Immunopathogenesis of Hepatitis B Virus

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Chronic hepatitis B virus (HBV) infection is a global public health issue. There are >250 million people chronically infected with HBV, and these chronic carriers are at high risk of developing end-stage liver diseases and hepatocellular carcinoma. Patients with chronic hepatitis B (CHB) usually acquire the virus perinatally, while most patients infected during adulthood develop acute hepatitis B (AHB), which usually results in viral clearance. HBV infection is noncytopathic, and liver injury is mostly contributed by host immune responses. The virus is stealthy, since the infection rarely induces type I interferon response in the early phase. In AHB, viral infection is detected and restrained by the innate immune response, which is followed by a strong and robust adaptive immune response and accompanied by viral clearance. In patients with CHB, both innate and adaptive immune responses are weak and thus rarely lead to viral clearance. Interferon α and nucleos(t)ide analogues are 2 classes of approved antiviral therapies. The former treatment activates nature killer (NK) cells and NK T cells, which partially enhances the innate immune response, while the later treatment suppresses viral replication by inhibiting reverse transcriptase, which may restore the HBV-specific adaptive immune response. However, single or combined treatment are still far from achieving seroclearance of HBV surface antigen. Although the treatment response is unsatisfactory in current clinical trials using several immunomodulators for boosting antiviral immunity, immunotherapy that is able to induce immune surveillance is still the most promising modality for HBV cure in the future.

Keywords. HBV; acute hepatitis B; chronic hepatitis B; immunotherapy.

INTRODUCTION TO HEPATITIS B VIRUS (HBV) IMMUNOPATHOGENESIS

HBV is a noncytopathic enveloped virus with a partially double-stranded circular form DNA genome and belongs to the Hepadnaviridae family. This virus infects humans and chimpanzees only, and it forms covalently closed circular DNA (cccDNA) in hepatocytes, which is a stable template for viral replication and the key for viral persistence [1]. There are >250 million individuals with chronic HBV infection, and these chronic carriers are at high risk of developing end-stage liver diseases and liver cancer [2].

HBV-infected adults usually develop self-limited and transient hepatitis, and 95% of the infections end with viral clearance and establishment of protective antibodies. However, most neonates acquiring HBV infection perinatally develop persistent infection [2]. The natural course of chronic HBV infection can be divided into 4 chronological phases based on the virus-host interactions [1, 2]. The immune-tolerant phase is characterized by active replication of HBV, HBV e antigen (HBeAg) positivity, and a normal-to-low serum alanine aminotransferase (ALT) level. The second stage is the immune clearance phase, in which HBeAg-positive patients have elevated serum ALT levels and decrease in the serum HBV DNA load. In the low-replication or residual phase, patients lose HBeAg and gain antibody to HBeAg (anti-HBe), with remission of liver disease; this is also designated as the inactive carrier state. However, about 20%–30% of people in the inactive carrier state may have a viral relapse and enter the reactivation phase (ie, the HBeAg-negative hepatitis phase) during follow-up, which is now recognized as a variant of immune clearance phase [1, 2].

Although HBV has been known for >5 decades, its immunopathogenesis remains poorly defined. The liver injury caused by HBV infection is principally contributed by immune responses, and the immune-related liver damage is induced by active viral replication [3, 4]. It is suggested that HBV-specific cytotoxic T lymphocytes (CTLs) initially induce the death of virus-infected hepatocytes. However, CTLs are unable to mediate complete eradication of the virus, and then subsequently recruit HBV-nonspecific inflammatory cells, including bystander T cells, natural killer (NK) cells, and neutrophils, that inevitably cause the immunopathology of CHB [5, 6]. However, it is unclear why the repetitive immune responses rarely clear the virus and why immune-tolerant patients do not develop liver damage despite active viral replication during the immune-tolerant phase. On the contrary, the role of HBV-specific immune response is clearer in acute hepatitis B (AHB). This literature review delineates how the immune response controls AHB but fails to combat CHB. Comparing the difference will facilitate understanding of disease progression and the development of therapeutic strategies for CHB.
AHB

Studies in chimpanzees with acute HBV infection showed that HBV, unlike hepatitis C virus, barely induced type I interferon (IFN) responses [7], although IFNs were shown to suppress HBV replication efficiently in the transgenic mouse model [8–10]. Thus, HBV is referred to as a stealth virus that replicates in hepatocytes without induction of a significant early innate immune response [11]. During acute HBV infection in humans and chimpanzees, reductions in the HBV DNA load and antigens in serum usually coincide with or precede liver damage, defined by an elevated level of serum ALT and T-cell infiltration [12–15], which suggests that there is an earlier, noncytopathic mechanism controlling HBV infection. In patients with AHB, the analysis of peripheral blood mononuclear cells (PBMCs) has shown that the activity of NK and NK T (NKT) cells peaked earlier than that of HBV-specific T cells [16]. The CD1d-restricted NKT cells were demonstrated to cause AHB in an HBV transgenic mouse model [17]. In addition, interferon γ (IFN-γ), which is secreted mainly by T cells, NKT cells, and NK cells, is an important cytokine for inhibiting HBV replication noncytopathically in the HBV transgenic mouse model [18, 19], and a surge in the IFN-γ level was found to coincide with a reduction in the level of serum HBV DNA during AHB in chimpanzees [7]. A recent ex vivo study further demonstrated that both IFN-γ and tumor necrosis factor α (TNF-α) stimulation could destabilize existing cccDNA through activation of APOBEC3A and APOBEC3B [20]. Taking these lines of evidence together, it is believed that NK cells and NKT cells may play an important role in early viral control of AHB through IFN-γ secretion.

There are several studies exploring the HBV-specific CTL response in patients with AHB [12, 21, 22]. Patients with AHB usually generated stronger polyclonal T-cell responses against HBV antigens, especially HBV core antigen and HBeAg, as compared to patients with CHB who developed only a monoclonal or even an undetectable T-cell response [12, 21, 23]. The CD4+ T-cell response could facilitate the development of neutralizing antibodies to HBV surface antigen (anti-HBs) [24, 25] and the induction of CTL responses. CD8+ T cells contribute to the production of antiviral cytokines (eg, IFN-γ) and perform cytotoxic activities to eliminate virus-infected hepatocytes, which not only clears HBV-infected cells but also induces immunopathology [14, 15].

In patients with AHB, the production of cytokines during the innate immune response, including type I IFN, interleukin 15, and IFN-λ1, was impaired during the early stage of infection and peaked after the serum HBV DNA level had declined, and the NK cell response was also delayed. The production of the immunosuppressive cytokine interleukin 10 coincided with an increase in the HBV DNA level in patients, which was inversely correlated with the intensity of HBV-specific T-cell responses [26]. The above data suggest that the innate immune responses are not sufficient to fully eliminate HBV during natural infections and that T-cell responses, although they may induce immunopathology, are required for control of acute HBV infection and contribute to the development of long-term immune surveillance against HBV.

CHB

Innate Immune Response in CHB

In contrast to AHB, NK cells in patients with CHB have been shown to express an inhibitory phenotype with blunt functional responses [27] and to mediate virus-specific CD8+ T-cell depletion through a death receptor pathway [28]. Granulocytic myeloid-derived suppressor cells (MDSCs) also play an important role in maintenance of the immune-tolerant stage of CHB, partially through arginase-mediated T-cell suppression [29]. Several treatment modalities have been proposed to enhance innate immunity for the treatment of CHB. Interferon alfa, a type I IFN, is one of two approved treatment modalities for CHB. Interferon alfa treatment has been shown to induce the proliferation and expansion of CD56bright NK cell numbers in peripheral blood, which is accompanied by augmentation of cytotoxicity function and IFN-γ expression [30–32]. The improved cell number and function of NK cells were associated with a decreased serum HBV surface antigen (HBsAg) level. However, interferon alfa–based therapy is far from an ideal modality because its overall responsive rate is about 30%, and it rarely reaches HBsAg seroconversion in Asian patients with CHB [33].

In addition to interferon alfa, several Toll-like receptor (TLR) agonists have been tested for the treatment of CHB in animal models and in humans since they are able to boost innate immune response. In a mouse model of persistent HBV infection, systemic administration of TLR9 agonist induced formation of intrahepatic myeloid-cell aggregates for T-cell population expansion in the liver, which facilitated intrahepatic expansion of HBV-specific CD8+ T cells derived from the HBV vaccine and clearance of the persistent HBV infection [34]. TLR7 agonist has been shown to induce a robust IFN response in plasmacytoid dendritic cells and to suppress HBV replication effectively in chronically infected chimpanzees [35]. This oral agonist has been explored in a phase I clinical trial. However, no significant change in the HBV DNA level or HBsAg level was observed in 49 treatment-naive patients or 51 patients with virologically suppression [36]. The unsatisfactory result of this clinical trial suggests that stimulation of the innate immune response alone is not sufficient to cure HBV in humans.

Adaptive Immune Response in CHB

How the Virus Escapes the Host Adaptive Immune Response

Adaptive immune responses, particularly the HBV-specific CD8+ T-cell response, have been suggested to play an important
role in viral clearance. This concept was supported by data from the chimpanzee model and the HBV transgenic mouse model [15, 37]. In patients with AHB, the present of strong and multiple CTL response in peripheral blood reinforced the concept [22, 38]. In contrast, the functional HBV-specific CTL response was barely detectable in the peripheral blood of patients with CHB [39].

Viral persistence after HBV infection depends on the age of exposure and the liver microenvironment. It causes self-limited and transient hepatitis in most adult patients, whereas 90% of neonates who acquire HBV infection perinatally develop persistent infection [14]. It has been demonstrated in an HBV transgenic mouse model that Kupffer cells in the nontransgenic offspring, which have been exposed to HBeAg from the mother, expressed a higher level of programmed cell death protein ligand 1 (PD-L1) and could jeopardize the function of CTLs [40]. In other words, adults never exposed to HBeAg could generate efficient immunity to control HBV infection in the first place. A recent study showed that the IFN-γ–mediated release of CXCL9 by Kupffer cells would augment the retention of HBV-specific CD4+ T cells through CXCR3 and promote their apoptosis, which facilitated the antigen-specific tolerance during HBV persistence [41].

Once chronic infection has been established, the patient may experience repetitive liver damage, characterized by an elevated serum ALT level. It is still unclear how the adaptive immune response leads to liver damage but does not completely clear the virus. The comparison of liver-infiltrating HBV-specific CD8+ T cells between patients with and those without a high viral load accompanied with liver damage revealed a high frequency of intrahepatic HBV-specific CD8+ T cells in patients without hepatic immunopathology. In patients with active hepatitis, virus-specific CD8+ T cells were less frequent among liver infiltrating CD8+ T cells, but the absolute number was similar, owing to the large amount of cellular infiltration [42].

The other study also showed that the HBV-specific CTLs were also present in PBMCs from immune-tolerant patients [39]. These data suggest that HBV-specific CTLs exist in patients with CHB but that they are not quantitatively and qualitatively strong enough to clear the virus. Analysis of HBV-specific CD8+ T cells in the liver and the peripheral blood showed that intrahepatic cells expressed higher levels of programmed cell death protein 1 (PD-1) than their peripheral counterparts [43, 44], which suggests that these CTLs would undergo exhaustion in a liver microenvironment with cognate antigen expression. Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is also highly expressed on HBV-specific CTLs among PBMCs from patients with CHB [45]. In addition to expression of multiple coinhibitory molecules on CD8+ T cells, a recent study based on an analysis of PBMCs from patients with CHB showed that exhausted HBV-specific CD8+ T cells have substantial downregulation of various cellular processes centered on extensive mitochondrial alterations [46]. Although most of these data are based on cross-sectional studies and HBV-specific CD8+ T cells were collected from PBMCs, these results suggest that there are multiple defects in HBV-specific CTLs and that their function is markedly impaired at multiple aspects.

How the Current Therapy Improves Adaptive Immune Response

Interferon alfa and nucleos(t)ide analogues (NAs) are 2 classes of approved antiviral therapies and play different roles in rescuing exhausted HBV-specific CTLs. Although interferon alfa has been viewed as an immune modulator, the clinical data show that it exerts minimal effect on HBV-specific CTLs [47]. NAs inhibit viral reverse transcription and profoundly suppress viral replication [1]. Although suppression of viral replication does not affect the expression of viral antigens, several studies have shown the NA treatment partially restores HBV-specific CTL functions. It has been shown that long-term NA treatment could restore functions of HBV-specific T cells [48, 49]. However, it only achieved partial restoration and did not lead to HBsAg clearance, which suggests that more treatment modalities are needed to achieve functional cure for HBV infection.

In addition to their role in T-cell responses, neutralizing antibodies produced by B cells are believed to play a role in control of HBV infections. It is clear that neutralizing anti-HBs can prevent HBV infection in general population, but its role is unclear in patients with chronic HBV infection. Indirect evidence from a study in patients with lymphoma receiving treatment with rituximab, an antibody the targets CD20 and thus specifically depletes B cells, revealed that depletion of B cells is associated with a higher risk of HBV reactivation not only in HBV carriers but also in patients with resolved HBV infection (ie, those who are positive for antibody to HBV core antigen but negative for HBsAg), who have been regarded as achieving functional cure [50]. HBsAg seroreversion and HBV reactivation (HBV DNA load, >2000 IU/mL) occur in 10.3% and in 17.9% of patients with resolved HBV infection, respectively [50]. Although how the B cells control the virus has not been well studied, B cells are believed to play a role in controlling the virus, and this may provide an important clue for future HBV cure.

IS IT POSSIBLE TO CURE CHB VIA IMMUNOTHERAPY?

Current treatment modalities rarely clear HBsAg. It is thus important to develop new strategies to clear the virus. Among all of the novel strategies, the killing of HBV-infected hepatocytes via CTL-based immunotherapy has the most promise. A therapeutic strategy combining NA treatment to suppress viral replication and to prevent uninfected hepatocytes from HBV infection and restoration of the HBV-specific CTL response to clear the infected hepatocytes seems attractive. It has been shown that there is low abundance of HBV-specific T cells in the peripheral blood of patients with CHB. The frequency of HBV-specific CD8+ T cells varies between the different stages.
of infection. It can peak to 2% of the total CD8+ T cells within 2 weeks of acute infection but is usually <0.1% during chronic stage [51]. It is very difficult to clear virus by relying on such a small number of CTLs; moreover, they have multiple functional defects. Instead of manipulating these existing HBV-specific CTLs, generating new HBV-specific CTLs via a therapeutic vaccine is a more reasonable strategy. So far, there have been 2 clinical trials using different therapeutic vaccines in addition to NAs to treat patients with CHB, but results for both were not satisfactory [52, 53]. Neither one induced HBsAg clearance or decline effectively. The failure could be attributed to an inadequate or nonsustained immune response exerted by therapeutic vaccines. If the nonsustained immune response is induced by immune checkpoint, such as engagement of PD-1 and PD-L1 between HBV-specific CTLs and liver-resident cells, on the addition of an immune checkpoint inhibitor, such as anti–PD-1, may be beneficial for improving the efficacy of treatment.

A recent phase 1 clinical trial was conducted to explore whether durable control of CHB can be achieved via treatment with nivolumab, a PD-1 inhibitor, with or without a therapeutic vaccine in HBsAg-negative patients with CHB and virologic suppression. The results showed that HBsAg loss was achieved in 1 of 12 patients receiving 1 dose of 0.3 mg/kg nivolumab (about 10% of the suggested dose), while none achieved HBsAg loss in patients receiving the combinational therapy [54]. Although it is too early to conclude that the combinational therapy did not achieve a better treatment response in the phase 1 study, anti–PD-1 treatment seems to play a role in curing CHB.

In the future, a successful treatment strategy may rely on combination of an effective therapeutic vaccine and an immune checkpoint inhibitor, which has been demonstrated in a wood-chuck model [55]. Although the current results of immunotherapy for CHB are not satisfactory, it would be the most attractive way to clear the virus, if we could select the appropriate patient population with optimal study designs.

**CONCLUSION**

HBV is a stealth virus that barely induces innate immunity in the early phase of infection. In AHB, innate immune responses could be established, followed by strong and robust adaptive immune responses, which both contribute to viral clearance. The noncytopathic pathway mediated by IFN-γ secreted by NK cells, NKT cells, and T cells contribute to the early control of HBV infection before emergence of the CTL response. CD4+ T cells facilitate the production of neutralizing antibodies and the induction of CTL responses, whereas CD8+ T cells contribute to the production of antiviral cytokines (eg, IFN-γ) and perform cytotoxic activities to eliminate virus-infected hepatocytes, which also induce immunopathology (Table 1). In patients with CHB, both innate and adaptive immune responses are weak. Suppressive cell populations and molecules (eg, NK cells, granulocytic MDSCs, Kupffer cells, PD-1, and CTLA-4) are key components and contribute to the chronicity of HBV infection (Table 1). Although interferon and NAs, the available treatment modalities, partially restore innate and adaptive immune responses, respectively, their use individually or in combination is still far from achieving HBsAg loss. Although the treatment response is unsatisfactory in current clinical trials using several immunomodulators, immunotherapy is the most promising treatment modality for HBV cure in the future.

| Table 1. Immune Effector Cells and Molecules Associated With the Immunopathogenesis of Acute Hepatitis B (AHB) and Chronic Hepatitis B (CHB) |
|---------------------------------|-------------------|-------------------|---------------------------------|-------------------|
| **Effector Cell or Molecule**   | **Disease Stage** | **Model(s)**      | **Mechanisms**                  | **Reference(s)**  |
| NK cells and NKT cells         | Active CHB        | Human, peripheral blood | Induction of apoptosis of HBV-specific CD8+ T cells through TRAIL-R2 signaling | [28] |
| CD8+ cytotoxic T lymphocytes    | AHB               | Human, peripheral blood | Early viral control by IFN-γ production | [16] |
| CD4+ helper T cells            | AHB               | Mouse; human, peripheral blood | Assisting development of neutralizing anti-HBs antibody | [24, 25] |
| CD1d-restricted NKT cells      | AHB               | Human, peripheral blood | Eliminating HBV nucleocapsid particles; destabilizing existing cccDNA through APOBEC3A and APOBEC3B | [20] |
| IFN-γ and TNF-α                | AHB               | Human, peripheral blood | Eliminating HBV nucleocapsid particles; destabilizing existing cccDNA through APOBEC3A and APOBEC3B | [20] |
| NK cells                       | Active CHB        | Human, peripheral blood | Induction of apoptosis of HBV-specific CD8+ T cells through TRAIL-R2 signaling | [28] |
| Granulocytic MDSCs             | Inactive CHB      | Human, peripheral blood | Suppression of T cell-mediated immunopathology through arginase I | [29] |
| Kupffer cells                  | Tolerance stage, CHB | Mouse           | Expression of PD-L1 and induction of T-cell exhaustion | [40] |
| CXCL9-CXCR3 axis               | CHB               | Mouse            | Retention of HBV-specific CD4+ T cells in the liver; contribution of apoptosis of virus-specific CD4+ T cells partially via CTLA-4 | [41] |
| B cells                        | CHB               | Human            | Suppression of HBV reactivation in HBV carrier and in resolved patients | [50] |
| PD-1 and CTLA-4                | CHB               | Human peripheral blood, human liver | Induction of T cell exhaustion | [43–45] |

**Abbreviations:** anti-HBs, antibody to hepatitis B virus surface antigen; cccDNA, covalently closed circular DNA; CTLA-4, cytotoxic T lymphocyte antigen 4; HBV, hepatitis B virus; IFN-γ, interferon γ; IL-4, interleukin 4; MDSC, myeloid-derived suppressor cell; NK cell, natural killer cell; NKT cell, natural killer T cell; PD-L1, programmed cell death protein ligand 1; PD-1, programmed cell death protein 1; TNFα, tumor necrosis factor α.
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