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

Ze-Qian Wu¹²,³, Lei Tan⁴, Wei-Qiang Gan¹²,³, Zhi-Shuo Mo¹²,³, Da-Biao Chen¹²,³, Pei-Pei Wang¹²,³, Qi-Yi Zhao¹²,³, Dong-Ying Xie¹²,³, Zhi-Liang Gao¹²,³

¹Department of Infectious Diseases, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China; ²Guangdong Key Laboratory of Liver Disease Research, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China; ³Key Laboratory of Tropical Disease Control (Sun Yat-Sen University), Ministry of Education, Guangzhou, China; ⁴Department of Medical Ultrasonic, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, China

Contributions: (I) Conception and design: ZQ Wu, L Tan, ZL Gao; (II) Administrative support: QY Zhao, DY Xie, ZL Gao; (III) Provision of study materials or patients: ZS Mo, DB Chen, PP Wang; (IV) Collection and assembly of data: ZQ Wu, L Tan; (V) Data analysis and interpretation: ZQ Wu, L Tan; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

*These authors contributed equally to this work.

Correspondence to: Zhi-Liang Gao. Department of Infectious Diseases, The Third Affiliated Hospital of Sun Yat-sen University, No. 600 Tianhe Road, Guangzhou 510630, China. Email: gaozl@mail.sysu.edu.cn.

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 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)

Submitted Dec 07, 2020. Accepted for publication Mar 04, 2021.
doi: 10.21037/atm-21-409

View this article at: http://dx.doi.org/10.21037/atm-21-409

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
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),
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-α treatment (30,31), the memory B cells and effector B
cells of CHB patients increased significantly; however, Peg-IFN-α treatment did not affect 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-α 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

### 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.
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-α 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 of 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.

Acknowledgments

Funding: National Science and Technology Major Project (2018ZX10302204-002); The National Natural Science Foundation of China (81672701); Outstanding Graduate Student Innovation and Development Project (19ykyjs04);
National Science and Technology Major Project (2017ZX10302201-006-002).

Footnote

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

Data Sharing Statement: Available at http://dx.doi.org/10.21037/atm-21-409

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.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. Tiollais P, Pourcel C, Dejean A. The hepatitis B virus. Nature 1985;317:489-495.
2. Chan SL, Wong VW, Qin S, et al. Infection and Cancer: The Case of Hepatitis B. J Clin Oncol 2016;34:83-90.
3. Nelson PK, Mathers BM, Cowie B, et al. Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. Lancet 2011;378:571-83.
4. Shin EC, Sung PS, Park SH. Immune responses and immunopathology in acute and chronic viral hepatitis. Nat Rev Immunol 2016;16:509-23.
5. Zhou X, Pan H, Yang P, et al. Both chronic HBV infection and naturally acquired HBV immunity confer increased risks of B-cell non-Hodgkin lymphoma. BMC Cancer 2019;19:477.
6. Yang JD, Roberts LR. Hepatocellular carcinoma: A global view. Nat Rev Gastroenterol Hepatol 2010;7:488-58.
7. Kwon H, Lok AS. Hepatitis B therapy. Nat Rev Gastroenterol Hepatol 2011;8:275-84.
8. Yeh ML, Peng CY, Dai CY, et al. Pegylated-interferon alpha therapy for treatment-experienced chronic hepatitis B patients. PLoS One 2015;10:e0122259.
9. Ren H, Huang Y. Effects of pegylated interferon-α based therapies on functional cure and the risk of hepatocellular carcinoma development in patients with chronic hepatitis B. J Viral Hepat 2019;26 Suppl 1:5-31.
10. Rosser EC, Mauri C. Regulatory B cells: origin, phenotype, and function. Immunity 2015;42:607-12.
11. Burton AR, Pallett LJ, McCoy LE, et al. Circulating and intrahepatic antiviral B cells are defective in hepatitis B. J Clin Invest 2018;128:4588-603.
12. Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. Annu Rev Immunol 1995;13:29-60.
13. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nat Rev Immunol 2005;5:215-29.
14. Wang Q, Sachse P, Semmo M, et al. T- and B-cell responses and previous exposure to hepatitis B virus in 'anti-HBc alone' patients. J Viral Hepat 2015;22:1068-78.
15. Cannizzo ES, Tincati C, Binda F, et al. Unconventional T cells in chronic hepatitis B patients on long-term suppressive therapy with tenofovir followed by a Peg-IFN add-on strategy: A randomized study. J Viral Hepat 2018;25:381-90.
16. Vanwolleghem T, Groothuismink ZMA, Kreeeft K, et al. Hepatitis B core-specific memory B cell responses associate with clinical parameters in patients with chronic HBV, J Hepatol 2020,73:52-61.
17. Matsushita T, Kobayashi T, Mizumaki K, et al. BAFF inhibition attenuates fibrosis in scleroderma by modulating the regulatory and effector cell balance. Sci Adv 2018;4:eaa9944.
18. Mauri C, Menon M. Human regulatory B cells in health and disease: therapeutic potential. J Clin Invest 2017;127:772-9.
19. Fanning GC, Zoulil M, Hou J, et al. Therapeutic
strategies for hepatitis B virus infection: towards a cure. Nat Rev Drug Discov 2019;18:827-44.
20. Law MF, Ho R, Cheung CK, et al. Prevention and management of hepatitis B virus reactivation in patients with hematological malignancies treated with anticancer therapy. World J Gastroenterol 2016;22:6484-500.
21. Kusumoto S, Tanaka Y, Suzuki R, et al. Monitoring of Hepatitis B Virus (HBV) DNA and Risk of HBV Reactivation in B-Cell Lymphoma: A Prospective Observational Study. Clin Infect Dis 2015;61:719-29.
22. Colucci M, Carsetti R, Serafinelli J, et al. Prolonged impairment of immunological memory after anti-CD20 treatment in pediatric idiopathic nephrotic syndrome. Front Immunol 2019;10:1653.
23. Kusumoto S, Arcaini L, Hong X, et al. Risk of HBV reactivation in patients with B-cell lymphomas receiving obinutuzumab or rituximab immunochemotherapy. Blood 2019;133:137-46.
24. Hou J, Wang G, Wang F, et al. Guideline of Prevention and Treatment for Chronic Hepatitis B (2015 Update). J Clin Transl Hepatol 2017;5:297-318.
25. Wright TL, Lau JY. Clinical aspects of hepatitis B virus infection. Lancet 1993;342:1340-4.
26. Visentini M, Casato M. HBV messing with the B-cell genome leads to DLBCL. Blood 2018;131:2602-3.
27. Valats JC, Tuaillon E, Funakoshi N, et al. Investigation of memory B cell responses to hepatitis B surface antigen in health care workers considered as non-responders to vaccination. Vaccine 2010;28:6411-6.
28. Vanwolleghem T, Groothuismink ZMA, Kreeft K, et al. Hepatitis B core-specific memory B cell responses associated with clinical parameters in patients with chronic HBV. J Hepatol 2020;73:52-61.
29. Tseng TC, Kao JH, Chen DS. Peginterferon α in the treatment of chronic hepatitis B. Expert Opin Biol Ther 2014;14:995-1006.
30. ter Borg MJ, Hansen BE, Bigot G, et al. ALT and viral load decline during PEG-IFN alpha-2b treatment for HBsAg-positive chronic hepatitis B. J Clin Virol 2008;42:160-4.
31. Liaw YF. Clinical utility of HBV surface antigen quantification in HBV e antigen-negative chronic HBV infection. Nat Rev Gastroenterol Hepatol 2019;16:631-41.
32. Oliviero B, Varchetta S, Paudice E, et al. Natural killer cell functional dichotomy in chronic hepatitis B and chronic hepatitis C virus infections. Gastroenterology 2009;137:1151-60, 1160.e1-7.
33. Thomas E, Yoneda M, Schiff ER. Viral hepatitis: past and future of HBV and HDV. Cold Spring Harb Perspect Med 2015;5:a021345.
34. Bing X, Linlang L, Keyan C. Decreased Breg/Th17 Ratio Improved the Prognosis of Patients with Ulcerative Colitis. Can J Gastroenterol Hepatol 2018;2018:5760849.
35. Fillatreau S. Regulatory functions of B cells and regulatory plasma cells. Biomed J 2019;42:233-42.
36. Das S, Sutoh Y, Cancro MP, et al. Ancient BCMA-like Genes Herald B Cell Regulation in Lampreys. J Immunol 2019;203:2909-16.
37. Das S, Bar-Sagi D. BTK signaling drives CD1d(hi)CD5(+) regulatory B-cell differentiation to promote pancreatic carcinogenesis. Oncogene 2019;38:3316-24.
38. Dambuza IM, He C, Choi JK, et al. IL-12p35 induces expansion of IL-10 and IL-35-expressing regulatory B cells and ameliorates autoimmune disease. Nat Commun 2017;8:719.
39. Sakkas LI, Mavropoulos A, Perricone C, et al. IL-35: a new immunomodulator in autoimmune rheumatic diseases. Immunol Res 2018;66:305-12.

(English Language Editor: L. Huleatt)