Humoral Immune Responses and Hepatitis B Infection

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Keywords

Hepatitis B · Chronic hepatitis B virus · Immune response · T follicular helper cells · Antibody · Interleukin-21

Abstract

Background: Chronicity or seroclearance of hepatitis B virus (HBV) antigens is determined by the host immune responses. Current approaches to treat HBV patients are based on inhibition of replication using different antivirals (nucleoside or nucleotide analogs) as monotherapy, or along with immune modulators as combination therapy is being used worldwide for reducing the viral load. Understanding the role of immune cellular therapies with currently available treatments for persistent viral-mediated responses in HBV patients is unexplored. However, the generation of antibodies against a surface (HBs) and envelop (HBe) antigen of hepatitis B remains an issue for future studies and needs to be explored. **Summary:** Humoral immunity, specifically T follicular helper (TFh) cells, may serve as a target for therapy for HBsAg seroconversion. In this review, we have been engrossed in the importance and role of the humoral immune responses in CHBV infection and vertical transmission. **Key Message:** TFh cells have been suggested as the potential target of immunotherapy which lead to seroconversion of HBe

and HBs antigens of HBV. HBsAg seroconversion and eradication of covalently closed circular DNA are the main challenges for existing and forthcoming therapies in HBV infection.

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Introduction

Chronic hepatitis B virus (CHBV) infection is a significant health problem with a projected 240 million carriers worldwide. Around half a million people die each year because of the advancement of the infection to liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [1–3]. This is a bigger problem for Asia as the continent contributes to the majority of the global HBV infections. India has approximately 3–4% HBV carriers among 1.35 billion [4] and considered as the second major pool of HBV patients [5].

Maternofetal transmission is one of the main causes of the persistence of the HBV and the development of chronicity [6]. The prevalence of CHBV among pregnant women is witnessed to be 0.82% and found to be associated with the risk of mother-to-child transmission [7]. Chronicity of HBV infection is inversely proportional to

the age, and newborns have higher chance of getting a chronic infection (90–95%) as compared to adults (5–10%) [8]. While in many countries vaccination has decreased the high burden of HBV and related complications, the currently available therapies for CHBV patients are inadequate. The currently recommended nucleoside analog therapy has little effect on HBsAg levels, HBsAg loss, and depletion of cccDNA [9]. Another major problem with long-term nucleoside analog therapy is emergence of resistance [10].

In the past 2 decades, HBsAg seroconversion is rare, and the pool of cccDNA in the nucleus is a major reason for the persistence of HBV. A number of studies addressed the issues pertaining to epidemiology, genotypes, viral mutations, and immunology of HBV [11]. The CHBV patients showed an impaired immune profile [12, 13], especially dysfunctional HBV-specific CD8⁺ T-cell responses [14]. T follicular helper (TFh) cell is a subset of the helper T-cell population and has shown a crucial role in the seroconversion of HBe and HBsAg in CHBV-infected patients [15, 16].

Recently, our group showed that B and TFh cells have also emerged as an essential player in preventing viral expansion within the host and involved in HBeAg [17] or HBsAg seroconversion along with CD8 T-cell responses [13]. The generation of antibodies against HBsAg or HBeAg seroconversion remains to be explored. Limited but compelling experimental evidence suggests that modulation of TFh cells can be an exciting prospect for achieving seroconversion of HBsAg in CHBV infections. In this review, we make a case for the role of TFh and B cells in seroconversion and their importance in CHBV infection.

For this review, PubMed was searched to summarize the available studies in the area of hepatitis B humoral immunity. Only published studies in English were reviewed and summarized with data. Importance has been given to well-established studies with high citation.

Hepatitis B Infection

HBV is a partially double-stranded DNA virus which is noncytopathic, but hepatotoxicity of the liver occurs due to vigorous proinflammatory immune cell activation in response to viral infection. Being a hepatotropic virus, HBV enters the blood and then replicates in the liver. Although the accurate mechanism of HBV entry into hepatocytes remains unclear, recent studies documented that HBV pre-S protein facilitates entry into the hepatocytes through the asialoglycoprotein receptor and sodium tau-

rocholate co-transporting polypeptide [18–20]. The fate of HBV infection is a result of a key equilibrium between viral replication and host immunity. Most individuals who get HBV infection in adulthood develop icteric acute hepatitis due to vigorous immune response against viruses resulting in self-limiting illness. Less than 1% of them develop acute liver failure requiring intensive monitoring and early liver transplantation [21, 22].

Virological factor and host immune responses govern one of the 3 possible outcomes of the HBV infection: (1) immune tolerance, (2) immune activation, and (3) inactive carrier stage. However, there are abundant similarities and interindividual inconsistencies in these stages. According to EASL 2017 guidelines, “CHBV infection can be classified into 5 phases (I) HBeAg-positive chronic infection, (II) HBeAg-positive chronic hepatitis, (III) HBeAg-negative chronic infection, (IV) HBeAg-negative chronic hepatitis, and (V) HBsAg-negative phase” [23].

On the basis of the presence and disappearance of HBV antigens (HBsAg, HBeAg, and HBcAg) and antibodies (anti-HBs, anti-HBe, and anti-HBc), different phases of CHBV are described (Fig. 1). The immune tolerant phase is characterized by HBeAg positivity, high HBV DNA levels, normal or low levels of alanine aminotransferases (ALT), and minimal liver necroinflammation hardly leading to any progression to fibrosis [24, 25]. However, due to sequential accumulation of HBV virions or mutation in the core promoter, few patients develop “HBeAg-negative CHB infection” which is also characterized by inconsistent levels of ALT and HBV DNA >2,000 IU/mL but with active hepatitis [26–29].

The immune tolerant phase is followed by the “immune active” phase, characterized by HBeAg positivity, high serum HBV DNA levels, persistent or intermittent increase in ALT levels, moderate or severe liver necroinflammation, and more progression to fibrosis. The extent of liver damage is directly related to the duration of this phase. The immune active phase ends with the seroconversion of HBeAg and the formation of anti-HBeAb.

The immune active phase is followed by “immune inactive HBV carrier state” which can be identified by very low or undetectable serum HBV DNA levels (below 2,000 IU/mL) and normal ALT. The disease remains stable in this phase due to a very low but ongoing risk of liver-related complications. The spontaneous HBeAg clearance rate varies from 3 to 12% annually, but a rate of HBsAg seroconversion is very rare, only ~1% [30, 31]. Even after the disappearance of HBsAg, cccDNA still persists in the liver but with a very little risk of further progression of liver disease [32].

Fig. 1. Infection of HBV is majorly categorized into 2 types: acute and chronic. **a** Acute HBV infection: HBsAg is the first to be detected in the circulation even 2–6 weeks earlier to the appearance of the symptoms of hepatitis. Presence of HBsAg together with anti-HBc IgM is considered the hallmark of acute HBV infection. HBeAg can be seen for approximately 6 months after the acute infection. After that, IgM disappears, and anti-HBc IgG is present lifelong. **b** Phases of CHBV infection: on the basis of HBV DNA, HBeAg, and ALT levels. HBV, hepatitis B virus; CHBV, chronic hepatitis B virus; ALT, alanine aminotransferase.

HBV Adaptive Immune Responses and Therapy

In adaptive immunity, CD8⁺ T cells are considered as one of the most important players in HBV infection, which act through cytolytic or noncytolytic mechanisms via the secretion of IFN- γ and TNF- α [33]. However, defective virus-specific responses by CD8 cells have been observed in CHBV patients [34]. Even in the presence of profound DC activation and antigen presentation, HBV-specific CD8⁺ T-cell exhaustion under continuous exposure to HBV antigens is a major challenge to clear the virus. Regulatory T cells, which are known for downregulation of inflammatory immune responses and maintaining the immune homeostasis, have been shown to fail during chronic HBV infection [35, 36]. Regulatory T cells maintain immunosuppressive functions by secreting the inhibitory cytokines such as TGF- β , IL-10, and IL-35 and suppress the activation of antigen-specific or nonspecific proliferation in most of the function of the immune cells [37, 38]. The current strategy for the restoration of the function of T cells, blocking or neutralizing the inhibitory receptors (CTLA-4, PD-1, and TIM-3) and cytokines (IL-10 and IL-35) may be effective therapeutic targets in HBV.

Currently available treatments of CHB include standard IFN- α , Peg-IFN- α 2a, Peg-IFN- α 2b, and NA (nucleoside analogs [lamivudine, telbivudine, emtricitabine,

and entecavir] and nucleotide analogs [adefovir and tenofovir]). A comparison of these antiviral therapies fails to show the superiority of one therapy over another with the aim of risk reduction in liver-related complications [20, 39, 40]. The current, underexploration therapies and immune modulators have been discussed in Table 1.

Mechanisms of HBeAg and HBsAg Seroconversion

B cells do not have critical roles in the elimination of viral particles directly [41]. However, B- and TFh-cell responses have been shown to play an important role in preventing viral expansion within the host and also the main players in HBe or HBsAg seroconversion.

TFh cells reside in the secondary lymphoid organ and help in the B-cell differentiation through the secretion of IL-21 [42, 43]. TFh cells express distinctive markers, including the transcriptional repressor Bcl6, the chemokine, and the costimulatory molecules such as CXCR5, ICOS, and CD40L (Fig. 2). Translocation of TFh cells toward the CXCL13-rich germinal center is mediated by the CXCR5 expression [44, 45]. TFh cells have been emerged as a crucial player in B-cell differentiation to form antibody-producing plasma cells that offer lifelong protection [46–48]. It is observed that the absence of TFh cells

Table 1. Current underexploration therapies and immune modulators

S. No.	Therapy	Intervention/modality	Mode of action	References
1. First-line interventions	NAs	LMV, adefovir, entecavir, telbivudine, and TFV	Interfere with the replication of HBV without targeting cccDNA	Krause et al. [83] and Woo et al. [84]
	Interferon therapy	IFN- α or pegylated-IFN- α	Antiviral and immunomodulatory	
2. Under exploration	Egress blockades	Nucleic acid-based polymers	Blocks the release of HBsAg	Spyrou et al. [85]
	Assembly blockades	CpAMs	Noncapsid core polymers	
		Capsid assembly modulators	Formation of morphologically normal capsids lacking viral nucleic acid	Lahlali et al. [86]
	Monoclonal antibodies as checkpoint inhibitors	Targeting immunoinhibitory molecules like PD-1, CTLA-4, Tim-3	Restoration of T-cell response	
3. Future modalities	Gene-targeting strategies	TALENs, ZFNs, or CRISPR-Cas9	Cleavage of cccDNA	Fanning et al. [88]
4. Epigenetic modulators of transcription		HBx-DDB1-targeting drugs	Restoration of smc5 function and inhibition of viral transcription	Sekiba et al. [89] and Maepa et al. [90]
		RNA interference, ASOs, and ribonucleic acid enzymes (ribozymes)	Transcription inhibition	
5. Therapeutic vaccination (combination therapy of antiviral drug and antibody-mediated immunotherapy)	Mixture of 2 monoclonal antibodies	HBV-AB ^{XTL} + lamivudine	Triggering B-cell response	Ma et al. [91]
	TLR agonist	TLR 7/9 ligands as adjuvant	Enhancing germinal center response	Lucifora et al. [92]
	PRRs ligands	CpG-loaded virus-like particles derived from HBcAg	Enhances T-cell priming and antigen presenting activity of B cells	Storni et al. [93]
6. Cytokine therapy		IL-12	Functional restoration of exhausted T cells	Shirazi et al. [94]
		IL-18	Stimulate NK, NKT cells, and T cells to secrete INF- γ	Kimura et al. [95]
		IFN- γ and TNF- α	Suppression of viral replication without cytotoxicity	Xia et al. [96]
		IL-6 and TGF-B	Inhibitory to viral replication	Schon et al. [97]
7. Cell-based therapy		Autologous DCs pulsed with HbcAg18–27 peptide (FLPSDFPSPV) and the HBV pre-s244–53 peptide (SILSKTGDPV)	Render HBV DNA undetectable	Dou et al. [98]
8. T-cell engineering		Targeting CD8 T cell	Replacement of exhausted CD8+ T cells	Boni et al. [99] and Kruse et al. [100]
		TCR-redirected T cells	Modified α and β chains of the TCR complex for high affinity recognition of HLA class I-dependent viral or tumor epitopes	
		CAR T cells	Constitutive signaling for effector T-cell activation and HLA class I-independent antigen recognition	

NAs, nucleos(t)ide analogs; LMV, lamivudine; TFV, tenofovir; HBV, hepatitis B virus; CpAMs, core protein allosteric modulators; TALENs, transcription activator-like effector nucleases; ZFNs, zinc-finger nucleases; ASOs, antisense oligonucleotides; CAR, chimeric antigen receptor.

Fig. 2. Phenotypic marker and characterization of Tfh cells: Tfh cells can be distinguished on the basis of transcription factors (BCL6 and cMAF), costimulatory marker (ICOS), and by secretion of the key cytokine IL-21. Tfh, T follicular helper.

at the onset of infection reduces the overall humoral response and prevents viral clearance [43, 49].

High-affinity class-switched antibodies are essential in clearing and establishing long-lasting humoral immunity against HBV infection and effective vaccination. It is observed that in response to HBV vaccination, antibody secretion was influenced by genetic polymorphism of CXCR5 and CXCL13 [50].

Importance of IL-21 Cytokine

Tfh cells have a pleiotropic function and secrete many cytokines such as IL-4, IL-2, IL-6, IFN- γ , IL-17, and IL-21. Production of IL-21 by Tfh cells was first described in context of tonsils and was shown to have a specific role in B-cell differentiation [51–54]. Further studies reiterated that IL-21 endows Tfh cells to exert B-cell helper functions (Fig. 3) [42, 43, 55]. Therefore, IL-21 has emerged as an essential cytokine for the maturation of B cells and the clearance of chronic viral infections [56, 57]. In HBV pathogenesis, mainly inhibitory cytokines are involved, but now it was observed that IL-21 cytokine has a key role in the therapeutic augmentation of immune responses against HBV [58]. Therefore, IL-21 has emerged

as an important cytokine in the maturation of B cells and the clearance of chronic viral infections [56, 57]. Recent studies by McGuire et al. [59] and Weinstein et al. [60] showed that IL-21 is secreted by Tfh before IL-4, suggesting that B-cell somatic hypermutation is through IL-21, and plasma cell differentiation is through IL-4 [59, 60].

T- and B-Cell Interaction within Germinal Center in HBV

T- and B-cell interaction initiates the extra follicular foci or germinal center formation in the secondary lymphoid organ. B-cell clones with high affinity receive maximum ICOSL, CD40, and IL-21 signals, which lead to differentiation and existence into long-lived plasma cells [61–64]. Therefore, the effectiveness of Tfh- and B-cell communication in the germinal center is critical for the expansion of memory B cells and formation of plasma cells (Fig. 4). Recent information has also emphasized the role of DCs localized in the interfollicular zone in priming the early-stage Tfh cells [65].

The Importance of HBeAg and HBsAg Seroconversion in Hepatitis B Infections

Seroconversion of HBeAg and HBsAg is critical for controlling the pathogenesis of CHBV infection, whereas HBeAg seroconversion is an indicator of inhibition of viral replication, and HBsAg seroconversion is considered the functional cure of the disease. HBeAg is considered the marker of replication and seroconversion of HBeAg, and the formation of anti-HBeAb is a critical incident in the history of CHB infection. Anti-HBeAg formation or seroconversion of HBeAg is associated with the reduction of HBV DNA. While spontaneous HBeAg seroconversion rate has been described to be between 8 and 15% [66, 67], HBsAg seroconversion is rare, and only 1–2% patients achieve this [31, 66, 67].

Earlier seroconversion of HBeAg in CHB patients is found to be associated with an increased chance of sustained remission, as well as slower pathogenesis of the liver disease [68, 69]. There are many factors such as sex, age, and the degree of liver disease that can affect the rate of HBeAg seroconversion. Patients with older history, female carriers, and subjects with the ALT levels higher than 5 times the upper limit of normal are more likely to clear HBeAg. Peg-IFN- α therapy results in sustained HBeAg seroconversion rates of up to 32 and 48%, respec-

Fig. 3. A history of T cells' help to B cells: the principal signs came from Claman and partners in 1966 [54], supported by a trio of consecutive studies by Miller and Mitchell in 1968.

Fig. 4. T- and B-cell interaction within the germinal center in HBV: circulating HBV proteins being sensed by circulating DCs. These cells carry HBV antigens and present through MHC to naïve CD4⁺ T cells and help in T-cell differentiation towards TFh cells. TFh cells drift from the T-cell zone to the B-cell zone in the germinal center, where follicular DCs, TFh, and B cells interact and help in affinity maturation of B cells. The B cells which failed to interact with TFh cells are recycled back into the dark zone where proliferation and somatic hypermutations in B cells take place. Once the B cell gains specificity for a particular HBV antigen, they become plasma cells secreting antigen-specific antibodies on exit from the germinal center. HBV, hepatitis B virus.

tively, when assessed at weeks 24 and 48 after treatment. In contrast, HBeAg seroconversion rates have been considerably lower (12–15%) with nucleotide analogues [70, 71].

The clearance of HBsAg is found to be associated with minimal risk of disease progression such as cirrhosis, decompensation, and HCC. But, if cirrhosis has developed before the disappearance of HBsAg, patients will be at risk of HCC. Long-term NA therapy decreased the viral load, but it is only effective in a minority of CHB patients for seroconversion of HBsAg [72, 73].

The Role of TFh Cells in Inducing HBeAg and HBsAg Seroconversion

The presence of HBeAg and HBsAg is considered as a marker of HBV replication and infection. Therefore, the secretion of antibodies against the HBV antigen, specifically HBe and HBsAg, is considered a favorable outcome of CHBV infection. Collected evidence has believed the liver worked like other secondary lymphoid organs, and it supports the priming of naïve T cells for differentiation of effector T cells [74–76].

Recently, studies using animal models of HBV have demonstrated that the immune response depends upon the age, and young age is found to be associated with effective immune stimulation and antiviral immunity [58, 77]. Furthermore, the hepatic lymphoid structures had the ability to take care of the differentiation as well as the maturation of B-cell-mediated responses and the priming of T cells to become TFh cells [77]. The animal model study has also found that the presence of IL-21-secreting TFh cells and IgG-expressing B cells in the liver is essential for effective priming of the humoral immune response leading to antibodies against HBsAg [58]. Adult mice, lacking IL-21R on the donor splenocytes, were found incapable to producing antibodies against HBsAg, and subsequent persistence of HBV occurs [58].

Another longitudinal study on a cohort of telbivudine-treated patients revealed that seroconversion of HBeAg was related to increase in IL-21 concentrations in the circulation at week 12 and considered as an independent predictor for HBV clearance [56, 78]. Other studies also supported indirectly where they showed that the disappearance of HBeAg is linked with higher CD4+ T-cell responses in HBeAg-positive patients after adefovir dipivoxil treatment [79]. Recent studies from our groups and others showed that immune active CHB patients had higher occurrences of peripheral blood TFh cells and IL-

21 levels and suggested that it could play a key role in HBe and HBs seroconversion [13, 46, 56, 80].

Role of Humoral Immunity in Hepatitis B Vertical Transmission

Mother-to-baby vertical transmission of HBV is one of the leading causes of CHBV infection in the Association of Southeast Asian Nations (ASEAN) countries. However, there are limited studies available on the role or association of maternal and newborn immunity in vertical transmission of HBV. According to the observation of Shrivastava et al. [81], hepatitis B surface antigen-positive infants showed higher numbers of immature transitional B cells at birth, which normalized a year after immunization. In this study, the authors conclude that the immature B-cell response to neonatal HBV exposure is associated with maternal-child transmission of HBV. Recently, we for the first time reported the role of maternal immunity, specifically humoral immunity, in mother-to-baby vertical transmission [82]. We found that mothers who did not transfer the virus vertically have higher TFh and plasma B-cell frequencies, as well as IL-21 levels in circulation compared to a mother who did so. Interestingly, we also observed that newborns showed immune imprints of their mothers (positive newborn born from HBV-infected mother compared to negative newborns born from HBV-positive mothers) [82]. While these observations testify for the importance of humoral immune status of the mother baby pair, studies with larger cohorts are needed to substantiate the findings.

Challenges Ahead and Conclusion

Targeting and elimination the HBV cccDNA which acts as a template for the transcription and persistence of HBV RNAs and seroconversion of HBsAg is considered a complete cure of HBV infection. It is well documented that cDNA acts as a reservoir of HBV and persists in the nucleus. The current treatment for CHBV patients is antivirals and traditional immune modulators. However, they have their own limitations such as drug resistance, flu-like symptoms, and other side effects. For the functional cure of HBV clearance, there is a need to introduce rationale-based newer immune modulators and cytokine adjuvants which induce protective host immune responses for seroconversion and target cccDNA and cellular host proteins for viral entry and replication. Research

from the past 2 decades has provided clues of the role of humoral immune response in HBV infection.

However, major knowledge gaps, specifically in TFh cell biology, remain. On the basis of the current understanding of available data in animal models and in CHBV patients, we conclude that IL-21-producing TFh cells represent a major mediator along with CD8 T and innate immunity for the induction and maintenance of HBeAg and HBsAg seroconversion and antigen clearance. There is a need to have a larger cohort of human studies for understanding the role of TFh biology in CHBV infection. It is also important for understanding the mechanism and their role in HBs and HBeAg seroconversion. The knowledge related to humoral responses in HBV vertical transmission and the understanding of the failure of HBV vaccination in HBV-infected babies born from HBV-infected mothers is also limited.

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Conflict of Interest Statement

There are no conflicts of interest among the authors.

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Author Contributions

A.K.V. conceptualized and drafted the manuscript, M.I. and G.G. were responsible for figure draft and literature survey, and A.K.S. and N.T. critically revised and edited the manuscript.

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