Original Article

The relationship between the clearance of HBsAg and the remodeling of B cell subsets in CHB patients treated with Peg-IFN-α

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Background: The seroconversion of the hepatitis B antigen is the ideal outcome for long-acting interferon-pegylated interferon-α (Peg-IFN-α) treatment among patients with chronic hepatitis B (CHB). B-cell response plays an important role in the process of hepatitis B antigen clearance, but the specific mechanism by which B-cell improve hepatitis B virus (HBV) is still unclear.

Methods: A total of 103 CHB patients participated in this study. The patients received 24 weeks of Peg-IFN-α treatment. Flow cytometry was used to detect B-cell surface markers' cluster of differentiation cluster of differentiation CD19, CD24, and CD27 in the peripheral blood mononuclear cells (PBMCs) of CHB patients before and after 24 weeks of Peg-IFN-α treatment.

Results: After 24 weeks of Peg-IFN-α treatment, the content of memory B cells ($CD19^+CD27^+$) and effector B cells ($CD19^+CD38^+$) increased significantly. Further analysis showed that the clearance of the hepatitis B antigen was correlated with the change value, ΔT, of plasma cells before and after treatment. The B-cell subsets ($CD19^+CD24^+$; $CD19^+CD40^+$; $CD19^+CD40^+$; $CD19^+CD80^+$), was also tested and the results showed that $CD19^+CD24^+$ and $CD19^+CD80^+$ content also increased significantly after treatment.

Conclusions: After Peg-IFN-α treatment, the B-cell subsets of CHB patients are remodeled. Thus, Peg-IFN-α treatment appears to play an important role in the remodeling of B cell subsets and the clearance of HBV antigens. The results of this study provide a theoretical basis and guidance for the clinical treatment of CHB.

Keywords: Peg-IFN-α; B-cell; chronic hepatitis B (CHB); hepatitis B virus (HBV)

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Introduction

Hepatitis B virus (HBV) infection is a global public health problem (1). Over 30% of the world's population (2,3) has serological evidence of past or current HBV infection. HBV infection can inhibit the human immune response, causing immune tolerance (4,5), and leading to chronic HBV infection. Additionally, patients with chronic hepatitis B (CHB) are at increased risk of liver cirrhosis and liver

Table 1 Baseline characteristics of CHB patients

Variable	Value
Number	103
Sex (M/F)	90/13
Age (years)	42.8±8.26
HBsAg	434.20 (219.50, 714.30)
Anti-HBs (IU/mL)	2.0 (2.0, 2.0)
HBV DNA (copies/mL)	100.0 (20.0, 100.0)
HBV RNA (copies/mL)	108.0 (0.0, 6,750.0)
ALT	26.0 (21.0, 36.0)

CHB, chronic hepatitis B; ALT, alanine aminotransferase.

cancer (2,6). At present, the common clinical treatment (7) for CHB is antiviral therapy. Interferon (8,9) and nucleoside analogues (10) are two common major antiviral drugs. The outcome of clinical cure for CHB is generally defined as a continuous elimination of the viral surface antigen. The seroconversion of the antigen should be accompanied by alanine aminotransferase (ALT) recovery and the improvement of liver tissue lesions. However, due to the continuous replication of and difficulty in eliminating the HBV circular covalently closed deoxyribonucleic acid (cccDNA), the therapeutic effects of CHB is not ideal. Compared to nucleoside drugs, interferon drugs can effectively enable patients to achieve hepatitis B surface antigen (HBsAg) seroconversion, which is the clinical endpoint of the ideal treatment. However, the immune regulation mechanism of hepatitis B patients treated with interferon is not yet fully understood (11,12).

To eliminate HBV, the body needs to produce an effective immune response (12,13). It is currently believed that T-cell-mediated cellular immune (14) response plays a leading role in eliminating viral infections (15). However, in recent years, the humoral immune response (which is based on neutralizing antibodies) in the resistance and elimination of the HBV infection has been the subject of increasing attention from researchers (16). Studies have shown that in CHB virus infection, the ability of B-cell differentiation *in vivo* (17,18) is significantly enhanced, but the proliferation ability is significantly reduced. Additionally, in CHB patients, the B-cell immune response is related to the clinical stage (19), which suggests that B-cell response plays an important role in treating HBV infection. However, currently, it is not yet clear how B cells exert their effects (20-23). To understand the role of B cells in clearing the HBV, flow cytometry was used to detect changes in the levels of cluster of differentiation (CD)19, CD24, CD27, CD40 and CD80 B cells in the peripheral blood mononuclear cells (PBMCs) of CHB patients after 24 weeks of pegylated interferon (Peg-IFN-α) treatment. We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/atm-21-409).

Methods

Patients

The data of patients treated with Peg-IFN-α who participated in the "Clinical Cure of Chronic Hepatitis B (Everest) Project" were collected. The course of treatment was 24 weeks, and the patients were managed according to the relevant recommendations of "Guideline of Prevention and Treatment for Chronic Hepatitis B (2015 Update) (24)." *Table 1* sets out the baseline information of the participating CHB patients. In the course of the Peg-IFN-α treatment, HBsAg seroconversion occurred, and observations were discontinued if HBsAg clearance (HBsAg <0.05 IU/mL) was confirmed in two consecutive examinations. Any patient with HBsAg clearance (HBsAg <0.05 IU/mL) was classified as "cured".

To participate in the study, patients had to meet the following inclusion criteria: (I) have a clinical diagnosis of CHB, whose diagnostic criteria complied with Guideline of Prevention and Treatment for Chronic Hepatitis B (2015 Update) (24) (II) be aged between 18 and 65 years; (III) have undergone nucleoside analogue (NAs) treatment for more than 1 year (HBsAg ≤1,500 IU/mL, HBeAg-negative and HBV DNA <100 IU/mL); (IV) have no contraindications to interferon treatment; and (V) be willing to receive Peg-IFN-α treatment and sign an informed consent form.

In terms of the exclusion criteria, patients were excluded from the study if they had a human immunodeficiency virus infection, had decompensated liver cirrhosis, had liver failure, had liver cancer, had undergone an organ transplantation, had an autoimmune disease, had a severe heart, brain, kidney disease or nervous system disease, were pregnant or planning a pregnancy, and/or the doctor in

charge believed that in the given circumstances interferon should not be used. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The research protocol was approved by the ethics committee of The Third Affiliated Hospital of Sun Yat-sen University ([2019]02-527-01), and all patients provided informed consent.

Clinical observation indicators

At 0 weeks, 12 weeks, 24 weeks, the patients were evaluated and blood samples were collected to complete the laboratory testing. The lower limit of HBsAg detection was 0.05 IU/mL. To test liver function a Hitachi 7600 automatic analyzer was used (the reagents were purchased from Guangdong Mike Biology Co., Ltd.).

Phenotypic analysis

To analyze the B-cell subsets, PBMCs were stained with fluorochrome-labelled anti-human $CD27^+$APC, $CD19^+$APC-Cy7, $CD24^+$PC7 (Biolegend Biosciences, San Jose, CA, USA). Flow cytometry was used to analyze antibody-stained cells using FACSCANTOII and Diva software (BD Biosciences).

Statistical analysis

Variables are presented as means and standard deviations (SDs). All analyses were conducted using SPSS software. Spearman correlations and *t*-tests were used in this study.

Results

The modulation of the B-cell subsets among CHB patients treated with Peg-IFN-α

First, this study sought to explore the modulatory effects of Peg-IFN-α on chronic CHB patients, and the effects of this treatment on the B cells of these patients. B-cell frequency in the circulation of patients was determined after 24 weeks of Peg-IFN-α treatment (see *Figure 1A*). The frequencies of the following subsets of B cells, including the activated B cells ($CD27^+CD19^+$), plasmablasts ($CD38^+CD19^+$), and three categories of Breg cells ($CD40^+CD19^+$, $CD80^+CD19^+$, $CD24^+CD19^+$) were also detected. The results showed that the frequencies of B cells increased with the treatment of Peg-IFN-α (see *Figure 1B*).

After 24 weeks of Peg-IFN-α treatment, the level of HBsAg was associated with active memory B cells and plasmablasts

Due to the significant role of HBsAg in the development of what in CHB patients, the concentration of the HBsAg was determined. As *Table 2* shows, the level of HBsAg decreased distinctly among CHB patients after 24 weeks of Peg-IFN-α treatment.

This study also sought to examine the effects of changes in the level of HBsAg and whether this had any related effects on the B cells. Thus, the effects of the subsets of B cells among CHB patients following Peg-IFN-α treatment were also examined. As anticipated, the components of the B cells subsets changed following Peg-IFN-α treatment. As *Table 2* shows, the active memory B cells ($CD19^+CD27^+$) increased after 24 weeks of Peg-IFN-α treatment. Further, the effector B cell ($CD19^+CD38^+$) also increased after treatment of Peg-IFN-α. Thus, Peg-IFN-α appears to have a protective effect on CHB patients, and the improvement in the HBsAg could be related to the effect of Peg-IFN-α on active memory B cells and plasmablasts.

After 24 weeks of treatment with Peg-IFN-α, the changed value (ΔA) of HBsAg was related to the changed value (ΔT) of the B-cell subsets

Based on the above findings, we queried whether the HBsAg improvement was related to the change in the active memory B cell and effector B cell. Specifically, we explored the relationship between the level of HBsAg and these two kinds of B-cell subsets using a statistical method. The results showed that the level of HBsAg was negatively related to the active memory B cell (r=–0.24, P=0.015) (see *Table 3*); that is, the results showed that when the level of the memory B cell increased, the concentration of HBsAg decreased. Further, the changed value between the baseline HBsAg and the HBsAg after 24 weeks of treatment was also negatively related to the changed valued of effector B cells (r=–0.235, P=0.017) (see *Table 4*). Thus, it appears that a decrease in the HBsAg might be associated with the improvement in active memory B cells and effector B cells treated with Peg-IFN-α.

Breg cell changes in CHB patients following treatment with Peg-IFN-α

Notably, we found that a kind of unfavored B cell (25),

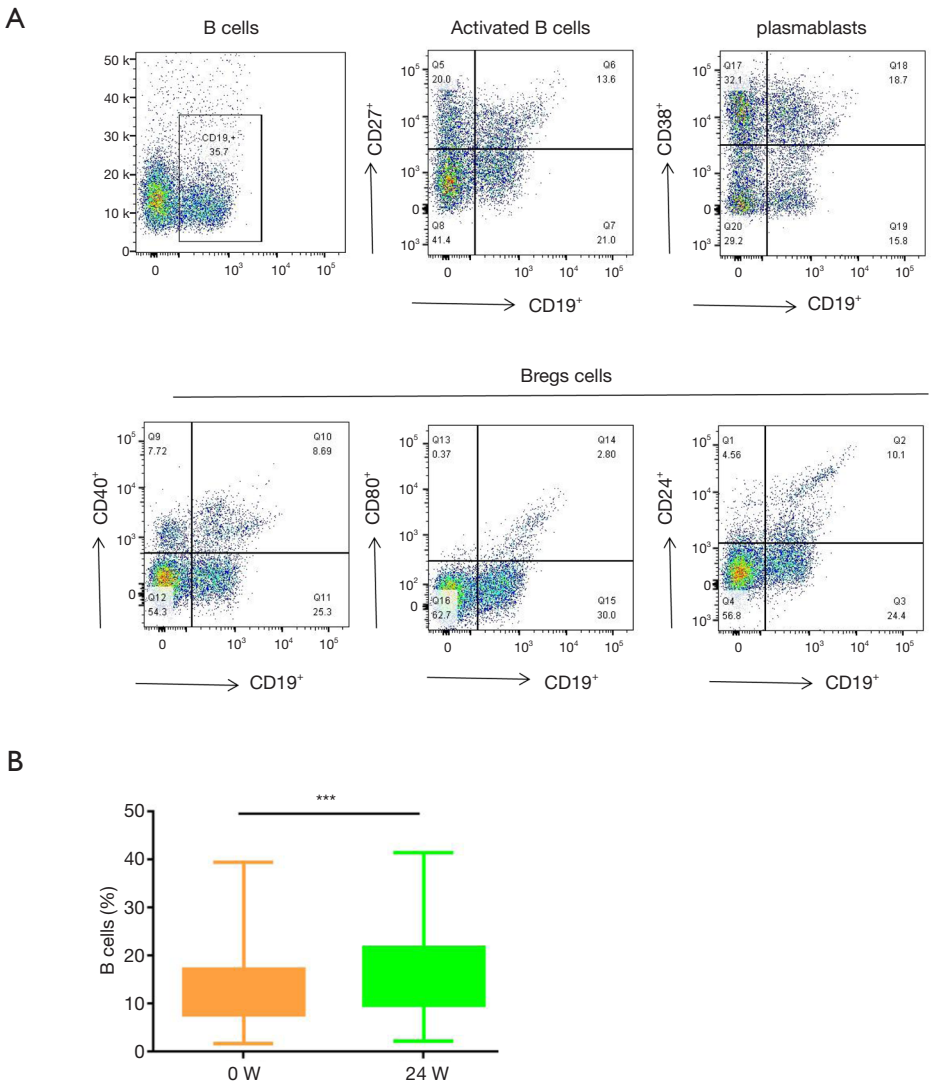

Figure 1 Change of B cell subsets after Peg-IFN-α treatment. (A) The subsets of B cells following treatment with Peg-IFN-α. B-cell gating strategy. The peripheral B-cell subsets were classified according to the most common lineage/differentiation markers of CD19, CD24, CD27, CD80. The B-cell subsets were defined as activated B cells ($CD19^+CD27^+$), plasmablasts ($CD19^+CD38^+$), and Breg cells ($CD19^+CD40^+$, $CD19^+CD80^+$, and $CD19^+CD24^+$). (B) The frequency of total B cells at week 0 and week 24. ***, P<0.0001

named a Breg cell, changed following treatment with Peg-IFN-α. Specifically, after Peg-IFN-α treatment, the number of Breg cells increased. As *Table 5* shows, compared to Week 0, two subgroups of Breg cell ($CD19^+CD24^+$/$CD19^+CD80^+$) all increased during Peg-IFN-α treatment (P<0.05). It may be that this type of B cell subgroup has a negative response by Peg-IFN-α treatment; however, further research needs to be conducted to identify this mechanism.

Discussion

The seroconversion of the HBsAg is an ideal endpoint for the treatment of HBV infection (26). The immune mechanism of the body in clearing the HBV is still unclear; however, B cells (4,27-29) play a vital role in the process of clearing HBV. This research showed that with f Peg-IFN-a treatment (30,31), the memory B cells and effector B

Table 2 Comparison of B cells and hepatitis B surface antigens

Subsets of B cells	0 W	24 W	P
$CD19^+CD27^+$	1.48 (0.72, 2.51)	2.34 (1.34, 3.38)	<0.001
$CD19^+CD38^+$	5.12 (2.67, 9.06)	8.75 (4.85, 14.90)**	<0.001
HBsAg	434.20 (219.50, 714.30)	19.34 (0.49, 391.00)**	<0.001

**, P<0.001.

Table 3 Partial correlation analysis between B-cell phenotype and hepatitis B surface antigen level at 24 weeks

Subsets of B cells	HBsAg	
	r	P
$CD19^+CD27^+$	–0.241	0.015
$CD19^+CD38^+$	–0.270	0.006

Table 4 Correlation analysis between the changed value (ΔA) of HBsAg and the changed value (ΔT) of the subsets of B cell and plasma cells after 24 weeks of Peg-IFN-α treatment

Subsets of B cells	HBsAg (ΔA)	
	r	P
$CD19^+CD27^+$ ΔT	–0.153	0.122
$CD19^+CD38^+$ ΔT	–0.235	0.017

ΔA: the value of HBsAg at week 24; ΔA: the value of HBsAg at week 0.

Table 5 Comparison of Breg cells and hepatitis B surface antigens

Subsets of B cells	0 W	24 W	P
$CD19^+CD24^+$	2.20 (1.05, 3.48)	5.33 (2.96, 9.30)**	<0.001
$CD19^+CD40^+$	8.25 (4.83, 12.70)	11.00 (5.69, 16.40)	0.133
$CD19^+CD80^+$	1.94 (0.87, 3.56)	2.76 (1.74, 7.14)**	<0.001
HBsAg	434.20 (219.50, 714.30)	19.34 (0.49, 391.00)**	<0.001

**, P<0.001.

cells of CHB patients increased significantly; however, Peg-IFN-a treatment did not affect a subsets of B cells. Notably, the present study showed that the clearance of the HBsAg was correlated with the change ΔT value of effector B cells. Thus, the ΔT value of effector B cells could be an effective way to assess the treatment of CHB. Additionally, somewhat unexpectedly, the present study showed that the content of the three types of Breg cells increased significantly after Peg-IFN-a treatment, indicating that Breg cells exerted an immune negative regulation and might be involved in the body's immune response to clear the HBsAg. This surprising finding provides new insights into the immune regulation mechanism of the body in clearing the HBV.

The occurrence and development of CHB are closely related to the body's immune system and immune response, especially the specific immune response that is involved in the elimination of the HBsAg through the secretion of protective antibodies. Given the major role of B cells in the process of antibody secretion, an understanding of changes in B cells during HBV infection will play an important role in exploring the specific immune mechanism by which the HBV antigen can be eliminated. Our study found

that after interferon treatment, activated memory B cells ($CD19^+CD27^+$) and effector cells increased significantly in HBV patients, suggesting that memory B cells may be involved in the immune response. This is consistent with the findings of Oliviero *et al.* (32). Thus, interferon therapy enhances B cell immunity, and the changes of B cell subsets may be able to predict responses of CHB patients to Peg-IFN-a therapy. The content of activated memory B cells ($CD19^+CD27^+$) and effector cells did increase among patients; however, there was no difference in the subgroup of B cells between cured patients and uncured patients after treatment. Thus, the protective effect of the B-cell response in CHB patients might reduce hepatitis B. Such findings are consistent with those of Thomas *et al.* (33).

In recent years, research has shown that a class of B-cell subgroups (Breg cells) has immune-suppressive functions. Similar to helper T cells, they release various cytokines in some autoimmune diseases and tumors that are involved in immune regulation. Previous studies have shown that Breg cells (34) exist in the human body and are involved in the body's negative immune regulation. Specifically, these cells secrete IL-10 and other cytokines by B cells which isolated from the peripheral blood of healthy people (35). Fillatreau *et al.* (35) confirmed that the stimulation of homologous antigens and functional B-cell receptors could promote the generation of Breg cells. Under the action of endogenous antigens, B lymphocytes or plasma cells can also differentiate into Breg cells due to antigen stimulation, and exert an immune balance effect on the body through negative immune regulation.

To date, very few studies have been conducted on Breg cells in relation to chronic HBV infection. The immune characteristic of chronic HBV infection is the lack or the exhaustion of a virus-specific T-cell function. It has been reported in the literature that the mechanisms causing HBV immune failure include sustained high viral replication, viral gene mutations and other viral factors, and immune regulatory factors, such as a programmed death-1(PD-1) pathway, high Treg-cell expression, and the immune imbalance of Treg/Th17. However, Breg cells can also inhibit the T-cell immunity of CHB patients through a variety of mechanisms, and thus prevent the body from producing an effective immune response to clear the virus. Das *et al.* (36,37) found that during chronic HBV infection, the content of Breg cells secreting IL-10 in the peripheral blood of CHB patients increased. Blocking IL-10 *in vitro* can restore the function of HBV-specific T cells. Other studies (10,38,39) have confirmed that IL-35-secreting Breg cells and IL-35 are also involved in the process of chronic HBV infection; however, the phenotype of the IL-35 secreting Breg cells is not yet clear. This study detected changes in the content of three types of Breg cells, including $CD19^+$ and $CD24^+$, $CD19^+$ and $CD40^+$, and $CD19^+$ and $CD80^+$ in CHB patients. The content of these three types of Breg cells increased significantly before and after treatment, suggesting that these three types of Breg cells might be involved in the clearance of the hepatitis B antigen immunomodulation. Thus, we speculate that after interferon treatment, the immune response of CHB patients should improve. To prevent excessive immune damage, the proportion of negatively regulated Breg cells increases, thereby suppressing the antiviral immune response, which is not conducive to the removal of the virus. The specific mechanism needs to be further explored.

In summary, the present study showed that, after interferon treatment, the immune response of B cells showed no correlation with patients' prognoses; however, the clearance of the hepatitis B antigen was found to be related to a change in plasma cells. Further, it appears that three types of Breg cells may be involved in the human immune response.

Conclusions

In this study, the results showed that content of memory B cells ($CD19^+$ $CD27^+$) and effector B cells increased after 24 weeks of treatment. Thus, Peg-IFN-α therapy appears to enhance B-cell immunity, which in turn might be able to predict the response of CHB to interferon therapy. Additionally, the change value of the hepatitis B antigen (ΔA) was correlated with the change value of effector B-cell content (ΔT). Thus, effector B cells may be involved in the clearance of the hepatitis B antigen. Notably, there was also a corresponding increase in the content of the three types of Breg cells. Thus, Breg cells, which are negative immune regulators, may be involved in the body's immune response. This research provides guidance for the clinical treatment of CHB and provides new insights into the immune response mechanism of the body in clearing the HBV antigen.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at http://dx.doi.org/10.21037/atm-21-409

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm-21-409). 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. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The research protocol was approved by the ethics committee of The Third Affiliated Hospital of Sun Yat-sen University ([2019]02-527-01), and all patients provided informed consent.

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