B Cell-mediated Humoral Immunity in Chronic Hepatitis B Infection

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Abstract

B cell-mediated humoral immunity plays a vital role in viral infections, including chronic hepatitis B virus (HBV) infection, which remains a critical global public health issue. Despite hepatitis B surface antigen-specific antibodies are essential to eliminate viral infections, the reduced immune functional capacity of B cells was identified, which was also correlated with chronic hepatitis B (CHB) progression. In addition to B cells, T follicular helper (Tfh) cells, which assist B cells to produce antibodies, might also be involved in the process of anti-HBV-specific antibody production. Here, we provide a comprehensive review of the role of various subsets of B cells and Tfh cells during CHB progression and discuss current novel treatment strategies aimed at restoring humoral immunity. Understanding the mechanism of dysregulated B cells and Tfh cells will facilitate the ultimate functional cure of CHB patients.

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Introduction

Hepatitis B virus (HBV) infection remains a significant cause of liver cirrhosis and hepatocellular carcinoma globally, especially in developing countries like China. In 2015, the World Health Organization (WHO) estimated that 257 million individuals live with HBV infection worldwide,1 resulting in 887,000 yearly deaths, mostly due to HBV infection-related hepatocellular carcinoma and cirrhosis.3–5

The challenge to CHB treatment is the failure to clear covalently closed circular DNA (referred to as cccDNA), which can give the virus the capacity to evade the host immune system, making a complete sterilizing cure unlikely to be feasible.6 On the other hand, a functional cure is defined as a sustained clearance of hepatitis B surface antigen (HBsAg) with or without seroconversion to anti-HBs antibodies after a finite course of therapy, but with the persistence of residual cccDNA. The functional cure of CHB has been considered as a feasible clinical treatment goal, which is correlated with improved clinical outcomes.9 Nevertheless, only a small proportion of patients reach this milestone.10,11

The complex interaction between HBV and the host immune system drives the process of chronic HBV infection, in which the anti-HBV adaptive immune system processes the clearance of HBV. Despite T cell responses having been well-studied in HBV infection, the beneficial biological function of B cells for functional cure of HBV has been consistently neglected. In addition, T follicular helper (Tfh) cells which regulate the B cell-mediated humoral immune responses have been identified as phenotypically distinct, leading to humoral immunity deficiency in patients with CHB.12 Hence, in this review, we will discuss the role of B cell-mediated humoral responses during chronic HBV infection and the current promising treatment strategies to induce robust anti-HBV humoral responses (Fig. 1).

Protective role of antibody in HBV control and clearance

B cell-mediated humoral immune responses are essential for HBV control and clearance. Universal vaccination against HBV has remarkably decreased HBV infection rate, since anti-HBsAg antibodies (i.e. anti-HBs) induced by immunization could prevent HBV infection.13 It is considered that those individuals with an anti-HBs concentration of ≥10 mIU/mL were immune against HBV infection, while those with an anti-HBs concentration of <10 mIU/mL might re-

Keywords: Chronic hepatitis B (CHB); B cell; T follicular helper (Tfh) cells; Antibodies; Therapeutics.

Abbreviations: HBV, hepatitis B virus; CHB, Chronic hepatitis B; Tfh, T follicular helper; HBsAg, hepatitis B surface antigen; APCs, professional antigen-presenting cells; IA, immune active; IT, immune tolerant; TLR, Toll-like receptors; PD-1, programmed cell death receptor-1; atMBCs, atypical memory B cells; Bregs, regulatory B cells; Tregs, regulatory T-cells; IL-10, interleukin-10; GCs, germinal centers; CXCR, chemokine receptor.

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require an additional booster vaccine dose.14–16 The specific antibodies against different HBV protein components are one of the major approaches for B cells to be involved in anti-HBV infection, such as antibody to hepatitis B core antigen (anti-HBc), antibody to hepatitis B e antigen (anti-HBe) and anti-HBs. Anti-HBc and anti-HBe serve as diagnostic biomarkers for HBV infection, while anti-HBs antibody is the only antibody that can specifically recognize and bind to HBsAg,17,18 thus serving an important role in HBsAg clearance.19 First, anti-HBs can not only block HBV entry by binding to free HBV viral particles as protective neutralizing antibodies to reduce viral load in vivo20–22 but it also can mediate antigen-dependent cellular cytotoxicity and antigen-dependent cellular phagocytosis to clear infected cells.23 HBV reactivation and hepatitis are well recognized complications that occur in patients who have undergone cytotoxic chemotherapy or immunosuppressive therapy.24 For example, high incidence of HBV reactivation was observed in lymphoma patients who were HBsAg-negative/anti-HBc-positive with or without anti-HBs and receiving rituximab-containing chemotherapy.25 Negative anti-HBs at baseline is an independent risk factor for HBV reactivation in patients with resolved CHB, compared with higher titer of anti-HBs ≥100 mIU/mL.26 Moreover, adoptive transfer of HBV-specific humoral and cellular immunity, which might be responsible for the delay of reinfection and a reduction of viral load.27 Therefore, anti-HBs is essential to alleviate disease advancement and prevent reinfection during CHB.

Several neutralizing monoclonal antibodies (referred to as mAbs) specific to HBsAg have been reported. For example, human mAbs including 2H5-A1428 and BcI.18729 that block the engagement of HBsAg to sodium taurocholate co-transporting polypeptide potently neutralize HBV in vitro. In addition, they could decrease viremia in vivo in an HBV mouse model. E6F6 that recognizes an evolutionarily conserved epitope (GPCK(R)TCT) not only prevented initial HBV infection and reduced the viral dissemination in human-liver-chimeric mice but also facilitated the restora-
tion of anti-HBV T cell response in hydrodynamic infection-based HBV carrier mice. Furthermore, in vivo delivery of a DNA-encoded monoclonal antibody plasmid can efficiently neutralize HBV virus in vitro. These antibodies can serve as a promising immunotherapeutic regimen or immunomodulation for HBV infection.

Regarding the traditional role of antibody production, B cells also may play a vital role as professional antigen-presenting cells (APCs) during CHB infection. Compared to the classical non-B cell APCs, HBCAg-specific B cells might efficiently serve as a primary source of APCs for native HBsAg-reactive T cells. In addition, B cells can induce an HBCAg-specific cytotoxic T lymphocytes (CTLs) response and further promote immune tolerance by the cross-presentation of HBCAg on major histocompatibility complex-I (i.e. MHC-I) to specific CD8+ T cells. At the same time, HBSAg is a special exogenous antigen, which can be involved in MHC-I molecules expressed on B cells.37,38

**Immune dysfunction of B cells during CHB infection**

HBV infection has exerted a significant impact on the global B cell compartment and HBV-specific antibody secretion. Global peripheral B cells were activated with reduced functional capacity, while anti-HBs-secreting B cells were rarely detected. Additionally, although total immunoglobulin G (IgG) in the serum among HBV patients is remarkably greater than that of healthy controls, the absence of HBV-specific antibodies was observed. B cell hyperactivation, differentiation disorder, activation of inhibitory signal and regulatory B cells may contribute to immune dysfunctions observed in CHB patients.44,45 A hallmark of chronic hepatitis infections, such as hepatitis C virus is the presence of immune exhausted virus-specific CD8+ T cells, characterized by their inability to secrete antiviral cytokines and an upregulation of inhibitory receptors such as programmed cell death receptor-1 (referred to as PD-1). B cell hyperactivation is characterized by enhanced expression of activation markers with displayed impaired function, especially in patients at immune active (IA) and immune tolerance (IT) stage. Overall, the mechanism of the hyperactivation of B cells remains to be clarified. Xu et al. reported that the B cell hyperactivation could be induced by increased interferon (IFN)-α and sCD40 ligands in IA patients. The increased activation of CD71 and CD69 expressed on B cell accounts for the B cell hyperactivation. A high level of Toll-like receptor (TLR) 9 expression likely contributes to the functional hyperactivation of B cells in CHB patients. A recent study revealed that B cells from CHB patients had a markedly reduced capacity to generate CD39/CD73-dependent extracellular adenosine and exhibited increased activation markers after adenosine-production blockade, suggesting CD39/CD73/adenosine pathway might contribute to B cell hyperactivation.

The frequency of HBSAg-specific B cells was comparable in both CHB patients and immunized healthy individuals, while anti-HBs in CHB patients were detected at low level or were even undetectable. In CHB patients, there was a unique population of B cell subsets with high levels of inhibitory receptors, including PD-1, which resemble CD21−CD27− atypical memory B cells (referred to as atMBCs). These atMBCs had elevated level of defective signals, which might be responsible for defective capacity of survival, cytokine production and differentiation into antibody-secreting cells. Such atMBCs were found to be expanded in CHB patients and further upregulated quickly in the HBSAg-specific compartment, which might reduce anti-HBs secretion and enhance B cell hyperactivation in CHB patients. In addition, the transcription factor T-bet was also upregulated in CD21− B cells during murine and human HBV infections, which may be correlated with the inadequate production of HBSAg-specific B cells among CHB patients. Moreover, chemokine receptor 3 (CXCR3), Fc receptor-like 4 (FCR4L) and FCR75 are upregulated in B cells and associated with B cell immune dysfunction during HBV infection.

A regulatory subset of B cells (regulatory B cells, Bregs) is elevated in CHB patients, which has been reported to inhibit liver inflammation and immune disorders in mouse models. Previous studies showed that the frequency of Bregs had a significant correlation with alanine aminotransferase (ALT) and glutamic oxaloacetic transaminase (AST). Furthermore, CHB patients in the IA phase exhibit increased Bregs due to inflammatory responses. However, the underlying mechanism of the Bregs’ elevation during CHB infection remains unclear. Bregs could suppress CD8+ T cell responses, which might serve a pathogenic role by secreting Interleukin-10 (IL-10), enhancing the function of regulatory T-cells (Tregs), and suppressing T cell from secreting proinflammatory cytokines in various autoimmune diseases. During CHB infection, Bregs have a crucial role in suppressing antiviral immune response by producing IL-10. Notably, in HBeAg-negative chronic HBV patients, serum IL-10 level was correlated with high virus load and advanced liver inflammation, while blockade of IL-10 could improve vaccine efficacy and disease resolution in CHB patients. A recent study elegantly characterized the phenotype and functional impairment of HBSAg-specific B cells and HBCAg-specific B cells. Of note, B cell response against HBSAg and HBCAg is different during CHB infection. HBCAg-specific B cells are present at higher frequency than HBSAg-specific B cells. Further, HBCAg-specific B cells are class-switched memory B cells and secrete antibodies, while HBSAg-specific B cells failed to mature efficiently into antibody secreting cells. The transcriptomic analysis showed that HBV-specific B cells had an mRNA expression pattern that differs from global memory B cells and express cross-presentation and innate immune genes, suggesting additional roles of HBV-specific B cells beyond the production of antibodies.

**Multifunctional roles of Tfh cell subsets in CHB infection**

T follicular helper (Tfh) cells are a unique subset of CD4+ T cells, which can directly help B cells secrete antibodies in germinal centers (referred to here as GCs). By colocalizing with B cells and expressing costimulatory signals as well as various cytokines, Tfh cells directly interact with B cells, facilitate B cell differentiation into long-lived plasma cells and memory B cells with high affinity, and facilitate the formation of GCs. Peripheral CD4+CXCR5+ T cells are considered as circulating memory CD4+ Tfh cells. Peripheral circulating memory Tfh cells had similar phenotypic and functional properties as Tfh cells in the GC, known as GC Tfh cells, such as enhanced expression of CXCR5, stimulation of B cell maturation, terminal differentiation of B cells into antibody-producing plasma cells, and isotype switching. By the dominant transcriptional factors and cytokines, the circulating human memory Tfh cells in HBsAg-negative CHB patients were divided into three subsets: Tfh1 (CXCR3+CXCRI6−); Tfh2 (CXCR3+CXCRI6−); and Tfh17 (CXCR3−CXCRI6−). It is considered that blood memory Tfh2 and Tfh17 cells can induce naive B cells to produce IgGs. Interestingly, Tfh2 cells can preferentially induce the secretion of IgG and IgE, while Tfh17 cells can effectively promote IgG and especially IgA secretion, while Tfh1 cells enhance protective antibody responses, making the memory B cells differentiate into effector B cells.!
It has been well established that Tfh cells have an essential role in various infectious diseases, such as *Plasmodium vivax* infection, acute malaria, CHB, human immunodeficiency virus, and tuberculosis. Indeed, Tfh cells also play a vital role during CHB progression. The frequency of circulating Tfh cells (CXCR5+CD4+ T cells, cTfh cells) was correlated with the serum levels of ALT and AST, suggesting that cTfh cells may be involved in HBV-specific immune responses. Further evidence showed that CHB patients have a significant increase of Tfh cells compared to healthy controls. The frequency of CD4+CXCR5+ T cells in IA patients was higher than that of IT patients and healthy individuals, suggesting high frequency of CD4+CXCR5+ Tfh cells could be a biomarker to assess the immune status of CHB patients. cTfh cells secrete IL-21 to facilitate HBeAg seroconversion. On the other hand, HBSAg is a T cell-dependent antigen, and seroconversion of HBSAg also requires the assistance of Tfh cells. A unique group of CXCR5+CD8+ T cells with minimal levels of inhibitory receptors exerted its potent cytotoxicity to control viral replication by migrating into B cells follicles during CHB. A subset of CD25+FOXP3+ Treg-like cells in Tfh cells that was enriched in patients, known as follicular regulatory T (referred to as TFR) cells, could suppress helper function of Tfh cells. In a mouse model with persistent HBV infection, the function of HBsAg-specific cTfh cells was blocked by Treg cells, whereas the depletion of Treg cells could restore the cTfh function. Moreover, a group of type 1 regulatory T (i.e. Tr1)-like cells migrated from the liver to the draining lymph node and can inhibit peripheral anti-HBV immunity by negatively regulating GC B cells and Tfh cells.

**Novel CHB treatment strategies targeting B cells**

The widely used clinical standard first-line antiviral therapeutics for chronic HBV infection include IFNs and nucleoside analogs (commonly known as NAs). IFNs have a strong antiviral effect and immune-mediated function, which promotes antiviral innate and adaptive immunity. Based on the genetic, structural and functional characteristics and their receptors on the cell surface, the IFN family is classified into three major types: type-I; type-II; and type-III. Type-I IFNs (IFN-α, IFN-β, IFN-ε, IFN-κ, and IFN-ω) has been approved for the treatment of CHB infection. Pegylated-IFN-α eliminates the production of HBsAg and is well tolerated in HBeAg-negative CHB patients. In addition to the previously reported efficiency of pegylated-IFN on T cells and natural killer cells, B cells may also play an essential role in this process. Pegylated-IFN-α treatment might exert the immunomodulatory effect by remodeling B cell compartments, which was correlated with a sustained increase in sCD30 levels and decrease of plasma HBsAg.

TLR agonists and checkpoint inhibitors are an emerging treatment strategy for CHB patients. TLR7 is highly expressed on B cells and has been proven to inhibit antibody production. As an oral agonist of TLR7, GS9620 is currently in clinical assessment to treat CHB patients. Preclinical study showed that GS9620 treatment significantly induced an intrahepatic transcriptional profile enriched with CD8+ T cells and B cells, contributing to clearance of HBV in a chimpanzee model. Also, TLR9 agonists such as CPG 7909 or 1018 ISS co-administered with HBSAg induced robust antibody responses among CHB patients. Therefore, combined immunotherapeutic agents might be necessary to restore B cell function and induce the desired B cell antibody response.

HBV therapeutic vaccines have also emerged as a promising treatment strategy to induce robust humoral responses by activating B cells. For example, the ferritin nanoparticle vaccine that delivers preS1 to specific myeloid cells, including SIGNR1+ dendritic cells, that activate Tfh cells and lymphatic sinus-associated SIGNR1+ macrophages that can activate B cells. Furthermore, a recent study developed a B cell epitope-based vaccine, which was able to suppress serum HBsAg and HBV DNA by inducing SEQ13-specific antibody response.

**Conclusion**

During the pathogenesis of CHB, defective HBV-specific B cells and antibodies were identified, in which global B cells were dysfunctional; whereas, HBV-specific antibodies were found to be insufficient and might be functionally limited. Tfh cells residing in peripheral blood, spleen and liver are pivotal to facilitate the seroconversion of HBeAg and HBsAg. Novel hepatitis B treatment strategies targeting B cells might facilitate the recovery of B cell function and develop the desired B cell responses, leading to functional cure of CHB.

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**Conflict of interest**

The authors have no conflict of interests related to this publication.

**Author contributions**

Study conception and design (YC, CW, SY), drafted the first manuscript (SY, YC, YL, GW, GC, RI, DW, GC, RH, XT, JX, CC). All authors approved the final version of the article, including the authorship list.

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