



Changes in circulating follicular helper T cells in peripheral blood of patients with acute hepatitis C virus infection

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Background: To investigate the changes in circulating follicular helper T cells (cTfh) in peripheral blood of patients with acute hepatitis C virus (HCV) infection and its correlation with symptom severity of the disease.

Methods: A total of 105 subjects were enrolled in this study, including 35 healthy people, 35 patients with acute HCV infection and 35 acute HCV patients with antiviral therapy. Flow cytometry was used to detect the expression of molecular markers related to the surface of cTfh cells, such as CD69, HLA-DR and CD57, and ELISA was used to detect the secretion of cytokines (IL-21, IL-4) in each group. The relationship between these markers and disease was analyzed statistically.

Results: Flow cytometry analysis demonstrated that the percentage of CD69 in peripheral blood of patients with acute hepatitis C infection was (18.90%±9.29%) significantly higher than that of normal control group (5.10%±4.21%) and antiviral treatment group (11.50%±5.38%). The difference was statistically significant ($P<0.05$). The results of HLA-DR and CD57 were consistent with CD69. The serum levels of IL-4 and IL-21 in the acute HCV infection group were significantly higher than those in the treatment and the control groups. After antiviral treatment, IL-4 and IL-21 levels significantly decreased but remained higher than those in the control group. This showed that antiviral treatment was effective, and the difference was statistically significant ($P<0.05$). The ratio of cTfh in infected group was negatively correlated with HCV RNA content ($r=-0.6858$, $P=0.0028$).

Conclusions: Antiviral immune response in patients with acute hepatitis C infection may be related to the proportion of T helper cells in peripheral blood circulation follicles. Dynamic detection of the number of T helper cells in clinical practice is conducive to identifying more effective methods to treat acute hepatitis C infection, which has important theoretical and clinical significance.

Keywords: Circulating follicular helper T; acute hepatitis C; IL-4; IL-21

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Introduction

Acute hepatitis C is a form of early hepatitis C infection, primarily transmitted by blood and presents a significant burden to the health of sufferers. In patients with acute HCV infection, only ~15% of patients can achieve curity spontaneously without anti-viral treatment, and the other,

85% of patients develop chronic HCV infection. Some patients develop more serious diseases, such as hepatic cirrhosis. Currently, the alanine aminotransferase (ALT), hepatitis C virus antibody (anti-HCV) and hepatitis C virus ribonucleic acid (HCV RNA) are utilized in the diagnosis of acute hepatitis C infection and the monitoring of the

progress and the therapeutic efficacy of acute hepatitis C infection.

Previous studies have shown that classical immune cells, such as CD8⁺ T cells, play an important role in acute and chronic HCV infection (1-3). However, there is still a lack of research on some newly emerging immune cell populations. T follicular helper cells (Tfh) are a newly discovered group of immune cells, located in lymphoid follicles. Their main function is to regulate antibody production, generated by B cells. However, the research progress of Tfh cells in clinical diseases is very slow, due to the difficulties in obtaining samples of lymphoid follicles in human tissues (3,4). In 2011, Morita *et al.* demonstrated the functional equivalence of CD4⁺ CXCR5⁺ Tfh cells in peripheral blood (cTfh) with Tfh cells in lymphoid follicles. Thus way, it provides a new and viable way to study acute HCV infection by investigating the changes of cTfh cells in peripheral blood.

Previous studies demonstrate the involvement of cTfh in chronic HCV infection (5-10). However, few studies have reported the role of cTfh cells in acute HCV infection. This study further clarifies the changes of cTfh before and after the anti-viral therapy. The results of this study will help to provide important theoretical and practical significance for clinical exploration of more effective treatment methods.

We present the following article in accordance with the STROBE reporting checklist (available at <http://dx.doi.org/10.21037/apm-20-1179>).

Methods

Study subjects

A total of 105 subjects were enrolled in this study. Subjects were divided into three groups, including 35 healthy people, 35 patients with acute HCV infection and 35 acute HCV patients with antiviral therapy. patients with acute hepatitis C infection were selected from the Digestive Department of Affiliated Hospital of Jiangnan University from August 2018 to May 2019, 35, including 48 males and 22 females, aged 25–66 years. All the acute HCV patients were in accordance to the diagnostic criteria for acute HCV infection as follows: history of blood transfusion, history of blood products or a history of exposure to hepatitis C virus. Clinical manifestations: general fatigue, nausea and right-quarter rib pain, mild hepatomegaly, splenomegaly in some patients, etc. Laboratory tests: The serum anti-HCV and HCV RNA were positive and the serum ALT value was

more than 10 times higher than that of normal persons, or the serum anti-HCV was negative, while the HCV RNA test was positive

In the control group, 35 health examinees were selected at the same period, including 24 males and 11 females, aged 20–66 years. All the healthy subjects had no history of other diseases, and had no abnormal indexes in liver imaging and serology.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of Affiliated Hospital of Jiangnan University. All participants in the study signed the informed consent form.

Detection of surface-related molecules in cTfh by flow cytometry

CD4⁺CXCR5⁺T cells with CD69⁺/CD57⁺/HLA-DR⁺ were identified as cTfh. Surface-related molecules were detected with BD Pharmingen™ Flow Kit. Whole blood was collected from participants of all three groups. One hundred μL of each anticoagulant blood sample were added to respective flow sampling tubes. Anti-CD4 FITC (5 μL), CXCR5 ALEXA 647 and CD69 PE were added prior to incubating at room temperature for 15 minutes. Hemolysin (2 mL) was added before incubation at room temperature for 15 minutes; PBS (2 mL, PH7.2) was added prior to centrifugation at 800 xg for 5 minutes. The supernatant was subsequently isolated. Approximately 500 μL PBS was added to the supernatant prior to detection via flow cytometry. Preparation of samples for CD57 and HLA-DR detection were performed using the same method as CD69. Data analysis was conducted with (BD Pharmingen™) software.

Cytokine expression by ELISA Kit

Venous blood collected from hepatitis C infection, treatment and control groups were stored at room temperature for 10–20 minutes to allow for coagulation. Samples subsequently underwent centrifugation for approximately 10 minutes (1,000 xg). The supernatant should be carefully collected and tested as soon as after extraction. If the test can not be carried out immediately, the specimens can be stored at –20 °C for examination. The test was performed in accordance with manufacturer's instructions. The regression equation of the standard curve was obtained with ELISA Calc using the concentration and OD value. The logistic curve (four parameters) was used

to fit the model, prior to determining cytokine expression levels in each group.

Analysis of correlation between cTfh ratio and HCV RNA content in hepatitis C infection group and treatment group

Pearson correlation analysis was used to analyze the relationship between cTfh and HCV RNA content in the infected group, expressed as a proportion. This provides the theoretical basis for evaluating treatment efficacies for acute hepatitis C infection.

Statistical processing

All experimental data were processed and analyzed by IBM® SPSS23 statistical software. The statistical data is expressed as mean \pm standard deviation ($\bar{x} \pm s$). The data between the two groups were compared by *t* test, with $P < 0.05$ as the statistical difference.

Results

Detection of surface-related molecular markers of cTfh by flow cytometry

Figure 1 illustrates the results of flow cytometry. The percentage of CD69 in the acute HCV infection group was (18.90% \pm 9.29%) higher than the control group (5.10% \pm 4.21%) and antiviral treatment group (11.50% \pm 5.38%). The percentage of cTfh cells decreased significantly after antiviral treatment, but remained higher than the control group ($P < 0.05$).

Detection of cytokine expression by ELISA Kit

The results of cytokine detection are shown in Table 1. ELISA detection of cytokine expression by cTfh cells in acute HCV infection, treatment and control groups showed significantly higher levels of IL-4 and IL-21 in acute HCV infection group compared to the treatment and control groups. Levels of IL-4 and IL-21 decreased significantly, post treatment but they remained higher than those of the control group.

CD4+CXCR5+cTfh ratio and HCV RNA content in infected group

Pearson correlation analysis showed that the proportion of cTfh (%) in infected group was negatively correlated with

HCV RNA content (Log10) ($r = -0.6858$, $P = 0.0028$). The results are shown Figure 2.

Discussion

Acute hepatitis C in adults is an infectious disease, mediated by HCV that causes a significant burden to the patients' health. Studies have shown that the pathogenesis of HCV infection mainly involves immune mediation and direct damage caused by HCV, which are divided into viral and host factors. Viral factors include viral replication ability, genotype, immunogenicity of viral polypeptide. Host factors encompass innate and cellular immune responses, along with humoral immunity.

Previous studies establish Th1-specific cytokines in cellular immunity as vital in controlling acute HCV infection (11-13). Recent international immunological research shows that cTfh plays a key role in the pathogenic factors of chronic HCV infection. However, little is known about the changes associated with cTfh cells in acute HCV infection. This study analyzes the role of cTfh cells in the pathologic process of acute HCV infection, providing new information to aid in novel discoveries.

Ctfh cells are a newly identified group of immune cells (13) which play a key role in many viral-related infection mechanisms (9). These cells are fast becoming a focus in the study of hepatitis infections. They are characterized by the presence of CXCR5, CD69, HLA-DR, CD57 chemokine receptors and secrete cytokines IL-21 and IL-4, which play an important role in the development and formation of cTfh cells. The results of Spaan *et al.* (14) showed that the expression of ICOS + Tfh cells and cytokine IL-21 increased significantly in the course of acute HCV infection, suggesting that cTfh cells play a key role in the regulation of immune response to acute HCV infection. Alternatively, IL-21 receptors are widely distributed in B cells and cTfh cells, which can positively promote the expression of CXCR5 in patients with acute hepatitis C through autocrine signaling (9,15,16).

The results of this study showed that the proportion of CD69, CD57 and HLA-DR in the acute hepatitis C infection group was significantly higher than the control and the antiviral treatment groups. After antiviral treatment, the proportion of cTfh cells decreased significantly, but remained higher than the control group. This indicates that antiviral treatment was effective. The hepatitis C infection group showed greater cytokine release, with higher Serum IL-4 than that in the control group. The contents of

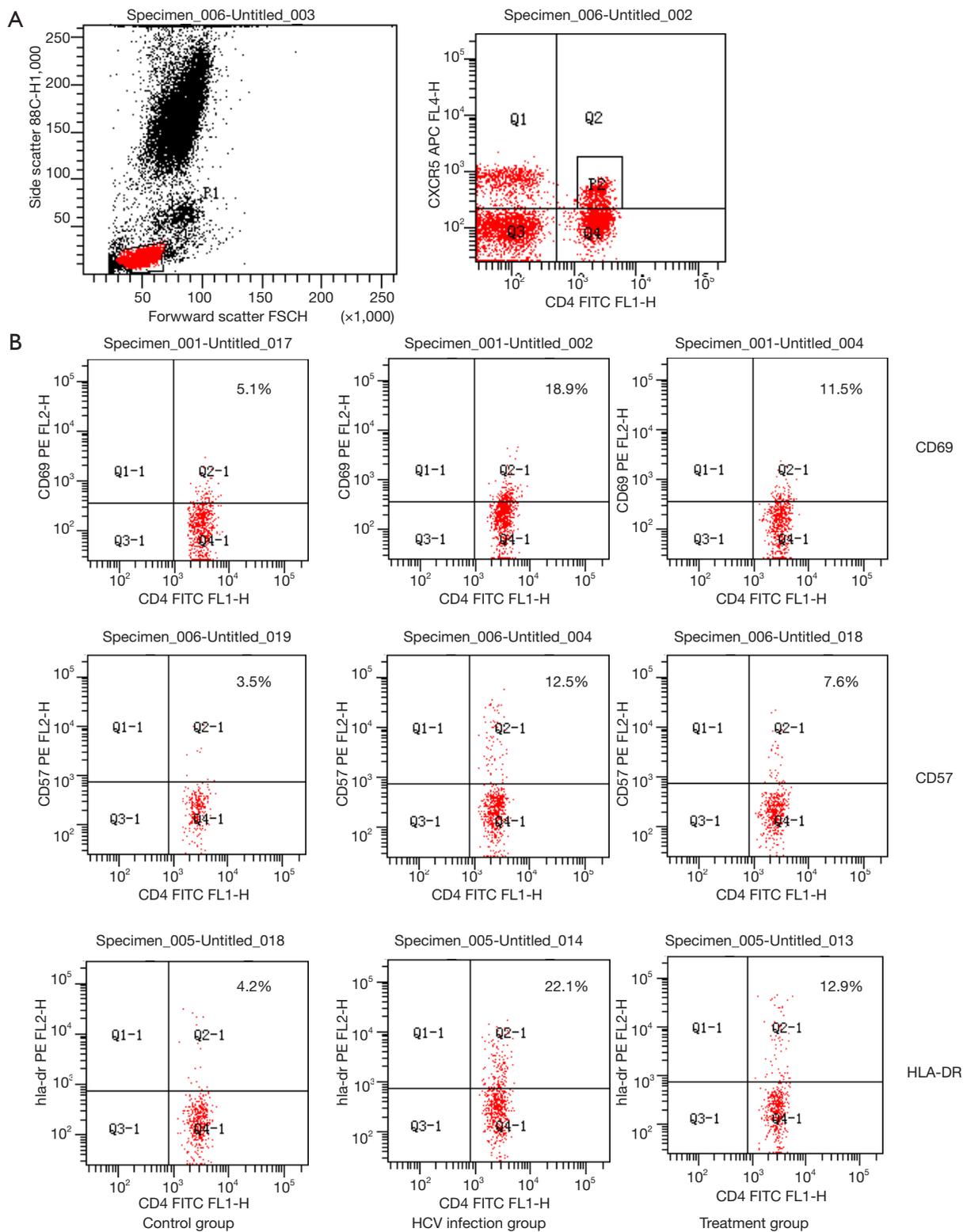
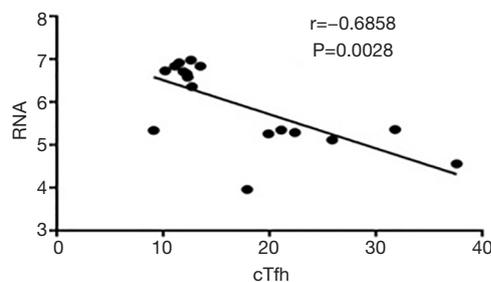


Figure 1 Detection of cTfh in each group by flow cytometry. (A) Gating strategy; (B) detection of cTfh in each group. cTfh, circulating follicular helper T cells; HCV, hepatitis C virus.

Table 1 Detection and analysis of serum contents of IL-4 and IL-21 (ng/L)

Group	Number of cases	IL-4	IL-21
Infection group	35	112.76±6.13*	134.32±7.28*
Treatment group	35	78.87±4.25*	90.21±6.47*
Control group	35	18.08±3.19	28.338±4.52
P value		P<0.05	P<0.05

Compared with normal control group, *P<0.05.

**Figure 2** Analysis of the correlation between the ratio of cTfh (%) and HCV RNA content (Log_{10}). cTfh, circulating follicular helper T cells; HCV, hepatitis C virus.

IL-21 and IL-4 in the treatment group and the control group decreased significantly after antiviral treatment, but remained higher than those in the control group. These results demonstrate that cTfh cells and cytokines secreted by cTfh cells play an important role in the process of eliminating acute HCV in the body. Regulating the proportion of related markers of cTfh cells will play an important role in the treatment of the disease.

There were some limitations in the current study. Firstly, the case size enroll in this study was small. Secondly, the level of IL-21 in cTfh cells were not be detected directly. Thirdly, the follow-up data was lacking in this study. Lastly, the underlying mechanism has not been well explored. All these may lead to the statistical bias of the results.

In conclusion, from an acute HCV infection standpoint, this study further clarified the specific pathogenesis of CD69, CD57, HLA-DR and its secreted cytokines IL-4, IL-21 in acute hepatitis C, providing an important foundation for further pre-clinical studies and more effective clinical exploration. This provides theoretical and practical basis for clinical treatment of acute hepatitis C infectious diseases.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at <http://dx.doi.org/10.21037/apm-20-1179>

Data Sharing Statement: Available at <http://dx.doi.org/10.21037/apm-20-1179>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/apm-20-1179>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All participants in the study signed the informed consent form. This study was approved by the Ethics Committee of Affiliated Hospital of Jiangnan University.

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