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# Immune Control of Hepatitis B Virus

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Viral persistence · Hepatitis B virus clearance · Immunopathogenesis · Innate immunity · Adaptive immunity

**Abstract**
Human hepatitis B virus (HBV) infects the liver of humans or humanoid primates. In humans, HBV infection often causes an inflammatory liver disease – hepatitis B. The virus is transmitted by perinatal, percutaneous and sexual exposure, as well as by close person-to-person contact. The latter occurs especially among young children, presumably by open cuts or sores. Vertical transmission from mothers to their neonates, or infection during the first year of life, results in persistent often lifelong infection in >90% of cases. In contrast, infection during adulthood is cleared in >95% of cases, and results in lifelong protective immunity. While a correlation between the strength of HBV-specific T cell responses and virus clearance has been established, factors determining the strength of a T cell response as well as factors shifting the balance from immune tolerance to immune clearance are hardly understood. The innate immune response, early adaptive B and T cell responses, regulatory T cells, the liver microenvironment, and the peculiar properties of hepatocytes and nonparenchymal liver cells to present antigen seem to play a role. Understanding this complex interplay requires systematic immune monitoring of well characterized human cohorts, but also experimental approaches using primary human cells and genetically modified mouse models. Using these models, we begin to understand the immune recognition of HBV and how it influences the outcome of HBV infection. In this paper we review the current knowledge about virus-host interactions and how it influences the outcome of HBV infection and describe the immune signatures associated with clinical recovery and/or persistent infection.


## The Clinical Course of Hepatitis B Virus Infection

Hepatitis B virus (HBV) infection in immunocompetent adults usually results in a self-limited, transient liver disease, and about 95% clear acute virus infection. In only 3–5% of adults the virus persists for more than 6 months and cause chronic infection. Conversely, vertical transmission from mothers to their children or horizontal transmission during early childhood results in chronic infection in about 90% of exposed individuals. Persistent HBV infection is associated with varying degrees of chronic liver disease, which often progresses to liver cir-

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rhosis and hepatocellular carcinoma. The outcome of HBV infection as well as the pathogenesis of liver diseases are immune-mediated and thus determined by the virus-host interaction [1].

In general, both arms of the immune system, the innate and the adaptive immune response, are involved in the control and elimination of a viral infection. Efficient control of HBV infection requires an effective adaptive CD4+ and CD8+ T cell response. Protective immunity, however, requires a B cell response which guarantees the production of sufficient amounts of neutralizing antibodies [2].

## Innate Immune Responses

Innate immune responses are crucial for early containment of viral infections and favor a timely and efficient induction of virus-specific adaptive responses. Innate immune responses are essential for elimination of the virus, but also contribute to immunopathology [3]. There is, however, an ongoing debate about the role of the innate immune system and how adaptive immunity is triggered. While flow cytometry now allows sophisticated analysis of T cell responses, innate immune responses characterized by complex signaling and effector cell networks are more difficult to analyze. In addition, HBV infection is often missed during its early phase of infection and in most cases is only diagnosed several weeks later when the expected peak of innate immunity has ceased. In recent years, there is emerging evidence that the innate immune system plays an important role in influencing both the outcome of acute HBV infection and the pathogenesis of liver disease.

The innate immune system is the host's first defense mechanism against viral infections, able to limit the extent of virus spread early during infection and to eliminate infected cells. Systemic innate immune responses may involve macrophages and dendritic cells (DC) as well as natural killer (NK) cells. A number of cells may be involved in local innate immune responses towards HBV in the liver, including hepatocytes and nonparenchymal liver cells such as liver resident macrophages (Kupffer cells), liver sinusoidal endothelial cells (LSEC), liver-resident myofibroblasts (Ito cells) and liver resident DC, together with intrahepatic lymphocytes such as NK cells and natural killer T (NKT) cells. The large number of cells potentially involved in innate immunity against HBV demonstrates the complexity of this response.

### Recognition of HBV by Pattern Recognition Receptors

Innate immune cells detect pathogens via pattern recognition receptors, including Toll-like receptors (TLR), retinoic acid-inducible gene I-(Rig-I)-like helicases and NOD-like receptors. Pathogen recognition receptors respond to distinct pathogen-associated or danger-associated molecular patterns. In general, activation of these receptors induces production of NF-κB-induced proinflammatory cytokines and, in most cases, IFN and signals that recruit immune cells and activate an inflammatory response. In addition, they are required to induce adaptive immune responses [4].

In the early phase of most viral infections, innate immune responses are mediated mainly by pattern recognition and activation of NK and NKT cells and are characterized by the release of antiviral cytokines such as type I (IFN-α/IFN-β) and type III IFN (IFN-λ) by infected cells. Type I IFN act either in a direct manner inhibiting viral replication or in an indirect manner recruiting and activating antigen-presenting cells (e.g. Kupffer cells, DC).

Type I IFN can inhibit transcriptional and posttranscriptional steps of the HBV replication cycle [5], whereas IFN-γ acts at a posttranscriptional step [5–7]. IFN-α/β is capable of inhibiting the formation of new HBV capsids, may cause destabilization of existing capsids and mediates degradation of preformed HBV RNA [8–10]. IFN-λ, produced in certain tissues including the liver, has also been shown to have activity against HBV in the HBV-transgenic mouse model [11, 12].

In accordance, administration of specific TLR ligands resulted in significant inhibition of viral replication in an IFN-dependent manner within 24 h in HBV transgenic mice [8, 9] and cultured nonparenchymal liver cells [13]. After baculovirus-mediated HBV genome transfer, an IFN response was observed in HepaRG cells [14].

After infection of primary human or chimpanzee liver cells, however, HBV does not induce an IFN response [15, 16]. In a recent study, we investigated whether HBV is detected at all in the liver. HBV is recognized by non-hepatocytes in the liver, precisely by liver macrophages, the Kupffer cells, rapidly (within 3 h) after contact [15]. In contrast to Kupffer cells, hepatocytes do not recognize viral particles (Broxtermann and Protzer, unpubl. results). Notably, Kupffer cells are not infected by HBV, and virus replication was not necessary to induce the pattern response [15]. Recognition of HBV leads to activation of NF-κB and to subsequent production of IL-6

and proinflammatory cytokines such as TNF-α, IL-8 and proIL-1β, but may also trigger the production of IL-10. Interestingly, we did not detect any induction of a type I IFN response although this is usually observed during viral infections [17, 18]. In addition, the inflammasome was not activated (Broxtermann and Protzer, unpubl.).

Once HBV has infected its target cell, visibility for the cellular innate sensing machinery is low due to its peculiar replication strategy. Firstly, HBV sequesters its transcriptional template, the covalently closed circular DNA, in the nucleus, where it forms a minichromosome and is covered by histones [19]. From HBV covalently closed circular DNA capped and polyadenylated viral mRNAs are transcribed resembling the structure of normal cellular transcripts. Pregenomic HBV RNAs are immediately packed into viral capsids where they are reverse-transcribed into new HBV genomes. By this, the virus shields the single-stranded RNA intermediates, which are generally strong activators of type I interferons from innate immune recognition [16, 20]. Accordingly, hepatocytes did not respond to either HBV infection or its replication in our study [15].

Studies indicate that HBV capsids are recognized by TLR-2 [21]. However, minute amounts of contaminating lipopolysaccharide or other TLR ligands may influence pattern recognition [22]. Under normal conditions, only enveloped virions are released from HBV-infected hepatocytes, and capsids are shielded by the viral envelope. If infected hepatocytes undergo apoptosis, however, non-enveloped capsids are released [23] and can activate pattern recognition receptors and trigger adaptive immunity.

Clinical studies revealed an upregulation of TLR-2 and increased TNF-α secretion in HBeAg-negative patients in the immune active phase of chronic HBV infection – as expected upon TLR-2 activation [24, 25]. In HBeAg-positive patients without obvious liver disease, TLR-2 was upregulated on hepatocytes and Kupffer cells, but also on peripheral monocytes, whereas it was downregulated in HBeAg-negative chronic hepatitis B [25]. In a recent paper, Lang et al. [26] indicated that HBeAg (but not HBV core protein) colocalizes with Toll/IL-1 receptor (TIR)-containing proteins such as TLR-2 inside a cell and disrupts homotypic TIR/TIR interaction critical for TLR-mediated signaling. Thereby, HBeAg specifically suppressed TIR-mediated activation of the prototypic inflammatory transcription factor NF-κB and of the IFN-β promoter and prevents activation of TLR-2.

### Innate Immune Response to HBV in Humans

When analyzing the immune response in acutely infected chimpanzees, Wieland and Chisari [16] did not observe significant alterations of the hepatic transcription profile during early HBV infection. Thus, HBV does not seem to induce either an IFN response or a strong innate immune response in the liver. All chimpanzees showed a self-limited infection, and HBV clearance was associated with a strong adaptive immune response. Therefore, the authors suggest that HBV may act as a 'stealth virus' capable of evading the first line of host defense. Due to the lack of liver biopsy samples during the acute phase of HBV infection, these analyses could not be confirmed in the human setting. However, the lack of a detectable innate immune response in chimpanzees may also reflect the better adaptation of HBV resulting in a milder course of liver disease in these primates.

Patients with symptomatic acute hepatitis B have long been known to have elevated plasma levels of proinflammatory cytokines [27]. During flares of chronic hepatitis B, IL-8 and IFN-α peak in the serum [28]. Longitudinal analysis of circulating NK cells revealed an increased frequency during the incubation phase of HBV infection declining with decreasing viral load [29]. Recently, Fisicaro et al. [30] confirmed an induction of NK and NKT cell responses before the onset of adaptive T cell responses in acute hepatitis B and concluded that the human innate immune system is able to sense HBV infection in vivo.

Dunn et al. [31] addressed this question in a cohort of acutely HBV-infected patients monitored during the presymptomatic phase. Neither type I nor type III IFN (including IFN-λ) nor IL-15 were induced, but IL-10 was. NK cell activation and IFN-γ production were probably mitigated due to high IL-10 production observed at the peak of viremia.

Another study determined the kinetics of cytokine and chemokine induction in plasma samples of HBV- and HIV-infected patients, collected during the initial phases of infection. Data revealed marked differences with a rapid induction of classic innate cytokines in acute HIV infection, but remarkably little changes in plasma cytokine and chemokine levels in acute HBV infection. However, a weak innate immune response (e.g. production of IFN-α, TNF-α, IL-15 and IL-6) against HBV was detectable in a subset of patients [32].

Although induction of innate immune responses by HBV remains a controversial issue, the data summarized

above suggest that HBV rather than being a complete stealth virus is recognized by the innate immune system very early during infection in humans.

### NK Cells in HBV Infection

The innate arm of the immune system contributes to control of HBV infection by different means: DC and macrophages can phagocytose HBV particles, LSEC may engulf them by macropinocytosis, antiviral cytokines are released and block HBV replication, and NK or NKT cells can eliminate HBV-infected cells.

NK and NKT cells can rapidly be recruited to the site of virus infection and have the potential to recognize infected cells very early and in an antigen-independent fashion. NK cells are cytotoxic and may kill HBV-infected hepatocytes mainly via the TNF-related apoptosis-inducing ligand (TRAIL) [28]. In addition, they produce cytokines and chemokines that have antiviral activity and recruit inflammatory cells into the infected tissue. Cytokines produced by NK cells either have direct antiviral (IFN-γ, TNF-α) or immunomodulatory activity (e.g. IL-3, granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor) [33]. Thus, NK cells play a dual role in controlling HBV by inhibiting virus replication in a noncytopathic fashion and by TRAIL-induced killing of HBV-infected hepatocytes – the latter at the expense of liver damage.

NKT cells, a T lymphocyte subset expressing classic NK cell markers, produce IFN-γ in response to NK cell stimulation [33] and also after recognition of stress signals on infected hepatocytes [34, 35].

Several studies have revealed a central role for NK and NKT cells during innate immune response to acute HBV infection. NK cells inhibit HBV replication upon activation in HBV transgenic mice [34]. In the same model, a 10- to 12-fold increase in NK cell numbers in the intrahepatic cellular infiltrate is seen during acute inflammation [36]. Nonclassical NKT cells become activated in acute HBV infection by the NKG2D receptor and are crucial in the immune response to HBV and induction of liver injury during acute hepatitis [35, 37].

Recruitment and activation of NK and NKT cells appear to be mediated mainly by cytokines IL-12 and IL-18 and the chemokine CCL3 released by antigen-presenting cells in response to type 1 IFN [38, 39]. However, there is also evidence that NK and NKT cells both may be activated directly by HBV-infected hepatocytes [34, 39]. Production of IFN-γ and TNF-α by activated NK and NKT cells leads to significant inhibition of HBV replication and to recruitment of virus-specific and nonspecific cells [34, 38]. NK cells contribute to liver injury during chronic HBV infection through TRAIL-mediated death of hepatocytes [28].

However, a dysfunctional cytokine production by NK cells has been observed in chronic HBV patients, which may contribute to virus persistence and chronic liver immunopathology [40]. HBV can even counteract NK-mediated killing because HBV capsids can inhibit TRAIL-induced apoptosis, probably favoring virus persistence [41].

### Activation of Antigen Presentation in HBV Infection

Dendritic cells are the most potent antigen-presenting cells, exclusively capable of stimulating naïve T cells. In humans, they are classified into two main subtypes: myeloid DC producing IL-12 and IL-15 upon activation, which in turn stimulates IFN-γ secretion by NK cells and promotes the differentiation of CD4 and CD8 T cells; and plasmacytoid DC secreting large quantities of type 1 IFN, which support activation of NK-mediated cytotoxicity (summarized in [42]).

In acute HBV infection, DC as well as other antigen-presenting cells recruit inflammatory cells to the liver, activate NK cells and prime T cells. In the HBV transgenic mouse model, activation of antigen-presenting cells, including DC, led to profound inhibition of HBV replication [43]. To activate virus-specific T cell responses and cytotoxicity in the liver, however, antigen presentation by LSEC is crucial (Stabenow and Knolle, manuscript submitted).

Although HBV neither productively infects DC nor expresses antigens in DC [44], there is evidence that the virus may disturb DC function to facilitate its persistence. However, this is controversial and several investigators have failed to detect functionally impaired DC in HBV-infected patients [45, 46]. On the other hand, studies describe an impaired allostimulatory capacity of DC upon HBV inoculation [47], and an upregulation of IL-10 and suppression of IL-12 and IFN-α production have been shown for myeloid DC and plasmacytoid DC isolated from HBV chronically infected patients [48]. It has been described that reduction of TLR-9 expression by plasmacytoid DC in chronically infected patients correlates with impaired IFN-α production [49]. Hereby, HBV surface antigen (HBsAg) contact may cause functional impairment of DC [50, 51].

Once activated directly by viral infection or indirectly by NK, NKT or T cell-derived cytokines, macrophages produce cytokines with direct antiviral activity (IFN-α/β, TNF-α) as well as cytokines with immunoregulatory functions (IL-1, IL-6, IL-8, IL-10, IL-12, GMCSF). Kupffer cells, the resident macrophages of the liver, perform a number of functions including phagocytosis, pattern recognition and antigen presentation, and they secrete a number of proinflammatory mediators. Kupffer cells express TLR-2, TLR-3 and TLR-4 and produce IL-12 and IL-18, which in turn can stimulate NK cells to produce IFN-γ [52].

Liver resident Kupffer cells and peripheral macrophages can trigger a cascade of inflammatory events bridging innate and adaptive immune responses. Kupffer cells produce in a type I IFN-dependent manner chemokines such as CCL2, which in turn recruit circulating macrophages into the liver, where they produce CCL3 to attract IFN-γ-secreting NK cells [53]. This, however, has not been observed during HBV infection [15, 16]. Upon contact with HBV, they secrete proinflammatory cytokines. Among these, IL-6 has proven to have dominant antiviral activity. In hepatocytes, Kupffer cell-derived IL-6 activates a STAT-3-dependent acute phase response, and also activates MAP kinases. Activation of MAP kinases ERK and JNK decreased the levels of hepatocyte nuclear factors 1α and 4α. Thereby, IL-6 controls transcription of HBV RNAs by decreasing its essential transcription factors [15].

The precise role of Kupffer cells in HBV infection is still under investigation. In an HBV transgenic mouse model, Kupffer cells produce the chemokines CXCL9 and CXCL10, which assist recruitment of inflammatory cells into the liver [54]. Our own investigations show that Kupffer cells rapidly take up HBV virions in liver tissue section, but apparently do not degrade them (Esser and Protzer, unpubl. results). The precise role of Kupffer cells as antigen-presenting cells in the HBV-infected liver and the role of Kupffer cells during immune pathogenesis, however, have still not been carefully investigated.

## Adaptive Immune Responses in HBV Infection

Although there is increasing evidence that an impairment of the innate immune response contributes to chronicity, the hallmark of chronic HBV infection is the lack of a robust HBV-specific CD8+ and CD4+ T cell response [3, 55].

HBV-specific adaptive immune responses include CD4+ T helper cells, cytotoxic CD4+ and CD8+ T cells and B cells responsible for a neutralizing antibody response. CD4+ T cells are robust cytokine producers, required for cross-priming of CD8+ T cells and T cell-dependent B cell responses. Cytotoxic T cells clear HBV-infected hepatocytes and reduce the level of free virus, while the humoral immune response neutralizes free virus and prevents virus spread.

There is a clear correlation between timing, strength and specificity of the adaptive cellular immune response and the outcome of infection (summarized in [2]). HBV-specific CD4+ and CD8+ T cell responses become detectable 7–10 weeks after infection – significantly before the onset of symptoms [29]. Antiviral T cell responses are followed by humoral immune responses, which become detectable 10–12 weeks after infection [56].

This time interval between infection and induction of HBV-specific adaptive immune responses are in sharp contrast to what is known from other viral infections [57]. HBV-DNA and HBV antigens are undetectable in serum and the liver during the first 4–6 weeks after infection [56]. The lack of antigens in the early phase of infection is thought to be responsible for the delayed induction of HBV-specific adaptive immune responses.

### CD4+ T Cell Responses in Acute and Chronic Infection

HBV-specific CD4+ T cells are required to promote efficient cytotoxic CD8+ T cell responses and T cell-dependent B cell responses. A vigorous CD4+ T helper cell response against multiple epitopes within the HBV core protein (HBcAg) is detectable in the peripheral blood of almost all patients with self-resolving acute hepatitis B infection [58–60]. Most epitopes are shared with the secreted hepatitis B e antigen (HBeAg) due to the wide protein overlap. HBV surface protein (HBsAg) in contrast does not induce equally strong CD4+ T cell responses [58, 61]. However, the lower frequency of HBsAg-specific T cells is not due to a general weak immunogenicity of HBsAg, as vaccine recipients mount a strong and multispecific CD4+ T cell response against HBsAg [61, 62].

HBcAg-specific CD4+ T cell responses are detected within weeks after infection during the incubation phase of acute hepatitis B [29]. The cytokine profile of HBcAg-specific CD4+ T cells in self-resolving acute HBV infection is characterized by production of the Th1-type cytokines IFN-γ and TNF-α, suggesting that CD4+ T cell

responses may contribute to liver cell injury. When symptoms start, those CD4+ T cells are still present, but to a much lower frequency [29]. The number of HBcAg-specific CD4+ T cells further decreases after clinical resolution of infection [29, 63]. Therefore, HBcAg-specific CD4+ T cell responses are probably essential for the effective control of viremia and seem to be associated with HBV clearance.

Although recovery from acute HBV infection is clearly correlated with HBcAg-specific CD4+ T cells, CD4+ T cells directed against epitopes within HBV polymerase and the regulatory HBx protein may also play an important role. Up to now only polymerase epitopes have been identified in patients with self-limiting HBV infection, which elicit a CD4+ T cell response comparable to HBcAg-specific T cells [64].

There are clear differences regarding CD4+ T cell responses between patients with chronic infection and those who recover from HBV infection. T helper cell responses of chronically infected patients are weaker or even undetectable with a narrowly focused epitope specificity [58]. When HBV-specific T cell responses were followed from the time of infection until the onset of chronicity, a multispecific CD8+ T cell response in the absence of a corresponding CD4+ T cell response was detected [65]. These data suggest that the absence of a robust CD4+ T cell response prevents the maturation of a fully functional CD8+ T cell response able to control HBV infection.

In chimpanzees, the depletion of CD4+ T cells before HBV infection precluded T cell priming and caused persistent infection with minimal immunopathology [66]. Inoculation of different doses of HBV revealed that the kinetics of viral spread and CD4+ T cell priming determined the outcome of HBV infection [66].

Due to the low frequency of circulating HBV-specific T cells, there is only little data about differentiation, maturation and function of CD4+ T cells during the natural course of HBV infection. In addition, the role of CD4+ regulatory T cells ($T_{reg}$; see below) and the contribution of direct and indirect antiviral effects of CD4+ T cells need further studies.

### CD8+ T Cell Responses in Acute and Chronic Infection

During acute HBV infection, the development of a strong CD8+ T cell response directed against multiple viral antigens is associated with the resolution of HBV infection [2]. Cytotoxic CD8+ T cells directed against several epitopes within HBcAg, HBsAg, HBV polymerase and HBx protein have been identified. These CD8+ T cell responses persist for decades after clinical recovery from acute infection and remain present after spontaneous resolution of chronic infection [67–69].

In contrast, a weak and narrowly focused CD8+ T cell response unable to control HBV replication correlates with chronic infection and progressive liver injury [2]. Depletion experiments in chimpanzees demonstrate that CD8+ T cells are the main effector cells responsible for viral clearance and disease pathogenesis during acute HBV infection [70].

A series of elegant studies using adoptive T cell transfer in HBV transgenic mice and chimpanzees revealed that CD8+ T cells serve a dual function: they eliminate HBV-infected cells by their cytotoxic function, but also abolish HBV gene expression and replication in the liver without killing the hepatocytes. The second function, which is antiviral but noncytolytic, is mediated by the secretion of antiviral cytokines such as IFNs and TNF-α [71–73].

In HLA-A2 positive carriers, the HBcAg 18–27 epitope is regarded as the immunodominant CD8+ T cell epitope. This response, however, becomes undetectable in chronically infected patients with a viral load of $>10^7$ copies/ml [74]. In contrast, HBsAg and polymerase-specific CD8+ T cell responses remain detectable suggesting a lack of antiviral functions [75].

In chronically infected patients, amino acid mutations within HBcAg epitope 18–27 have been described leading to a functional loss of the corresponding CD8+ T cell response, whereas mutations within HBsAg and polymerase epitopes are rare [74, 76, 77]. These data indicate a higher selection pressure by HBcAg-specific CD8+ T cells compared to T cell responses against other HBV antigens.

Intrahepatic CD8+ T cells seem to be impaired in patients with chronic HBV infection regardless of their specificity [78]. Compared to healthy donors, a large influx of nonantigen-specific CD8+ T cells into the liver was observed. These liver-associated CD8+ T cells show poor IL-2 production and proliferative capacity, but maintain their capacity to produce IFN-γ and TNF-α. Deficient IL-2 production hampers not only HBV-specific T cell proliferation, but may also confer protection against T cell apoptosis [79].

### Collapse of HBV-Specific T Cell Responses in Chronic Hepatitis B

The inability of the immune system to control HBV infection results in a collapse of virus-specific immunity. The collapse of HBV-specific T cell responses marked by qualitative and quantitative changes in CD8+ T cell responses is the most prominent characteristic of chronic HBV infection. In patients with chronic infection, the frequency and function of HBV-specific CD8+ T cells is inversely proportional to the viral load [74, 80, 81].

During persistent viral infections, T cells are continuously confronted with viral antigens [42, 82]. Such continued triggering of T cells may lead to T cell exhaustion, a phenomenon first described for chronic murine lymphocytic choriomeningitis virus infection [83], but also seems to apply for other persistent infections such as HBV and HCV [84]. As is known with lymphocytic choriomeningitis virus infection, sustained presence of viral antigens leads to a progressive decline in function and finally to a depletion of virus-specific T cells [85, 86]. Chronic HBV infection is also known to delete or tolerize antigen-specific T and B cells for a long time [87–89], but the mechanisms are still poorly understood.

T cell exhaustion is mediated by various regulatory mechanisms including the level of antigen exposure, expression of inhibitory receptors, immunosuppressive cytokines such as IL-10 and TGF-β, dependence on growth factors such as IL-2, and the presence of $T_{reg}$ (summarized in [82]).

The mechanisms underlying T cell exhaustion during chronic HBV infection are not fully understood. Exhaustion results in the failure to produce essential cytokines (often observed in a hierarchical manner; IL-2 > TNF-α > IFN-γ), the inability to expand following antigenic stimulation and a substantial loss of cytolytic activity [79, 80, 90].

Besides the ongoing encounters of T cells with high viral antigen loads, the collapse of HBV-specific T cell responses may be supported by additional mechanisms such as a direct immunosuppression by HBV proteins [50, 88, 91] and an impairment of antigen-presenting DC [46, 47, 92].

Deletion of certain HBV-specific CD8+ T cells is responsible for the most severe quantitative changes during antiviral immune response. Microarray analysis revealed that proapoptotic genes are upregulated in HBV-specific T cells from chronically infected patients as when compared to individuals with self-resolving infection [79]. In particular, the proapoptotic protein Bcl2-interacting mediator (Bim) was highly upregulated, and mediated apoptosis of CD127low HBV-specific CD8+ T cells in chronically infected patients [93]. Remaining CD8+ T cells in those patients were CD127high and expressed high levels of the antiapoptotic protein Mcl1 known to counteract the proapoptotic effects of Bim [94].

Recently, much attention has been devoted to the relevance of inhibitory receptor expression on T cells during chronic infections, including PD-1, Tim-3, CTLA-4 and LAG-3 [82]. Many of these markers are also expressed on activated effector T cells early during acute infections. Sustained expression, however, seems to be associated with chronic viral infection.

Programmed death-1 receptor (PD-1), the most prominent inhibitory receptor, is expressed on activated T cells, while its two ligands (PD-L1 and PD-L2) are expressed on DC, LSEC and Ito cells in the liver [52]. It is well known from the lymphocytic choriomeningitis virus model, that T cell functions (i.e. cytokine production as well as proliferative and cytolytic capacity) can be restored by blocking the inhibitory PD-1 pathway. Similar results were obtained using a HBV transgenic mouse model to study the effect of PD-1 mediated T cell exhaustion [95] where blocking of PD-1 leads to increased numbers of cytokine-producing CD8+ T cells [96].

Undoubtedly, inhibitory receptors expressed during chronic HBV infection play a role in silencing CD8+ T cell responses. However, caution must be taken in targeting such immunomodulatory pathways, as many of these pathways are important for maintenance of immune homeostasis.

### The Role of $T_{reg}$

Negative regulation of CD8+ T cell responses during chronic HBV infection can also be mediated by immunosuppressive cytokines such as IL-10 and TGF-β. In recent years there has been increasing evidence for a role of $T_{reg}$ in maintaining HBV infection [97]. $T_{reg}$ represent an extremely diverse subpopulation of either CD4+ or CD8+ T cells characterized by the expression of several phenotypic markers. $T_{reg}$ capable of downregulating antigen-specific immune responses can act via direct cell-cell contact or by secretion of immunosuppressive cytokines, i.e. in an antigen-nonspecific fashion.

The impact of $T_{reg}$ on HBV pathogenesis has been analyzed in several studies, but the results are contradictory (reviewed in [98]).

Increased frequencies of circulating $T_{reg}$ have been reported in patients with chronic HBV infection in some studies [99, 100], whereas other studies have shown no correlation [101, 102]. HBV-specific $T_{reg}$ have also been found in chronically HBV-infected liver transplant recipients [103]. Similarly controversial data were obtained when $T_{reg}$ frequencies were evaluated in relation to viral load [99, 104].

Depletion of $T_{reg}$ increased the function of HBV-specific T cells from chronic HBV carriers as well as from individuals with resolved infection when tested in vitro [99, 101]. Whether this may be a valid therapeutic approach, however, remains open.

### Humoral Immune Responses

The function and role of T cells in HBV control is well established. To what extent the humoral immune response contributes to the control of chronic HBV infection is less clear.

HBV-specific antibodies are indicators for certain stages of these diseases. Antibodies against HBsAg (anti-HBs), detectable in patients who have recovered from acute hepatitis B and in HBV-vaccinated individuals serve as neutralizing antibodies, able to inhibit viral attachment and entry (reviewed in [2]). Induction of anti-HBs is sufficient to completely prevent infection. In patients who recover from acute infection, seroconversion to anti-HBs is generally considered to be a marker of disease resolution. Recovery from HBV infection results in lifelong protective immunity mediated by neutralizing anti-HBs antibodies. In chronic HBV infection, anti-HBs is undetectable in the serum. However between 0.5% and 0.8% of chronically infected individuals with sustained presence of the inactive hepatitis B phase will clear HBsAg every year [105].

Unlike anti-HBs, antibodies to HBV core protein (anti-HBc) are not protective. Anti-HBc can be readily detected during the early phase of infection and anti-HBc is present in virtually all patients who have ever been exposed to HBV. Its presence alone cannot be used to distinguish acute from chronic infection.

HBeAg-specific antibodies (anti-HBe) appear late during infection and indicate a favorable outcome of infection. In acute hepatitis, clinical recovery is associated with loss of serum HBeAg and seroconversion to anti-HBe. In chronically evolving hepatitis, clearance of serum HBeAg and development of anti-HBe marks the transition from high replicative to low replicative inactive hepatitis B [2]. Only 10–20% of chronically infected individuals will remain in the high replicative phase after seroconversion to anti-HBe [105], mainly due to the emergence of HBV mutants [106].

### Conclusion

Innate and adaptive immune responses act as two interlocking defense lines against HBV infection. Successful elimination of HBV appears to require initial virus suppression by the innate immune response accompanied by recruitment of effector T cells leading to activation of an adaptive immune response. The fine-tuning and the interaction of these responses as well as effective timing still need to be defined.

### Disclosure Statement

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