

Feature and significance of the ratios of peripheral blood T lymphocytes for patients with hepatitis B virus-related acute-on-chronic liver failure.

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Abstract

This study aims to investigate the changes and significance of the peripheral blood T lymphocytes for patients with Hepatitis B Virus (HBV) related acute-on-chronic liver failure. 50 patients with HBV related acute-on-chronic liver failure (liver failure group), 50 patients with Chronic Hepatitis B (CHB group), and 20 healthy persons taking physical examination in the corresponding period (healthy control group) were selected to determine the proportions of CD3⁺, CD4⁺, CD8⁺ T lymphocytes and CD4⁺CD25⁺ regulatory T cells (Tregs) in peripheral blood by flow cytometry. Compared with healthy control group, the proportions of peripheral blood CD3⁺, CD8⁺ T lymphocytes and CD4⁺CD25⁺ Tregs significantly decreased in liver failure group, while the proportion of CD4⁺ CD25⁺ Tregs significantly increased in CHB group. Taken together, these data suggest that the changes in peripheral blood T lymphocytes, CD8⁺ T lymphocytes and CD4⁺CD25⁺ Tregs are involved in HBV related acute-on-chronic liver failure.

Keywords: Hepatitis B virus-related acute-on-chronic liver failure, T lymphocytes, Regulatory T lymphocytes.

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Introduction

CD4⁺ CD25⁺ regulatory T cells (regulatory T cells, Tregs) are CD4⁺ T-lymphocyte subgroups with properties of autoimmune incompetence and immunosuppression, which could regulate immune response to Hepatitis B Virus (HBV) infection and thereby affect the progress of chronic hepatitis B by inhibiting the activation and proliferation of CD8⁺ T cells and the secretion of cytokines [1,2]. Our previous study showed that HBeAg (+) and HBeAg (-) HBV carriers had different proportion of CD4⁺CD25⁺ Tregs although their liver function was in the normal range [3]. HBV related Acute-on-Chronic Liver Failure (HBV-ACLF) has complex clinical syndromes, difficult treatment and high mortality, and the pathological mechanism remains unclear. Previous studies showed that during HBV-ACLF cytokine-induced immunologic injuries with the involvement of T lymphocytes played a vital role in the incidence of liver failure [4,5]. This study aimed to investigate the changes and significances of peripheral blood T lymphocytes for HBV-ACLF patients.

Material and Methods

Subjects

50 patients diagnosed as HBV-ACLF in early stage (liver failure group) and admitted to liver disease department, Second

Hospital of Nanjing City from January, 2010 to December, 2012 were selected. 50 patients diagnosed as CHB group in the corresponding period were selected. The diagnostic criteria for HBV-ACLF and CHB were described previously [6,7]. And 20 healthy outpatients (healthy control group) taking physical examination in the corresponding period were selected. The liver failure group had 50 patients, CHB group had 50 patients, and healthy control group had 20 patients. The comparisons of clinical characteristics for each group showed no significant differences ($P > 0.05$, Table 1). This study was conducted in accordance with the declaration of Helsinki and approved by the Ethics Committee of the Second Hospital of Nanjing. Written informed consent was obtained from all participants.

Diagnostic criteria

Clinical syndromes for early period of acute-on-chronic liver failure:

- Extreme fatigue, and severe gastrointestinal symptoms such as significant loss of appetite, vomiting, and abdominal distension
- Progressive deepening jaundice, total serum bilirubin (TBil) $\geq 171 \mu\text{mol/L}$
- Bleeding tendency, 30% < prothrombin activity (PTA) $\leq 40\%$
- No obvious hepatic encephalopathy or ascites appeared

Clinical syndromes for CHB were as follows: positive serum HBsAg, positive or negative HBeAg, positive or negative anti-HBe, positive HBV DNA, persistent or recurrent abnormal ALT, or hepatitis pathological changes in histological examination.

Exclusion criteria

Subjects were excluded if they had acute or subacute liver failure; chronic liver injuries caused by other liver disease including autoimmune, drugs, alcohol, toxic and parasites; primary liver cancer; other hepatitis; immune treatment history; participated in other clinical trials in previous three months; were pregnant or lactating women; were positive anti-HIV.

Detection of peripheral blood T lymphocytes

Following previous literatures [8,9], mononuclear cells in the peripheral blood were isolated by flow cytometry (BD Biosciences), about 10^5 cells were incubated with anti-CD3-Percp, anti-CD4-FITC, anti-CD25-PE reagent (RECKMAN COLILTER), or IgG-FITC, IgG-PE, IgG-Percp (RECKMAN COLILTER) as an isotype control. CD3⁺, CD4⁺ and CD25⁺ on

cell surface were detected by fluorescent antibody staining. CD4⁺ and CD25⁺ T cell subgroups were defined as phenotype of CD4⁺CD25⁺ Tregs to analyze the percentage of CD4⁺CD25⁺ Tregs.

Statistical analysis

Statistical analysis was performed by SPSS 16.0 software. The percentage of T lymphocyte subgroups was expressed as $x \pm s$. Comparison between groups was performed using independent samples t test. $P < 0.05$ indicated significant differences.

Results

Proportion of CD3⁺ T lymphocytes in peripheral blood in each group

Compared with healthy control group, the percentage of peripheral blood CD3⁺ T lymphocytes in CHB group had no significant difference ($P > 0.05$). The percentage of peripheral blood CD3⁺ T lymphocytes in the liver failure group decreased significantly compared with healthy control group and CHB group ($P < 0.01$, Table 2).

Table 1. Comparison of clinical data in different groups.

Group	Healthy control group	Chronic hepatitis B group	Liver failure group
n	20	50	50
Age (years)	34.51 ± 3.45	36.23 ± 4.76	33.62 ± 3.01
Gender (Male/Female)	3/7	13/37	15/35
ALT (U/L)	35.01 ± 6.37	87.73 ± 7.56	341.45 ± 50.41
AST (U/L)	32.92 ± 5.98	67.52 ± 6.69	145.59 ± 46.35
TBil (μmol/L)	17.62 ± 4.83	18.95 ± 3.85	327.78 ± 72.43
DBil (μmol/L)	8.01 ± 2.34	7.86 ± 2.13	127.81 ± 10.92
PTA (%)	98.27 ± 5.73	87.93 ± 8.09	35.95 ± 4.78
HBVDNA (lg10 copies/ml)	-	6.02 ± 2.62	5.87 ± 2.36
HBeAg (+/-)	-	22/28	24/26

Data were expressed as $x \pm s$. $P > 0.05$ for the comparison of all data between chronic hepatitis B group and liver failure group.

Table 2. Comparisons of the proportions of peripheral blood T lymphocytes.

Group	n	CD3 ⁺ T lymphocytes (%)	CD4 ⁺ T lymphocytes (%)	CD8 ⁺ T lymphocytes (%)	CD4 ⁺ /CD8 ⁺	CD4 ⁺ CD25 ⁺ Tregs (%)
Healthy control group	20	50.31 ± 12.09	50.01 ± 8.67	42.05 ± 9.26	1.32 ± 0.31	2.93 ± 1.31
Chronic hepatitis B group	50	49.72 ± 20.11	50.72 ± 10.35	41.95 ± 8.63	1.31 ± 0.52	3.47 ± 2.29 [#]
Liver failure group	50	35.48 ± 23.44 [*]	56.34 ± 14.47 [*]	37.66 ± 13.28 [*]	1.79 ± 1.23 [*]	0.72 ± 1.07 [*]

Data were expressed as $x \pm s$. [#] $P < 0.05$ compared with healthy control group, ^{*} $P < 0.05$ compared with healthy control group and chronic hepatitis B group.

Proportion of peripheral blood CD4⁺ and CD8⁺ T lymphocytes in each group

Compared with healthy control group, the proportion and percentage ratio of the peripheral blood CD4⁺ and CD8⁺ T lymphocytes in CHB group showed no significant difference ($P > 0.05$). In the liver failure group, the percentage of peripheral blood CD4⁺ T lymphocyte increased, while the percentage of CD8⁺ T lymphocyte decreased, and the ratio of CD4⁺ CD8⁺ T lymphocyte increased significantly ($P < 0.05$, Table 2).

Proportion of peripheral blood CD4⁺ CD25⁺ Tregs in each group

Compared with healthy control group, the proportion of peripheral blood CD4⁺ CD25⁺ Tregs T lymphocytes in CHB group increased significantly ($P > 0.05$). The proportion of peripheral blood CD4⁺ CD25⁺ Tregs T lymphocytes in the liver failure group declined significantly compared with healthy control group ($P < 0.01$, Table 2).

Discussion

Immunologic injuries are the main liver damage caused by HBV infection, and T lymphocytes play a major role in the immune pathogenesis. T cells not only destroy HBV infected hepatocytes, but also eliminate HBV by controlling HBV replication or non-cell lysis [10]. CD8⁺ T lymphocytes are Cytotoxic T Lymphocytes (CTL), while CD4⁺ T lymphocytes play supporting role [11]. CD4⁺ and CD25⁺ T cells, known as CD4⁺ CD25⁺ Tregs, are important immunomodulatory T lymphocyte subgroups *in vivo*, counting for 5-10% of peripheral CD4⁺ T lymphocyte [12]. CD4⁺ CD25⁺ Tregs are generated from the thymus, and then migrate into peripheral blood [13] and the surrounding tissues to prevent potential autoimmune response [14]. CD4⁺ CD25⁺ Tregs have properties of autoimmune impotence and immunosuppression, which could inhibit the natural and acquired immune systems to maintain immune tolerance and homeostasis [15].

A study reported that CD4⁺ CD25⁺ Tregs rate in CHB patients was higher than that in healthy control group and cured patients, suggesting that Tregs may perform immunologic effects by directly lowering immunologic epitope-specific CTL, leading to chronic persistence of HBV in the patients [16]. However, another study showed that the numbers of Tregs in asymptomatic virus carriers, active chronic infection period, previous infection and health control group were not different, and unrelated with Hepatitis B e Antigen (HBeAg) state, HBV viral load and various antiviral therapies [17]. Although the two studies showed contradictory results, they supported that CD4⁺ CD25⁺ Tregs could inhibit epitope-specific CTL proliferation and Interferon- γ (IFN- γ) secretion *in vitro*. Additional data indicated that the high percentage of Tregs was usually accompanied by high viral loads in CHB patients, which may explain the cause of uncontrolled viral replication [18]. The percentage of CD4⁺ CD25⁺ Tregs elevated in HBeAg (+) normal function livers, and once the

liver function became abnormal, the percentage declined and was positively correlated with HBV DNA [19]. However, the frequency of CD4⁺ CD25⁺ Tregs was not directly related with HBV-ACLF, and the link of CD4⁺ CD25⁺ Tregs with the severity of acute-on-chronic liver failure remains unclear.

A study showed that the percentage of CD4⁺ CD25⁺ Tregs in peripheral blood of patients with ACLF was significantly higher than that of the healthy individuals and CHB patients, and positively correlated with HBV DNA load in ACLF or CHB patients [8]. Other studies suggested that the ratios of CD3⁺ T lymphocytes and monocytes were related with the poor prognosis of acute-on-chronic liver failure [20]. A recent study showed that the percentage of CD4⁺ T lymphocytes reduced in HBV-ACLF patients, similar to septic shock [21].

In this study we showed that the peripheral blood CD4⁺ CD25⁺ Tregs in patients with CHB was significantly increased, but the percentages and ratios of CD8⁺ and CD4⁺ T lymphocytes had no difference compared with healthy control group, while the proportions of the total peripheral blood T lymphocyte-CD3⁺ T lymphocytes, CD8⁺ T lymphocytes and CD4⁺ CD25⁺ Tregs of HBV-ACLF patients were significantly decreased compared with healthy controls and CHB patients. These data indicated that the proportion of peripheral blood CD4⁺ CD25⁺ Tregs increased in CHB patients, but had no obvious effects on the regulation of CD8⁺ T lymphocytes. Once HBV-ACLF develops, the peripheral blood effector T lymphocytes such as T lymphocytes, CD8⁺ T lymphocytes and CD4⁺ CD25⁺ Tregs would be at a low level state, suggesting that T lymphocytes involved in immune regulation are in the "failure" state during the process of HBV-ACLF.

This study only examined the proportion changes of peripheral blood T lymphocytes in HBV-ACLF patients, the function and features such as the secreted cytokine profiles characteristic of peripheral blood T lymphocytes needed to be further studied. Nevertheless, the results in this study indicate that once the liver failure (HBV-ACLF) occur due to CHB, T lymphocytes in CHB patients would be in distinctly different states, which provides a rational for exploring the role of T lymphocytes, especially CD4⁺ CD25⁺ Tregs, in the pathogenesis of HBV-ACLF and specific targeting of these T-cell populations as novel therapy strategies for HBV-ACLF [22].

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