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Analysis of the relationship between peripheral blood T lymphocyte subsets and HCV RNA levels in patients with chronic hepatitis C

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ABSTRACT. We investigated the relationship between peripheral blood T lymphocyte subsets and hepatitis C virus (HCV) RNA levels in patients with hepatitis C. Samples from 69 chronic hepatitis C (CHC) patients and 20 healthy controls were analyzed using quantitative polymerase chain reaction (PCR) to detect HCV RNA and flow cytometry to determine the expression levels of CD3, CD4, and CD8 in lymphocytes. The percentage of CD4+ T cells (42.87 ± 6.11%) and the ratio of CD4+/CD8+ (1.34 ± 0.25) in these patients were significantly lower than those in the healthy control group (49.55 ± 6.68%, 1.82 ± 0.11, respectively) ($P < 0.01$, $P < 0.01$), while the percentage of CD8+ T cells (32.78 ± 5.48%) was higher than that in the control group (27.35 ± 4.32%) ($P < 0.01$). There was no significant difference in the percentage of CD3+ T cells between the two groups ($P > 0.05$). With the increase in HCV RNA replication, the percentage of CD8+ T cells increased gradually, while the CD4+ T cell percentage and CD4/CD8 ratio decreased. The change in the percentage of T lymphocyte subsets may be one of the reasons for

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persistent HCV infection, and the high expression levels of HCV RNA might be the reason for the low frequency of CD4+ T lymphocytes in patients with chronic HCV.

Key words: Chronic hepatitis C; Hepatitis C virus; HCV RNA; T lymphocyte subsets

INTRODUCTION

The hepatitis C virus (HCV) is the cause of one of the world's most serious public health care issues. About 200 million people are infected with HCV and 54-86% of the infected patients progress to chronic infection within 6 months, subsequent to persistent infection. HCV causes chronic hepatitis C (CHC); there is a high prevalence of the disease in China with an infection rate of 3.2% (Post et al., 2009). The relationship between the course of the disease and the number and function of T lymphocyte subsets has proven important in many studies (Chen et al., 2009). The mechanisms involved in chronic HCV infection, especially the immune responses induced by host immune factors, have become a topic of great interest for researchers. We detected the reproduction and load levels of HCV RNA in the T lymphocyte subsets of peripheral blood serum from CHC patients. We also analyzed the relationship between HCV RNA levels and T lymphocyte subsets to investigate immune dysfunction in hepatitis C patients, with the aim of providing a certain theoretical basis for the pathogenesis and prevention of hepatitis C.

MATERIAL AND METHODS

General information

Sixty-nine adult CHC patients (23 males and 46 females, aged 17-67 years, mean age 45.8 ± 13.8 years) from our hospital's outpatient and in-patient departments were selected as the treatment group between February 2011 and July 2013. Their diagnoses were all in line with the 2011 "Hepatitis C Prevention Guide" diagnostic criteria (EASL, 2011), and patients who also had other diseases (including other viral hepatitis, autoimmune diseases, alcoholic liver disease, cirrhosis, cancer, metabolic diseases, and underlying diseases) were excluded. Furthermore, none of the selected patients had received antiviral or immunomodulatory medications in the 6 months prior to the test. The subjects were divided into three groups: A (high-load group, HCV RNA viral load >1.00 x 10^5 copies/mL); B (low-load group, HCV RNA viral load >1.00 x 10^5 copies/mL); and C (RNA of less than 1.00 x 10^3 copies/mL). Group D comprised 20 age- and gender-matched healthy controls (aged 36-59 years, mean age 44.6 ± 6.6 years). All the liver and kidney function indicators in the experimental and control groups were normal.

Cells, instruments, and reagents

Flow cytometry was carried out using a COULTER Epics XL MCL flow cytometer

produced by the Beckman Coulter Company. Fluorescent-labeled D3/CD4/CD8 monoclonal antibodies and red blood cell lysing liquid were selected from original OptiClone reagents. The polymerase chain reaction (PCR) amplification was carried out using a LightCycler fluorogenic quantitative nucleic acid amplifier. The HCV nucleic acid quantification kit was selected from kits produced by the Shanghai Kehua Bio-engineering Co., Ltd.

HCV RNA quantification method

The real-time fluorescence quantitative PCR was carried out in strict accordance with manufacturer instructions, and HCV RNA detection is indicated by copies/mL.

Detection of T lymphocyte subsets

We collected 1 mL peripheral venous blood from each CHC patient and healthy control subject in ethylenediaminetetraacetic acid (EDTA)-K2 tubes. Tri-labeled monoclonal antibody (20 μL) (CD4-PE/CD8-ECD/CD3-PC5) was then added to the clean test tubes. Another 100 mL anticoagulated venous blood was added and mixed before incubation for 20 min at 25°C in the dark. Erythrocytolysis reagent (250 μL) was added to the tube and dissolved for 20 min. We then centrifuged the samples at 1200 revolutions/min for 5 min and removed the supernatant; the samples were washed twice with phosphate-buffered saline, and the cells were suspended in 500 mL phosphate-buffered saline. Lymphocyte counts of over 10,000 per sample were detected by flow cytometry.

Statistical analysis

Data are reported as means ± standard error. The enumeration data are reported as percentages. Mean comparison in groups was conducted using analysis of variance, and the enumeration data were compared using the Fisher test. The relationships between data were subjected to Spearman rank correlation analysis. Statistical analysis was carried out by the SPSS software.

RESULTS

Distribution of T cell subsets in the CHC patients' peripheral blood

A comparison of the T cell subsets in the CHC patients' peripheral blood and in the healthy controls is given in Table 1 and Figure 1A and B.

Correlation analysis between the HCV RNA load and T cell subsets in CHC patients

According to the comparison between the groups shown in Table 2, there was no remarkable correlation between the percentage of CD3+ T cells and the HCV-RNA concentration by Spearman's rank correlation analysis ($r = 0.068$, $P = 0.576$). The percentages of the CD4+ and CD8+ T cells, and the CD4/CD8 ratios were related to HCV concentration ($r = 0.477$, $P < 0.01$; $r = 0.599$, $P < 0.01$; $r = 0.896$, $P < 0.01$). With increasing HCV RNA concen-

tration, the percentage of CD8+ T cells increased gradually, whereas the CD4+ T cell percentage and the CD4/CD8 ratio decreased gradually (Figure 2).

Table 1. Distribution of T cell subsets in the peripheral blood of chronic hepatitis C (CHC) patients and healthy controls

	CHC patients (N = 69)	Healthy controls (N = 20)	*t*	P
CD3+ (%)	76.72 ± 8.75	77.25 ± 11.13	0.474	0.636
CD4+ (%)	42.87 ± 6.11	49.55 ± 6.68	4.212	0.000
CD8+ (%)	32.78 ± 5.48	27.35 ± 4.32	4.071	0.000
CD4+/CD8+	1.34 ± 0.25	1.82 ± 0.11	8.454	0.000

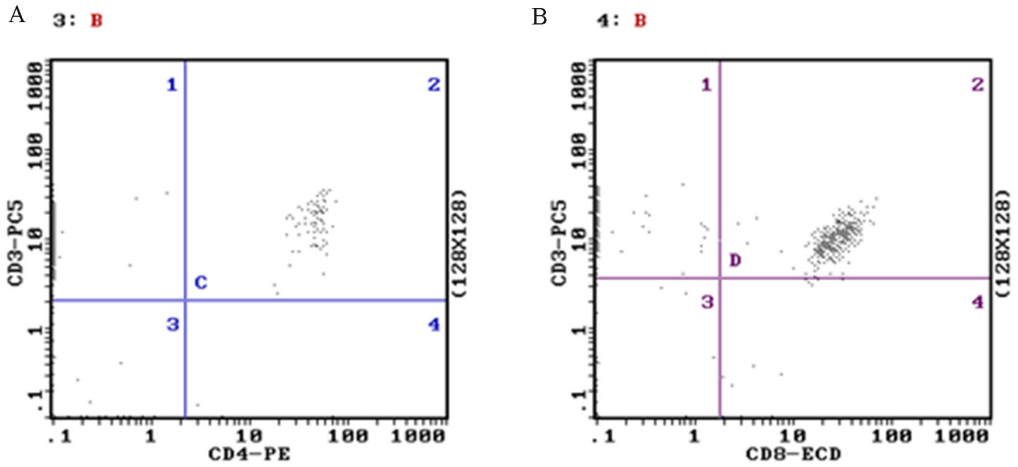

Figure 1. Scatter plots of CD3/CD4 and CD3/CD8 cells in the chronic hepatitis C patients. The CD4+ T cells decreased, the CD8+ T cells increased, and the CD4/CD8 ratio was significantly lower than that in the healthy controls.

Table 2. Distribution of T cell subsets in the peripheral blood of chronic hepatitis C patients at different hepatitis C virus RNA concentrations.

	Group	Number of subjects	CD3+ (%)	CD4+ (%)	CD8+ (%)
Group A	24	76.25 ± 9.23	39.01 ± 4.89	37.21 ± 5.24	1.06 ± 0.11
Group B	23	75.65 ± 9.14	43.74 ± 5.72	31.87 ± 4.00	1.38 ± 0.13
Group C	22	75.05 ± 8.90	46.14 ± 5.66	28.91 ± 3.41	1.60 ± 0.08
Group D	20	77.00 ± 10.65	49.55 ± 6.68	27.35 ± 4.32	1.82 ± 0.11
		qAB = 0.598, P = 0.829	qAB = 4.70, P = 0.006	qAB = 5.339, P < 0.01	qAB = 0.319, P < 0.01
		qAC = 1.205, P = 0.667	qAC = 7.09, P < 0.01	qAC = 8.299, P < 0.01	qAC = 0.539, P < 0.01
		qAD = 0.75, P = 0.794	qAD = 10.51, P < 0.01	qAD = 9.858, P < 0.01	qAD = 0.762, P < 0.01
		qBC = 0.607, P = 0.830	qBC = 2.397, P = 0.164	qBC = 2.96, P = 0.024	qBC = 0.220, P < 0.01
		qBD = 1.348, P = 0.643	qBD = 5.81, P = 0.001	qBD = 4.52, P = 0.001	qBD = 0.444, P < 0.01
		qCD = 1.955, P = 0.506	qCD = 3.413, P = 0.057	qCD = 1.56, P = 0.246	qCD = 0.223, P < 0.01
F		0.165	13.165	22.857	192.65
P		0.920	0.00	0.00	0.00

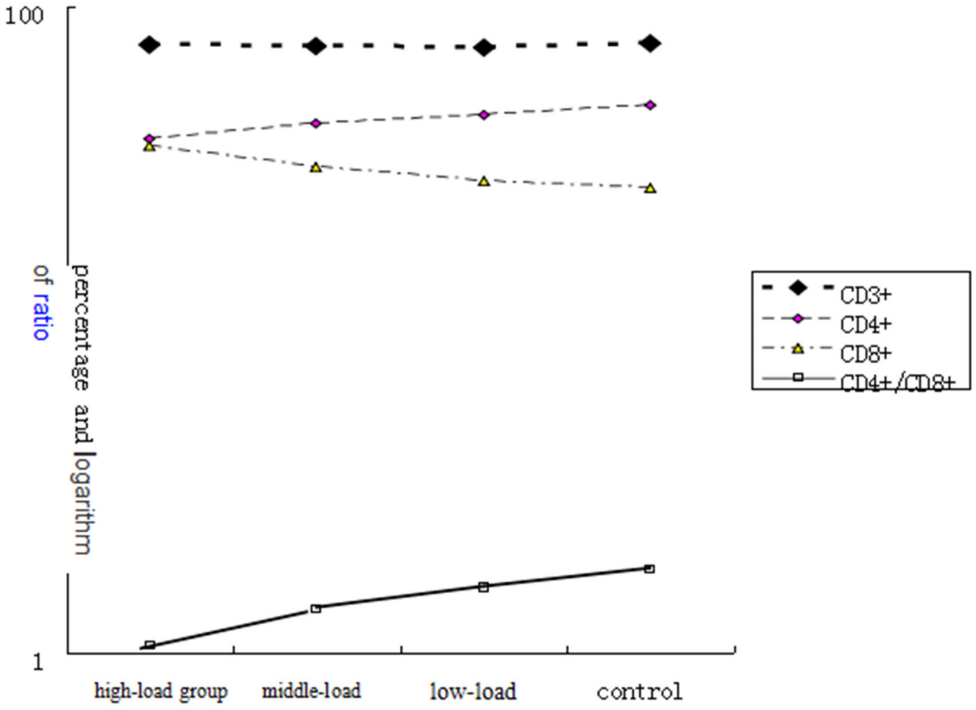

Figure 2. Correlation between the hepatitis C virus RNA load and T cell subsets in chronic hepatitis C patients.

DISCUSSION

It is currently believed that the cellular immune response plays a major role in disease progression and prognosis in CHC patients (Hiroishi et al., 2008; Satake et al., 2008). Lymphocytes are the main immune cells and form the foundation of the immune response, and their relative numbers reflect the level of immunity to some extent. It is known that the liver has the ability to compensate for the injuries it receives, and when HCV invades the liver, the liver injury is usually not manifested in an increase in the serum biochemical indices. However, immune dysfunction plays an important role in causing liver injury (Dong et al., 2011). Keeping the proportion of peripheral blood T lymphocyte subsets relatively stable is important for maintaining an organism's normal immune function. T lymphocyte counts and the ratio between subsets are the main indictors of an organism's level of immunity. CD3+ T cells (total T cells) and CD4+ T cells (helper T cells) not only have auxiliary and inducing roles, but also secrete lymphokines, initiate delayed hypersensitivity reactions, and induce activation of macrophages, subsequently causing liver injury (Satake et al., 2008). CD4+ T cells can also boost the proliferation and differentiation of B cells, cytotoxic T lymphocytes, and other immune cells, and modulate humoral and cellular immunity. CD8+ T cells (cytotoxic T cells) comprise cytotoxic T lymphocytes and a few T suppressor cells. The two kinds of cell are present in

a certain ratio under normal circumstances, which maintains the organism's normal cellular immune function.

Our experiments showed that the number of CD8+ cells in the CHC patients was higher than in the healthy control group ($P < 0.01$), while the number of CD4+ cells and the CD4+/CD8+ ratio in the patients were both lower than in the control group ($P < 0.01$). There was a significant difference in the CD3+ T cell counts between the CHC patients and the healthy controls ($P > 0.05$), which is consistent with the Chinese researchers' results (Wang et al., 2009; Liang et al., 2012). The change in the proportion of peripheral blood T lymphocyte subsets after HCV infection may be the cause of chronic HCV infection. The pathogenesis of HCV is still not very clear. Currently, many researchers believe that cellular immunity may constitute the main pathogenesis of HCV. In CHC patients, sensitized CD8+ T cells, with help from antigen presenting cells, attack and destroy liver cells. It has recently been discovered that many kinds of inhibitory receptors are expressed in HCV-specific CD8+ T cells (Liu et al., 2010), and antigen-specific CD4+ T cells play an important role in HCV clearance and pathogenesis. The ratio of these two kinds of cell changes and may cause a failure in the coordination between them, which results in chronic persistent infection (Bengsch et al., 2010). CD4+ T cells play an important role in activating antigen-presenting cells and inducing the CD8+ T cell immune response. A strong and enduring CD4+ T cell immune response can enhance an organism ability to rid itself of viruses. The depletion of CD4+ T cells in a HCV-infected chimpanzee can cause chronic HCV infection, even with normal CD8+ T cellular immunity (Accapezzato et al., 2004; Spangenberg et al., 2005; McCaughan and Bowen, 2011). Some researchers believe that the coordination between CD4+ and CD8+ T cells can control viral infection effectively; without a lasting and comprehensive CD4+ T cell response it is usually impossible to effectively eliminate a virus, which results in chronic infection (Sugimoto et al., 2003).

The serum HCV RNA content reflects the degree of active HCV replication and disease evolution. It also confirms the presence of HCV in gene diagnosis and is an indicator of HCV infectivity. There is a positive correlation between RNA load in the serum and HCV RNA levels in the liver (Lemon et al., 2010). Detection of serum HCV RNA can be used as a direct indication of hepatitis C viremia. We also analyzed the load of HCV RNA in the sera of CHC patients, and studied the relationship between the degree of virus replication and cellular immunity. The results showed that the percentage of CD4+ T cells and the CD4+/CD8+ ratio decreased, apparently with an increase in the virus copy number ($P < 0.01$). However, the percentage of CD8+ T cells was proportional to the increase in HCV RNA copies, which shows that immune dysfunction leads to increased levels of circulating virus. A low immune response makes it difficult for the organism to remove the virus. According to our tests, the RNA-negative group showed significant differences in the percentage of CD4+ and CD8+ T cells compared with the high-load and low-load groups ($P < 0.01$). There was no significant difference compared with the healthy control group ($P > 0.05$), but the CD4+/CD8+ ratio was significantly lower than that in the healthy control group. Immune function change is primarily manifested in the CD4/CD8 ratio reduction after HCV infection, implying that the continual reduction of this ratio is highly relevant to persistent HCV infection and the number of viral copies.

In summary, the change in the proportion of T lymphocyte subsets in the peripheral blood of CHC patients may be one of the important causes of chronic HCV infection. The increase in HCV RNA replication further aggravates the imbalance of T cell subsets. The dynamic changes in the CD4+/CD8+ ratio can be a timely indicator of a change of cellu-

lar immune function in clinical HCV infections, and can strengthen the clinical prediction. Therefore, the combined detection of serum HCV RNA and T lymphocyte subpopulations in the peripheral blood of patients with chronic HCV infection is valuable for observing viral replication and judging cellular immunity.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Accapezzato D, Francavilla V, Paroli M, Casciaro M, et al. (2004). Hepatic expansion of a virus-specific regulatory CD8(+) T cell population in chronic hepatitis C virus infection. *J. Clin. Invest.* 113: 963-972.

Bengsch B, Seigel B, Ruhl M, Timm J, et al. (2010). Coexpression of PD-1, 2B4, CD160 and KLRG1 on exhausted HCV-specific CD8+ T cells is linked to antigen recognition and T cell differentiation. *PLoS Pathog.* 6: e1000947.

Chen SL, Bian J, Zhang F and Zhao HN (2009). Cellular immune function in patients with chronic hepatitis C. *Yixue Yanjiu yu Jiaoyu* 26: 8-10.

Dong XH, Zhou X, Zhou LP and Zheng Y (2011). Relationship between serum HCV RNA in patients with hepatitis C between TNF-α and its correlation with the degree of liver injury research. *Xiandaiyixuejianyanzazhi* 26: 21-27.

EASL (European Association for the Study of the Liver) (2011). EASL Clinical Practice Guidelines: Management of hepatitis C virus infection. *J. Hepatol.* 55: 245-264.

Hiroishi K, Ito T and Imawari M (2008). Immune responses in hepatitis C virus infection and mechanisms of hepatitis C virus persistence. *J. Gastroenterol. Hepatol.* 23: 1473-1482.

Lemon SM, McKeating JA, Pietschmann T, Frick DN, et al. (2010). Development of novel therapies for hepatitis C. *Antiviral Res.* 86: 79-92.

Liang Q, Jiao YM and Ji YX (2012). Patients with chronic hepatitis C DNT cells and T cell subsets. *Shouduyikedaxuexueba.* 33: 214-217.

Liu YG, Wang TR and Li GL (2010). HCV infection of peripheral blood T lymphocyte subsets. *Sichuanyixue.* 31: 241-242.

McCaughan GW and Bowen DG (2011). Pathogenesis of cholestatic hepatitis C. *J. Hepatol.* 54: 392-394.

Post J, Ratnarajah S and Lloyd AR (2009). Immunological determinants of the outcomes from primary hepatitis C infection. *Cell Mol. Life Sci.* 66: 733-756.

Satake S, Nagaki M, Kimura K, Naiki T, et al. (2008). Significant effect of hepatitis C virus specific CTLs on viral clearance in patients with type C chronic hepatitis treated with antiviral agents. *Hepatol. Res.* 38: 491-500.

Spangenberg HC, Viazov S, Kersting N, Neumann-Haefelin C, et al. (2005). Intrahepatic CD8+ T-cell failure during chronic hepatitis C virus infection. *Hepatology* 42: 828-837.

Sugimoto K, Stadanlick J, Ikeda F, Brensinger C, et al. (2003). Influence of ethnicity in the outcome of hepatitis C virus infection and cellular immune response. *Hepatology* 37: 590-599.

Wang JP, Zhang Y and Nie Q (2009). Impact of PD-1 molecule on T cells in patients with chronic hepatitis C immune function. *Ganzang* 14: 200-203.