity	NS	NS

NS: not significant.
* $P < 0.05$, statistically significant.
** $P < 0.01$, statistically significant.

riers with normal ALT than in those with chronic hepatitis C. However, other authors described percentages of IFN-γ positive $CD4^+$ T cells [28] and IFN-γ production [27] did not differ between these two groups. Therefore, the question remains controversial.

Immunomodulatory functions of IFN-γ in HCV infection are not fully understood. Recently, Thimme et al. [30] reported IFN-γ mRNA to be detectable only in the livers of chimpanzees that cleared or controlled the disease, raising the possibility that IFN-γ might perform non-cytolytic antiviral effector functions during HCV infection. On the other hand, previous studies indicated the progressive liver injury seen in chronic HCV is associated with the upregulation of intrahepatic IFN-γ, a Th1-like cytokine and with down regulation of IL-10, a Th2-like cytokine [10]. In this study, the proportions positive for serum IFN-γ and IL-10, and serum levels of those cytokines did not differ between patients with HCV infection and controls. To gain further insight into Th-cell responsiveness in HCV infection, mitogen-induced production by peripheral mononuclear cells, which is considered to reflect the individual immunological competence, has been assessed. Most investigators have found that patients with HCV infection [31–33], especially with advanced liver disease [34], display deficient IFN-γ production by peripheral blood mononuclear cells on stimulation with various mitogens. However, patients with chronic viral hepatitis may display strong release of INF-γ by peripheral blood T cells [35]. In the present study, IFN-γ production was significantly lower in patients with HCV-infected chronic hepatitis and liver cirrhosis than in controls. However, IFN-γ production in patients with normal ALT was not significantly reduced, indicating for the first time that patients with normal ALT conserve the immunological competence for Th1-like cytokine production. Although variation between individuals in IFN-γ production of patients with normal ALT was observed, four of six showed moderate to strong IFN-γ production. We here strictly selected long-term patients with normal ALT to be comparable with those demonstrating chronic liver disease. One reason why we found a difference, while other investigators did not, might be because IFN-γ production was assayed via the whole blood induction method instead of by isolating peripheral mononuclear cells from peripheral blood. The advantage of the whole blood induction method is in evaluating immune competence which is reflective of the in vivo condition [36].

Although the mechanisms of IFN-γ production by peripheral whole blood in response to LPS stimulation are not fully understood, IL-12 is the main stimulatory cytokine. Kanto et al. [37] reported that dendritic cells from HCV-infected individuals produce lower levels of IL-12 in response to LPS compared to those from normal controls, and this may lead to low IFN-γ production by $CD4^+$ T cells. However, in the present study, there were no significant differences in IL-12p 70 and IL-12 p40 production between patients with HCV infection and controls (Fig. 2). Rather, the cases with detectable serum IL-12 p70 and serum levels of IL12 p40 were higher in HCV-infected patients with chronic hepatitis and liver cirrhosis than in the controls (Table 2). It has been speculated that down-regulation of IFN-γ production in HCV-infected chronic liver diseases is secondary to inhibition of IL-12 activity due to increased IL-12 p40 [38]. As for Th2 associated parameters, which inhibit development of the Th1 response, no significant variation was observed for IL-10 production and the ratios of Th-1 associated

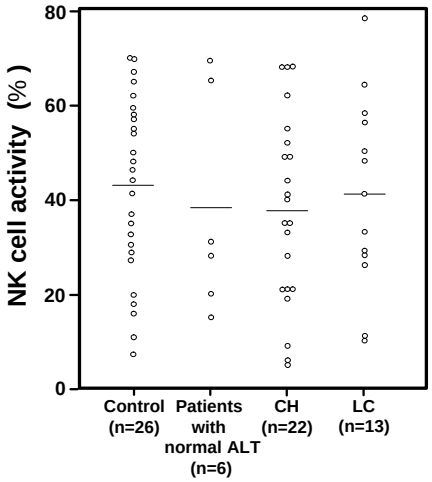

Fig. 4. NK cell activity in patients with normal ALT, HCV-infected individuals with chronic hepatitis, liver cirrhosis cases and age-matched controls. Small circles represents a single subject. Mean NK cell activity is illustrated by horizontal bars. Patients with normal ALT: patients with persistently normal serum alanine transferase levels. No significant difference was observed for NK cell activity between patients with HCV infection and controls.

IFN-γ/Th-2 associated IL-10 between patients with HCV infection and controls (Fig. 3). Concerning IFN-γ production by NK cells, Tseng and Klimpel reported that cross-linking of CD81 on NK cells by HCV envelope protein E2 resulted in reduced IFN-γ production in response to IL-12 in vitro [39]. However, in this study, NK cell activity was not different between patients with HCV infection and controls (Fig. 4). Our data thus suggest that deficiency in IFN-γ production observed in HCV-infected chronic liver diseases may be independent to deficiency in IL-12, abundance in IL-10 or weakened NK cell activity. Other immunoregulatory factors, such as IL-18, IL-15 and TGF-γ [9], might be involved in the down-regulation of IFN-γ production.

In order to cast light on possible mechanisms of preserved IFN-γ production in patients with normal ALT, factors, which were positively correlated in controls (Table 3), such as WBC, lymphocyte count, RBC, IL-12 p70, IL-12 p40 and IL-10 production, and negatively correlated serum IL-12 p40 levels, were also assessed in patients with normal ALT, and those with chronic hepatitis or liver cirrhosis (Table 1, Figs. 2 and 3). However, there were no significant intergroup differences, indicating no connection with IFN-γ production in patients with normal ALT. In this study, IFN-γ production was significantly lower in patients infected with genotype 1b than in those with 2a and 2b. Only two of six patients with normal ALT were infected with genotype 1b, which may thus, in part explain the preserved IFN-γ production, although, mean IFN-γ production in 1b infected patients with normal ALT was as high as that in 2a and 2b infected counterparts (data not shown). Westendorp [40] reported that genetic factors substantially influence production of cytokines induced by LPS and that the cytokine profile may be connected with the outcome of meningococcal diseases. Variation in IFN-γ production among patients with normal ALT and controls, as observed in our study, might be a reflection of genetic influences. Although it is generally thought that IFN-γ, a Th1-like cytokine, is associated with progression of liver injury [10], animal experiment revealed that IFN-γ reduces extracellular matrix deposition in liver fibrosis by inhibition of hepatic stellate cell activation [41]. Preserved IFN-γ production in patients with normal ALT may be interpreted, not as a hazardous expression, but as a good prognostic indicator.

In conclusion, our results suggest that preserved IFN-γ production in patients with normal ALT, in contrast to the reduction in chronic hepatitis and liver cirrhosis, may be related to a slow rate of disease progression. A further study with a large number of patients with persistently normal ALT levels is necessary to confirm the present results and to clarify the underlying mechanisms.

Acknowledgements

The authors thank Dr. Malcolm Moore for critical reading of the manuscript, Dr. Toshio Matsui for advice on statistics and Dr. Tadayoshi Takegoshi for supply of HCV-infected patients.

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