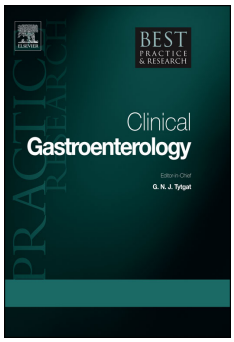
Accepted Manuscript



Hepatitis B virus infection and the immune response: The big questions

Lauke L. Boeijen, Ruben C. Hoogeveen, Andre Boonstra, Georg M. Lauer

PII: S1521-6918(17)30041-0

DOI: 10.1016/j.bpg.2017.05.003

Reference: YBEGA 1513

To appear in: *Best Practice & Research Clinical Gastroenterology*

Received Date: 1 April 2017

Revised Date: 26 April 2017

Accepted Date: 13 May 2017

Please cite this article as: Boeijen LL, Hoogeveen RC, Boonstra A, Lauer GM, Hepatitis B virus infection and the immune response: The big questions, *Best Practice & Research Clinical Gastroenterology* (2017), doi: 10.1016/j.bpg.2017.05.003.

Hepatitis B virus infection and the immune response: The big questions

Lauke L Boeijen[1], Ruben C Hoogeveen[1,2], Andre Boonstra[1,*], Georg M Lauer[2]

[1] Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Center Rotterdam, the Netherlands

[2] Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

***Corresponding author**

Andre Boonstra PhD

E-mail: p.a.boonstra@erasmusmc.nl

Keywords: Hepatitis B; NK cell; B cell; T cell; Kupffer cell; adaptive; innate; immune response

Word count:

Abstract: 150

Text (including references): 8127

Text (excluding references): 5173

Practice Points

- Clinical events during the natural history of chronic hepatitis B virus (HBV) are intricately linked to the host immune system, but the details of host-virus interactions remain insufficiently understood
- HBV is sensed by the immune system, but the ensuing antiviral responses are not sufficient to prevent chronicity in most newborns and some adults

Research Agenda

- To understand the basic mechanisms of HBV clearance, persistence and long lasting HBV cure by using the most advanced and detailed biomedical techniques, down to the single cell level.
- To gather more information on the intrahepatic, rather than the peripheral, immune populations, as their phenotype and function are very different
- To focus on longitudinal assessment of immune parameters, thereby excluding effects of cohort variation in HBV research

Abstract

Clinical events and the host immune response during hepatitis B virus (HBV) infection are intricately linked. Despite decades of research, important questions concerning the immunopathogenesis of chronic HBV infection remain unanswered. For example, it is unclear which immune parameters facilitate persistence, and if HBV can be completely cleared from the human liver. Recent technological breakthroughs now allow researchers to address these seemingly basic, but essential questions surrounding HBV immunity. It will be important to better define the molecular underpinnings of immune cell function and dysfunction during chronic disease and in controlled infection, with particular focus on the liver, as little information is available on the intrahepatic compartment. In the near future, it may be possible to solve some of the controversy surrounding the immune responses to HBV, and establish the features of both the innate and adaptive arms of the immune system required to achieve sustained control of HBV infection.

HBV and the immune response: The big questions

The natural history of HBV infection is exceptionally complex, and, not surprisingly, so are the host immune responses. For almost every clinical or virological change during the course of HBV infection, there are proven or suspected correlates in host immunity, some of which might represent the underlying cause of the observed alterations in disease, while others will be consequences of the change, for example in viral load. Recent advances in experimental and analytical capabilities now allow researchers to address seemingly basic but still unsolved questions surrounding HBV immunity. We have formulated several questions regarding host-virus interactions whose answers we deem most relevant for a better understanding of HBV immunopathogenesis and for the development of novel therapeutic strategies (Figure 1). After addressing these issues, we will highlight some of the progress that has already been made in the field of chronic HBV.

First is the question of what determines the different outcome in acute HBV infection, as the virus is spontaneously controlled in some subjects, while becoming chronic in others. Here we have to consider two completely distinct scenarios: adult HBV infection typically leads to HBV control, whereas natal infection results in chronicity in almost all cases, with declining but still very significant chronicity rates if infection occurs before the age of 5. However, not much is known of the immunological processes, resulting in these striking differences. We know much more about acute and chronic infection “after the fact”, i.e. in adult patients who have established chronic infection or successful HBV control. Studies during the early phase of infection, when the outcome is not yet determined, are much more difficult to undertake. The best opportunity for further insights will be studies in young children who present with early infection. Immunological studies in this population are feasible, but until recently had to be very limited in scope, in part because only small amounts of blood can be drawn for analysis. New technologies now enable analysis of low volume samples with high resolution and without the requirement of research facilities close to the clinic.

Immune responses of chronically infected adults have been performed for decades, but, as we will describe in this review, often have conflicting results. These variations are partly caused by methodological differences, but the variation among patient characteristics also has significant impact [1]. In addition to establishing the range and variation of immune cell parameters during frequent subtypes of chronic infection, we also need to know if, and to what degree, antiviral therapy is able to reconstitute those parts of the HBV immune response previously insufficient for functional control. At least based on the different antibody patterns observed in treated patients, it seems obvious that restoration of immunity is not uniform, and certainly in most subjects not sufficient for viral control in the absence of

therapy. But what exactly is restored, whether immune restoration is just a consequence of diminished viral replication that is lost in the absence of antiviral treatment, or whether restored immunity is independent or even the cause of viral suppression, needs to be evaluated in much more detail. In this context it is also important to define whether circulating viral proteins, such as HBs antigen (HBsAg), are indeed the key immunosuppressive agents that *in vitro* studies have suggested[2].

Another important question is what level of HBV control can actually be achieved through the host immune response. It is clear that even after HBsAg clearance from the blood, covalently closed circular DNA (cccDNA) can remain in hepatocytes indeterminately, evident from the well-documented cases of HBV reactivation in anti-HBsAg positive subjects undergoing immune-ablative therapies. Whether this is the dominant or even the only scenario in “resolved” HBV infection, or whether some patients are indeed able to completely clear HBV DNA based on an even more effective immune response, will require larger studies analyzing liver tissue for cccDNA in these populations.

Finally, greater insights need to be generated into what actually happens in the liver as the site of infection, since both the composition of immune populations as well as their functional and phenotypic profiles are different from what is observed in the blood. New technologies now allow analysis of rare immune populations in the liver, down to cells on the single cell level. Similarly, we should utilize the improved tools for the integrative analysis of cellular processes and immune functions in order to understand the immune response to HBV more holistically. Many components of the immune response, both innate and adaptive, have to act in concert in different scenarios of viral control and viral persistence. Below we will broadly summarize the current knowledge of both innate and adaptive arms of the immune responses to HBV infection**.** While the studies described below do not yet provide definitive answers, they are the foundation for future investigations into the issues raised above.

Innate immune responses to HBV

HBV is transmitted upon contact with blood or body fluids of an infected person. A minute amount of HBV virions in the bloodstream is sufficient for infection of hepatocytes [3]. The HBV virion enters the hepatocyte via the sodium taurocholate co-transporting polypeptide (NTCP), a bile receptor located on the basolateral membrane, contributing to its specificity to human or chimpanzee hepatocytes [4]. After the envelope protein mediates fusion of the viral and endosomal membranes, the capsid enters the cytoplasm and the viral DNA is released into the nucleus through nuclear pores. Upon import into the nucleus, HBV can integrate into the host genome or be present as non-integrated covalently closed circular DNA (cccDNA). The cccDNA molecule will serve as a template for replication leading to infection of more hepatocytes, and can persist even after HBsAg loss [5]. As circulating blood passes the liver, HBV can easily spread to other hepatocytes.

The detection of HBV by infected hepatocytes

The sensing of HBV and triggering of intracellular antiviral mechanisms can occur on the cell membrane by Toll-like receptors (TLR), in the endosome by TLR7 or TLR9, and in the cytoplasm by sensors, such as intracellular retinoic acid inducible gene I (RIG-I) and melanoma differentiation gene 5 (MDA5) upon ligation with viral proteins or nucleic acids. Although some controversy exists, it has been reported that TLR2, MDA-5 and RIG-I are involved in sensing of HBV. For the cytoplasmic sensor RIG-I, it was demonstrated that HBV pre-genomic RNA triggered its activation, resulting in the release of interferon (IFN)-λ but not type-I IFN by HBV infected primary human hepatocytes and hepatoma cell lines[6]. Release of IFN by hepatocytes and possibly other cells induces expression of hundreds of IFN-stimulated genes (ISGs) with potent antiviral activity. However, the HBV-induced IFN responses are weak [7, 8], which is reflected by the usual lack of clinical symptoms during the acute HBV infection. Also, early data from animal models showed that HBV does not induce the release of type-I IFN [9, 10]. The absence of symptoms and the modest IFN induction by HBV led to adaptation of the term ‘stealth virus’. However, IFN responses and ISG induction are present, albeit marginal compared to other chronic viruses [7, 8]. The use of cccDNA as a transcriptional template in the nucleus likely contributes to HBV’s capacity to limit detection in hepatocytes. Adding to this, viral proteins, like HBV polymerase and HBx protein, directly inhibit the cellular machinery that detects replication intermediates. It is currently unknown which pattern recognition receptor or signaling pathway is essential for early viral control *in vivo*, and perhaps more relevant to the majority of patients, to HBV persistence in humans.

As the human liver is the site of HBV replication and contains high viral protein concentrations, the most appropriate approach for addressing basic questions concerning HBV detection is to evaluate liver material. Unfortunately, such studies are rare. Lebosse et al. [11] analyzed RNA extracted from liver biopsies of chronic HBV patients and showed low intrahepatic IFNα expression relative to healthy controls, which was unaffected by viral replication. Previously, we analyzed liver biopsies from patients in specific clinical phases of chronic HBV. By comparing the transcriptome of liver and blood samples from patients in distinct clinical phases of HBV we found that, ISG are transcribed even in patients presumed to be 'immune tolerant' to HBV [12]. Transcription of catalytic polypeptide-like 3B, a protein related to cccDNA degradation [13], was increased in the immune tolerant phase, which suggests that the capacity to limit the establishment of high amounts of cccDNA may be different between phases, but not absent in any phase. These two studies using human liver tissue raise significant doubt about the labels 'stealth virus' and 'immune tolerant', as HBV is indeed sensed by the host immune system and antiviral responses are initiated, even as they are not sufficient to halt viral replication and spread in persons with chronic infection. Whether these differences of innate intrahepatic responses are caused by chronic HBV replication, or reflect immune characteristics that select patients for persistent infection, remains to be clarified using longitudinally acquired samples of human liver.

The interaction of antigen presenting cells and HBV

Intrahepatic leukocyte populations lining the sinusoidal lumen of the portal branches as well as hepatocytes are constantly in contact with huge amounts of bacterial antigen derived from the gut. Tolerance during exposure to high antigen loads is essential for host survival, and the relative tolerogenic milieu of the liver is well described[14]. Illustrative of the complex balance of the liver immune system is the fact that, despite its tolerant nature, HBV is cleared after transmission in the vast majority of infected individuals. For the development of treatment strategies, it is important to evaluate the leukocyte populations involved in this process. Liver residing antigen presenting cells, like Kupffer cells and dendritic cells (DC), could potentially modulate host immune responses to a phenotype enabling chronic viral infection, but limited information exists on the interaction between these cells and HBV (Figure 2). DC are crucial for their ability to efficiently present antigen to naïve CD4 T cells, or to CD8 T cells via cross-priming. At present, the impact of persistent HBV infection on the DC compartment is not fully clear. *In vitro* studies have demonstrated that the presence of viral antigen may limit DC functionality[15]. Both plasmacytoid and myeloid DC can present antigens to T cells, and can, depending on the cytokine milieu, skew T cell effector function towards tolerance or activation. Plasmacytoid DC are specialized in the production of high levels of type I and type III IFN, thereby potentially initiating innate immune responses to

HBV after recognition of viral nucleic acids via TLR7 or TLR9. However, based on *ex vivo* assays, DC function during chronic HBV patients may be hampered. The frequency of both myeloid DC and plasmacytoid DC is not changed in the majority of studies with patient material, but others have reported various functional differences compared to healthy controls [16-19]. Possibly via downregulation of TLR7 or TLR9, plasmacytoid DC seem to be less capable to produce IFNα, especially when DC are derived from patients with considerable liver inflammation [17, 20].

Kupffer cells are liver resident macrophages, and make up 15-20 % of the intrahepatic leukocytes [21]. We have previously shown that Kupffer cells can interact with HBsAg *in vivo*, which led to increased pro-inflammatory cytokine production compared to healthy controls[22]. However, HBV proteins like HBeAg may interfere with Kupffer activation by downregulation of TLR expression as shown *in vitro*[23]. In line with this finding, patient-derived peripheral monocytes were also found to have lower TLR2 expression compared to healthy controls[24]. Kupffer cells can play different roles in the presence of HBV antigens, as was illustrated in a rat model, where HBV causes Kupffer cells to produce more of the pro-fibrogenic and tolerogenic cytokine TGF-β1, as opposed to the pro-inflammatory cytokines IL-6, IL-1 and TNF[25]. To prevent continuous activation and excessive intrahepatic inflammation, Kupffer cells become refractory to subsequent endotoxin challenge, which may contribute to the tolerogenic liver environment. Furthermore, Kupffer cells can interact and influence other immune cells, either directly via cell-cell contact or indirectly via the activity of cytokines. Indirect activation of natural killer (NK) cells can take place via Kupffer cell-derived IL-12 and IL-18[22, 26].

NK cell function and phenotype in chronic HBV

NK cells can recognize and lyse infected cells, and may therefore be an important effector cell that plays a role during persistence of HBV. Indeed, the frequency and function of NK cells in peripheral blood of chronic patients has been studied extensively, and it seems clear that the balance of NK cell stimulatory and inhibitory signals significantly impacts their activity, which in turn correlates with patient outcomes during the natural history of chronic HBV. One of the major effector functions of NK cells, the production of the antiviral and anti-fibrotic cytokine IFNɣ, is reported to be unaffected during chronic HBV by some studies [27, 28] but reduced in others[25, 29, 30]. IFNɣ release by NK cells mediates the non-cytolytic clearance of HBV by virus-specific CD8 T cells[31, 32]. Another beneficial effect may be that IFNɣ induces the production of the antiviral APOBEC proteins A3A and A3B directly affecting the integrity of cccDNA [33]. In a transgenic mouse model, the potential of IFNɣ was dramatically demonstrated by injection of the NKT cell activating agent alpha-galactosylceramide, which stopped HBV from replicating, and led to infiltration of NK cells

into the liver[34]. In humans, however, alpha-galactosylceramide affected HBV DNA levels in only a subset of human chronic HBV patients, and the treatment was not well tolerated [35]. During chronic HBV infection, the cytotoxic capacity of NK cells seems to remain intact[36], which together with the impaired cytokine production as described by some studies, suggests the existence of a functional dichotomy of NK cells in chronic HBV. However, given the conflicting reports in the literature, it is obvious that more detailed and more controlled studies need to be performed. A recent study performed by our group compared NK cell phenotype and function in blood among HBV patients in different clinical phases. Interestingly, despite big differences in viral load and ALT levels between patients, the NK cell compartment demonstrated only subtle differences between the patients cohorts[37]. Concerning their activation, a combination of IL-12, IL-15 and IL-18 secreted by other intrahepatic immune cell populations, including activated Kupffer cells, seems to be the most likely mechanism. Direct contact with virally infected cells or HBV DNA in the blood may be a less effective stimulus for NK cells, based on work by Bonoroni et al., who describe no correlation between viral load and peripheral NK activation [38], which is in line with our previous study[37]. Also supporting this finding is the observation that tenofovir-induced viral load reduction did not significantly alter intrahepatic NK cell activation as demonstrated using the analysis of fine-needle aspirate liver biopsies, even after six years of viral suppression[39]. The capacity of intrahepatic NK cells to inhibit fibrogenesis through IFNɣ production [40], or by killing stellate cells that are driving collagen syntheses in the liver [41], may also be relevant for clinical outcomes. However, in contrast to the above described beneficial effects of NK cells, their activation may alternatively facilitate HBV persistence, as was recently illustrated by the finding that HBV-specific CD4 T cells expressing death ligands (not seen in healthy controls) can be targets for NK cell mediated killing [42]. In a similar fashion, NK cells have been shown to kill HBV-specific CD8 T cells.

Adaptive immune responses during chronic HBV

Adaptive immune responses by virus-specific CD4 and CD8 T cells, B cells and antibodies are indispensable for HBV control[43]. These responses develop relatively late in HBV infection compared to other viral infections: at ten to twelve weeks post exposure[44]. This late emergence of adaptive immunity is thought to be a consequence of the stealthy nature of early HBV infection, with low viral replication and minimal to no activation of innate immunity. This delayed response is not per se a factor in HBV chronicity, as it is also observed in the vast majority of adult patients who go on to control the virus spontaneously. While we have learned many important details about how adaptive immune responses emerge and evolve from the early phase of infection throughout viral resolution or chronic

viremia[43], much remains to be determined in order to fully appreciate the exact mechanisms driving successful and failing adaptive immune responses targeting HBV. This is especially true as our appreciation of the complexity and diversity of different players of the adaptive immune response, mostly defined in animal models, has grown faster than our ability to translate such integrative concepts into human infection[45].

Detection of HBV-specific CD4 T cells

Virus-specific CD4 T lymphocytes are key regulators of both efficient B cell/antibody and CD8 T cell responses and thus are thought to be essential to the control of most viral infections [46]. Unfortunately, virus-specific CD4 T cells are also notoriously difficult to study, as they are rather low in frequency and complex in their phenotypes and function. Overall, our understanding of the HBV-specific CD4 response remains incomplete. The seminal chimpanzee studies defining the importance of CD4 T cells cells by analyzing the impact of CD4 T cell depletion on the course of infection are not straightforward in their findings, as depletion of CD4 T cells before infection leads to chronic HBV infection, while depletion just before the rise of viremia and liver enzymes did not alter the natural course of infection[46, 47]. These findings support a critical role of CD4 T cells in the control of HBV infection, but also raise questions about the exact role of T cell help and its mode of action. Generally, HBV-specific CD4 T cells appear in the blood seven to ten weeks after infection, in parallel with the emergence of HBV-specific CD8 T cells and antibodies[44, 48]. The CD4 T cells mostly target epitopes in HBV core, though minor responses against surface, polymerase and x protein have also been described[49-52]. In patients with acute or controlled HBV infection, CD4 responses are more broadly directed and more vigorous, compared to patients with established chronic viremia. Functionally, HBV-specific CD4 T cells have been shown to predominantly secrete Th1 type cytokines[53], though overall studies assessing the phenotype and function of the cells directly *ex vivo* are very few and rather limited. In this context it is important to remember that most of these studies were performed in the era when standard proliferation and ELISpot assays where the only means to assessing virus-specific CD4 T cell responses, and as we have learned from other infections such as with HCV, these functional assays might miss significant parts of the response[54]. In addition, HBV-specific CD4 T cells at the site of infection might be quite different than those analyzed in the blood. Indeed, after *in vitro* expansion from liver biopsies obtained in patients with chronic infection, intrahepatic CD4 T cells have shown a distinct functional profile compared to those derived from blood, with the secretion of IL-4 and IL-5 in addition to Th1 type cytokines[55]. A reassessment of HBV-specific CD4 T cell responses using current methodologies, i.e. HLA class II tetramers, for direct *ex vivo* phenotyping and flow based cell sorting, followed by omics analyses, seems imperative in order to obtain a more detailed

understanding of this central component of the adaptive immune response. These methods enable analysis of single cells, and thus might allow *ex vivo* studies even in infection at young age, for which we currently have no significant data about the CD4 T cell response.

Detection of HBV-specific CD8 T cells

In contrast to CD4 T cells, CD8 T cell responses have been studied much more widely and in greater detail, including direct *ex vivo* analyses[56]. They are essential for HBV control, as their depletion invariantly led to chronicity in chimpanzees[46]. HBV-specific CD8 T cells become readily detectable six to eight weeks after infection in adults, and their appearance coincides with a decrease in viral load that is observed even before the onset of liver injury[44], supporting the contribution of non-cytolytic elimination of intracellular virus to viral control. CD8 lymphocytes targeting all HBV proteins have been identified[43] and shown to have cytolytic as well as non-cytolytic effector functions[57]. Non-cytolytic effector functions could be especially relevant, as hepatocytes have been shown to be quite resilient to cell-mediated killing *in vitro*[58]. While the exact immunological determinants of CD8 mediated HBV control are difficult to define with certainty in humans, the development of a sustained, broad and polyclonal CD8 T cell response that is highly functional is seen as *conditio sine qua non* for HBV control. To what degree such a CD8 response is dependent on other arms of the immune response, be it CD4 T cell help or a preceding innate response, is not fully clear.

Importantly, there is also a good amount of literature on intrahepatic CD8 T cells targeting HBV, mostly from chronic infection. Detection of HBV-specific CD8 T cells by tetramer staining of cultured intrahepatic lymphocytes revealed that the frequencies of virus specific CD8 T cells were inversely correlated with the degree of liver injury[59], supporting the hypothesis that specific responses might be not only important for disease resolution, but also for protection from progressive liver disease. Another pioneering study using fine needle aspirate biopsies to analyze intrahepatic lymphocytes during acute infection provided longitudinal characterization of HBV-specific CD8 T cells directly *ex vivo* without *in vivo* expansion. HBV-specific CD8 T cells were highly enriched in the liver during the acute phase of infection and remained detectable after HBsAg seroconversion and full clinical recovery[60]. A similar approach was also employed in chronically infected patients; revealing that virus specific CD8 T cells were most readily detected during the inactive carrier phase[61]. Altogether, current evidence illustrates differences in phenotype and function between peripheral and intrahepatic lymphocytes, and during different stages of HBV infection. Since one can now analyze cells from liver biopsies or fine needle aspirates with

much more powerful analytic tools, this opportunity should be used to deepen our understanding of the CD8 T cell response in acute and chronic infection, and during therapy.

Chronic HBV and functional T cell impairment

Once patients are chronically infected, especially in those patients exposed in early childhood, there is usually an extended phase that has been described as immune tolerant, with high levels of viremia but no or very little liver disease. It has been postulated that during this phase T cell responses are mostly absent and/or non-functional. Recent data indicate that this is not the case, and instead T cells are readily detectable in these patients and seem, if anything, more and not less functional than those in later, so called immune active, stages of disease[12]. This “immune tolerant” phase of HBV might hold more surprises and certainly warrants further investigation. During later stages of chronic infection, the repertoire of detectable HBV-specific T cells is limited and their frequencies are rather low, at least in the blood, and especially so in patients with high viral loads[62]. Preserved HBV-specific CD8 T cells become gradually more functionally impaired, or exhausted, which is thought to be driven by persistent antigen exposure. 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 and 2B4, reduced proliferative capacity and poor effector functions such as reduced IFNɣ and IL-2 secretion [63-65]. The exhausted state is also associated with distinct expression patterns of transcription factors, compared to functional effector or memory T cells, most notably low expression of T-bet[66] The cells seem also increasingly susceptible to apoptosis through upregulation of Bim and TRAIL-R2[67, 68], two key regulators of cell death. Whether the functional exhaustion of HBV-specific CD8 T cells is the sole or dominant contributor to CD8 T cell failure, or whether the virus can also escape through the generation of viral escape sequence variants, like in HIV and HCV infection, is currently an open question. HBV as a DNA virus is much more genetically stable compared to HIV and HCV, and early studies supported the idea that HBV displayed little variability that could be linked to immune pressure[69]. However, a recent study revealed viral variation compatible with escape mutations in both the core and envelope sequence and to a lesser extent in the polymerase sequence of HBV[70, 71]. Future studies in this area should further define the mechanisms of T cell exhaustion and whether exhausted T cells in chronic HBV infection could potentially be reinvigorated through immunotherapeutic interventions. In addition, we need to define the contribution of viral variants to the failure of HBV adaptive immunity.

Functional T-cell restoration under treatment

If T cell exhaustion is principally maintained by persistent antigen exposure, long-term therapy with successful control of viremia, should, to some degree, be capable of restoring T cell function. This would be especially relevant for immunotherapeutic approaches that most likely will be applied in the context of antiviral therapy. This has been studied in some detail, though the complexity of antiviral treatments, but also of the course of chronic HBV infection itself, makes interpretation of the data challenging [72-75]. Boni et al compared nucleos(t)ide treated chronic HBV infected patients to those with untreated or resolved infection. PBMC were analyzed either directly *ex vivo* with class I dextramers or for their proliferative capacity and cytokine production after *in vitro* expansion. *Ex vivo* analysis of virus-specific T cells suggested continued impairment even after long-term treatment, though after *in vitro* expansion some functional properties were partially restored, indicating some improvement of the T cell populations[73]. A similar study design has also been performed in IFNα treated chronic HBV patients and failed to detect improved T cell function, at least in terms of cytokine production[74]. Whether the modest T cell recovery is due to the remaining high levels of HBsAg in serum, despite the control of viral replication, is an important and controversial question. While it is widely assumed that surface antigen has a directly negative effect on T cell function, this hypothesis is mostly based on *in vitro* studies using high doses of recombinant proteins. One wonders how this effect mediated by circulating proteins could be specific to HBV-specific immune cells as there is no experimental evidence that adaptive immune responses to other pathogens are similarly impaired as those targeting HBV. Clinically chronic HBV patients are also not showing signs of significant immune impairment. Further studies analyzing the impact of circulating HBV antigens on HBV immune responses *in vivo* are needed, together with a more detailed and comprehensive assessment of the integrated adaptive immune response in well-defined longitudinal cohorts undergoing structured antiviral treatment and treatment interruptions.

Regulatory T cells

Regulatory T (Treg) cells are T cells that can regulate the local immune response via cell-cell contact or via secretion of cytokines, such as TGF-β or IL-10. Treg cells play a central role in immunological tolerance to self- and foreign antigens by suppressing activation, proliferation and effector functions of a wide range of lymphocyte subsets[76]. The best-known are CD25+FoxP3+CD4 natural Treg cells that directly inhibit other T cells. In addition, an increasing number of other regulatory T cell types have been described, including CD8 T cells with inhibitory functions. Most studies in HBV have focused on natural CD4 Treg cells in the peripheral blood, where these cells usually constitute between 3 and 10% of the total CD4 T cell population. Confusingly, while some studies have found increased intrahepatic and peripheral Treg cell levels in chronic HBV compared to healthy individuals and self-

limited HBV infection, others did not, similar to results in HCV infection[77-82]. This is complicated by different methods to define the Treg populations, which have evolved over time from simple staining of CD25 antigen to increasingly more complex and specific combinations of phenotypic markers, including FoxP3, PD-1 and CD127. Given their local mode of action, the presence and functionality of intrahepatic Treg cells should be most consequential, but almost all data is from the blood. It remains to be seen whether their main role is enabling chronic infection or rather protection from active liver disease in the context of long-term viremia.

B cells and antibodies

HBV-specific antibodies are clearly able to provide sterilizing immunity after vaccination. Clinically, different antibody profiles are important for the diagnosis and characterization of acute and chronic HBV infection. Much less is known about the relative contribution of B cells and HBV antibodies to viral control once infection has been established. B cells have been reported to display an activated phenotype and seem functionally intact, even at later stages of infection. We also recently demonstrated that blood gene signatures indicative of B cell responses were highly active during the immune active phase in chronic HBV patients, using a systems biology approach [12]. HBV antibodies target the surface, polymerase, core and x proteins of HBV, and appear ten to twelve weeks after infection. Detection of antibodies against HBsAg is the clinical correlate of protective immunity, but HBs antibodies likely contribute to control of viremia even in chronic infection where they are not detected by the standard antibody assays as they form immune complexes that prevent viral attachment and entry. In contrast, the core antibody (anti-HBc) is detectable in all stages of infection and considered not to mediate viral control, though the passive immunization of anti HBc/HBe does seem to prolong the incubation period in chimpanzees[83]. Overall a better characterization of how both B cells and antibody responses contribute to viral control during acute and chronic infection is urgently needed, as most likely a concerted effort by T cells and antibodies will offer the highest likelihood of effective HBV control. In this context it should also be noted that treatment with immunomodulatory drugs can lead to reactivation of controlled HBV infection[84]. The classic example is rituximab, a monoclonal antibody targeting CD20-expressing B cells, thus eliminating B cells and suppressing antibody production. It is not clear, however, whether the effect on antibodies is the sole or main cause for HBV reactivation, as rituximab treatment also impacts CD4 T cells and potentially indirectly also CD8 T cell memory[84, 85]. A detailed characterization of virus-specific immune responses during treatment with such agents should reveal important insights into immunological changes that might lead to diminished HBV control.

Summary

Despite numerous immunological studies performed in HBV infected patients several key questions remain unanswered. For decades, the lack of HBV models facilitating replication of human strains hampered scientific progress, and even patient data can be misleading due to the huge variation among study cohorts of patients with chronic infection. Through careful patient selection and the use of modern biomedical techniques, like genomics and proteomics, researchers can now tackle basic questions regarding HBV immunopathogenesis. The aim should be to determine immune parameters associated with persistence, clearance and recurrence of HBV. Also, the mechanisms of recognition of viral antigens in chronic infections by hepatocytes in vivo remain unclear, with a particular need for ex vivo assays. Potential antiviral effector cells like Kupffer cells, natural killer cells and dendritic cell populations may be less functional during chronic infection, possibly leading to infrequent and exhausted HBV-specific T cells in adults. By the use of modern techniques, the function and phenotype of both peripheral and intrahepatic lymphocyte populations as well as hepatocytes can be determined, which may aid in the rational design of immunotherapeutic strategies.

Acknowledgments:

LLB and AB are supported by the Virgo consortium, funded by the Dutch government project number FES0908.

Role of the funding source:

The sponsors had no involvement in the content of the review

Conflict of interest:

The authors report no potential conflicts of interest.

468 **Figure Legends**

469

470 Figure 1: Important questions concerning the clinical events and immunopathogenesis of
471 chronic HBV infection remain unanswered.

472

473 Figure 2: The persistence of HBV infections is determined by the complex interactions of
474 multiple leukocytes.

References

[1] Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol. 2008;48:335-52.

[2] Cheng J, Imanishi H, Morisaki H, Liu W, Nakamura H, Morisaki T, et al. Recombinant HBsAg inhibits LPS-induced COX-2 expression and IL-18 production by interfering with the NFkappaB pathway in a human monocytic cell line, THP-1. J Hepatol. 2005;43:465-71.

[3] Komiya Y, Katayama K, Yugi H, Mizui M, Matsukura H, Tomoguri T, et al. Minimum infectious dose of hepatitis B virus in chimpanzees and difference in the dynamics of viremia between genotype A and genotype C. Transfusion. 2008;48:286-94.

[4] 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.

[5] Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, et al. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. Gastroenterology. 2004;126:1750-8.

[6] Sato S, Li K, Kameyama T, Hayashi T, Ishida Y, Murakami S, et al. The RNA sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus. Immunity. 2015;42:123-32.

[7] Dunn C, Peppa D, Khanna P, Nebbia G, Jones M, Brendish N, et al. Temporal analysis of early immune responses in patients with acute hepatitis B virus infection. Gastroenterology. 2009;137:1289-300.

[8] Guo JT, Sohn JA, Zhu Q, Seeger C. Mechanism of the interferon alpha response against hepatitis C virus replicons. Virology. 2004;325:71-81.

[9] Seeger C, Mason WS. Hepatitis B virus biology. Microbiol Mol Biol Rev. 2000;64:51-68.

[10] Wieland S, Thimme R, Purcell RH, Chisari FV. Genomic analysis of the host response to hepatitis B virus infection. Proc Natl Acad Sci U S A. 2004;101:6669-74.

[11] Lebosse F, Testoni B, Fresquet J, Facchetti F, Galmozzi E, Fournier M, et al. Intrahepatic innate immune response pathways are downregulated in untreated chronic hepatitis B patients. J Hepatol. 2016.

[12] Vanwolleghem T, Hou J, van Oord G, Andeweg AC, Osterhaus AD, Pas SD, et al. Re-evaluation of hepatitis B virus clinical phases by systems biology identifies unappreciated roles for the innate immune response and B cells. Hepatology. 2015;62:87-100.

[13] Lucifora J, Xia Y, Reisinger F, Zhang K, Stadler D, Cheng X, et al. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science. 2014;343:1221-8.

[14] Crispe IN. Immune tolerance in liver disease. Hepatology. 2014;60:2109-17.

[15] Lan S, Wu L, Wang X, Wu J, Lin X, Wu W, et al. Impact of HBeAg on the maturation and function of dendritic cells. Int J Infect Dis. 2016;46:42-8.

[16] Beckebaum S, Cicinnati VR, Dworacki G, Muller-Berghaus J, Stolz D, Harnaha J, et al. Reduction in the circulating pDC1/pDC2 ratio and impaired function of ex vivo-generated DC1 in chronic hepatitis B infection. Clin Immunol. 2002;104:138-50.

[17] van der Molen RG, Sprengers D, Binda RS, de Jong EC, Niesters HG, Kusters JG, et al. Functional impairment of myeloid and plasmacytoid dendritic cells of patients with chronic hepatitis B. Hepatology. 2004;40:738-46.

[18] Tavakoli S, Mederacke I, Herzog-Hauff S, Glebe D, Grun S, Strand D, et al. Peripheral blood dendritic cells are phenotypically and functionally intact in chronic hepatitis B virus (HBV) infection. Clin Exp Immunol. 2008;151:61-70.

[19] 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;143:1586-96 e8.

[20] Woltman AM, Op den Brouw ML, Biesta PJ, Shi CC, Janssen HL. Hepatitis B virus lacks immune activating capacity, but actively inhibits plasmacytoid dendritic cell function. PLoS One. 2011;6:e15324.

[21] Yamamoto T, Kaizu C, Kawasaki T, Hasegawa G, Umezu H, Ohashi R, et al. Macrophage colony-stimulating factor is indispensable for repopulation and differentiation of Kupffer cells but not for splenic red pulp macrophages in osteopetrotic (op/op) mice after macrophage depletion. Cell Tissue Res. 2008;332:245-56.

[22] Boltjes A, van Montfoort N, Biesta PJ, Op den Brouw ML, Kwekkeboom J, van der Laan LJ, 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;211:1268-78.

[23] Wu J, Meng Z, Jiang M, Pei R, Trippler M, Broering R, et al. Hepatitis B virus suppresses toll-like receptor-mediated innate immune responses in murine parenchymal and nonparenchymal liver cells. Hepatology. 2009;49:1132-40.

[24] Visvanathan K, Skinner NA, Thompson AJ, Riordan SM, Sozzi V, Edwards R, et al. Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein. Hepatology. 2007;45:102-10.

[25] Li H, Zheng HW, Chen H, Xing ZZ, You H, Cong M, et al. Hepatitis B virus particles preferably induce Kupffer cells to produce TGF-beta1 over pro-inflammatory cytokines. Dig Liver Dis. 2012;44:328-33.

[26] Tu Z, Bozorgzadeh A, Pierce RH, Kurtis J, Crispe IN, Orloff MS. TLR-dependent cross talk between human Kupffer cells and NK cells. J Exp Med. 2008;205:233-44.

[27] Zhang Z, Zhang S, Zou Z, Shi J, Zhao J, Fan R, et al. Hypercytolytic activity of hepatic natural killer cells correlates with liver injury in chronic hepatitis B patients. Hepatology. 2011;53:73-85.

[28] Zhao J, Li Y, Jin L, Zhang S, Fan R, Sun Y, et al. Natural killer cells are characterized by the concomitantly increased interferon-gamma and cytotoxicity in acute resolved hepatitis B patients. PLoS One. 2012;7:e49135.

[29] Peppa D, Micco L, Javaid A, Kennedy PT, Schurich A, Dunn C, et al. Blockade of immunosuppressive cytokines restores NK cell antiviral function in chronic hepatitis B virus infection. PLoS Pathog. 2010;6:e1001227.

[30] Lunemann S, Malone DF, 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;209:1362-73.

[31] Phillips S, Chokshi S, Riva A, Evans A, Williams R, Naoumov NV. CD8(+) T cell control of hepatitis B virus replication: direct comparison between cytolytic and noncytolytic functions. J Immunol. 2010;184:287-95.

[32] Zheng M, Sun R, Wei H, Tian Z. NK Cells Help Induce Anti-Hepatitis B Virus CD8+ T Cell Immunity in Mice. J Immunol. 2016;196:4122-31.

[33] Kimura K, Kakimi K, Wieland S, Guidotti LG, Chisari FV. Interleukin-18 inhibits hepatitis B virus replication in the livers of transgenic mice. J Virol. 2002;76:10702-7.

[34] Kakimi K, Guidotti LG, Koezuka Y, Chisari FV. Natural killer T cell activation inhibits hepatitis B virus replication in vivo. J Exp Med. 2000;192:921-30.

[35] Woltman AM, Ter Borg MJ, Binda RS, Sprengers D, von Blomberg BM, Scheper RJ, et al. Alpha-galactosylceramide in chronic hepatitis B infection: results from a randomized placebo-controlled Phase I/II trial. Antivir Ther. 2009;14:809-18.

[36] Rehermann B. Natural Killer Cells in Viral Hepatitis. Cell Mol Gastroenterol Hepatol. 2015;1:578-88.

[37] de Groen RA, Hou J, van Oord GW, Groothuismink ZM, van der Heide M, de Knegt RJ, et al. NK cell phenotypic and functional shifts coincide with specific clinical phases in the natural history of chronic HBV infection. Antiviral Res. 2017;140:18-24.

[38] Bonorino P, Ramzan M, Camous X, Dufeu-Duchesne T, Thelu MA, Sturm N, et al. Fine characterization of intrahepatic NK cells expressing natural killer receptors in chronic hepatitis B and C. J Hepatol. 2009;51:458-67.

[39] Tjwa ET, Zoutendijk R, van Oord GW, Boeijen LL, Reijnders JG, van Campenhout MJ, et al. Similar frequencies, phenotype and activation status of intrahepatic NK cells in chronic HBV patients after long-term treatment with tenofovir disoproxil fumarate (TDF). Antiviral Res. 2016;132:70-5.

[40] Fasbender F, Widera A, Hengstler JG, Watzl C. Natural Killer Cells and Liver Fibrosis. Front Immunol. 2016;7:19.

583 [41] Radaeva S, Sun R, Jaruga B, Nguyen VT, Tian Z, Gao B. Natural killer cells ameliorate
584 liver fibrosis by killing activated stellate cells in NKG2D-dependent and tumor necrosis factor-
585 related apoptosis-inducing ligand-dependent manners. Gastroenterology. 2006;130:435-52.
586 [42] Huang WC, Easom NJ, Tang XZ, Gill US, Singh H, Robertson F, et al. T Cells Infiltrating
587 Diseased Liver Express Ligands for the NKG2D Stress Surveillance System. J Immunol.
588 2017;198:1172-82.
589 [43] Bertoletti A, Ferrari C. Adaptive immunity in HBV infection. J Hepatol. 2016;64:S71-83.
590 [44] Webster GJ, Reignat S, Maini MK, Whalley SA, Ogg GS, King A, et al. Incubation phase
591 of acute hepatitis B in man: dynamic of cellular immune mechanisms. Hepatology.
592 2000;32:1117-24.
593 [45] Thomas E, Liang TJ. Experimental models of hepatitis B and C - new insights and
594 progress. Nat Rev Gastroenterol Hepatol. 2016;13:362-74.
595 [46] Thimme R, Wieland S, Steiger C, Ghrayeb J, Reimann KA, Purcell RH, et al. CD8(+) T
596 cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus
597 infection. J Virol. 2003;77:68-76.
598 [47] Asabe S, Wieland SF, Chattopadhyay PK, Roederer M, Engle RE, Purcell RH, et al. The
599 size of the viral inoculum contributes to the outcome of hepatitis B virus infection. J Virol.
600 2009;83:9652-62.
601 [48] Ferrari C. HBV and the immune response. Liver Int. 2015;35 Suppl 1:121-8.
602 [49] Penna A, Artini M, Cavalli A, Levrero M, Bertoletti A, Pilli M, et al. Long-lasting memory T
603 cell responses following self-limited acute hepatitis B. J Clin Invest. 1996;98:1185-94.
604 [50] Mizukoshi E, Sidney J, Livingston B, Ghany M, Hoofnagle JH, Sette A, et al. Cellular
605 immune responses to the hepatitis B virus polymerase. J Immunol. 2004;173:5863-71.
606 [51] Jung MC, Stemler M, Weimer T, Spengler U, Dohrmann J, Hoffmann R, et al. Immune
607 response of peripheral blood mononuclear cells to HBx-antigen of hepatitis B virus.
608 Hepatology. 1991;13:637-43.
609 [52] Penna A, Bertoletti A, Cavalli A, Valli A, Missale G, Pilli M, et al. Fine specificity of the
610 human T cell response to hepatitis B virus core antigen. Arch Virol Suppl. 1992;4:23-8.
611 [53] Penna A, Del Prete G, Cavalli A, Bertoletti A, D'Elios MM, Sorrentino R, et al.
612 Predominant T-helper 1 cytokine profile of hepatitis B virus nucleocapsid-specific T cells in
613 acute self-limited hepatitis B. Hepatology. 1997;25:1022-7.
614 [54] Lokhande MU, Thimme R, Klenerman P, Semmo N. Methodologies for the Analysis of
615 HCV-Specific CD4(+) T Cells. Front Immunol. 2015;6:57.
616 [55] Bertoletti A, D'Elios MM, Boni C, De Carli M, Zignego AL, Durazzo M, et al. Different
617 cytokine profiles of intraphepatic T cells in chronic hepatitis B and hepatitis C virus infections.
618 Gastroenterology. 1997;112:193-9.

[56] Nitschke K, Luxenburger H, Kiraithe MM, Thimme R, Neumann-Haefelin C. CD8+ T-Cell Responses in Hepatitis B and C: The (HLA-) A, B, and C of Hepatitis B and C. Dig Dis. 2016;34:396-409.

[57] Guidotti LG, Ishikawa T, Hobbs MV, Matzke B, Schreiber R, Chisari FV. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. Immunity. 1996;4:25-36.

[58] Kafrouni MI, Brown GR, Thiele DL. Virally infected hepatocytes are resistant to perforin-dependent CTL effector mechanisms. J Immunol. 2001;167:1566-74.

[59] Maini MK, Boni C, Lee CK, Larrubia JR, Reignat S, Ogg GS, et al. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. J Exp Med. 2000;191:1269-80.

[60] Sprengers D, van der Molen RG, Kusters JG, De Man RA, Niesters HG, Schalm SW, et al. Analysis of intrahepatic HBV-specific cytotoxic T-cells during and after acute HBV infection in humans. J Hepatol. 2006;45:182-9.

[61] Sprengers D, van der Molen RG, Kusters JG, Hansen B, Niesters HG, Schalm SW, et al. Different composition of intrahepatic lymphocytes in the immune-tolerance and immune-clearance phase of chronic hepatitis B. J Med Virol. 2006;78:561-8.

[62] Webster GJ, Reignat S, Brown D, Ogg GS, Jones L, Seneviratne SL, et al. Longitudinal analysis of CD8+ T cells specific for structural and nonstructural hepatitis B virus proteins in patients with chronic hepatitis B: implications for immunotherapy. J Virol. 2004;78:5707-19.

[63] Raziorrouh B, Schraut W, Gerlach T, Nowack D, Gruner NH, Ulsenheimer A, et al. The immunoregulatory role of CD244 in chronic hepatitis B infection and its inhibitory potential on virus-specific CD8+ T-cell function. Hepatology. 2010;52:1934-47.

[64] Schurich A, Khanna P, Lopes AR, Han KJ, Peppa D, Micco L, et al. Role of the coinhibitory receptor cytotoxic T lymphocyte antigen-4 on apoptosis-Prone CD8 T cells in persistent hepatitis B virus infection. Hepatology. 2011;53:1494-503.

[65] Nebbia G, Peppa D, Schurich A, Khanna P, Singh HD, Cheng Y, et al. Upregulation of the Tim-3/galectin-9 pathway of T cell exhaustion in chronic hepatitis B virus infection. PLoS One. 2012;7:e47648.

[66] Kurktschiev PD, Raziorrouh B, Schraut W, Backmund M, Wachtler M, Wendtner CM, et al. Dysfunctional CD8+ T cells in hepatitis B and C are characterized by a lack of antigen-specific T-bet induction. J Exp Med. 2014;211:2047-59.

[67] Lopes AR, Kellam P, Das A, Dunn C, Kwan A, Turner J, et al. Bim-mediated deletion of antigen-specific CD8 T cells in patients unable to control HBV infection. J Clin Invest. 2008;118:1835-45.

[68] Peppa D, Gill US, Reynolds G, Easom NJ, 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.

[69] Rehermann B, Pasquinelli C, Mosier SM, Chisari FV. Hepatitis B virus (HBV) sequence variation of cytotoxic T lymphocyte epitopes is not common in patients with chronic HBV infection. J Clin Invest. 1995;96:1527-34.

[70] Kefalakes H, Budeus B, Walker A, Jochum C, Hilgard G, Heinold A, et al. Adaptation of the hepatitis B virus core protein to CD8(+) T-cell selection pressure. Hepatology. 2015;62:47-56.

[71] Desmond CP, Gaudieri S, James IR, Pfafferott K, Chopra A, Lau GK, et al. Viral adaptation to host immune responses occurs in chronic hepatitis B virus (HBV) infection, and adaptation is greatest in HBV e antigen-negative disease. J Virol. 2012;86:1181-92.

[72] de Niet A, Stelma F, Jansen L, Sinnige MJ, Remmerswaal EB, Takkenberg RB, et al. Restoration of T cell function in chronic hepatitis B patients upon treatment with interferon based combination therapy. J Hepatol. 2016;64:539-46.

[73] Boni C, Laccabue D, Lampertico P, Giuberti T, Vigano M, Schivazappa S, et al. Restored function of HBV-specific T cells after long-term effective therapy with nucleos(t)ide analogues. Gastroenterology. 2012;143:963-73 e9.

[74] Penna A, Laccabue D, Libri I, Giuberti T, Schivazappa S, Alfieri A, et al. Peginterferon-alpha does not improve early peripheral blood HBV-specific T-cell responses in HBeAg-negative chronic hepatitis. J Hepatol. 2012;56:1239-46.

[75] Sprinzl MF, Russo C, Kittner J, Allgayer S, Grambihler A, Bartsch B, et al. Hepatitis B virus-specific T-cell responses during IFN administration in a small cohort of chronic hepatitis B patients under nucleos(t)ide analogue treatment. J Viral Hepat. 2014;21:633-41.

[76] Manigold T, Racanelli V. T-cell regulation by CD4 regulatory T cells during hepatitis B and C virus infections: facts and controversies. Lancet Infect Dis. 2007;7:804-13.

[77] Franzese O, Kennedy PT, Gehring AJ, Gotto J, Williams R, Maini MK, et al. Modulation of the CD8+-T-cell response by CD4+ CD25+ regulatory T cells in patients with hepatitis B virus infection. J Virol. 2005;79:3322-8.

[78] Stoop JN, van der Molen RG, Baan CC, van der Laan LJ, Kuipers EJ, Kusters JG, et al. Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. Hepatology. 2005;41:771-8.

[79] Xu D, Fu J, Jin L, Zhang H, Zhou C, Zou Z, et al. Circulating and liver resident CD4+CD25+ regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. J Immunol. 2006;177:739-47.

[80] Yang G, Liu A, Xie Q, Guo TB, Wan B, Zhou B, et al. Association of CD4+CD25+Foxp3+ regulatory T cells with chronic activity and viral clearance in patients with hepatitis B. Int Immunol. 2007;19:133-40.

[81] Peng G, Li S, Wu W, Sun Z, Chen Y, Chen Z. Circulating CD4+ CD25+ regulatory T cells correlate with chronic hepatitis B infection. Immunology. 2008;123:57-65.

693 [82] Stoop JN, Claassen MA, Woltman AM, Binda RS, Kuipers EJ, Janssen HL, et al.
694 Intrahepatic regulatory T cells are phenotypically distinct from their peripheral counterparts in
695 chronic HBV patients. Clin Immunol. 2008;129:419-27.
696 [83] Stephan W, Prince AM, Brotman B. Modulation of hepatitis B infection by intravenous
697 application of an immunoglobulin preparation that contains antibodies to hepatitis B e and
698 core antigens but not to hepatitis B surface antigen. J Virol. 1984;51:420-4.
699 [84] Paul S, Dickstein A, Saxena A, Terrin N, Viveiros K, Balk EM, et al. Role of Surface
700 Antibody in Hepatitis B Reactivation in Patients with Resolved Infection and Hematologic
701 Malignancy: A Meta-Analysis. Hepatology. 2017.
702 [85] Palanichamy A, Jahn S, Nickles D, Derstine M, Abounasr A, Hauser SL, et al. Rituximab
703 efficiently depletes increased CD20-expressing T cells in multiple sclerosis patients. J
704 Immunol. 2014;193:580-6.

705