Review

JOURNAL OF HEPATOLOGY

Hepatitis B surface antigen seroclearance: Immune mechanisms, clinical impact, importance for drug development

Issam Tout[1,2], Dimitri Loureiro[1,2], Abdellah Mansouri[1,2], Vassili Soumelis[3,4], Nathalie Boyer[1,2], Tarik Asselah[1,2,*]

Summary

HBsAg seroclearance occurs rarely in the natural history of chronic hepatitis B (CHB) infection and is associated with improved clinical outcomes. Many factors are associated with HBsAg seroconversion, including immune and viral factors. However, the immune mechanisms associated with HBsAg seroclearance are still difficult to elucidate. HBsAg seroclearance is the ideal aim of HBV treatment. Unfortunately, this goal is rarely achieved with current treatments. Understanding the mechanisms of HBsAg loss appears to be important for the development of curative HBV treatments. While studies from animal models give insights into the potential immune mechanisms and interactions occurring between the immune system and HBsAg, they do not recapitulate all features of CHB in humans and are subject to variability due to their complexity. In this article, we review recent studies on these immune factors, focusing on their influence on CHB progression and HBsAg seroconversion. These data provide new insights for the development of therapeutic approaches to partially restore the anti-HBV immune response. Targeting HBsAg will ideally relieve the immunosuppressive effects on the immune system and help to restore anti-HBV immune responses.

Keywords: Chronic HBV infection; HBsAg seroclearance; Immune system; Interferon; Nucleos(t)ide analogues; HBV therapy.

Received 4 December 2019; received in revised form 3 April 2020; accepted 7 April 2020; available online 22 April 2020

Introduction

HBV infection represents a global health problem with approximately 257 million people chronically infected.[1] Chronic HBV infection (CHB) is a major contributor to the development of cirrhosis, hepatocellular carcinoma (HCC) and liver-related death worldwide.[2–5] Currently, 2 approved therapeutic strategies are available, pegylated interferon (PEG-IFN) or nucleos(t)ide analogues (NAs), which suppress HBV replication and slow disease progression. However, these treatments do not generally lead to cure.[3–5] The ultimate endpoint of chronic HBV treatment is sustained HBsAg loss with or without seroconversion to hepatitis B surface antibody (anti-HBs). Seroclearance and conversion of HBsAg represent the most important outcomes for CHB trials because they represent immunity to HBV and indicate a better prognosis.[6] However, due to the persistence of intrahepatic, covalently closed circular HBV DNA (cccDNA),[7] HBsAg seroclearance and conversion to anti-HBsAg is a rare event in CHB. It can occur spontaneously, but the reported rates have been variable, as they are affected by a myriad of patient characteristics such as age, cirrhosis, HBeAg status, HBV DNA level, and serum HBsAg level.[8,9] Prior cohort studies, from Southeast Asia, showed that the annual HBsAg seroclearance rate can range from a low of 0.12% to a high of 2.38%.[8,10] The host immune response to HBV is closely related to the natural course of HBV infection. CHB has been associated with the exhaustion of T and B cell responses,[11,12] with HBsAg being a major contributor to the immunopathogenesis of CHB. This review focuses on the immune factors affected by HBsAg and the immunological changes associated with HBsAg seroclearance which could be potential targets for immunotherapy.

Molecular virology and structure of HBsAg

HBV is a small hepatotropic enveloped DNA virus belonging to the *Hepadnaviridae* family.[13] HBV virions have an icosahedral nucleocapsid formed by HBV core proteins (HBcAg) containing the partly double-stranded DNA in relaxed circular conformation (rcDNA). The nucleocapsid is surrounded by the viral envelop composed of 3 types of HBsAg: L-(large), M-(middle) and S-(small) HBsAg.[13] HBsAg plays a crucial role in the attachment to the hepatocyte via the high affinity interaction between the 75 amino acids on the N-terminus of L-HBsAg (PreS1 domain) and the 157 to 165 residues on the HBV receptor, the human sodium taurocholate co-transporting polypeptide receptor (hNTCP, SLC10A1) that enables hepatocyte infection.[14–16] HBV entry is followed by the translocation of the nucleocapsid into the nucleus of infected cells. Then, rcDNA is remodelled by host factors into a cccDNA minichromosome and serves as a template for the transcription of all HBV viral transcripts including HBsAg.[17] Interestingly, cccDNA is not the only source

[1]*University Paris Diderot, Sorbonne Paris Cité, CRI, UMR 1149, Inserm, F-75018 Paris, France;*
[2]*Department of Hepatology, AP-HP Hôpital Beaujon, Clichy 92110, France;*
[3]*Hôpital Saint Louis, Saint Louis Research Institute, Paris, France;*
[4]*INSERM U976, Human Immunology, Physiopathology and Immunotherapy, Paris, France*

* Corresponding author. Address: Viral Hepatitis, INSERM, UMR 1149, Hôpital Beaujon, 100 Boulevard du General Leclerc, Clichy 92110, France. Tel.: +33 (0) 140875579, fax: +33 (0) 147309440.

E-mail address: tarik.asselah@aphp.fr (T. Asselah).

https://doi.org/10.1016/j.jhep.2020.04.013

of HBsAg production, as HBV integration into the host genome can lead to cancer development and HBsAg secretion.[18] Thus, it is possible to block HBV transcription (cccDNA) without affecting HBsAg quantification. HBsAg proteins are encoded by 2 viral transcripts, PreS1 and PreS2/S mRNA (2.4 and 2.1 kb), which are transcripts from 1 of the 4 overlapping open reading frames (ORFs) in the HBV genome.[19] L- and M-HBsAg proteins are produced respectively from PreS1 and PreS2/S mRNA. The presence of a second weak start codon on the PreS2/S mRNA leads to S-HBsAg translation.[20] HBsAg proteins differ in their N-terminus but share a common S domain with 4 putative transmembrane domains on their C-terminus. S-HBsAg is the smallest HBsAg with 227 amino acids and contains only the S domain. M-HBsAg contains on their N-terminus an additional domain, the PreS2 domain, with an extension of 55 amino acids. L-HBsAg proteins have the PreS2 and PreS1 domains at their N-terminus. Additionally, the N-terminus of M-HBsAg ends with an acetylate group and L-HBsAg with an myristate group at glycine 2.[21,22] Fig. 1 shows the different viral transcripts as well as the structure of the 3 HBsAg proteins. Myristoylation of L-HBsAg at the N-terminus is important for NTCP receptor attachment.[23,24] After their translation, HBsAg proteins accumulate in the endoplasmic reticulum (ER) where they form agglomerates via covalent disulfide bridges with different cysteines in their S domain.[25] Surface proteins are required for the capsid envelopment, except M-HBsAg which is not essential for virion morphogenesis and secretion.[26,27] L- and M-HBsAg can act as transcriptional activators and are able to activate some promoters.[28] S and PreS1 domains interact with HBcAg leading to virion secretion.[29] The excessive production of HBsAg leads to the production of non-infectious spherical and filamentous subviral particles (SVPs) and HBV infectious particles. Fig. 2 shows the various roles of HBsAg within the HBV replication cycle.

HBsAg detection and quantification

Circulating HBsAg levels may reflect cccDNA transcription and act as an additional marker of on-treatment efficacy.[30] The availability of standardised commercial assays has renewed interest in quantitative serum HBsAg as a biomarker to stratify the risk of disease progression, relapse, and predict treatment response.[31] Two assays are currently used in HBV diagnostics to detect epitopes in the "a" determinant region of HBsAg, a highly conformational, hydrophilic domain from positions 124 to 147.[32] The most widely used is the Architect HBsAg QT (Abbott Diagnostics) assay, an automated chemiluminescent microparticle immunoassay based on a calibration curve standardised by the World Health Organization.[33] It can measure, in 2 steps, HBsAg concentrations from 0.05 to 250 IU/ml with a sensitivity of 99.8% and a specificity of 95%. The other HBsAg quantification assay is the automated Roche Diagnostics Elecsys® HBsAg II screening assay, which is able to quantify HBsAg concentrations from 0.05 to 52,000 UI/ml with a high specificity (>99.8%).[34]

Recently, serum HBV pregenomic (pg)RNA (henceforth referred to as HBV RNA) has been proposed as a new biomarker for cccDNA,[35] especially in virally suppressed patients with low detectable HBV DNA under NA therapy. Methods for the quantitative detection of HBsAg have been widely used in antiviral efficacy prediction. However, many factors may make it difficult to use serum HBsAg as a surrogate for the transcriptional activity of cccDNA, including HBV DNA integration[13] and HBsAg retention related to long-term NA treatment.[36] Unlike HBsAg, HBV RNA is derived only from cccDNA, and its quantification is not affected by viral antigens or antibody complexes and therefore, it may more accurately reflect the transcriptional activity of cccDNA. A study of 291 treatment-naïve patients with CHB showed the "superiority" of serum HBV RNA compared to HBV DNA and HBsAg for differentiating the 'HBeAg-negative reactive' phase, as serum HBV RNA levels increased in case of reactivation.[37] Numerous studies showed a strong to moderate correlation between HBsAg and serum HBV RNA, except in HBeAg-negative patients, where the correlation was weak.[37,38]

Finally, serum hepatitis B core-related antigen (HBcrAg) is a surrogate marker of both intrahepatic cccDNA and its transcriptional activity.[39]

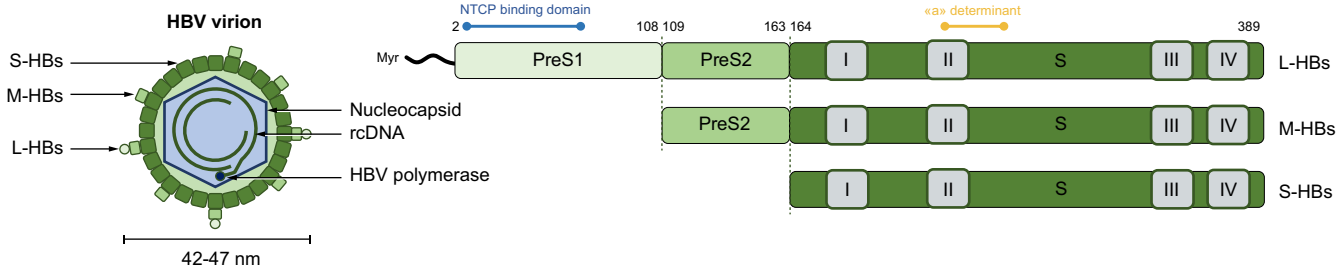

Fig. 1. Schematic model of Dane particle and HBsAg proteins. HBV genome is housed in a capsid structure formed by core (HBcAg) proteins surrounded by 3 different HBsAg, L-, M- and S-HBsAg. All HBsAg share the S domain which contains 4 putative transmembrane domains. M-HBsAg contains an additional PreS2 domain and L-HBsAg have both PreS1 and PreS2 domains. The NTCP binding domain is present in the PreS1 domain and is important for HBV infection. The "a" determinant is immunogenic in its S domain which is important for antibody neutralisation. NTCP, sodium taurocholate co-transporting polypeptide.

HBsAg seroclearance: prevalence and clinical significance

Spontaneous HBsAg seroclearance, defined as the loss of serum HBsAg on 2 occasions at least 6 months apart and remaining absent up to the last visit,[40] is a rare event in the natural history of CHB infection. Interestingly, HBsAg seroclearance is less common in cases of perinatally acquired HBV infection than in immune-competent adults who are infected later in life. HBsAg seroclearance is rarely observed when CHB is established, probably due to the induction of tolerance. In a systematic review and pooled meta-analysis, the annual incidence of HBsAg seroclearance was 1–2% worldwide.[41] Similarly, another meta-analysis found a low rate of HBsAg seroclearance in untreated and treated patients (pooled annual rate approximately 1%).[42]

The natural course of CHB infection is described by 5 distinct phases.[3–5] First, the "HBeAg-positive chronic HBV infection" phase consisting of high HBV DNA levels, HBeAg positivity, and normal alanine aminotransferase (ALT) levels. The second phase "HBeAg-positive chronic hepatitis B" is characterised by high levels of HBV DNA and ALT and moderate to severe liver necroinflammation. Most patients can achieve HBeAg seroconversion and enter the third phase, "HBeAg-negative chronic HBV infection", with positive anti-HBe, undetectable/low (<2,000 IU/ml) HBV DNA levels and normal ALT levels.[2] Other patients can progress to the fourth phase, "HBeAg-negative chronic hepatitis B" (reactivation), characterised by moderate to high levels of serum HBV DNA and ALT. The fifth phase, the HBsAg-negative phase is characterised by serum negative HBsAg with or without anti-HBs.

Of note, spontaneous HBsAg loss occurred rarely[40,43] and mainly in inactive carriers[43–45] usually more than 10 years after they had entered inactive phase.[46] Interestingly, HBsAg seroclearance was associated with a lower baseline HBV DNA level (6.61 $\log_{10}$ IU/ml *vs.* 7.71 $\log_{10}$ IU/ml) and a lower baseline HBsAg level (2.74 $\log_{10}$ IU/ml *vs.* 3.90 $\log_{10}$ IU/ml). HBsAg seroclearance was not associated with gender, HBV genotype or treatment history. Heterogeneity was substantial across the studies. HBsAg seroclearance is associated with a reduced risk of HCC compared with patients who are HBsAg-persistent carriers.[47] In a population-based prospective study, 1–2% (8/652) of participants with HBsAg seroclearance developed HCC over a 9-year surveillance programme.[47] The cumulative HCC incidence rate was significantly lower in patients with HBsAg seroclearance than in HBsAg-persistent carriers but was slightly higher than in HBV-uninfected controls. In another study, among 20,263 patients with CHB, those with NA-induced HBsAg seroclearance with complete viral suppression had a lower risk of HCC (but not hepatic events) than those only achieving complete viral suppression with long-term NA treatment.[48]

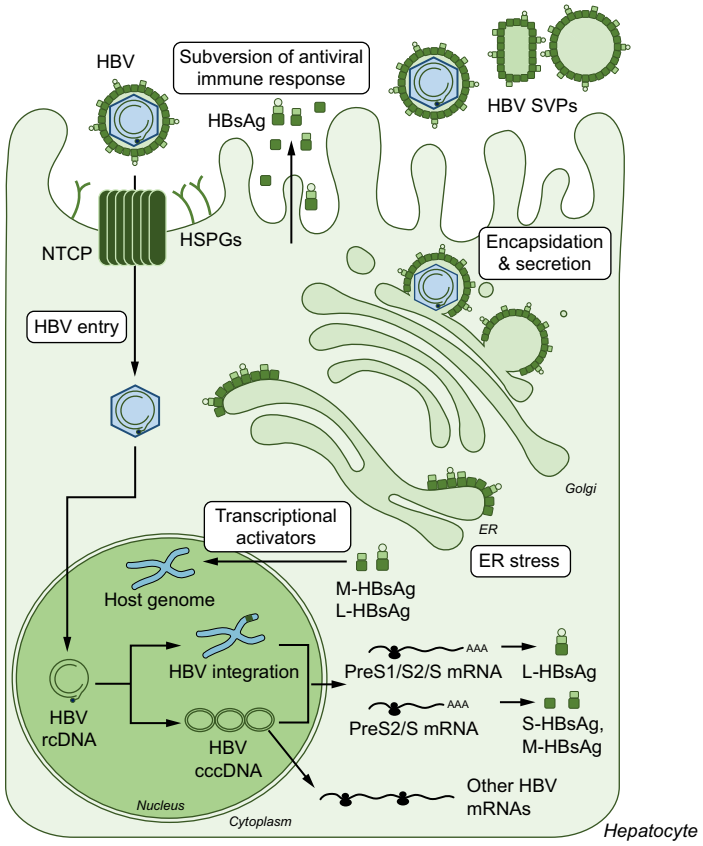

Fig. 2. HBV replication cycle and main effects in infected hepatocytes. HBsAg is produced from both cccDNA transcripts and HBV integration in the host genome. M- and L-HBsAg can act as transcriptional activators for host genes. HBsAg proteins are accumulated in ER and can induce the activation of cellular stress pathways. HBsAg plays a crucial role in HBV encapsidation and virions secretion. Secreted HBsAg can subvert the antiviral immune response. cccDNA, covalently closed circular DNA; ER, endoplasmic reticulum; HSPG, heparan sulphate proteoglycan; L-HBsAg, large HBsAg; M-HBsAg, medium HBsAg; NTCP, sodium taurocholate co-transporting polypeptide; rcDNA, relaxed circular DNA; S-HBsAg, small HBsAg; SVP, sub-viral particle.

Key point

HBsAg seroclearance is a rare event in the natural history of CHB.

Immune cells and HBsAg

In CHB, liver necroinflammation which is the driver for fibrosis, is affected by a dynamic imbalance of HBV, liver cells, and the host's immune system. Although many useful immunological insights into HBV pathogenesis have been made by studying peripheral blood, a large proportion of relevant responses are enriched in the liver as tissue-resident immune subsets play vital roles in front-line immunosurveillance in the liver and other organs. Liver biopsies and fine needle aspiration have enabled the identification of tissue- and liver-resident immune cells, not seen in the peripheral blood, including HBV-specific programmed cell death protein 1 $(PD\text{-}1)^{hi}$ T cells and natural killer (NK) cells, together with PD-L1 (or CD274)-expressing hepatocytes. A preferential accumulation of $PD\text{-}1^{hi}$ $Tbet^{hi}$ atypical memory B cells

Key point

HBsAg seroclearance is associated with a reduced risk of HCC.

(atMBCs) in the liver was shown compared to the peripheral blood.[12] The liver is also enriched with several innate-like populations such as mucosal-associated invariant T cells and liver sinusoidal endothelial cells.

HBV particles can inhibit innate immune responses in hepatocytes, leading to decreased expression of antiviral cytokines.[49] HBsAg is involved in immune evasion processes which are presented in Table 1. Many immune cells contribute to the immunopathogenesis of HBV infection, such as NK cells, cytotoxic T lymphocytes (CTLs), dendritic cells (DCs), memory and plasma B cells, and myeloid-derived suppressor cells (MDSCs), among others.[50–56] However, the immune mechanisms underlying HBsAg loss have not been studied in detail. Understanding the cellular basis of these immune interactions may help in the development of improved strategies for viral clearance. We will discuss the role of innate and adaptive immune cells in CHB, as well as reviewing their interactions and correlations with HBsAg (Table 1).

Innate immune cells

Kupffer cells

Kupffer cells (KCs) are the resident macrophages of the liver,[68] accounting for approximately 20% of liver parenchymal cells. Their most important function is the removal of toxins from the circulating blood, but KCs can also effectively remove viruses, bacteria, and other pathogens mostly via tumour necrosis factor-α (TNF-α), interleukin-1 (IL)-1, IL-6, oxygen free radicals and the inflammasome. HBV and HBsAg can abrogate absent in melanoma 2 (AIM2) inflammasome responses by deregulating IRF7 (interferon regulatory factor 7) expression and binding on the *AIM2* promoter in human KCs.[57]

Furthermore, KCs directly interacted with HBsAg *in vivo* and *in vitro*[58] which induced the production of pro-inflammatory cytokines, such as TNF-α, IL-6, and CXCL8 (C-X-C motif chemokine ligand 8).

HBsAg-induced cytokine production by KCs and monocyte-derived macrophages and subsequent NK cell activation may be an early event in viral containment, potentially supporting the induction of HBV-specific immunity upon HBV infection.

Dendritic cells

DCs are crucial immune sentinels which orchestrate antiviral immunity. They can detect viruses and their components through multiple pattern recognition receptors (PRRs). DCs then produce large quantities of antiviral cytokines, especially type I and type III IFNs, and cooperate with other immune effectors, as well as performing cross-presentation and priming virus-specific cytotoxic T cells.[69] Myeloid or conventional dendritic cells (cDCs) that express Toll-like receptor (TLR)3, TLR4 and TLR8 can be distinguished from plasmacytoid dendritic cells (pDCs) which express mainly TLR7 and TLR9.[70–72] In CHB infection, functional perturbations in DCs have been described in numerous studies.[51,60–63,73,74] Moreover, two recent studies showed that HBsAg can affect the maturation of DCs[75] and that HBV subverts DCs in both the blood and liver.[76] A study on the peripheral blood mononuclear cells of patients undergoing HBsAg seroclearance showed increased DC frequencies with enhanced expression of TLRs, as well as increased $CD8^+$ T cell and plasma B cell frequencies, suggesting that DCs may play a crucial role in HBsAg seroclearance.[77]

NK cells

NK cells are important immune lymphocytes in the liver, accounting for approximately one-third of intrahepatic lymphocytes.[78] NK cell receptors have activating or inhibitory properties upon engagement by molecules on the surface of target cells.

Table 1. HBsAg effects on immune cells.

Immune cell type	HBsAg effects	Reference(s)
Kupffer cell	Directly interacts with KCs *in vivo* and *in vitro* ↓ the AIM2-inflammasome and ↓ the production of IL-1β	57,58
Monocyte/macrophage	↓ TLR2 and c-Jun N-terminal protein kinase (JNK), thus ↓ production of IL-12	59
mDC	↓ frequency and function	60,61
pDC	↓ TLR9-mediated activation and IFN-α production	51,52,62,63
PBMC	↓ the interaction of MVP with MyD88 in infected cells, thus ↓ type I IFN responses	56
NK cell	↓ cytolytic activity	51
T cell	Exhaustion of CD4+ and CD8+ T cells by ↑ of PD-1 and Lag-3 expression	64,65
B cell	↓ TLR9 expression and B cell related functions	66
Monocyte/MDSC	↑ the differentiation and ↑ the expansion of monocytes into MDSCs	67

AIM2, absent in melanoma 2; IFN, interferon; IL-, interleukin; KC, Kupffer cell; LAG-3, lymphocyte-activation gene 3; mDC, myeloid dendritic cell; MDSC, myeloid-derived suppressor cell; MVP, major vault protein; Myd88, Myeloid differentiation primary response 88; NK cell, natural killer cell; PBMC, peripheral blood mononuclear cell; PD-1, programmed cell death protein 1; pDC, plasmacytoid dendritic cell; TLR, Toll-like receptor.

The balance between these signals controls immediate effector functions: cytotoxicity and IFNγ secretion. They have an important role in the defence against intracellular pathogens and tumours[79] and they play a crucial antiviral role during HBV infection.[80–82] During CHB, NK cells have been shown to express an inhibitory phenotype with blunt functional responses[83] and to mediate virus-specific CD8$^+$ T cell depletion through a death receptor pathway.[84] Furthermore, HBV can generate suppressive monocytes, which initiate regulatory NK cell differentiation resulting in T cell inhibition.[85] Changes in the NK cell phenotype may predict efficient immune reconstitution before anti-HBsAg seroconversion. Furthermore, induction of the proliferation and expansion of CD56 bright NK cell numbers in peripheral blood and increased cytotoxicity and IFNγ expression were associated with decreased serum HBsAg levels.[86–88] Increased NK cell function is associated with active hepatitis and HBsAg seroclearance following structured NA cessation.[89] However, the role of NK cells in the process of HBsAg loss is still unknown.

Adaptive immune cells

Cytotoxic CD8$^+$ T cells

HBV-specific CD8$^+$ T cell responses have been suggested to play an important role in viral clearance. In contrast to CD4$^+$ T cells, CD8$^+$ T cell responses have been studied more widely. The presence of strong and multiple CTL responses in the peripheral blood of patients with acute hepatitis B reinforced this concept,[90,91] while such responses were barely detectable in the peripheral blood of patients with CHB.[92] T cell exhaustion has been documented in the literature: CTLs are unable to mediate complete eradication of the virus, and they subsequently recruit HBV-non-specific inflammatory cells, including bystander T cells, NK cells, and neutrophils, that inevitably cause the immunopathology of CHB.[93] The low clearance rate of HBsAg is possibly related to a weak CTL response to HBsAg. CD8$^+$ T cell exhaustion in chronic HBV infection mirrors that described in other chronic viral infections in mice and humans, with the sustained expression of inhibitory receptors, such as PD-1, TIM-3 (T cell immunoglobulin and mucin-domain containing-3) and CD244 (2B4), reduced proliferative capacity and poor effector functions (reduced IFNγ and IL-2 secretion).[64,94] Inhibition of indoleamine 2,3-dioxygenase activity enhances the HBsAg-specific Tc1 immune response and induction of CTLs after immunisation with HBsAg, α-GalCer, IL-2, and IL-12β, which are vital cytokines for inducing the antigen-specific Tc1 response.[95,96] A genome-wide expression profiling of exhausted HBV-specific CD8$^+$ T cells from patients with CHB revealed an extensively downregulated gene-expression programme when compared to functionally competent CD8$^+$ T cells from patients who spontaneously resolved infection.[97] Among the various dysregulated processes, mitochondrial function seemed to be extensively defective, and its restoration by mitochondria-targeted antioxidants elicited functional T cell reconstitution. These results may lead to novel strategies for improving HBsAg clearance.

Key point

HBsAg contributes to the deregulation of both innate and adaptive immune cells.

Th CD4$^+$ cells

Virus-specific CD4$^+$ T cells are key regulators of both efficient B cell/antibody and CD8$^+$ T cell responses.[98,99] However, in CHB infection, T cell responses are described as hardly detectable and display a functionally exhausted phenotype.[65,100] T cells of patients with subsequent HBsAg loss, following cessation of NA treatment, had a less exhausted and more activated phenotype, compared to patients with retained HBsAg.[101] T cells expressed low levels of PD-1 and KLRG1 (killer cell lectin like receptor G1). Furthermore, PD-1$^+$ CD8$^+$ T cells positively correlated with HBsAg levels at baseline. In HBV-infected mice and blood from patients with CHB, a T follicular helper (TFH)-cell response to HBsAg is required for HBV clearance, and blocking regulatory T (Treg) cell activity restored the ability of TFH cells to clear HBV infection.[102] Circulating PD-1^{hi} CXCR5$^+$ CD4$^+$ T cells were associated with decreased HBsAg levels in patients with CHB receiving PEG-IFNα therapy.[103] Indeed, HBV-specific IFNγ producing CD4$^+$ T cells are associated with viral clearance in patients with CHB and their frequency was positively correlated with the decrease of HBsAg.[104] Finally, in a cohort of 209 patients with CHB, there was a strong negative correlation between IL-4-secreting CD4$^+$ T cells and quantitative HBsAg levels.[105]

B cells

B cells are major contributors in the humoral immune response and have dual roles: being professional antigen-presenting cells, they recognise antigens and prime T cells. They also differentiate into memory cells and antibody-producing plasma cells which are responsible for the production of antibodies. B cells play a vital role during HBV infection by secreting anti-HBsAg, a sign of the resolution of the infection. Conversely, HBsAg could disrupt the mechanisms of innate and adaptive immunity and result in the suppression of immune responses against HBV.[66] However, while the quantity and function of HBV-specific T cells have been clearly defined in patients with CHB,[97,106] less attention has been paid to the role of neutralising anti-HBsAg and detailed characterisation of the anti-HBV-specific B cell response is lacking. In addition, B cell-depleting drugs, such as the anti-CD20 antibody rituximab, induce HBV reactivation in HBV carriers and even in patients with “resolved” HBV in whom nuclear HBV cccDNA persists as a viral reservoir for decades.[107] B cells from patients with CHB had a reduced proliferative capacity and were incapable of

producing anti-HBsAg upon stimulation. This functional defect reverted after HBsAg loss.[54,108] Recently, two studies were able to characterise HBsAg-specific B cells. While B cells were present in similar frequencies in chronic and resolved infections, they were unable to mature into anti-HBsAg-secreting cells in CHB. Instead, they presented functional alterations that resemble atMBCs, with low expression of CD21 and CD27 and high expression of PD-1 and T-bet.[12,55] The function of these HBsAg-specific B cells can be partially restored *in vitro* by PD-1 blockade.[55] These results suggest that B cell dysfunction rather than antibody depletion is the main reason for the lack of anti-HBsAg; thus, B cells are a possible target for novel antiviral strategies.

Regulatory cells

Treg cells are a specialised subpopulation of T cells that act to suppress T cell proliferation and cytokine production, thereby maintaining homeostasis. HBsAg can enhance Treg cell activity and mDC costimulatory molecules and can suppress monocyte activation and pDC function.[109] Monocytic MDSCs are strong inhibitors of the T cell response.[110] A recent study found that HBsAg levels were positively correlated with monocytic MDSC frequency in patients with CHB and that HBsAg can maintain HBV persistence by increasing the differentiation of monocytes into monocytic MDSCs.[67] The polarisation of MDSCs by HBsAg can restrain the activation of T cells in CHB infection.[67] Despite these data, several key questions remain unanswered. The ideal would be to determine immune parameters associated with persistence, clearance and recurrence of HBV and to study the function and phenotype of both peripheral and intrahepatic lymphocyte populations, as well as hepatocytes, which may aid in the rational design of immunotherapeutic strategies.

Key point

The decrease of HBsAg can potentially restore anti-HBV immune responses.

Cytokines and HBsAg

Cytokines act as key coordinators of the inflammatory responses involved in HBV pathogenesis. IL-6 is produced by a variety of cells and is involved in many biological processes, including induction of cell differentiation, generation of B immunoglobulin, promotion of T cell proliferation. A recent study showed that IL-6 polymorphisms 572G/C and -597G/A are significantly associated with CHB risk.[111] Furthermore, Bouezzedine *et al.* show that IL-6 can strongly inhibit HBsAg secretion which was confirmed by observation of a severe reduction in cccDNA after IL-6 treatment.[112] IL-12 also has numerous roles: promoting the differentiation of Th1 cells, enhancing NK cell cytotoxicity and activation of IFNγ pathways, and rescuing the antiviral function of exhausted HBV-specific T cells.[113] Direct interaction of HBsAg with human monocytes and macrophages regulates the production of IL-12.[67]

TLRs and HBsAg

The early and non-specific detection of pathogens generally occurs at subcellular/molecular levels, via the recognition of pathogen-associated molecular patterns by innate immunity sensors, also called PRRs expressed in various types of epithelial/endothelial cells, as well as professional and non-professional immune cells.[114] Among PRRs, TLRs belong to a conserved family of transmembrane glycoprotein receptors capable of sensing a wide variety of pathogen- and damage-associated molecular patterns. Viral proteins have been suggested to interfere with innate signalling pathways in hepatocytes and immune cells. In fact, HBsAg is capable of impairing the activation of all TLR pathways.[115] HBsAg downregulates TLR9 in pDCs leading to the inhibition of IFNα production.[74,116] In B cells, HBsAg has been shown to downregulate TLR9 expression and function, leading to deficient TLR9-mediated B cell responses.[66] Moreover, HBsAg can selectively inhibit TLR2 ligand–induced IL-12 production in monocytes/macrophages by interfering with JNK (c-Jun N-terminal protein kinase) activation.[59] These data may suggest that strategies targeting HBsAg production or secretion may lead to the restoration of an efficient immune response in patients with CHB and therefore combination therapy should be considered. Fig. 3 represents the proposed immune mechanisms of HBsAg seroclearance, from decreased HBsAg levels to the potential restoration of anti-HBV immune responses.

HBsAg seroclearance in animal models

Animal models are important tools to explore mechanisms of HBV immunopathogenesis and to evaluate new therapies. For example, chimpanzees chronically infected with HBV were used to test the efficacy of GS-9620, a TLR7 agonist.[117] A 100-fold decline in viral load and a dramatic drop in the number of HBsAg-positive cells was observed during therapy. A phase II study with GS-9620, in virally suppressed patients on NAs showed evidence of immune activation but no significant decline in HBsAg.[118] Another study assessed the efficacy of ARC-520, an RNAi antiviral targeting HBV transcripts including HBsAg, in combination with NAs, and reported a profound reduction in HBsAg, with a maximum reduction of more than 2 logs.[18] Additionally, a novel long-acting modified IFNα (PASylated-IFNα) induced anti-HBsAg seroconversion in HBV-transgenic mice after 3 weeks.[119] Recently, GLP-26, a capsid assembly modulator, was tested in a humanised mouse model in combination with entecavir (ETV) and induced a decrease in viral load and viral antigens that was sustained for up to 12 weeks after treatment cessation.[120]

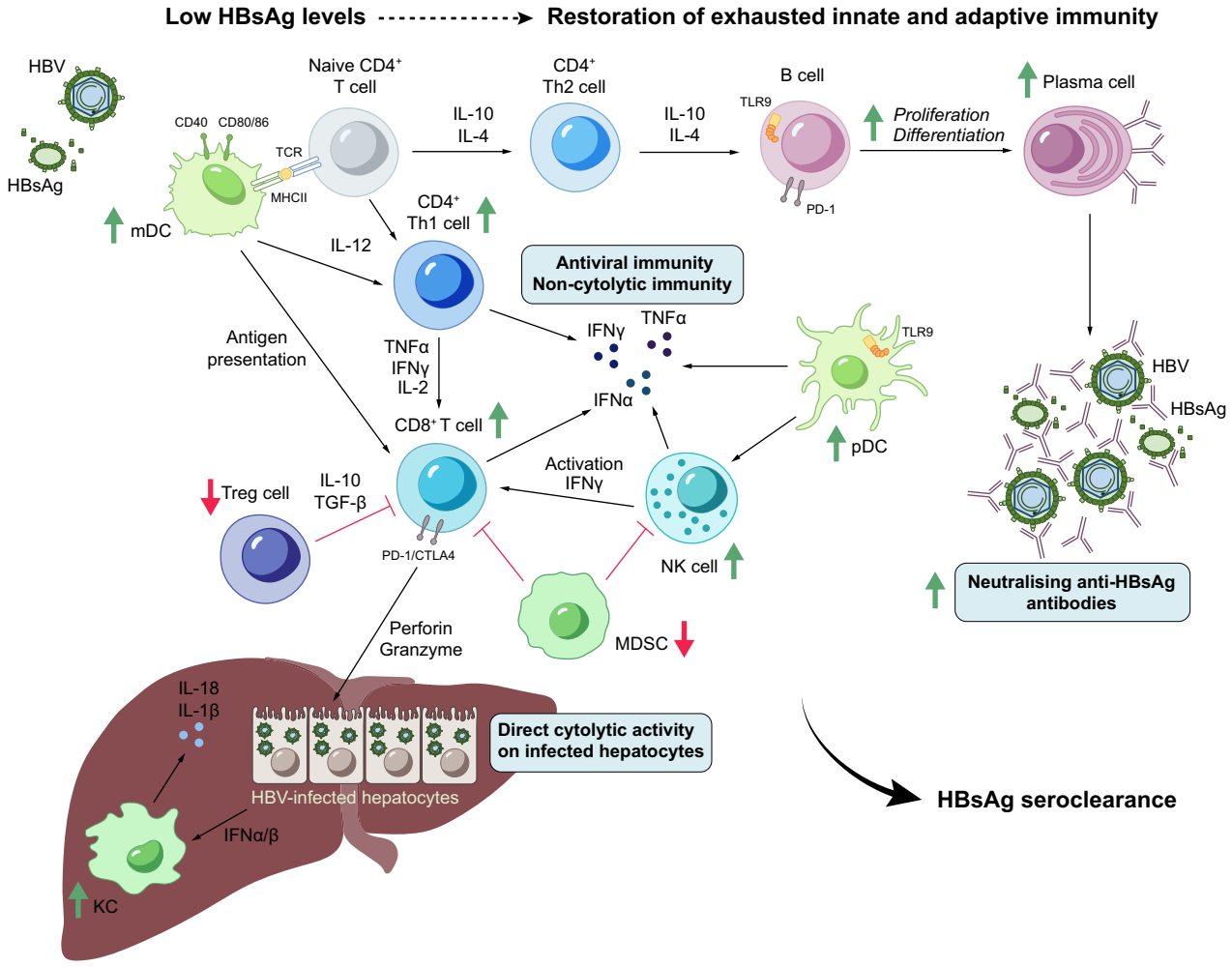

Fig. 3. Proposed immune mechanisms for HBsAg seroclearance. Decreased HBsAg levels could facilitate the recovery of the host's immune system. mDCs regain their antigen-presenting capacities to activate T cells, as well as TNFα secretion. pDCs restore TNFα and IFNγ secretion and activate NK cells. Restoration of NK cell effector functions: cytotoxicity and IFNγ secretion, activation of T cells. Recovery from T cell exhaustion: restored proliferation, increase in HBsAg-specific CTLs, direct cytolytic activity on infected hepatocytes and IFNs secretion. Suppression of excessive functions of Treg cells and MDSCs. KCs induce cytokine production, have restored inflammasome functions and activate NK cells. Restoration of functional HBsAg-specific plasmocytes secreting neutralising anti-HBsAg. Upward green arrows signify a restoration of function and/or frequency while downward red arrows signify a decrease in function and/or frequency. Anti-HBsAg, hepatitis B surface antigen antibodies; CTLA4, cytotoxic T lymphocyte-associated protein 4; IFN, interferon; IL-, interleukin; KC, Kupffer cell; mDC, myeloid dendritic cell; MDSC, myeloid-derived suppressor cell; NK cell, natural killer cell; PD-1, programmed cell death protein 1; pDC, plasmacytoid dendritic cell; TCR, T cell receptor; TGF-β, transforming growth factor-β; Th, T helper; TNF-α, tumour necrosis factor alpha; Treg, regulatory T.

HBsAg seroclearance and current therapies

The goal of treatment is to improve survival, by preventing the risk of end-stage liver disease and HCC, and to improve quality of life. However, it is difficult to demonstrate improvements in survival and surrogate markers are needed. HBsAg seroclearance is a surrogate marker of survival and is therefore the ideal endpoint for treatment. Current treatments include PEG-IFN, which have been shown to exert dual actions, including an immunomodulatory effect and minimal direct antiviral activity against HBV or NAs such as lamivudine, telbivudine, adefovir dipivoxil, ETV, tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF), which directly target the reverse transcription functions of the viral polymerase, and thus suppress HBV replication effectively, when used as monotherapy. Both approaches offer limited efficacy in achieving HBsAg loss.[121] PEG-IFN has the advantage of inducing sustained response after a defined course of treatment (usually 48 weeks), although response rates and tolerability are poor. NAs require life-long administration because they do not eliminate the persistent cccDNA within the infected hepatocytes. Among NAs, ETV, TDF and TAF are preferred as first choice therapy because of their high antiviral potency and the low risk of resistance. A significantly greater proportion of patients receiving TDF plus PEG-IFN for 48 weeks had HBsAg loss than those receiving TDF or PEG-IFN alone, and this combination is

suitable in a subset of patients.[122] However, the observed rate of HBsAg loss in the study was lower than that assumed in the study design, reducing the power for comparison between groups. Finally, prolonged follow-up of patients who had not restarted TDF treatment would be required to determine the long-term benefits of response and durability of outcome. Further studies are required to identify the optimal combination regimen that would allow more patients to achieve and sustain HBsAg loss.

HBsAg seroclearance during IFN therapy

A 48-week treatment with PEG-IFN has the potential to elicit immune control of HBV infection, leading to higher rates of HBeAg seroconversion (than achieved with NAs) and the possibility of viral suppression after stopping treatment, with HBsAg loss in a proportion of patients who maintain undetectable HBV DNA. After PEG-IFN, sustained off-therapy virological response (SVR) is defined as serum HBV DNA levels <2,000 IU/ml after the end of therapy. In HBeAg-negative patients with CHB, a phase III trial evaluating PEG-IFN monotherapy reported SVR rates of 44% at 6 months and 28% at 3 years after the end of therapy.[123] In HBeAg-negative patients with genotype D or E, PEG-IFN was less effective with an SVR of around 20%. The rate of HBsAg loss progressively increased after PEG-IFNα discontinuation, from 3% at month 6, to 9% at year 3, and to 12% at year 5 in the registrational trial. Overall, among sustained responders, approximately 30% clear HBsAg in the long-term.

Key point

Combination of antivirals and immune therapy is crucial for drug development.

HBsAg loss rates increase after the end of PEG-IFNα therapy in initially HBeAg-positive patients with SVR.[124] Of the patients with an initial HBeAg seroclearance, 30% experienced HBsAg seroclearance after 3 years of follow-up. The sustainability of HBsAg loss and seroconversion after PEG-IFNα have been documented.[122]

HBsAg loss was evaluated in patients receiving the combination of TDF and PEG-IFNα-2a for a finite duration in a randomised trial, and compared to TDF monotherapy and PEG-IFN monotherapy.[122] A total of 740 patients with CHB were randomly assigned to receive TDF plus PEG-IFN for 48 weeks, TDF plus PEG-IFN for 16 weeks followed by TDF for 32 weeks, TDF for 120 weeks, or PEG-IFN for 48 weeks. At week 72, 9% of patients in the group receiving TDF plus PEG-IFN for 48 weeks had HBsAg loss compared with less than 3% in the other groups.

HBsAg seroclearance during NA therapy

ETV and TDF are potent HBV inhibitors with a high barrier to resistance and should be used as first-line monotherapies.[125] More than 95% of patients treated with the highly potent TDF and ETV achieve virological undetectability. NAs are administered orally, tolerability is favourable and efficacy is good.[126] In a mainly Caucasian population of HBeAg-positive patients, HBsAg loss was around 10% after 5 years of TDF and was more likely to occur in Caucasian patients.[127] No HBsAg loss was observed after 2 years of TDF or ETV. Table 2 represents the spontaneous and after treatment HBsAg seroclearance rates reported to date (Table 2).

HBsAg decrease as a predictor of treatment response

To identify responders in the early phase of PEG-IFNα-2a therapy, decreased serum quantitative HBsAg is a validated on-treatment marker predicting sustained off-treatment response.[138] In a proof of concept study of 48 HBeAg-negative patients receiving PEG-IFNα-2a, a decrease of 0.5 and 1 $\log_{10}$ IU/ml of serum HBsAg levels at weeks 12 and 24 of therapy had a 90% negative predictive value and a 89% positive predictive value for week 12 and 97% negative predictive value and a 92% positive predictive value for sustained response at week 24, respectively.[139] This was the first study to suggest that the early kinetics (week 12) of HBsAg might differentiate sustained responders from non-responders to PEG-IFN.

The role of HBsAg in NA cessation

Interestingly, in a retrospective study, Chen *et al.* evaluated the role of HBsAg quantification in predicting HBsAg loss and HBV relapse.[140] End of treatment HBsAg levels of <300 IU/ml, 300–1,000 IU/ml and >1000 UI/ml in HBeAg-positive patients were associated with sustained HBeAg loss in 55.6%, 7.7% and 3.3%, respectively. End of treatment

Table 2. Spontaneous and after treatment HBsAg seroclearance.

Mode	Treatment	Therapy duration (weeks)	Follow-up duration (weeks)	Number of participants (n)	HBsAg seroclearance (%)	Reference(s)
Spontaneous	n.a.	n.a.	260–520	1,965–42,588	4.03–8.1	8,41
Under current treatment (monotherapy)	ETV	52–260	52–260	146–709	1.4–5.1	128–130
	TDF	48–260	48–260	266–585	3–11	127,131,132
	TAF	96	96	576	1	133
	PEG-IFN	48–52	48–52	136–177	4–7	123,134
Combination therapy	ETV+TDF	92–96	96	57–197	1.7–3.6	135,136
	PEG-IFN+ETV	48	48	85	1.17	137
	PEG-IFN+TDF	48	72	186	9.1	122

ETV, entecavir; n.a., not applicable; PEG-IFN, pegylated interferon; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

HBsAg cut-offs <120 IU/ml, 120–1,000 IU/ml and >1,000 IU/ml in HBeAg-negative patients were associated with HBsAg loss in 79.2%, 14.3% and 0%, respectively, and HBsAg cut-offs <200 IU/ml, 200–1,000 IU/ml and >1,000 IU/ml were associated with an SVR in 93%, 11.1% and 15.4%, respectively. Similar results were reported in patients whose treatment was discontinued; an end of treatment HBsAg level <100 IU/ml was associated with high SVR, while >1,000 IU/ml was associated with a 1-year post-treatment relapse in 70%.[141] A systemic review to summarise the role of HBsAg in NA cessation among Asian patients with CHB showed that HBsAg loss ranged between 21.1–58-8% in patients with HBsAg <100 IU/ml, compared to 3.3–7.4% in patients with HBsAg >100 IU/ml.[142]

HBsAg seroclearance and novel therapies

Several strategies, including antivirals targeting various stages of the HBV replication cycle (HBV entry, viral replication, cccDNA production, and viral protein expression), as well as immunotherapeutic agents, are being explored in experimental models or have reached clinical testing, which may have the potential to complement PEG-IFN- and NA-based therapy.[17,143–145] Strategies inhibiting viral gene expression either through cccDNA transcription or viral mRNA translation can decrease serum HBsAg levels. Nucleic acid-based polymers (NAPs) are a new class of broad-spectrum antiviral compounds which act against HBV infection by blocking the release of HBsAg from infected hepatocytes.[146] This pharmacological activity blocks replenishment of HBsAg in the circulation, enabling host-mediated clearance and has important clinical significance as it may potentiate the ability of immunotherapies (resumed in Fig. 4) to restore functional control of HBV infection. Removal of HBsAg would enhance the effect of PEG-IFNα-2a and could lead to favourable immunological activation, the appearance of free anti-HBsAg and the clearance of HBV virions in the blood. Among HBsAg-targeting antiviral strategies (Fig. 5), NAPs are being investigated in preclinical evaluations and in several clinical trials that have evaluated the activity of REP 2139, REP 2055 and REP 2165 in monotherapy and in combination with immunotherapy.[146–149] Out of 12 patients, a substantial reduction of HBsAg and seroconversion to anti-HBsAg was observed in response to REP 2139-Ca in 9 patients (NCT02646189).[147] Recently, an open label, randomised, controlled, phase II study

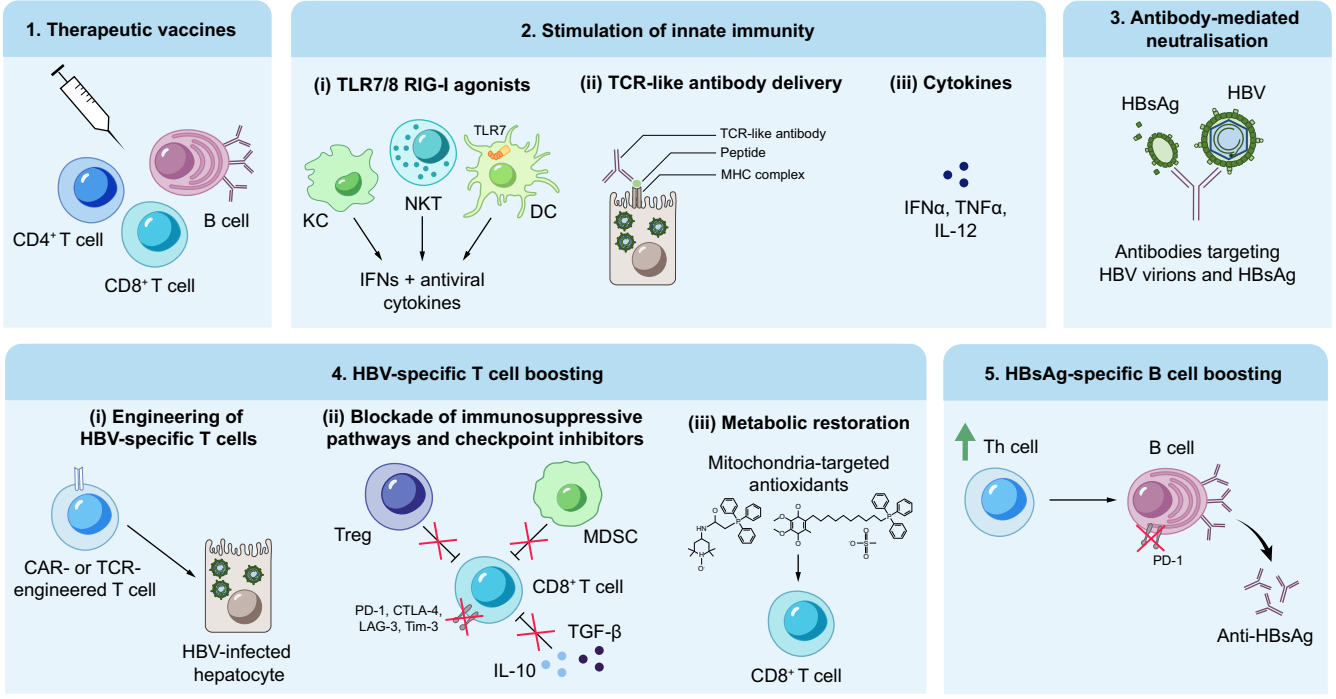

Fig. 4. Developed immune-based approaches to clear HBV. (1) Therapeutic vaccination could restore dysfunctional T and B cell responses during CHB. (2) Stimulation of innate immunity by (i) TLRs and RIG-I agonists leading to the activation of hepatocytes, intrahepatic dendritic, NK and mucosal-associated invariant T cells. (ii) TCR-like antibodies allow direct recognition of HBV-infected hepatocytes. (iii) Cytokines inhibit HBV replication. (3) Antibody-mediated neutralisation could prevent HBV infection of hepatocytes and reduce HBsAg circulating levels. (4) Anti-HBV T cell boosting by (i) T cell engineering. (ii) and (iii) Checkpoint blockade, modulation of regulatory cells and metabolic modulation. (5) Functional maturation of dysfunctional HBsAg-specific B cells by boosting Th cells or anti-PD-1 therapy. CTLA4, cytotoxic T lymphocyte-associated protein 4; DC, dendritic cell; IFN, interferon; IL-, interleukin; KC, Kupffer cell; LAG-3, lymphocyte-activation gene 3; NKT cell, natural killer T cell; PD-1, programmed cell death protein 1; RIG-1, retinoic acid-inducible gene 1; TCR, T cell receptor; TGF-β, transforming growth factor-β; Th, T helper; Tim-3, T cell immunoglobulin and mucin-domain containing-3; TNF-α, tumour necrosis factor alpha; Treg, regulatory T.

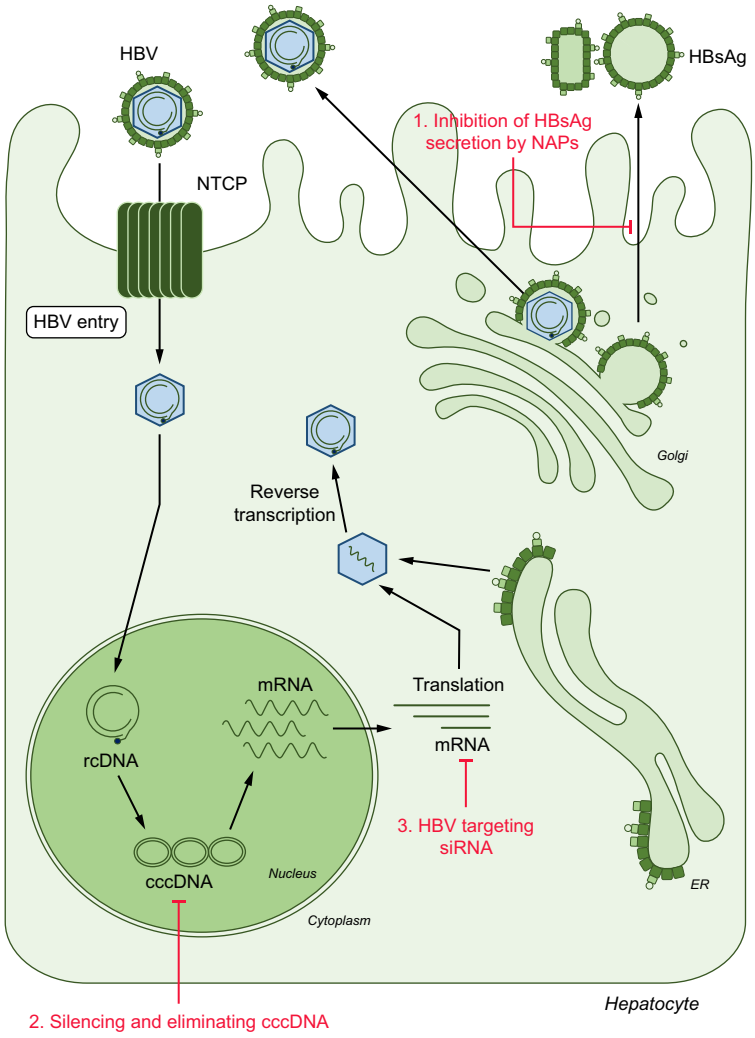

Fig. 5. HBsAg-targeting antiviral approaches. Schematic representation of HBsAg-targeting strategies. HBV replication cycle is shown, and different approaches are developed to reduce high levels of HBsAg in patients with CHB. (1) Inhibition of HBsAg release by NAPs. (2) Silencing and eliminating cccDNA. Targets against cccDNA include antiviral cytokines (PEG-IFN), blockade of rcDNA and epigenetic regulation to undergo its degradation. Technologies such as CRISPR/Cas9 are being utilised to eliminate cccDNA along with the use of histone deacetylase inhibitors. (3) Suppression of HBsAg expression by HBV-targeting siRNA. cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; ER, endoplasmic reticulum; NAPs, nucleic acid polymers; NTCP, sodium taurocholate co-transporting polypeptide; PEG-IFN, pegylated interferon; rcDNA, relaxed circular DNA; siRNA, small interfering RNA.

reported on the use of 2 different NAPs, REP 2139-Mg or REP 2165-Mg in association with PEG-IFN and TDF in 40 patients with CHB. No severe adverse events were reported. Efficacy was impressive with around half of the patients achieving HBsAg seroconversion.[149] These interesting results need to be confirmed in larger studies. Furthermore, the potential for cytotoxicity resulting from intracellular retention of HBsAg should be investigated and the problem of NAPs accumulating in the liver should be addressed.

Conclusion

HBsAg seroclearance is a rare event in the natural history of HBV and it is associated with reduced risk of HCC. Current antiviral therapies using PEG-IFN and NAs can suppress HBV replication and improve the prognosis of CHB, but they fail to clear HBsAg. Several guidelines proposed HBsAg seroclearance as a crucial surrogate marker of thorough HBV clearance. HBsAg may contribute to the impairment of innate and adaptive immunity and the exhaustion of T cell and B cell responses. Therefore, a reduced serum HBsAg level could facilitate the recovery of the host's immune system. Numerous immune cells have been shown to interact with HBsAg, contributing to the immunopathogenesis of CHB, but the immune mechanisms underlying HBsAg seroclearance are still unclear. A better understanding of the interaction between HBsAg and these immune factors could contribute to the development of effective immunotherapies.

The presence of cccDNA and integrated DNA on one hand, and the particular liver microenvironment and immune deregulations induced by chronic infection on another hand, are key challenges for antiviral approaches.[144] Overall, the safety and efficacy of the newly developed strategies, whether antivirals or immune based, need to be tested, and their ability to remove HBsAg needs to be investigated. Strategies focusing on reducing HBsAg by siRNA, NAPs or antibody-mediated neutralisation could be crucial in the restoration of effective immune responses. Developing effective combination therapies that target HBsAg may further induce the appearance of an anti-HBV-specific immune response and lead to a functional cure for CHB.

Abbreviations

ALT, alanine aminotransferase; anti-HBsAg, hepatitis B surface antigen antibodies; AST, aspartate aminotransferase; AIM2, absent in melanoma 2; atMBC, atypical memory B cells; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; CTL, cytotoxic T cell; CTLA4, cytotoxic T lymphocyte-associated protein 4; DCs, dendritic cells; ETV, entecavir; HCC, hepatocellular carcinoma; hNTCP, human sodium taurocholate co-transporting polypeptide; HSPG, heparan sulphate proteoglycan; IFN, interferon-; IL-, interleukin-; KC, Kupffer cells; LAG-3, lymphocyte-activation gene 3; L-HBsAg, large HBsAg; mDC, myeloid dendritic cell; MDSCs, myeloid-derived suppressor cells; M-HBsAg, medium HBsAg; MVP, major vault protein; MyD88, myeloid differentiation primary response 88; NA, nucleoside analogue; NAP, nucleic acid polymer; NK cells, natural killer cells; ORF, open reading frames; PBMCs, peripheral blood mononuclear cells; PD-1, programmed cell death protein 1; pDC, plasmacytoid dendritic cell; PEG-IFN, pegylated-interferon; pgRNA, pregenomic RNA; PRRs, pattern recognition receptors; rcDNA, relaxed circular DNA; RIG-1, retinoic acid-inducible gene 1; S-HBsAg, small HBsAg; siRNA, small interfering RNA; SVPs, sub-viral particles; TAF, tenofovir

alafenamide; T-bet, T-box protein expressed in T cells; TCR, T cell receptor; TDF, tenofovir disoproxil fumarate; TFH, T follicular helper cell; TFV, tenofovir; TGF-β, transforming growth factor-β; Th, T helper; Tim-3, T cell immunoglobulin and mucin-domain containing-3; TLR, toll-like receptor; TNF-α, tumour necrosis factor alpha; Treg, regulatory T.

Financial support
The authors received no financial support to produce this manuscript.

Conflict of interest
Tarik Asselah has acted as a speaker and investigator for Janssen, Gilead, Roche, and Merck. Nathalie Boyer has acted as a speaker and investigator for Janssen, Gilead, Roche and Merck. Issam Tout, Dimitri Loureiro, Abdellah Mansouri and Vassili Soumelis declare no competing interests.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions
TA designed and supervised the manuscript. IT and TA prepared the manuscript. All the authors contributed to the drafting of the review, the critical revision of the manuscript and the final approval of the version.

Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2020.04.013.

References
[1] World Health Organization, Global Hepatitis Programme. Global Hepatitis Report, 2017. Geneva: World Health Organization; 2017.
[2] Liaw Y-F, Chu C-M. Hepatitis B virus infection. Lancet 2009;373:582–592.
[3] European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu, European Association for the Study of the Liver. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67:370–398.
[4] Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HLY, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 2016;10:1–98.
[5] Terrault NA, Lok ASF, McMahon BJ, Chang K-M, Hwang JP, Jonas MM, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 2018;67:1560–1599.
[6] Ahn SH, Park YN, Park JY, Chang H-Y, Lee JM, Shin JE, et al. Long-term clinical and histological outcomes in patients with spontaneous hepatitis B surface antigen seroclearance. J Hepatol 2005;42:188–194.
[7] Yuen M-F, Wong DK-H, Fung J, Ip P, But D, Hung I, et al. HBsAg seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology 2008;135:1192–1199.
[8] Chu C-M, Liaw Y-F. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. Hepatology 2007;45:1187–1192.
[9] Kim G-A, Lim Y-S, An J, Lee D, Shim JH, Kim KM, et al. HBsAg seroclearance after nucleoside analogue therapy in patients with chronic hepatitis B: clinical outcomes and durability. Gut 2014;63:1325–1332.
[10] Liu J, Yang H-I, Lee M-H, Lu S-N, Jen C-L, Wang L-Y, et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community-based follow-up study. Gastroenterology 2010;139:474–482.
[11] Park J-J, Wong DK, Wahed AS, Lee WM, Feld JJ, Terrault N, et al. Hepatitis B virus–specific and global T-cell dysfunction in chronic hepatitis B. Gastroenterology 2016;150:684–695.e5.
[12] Burton AR, Pallett LJ, McCoy LE, Suveizdyte K, Amin OE, Swadling L, et al. Circulating and intrahepatic antiviral B cells are defective in hepatitis B. J Clin Invest 2018;128:4588–4603.
[13] Seeger C, Mason WS. Molecular biology of hepatitis B virus infection. Virology 2015;0:672–686.
[14] Glebe D, Urban S, Knoop EV, Cag N, Krass P, Grün S, et al. Mapping of the hepatitis B virus attachment site by use of infection-inhibiting preS1 lipopeptides and tupaia hepatocytes. Gastroenterology 2005;129:234–245.
[15] Blanchet M, Sureau C. Infectivity determinants of the hepatitis B virus pre-S domain are confined to the N-terminal 75 amino acid residues. J Virol 2007;81:5841–5849.
[16] Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. ELife 2012;1:e00049.
[17] Asselah T, Loureiro D, Boyer N, Mansouri A. Targets and future direct-acting antiviral approaches to achieve hepatitis B virus cure. Lancet Gastroenterol Hepatol 2019;4:883–892.
[18] Wooddell CI, Yuen M-F, Chan HL-Y, Gish RG, Locarnini SA, Chavez D, et al. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. Sci Transl Med 2017;9:eaan0241.
[19] Heermann KH, Goldmann U, Schwartz W, Seyffarth T, Baumgarten H, Gerlich WH. Large surface proteins of hepatitis B virus containing the pre-s sequence. J Virol 1984;52:396–402.
[20] Cattaneo R, Will H, Hernandez N, Schaller H. Signals regulating hepatitis B surface antigen transcription. Nature 1983;305:336–338.
[21] Schmitt S, Glebe D, Alving K, Tolle TK, Linder M, Geyer H, et al. Analysis of the pre-S2 N- and O-linked glycans of the M surface protein from human hepatitis B virus. J Biol Chem 1999;274:11945–11957.
[22] Persing DH, Varmus HE, Ganem D. The preS1 protein of hepatitis B virus is acylated at its amino terminus with myristic acid. J Virol 1987;61:1672–1677.
[23] Meier A, Mehrle S, Weiss TS, Mier W, Urban S. Myristoylated PreS1-domain of the hepatitis B virus L-protein mediates specific binding to differentiated hepatocytes. Hepatology 2013;58:31–42.
[24] De Falco S, Ruvo M, Verdoliva A, Scarallo A, Raimondo D, Raucci A, et al. N-terminal myristylation of HBV preS1 domain enhances receptor recognition. J Pept Res 2001;57:390–400.
[25] Mangold CM, Unckell F, Werr M, Streeck RE. Secretion and antigenicity of hepatitis B virus small envelope proteins lacking cysteines in the major antigenic region. Virology 1995;211:535–543.
[26] Ueda K, Tsurimoto T, Matsubara K. Three envelope proteins of hepatitis B virus: large S, middle S, and major S proteins needed for the formation of Dane particles. J Virol 1991;65:3521–3529.
[27] Fernholz D, Stemler M, Brunetto M, Bonino F, Will H. Replicating and virion secreting hepatitis B mutant virus unable to produce preS2 protein. J Hepatol 1991;13(Suppl 4):S102–S104.
[28] Hildt E, Saher G, Bruss V, Hofschneider PH. The hepatitis B virus large surface protein (LHBs) is a transcriptional activator. Virology 1996;225:235–239.
[29] Poisson F, Severac A, Hourioux C, Goudeau A, Roingeard P. Both pre-S1 and S domains of hepatitis B virus envelope proteins interact with the core particle. Virology 1997;228:115–120.
[30] Chan HL-Y, Wong VW-S, Tse AM-L, Tse C-H, Chim AM-L, Chan H-Y, et al. Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. Clin Gastroenterol Hepatol 2007;5:1462–1468.
[31] Martinot-Peignoux M, Asselah T, Marcellin P. HBsAg quantification to optimize treatment monitoring in chronic hepatitis B patients. Liver Int 2015;35(Suppl 1):82–90.
[32] Wu C, Deng W, Deng L, Cao L, Qin B, Li S, et al. Amino acid substitutions at positions 122 and 145 of hepatitis B virus surface antigen (HBsAg) determine the antigenicity and immunogenicity of HBsAg and influence in vivo HBsAg clearance. J Virol 2012;86:4658–4669.
[33] Deguchi M, Yamashita N, Kagita M, Asari S, Iwatani Y, Tsuchida T, et al. Quantitation of hepatitis B surface antigen by an automated chemiluminescent microparticle immunoassay. J Virol Methods 2004;115:217–222.

[34] Mühlbacher A, Weber B, Bürgisser P, Eiras A, Cabrera J, Louisirirotchanakul S, et al. Multicenter study of a new fully automated HBsAg screening assay with enhanced sensitivity for the detection of HBV mutants. Med Microbiol Immunol 2008;197:55–64.
[35] Wang J, Shen T, Huang X, Kumar GR, Chen X, Zeng Z, et al. Serum hepatitis B virus RNA is encapsidated pregenome RNA that may be associated with persistence of viral infection and rebound. J Hepatol 2016;65:700–710.
[36] Warner N, Locarnini S. Can antiviral therapy for chronic hepatitis B enhance the progression to hepatocellular carcinoma? Antivir Ther 2009;14:139–142.
[37] Liu Y, Jiang M, Xue J, Yan H, Liang X. Serum HBV RNA quantification: useful for monitoring natural history of chronic hepatitis B infection. BMC Gastroenterol 2019;19:53.
[38] Liu S, Zhou B, Valdes JD, Sun J, Guo H. Serum hepatitis B virus RNA: a new potential biomarker for chronic hepatitis B virus infection. Hepatology 2019;69:1816–1827.
[39] Testoni B, Lebossé F, Scholtes C, Berby F, Miaglia C, Subic M, et al. Serum hepatitis B core-related antigen (HBcrAg) correlates with covalently closed circular DNA transcriptional activity in chronic hepatitis B patients. J Hepatol 2019;70:615–625.
[40] Liaw YF, Sheen IS, Chen TJ, Chu CM, Pao CC. Incidence, determinants and significance of delayed clearance of serum HBsAg in chronic hepatitis B virus infection: a prospective study. Hepatology 1991;13:627–631.
[41] Zhou K, Contag C, Whitaker E, Terrault N. Spontaneous loss of surface antigen among adults living with chronic hepatitis B virus infection: a systematic review and pooled meta-analyses. Lancet Gastroenterol Hepatol 2019;4:227–238.
[42] Yeo YH, Ho HJ, Yang H-I, Tseng T-C, Hosaka T, Trinh HN, et al. Factors associated with rates of HBsAg seroclearance in adults with chronic HBV infection: a systematic review and meta-analysis. Gastroenterology 2019;156:635–646.e9.
[43] Wu TT, Hsu HC, Chen DS, Sheu JC, Su IJ, Chen SL, et al. Clearance of hepatitis B surface antigen (HBsAg) after surgical resection of hepatocellular carcinoma. J Hepatol 1987;4:45–51.
[44] Chan HL-Y, Wong GL-H, Tse C-H, Chan H-Y, Wong VW-S. Viral determinants of hepatitis B surface antigen seroclearance in hepatitis B e antigen-negative chronic hepatitis B patients. J Infect Dis 2011;204:408–414.
[45] Habersetzer F, Moenne-Loccoz R, Meyer N, Schvoerer E, Simo-Noumbissie P, Dritsas S, et al. Loss of hepatitis B surface antigen in a real-life clinical cohort of patients with chronic hepatitis B virus infection. Liver Int 2015;35:130–139.
[46] Chu C-M, Liaw Y-F. Hepatitis B surface antigen seroclearance during chronic HBV infection. Antivir Ther 2010;15:133–143.
[47] Song C, Zhu J, Ge Z, Yu C, Tian T, Wang H, et al. Spontaneous seroclearance of hepatitis B surface antigen and risk of hepatocellular carcinoma. Clin Gastroenterol Hepatol 2019;17:1204–1206.
[48] Yip TC-F, Wong GL-H, Chan HL-Y, Tse Y-K, Lam KL-Y, Lui GC-Y, et al. HBsAg seroclearance further reduces hepatocellular carcinoma risk after complete viral suppression with nucleos(t)ide analogues. J Hepatol 2019;70:361–370.
[49] Luangsay S, Gruffaz M, Isorce N, Testoni B, Michelet M, Faure-Dupuy S, et al. Early inhibition of hepatocyte innate responses by hepatitis B virus. J Hepatol 2015;63:1314–1322.
[50] Kondo Y, Shimosegawa T. Significant roles of regulatory T cells and myeloid derived suppressor cells in hepatitis B virus persistent infection and hepatitis B virus-related HCCs. Int J Mol Sci 2015;16:3307–3322.
[51] Martinet J, Dufeu-Duchesne T, Bruder Costa J, Larrat S, Marlu A, Leroy V, et al. Altered functions of plasmacytoid dendritic cells and reduced cytolytic activity of natural killer cells in patients with chronic HBV infection. Gastroenterology 2012;143:1586–1596.e8.
[52] Shi B, Ren G, Hu Y, Wang S, Zhang Z, Yuan Z. HBsAg inhibits IFN-α production in plasmacytoid dendritic cells through TNF-α and IL-10 induction in monocytes. PLoS One 2012;7:e44900.
[53] Kalathil S, Lugade AA, Miller A, Iyer R, Thanavala Y. Higher frequencies of GARP(+)CTLA-4(+)Foxp3(+) T regulatory cells and myeloid-derived suppressor cells in hepatocellular carcinoma patients are associated with impaired T-cell functionality. Cancer Res 2013;73:2435–2444.
[54] Xu X, Shang Q, Chen X, Nie W, Zou Z, Huang A, et al. Reversal of B-cell hyperactivation and functional impairment is associated with HBsAg seroconversion in chronic hepatitis B patients. Cell Mol Immunol 2015;12:309–316.
[55] Salimzadeh L, Le Bert N, Dutertre C-A, Gill US, Newell EW, Frey C, et al. PD-1 blockade partially recovers dysfunctional virus-specific B cells in chronic hepatitis B infection. J Clin Invest 2018;128:4573–4587.
[56] Liu S, Peng N, Xie J, Hao Q, Zhang M, Zhang Y, et al. Human hepatitis B virus surface and e antigens inhibit major vault protein signaling in interferon induction pathways. J Hepatol 2015;62:1015–1023.
[57] Zannetti C, Roblot G, Charrier E, Ainouze M, Tout I, Briat F, et al. Characterization of the inflammasome in human Kupffer cells in response to synthetic agonists and pathogens. J Immunol 2016;197:356–367.
[58] Boltjes A, van Montfoort N, Biesta PJ, Op den Brouw ML, Kwekkeboom J, van der Laan LJW, et al. Kupffer cells interact with hepatitis B surface antigen in vivo and in vitro, leading to proinflammatory cytokine production and natural killer cell function. J Infect Dis 2015;211:1268–1278.
[59] Wang S, Chen Z, Hu C, Qian F, Cheng Y, Wu M, et al. Hepatitis B virus surface antigen selectively inhibits TLR2 ligand-induced IL-12 production in monocytes/macrophages by interfering with JNK activation. J Immunol 2013;190:5142–5151.
[60] van der Molen RG, Sprengers D, Binda RS, de Jong EC, Niesters HGM, Kusters JG, et al. Functional impairment of myeloid and plasmacytoid dendritic cells of patients with chronic hepatitis B. Hepatology 2004;40:738–746.
[61] Op den Brouw ML, Binda RS, van Roosmalen MH, Protzer U, Janssen HLA, van der Molen RG, et al. Hepatitis B virus surface antigen impairs myeloid dendritic cell function: a possible immune escape mechanism of hepatitis B virus. Immunology 2009;126:280–289.
[62] Woltman AM, Op den Brouw ML, Biesta PJ, Shi CC, Janssen HLA. Hepatitis B virus lacks immune activating capacity, but actively inhibits plasmacytoid dendritic cell function. PLoS One 2011;6:e15324.
[63] Xu Y, Hu Y, Shi B, Zhang X, Wang J, Zhang Z, et al. HBsAg inhibits TLR9-mediated activation and IFN-alpha production in plasmacytoid dendritic cells. Mol Immunol 2009;46:2640–2646.
[64] Ye B, Liu X, Li X, Kong H, Tian L, Chen Y. T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. Cell Death Dis 2015;6:e1694.
[65] Dong Y, Li X, Zhang L, Zhu Q, Chen C, Bao J, et al. CD4+ T cell exhaustion revealed by high PD-1 and LAG-3 expression and the loss of helper T cell function in chronic hepatitis B. BMC Immunol 2019;20:27.
[66] Tout I, Gomes M, Ainouze M, Marotel M, Pecoul T, Durantel D, et al. Hepatitis B virus blocks the CRE/CREB complex and prevents TLR9 transcription and function in human B cells. J Immunol 2018;201:2331–2344.
[67] Fang Z, Li J, Yu X, Zhang D, Ren G, Shi B, et al. Polarization of monocytic myeloid-derived suppressor cells by hepatitis B surface antigen is mediated via ERK/IL-6/STAT3 signaling Feedback and restrains the activation of T cells in chronic hepatitis B virus infection. J Immunol 2015;195:4873–4883.
[68] Naito M, Hasegawa G, Takahashi K. Development, differentiation, and maturation of Kupffer cells. Microsc Res Tech 1997;39:350–364.
[69] Patente TA, Pinho MP, Oliveira AA, Evangelista GCM, Bergami-Santos PC, Barbuto JAM. Human dendritic cells: their heterogeneity and clinical application potential in cancer immunotherapy. Front Immunol 2018;9:3176.
[70] Hémont C, Neel A, Heslan M, Braudeau C, Josien R. Human blood mDC subsets exhibit distinct TLR repertoire and responsiveness. J Leukoc Biol 2013;93:599–609.
[71] Sittig SP, Bakdash G, Weiden J, Sköld AE, Tel J, Figdor CG, et al. A comparative study of the T cell stimulatory and polarizing capacity of human primary blood dendritic cell subsets. Mediators Inflamm 2016;2016:3605643.
[72] Collin M, Bigley V. Human dendritic cell subsets: an update. Immunology 2018;154:3–20.
[73] van Montfoort N, van der Aa E, van den Bosch A, Brouwers H, Vanwolleghem T, Janssen HLA, et al. Hepatitis B virus surface antigen activates myeloid dendritic cells via a soluble CD14-dependent mechanism. J Virol 2016;90:6187–6199.
[74] Vincent IE, Zannetti C, Lucifora J, Norder H, Protzer U, Hainaut P, et al. Hepatitis B virus impairs TLR9 expression and function in plasmacytoid dendritic cells. PLoS One 2011;6:e26315.
[75] Li X, Zhou L, Gu Y, Chen L, Gu L, Huang Y. Quantative HBsAg level correlates dendritic cells maturation in chronic hepatitis B patients. Clin Res Hepatol Gastroenterol 2020;44(3):321–328.

[76] Ouaguia L, Leroy V, Dufeu-Duchesne T, Durantel D, Decaens T, Hubert M, et al. Circulating and hepatic BDCA1+, BDCA2+, and BDCA3+ dendritic cells are differentially subverted in patients with chronic HBV infection. Front Immunol 2019;10:112.
[77] Vyas AK, Sharma BC, Sarin SK, Trehanpati N. Immune correlates of hepatitis B surface antigen spontaneous seroconversion in hepatitis B e antigen negative chronic hepatitis B patients. Liver Int 2018;38:38–49.
[78] Norris S, Collins C, Doherty DG, Smith F, McEntee G, Traynor O, et al. Resident human hepatic lymphocytes are phenotypically different from circulating lymphocytes. J Hepatol 1998;28:84–90.
[79] Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol 2008;9:503–510.
[80] Zhang J, Chen Q, Feng H. Relationship between chronic hepatitis B virus infection and nature killer cells. Viral Immunol 2019;32:263–268.
[81] Guidotti LG, Borrow P, Hobbs MV, Matzke B, Gresser I, Oldstone MB, et al. Viral cross talk: intracellular inactivation of the hepatitis B virus during an unrelated viral infection of the liver. Proc Natl Acad Sci U S A 1996;93:4589–4594.
[82] Xia Y, Stadler D, Lucifora J, Reisinger F, Webb D, Hösel M, et al. Interferon-γ and tumor necrosis factor-α produced by T cells reduce the HBV persistence form, cccDNA, without cytolysis. Gastroenterology 2016;150:194–205.
[83] Lunemann S, Malone DFG, Hengst J, Port K, Grabowski J, Deterding K, et al. Compromised function of natural killer cells in acute and chronic viral hepatitis. J Infect Dis 2014;209:1362–1373.
[84] Peppa D, Gill US, Reynolds G, Easom NJW, Pallett LJ, Schurich A, et al. Up-regulation of a death receptor renders antiviral T cells susceptible to NK cell-mediated deletion. J Exp Med 2013;210:99–114.
[85] Li H, Zhai N, Wang Z, Song H, Yang Y, Cui A, et al. Regulatory NK cells mediated between immunosuppressive monocytes and dysfunctional T cells in chronic HBV infection. Gut 2018;67:2035–2044.
[86] Micco L, Peppa D, Loggi E, Schurich A, Jefferson L, Cursaro C, et al. Differential boosting of innate and adaptive antiviral responses during pegylated-interferon-alpha therapy of chronic hepatitis B. J Hepatol 2013;58:225–233.
[87] Tan AT, Hoang LT, Chin D, Rasmussen E, Lopatin U, Hart S, et al. Reduction of HBV replication prolongs the early immunological response to IFNα therapy. J Hepatol 2014;60:54–61.
[88] Gill US, Peppa D, Micco L, Singh HD, Carey I, Foster GR, et al. Interferon alpha induces sustained changes in NK cell responsiveness to hepatitis B viral load suppression in vivo. PLoS Pathog 2016;12:e1005788.
[89] Zimmer CL, Rinker F, Höner Zu Siederdissen C, Manns MP, Wedemeyer H, Cornberg M, et al. Increased NK cell function after cessation of long-term nucleos(t)ide analogue treatment in chronic hepatitis B is associated with liver damage and HBsAg loss. J Infect Dis 2018;217:1656–1666.
[90] Maini MK, Boni C, Ogg GS, King AS, Reignat S, Lee CK, et al. Direct ex vivo analysis of hepatitis B virus-specific CD8(+) T cells associated with the control of infection. Gastroenterology 1999;117:1386–1396.
[91] Penna A, Artini M, Cavalli A, Levrero M, Bertoletti A, Pilli M, et al. Long-lasting memory T cell responses following self-limited acute hepatitis B. J Clin Invest 1996;98:1185–1194.
[92] Kennedy PTF, Sandalova E, Jo J, Gill U, Ushiro-Lumb I, Tan AT, et al. Preserved T-cell function in children and young adults with immune-tolerant chronic hepatitis B. Gastroenterology 2012;143:637–645.
[93] Rehermann B. Pathogenesis of chronic viral hepatitis: differential roles of T cells and NK cells. Nat Med 2013;19:859–868.
[94] Reignat S, Webster GJM, Brown D, Ogg GS, King A, Seneviratne SL, et al. Escaping high viral load exhaustion: CD8 cells with altered tetramer binding in chronic hepatitis B virus infection. J Exp Med 2002;195:1089–1101.
[95] Ito H, Ando T, Ando K, Ishikawa T, Saito K, Moriwaki H, et al. Induction of hepatitis B virus surface antigen-specific cytotoxic T lymphocytes can be up-regulated by the inhibition of indoleamine 2, 3-dioxygenase activity. Immunology 2014;142:614–623.
[96] Ito H, Ando K, Ishikawa T, Nakayama T, Taniguchi M, Saito K, et al. Role of Valpha14+ NKT cells in the development of Hepatitis B virus-specific CTL: activation of Valpha14+ NKT cells promotes the breakage of CTL tolerance. Int Immunol 2008;20:869–879.
[97] Fisicaro P, Barili V, Montanini B, Acerbi G, Ferracin M, Guerrieri F, et al. Targeting mitochondrial dysfunction can restore antiviral activity of exhausted HBV-specific CD8 T cells in chronic hepatitis B. Nat Med 2017;23:327–336.
[98] Milich DR, McLachlan A, Thornton GB, Hughes JL. Antibody production to the nucleocapsid and envelope of the hepatitis B virus primed by a single synthetic T cell site. Nature 1987;329:547–549.
[99] Böcher WO, Herzog-Hauff S, Herr W, Heermann K, Gerken G, Meyer Zum Büschenfelde KH, et al. Regulation of the neutralizing anti-hepatitis B surface (HBs) antibody response in vitro in HBs vaccine recipients and patients with acute or chronic hepatitis B virus (HBV) infection. Clin Exp Immunol 1996;105:52–58.
[100] Boni C, Fisicaro P, Valdatta C, Amadei B, Vincenzo PD, Giuberti T, et al. Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. J Virol 2007;81:4215–4225.
[101] Rinker F, Zimmer CL, Höner Zu Siederdissen C, Manns MP, Kraft ARM, Wedemeyer H, et al. Hepatitis B virus-specific T cell responses after stopping nucleos(t)ide analogue therapy in HBeAg-negative chronic hepatitis B. J Hepatol 2018;69:584–593.
[102] Wang X, Dong Q, Li Q, Li Y, Zhao D, Sun J, et al. Dysregulated response of follicular helper T cells to hepatitis B surface antigen promotes HBV persistence in mice and associates with outcomes of patients. Gastroenterology 2018;154:2222–2236.
[103] Zhang L, Li H, Ren H, Hu P. Circulating PD-1hiCXCR5+CD4+ T cells are associated with a decrease in hepatitis B surface antigen levels in patients with chronic hepatitis B who are receiving peginterferon-α therapy. Mol Immunol 2018;103:270–278.
[104] Wang H, Luo H, Wan X, Fu X, Mao Q, Xiang X, et al. TNF-α/IFN-γ profile of HBV-specific CD4 T cells is associated with liver damage and viral clearance in chronic HBV infection. J Hepatol 2020;72:45–56.
[105] Gu Y, Lian Y, Gu L, Chen L, Li X, Zhou L, et al. Correlations between cytokines produced by T cells and clinical-virological characteristics in untreated chronic hepatitis B patients. BMC Infect Dis 2019;19:216.
[106] Bengsch B, Martin B, Thimme R. Restoration of HBV-specific CD8+ T cell function by PD-1 blockade in inactive carrier patients is linked to T cell differentiation. J Hepatol 2014;61:1212–1219.
[107] Loomba R, Liang TJ. Hepatitis B reactivation associated with immune suppressive and biological modifier therapies: current concepts, management strategies, and future directions. Gastroenterology 2017;152:1297–1309.
[108] Oliviero B, Cerino A, Varchetta S, Paudice E, Pai S, Ludovisi S, et al. Enhanced B-cell differentiation and reduced proliferative capacity in chronic hepatitis C and chronic hepatitis B virus infections. J Hepatol 2011;55:53–60.
[109] Kondo Y, Ninomiya M, Kakazu E, Kimura O, Shimosegawa T. Hepatitis B surface antigen could contribute to the immunopathogenesis of hepatitis B virus infection. ISRN Gastroenterol 2013;2013:935295.
[110] Kong X, Sun R, Chen Y, Wei H, Tian Z. γδT cells drive myeloid-derived suppressor cell-mediated CD8+ T cell exhaustion in hepatitis B virus-induced immunotolerance. J Immunol 2014;193:1645–1653.
[111] Riazalhosseini B, Mohamed Z, Apalasamy YD, Shafie NS, Mohamed R. Interleukin-6 gene variants are associated with reduced risk of chronicity in hepatitis B virus infection in a Malaysian population. Biomed Rep 2018;9:213–220.
[112] Bouezzedine F, Fardel O, Gripon P. Interleukin 6 inhibits HBV entry through NTCP down regulation. Virology 2015;481:34–42.
[113] Schurich A, Pallett LJ, Lubowiecki M, Singh HD, Gill US, Kennedy PT, et al. The third signal cytokine IL-12 rescues the anti-viral function of exhausted HBV-specific CD8 T cells. PLoS Pathog 2013;9:e1003208.
[114] Crump KE, Sahingur SE. Microbial nucleic acid sensing in oral and systemic diseases. J Dent Res 2016;95:17–25.
[115] Jiang M, Broering R, Trippler M, Poggenpohl L, Fiedler M, Gerken G, et al. Toll-like receptor-mediated immune responses are attenuated in the presence of high levels of hepatitis B virus surface antigen. J Viral Hepat 2014;21:860–872.
[116] Xu N, Yao H-P, Lv G-C, Chen Z. Downregulation of TLR7/9 leads to deficient production of IFN-α from plasmacytoid dendritic cells in chronic hepatitis B. Inflamm Res 2012;61:997–1004.
[117] Lanford RE, Guerra B, Chavez D, Giavedoni L, Hodara VL, Brasky KM, et al. GS-9620, an oral agonist of toll-like receptor-7, induces prolonged suppression of hepatitis B virus in chronically infected chimpanzees. Gastroenterology 2013;144:1508–1517. 1517.e1–10.
[118] Janssen HLA, Brunetto MR, Kim YJ, Ferrari C, Massetto B, Nguyen A-H, et al. Safety, efficacy and pharmacodynamics of vesatolimod (GS-9620) in virally suppressed patients with chronic hepatitis B. J Hepatol 2018;68:431–440.

[119] Xia Y, Schlapschy M, Morath V, Roeder N, Vogt EI, Stadler D, et al. PASylated interferon α efficiently suppresses hepatitis B virus and induces anti-HBs seroconversion in HBV-transgenic mice. Antivir Res 2019;161:134–143.
[120] Amblard F, Boucle S, Bassit L, Cox B, Sari O, Tao S, et al. Novel hepatitis B virus capsid assembly modulator induces potent antiviral responses in vitro and in Humanized mice. Antimicrob Agents Chemother 2020;64:e01701–e01719.
[121] Schinazi RF, Ehteshami M, Bassit L, Asselah T. Towards HBV curative therapies. Liver Int 2018;38(Suppl 1):102–114.
[122] Marcellin P, Ahn SH, Ma X, Caruntu FA, Tak WY, Elkashab M, et al. Combination of tenofovir disoproxil fumarate and peginterferon α-2a increases loss of hepatitis B surface antigen in patients with chronic hepatitis B. Gastroenterology 2016;150:134–144.e10.
[123] Marcellin P, Lau GKK, Bonino F, Farci P, Hadziyannis S, Jin R, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2004;351:1206–1217.
[124] Lau GKK, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682–2695.
[125] Papatheodoridis GV, Sypsa V, Dalekos G, Yurdaydin C, van Boemmel F, Buti M, et al. Eight-year survival in chronic hepatitis B patients under long-term entecavir or tenofovir therapy is similar to the general population. J Hepatol 2018;68:1129–1136.
[126] Marcellin P, Asselah T. Long-term therapy for chronic hepatitis B: hepatitis B virus DNA suppression leading to cirrhosis reversal. J Gastroenterol Hepatol 2013;28:912–923.
[127] Marcellin P, Buti M, Krastev Z, de Man RA, Zeuzem S, Lou L, et al. Kinetics of hepatitis B surface antigen loss in patients with HBeAg-positive chronic hepatitis B treated with tenofovir disoproxil fumarate. J Hepatol 2014;61:1228–1237.
[128] Chang T-T, Lai C-L, Kew Yoon S, Lee SS, Coelho HSM, Carrilho FJ, et al. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. Hepatology 2010;51:422–430.
[129] Gish RG, Chang T-T, Lai C-L, de Man R, Gadano A, Poordad F, et al. Loss of HBsAg antigen during treatment with entecavir or lamivudine in nucleoside-naïve HBeAg-positive patients with chronic hepatitis B. J Viral Hepat 2010;17:16–22.
[130] Chang T-T, Gish RG, de Man R, Gadano A, Sollano J, Chao Y-C, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. N Engl J Med 2006;354:1001–1010.
[131] Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet Lond Engl 2013;381:468–475.
[132] Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. N Engl J Med 2008;359:2442–2455.
[133] Agarwal K, Brunetto M, Seto WK, Lim Y-S, Fung S, Marcellin P, et al. 96 weeks treatment of tenofovir alafenamide vs. tenofovir disoproxil fumarate for hepatitis B virus infection. J Hepatol 2018;68:672–681.
[134] Janssen HLA, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, et al. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. Lancet Lond Engl 2005;365:123–129.
[135] Petersen J, Ratziu V, Buti M, Janssen HLA, Brown A, Lampertico P, et al. Entecavir plus tenofovir combination as rescue therapy in pre-treated chronic hepatitis B patients: an international multicenter cohort study. J Hepatol 2012;56:520–526.
[136] Lok AS, Trinh H, Carosi G, Akarca US, Gadano A, Habersetzer F, et al. Efficacy of entecavir with or without tenofovir disoproxil fumarate for nucleos(t)ide-naïve patients with chronic hepatitis B. Gastroenterology 2012;143:619–628.e1.
[137] Brouwer WP, Xie Q, Sonneveld MJ, Zhang N, Zhang Q, Tabak F, et al. Adding pegylated interferon to entecavir for hepatitis B e antigen-positive chronic hepatitis B: a multicenter randomized trial (ARES study). Hepatology 2015;61:1512–1522.
[138] Martinot-Peignoux M, Lapalus M, Asselah T, Marcellin P. HBsAg quantification: useful for monitoring natural history and treatment outcome. Liver Int 2014;34(Suppl 1):97–107.
[139] Moucari R, Mackiewicz V, Lada O, Ripault M-P, Castelnau C, Martinot-Peignoux M, et al. Early serum HBsAg drop: a strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology 2009;49:1151–1157.
[140] Chen C-H, Lu S-N, Hung C-H, Wang J-H, Hu T-H, Changchien C-S, et al. The role of hepatitis B surface antigen quantification in predicting HBsAg loss and HBV relapse after discontinuation of lamivudine treatment. J Hepatol 2014;61:515–522.
[141] Liang Y, Jiang J, Su M, Liu Z, Guo W, Huang X, et al. Predictors of relapse in chronic hepatitis B after discontinuation of anti-viral therapy. Aliment Pharmacol Ther 2011;34:344–352.
[142] Liu J, Li T, Zhang L, Xu A. The role of hepatitis B surface antigen in nucleos(t)ide analogues cessation among Asian patients with chronic hepatitis B: a systematic review. Hepatology 2019;70:1045–1055.
[143] Bertoletti A, Le Bert N. Immunotherapy for chronic hepatitis B virus infection. Gut Liver 2018;12:497–507.
[144] Fanning GC, Zoulim F, Hou J, Bertoletti A. Therapeutic strategies for hepatitis B virus infection: towards a cure. Nat Rev Drug Discov 2019;18:827–844.
[145] Boni C, Janssen HLA, Rossi M, Yoon SK, Vecchi A, Barili V, et al. Combined GS-4774 and tenofovir therapy can improve HBV-specific T-cell responses in patients with chronic hepatitis. Gastroenterology 2019;157:227–241.e7.
[146] Vaillant A. Rep 2139: antiviral mechanisms and applications in achieving functional control of HBV and HDV infection. ACS Infect Dis 2019;5:675–687.
[147] Bazinet M, Pântea V, Cebotarescu V, Cojuhari L, Jimbei P, Albrecht J, et al. Safety and efficacy of REP 2139 and pegylated interferon alfa-2a for treatment-naive patients with chronic hepatitis B virus and hepatitis D virus co-infection (REP 301 and REP 301-LTF): a non-randomised, open-label, phase 2 trial. Lancet Gastroenterol Hepatol 2017;2:877–889.
[148] Al-Mahtab M, Bazinet M, Vaillant A. Safety and efficacy of nucleic acid polymers in monotherapy and combined with immunotherapy in treatment-naive Bangladeshi patients with HBeAg+ chronic hepatitis B infection. PLoS One 2016;11:e0156667.
[149] Bazinet M, Pântea V, Placinta G, Moscalu I, Cebotarescu V, Cojuhari L, et al. Safety and efficacy of 48 Weeks REP 2139 or REP 2165, tenofovir disoproxil, and pegylated interferon alfa-2a in patients with chronic HBV infection naïve to nucleos(t)ide therapy. Gastroenterology 2020;158(8):2180–2194.