

LETTER TO THE EDITOR

Apoptosis is related to imbalance of Th1/Th2-type cytokine in peripheral blood mononuclear cells of patients with chronic hepatitis B

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Chronic hepatitis B (CHB) constitutes a major health problem worldwide, yet the mechanism of hepatitis B virus (HBV) infection remains poorly understood. It is believed that hepatocellular injury in patients with HBV infection leads to activation of immune system (1). The host immune responses, particularly those mediated by T lymphocytes, are essential for the control of disease progression and restriction of virus replication in hepatic tissues (2). Maturated T cells lead to the development of CD4+ and CD8+ single positive T cells which have diverse functions in the peripheral immune system. CD4+ T helper cells (Th) can be further subdivided into Th1 and Th2 cells, based on their cytokine profiles (3). Abnormal expression of Th1- and Th2-type cytokines is known as a major reason of CHB progression (4). Thus, it is crucial to have a better understanding of T cell response for more effective control of HBV infection (5).

Activation-induced cell apoptosis, a process in which activated T-cell receptor stimulates T cells to undergo cell death, has been found in both lymphocytes and hepatocytes in patients with HBV infection, and is an important mechanism to eliminate auto-reactive T cells and to ensure

tolerance (6). It is deemed as one of the important reasons for persistent infection of HBV. The objective of this study was to determine whether immune deficiency in patients with CHB might be related to activation-induced cell apoptosis by assessing the expression of Th1/Th2-type cytokines and apoptosis of peripheral blood mononuclear cells (PBMCs) from patients with CHB.

Forty-five (n = 45) patients and 22 healthy volunteers (Table) were enrolled in this pilot study. PBMCs were isolated from fresh heparinized blood by Ficoll-Paque density gradient centrifugation. PBMC apoptosis was analyzed by using flow cytometer after induction by phytohemagglutinin P (PHA-P). Apoptosis of PBMCs from patients with CHB was significantly higher than that from healthy volunteers (37.52 ± 10.21 vs 16.54 ± 7.24 , $P < 0.01$). Activated T cells release different endogenous cytokines, with IFN- γ , TNF- α , IL-1, IL-2, and IL-12 mainly from Th1, and IL-4, IL-13, IL-5, IL-6, and IL-10 from Th2. The levels of IFN- γ and IL-4 representing Th1 and Th2 activation respectively from the supernatant of PBMCs were measured by ELISA. The IFN- γ levels in patients with CHB (65.7 ± 25.2 ng/l) were significantly lower than that from the healthy volunteers (156.4 ± 38.8 ng/l) ($P < 0.01$) and inversely correlated with the rate of apoptosis of PBMCs ($r = -0.647$, $P < 0.01$). However, IL-4 levels from the patients (165.3 ± 34.1 ng/l) were significantly higher in comparison to those in the control group (78.4 ± 25.3 ng/l) ($P < 0.01$) and positively correlated with the rate of apoptosis of PBMCs ($r = 0.598$, $P < 0.01$).

Since PBMCs are composed mainly of lymphocytes, hence, the apoptosis detected in PBMCs may primarily

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Abbreviations: Th1/Th2 = T helper cells 1/2; CHB = chronic hepatitis B; PBMCs = peripheral blood mononuclear cells; PHA-P = phytohemagglutinin P; IFN- γ = interferon- γ ; IL = interleukin; HBV = hepatitis B virus; 7-AAD = 7-aminoactinomycin D; TCR = T-cell receptor; TNF- α = tumor necrosis factor alpha

Table

Group	n	Apoptosis (%)	IFN- γ (ng/l)	IL-4 (ng/l)
Healthy control	22	16.54 \pm 7.24	156.4 \pm 38.8	78.4 \pm 25.3
Patients	45	37.52 \pm 10.21*	65.7 \pm 25.2*	165.3 \pm 34.1*

*P <0.01.

reflect apoptosis of peripheral lymphocytes. In the present study, the percentage of PBMC apoptosis from CHB patients was significantly higher than that from healthy control, suggesting that activation-induced cell apoptosis in the course of CHB might lead to functional defect of lymphocytes that is possibly associated with decreased HBV clearance and immune tolerance to HBV (7).

The specific immune responses are mainly mediated by Th1 and Th2 which release different cytokines. While the cytokines released by Th1 are mainly involved in the cellular immune response, the cytokines released by Th2 group are important for humoral immunity (8). The balance between Th1 and Th2 related cytokines plays a crucial role in the regulation of the immune status. Disturbance of such balance results in immune disorders as well as in defects of immune defenses (9). It has been proposed that imbalance of Th1/Th2 mediated immune response might be an underlying mechanism of CHB (10, 11).

In our study, significantly low IFN- γ levels and high IL-4 levels were noted in PBMCs from the patients of CHB as compared to the healthy control. These results suggested that apoptosis mainly occurs in Th1 cells so that disturbance of Th1/Th2 related cytokines might lead to defected specific cell immunity response against HBV and subsequently persistent chronic HBV infection. Our findings are consistent with others. Hou *et al.* (12) reported significantly lower IFN- γ in cultured PBMCs from CHB than that from the controls. Ji *et al.* (13) demonstrated lower levels of IL-12 in CHB patients. Thus, measurement of Th1/Th2 cytokines might be useful for monitoring the inflammatory response in CHB.

To conclude, the findings of our study support the hypothesis of Th1/Th2 cytokine imbalance and demonstrate elevated levels of IL-4 in concert with decreased levels of IFN- γ in CHB. These findings will aid a better understanding of changes in immunologic responses to CHB and contribute to the development of new management strategies for better clinical outcomes. Future studies are needed to evaluate the restoration of the Th1/Th2 balance as a biomarker for treatment effectiveness of CHB.

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