Telbivudine decreases proportion of peripheral blood $CD4^+CD25^+CD127^{low}$ T cells in parallel with inhibiting hepatitis B virus DNA

ZEHUI YAN[1], JIJUN ZHOU[1], MENGJUN ZHANG[2], XIAOLAN FU[2], YUZHANG WU[2] and YUMING WANG[1]

[1]Institute of Infectious Diseases, Southwest Hospital, Third Military Medical University;
[2]Institute of Immunology, Third Military Medical University, Chongqing 400038, P.R. China

Received July 11, 2013; Accepted February 25, 2014

DOI: 10.3892/mmr.2014.2042

Abstract. Regulatory T cells (Treg) have significant roles in the immunopathology of patients with chronic hepatitis B (CHB) and exhibit an evident correlation with antiviral immunity when antiviral therapy is applied. In order to investigate how circulating Tregs are affected by telbivudine treatment and its significance in patients with CHB, peripheral blood mononuclear cells (PBMCs) were isolated and the proportions of circulating cluster of differentiation (CD)$4^+CD25^+CD127^{low}$ and $CD8^+CD25^+$ T cells of CHB patients prior to and during the three or six months of treatment were assessed and detected by flow cytometric analysis. The levels of forkhead/winged helix transcription factor (Foxp3) mRNA were also quantified using quantitative polymerase chain reaction. A significantly higher percentage of $CD4^+CD25^+CD127^{low}$ and $CD8^+CD25^+$ T cells in the PBMCs of patients with CHB were identified compared with that of healthy individuals. Patients with CHB also demonstrated significantly higher levels of Foxp3 mRNA compared with that of healthy individuals. Following six months of telbivudine treatment, the proportion of circulating $CD4^+CD25^+CD127^{low}$ and $CD8^+CD25^+$ T cells and the relative levels of Foxp3 mRNA in patients with CHB was comparable to the proportion in healthy individuals. The proportions of circulating peripheral blood $CD4^+CD25^+CD127^{low}$ T cells were paralleled with its HBV DNA inhibition. The results of the present study indicate that telbivudine treatment reduces HBV DNA levels rapidly and indirectly affects the immune system by downregulating the proportion of circulating Treg markedly, which may be beneficial to restore the antiviral immune response.

Correspondence to: Professor Yuming Wang, Institute of Infectious Diseases, Southwest Hospital, the Third Military Medical University, 30 Gao Tan Yan Street, Sha Ping Ba, Chongqing 400038, P.R. China
E-mail: wym417@163.com

Key words: chronic hepatitis B, regulatory T cells, telbivudine

Introduction

Hepatitis B virus (HBV) infection is a serious public health problem affecting more than 400 million individuals worldwide (1). During HBV infection, the host immune responses, particularly the cellular immune response, mediate clearance of HBV infection and the pathogenesis of liver injury is dependent on the balance between HBV replication and the viral-specific cytotoxic T-lymphocyte (CTL) response (2). In patients with an acute self-limiting HBV infection, the $CD4^+$ and $CD8^+$ T-cell response with T helper 1 type cytokine profile is crucial for the control of the infection (3,4). However, patients with chronic hepatitis B (CHB) often exhibit impairment of HBV-specific T-cell activity, which is characterized by a weak immune response to HBV and lack of the vigorous specific $CD4^+$ and $CD8^+$ T-cell response (4,5).

The precise mechanisms responsible for this impaired T-cell response to the chronicity of HBV infection are not fully understood. One scenario is the potential role of host-mediated immunosuppressive mechanisms that may be activated following persistent antigenic exposure (6). Previously, regulatory T cells (Tregs) were focused to have an indispensable role in maintaining immunological unresponsiveness to antigens (7,8), as they have a prominent role in immunoregulation and tolerance. During the acute-resolved HBV infection, circulating Treg frequency is low in the acute phase, but is significantly increased during the convalescent phase and then returns to a normal level along with disease resolution (9). In patients with CHB, the increased Treg frequencies in the peripheral blood and liver may significantly suppress HBV-specific CTL responses; however, they are found to be decreased upon hepatitis B e antigen (HBeAg) seroconversion (10,11). Furthermore, the decreased frequency of hepatitis B c antigen (HBcAg)-specific Tregs accompanied by increased HBcAg peptide-specific CTLs partially account for the acute exacerbation in patients with CHB (12).

It has been suggested that Treg can be induced through a repetitive stimulation of T cells by high concentrations of antigen for longer periods of time (13). The high viral load present in peripheral blood of HBV patients may possibly provide such a stimulus. For CHB, two major types of

Table I. Basic characteristics of the participants.

Characteristic	Chronic hepatitis B patients, n=22	Healthy blood donors, n=19
Gender, male/female	16/6	14/5
Age, years	36±6.5	38.6±12.1
HBsAg	22	0
Anti-HBs IgG	0	19
HBeAg positive	22	0
Serum HBV DNA load	>6 $\log_{10}$ copies/ml	Not detectable
TBil, μmol/l	29.6 (17.4-52.8)	8.4 (3.9-16.5)
ALT IU/l	143.2 (95.1-224.3)	17.5 (11.0-27.3)
AST IU/l	132.1 (103.2-198.5)	14.2 (9.5-23.2)

Data are expressed as the median (range) or n (%), unless otherwise indicated. TBil, total bilirubin (normal total bilirubin range, 1.0-21.0 μmol/l); ALT, serum alanine aminotransferase (normal ALT range, 3-50 U/l); AST, aspartate aminotransferase (normal AST range =3-40 U/l); HBV, hepatitis B virus; IgG, immunoglobulin G; HBs, hepatitis B surface; HBe, hepatitis B e; Ag, antigen.

antiviral drugs are widely used: Nucleotide analogs (including lamivudine, defovir, entecavir, tenofovir and telbivudine) and interferon (conventional and pegylated interferon alfa) (14,15). During the course of pegylated interferon treatment, the decline of circulating Tregs together with a partial recovery of the immune responses was able to predict favorable responses (16,17). The frequency of HBV-specific CTL in the peripheral blood of responders was significantly higher compared with that of non-responders following lamivudine treatment (18-20). Previous studies have also reported that adefovir-induced viral load reduction results in a decline of circulating Treg together with a partial recovery of the immune response, as indicated by a decrease in percentages of Treg and an increased HBV-specific proliferation (21). Concomitantly with a quantitative reduction in viral replication, the frequency of $CD4^+$ T cells and the $CD4^+/CD8^+$ ratio increased during effective telbivudine therapy (22). These findings indicated that effective antiviral treatment can sustain the inhibition of viral replication and antigen production, which may potentially result in a decrease of Treg induction, leading to restoration of the immune response. However, this hypothesis was challenged by a recent report in which a greater increase in HBcAg-specific Tregs was correlated with a higher rate of sustained responsiveness to antiviral therapy in patients with CHB (23).

Telbivudine is a novel orally bioavailable antiviral drug with high potency and selectivity against HBV (24). Multinational studies have demonstrated the superiority of telbivudine over lamivudine for the treatment of CHB, particularly in terms of viral load reduction, serum alanine aminotransferase (ALT) normalization, HBeAg loss and reduced viral resistance (25-28). Certain studies indicated that the rapid inhibition of the HBV load resulting from continued telbivudine treatment may cause a restoration of the impaired T cell response in patients with CHB (22).

In the present study, it was hypothesized that patients with CHB have a higher proportion of Treg compared with healthy controls and that the inhibition of viral replication by continued telbivudine treatment can reduce proportions of Treg in the peripheral blood and enhance the antiviral immune response. To evaluate the effects of telbivudine and its correlation with the HBV DNA load in patients with CHB, the proportions of peripheral blood $CD4^+CD25^+CD127^{low}$ and $CD8^+CD25^+$ T cells, and the associated mRNA levels of Forkhead/winged helix transcription factor (FoxP3, it is a transcription factor specifically expressed by $CD4^+CD25^+$ Treg cells), were examined and detected in 22 patients with CHB undergoing telbivudine treatment.

Patients and methods

Study participants. A total of 22 unrelated patients (16 males and 6 females; age, 36±6.5 years) who were diagnosed with chronic hepatitis B and received antiviral treatment with telbivudine (600 mg orally per day) between October 2009 and December 2010 at Southwest Hospital (Chongqing, China) were recruited for the present study. Nineteen age- and gender-matched healthy blood donors were selected as the control group. The diagnostic criteria of CHB were based on the related literature recommended by the Chinese Medical Association (29). All the patients were selected according to the following criteria: (i) All hepatitis B surface antigen (HBsAg) carriers were positive for both HBsAg and antibody to HBV core antigen of the immunoglobulin G (IgG) type for at least 12 months; (ii) HBeAg-positive; (iii) age between 18 and 60 years; (iv) HBV DNA≥6$\log_{10}$ copies/ml; (v) ALT levels ≥2 fold the upper limit of that of normal patients and (vi) no antiviral, immune suppressive or immunomodulatory treatment during the last six months. Patients who were pregnant and co-infected with the human immunodeficiency virus, hepatitis A, C or D and patients with other types of hepatitis were excluded from the present study.

For the patients with CHB, clinical, biochemical, virological and immunological parameters were assessed in the study at three fixed time-points (baseline, three and six months). The clinical characteristics at baseline of the 22 patients are shown in Table I. Peripheral blood mononuclear cells (PBMCs) were obtained at baseline and subsequent to three and six months of telbivudine treatment. All the subjects provided informed consent to participate in the study (Trial Registration:

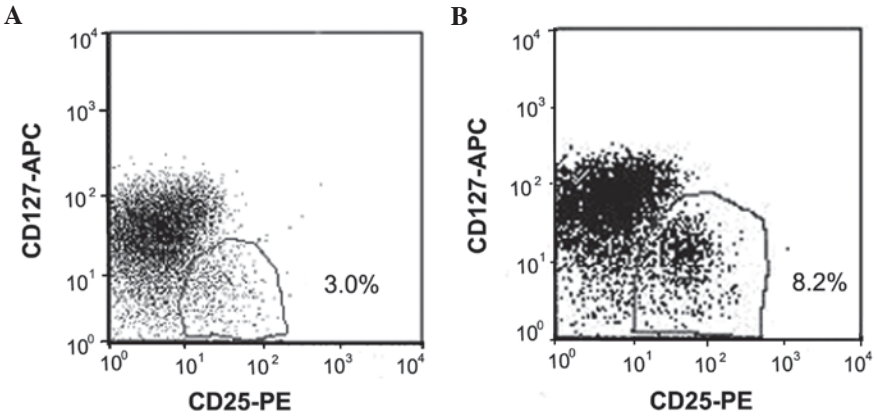

Figure 1. Proportion of peripheral blood $CD4^+CD25^+CD127^{low}$ T cells among $CD4^+$ T cells in patients with CHB and healthy controls. (A) Healthy control, (B) CHB. CHB, chronic hepatitis B; CD, cluster of differentiation.

Chinese Clinical Trial Registry ChiCTR-TRC-12001987), as approved by the ethical committee of the Southwest Hospital (Chongqing, China).

Biochemical and virological assessment. Routine liver function tests included ALT, aspartate aminotransferase and total bilirubin. These assays were performed with routine automated techniques (upper limit of normal: 50 U/l, 40 U/l and 21.0 μmol/l, respectively; HITACHI L-7600; Hitachi Medical Corp., Hitachi, Japan). HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc), anti-HAV, anti-hepatitis C virus (HCV), anti-hepatitis D virus (HDV) and anti-human immunodeficiency virus (HIV) were determined by commercial enzyme immunoassay kits using the Abbott IMX system (Abbott Laboratories, North Chicago, IL, USA). Serum HBV DNA levels were quantified using the Roche Amplicor Monitor assay (the limit threshold of the assay was 400 copies/ml) according to the manufacturer's instructions (Roche, Branchburg, NJ, USA).

Isolation of the PBMCs and flow cytometric analysis. PBMCs from patients with CHB and healthy donors were isolated from heparinized whole blood by density gradient centrifugation using Ficoll-Histopaque (Sigma, St. Louis, MO, USA). The cells were washed twice with RPMI 1640 (Bio Whittaker, Verviers, Belgium) and were frozen in RPMI 1640 containing 20% fetal calf serum (Hyclone, Logan, UT, USA) and 10% dimethyl sulfoxide. PBMCs from different time-points were stored at -135˚C for further analysis.

When the flow cytometric analysis was performed, the frozen PBMCs were washed once in phosphate-buffered saline (PBS) containing 0.3% bovine serum albumin and stained with fluorescently-labeled antibodies for the surface markers CD4-fluorescein isothiocyanate, CD25-phycoerythrin (PE), CD127-Alexa Fluor 647 and CD8- APC-Alexa Flour 750 (BD Biosciences PharMingen, San Diego, CA, USA) for 20 min at 4˚C. The cells were then washed twice with PBS containing 1% fetal calf serum, immediately analyzed using a FACScan flow cytometer (Becton-Dickinson, Franklin Lanes, NJ, USA) and analyzed using Cell Quest software (FACScalibur™, CELLQuest Pro™ software, Beckton-Dickinson).

Forkhead/winged helix transcription factor (FoxP3) mRNA quantification. RNA was isolated from the PBMC samples of all patients and controls using TRIzol® solution (Roche). The FoxP3 mRNA levels were quantified using a BioRad IQTM5 multicolor quantitative polymerase chain reaction (qPCR) detection system (BioRad, Hercules, CA, USA) with SYBR® Green I probes (Toyobo, Osaka, Japan), using β-actin as an internal control. Primers used for qPCR of FoxP3 and β-actin mRNAs were as follows: FoxP3 forward, 5'-AAGGAAAGGAGGATGGACG-3' and reverse, 3'-CAGGCAAGACAGTGGAAACC-5'; β-actin forward, 5'-CGTGGACATCCGCAAAGAC-3' and reverse, 3'-CTCGCTCCAACCGACTGCT-5'. Data from qPCR using SYBR Green I probes were analyzed by the standard curve method as described previously (30).

Statistical analysis. Statistical analysis was performed using SPSS software (version 9.0; SPSS Inc., Chicago, IL, USA). Continuous data are presented as the mean ± standard deviation, unless specified otherwise, and the significance was analyzed with the t-test. Flow cytometry and FoxP3 data were analyzed using the Mann-Whitney U test. All flow cytometry and functional data were compared to the levels at baseline using a Wilcoxon matched pairs signed rank sum test. $P>0.05$ was considered to indicate a statistically significant difference.

Results

The proportion of circulating Treg is increased in CHB patients and can be decreased during telbivudine treatment. To demonstrate the importance of Treg in chronic HBV infection, the proportion of Treg present in the peripheral blood of patients with CHB (n=22) and healthy individuals (n=19) was first detected and compared. Figure 1 shows the typical dot plots of the proportion of peripheral blood $CD4^+CD25^+CD127^{low}$ T cells among $CD4^+$ T cells for PBMCs from a representative patient with CHB (Fig. 1A) and a healthy individual (Fig. 1B). Typical dot plots of the proportion of the peripheral blood $CD8^+CD25^+$ T cells among $CD8^+$ T cells from a representative patient with CHB (Fig. 2A) and a healthy individual (Fig. 2B) are shown in Figure 2. As Figure 3 shows, patients with CHB demonstrated a significantly higher percentage

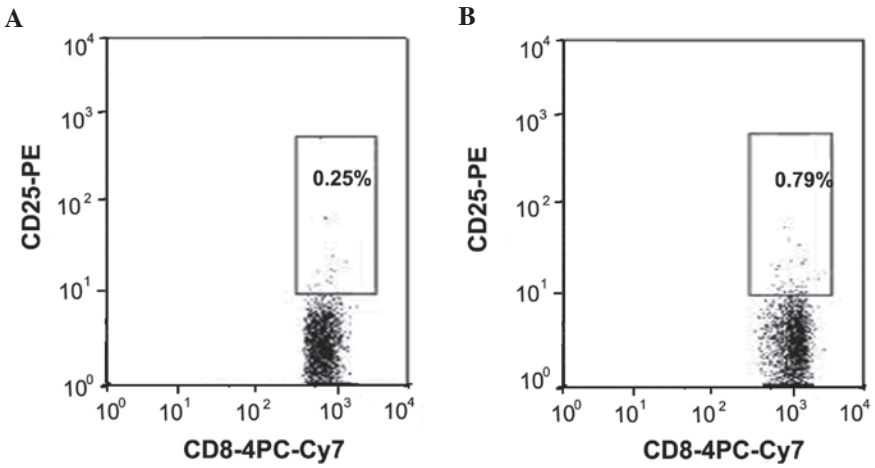

Figure 2. Proportion of peripheral blood $CD8^+CD25^+$ among $CD8^+$ T cells in (A) a representative patient with CHB and (B) a healthy control. CHB, chronic hepatitis B; CD, cluster of differentiation.

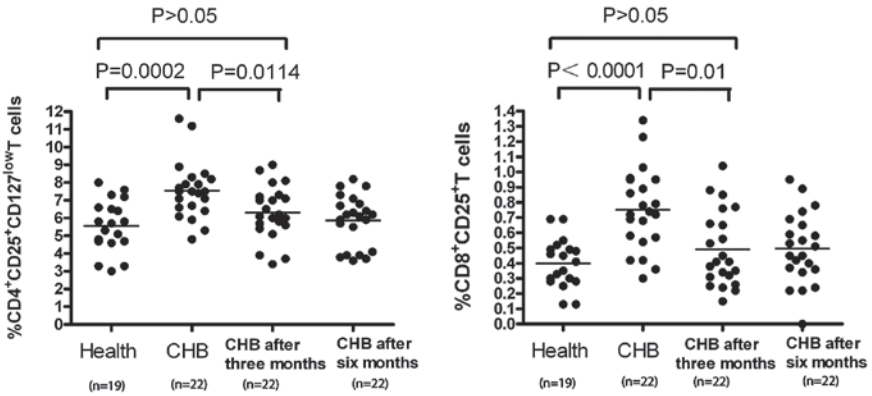

Figure 3. Proportion of peripheral blood $CD4^+$ $CD25^+$ $CD127^{low}$ and $CD8^+CD25^+$ T cells in patient subjects. CHB, chronic hepatitis B; CD, cluster of differentiation.

of $CD4^+CD25^+CD127^{low}$ T cells within their population of $CD4^+$ T cells (7.55±1.61%) compared with healthy controls in the peripheral blood (5.6±0.62%) (P=0.002). Patients with CHB also showed a significantly higher percentage of $CD8^+CD25^+$ T cells within their population of $CD8^+$ T cells (0.75±0.27%) compared with healthy controls (0.39±0.09%) (P<0.0001).

To assess the effect of treatment with telbivudine on the immune response, the proportion of Treg from patients' PBMCs were then compared at different time-points prior to and during antiviral treatment. Subsequent to three and six months of therapy, a significant decrease in the percentage of $CD4^+CD25^+CD127^{low}$ Treg (baseline, 7.55±1.61% vs. three months, 6.31±1.50% vs. six months, 5.86±1.44%, P=0.00114) was observed. Similarly, after three and six months of therapy, a significant decrease in the percentage of $CD8^+CD25^+$ Treg (baseline, 0.75±0.27% vs. three months, 0.49±0.25% vs. six months, 0.50±0.23%; P=0.01; Fig. 3) were also identified. Subsequent to six months of telbivudine treatment, the proportion of peripheral blood $CD4^+CD25^+CD127^{low}$ and $CD8^+CD25^+$ T cells in patients with CHB was comparable to the proportion of the healthy controls (P>0.05).

FoxP3 RNA expression is increased in patients CHB and can be decreased during telbivudine treatment. Since FoxP3 is considered to be the most specific marker for Treg, the relative FoxP3 mRNA levels of the peripheral blood samples from all the patients with CHB (one patient with three samples: At baseline, subsequent to three and six months of telbivudine treatment) and healthy controls were determined by qPCR. The FoxP3 mRNA levels in PBMCs in patients with chronic

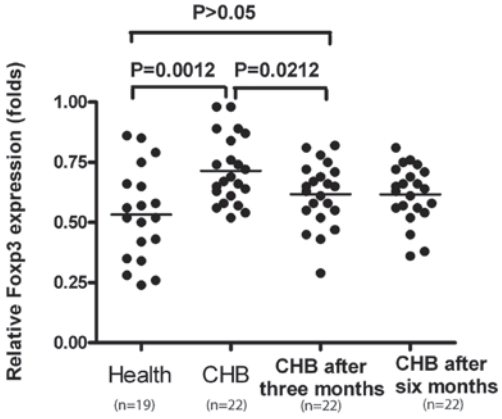

Figure 4. Levels of Foxp3 mRNA in PBMCs from subjects were detected by quantitative polymerase chain reaction. PBMCs, peripheral blood mononuclear cells; CHB, chronic hepatitis B; Foxp3, orkhead/winged helix transcription factor gene.

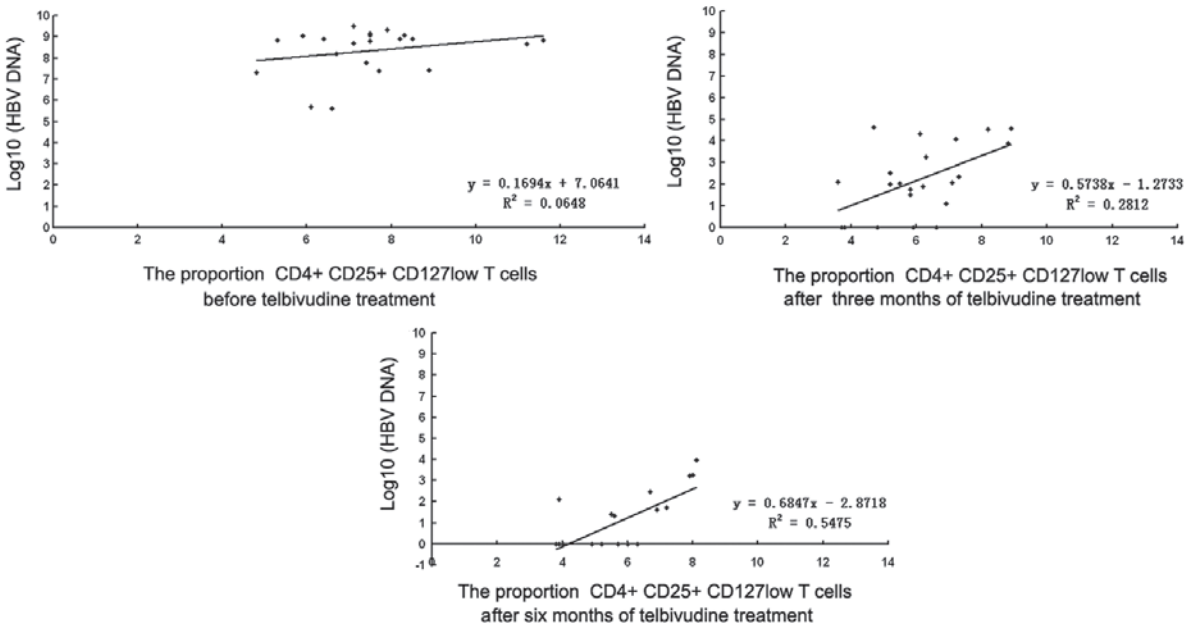

Figure 5. Correlation between the proportion of peripheral blood $CD4^+CD25^+CD127^{low}$ T cells and HBV DNA levels. HBV, hepatitis B virus; CD, cluster of differentiation.

HBV infection were significantly higher compared with those in the healthy controls (0.72±0.12 vs. 0.51±0.15; P=0.0012). Subsequent to three and six months of telbivudine treatment, the relative levels of Foxp3 mRNA in PBMCs from patients with CHB were significantly decreased from 0.72±0.12 at baseline to 0.62±0.13 (P=0.0212) and 0.61±0.12 after three and six months treatment, respectively (P=0.0231) (Fig. 4). Following telbivudine treatment, the relative levels of Foxp3 mRNA in PBMCs in patients with CHB were comparable to the levels in the healthy controls (P>0.05).

Correlation between Treg and clinical parameters. All 22 chronic patients with HBV in the present study were HBeAg-positive with ≥6log_{10} copies/ml HBV DNA and ≥2 times the upper limit of the normal serum ALT levels prior to telbivudine treatment. Subsequent to three months of telbivudine treatment, the HBV DNA levels of all 22 patients with CHB had dropped to 10^5 copies/ml or less, and those of 15 patients (15/22, 68.2%) dropped to 10^3 copies/ml or less; the serum ALT levels of 17 patients (17/22, 77.3%) dropped to 40 U/l or less, and two (9.1%) patients identified as HBeAg-negative. Subsequent to six months of telbivudine treatment, the ALT levels of all 22 patients with CHB had dropped to normal levels (40 U/l or less); the HBV DNA levels of all 19 patients with CHB (19/22, 86.4%) had dropped to 10^3 copies/ml or less, and four patients (18.2%) identified as HBeAg-negative. During treatment, no HBsAg loss was observed in the present study. Following telbivudine treatment, no correlation was observed between hepatic inflammation and the percentage of peripheral blood Treg, since the ALT levels of patients whose proportions of Treg were reduced to normal levels showed significant differences. Subsequent to six months of telbivudine treatment, the percentages of Treg of four HBeAg-negative patients were significantly decreased to normal levels. A weak correlation was observed between the percentage decrease in HBV DNA and that in the proportion of Treg after three (r=0.2812 and P<0.05) and six (r=0.5475 and P<0.05) months of telbivudine treatment (Fig. 5).

The proportions of peripheral blood $CD8^+CD25^+$ T cells among $CD8^+$ T cells in patients with CHB were low (0.1-1.3% at baseline). No correlation was observed between the viral load or hepatic inflammation (ALT) and the percentage of the peripheral blood $CD8^+CD25^+$ T cells. However, the percentages of $CD8^+CD25^+$ T cells in four patients exhibiting HBeAg loss were also significantly decreased in the present study.

Discussion

Consistent with the results of other previous studies (24-28), the results of the present study demonstrated telbivudine's marked potency to reduce HBV DNA levels. In the present study, continued telbivudine treatment was identified to be capable of rapidly reducing HBV DNA levels together with continuing improvement of biochemical and virological parameters through six months of treatment in patients with CHB.

Furthermore, the present study demonstrated the importance of Tregs in CHB. The frequency and functional properties of Tregs are significant due to increased numbers of Tregs, which may favor the development of chronic viral infections and influence the course of the disease and effectiveness of antiviral treatment. However, it had remained controversial whether circulating $CD4^+CD25^+$ Treg is frequency increased in patients with CHB and whether the frequency is correlated with HBV replication (31,32). The results of the present study indicated that patients with CHB infection displayed higher levels of Treg in their PBMCs compared with healthy controls. In addition, the relative FoxP3 mRNA levels were higher in PBMCs of patients with CHB. FoxP3 is a transcription factor specifically expressed by $CD4^+CD25^+$

Treg cells (33-35). A previous study showed that FoxP3 mRNA expression is relatively unique to the $CD4^+CD25^+$ cell population of PBMCs and measuring FoxP3 mRNA expression in $CD4^+$ cell populations or even total PBMCs is more practical compared with isolating the $CD4^+CD25^+$ cell population to evaluate $CD4^+CD25^+$ Treg activity and predict a clinical outcome. The higher relative levels of FoxP3 mRNA in patients with CHB in the present study indicate that patients with CHB have a higher percentage of Treg in the peripheral blood compared with healthy controls. This is in agreement with a previous study, which also identified a higher percentage of $CD4^+CD25^+FoxP3^+$ Treg in patients with CHB compared with healthy controls (36). However, an early study did not find the diversity of the Treg distribution among the asymptomatic carriers, patients with CHB and healthy individuals (11). The possible reason for this difference between different studies may be the different detection methods of Treg used in these studies, among various other specific circumstances. Certain studies have found that the virus-specific induction of Treg may result in two different consequences: It may have key roles in the process to prevent excessive immunopathological injury and it may also cause the establishment of persistent viral infection (37-41).

Furthermore, antiviral treatment with telbivudine was observed to be capable of downregulating the proportion of Treg in PBMCs and enhancing the antiviral immune response of patients with CHB. An abundance of experimental data has confirmed that $CD4^+CD25^+$ Tregs can suppress effective antiviral immune responses, in particular, in the chronic infections caused by the HIV (42) and HCV (43). A number of studies have shown that during the course of nucleotide analogs and interferon treatment, the decline of circulating Tregs together with a partial recovery of the immune responses can predict favorable responses (16,20-22). However, this standpoint was oppugned by a recent study in which greater increases of Tregs were correlated with a higher rate of sustained responsiveness to antiviral therapy (23). In the present study, the proportion of peripheral blood $CD4^+CD25^+CD127^{low}$ and $CD8^+CD25^+$ T cells in patients with CHB was observed to be decreased over three or six months of telbivudine treatment to a level comparable to that of the healthy controls. Concomitantly, the levels of Foxp3 mRNA in PBMCs from patients with CHB also decreased over three or six months of telbivudine treatment to a level comparable to that prior to treatment. The results indicate that the inhibition of viral replication reduced regulatory T cells and enhanced the antiviral immune response in chronic hepatitis B. It is hypothesized that patients effectively treated with telbivudine not only showed an enhanced reconstitution of the CD4 response, but also exhibited a significant enhancement in stimulation of HBV-specific CTL activity and reduced HBV serum titers, efficiently resulting in a significant increase in the frequency of CTL and a greater magnitude of cytokine production, which was partly proved previously (21,22,44).

Last and most importantly, the results of the present study indicated that the proportions of peripheral blood $CD4^+CD25^+CD127^{low}$ T cells were paralleled with its HBV DNA inhibition upon telbivudine treatment. The present study revealed a positive correlation between the $CD4^+CD25^+CD127^{low}$ Treg frequency and the serum HBV DNA levels, indicating that the upregulation of Tregs may be associated with an increase in HBV replication. This result is identical to those of previous studies (36,45). However, two earlier studies did not find any significant association between circulating Treg frequency and HBV DNA titer in patients with CHB (10,11). In addition, certain studies support an association between increased $CD4^+CD25^+$ Treg and HBeAg, as well as impaired viral clearance (36), while others did not find this correlation (10). By contrast, the present study did not identify any association of the HBeAg status with either $CD4^+CD25^+CD127^{low}$ or $CD8^+CD25^+$ T cell frequency. Differences in the methods, reagents and samples used in these studies may account for the discrepancies. Following telbivudine treatment, no correlation was observed between ALT and the percentage of the peripheral blood Treg in the present study, which was in agreement with a previous study (10). However, another study reported that Treg accumulated and expanded locally at the site of infection (46). Alltogether, the preliminary data of the current study indicated that the elevation in the number of circulating Tregs in patients with CHB decreased following antiviral treatment and the antigen-specific T-cell response to HBsAg was more significantly suppressed by Tregs. These results support the hypothesis that CHB infection leads to the induction of suppressive Tregs which inhibit antiviral immune responses.

In conclusion, the findings of the present study indicate that telbivudine treatment not only reduces serum HBV DNA levels rapidly, but is also beneficial to restore the antiviral immune response. Effective telbivudine treatment indirectly affects the immune system by downregulating the proportion of the peripheral blood $CD4^+CD25^+CD127^{low}$ and $CD8^+CD25^+$ T cells markedly, which may be predictive of the responsiveness to telbivudine therapy.

Acknowledgements

The present study was supported in part by the State Key Project Specialized for Infectious Diseases (no. 2012ZX10002-004).

References

1. Lai CL, Ratziu V, Yuen MF and Poynard T: Viral hepatitis B. Lancet 362: 2089-2094, 2003.
2. Guidotti LG and Chisari FV: Immunobiology and pathogenesis of viral hepatitis. Annu Rev Pathol 1: 23-61, 2006.
3. Rehermann B, Fowler P, Sidney J, *et al*: The cytotoxic T lymphocyte response to multiple hepatitis B virus polymerase epitopes during and after acute viral hepatitis. J Exp Med 181: 1047-1058, 1995.
4. Bertoletti A and Naoumov NV: Translation of immunological knowledge into better treatments of chronic hepatitis B. J Hepatol 39: 115-124, 2003.
5. Bertoletti A, D'Elios MM, Boni C, *et al*: Different cytokine profiles of intraphepatic T cells in chronic hepatitis B and hepatitis C virus infections. Gastroenterology 112: 193-199, 1997.
6. Zhang Z, Zhang JY, Wang LF and Wang FS: Immunopathogenesis and prognostic immune markers of chronic hepatitis B virus infection. J Gastroenterol Hepatol 27: 223-230, 2012.
7. Sakaguchi S, Yamaguchi T, Nomura T and Ono M: Regulatory T cells and immune tolerance. Cell 133: 775-787, 2008.
8. Miyara M and Sakaguchi S: Natural regulatory T cells: mechanisms of suppression. Trends Mol Med 13: 108-116, 2007.
9. Xu D, Fu J, Jin L, *et al*: Circulating and liver resident CD4+CD25+ regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. J Immunol 177: 739-747, 2006.
10. Stoop JN, van der Molen RG, Baan CC, *et al*: Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. Hepatology 41: 771-778, 2005.

11. Franzese O, Kennedy PT, Gehring AJ, *et al*: Modulation of the CD8+-T-cell response by CD4+ CD25+ regulatory T cells in patients with hepatitis B virus infection. J Virol 79: 3322-3328, 2005.
12. Feng IC, Koay LB, Sheu MJ, *et al*: HBcAg-specific CD4+CD25+ regulatory T cells modulate immune tolerance and acute exacerbation on the natural history of chronic hepatitis B virus infection. J Biomed Sci 14: 43-57, 2007.
13. Taams LS, Vukmanovic-Stejic M, Smith J, *et al*: Antigen-specific T cell suppression by human CD4+CD25+ regulatory T cells. Eur J Immunol 32: 1621-1630, 2002.
14. Liaw YF and Chu CM: Hepatitis B virus infection. Lancet 373: 582-592, 2009.
15. Dienstag JL: Hepatitis B virus infection. N Engl J Med 359: 1486-1500, 2008.
16. Sprengers D, Stoop JN, Binda RS, *et al*: Induction of regulatory T-cells and interleukin-10-producing cells in non-responders to pegylated interferon-alpha therapy for chronic hepatitis B. Antivir Ther 12: 1087-1096, 2007.
17. Rico MA, Quiroga JA, Subirá D, *et al*: Hepatitis B virus-specific T-cell proliferation and cytokine secretion in chronic hepatitis B e antibody-positive patients treated with ribavirin and interferon alpha. Hepatology 33: 295-300, 2001.
18. Maini MK, Reignat S, Boni C, *et al*: T cell receptor usage of virus-specific CD8 cells and recognition of viral mutations during acute and persistent hepatitis B virus infection. Eur J Immunol 30: 3067-3078, 2000.
19. Boni C, Penna A, Bertoletti A, *et al*: Transient restoration of anti-viral T cell responses induced by lamivudine therapy in chronic hepatitis B. J Hepatol 39: 595-605, 2003.
20. Tsai SL, Sheen IS, Chien RN, *et al*: Activation of Th1 immunity is a common immune mechanism for the successful treatment of hepatitis B and C: tetramer assay and therapeutic implications. J Biomed Sci 10: 120-135, 2003.
21. Stoop JN, van der Molen RG, Kuipers EJ, Kusters JG and Janssen HL: Inhibition of viral replication reduces regulatory T cells and enhances the antiviral immune response in chronic hepatitis B. Virology 361: 141-148, 2007.
22. Chen Y, Li X, Ye B, *et al*: Effect of telbivudine therapy on the cellular immune response in chronic hepatitis B. Antiviral Res 91: 23-31, 2011.
23. Koay LB, Feng IC, Sheu MJ, *et al*: Hepatitis B virus (HBV) core antigen-specific regulatory T cells confer sustained remission to anti-HBV therapy in chronic hepatitis B with acute exacerbation. Hum Immunol 72: 687-698, 2011.
24. Nash K: Telbivudine in the treatment of chronic hepatitis B. Adv Ther 26: 155-169, 2009.
25. Lai CL, Gane E, Liaw YF, *et al*: Telbivudine versus lamivudine in patients with chronic hepatitis B. N Engl J Med 357: 2576-2588, 2007.
26. Lai CL, Leung N, Teo EK, *et al*: A 1-year trial of telbivudine, lamivudine, and the combination in patients with hepatitis B e antigen-positive chronic hepatitis B. Gastroenterology 129: 528-536, 2005.
27. Lai CL, Gane E, Hsu CW, *et al*: Two-year results from the GLOBE trial in patients with hepatitis B: greater clinical and antiviral efficacy for telbivudine (LdT) vs. lamivudine. Hepatology 44 (Suppl 1): 222A, 2006.
28. Liaw YF, Gane E, Leung N, *et al*: 2-Year GLOBE trial results: telbivudine Is superior to lamivudine in patients with chronic hepatitis B. Gastroenterology 136: 486-495, 2009.
29. Chinese Society of Hepatology, Chinese Medical Association; Chinese Society for Infectious Diseases, Chinese Medical Association: Guideline on prevention and treatment of chronic hepatitis B in China (2005). Chin Med J (Engl) 120: 2159-2173, 2007.
30. Shaik GM, Dráberová L, Dráber P, Boubelík M and Dráber P: Tetraalkylammonium derivatives as real-time PCR enhancers and stabilizers of the qPCR mixtures containing SYBR Green I. Nucleic Acids Res 36: e93, 2008.
31. Lan RY, Cheng C, Lian ZX, *et al*: Liver-targeted and peripheral blood alterations of regulatory T cells in primary biliary cirrhosis. Hepatology 43: 729-737, 2006.
32. Li S, Gowans EJ, Chougnet C, Plebanski M and Dittmer U: Natural regulatory T cells and persistent viral infection. J Virol 82: 21-30, 2008.
33. Fontenot JD and Rudensky AY: A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. Nat Immunol 6: 331-337, 2005.
34. Fontenot JD, Gavin MA and Rudensky AY: Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 4: 330-336, 2003.
35. Hori S, Nomura T and Sakaguchi S: Control of regulatory T cell development by the transcription factor Foxp3. Science 299: 1057-1061, 2003.
36. Yang G, Liu A, Xie Q, *et al*: Association of CD4+CD25+Foxp3+ regulatory T cells with chronic activity and viral clearance in patients with hepatitis B. Int Immunol 19: 133-140, 2007.
37. Belkaid Y: Regulatory T cells and infection: a dangerous necessity. Nat Rev Immunol 7: 875-888, 2007.
38. Belkaid Y and Rouse BT: Natural regulatory T cells in infectious disease. Nat Immunol 6: 353-360, 2005.
39. Alatrakchi N and Koziel M: Regulatory T cells and viral liver disease. J Viral Hepat 16: 223-229, 2009.
40. Rushbrook SM, Hoare M and Alexander GJ: T-regulatory lymphocytes and chronic viral hepatitis. Expert Opin Biol Ther 7: 1689-1703, 2007.
41. Billerbeck E, Bottler T and Thimme R: Regulatory T cells in viral hepatitis. World J Gastroenterol 13: 4858-4864, 2007.
42. Aandahl EM, Michaëlsson J, Moretto WJ, Hecht FM and Nixon DF: Human CD4+ CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. J Virol 78: 2454-2459, 2004.
43. Cabrera R, Tu Z, Xu Y, *et al*: An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. Hepatology 40: 1062-1071, 2004.
44. Lau GK, Cooksley H, Ribeiro RM, *et al*: Impact of early viral kinetics on T-cell reactivity during antiviral therapy in chronic hepatitis B. Antivir Ther 12: 705-718, 2007.
45. Peng G, Li S, Wu W, Sun Z, Chen Y and Chen Z: Circulating CD4+ CD25+ regulatory T cells correlate with chronic hepatitis B infection. Immunology 123: 57-65, 2008.
46. Cao D, Malmström V, Baecher-Allan C, Hafler D, Klareskog L and Trollmo C: Isolation and functional characterization of regulatory CD25brightCD4+ T cells from the target organ of patients with rheumatoid arthritis. Eur J Immunol 33: 215-223, 2003.