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Circulating Th1 and Th2 cytokines in patients with hepatitis C virus infection

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THE imbalance of T-helper (Th) lymphocyte cytokine production may play an important role in immunopathogenesis of persistent hepatitis C virus (HCV) infection. To know whether an imbalance between Th1 and Th2 cytokines is present in chronic HCV infection, serum levels of Th1 cytokines, interferon gamma (IFN-γ) and interleukin (IL)-2, and Th2 cytokines, IL-4 and IL-10, were measured using enzyme-linked immunosorbent assay in this study. Eighteen individuals with chronic HCV infection, 11 healthy subjects as normal controls and 10 chronic HBV infected patients as disease controls were observed. The results showed that the levels of Th2 cytokines (IL-4 and IL-10) were significantly increased in chronic HCV infected patients compared with normal controls (IL-4: 30.49 ± 17.55 vs. 14.94 ± 13.73, pg/ml, $P<0.025$; IL-10: 50.30 ± 19.59 vs. 17.87 ± 9.49, pg/ml, $P<0.001$). Similarly, the levels of Th1 cytokine, IL-2, was also elevated in individuals with chronic HCV infection when compared with normal controls (IL-2: 118.53 ± 95.23 vs. 61.57 ± 28.70, pg/ml, $P<0.05$). However, Th1 cytokine IFN-γ level was not significantly changed during HCV infection (IFN-γ: 28.09 ± 15.65 vs. 24.10 ± 15.61, pg/ml, $P>0.05$). Furthermore, the elevated levels of Th2 cytokines are greater than Th1 cytokines in HCV infection. Thus, the study indicates that an enhanced Th2 responses are present during chronic HCV infection, which may partly be responsible for the persistence of HCV infection.

Key words: Hepatitis C virus, T-helper lymphocytes, Cytokines

Introduction

Hepatitis C virus (HCV) infection has been confirmed to be the major aetiologic agent for post-transfusion hepatitis all over the world. One of the characteristic features is at least half of the infection to become chronic.[1–3] The high mutational rate of the viral genome is considered to be responsible for persistent HCV infection.[3] The inability of the host immune function to eliminate an organism is also an important cause for persistentive form of infection.[4] However, little has been learned about the host cellular immune response to HCV. The role of the immunoregulatory cytokines in chronic HCV infection is recently being stressed.[5,6] In this study, to better know the profiles of cytokines production in individuals with chronic HCV infection, we measured serum levels of T-helper lymphocytes (Th)1 cytokines interferon gamma (IFN-γ), interleukin (IL)-2, and Th2 cytokines IL-4, IL-10 in such patients.

Subjects and Methods

Subjects

Eighteen patients with chronic HCV infection were included in this study. The diagnosis of chronic HCV infection was based on the seropositivity of HCV specific antibody and HCV-RNA for at least 6 months of ≥ 2 times detection. There was no serologic evidence of co-infection with other hepatotropic viruses. Other possible causes of hepatocellular injury, such as alcohol, drugs and autoimmune diseases were also excluded. No immunoregulatory agents were administered in recent 3 months before enrolment. The characteristics of 18 chronic HCV infected individuals were summarized in Table 1.

As normal controls, 11 healthy subjects who were negative for both serum anti-HCV and hepatitis B virus (HBV) markers were selected without a clinical history of hepatitis and without symptoms or signs of liver diseases (seven males and four females; mean age 32 years, range 20–47 years). Also, 10 chronic

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Table 1. Details of individuals with chronic HCV infection

Patient	Sex (F/M)	Age (years)	Blood transfusion history (time)	Anti-HCV	HCV-RNA	ALT(U/L)*
1	F	30	yes (1995)	+	+	150
2	F	23	no	+	+	<40
3	M	68	yes (1995)	+	+	138
4	M	40	yes (1993)	+	+	184
5	M	42	yes (1989)	+	+	66
6	F	27	yes (1990)	+	+	180
7	M	58	yes (1995)	+	+	158
8	M	49	yes (1992)	+	+	252.2
9	F	51	yes (1991)	+	+	<40
10	F	61	yes (1989)	+	+	<40
11	M	26	yes (1995)	+	+	60.5
12	M	44	yes (1992)	+	+	760
13	M	45	yes (1993)	+	+	48.5
14	F	28	yes (1994)	+	+	74.3
15	M	38	yes (1994)	+	+	742
16	M	43	yes (1994)	+	+	48
17	F	54	yes (1990)	+	–	71.7
18	F	66	yes (1990)	+	+	129.8

*ALT: alanine transaminase, normal value ≤40 U/l.

hepatitis B patients (seven males and three females, mean age 38 years, range 23–56 years) with no serologic evidence of HCV infection were recruited as disease controls, who had seropositivity of HBV markers and abnormal liver tests for more than 6 months.

Methods

Serologic detections of HBV markers, anti-HCV (a second-generation test) were carried out using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Sino-American Biotechnology Company, Louyan, China). The procedures were performed according to the manufacturer's instruction. Serum HCV-RNA was determined by reverse transcription polymerase chain reaction (RT-PCR). Briefly, RNA was extracted from the samples by single step method as described previously.[7] The amplification was done after an initial denaturation step (94°C, 5 min) by 32 cycles (94°C, 30 s; 58°C, 30 s; 72°C, 1 min) and 5 min at 72°C in a thermocycler (Perkin-Elmer Cetus, Changsha Branch, Changsha, China). The amplified cDNA fragments were analysed on a 1.5% agarose gel. The bands were visualized by ethidium bromide staining.

Quantification of serum cytokines was measured by ELISA using commercially available kits (Genzyme, Shenzheng Branch, Shenzheng, China). The sera were stored at –30°C until assay (in 3 months) and each sample was examined in duplicate. The assays were performed following the manufacturer's instruction.

Statistics

Data were analysed by non-parametric test and expressed as mean ± standard division.

Results

The serum levels of IFN-γ, IL-2, IL-4 and IL-10 were measured in patients with HCV or HBV infection and normal controls. As shown in Table 2, Th2 cytokines (IL-4 and IL-10) levels were significantly increased in chronic HCV infected patients compared with normal controls ($P<0.025$, $P<0.001$). Similarly, the levels of the Th1 cytokine, IL-2, was also elevated in individuals with chronic HCV infection when compared with normal controls ($P<0.05$). However, Th1 cytokine IFN-γ level was not significantly changed during HCV infection ($P>0.05$).

Table 2. The comparison of serum cytokines in three studied groups

	IFN-γ (pg/ml)	IL-2 (pg/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)
Control	24.10 ± 15.61	61.57 ± 28.70	14.94 ± 13.73	17.87 ± 9.49
HBV infection	32.48 ± 19.99	56.75 ± 20.04	21.44 ± 11.23	31.16 ± 17.15**
HCV infection	28.09 ± 15.65	118.53 ± 95.23*	30.49 ± 17.55**	50.30 ± 19.59***

*,**,***: *P* values <0.05, <0.025, <0.001 compared with normal controls respectively.

Table 3. The relation of cytokines and ALT in HCV infection

ALT (U/L)	IFN-γ (pg/ml)	IL-2 (pg/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)
>100	25.94 ± 14.77	132.36 ± 107.08	36.71 ± 21.74	51.97 ± 24.46
≤100	30.24 ± 16.20	104.71 ± 79.31	24.28 ± 8.11	48.63 ± 11.01

ALT: alanine transaminase, no significance difference in each group.

In chronic HBV infected patients, serum levels of IFN-γ, IL-4 and IL-10 were higher than those in normal controls, though only IL-10 level reached a statistical difference (Table 2). Furthermore, the elevated level of serum IL-10 was smaller in HBV infected patients compared with that in HCV infected patients.

The serum levels of IFN-γ, IL-2, IL-4 and IL-10 have not been found to be related to serum activity of alanine transaminase (ALT) in HCV infected individuals (Table 3).

Discussion

It has been demonstrated that Th lymphocytes may be subdivided into Th1 and Th2 cells based on the distinct patterns of cytokine production.[8] Th1 cells produce IFN-γ, IL-2 and lymphotoxin which promote cell-mediated effector responses; whereas Th2 cells produce IL-4, IL-5, IL-6 and IL-10 cytokines which influence B-cell development and can augment humoral responses. Cytokines released by one type of Th lymphocyte population can down-regulate the functions of another Th population. Th1 responses are associated with immunity or resistance to infection, while Th2 responses are associated with the progression or persistence of infection.[9,10] A shift of Th1 to Th2 responses has been implicated in the pathogenesis of some infectious diseases, such as human immunodeficiency virus infection, mycobacterial and protozoal diseases.[4,10]

Inflammatory cytokines play an important role in pathogenesis of hepatitis B.[11] However, the reports on the manner of cytokines in chronic HCV infection are rare. The current study showed that the increased production of Th1 (IFN-γ and IL-2) and Th2 (IL-4 and IL-10) cytokines are present in HCV infection, suggesting Th cells are activated *in vivo* due to HCV infection. This results is in agreement with another observation reported by Cacciarelli.[6] It is also noted that the elevated levels of Th2 cytokines are greater than Th1 cytokines in HCV infection. In view of negative regulation of Th2 cytokines for immune functions,[4] we consider that enhanced Th2 reaction is at least partly responsible for immunopathogenesis of chronic HCV infection. We also propose that enhanced Th2 responses in HCV infection may allow the human host to suppress the inflammatory/immune responses,[12,13] resulting in reducing the hepatic tissue injury through down-regulation of the inflammatory/immune reaction and leading to inability to eliminate the virus. This is one possible explanation why HCV infection tends to be a chronic condition. To further elucidate the role of these cytokines in immunopathogenesis of chronic HCV infection, the levels of mRNA expression for intrahepatic Th cytokines are currently performing.

Similarly, an increased production of IL-10 was observed for chronic HBV infection in the study. Based on the results in this study, enhanced Th2 responses, however, are obviously weaker in HBV infection than in HCV infection. It is possibly one of the causes that the chronic feature is more common in HCV infection than in HBV infection.

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