Review Article

The Role of Immune Cells in Chronic HBV Infection

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Abstract

Hepatitis B virus (HBV) infection is a major cause of chronic liver diseases that may progress to liver cirrhosis and hepatocellular carcinoma. Host immune responses are important factors that determine whether HBV infection is cleared or persists. After infection, viral replication occurs inside hepatocytes, and the secretion of infectious virions can take place at high rates for decades. Consequently, HBV DNA and viral proteins, like HBV early antigen (HBeAg) and HBV surface antigen (HBsAg), can be easily detected in serum. Chronic infection with HBV is the result of an ineffective antiviral immune response towards the virus. In this review, we discuss the role of immune cells in chronic HBV infection.

Introduction

Hepatitis B virus (HBV) infection is a major health problem, affecting around 350 million people worldwide, despite the availability of a prophylactic vaccine.[1] Although the majority of infected individuals clear the virus spontaneously, a fraction of patients is unable to clear the virus and develops a chronic form of hepatitis. The number of chronically infected patients has already reached over 240 million.[2] In time, these patients are at high risk of developing HBV-related diseases, such as liver cirrhosis and hepatocellular carcinoma (HCC), which account for 600,000 deaths annually.[3]

Chronicity of HBV is the result of a complex interaction between the replicating virus and an inadequate immune response.[4–6] After infection, viral replication takes place inside hepatocytes, and the secretion of infectious virions can occur at high rates for decades. Consequently, HBV DNA and viral proteins, like HBV early antigen (HBeAg) and HBV surface antigen (HBsAg), can be easily detected in serum. The levels of these clinical markers may fluctuate over time and are a reflection of disease activity and commonly used to define disease stage.[5,6]

The liver is an immune organ that contains diverse immunologically active cell types, including both lymphocytes and myeloid cells.[7] It has been puzzling for years why some patients develop chronic hepatitis while HBV infection resolves without clinical consequences in others, and why viral infection is not cleared in chronic carriers. Although these issues are unresolved, it has been speculated that aberrant immune tolerance in chronic hepatitis and HBV carriers plays an important role in impairing the immune responses for viral clearance.[8,9] Chronic infection with HBV is the result of an ineffective antiviral immune response towards the virus.[10–12] The exact mechanism by which HBV escapes immunity is still not known. The role of immune cells in chronic HBV infection will be discussed in this review.

HBV epidemiology in China

Infection with HBV and development of HCC are responsible for heavy disease burdens in China. In 2006, the Ministry of Health of China (MOH) estimated that, among Chinese aged 1 to 59 years as of 1992, the national prevalence of HBV infection and HBV carriers was 57.63% and 9.75%, respectively, which corresponded to 690 million infected people, 120 million carriers, and 20 million with chronic hepatitis (Table 1).[13] The disease burden of HBV is very large, even when compared to that of tuberculosis, which was only responsible for 1.4 million new cases in 2000.[14] Chronic hepatitis B (CHB) is one of the most serious infectious diseases in China.

To date, the MOH has taken several measures to address HBV. In its National Plan for Prevention and Treatment against Hepatitis B for 2006-2010, the MOH stated that CHB causes serious consequences for patients, their families, and society as a whole and that it is a major cause of poverty and a health issue of the highest priority. The prevalence of HBV infection in China is one of the highest in the world. In addition, China has the highest incidence of HCC (37.9 and 14.2 for males and females, respectively, per 100,000 world standard 340,000 cases), with 630,000 newly diagnosed cases in 2002.[15]

In China, HBV is the most important risk factor for the development of HCC. Although the HBV immunization program is expected to greatly reduce HCC incidence, a few more decades are required before an obvious decrease among the general population will be seen. With the high cost of the current antiviral therapy for CHB, HCC control among existing carriers depends on reducing risk factors that accelerate the development of HCC among carriers. Since the introduction of HBV immunization, control of HBV infection

Keywords: Immune cells; Natural killer cells; CD8$^+$ T cells; Hepatitis B virus.
Abbreviations: ADCC, antibody-dependent cytotoxicity; APCs, antigen presenting cells; CHB, chronic hepatitis B; CTLA-4, cytotoxic T lymphocyte antigen 4; DC, dendritic cells; HBeAg, HBV early antigen; HBsAg, HBV surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IFN-γ, interferon gamma; IL, interleukin; IPC, interferon producing cell; KCs, Kupffer cells; LPS, lipopolysaccharide; mDC, myeloid DC; MHC I, major histocompatibility complex 1; MOH, Ministry of Health of China; NK, natural killer; NKG2A, NK group 2A; PBMC, peripheral blood mononuclear cell; PD-1, programmed death-1; pDC1, precursor DC1; PD-L, PD ligand; TGF-β, transforming growth factor beta; Tim-3, T cell immunoglobulin- and mucin-domain-containing molecule-3; TLR2, toll-like receptor 2; Tregs, regulatory T cells.
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Table 1. Epidemiology survey of hepatitis B virus infection in 1992

Region	Province	HBsAg (%)
NorthEast	Heilongjiang, Jilin, Liaoning	10.71
NorthCentral	Neimenggu, Beijing, Tianjin, Hebei, Shanxi	5.53
East	Shandong, Jiangsu, Shanghai, Zhejiang, Anhui, Jiangxi, Fujian	9.94
SouthCentral	Henan, Hubei, Hunan, Guangdong, Guangxi	12.75
SouthWest	Xizang, Sichuan, Yunnan, Guizhou	8.90
NorthWest	Xinjiang, Gansu, Qinghai, Ningxia, Shangxi	8.68

HBsAg, hepatitis B surface antigen.

has been substantially progressing in China, which will lead to a dramatic decrease in the disease burden from HBV infection and HCC.[16] Therefore, understanding the role of immune cells in chronic HBV infection will become more and more important.

CD8$^+$ T cells

CD8$^+$ T cells are a major component of cellular adaptive immunity, and they normally mediate protection against intracellular pathogens and tumor cells. In order to be properly activated, CD8$^+$ T cells require at least two signals: first, the recognition of their cognate antigen presented by major histocompatibility complex 1 (MHC I) molecules on antigen presenting cells (APCs). This is mediated by the interaction between the antigen-specific T cell receptor and the peptide-MHC I complex. Second, additional costimulatory signals have to be provided by the same APC to prevent anergy. Different cytokine milieus may influence this activation process.[17–19]

In chronically HBV-infected individuals, virus-specific CD8$^+$ T cell responses are rarely detectable.[20–24] The profiles of HBV specific CD8$^+$ T cell responses depend on the stage of disease and are highly influenced by the level of HBV replication. Indeed, circulating multispecific HBV-specific CD8$^+$ T cell responses are predominantly detectable *ex vivo* in patients with low viral load. Furthermore, HBV-specific CD8$^+$ T cells may not or only insufficiently be primed by APCs and, consequently, may not expand upon antigen-encounter. Accordingly, HBV-specific CD8$^+$ T cells would be expected to display a naïve phenotype, characterized by high expression levels of CD45RA, CD27, CD28, and CCR7.[25]

CD8$^+$ T cell dysfunction in chronic HBV infection follows a well-established pattern with elevated expression of inhibitory molecules, such as programmed death-1 (PD-1),[26,27] cytotoxic T lymphocyte antigen 4 (CTLA-4),[28] Tim-3,[29,30] and 2B4 (CD244).[31] Furthermore, the expression of corresponding ligands, such as PD ligand (PD-L)1, has been shown to be increased on hepatocytes.[32] Therefore, highly viremic HBV-infected patients exhibit a more severely impaired CD8$^+$ T cell phenotype and more profound T cell dysfunction in the liver than in the blood.[27,33] Blockade of these inhibitory pathways may at least partially restore HBV-specific CD8$^+$ T cell functionality, as demonstrated *in vitro*.[31] The potential relevance of blocking the PD-1 pathway was shown in the HBV mouse model, where HBV-transgenic mice were treated with blocking antibodies for PD-L1 prior to the adoptive transfer of HBV-specific cytotoxic T cells.[34] Moreover, the lack of CD4$^+$ T cell help was shown to contribute to defective CD8$^+$ T cell function.[35] The increase in regulatory T cell (Treg) numbers[36–38] and immunosuppressive cytokines, such as interleukin (IL)-10 and transforming growth factor beta (TGF-β),[39] impaired virus-specific CD8$^+$ T cell responses. It has also been reported that increased intrahepatic arginase levels[40] and, hence, the lack of arginine, led to a functional silencing of CD8$^+$ T cells due to the downregulation of the CD3ζ-chain.[41]

T-cell exhaustion is regulated by a complex network of coexpressed inhibitory receptors that act synergistically and redundantly. Hence, a better understanding of the diverse inhibitory receptors involved in the regulation of exhausted T cells will pave the way for the development of more effective immunotherapeutic and prophylactic strategies for the treatment of chronic infectious diseases.[42] Recently, several studies have provided insight into the transcriptional mechanisms behind T-cell exhaustion in chronic infection. The transcription factor T-bet was shown to repress directly the expression of inhibitory receptor PD-1 and to sustain virus-specific CD8$^+$ T-cell responses during chronic infection, as its expression was downregulated in exhausted CD8$^+$ T cells in response to persisting antigen.[43] In addition, a critical role of T-bet for viral clearance was demonstrated for HBV infection, where T-bet deficiency was more characteristic of a chronic evolving infection.[44]

Regulatory T cells

Recent advances in the understanding of the properties of CD4$^+$CD25$^+$ Tregs have provided new insights into the mechanism by which immune tolerance is maintained or broken in various disease conditions.[45–47] Significant deficits in the number and function of these Tregs were found in autoimmune diseases and persistent infections.[48–51] These studies have demonstrated the importance of CD4$^+$CD25$^+$ Tregs in various immune-related diseases and have provided a clue for exploring the role of CD4$^+$CD25$^+$ Tregs in hepatitis B.[52,53]

It has been reported that immune tolerance in chronic hepatitis may be associated with CD4$^+$CD25$^+$ Tregs. The increased frequency of CD4$^+$CD25$^+$Foxp3$^+$ T cells was associated with serum levels of TGF-β, which is known to promote peripheral conversion of CD4$^+$CD25$^-$ T cells to CD4$^+$CD25$^+$Foxp3$^+$ Tregs. The gene products of HBV, such as the envelope proteins, may directly induce CD4$^+$CD25$^+$Foxp3$^+$ Tregs through antigen recognition. Similar situations with T cell receptor peptides or toll-like receptor 2 (TLR2) in the induction of CD4$^+$CD25$^+$ Tregs have been reported.[54] TGF-β serum levels correlated significantly with the expression of Foxp3 and the frequency and function of CD4$^+$CD25$^+$ Tregs in chronic hepatitis patients. On the other hand, CD4$^+$CD25$^+$ Tregs could secret IL-10 to inhibit NK cell and CD8$^+$ T cell antivirus response.[55]

The HBV-specific immune response could be suppressed by CD4$^+$CD25$^+$ Tregs in patients with HBV infection. Tregs not only from CHB patients but also those from patients with resolved HBV infection suppressed HBV specific CD8$^+$ T cells. However, it has been reported that the frequency of Tregs in CHB patients was significantly higher than that in

healthy controls and those with resolved HBV infection.[56] Therefore, the frequency of Tregs might contribute to the disease status of HBV infection. Another group reported that the frequency of $CD39^+$ Tregs correlated with the progression of HBV infection. Therefore, we should consider this minor subset of Tregs in CHB patients. Previously, we reported that HBcAg-specific IL-10 secreting $CD4^+CD25^+$ Tregs might contribute to the suppression of HBV-specific interferon gamma (IFN-γ) secreting $CD4^+$ T cells.[57] Tregs might contribute to the suppression of HBV-specific T cells. Therefore, treatment with a nucleos(t)ide analogue might affect the Tregs.

NK cells

NK cells represent the main effector cell population involved in innate immune responses against intracellular pathogens and abnormal cells.[58] They are enriched in the liver and account for one-third of the intrahepatic lymphocytes, compared to 5–15% in the peripheral blood.[59,60] Studies regarding phenotype and function of NK cells during chronic HBV infection have revealed, in part, conflicting results. Several reports have concluded that NK cells exhibit selective defects in their antiviral function. This functional dichotomy features conserved or enhanced cytolytic activity and diminished cytokine production that may contribute to viral persistence and implicate a role for NK cells in disease pathogenesis.[61–63] The mechanisms leading to this functional impairment are still not fully understood but are thought to be heterogeneous.

HBV infection may alter the activation status and receptor expression patterns on the surface of NK cells. Indeed, the expression of inhibitory receptors, such as NK group 2A (NKG2A), was shown to be elevated, while the activating receptors CD16 and NKp30 were downregulated.[63,64] These changes were correlated with serum HBV DNA load. In addition to classical NK cell receptors, other co-inhibitory molecules involved in immune responses may impair NK cell function. Notably, T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) has been shown to be upregulated on NK cells during HBV infection, and *in vitro* blockade of Tim-3 was able to enhance NK cell cytotoxicity.[65]

Impaired NK cell activation and function may also arise from modified expression patterns of ligands for inhibitory and activating NK cell receptors.[66] Furthermore, the immunosuppressive cytokine environment in chronic HBV infection, created through high levels of IL-10, may inhibit the ability of NK cells to produce IFN-γ,[67] as was previously shown in acutely infected patients. This defect persisted in patients with chronic HBV infection receiving antiviral therapy but was reversed *in vitro* by specific blockade of IL-10 and TGF-β.[67]

Chronic HBV infection is the key driving force for the development of hepatic cirrhosis and HCC. In developing countries, the risk for developing HBV-related HCC is 70%. The frequency, activation, and cytokine production of circulating NK cells were significantly reduced in CHB patients compared with healthy controls. Increased secretion of the immunosuppressive cytokine IL-10 in CHB patients suppressed NK cell function, which contributed to immune tolerance and facilitated viral persistence. Blocking IL-10 or administering antiviral therapy restored NK cell activation and IFN-γ production. Impaired NK cell function induced by persistent HBV infection and chronic inflammation contributed to the progression of HCC.[68]

B cells

B cells have been identified as potent regulators of T cell immune responses in studies of autoimmunity, infection, and cancer.[68–70] IL-10 is the primary mechanism by which B cells modulate other immune cells. These regulatory B cells (Bregs) are pathogenic in parasitic infections, subverting $CD4^+$ T cells toward a Th2 phenotype favorable to pathogen survival, and they can reduce Ag-specific $CD8^+$ T cell responses through production of IL-10 in murine viral infection.[71] In humans, it was recently demonstrated that analogous Breg subsets can suppress $CD4^+$ T cell proliferation and IFN-γ and TNF-α production by $CD4^+$ T cells as well as regulate TNF-α release by monocytes.[72] The suppressive effects were mediated by IL-10 and were independent of TGF-β.

There is little data available regarding the role of Bregs in human viral infection. A recent report longitudinally studied serum IL-10 levels in patients with CHB undergoing spontaneous disease flares. There was a close temporal correlation between IL-10 levels and fluctuations in viral load or liver inflammation. Blockade of IL-10 *in vitro* rescued polyfunctional virus-specific $CD8^+$ T cell responses. These data revealed that a novel IL-10-producing subset of B cells were able to regulate T cell immunity in CHB.[73]

Monocytes

Although circulating monocytes represent about 10% of leukocytes in human blood, relatively little is known on the effects of chronic viral infections on monocytes. Impaired monocyte functions have been reported in human immunodeficiency virus (HIV) infections,[74,75] and TLR responsiveness of monocytes has been obtained from patients with chronic hepatitis C virus (HCV) infections.[76,77] Monocytes can be divided into two distinct subpopulations based on the surface expression of CD14 and CD16. $CD14^{high}CD16^-$ monocytes make up the majority (80–90%) of blood monocytes and have been reported to produce relatively high IL-10 and low TNF levels, whereas the $CD14^+CD16^+$ subpopulation produces higher levels of the pro-inflammatory cytokines TNF and IL-1β.[78,79]

In chronic HBV, some studies reported modulation of the monocyte compartment as a result of the disease. Depending on the clinical phase of the chronic HBV infection, altered monocyte subset frequencies were reported.[80,81] Moreover, peripheral blood mononuclear cells (PBMCs) from HBeAg-positive patients produced less TNF and IL-6 upon stimulation with TLR2 agonists as compared to HBeAg-negative patients,[82] which may be due to lower expression of TLR2 in HBeAg-positive patients.[83] Furthermore, exposure of monocytes to HBsAg suppressed lipopolysaccharide (LPS)-induced TNF and IL-1β production *in vivo* and *in vitro*.[84,85] There was a link between HBsAg and decreased cytokine production induced by the TLR2 ligand (Pam3csk4) in PBMCs from CHB patients *in vivo*.[86] This finding demonstrated that HBsAg selectively inhibited Pam3csk4-stimulated IL-12 production. HBsAg inhibited the jun N-terminal kinase-mitogen activated protein kinase (JNK-MAPK) pathway, and it may be by this mechanism that HBV evades the immune system and maintains its persistence.[87]

Kupffer cells

Kupffer cells (KCs) are nonparenchymal cells that account for approximately 15% of the total liver cell population and constitute 80–90% of the tissue-resident macrophages in the whole body. Due to their intravascular (sinusoidal) localization, KCs have long been studied as scavenger cells that physiologically remove particulate material from the portal circulation.[88] In recent years, KCs have been implicated in the pathogenesis of an assortment of inflammatory liver diseases, including viral hepatitis.[89] Accordingly, the current dogma regarding the role of KCs in HBV pathogenesis considers these cells as important contributors to liver injury.[90]

The liver is continuously exposed to nonpathogenic antigens (from food) and to gut-derived LPS. LPS is a powerful stimulus for innate immunity through TLR ligation and activation of professional APCs. The elaboration of IL-10 by KCs is one mechanism by which to modulate the host response to proinflammatory cytokines (IL-12, IL-15, and IL-18) also secreted by KCs.[91] KCs are intravascular macrophages that are continuously exposed to, and tolerant of, bacterial TLR ligands, which are delivered via the portal circulation. By mimicking a bacterial TLR2 ligand and effectively blocking the TLR3-mediated, double-stranded RNA (dsRNA)-induced antiviral response, HCV core protein might exploit this unique aspect of immunity in the liver.[92] Like HCV, HBV is also a liver specific virus that produces a lot of viral proteins, DNAs, dsRNAs, and so on. They may interact with KCs through TLRs or other receptors and initiate liver damage or other immune cell responses.

DCs

Dendritic cells (DC) are considered to be professional antigen presenting cells that initiate primary immune responses, combining innate and adaptive immunity. DC plays an important role in activation of $CD8^+$ CTL and $CD4^+$ Th cells. Bioactive cytokines released by DC can stimulate NK cells, induce Th1 to produce cytokines and promote the proliferation of CTL.[93] At least two DC subsets have been found in human and mouse peripheral blood, named precursor DC1 and DC2 (pDC1 and pDC2). pDC1, also called myeloid DC (mDC) or antigen presenting cells type 1, expresses CD11c and CD1a, and its expression is mainly correlated with antigen ingestion and activation of naïve T cells. pDC2 or IFN producing cells (IPCs) express CD123, CD4, MHC-II molecules, and BDCA-2 antigen. During different stages of infection in patients with hepatitis B, the frequency and quantity of DC declined significantly, with maturation disorder and function defects, resulting in inadequate effective immune response to clear virus.[94,95]

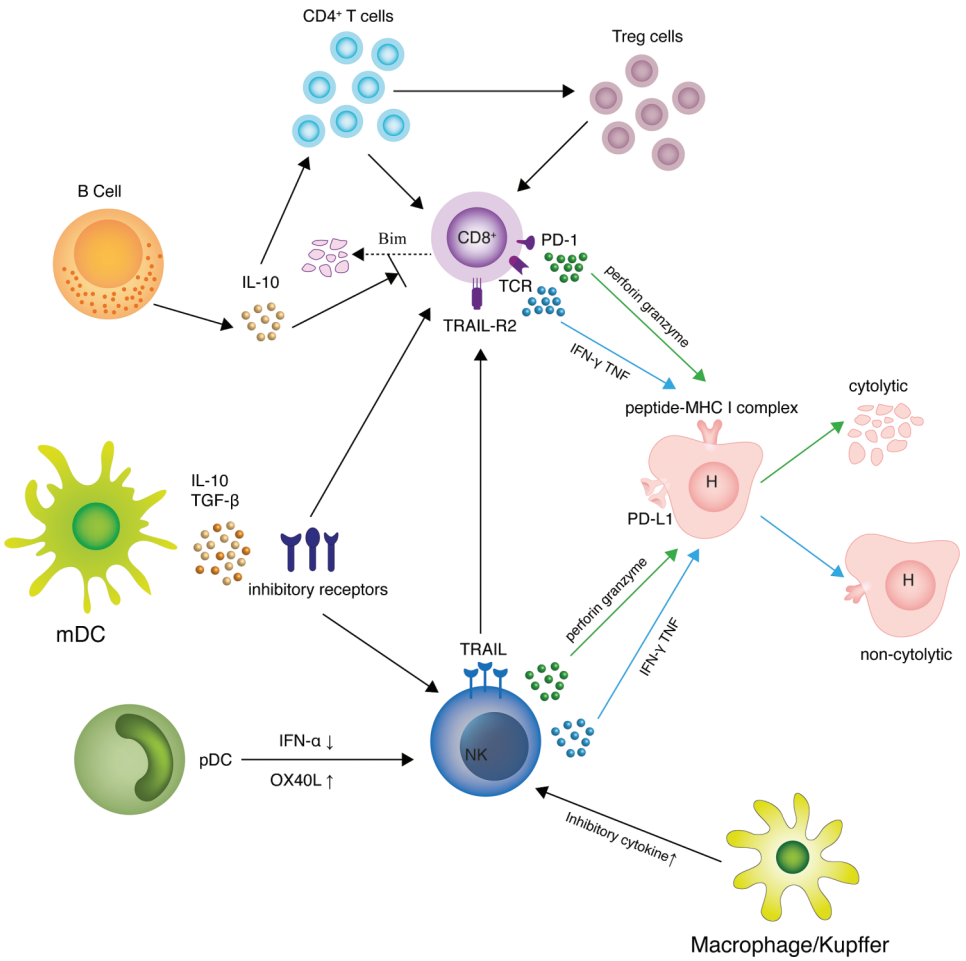

Fig. 1. Natural killer cells and hepatitis B virus (HBV)-specific $CD8^+$ T cells are centrally involved in the HBV immune response.

pDC2 is more sensitive than pDC1 when evaluating disease progress in patients with hepatitis B, with pDC2 declined in the early stage of infection and pDC1 declined later.

Broadly speaking, DCs are hematopoietically-derived cells that efficiently link the innate and the adaptive immune responses. Functionally, impaired DCs play important roles in suppressing host immune responses as well as facilitating viral persistence in chronic HBV infection.[96] HBV DNA can be recognized by DCs through TLR9, and HBsAg can be internalized by DCs through the mannose receptor.[97] DCs phagocytize hepatitis B virus, process them into antigenic peptides and present them to $CD4^+$ and $CD8^+$ T cells.[98] DCs activate antibody-dependent cytotoxicity (ADCC) cells and NK cells, which stimulate these cells to secrete immunosuppressive cytokines IL-10 and TGF-β, thereby assisting in the induction of Tregs with the participation of mDCs to destroy HBV-infected hepatocytes.[99] DCs undergo a process called maturation in the presence of inflammatory signals, such as proinflammatory cytokines, microbial products, and other endogenous signals.

Circulating blood pDC numbers seem to be unaffected by HBV, but functional deficits in pDCs from chronic HBV patients, including impaired IFN-α production, have been reported.[100] Recently, it was shown that patient-derived HBsAg could bind to human pDC *in vitro* and impair TLR9-induced IFN-α production by pDCs.[101] Although circulating and intrahepatic pDCs from patients with chronic HBV infection showed a more activated phenotype, their ability to respond to TLR-9 stimulation was significantly impaired. Moreover, patient-derived mature pDCs were poor activators of NK cell cytotoxic function due to impaired IFN-α secretion and reduced OX40 ligand expression. HBV seems not only to directly inhibit pDC maturation in a TLR9-dependent manner but also to abrogate the supporting function of monocytes regarding IFN-α production by pDCs.[102,103]

Taken together, these findings suggest that chronic HBV infection could lead to decreased DC function and inhibition of T cell activation. Therefore, the quantity and dynamic changes of DCs are closely related to immune status and reactivity of antiviral therapy. DC function may become a valid index for the assessment of disease progress and efficacy of antiviral therapy.

Conclusions

Recently, knowledge of the tolerogenic liver environment and the key cell populations in local hepatic immune regulation has rapidly grown. Despite these important insights, which are primarily based on studies performed in genetically modified mouse models, very little information is available about the specific role of these cell populations and the molecular mechanisms of hepatic immune surveillance in human viral infection, especially in chronic HBV infection.

Chronicity of HBV is the result of a complex interaction between the replicating virus and an inadequate immune response (Fig. 1). After infection, viral replication occurs in hepatocytes, and the secretion of infectious virions can take place for decades at high rates. Consequently, HBV DNA, as well as viral proteins like HBeAg and HBsAg, can be easily detected in serum. Immune cells in the liver respond to these stimuli differently and secrete special cytokines at different disease stages. Immune cells in the liver construct a complex net and cytokine map that is not only influenced by themselves but also other cell types. The cross-talk between immune cells and the mechanisms controlling virus replication have become more popular recently. A better understanding of the mechanisms leading to viral persistence may result in new therapeutic treatment strategies that aim to remedy the immune cell defects described, thereby augmenting functional responses and decreasing antigen-unspecific liver damage.

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Conflict of interest

None.

Author contributions

Writing the manuscript (HJL), collecting the data and references (NCZ, HXS, YY, AC), drawing the pictures (TYL), critically reviewing the manuscript (ZKT).

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