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Changes in the balance between Treg and Th17 cells in patients with chronic hepatitis B

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ABSTRACT

The purpose of this study was to explore the role of Treg cells, Th17 cells and cytokines associated with Treg/Th17 differentiation in the occurrence, development and outcome of chronic hepatitis B (CHB). To do so, we detected populations of Treg and Th17 cells and their associated cytokines in the peripheral blood of CHB patients. The populations of Treg cells ($CD4^+CD25^{high}CD127^{low}$ T cells) and Th17 cells ($CD3^+CD8^-IL\text{-}17^+$ T cells) were analyzed in 46 patients with low to moderate chronic hepatitis B (CHB-LM), 24 patients with severe chronic hepatitis B (CHB-S) and 20 healthy controls (HC) using flow cytometry. The levels of cytokines associated with Treg/Th17 differentiation, including IL-10, TGF-β1, IL-17 and IL-23, were measured by enzyme-linked immunosorbent assay (ELISA). Our study showed that the imbalance of Treg and Th17 cells might play an important role in the occurrence, development and outcome of CHB.

1. Background

Chronic hepatitis B (CHB) is one of the important public health problems worldwide (de Franchis et al., 2003). The occurrence, development and outcome of CHB are affected by the dynamic imbalance of hepatitis B virus (HBV), liver cells and the host's immune system. HBV invades human liver cells and then begins to replicate itself and release antigens, which induce the immune response. Many immune factors, such as $CD4^+CD25^+$ regulatory T (Treg) cells, T helper 17 (Th17) cells, T helper 1 (Th1) cells, T helper 2 (Th2) cells and procedural death factor-1 (PD-1), are involved in this process (Ariyasu et al., 2005). Some studies have shown that Th1 cells are significantly activated, while Th2 cells are inhibited in the immune response of CHB, which causes inflammation of the liver (Harrington et al., 2006). Nonetheless, the pathogenic mechanism of CHB remains unclear.

Treg cells play an anti-inflammatory role mainly through contact dependent suppression or releasing anti-inflammatory factors on other immune cells, such as CD4+ and CD8 + cells, natural killer (NK) cells and NKT cells, B cells and dendritic cells (DC). Thus, Treg cells are considered to be of great importance for maintaining self-tolerance, immune balance and the prevention of autoimmune diseases, allergies and other immune pathological conditions. Treg cells are closely associated with the development and progress of CHB (Peng et al., 2008).

$CD4^+$T cells secreting interleukin-17 (IL-17) are newly established T helper cell subsets (Th17), which are different from Th1 and Th2 cells. Th17 cells originate from the same naive cells with Treg cells, mainly secreting pro-inflammatory cytokines IL-17A, which are linked to inflammation and host antimicrobial immunity. There is evidence that circulating $IL\text{-}17^+$T cells are largely accumulated in the livers of CHB patients (Xue-Song et al., 2012).

Recently, we have become aware of the dysfunction of Treg cells and Th17 cells in disease. Th17 cells are characterized by their dependence on IL-23. Th17 cells play a strong pro-inflammatory role in many chronic inflammatory and autoimmune diseases (Xin et al., 2007). Treg cells can mediate immune tolerance and maintain immune balance by secreting IL-10 and transforming growth factor-β1 (TGF-β1), which regulate the function of other immune cells and the immune response actively and effectively (Lina et al., 2011). Th17 cells enhance inflammation of tissue, while Treg cells suppress inflammation. The imbalance between Treg and Th17 cells can lead to the occurrence and development of many chronic inflammatory diseases, autoimmune diseases and tumors (Hanidziar and Koulmanda, 2010; Maruyama et al., 2010; Zhang et al., 2010a). Many studies have shown that the imbalance of Treg

Abbreviations: CHB, Chronic hepatitis B; HBV, Hepatitis B virus; PD-1, Procedural death factor-1; Treg, T; Th17, T helper 17; NK, Natural killer; DC, Dendritic cells; IL-17, Interleukin-17; TGF-β1, Transforming growth factor-β1; CHB-LM, Low to moderate chronic hepatitis B; CHB-S, Severe chronic hepatitis B; HBsAg, Hepatitis B surface; ALT, Alanine aminotransferase; TBil, Total bilirubin; HAV, Hepatitis A virus; HCV, Hepatitis C virus; HDV, Hepatitis D virus; HC, Healthy controls; PBMCs, Peripheral blood mononuclear cells; PMA, Phorbol 12-myristate-13-acetate; Ion, Ionomycin; Mon, Monensin; AST, Aspartate aminotransferase; DBil, Direct bilirubin; HBeAg, Hepatitis B e Antigen; ETV, Entecavir; G-CSF, Granulocyte colony-stimulating factor; NF-kappa B, Nuclear factor kappa B; ICAM-1, Intercellular adhesion molecule-1.

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Table 1
Clinical characteristics of the population enrolled in the study.

Group	HC (n = 20)	CHB-LM (n = 46)	CHB-S (n = 24)
Sex (male, %)	14 (70.00%)	35 (76.01%)	19 (79.17%)
Age (years)	38.5 (21.1–59.9)	32 (22.4–51.3)	28.5 (21–42.5)
TBil (μmol/L)	9.0 (7.3–16.3)	18.1 (10.1–32.1)	144.8 (89.2–325.7)▼
DBil (μmol/L)	6.3 (5.3–11.9)	6.6 (4.1–14.6)	83.6 (20.0–188.5)▼
ALT (IU/L)	19.5 (7.2–36.9)	229.5 (41.7–570.5)	477.0 (67.5–2345.5)▼
AST (IU/L)	15 (9.2–31.9)	112.5 (37–424.1)	275 (71.5–1256.8)▼
HBsAg positive	0	46	24
HBeAg positive	0	26 (56.52%)	15 (62.50%)
HBeAb positive	0	20 (44.48%)	9 (37.50%)
HBcAb positive	0	46	24
HBVDNA (lg copies/mL)	ND	6.0 (4.5–6.9)	6.3 (4.8–6.9)

Numerical data are shown as the median (10th–90th percentile) and analyzed by the Mann–Whitney *U* test. Compare to the CHB-LM group, ▼$P < 0.05$. ND, not determined; HC, healthy controls; CHB-LM, low to moderate chronic hepatitis B; CHB-S, severe chronic hepatitis B.

and Th17 cells exists in patients with CHB (Hu et al., 2011; Huang et al., 2011; Niu et al., 2011). However, the role of the imbalance of Treg/Th17 cells and Treg/Th17-related cytokines in the mechanism of CHB and their relationships with the grade of liver injury remain unknown. More importantly, there is a paucity of data showing the change in the Treg/Th17 cells and their cytokines in CHB after anti-virus treatment.

In this study, we detected the change in the frequencies of Treg cells and Th17 cells and the levels of Treg/Th17-associated cytokines in patients with low to moderate chronic hepatitis B (CHB-LM) and patients with severe chronic hepatitis B (CHB-S) before and after anti-virus therapy. We also explored their roles in CHB, aiming to understand the pathogenesis of CHB and provide a basis for new therapeutic targets.

2. Materials and methods

2.1. Subjects

Informed consent was obtained from each patient included in the study, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the institution's human research committee. Blood samples were collected from 46 CHB-LM patients and 24 CHB-S patients who were hospitalized or followed up from July 2010 to August 2011 in the Department of Infectious Diseases of the First Hospital of Quanzhou, which is affiliated with Fujian Medical University. CHB-LM patients were defined as those who were positive for hepatitis B surface antigen (HBsAg) for more than six months and had an alanine aminotransferase (ALT) level <600 IU/L and a total bilirubin (TBil) level ≤51.3 μmol/L (Imamura et al., 2003). CHB-S patients were defined as those who were positive for HBsAg for more than six months and had an ALT level >600 IU/L and a TBil level >51.3 μmol/L (Imamura et al., 2003; Seddiki et al., 2006). No patients had received immunomodulating agents, such as thymosin and glucocorticoid hormones, or anti-virus therapy. Patients infected with hepatitis A

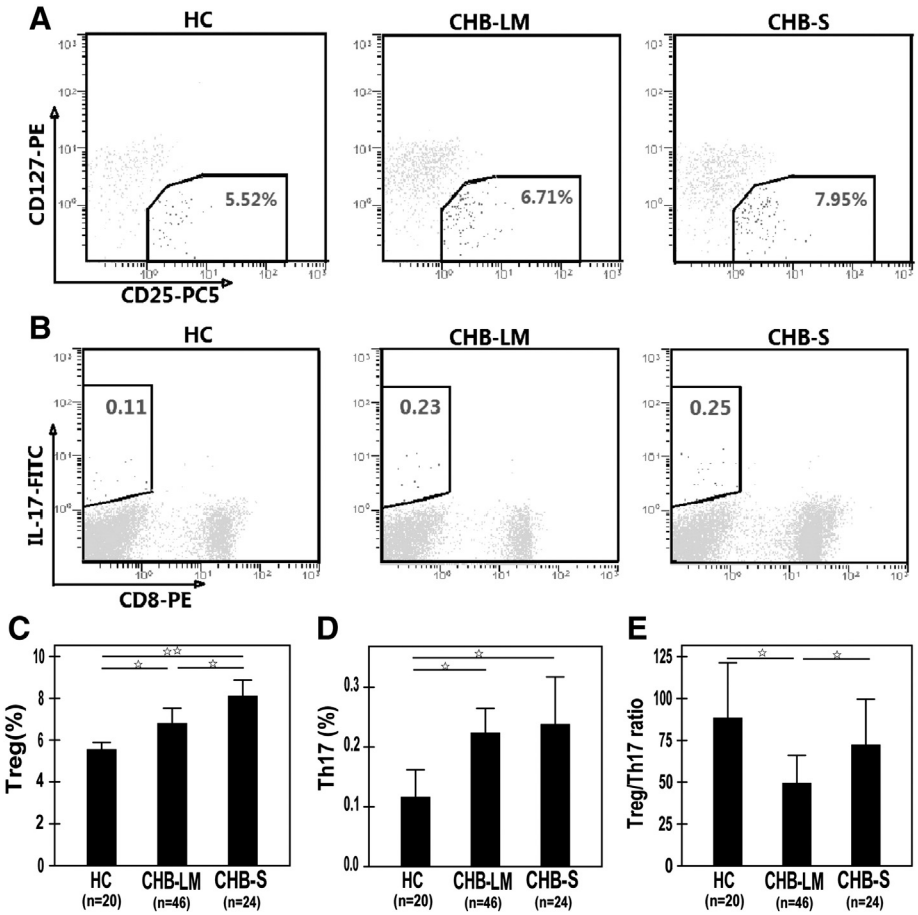

Fig. 1. Frequencies of Treg ($CD4^+CD25^+CD127^{low}$) and Th17 ($CD3^+CD8^-IL\text{-}17^+$) cells and the Treg/Th17 ratio in patients with CHB and healthy controls. Data are shown as the mean ± standard error and were analyzed by the Kruskal-Wallis H test and the Mann–Whitney *U* test. (A, B) Representative dot plots of Treg and Th17 cell staining in the CHB and HC groups. (C) Analysis of the percentage of $CD4^+CD25^{high}CD127^{low}$ T cells among $CD4^+$ T cells. (D) Analysis of the percentage of $CD3^+CD8^-IL\text{-}17^+$ T cells among $CD3^+$ T cells. (E) Analysis of the Treg/Th17 ratio. HC, healthy controls; CHB-LM, low to moderate chronic hepatitis B; CHB-S, severe chronic hepatitis B. ☆$P < 0.05$; ☆☆$P < 0.01$.