



# Role of Immunomodulators in Functional Cure Strategies for HBV

Benedikt Binder<sup>1,2</sup> · Maike Hofmann<sup>1,3</sup> · Robert Thimme<sup>1,3</sup>

Published online: 27 August 2020  
© The Author(s) 2020

## Abstract

**Purpose of Review** Chronic Hepatitis B Virus (HBV) Infection is a major global health burden. Currently, a curative therapy does not exist; thus, there is an urgent need for new therapeutical options. Viral elimination in the natural course of infection results from a robust and multispecific T and B cell response that, however, is dysfunctional in chronically infected patients. Therefore, immunomodulatory therapies that strengthen the immune responses are an obvious approach trying to control HBV infection. In this review, we summarize the rationale and current options of immunological cure of chronic HBV infection.

**Recent Findings** Recently, among others, drugs that stimulate the innate immune system or overcome CD8+ T cell exhaustion by checkpoint blockade, and transfer of HBV-specific engineered CD8+ T cells emerged as promising approaches.

**Summary** HBV-specific immunity is responsible for viral control, but also for immunopathogenesis. Thus, the development of immunomodulatory therapies is a difficult process on a thin line between viral control and excessive immunopathology. Some promising agents are under investigation. Nevertheless, further research is indispensable in order to optimally orchestrate immunostimulation.

**Keywords** Hepatitis B · Immunology · Immunotherapy · CD8+ T cells · Exhaustion · Checkpoint blockade

## Introduction

Despite the existence of an efficient vaccine, chronic Hepatitis B Virus (HBV) infection is a major global health burden with an approximate number of 257 million infected individuals and 887,000 deaths per year worldwide [1]. The risks of chronic HBV infection include progressive liver disease possibly leading to the development of liver fibrosis, cirrhosis, and ultimately hepatocellular carcinoma (HCC). The fact that HBV is a non-cytopathic virus implies that the liver inflammation as indicated by elevated serum liver enzymes in patients is caused by virus-mediated immunopathogenesis.

Current therapeutic options include treatment with pegylated interferon alpha (pegIFN $\alpha$ ) and nucleoside/nucleotide-analogues (NUCs). In the majority of cases, these drugs are capable of limiting hepatic inflammation and viral replication. Nevertheless, viral elimination is barely achieved and HCC development is still not completely abrogated [2, 3], implicating long-time treatment and consequent frequent medical attendance.

There are different definitions of cure in the context of HBV infection. *Partial cure* is defined as normalization of serum transaminases, low viral loads with the presence of hepatitis B surface antigen (HBsAg) in the blood and can be achieved by common therapies, e.g., NUC treatment. *Sterilizing* or *virological cure* involves the complete eradication of the virus, including of the *covalently closed circular deoxyribonucleic acid* (cccDNA), the viral persistence reservoir existing as a mini chromosome in hepatocytes, and of the HBV DNA that is integrated in the hosts nuclear DNA [4]. Therefore, sterilizing cure is hard to achieve. *Functional cure* implies the loss of HBsAg with or without seroconversion to anti-HBsAg. This is considered a reasonable and realistic goal of uprising immunomodulatory HBV therapies.

---

This article is part of the Topical Collection on *Hepatitis B*

✉ Robert Thimme  
robert.thimme@uniklinik-freiburg.de

<sup>1</sup> Department of Medicine II, University Hospital Freiburg, Hugstetter Straße 55, 79106 Freiburg, Germany

<sup>2</sup> IMM-PACT Clinician Scientist Programme, Faculty of Medicine, University of Freiburg, Freiburg, Germany

<sup>3</sup> Faculty of Medicine, University of Freiburg, Freiburg, Germany

## Immune Responses During Acute Resolving HBV Infection

Ninety-five percent of adults being infected with HBV are capable of clearing the virus spontaneously [5]. The immune response in these individuals is characterized by a strong and multispecific T and B cell response leading to life-long immunity [6–8]. The important role of B cells in viral control is highlighted by the high risk of HBV reactivation following B cell ablation applying the CD20 antibody Rituximab in patients with controlled HBV infection [9].

The importance of CD8+ T cells in the process of viral control has been shown by depletion studies, where depletion of CD8+ T cells after HBV infection of chimpanzees led to transient persistence of the virus, whereas depletion of CD4+ T cells after infection did not [10]. Nevertheless, CD4+ T cell depletion prior to infection did lead to viral persistence; most likely due to the lack of CD4+ T cell-mediated help for CD8+ T cells [11]. Therefore, virus-specific CD8+ T cells are seen as key players in controlling HBV infection. They can exert diverse effector functions including lysis of infected cells via Fas/FasL and the secretion of perforin and granzymes, as well as non-cytolytic effector functions via secretion of interferon gamma (IFN $\gamma$ ) and tumor necrosis factor (TNF) [10, 12–14]. Both cytolytic and non-cytolytic effector functions of CD8+ T cells seem to be important for viral control in HBV infection [10, 15, 16].

## Immune Failure in Viral Persistence and Chronic HBV Infection

Ninety percent of the perinatally infected patients and 5% of the adults are not able to clear the virus [5]. Instead, chronic HBV infection is established, accompanied by the aforementioned complications. In these patients, the immune response is weak and characterized by attenuated B and T cell responses.

One hallmark of chronic HBV infection is T cell failure, especially the so-called CD8+ T cell exhaustion. This T cell state is characterized by impaired proliferative capacity and effector functions and is associated with the expression of inhibitory molecules like *programmed death 1* (PD-1), *cytotoxic T lymphocyte-associated Protein 4* (CTLA-4), or *T cell immunoglobulin and mucin-domain containing-3* (TIM-3) [17]. It is further characterized by mitochondrial alterations and a distinct transcriptional profile including dysregulation of T-bet and upregulation of Eomesodermin (Eomes) [18, 19]. The role of the transcription factor *Thymocyte selection-associated high mobility group box protein* (TOX) as master regulator of T cell exhaustion has been highlighted by several studies [20–22]. However, its role in chronic HBV infection is unclear and needs to be investigated in future studies.

CD8+ T cell exhaustion has generally been linked to persisting antigen exposure, the lack of CD4+ T cell help and the immunosuppressive liver milieu, although the specific contribution of each of these mechanisms to HBV-related CD8+ T cell failure remains unclear [23].

Of note, CD8+ T cells targeting different epitopes differ in their state of exhaustion. Recently, for example, we could show that HBV polymerase-specific CD8+ T cells express molecular patterns suggestive of a more severe exhaustion compared with HBV core-specific CD8+ T cells [24]. CD8+ T cell exhaustion can partially be improved by antiviral therapy and is considered one of the main targets of immunotherapies [25].

Another mechanism of CD8+ T cell failure is the occurrence of viral escape mutations that avoid recognition by HBV-specific CD8+ T cells, therefore also limiting antiviral activity [26].

Not only CD8+ T cells but also B cells fail to exert sufficient antiviral functions. HBsAg-specific B cells obtained from chronically infected patients are impaired in producing antibodies. This dysfunction is also associated with the expression of PD-1 [27, 28].

The central role of a strong and well-orchestrated immune response in terms of viral control and HBV cure has driven attempts for new immunomodulatory drugs that aim to improve B and T cell functionality in order to gain control over the global burden of HBV.

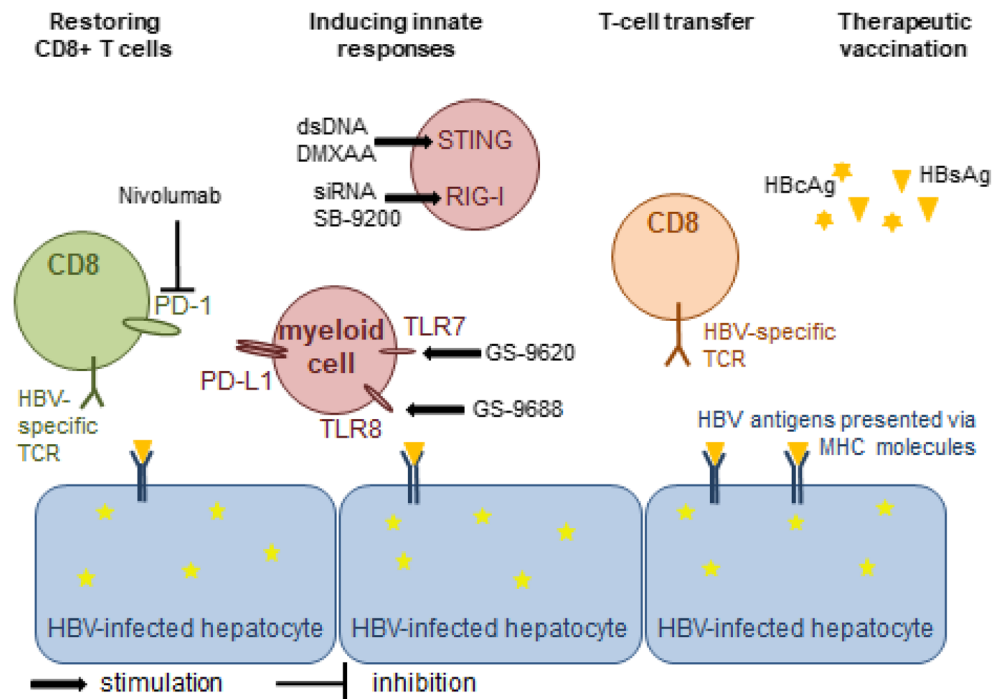
## Immunotherapeutic Options

The current immunotherapeutic approaches can be divided by their targets. The most promising approaches aim to stimulate the innate and/or adaptive immune system via direct agonists, checkpoint-blockade, or therapeutic vaccination, or work via T cell transfer (Fig. 1). An overview about the therapies currently under investigation in clinical trials is provided in Table 1.

### Stimulating the Innate Immune System

HBV is considered a stealth virus that does not induce innate immune responses in terms of induction of interferon-stimulated genes (ISGs) [29]. Therefore, stimulation of the innate immune system is expected to result in a more efficient anti-HBV immune response. Several pathways that are linked to innate immune functions lead to the activation of the transcription factor *nuclear factor kappa-light-chain-enhancer of activated B cells* (NF $\kappa$ B), hereby exerting antiviral activity. Therefore, these pathways are potential targets of HBV immunotherapy. Currently, the stimulation of toll-like receptors (TLRs), the cytoplasmic *stimulator of interferon genes* (STING), and of the pattern recognition receptor *retinoic*

**Fig. 1** Immunotherapeutical options in chronic HBV infection. The most promising approaches to immunological cure of chronic HBV infection are restoration of CD8+ T cells by checkpoint blockade, induction of innate immune responses via TLR agonists or activators of cytoplasmic molecules in myeloid cells, transfer of engineered CD8 T+ cells expressing HBV-specific T cell receptors and therapeutic vaccinations



*acid-inducible gene 1* (RIG-I) have been highlighted as promising approaches in this respect (Fig. 1).

TLRs are pattern recognition receptors that are expressed on myeloid cells, but also in lower amount on hepatocytes. Following stimulation, an innate immune response can be induced via *Myeloid differentiation primary response 88* (MyD88) and  $\text{NF}\kappa\text{B}$ . Several agonists of different TLRs have been tested upon their antiviral efficacy.

One agent under investigation is the TLR7 activator GS-9620 (Vesatolimod). In the woodchuck model of Hepatitis B, activation of TLR7 led to a reduction in the replication of woodchuck hepatitis virus (WHV), accompanied by reduction of woodchuck hepatitis surface antigen (WHsAg) levels and

by anti-WHs seroconversion in some animals [30]. In HBV-infected chimpanzees, treatment with GS-9620 led to ISG induction and activation of natural killer (NK) cells, followed by suppression of viral replication that lasted longer than the treatment itself [31]. However, despite induction of T- and NK-cell responses by GS-9620, antiviral effects could not be observed in humans [32, 33].

The TLR8 agonist GS-9688 (Selgantolimod) also showed antiviral effects in the woodchuck model, specifically reduction of viral load and anti-WHsAg seroconversion in some animals [34]. Other TLR agonists targeting TLR1/2/3 exert antiviral activity in vitro, as shown by inhibition of viral replication in HBV-infected primary human hepatocytes [35].

**Table 1** Overview about immunomodulatory therapies currently under investigation in clinical trials

Mechanism	Drug	Format	Target	Phase
Immunostimulation	GS-9620	Small molecule	TLR7 agonist	Phase 2
Immunostimulation	GS-9688	Small molecule	TLR8 agonist	Phase 2
Immunostimulation	SB9200	Small molecule	Activator of RIG-I + NOD2	Phase 2
Checkpoint blockade	Nivolumab	Monoclonal antibody	PD-1 blockade	Phase 2
Checkpoint blockade	HLX10	Monoclonal antibody	PD-1 blockade	Phase 2
Therapeutic vaccine	ChAdOx1-HBV	Adenoviral vector		Phase 1
Therapeutic vaccine	VVX001	preS domain		Phase 2
Therapeutic vaccine	CVI-HBV-002	L-HBsAg + L-pampo		Phase 2
Therapeutic vaccine	JNJ-64300535	Electroporation mediated DNA-vaccine		Phase 1
Therapeutic vaccine	GS-4774	Yeast-based vaccine		Phase 2
T-cell transfer		TCR redirected CD8+ T cells		Phase 1
T-cell transfer		Adoptive transfer of $\gamma\delta\text{T}$ cells		Early phase 1

Ligands for TLR3/4/5/7/9 inhibited HBV replication in a non-cytopathic manner in an HBV transgenic mouse model [36].

Further studies will need to prove antiviral effects in humans. Of note, the expression of TLR7 differs strongly in mice, woodchucks, and humans. Indeed, in humans, TLR7 and TLR8 are only weakly expressed on hepatocytes [37]. Depending on TLR expression, TLR agonists directly interact with the immune system or with hepatocytes as well. However, control of HBV replication by application of TLR agonists in mice was found to be mediated by non-parenchymal liver cells such as Kupffer cells and sinusoidal endothelial cells [38].

Another promising target is the cytoplasmic pattern recognition receptor RIG-I that can induce an antiviral response upon stimulation by ribonucleic acids (RNA) via NF $\kappa$ B and *Interferon regulatory factor 3* (IRF3) [39–41]. SB-9200 (Inarigivir) is an activator of RIG-I and *nucleotide-binding oligomerization domain-containing 2* (NOD2), which exerts antiviral activity in the WHV model, where a dose-dependent reduction of WHsAg could be observed. This effect could be boosted by subsequent therapy with the NUC entecavir [42]. Anti-HBV activity could also be observed after stimulation with modified *small interfering RNAs* (siRNAs) [43].

STING is another cytoplasmic target of HBV immunotherapy that also activates NF $\kappa$ B and has been shown to suppress HBV replication upon stimulation. Cytoplasmic DNA can be sensed by *cyclic guanosine monophosphate-adenosine monophosphate synthase* (cGAS), which then activates STING, leading to activation of NF $\kappa$ B and IRF3. This leads to an initiation of an innate immune response [44, 45]. In contrast to myeloid cells, human hepatocytes do not seem to express STING [46]. Nevertheless, activation of the cGAS-STING-pathway by HBV-derived double-stranded DNA or by the STING agonist *5,6-dimethylxanthenone-4-acetic acid* (DMXAA) in a hepatoma cell line leads to suppression of viral assembly, induction of ISG56, and inhibition of viral replication as measured by secretion of HBsAg [47, 48]. Furthermore, viral replication could be suppressed in vitro and in vivo in a mouse model dependent on transfection of an HBV replicating plasmid [49], thus making the cGAS-STING-pathway an interesting target for HBV immunotherapy.

### Induction of the Adaptive Immune System

Since HBV infection cannot be cleared due to an impaired immune response, which is mainly characterized by CD8+ T cell exhaustion, it is an obvious and also promising approach to try to restore CD8+ T cell functionality by overcoming the mechanisms of exhaustion (Fig. 1).

The predominant marker of exhaustion of HBV-specific CD8+ T cells is PD-1. Noteworthy, blockade of PD-1 resulted in the strongest increase in CD8+ T cell function in vitro, compared with other inhibitory receptors. This effect was

linked to intermediate T cell differentiation [50]. The effect of PD-1 blockade on HBV-specific CD8+ T cells from blood and liver of chronically infected patients could in some cases be boosted by the addition of CD137L [51, 52] and was shown to be more efficient in HBeAg+ patients [53]. But not only CD8+ T cells are affected by checkpoint inhibitors. Noteworthy, the function of HBsAg-specific B cells obtained from chronically infected individuals could also be partially restored by PD-1 blockade in vitro [27, 28].

In the woodchuck model, blockade of PD-L1 in combination with entecavir and therapeutic DNA vaccination led to suppression of viral replication and, in some animals, to viral elimination and seroconversion [54]. A first study in humans reported that checkpoint blockade with nivolumab in HBeAg-negative patients with suppressed viral load led to a decline of HBsAg in most patients and clearance in one patient [55••]. Monoclonal antibodies against PD-1 like nivolumab and HLX10 are currently under investigation in clinical trials (Table 1).

Another inhibitory receptor under investigation is CTLA-4. Blocking CTLA-4 with monoclonal antibodies resulted in an increased expansion of hepatic and peripheral blood IFN $\gamma$ -producing CD8+ T cells in vitro, indicating a possible role of other inhibitory molecules that may be tested in a clinical setting [56].

Clearly, when interfering with inhibitory pathways of virus-specific CD8+ T cells, it will be important to avoid the induction of excessive liver damage while exerting antiviral functions or killing infected hepatocytes. Fortunately, early studies with PD1 blockade did not observe significant side effects in patients with chronic HBV infection [55••].

### Therapeutic Vaccination

Instead of restoring compromised immune cells, therapeutic vaccinations aim to induce new immune responses by exposing the immune system to modified HBV antigens (Fig. 1). Inserting immunogenic components of HBV such as HBsAg or Hepatitis B core antigen (HBcAg) into the organism has the goal, similar to a preventive vaccination, to induce adaptive immune responses that can control the infection and ideally result in life-long immunity.

However, most studies thus far failed to show significant antiviral effects. For example, intramuscular application of HBsAg after a 6-month NUC treatment did not lead to a more frequent loss of HBsAg compared with a control group [57]. Similarly, a combination of HBsAg and HBcAg showed no benefit compared with pegIFN $\alpha$  at the end of therapy. However, HBV suppression under the detection level was more frequent in the vaccine group 24 weeks after the end of therapy [58]. Thus, these combined results indicate that the sole application of viral components is not sufficient to induce immune responses leading to viral control.

Other approaches work with yeast-based vaccines that are thought to have a stronger immunogenic potential. GS-4774 is

a yeast-based therapeutic vaccine engineered to express HBV antigens. In clinical trials, HBsAg reduction was not observed after treating HBV patients with viral load of > 2000 IU/ml. However, production of IFN $\gamma$ , TNF, and IL-2 by HBV-specific CD8+ T cells increased in these patients but did not reach comparable levels as in patients with acute self-limiting infections. Moreover, modulations in dendritic cells could not be observed [59]. In summary, the immunomodulatory effects of GS-4774 seem to be too mild to efficiently reduce HBV replication, and higher doses that may lead to a stronger activation of adaptive immune responses were not tolerated by the patients [59].

In a recent proof of principle-study in mice, a vaccine based on cell-permeable HBV capsids was used. Specifically, the preS1preS2 domain served as antigen and was able to induce a specific cellular immune response and neutralizing antibodies, followed by lysis of HBV-infected cells, although changes in viral loads were not reported [60]. Therefore, the antiviral efficacy of this method remains unclear. However, it still highlights an interesting concept that can be further analyzed in future studies.

Currently, the therapeutic vaccine candidates VVX001 (preS1 domain), CVI-HBV-002 (large HBsAg), JNJ-64300535 (DNA vaccine), and ChAdOx1-HBV (adenoviral vector) are under investigation in clinical trials (Table 1).

## T Cell Transfer

Besides restoring or inducing the hosts' immune responses, there is also the possibility to transfer fully functional HBV-specific T cells into the host (Fig. 1). Different methods of engineering CD8+ T cells to express HBV-specific T cell receptors (TCRs) have been established. Retroviral transduction of TCRs leads to a stable and permanent expression, thus exerting strong and durable antiviral responses, but also inducing severe tissue damage. Excessive immunopathology can be avoided by limiting the time span of TCR expression. This can be achieved by electroporation of the T cells and introduction of the messenger RNA (mRNA) of an HBV-specific TCR. Adversely, however, the transient TCR expression might also hinder long-lasting antiviral effects.

The concept of T cell transfer is based on the finding that chronically HBV-infected patients receiving allogeneic hematopoietic stem cell transplantation (alloHSCT) from individuals with HBV immunity can resolve the infection [61, 62]. However, the fact that alloHSCT is associated with numerous and severe side effects prohibits this procedure as a therapeutic option in clinical routine.

One approach is that CD8+ T cells are equipped with an HBV-specific chimeric TCR by retroviral transduction, resulting in so-called *chimeric antigen receptor T cells* (CAR T cells). This TCR is composed of an antibody fragment directed against epitopes in the HBsAg that is fused to an

intracellular domain of CD3 and the costimulatory molecule CD28. These engineered CD8+ T cells are able to recognize and lyse HBV-infected hepatocytes in vitro, thus potentially eradicating the viral persistence reservoir cccDNA [63]. In a model of HBV-transgenic mice, the antiviral effect could be confirmed and liver damage was only transient [64]. In HBV-infected human liver chimeric mice, HBsAg-specific CAR T cells were also able to reduce HBV replication, supporting the potential of this interesting immunological tool [65•].

Encouraging results have also been reported with T cells that have been electroporated with mRNA encoding for HBsAg- and HBcAg-specific TCRs. These cells could reduce HBV replication in a non-cytopathic manner by activation of *apolipoprotein B mRNA editing enzyme, catalytic polypeptide 3* (APOBEC3) in humanized mice and HBV-infected HepG2-hNTCP-cells, a model previously described as a tool to investigate HBV infection in vitro [15, 66]. In another study, electroporated T cells that were engineered to transiently express an HBsAg-specific TCR were able to reduce viral load accompanied by reversible increase of serum transaminases in HBV-infected human liver chimeric mice, thus highlighting a possible way to limit the risk of immunopathology by restricting the duration of T cell activity [67].

Engineered T cells are further investigated in the context of malignancies. One such example is HBV-related HCC, where antitumoral and antiviral functions can be combined. CD8+ T cells that were taken from a patient with HBV-associated HCC, genetically modified to express an HBsAg-specific TCR and then put back into the patient they were obtained from (so-called autologous TCR-redirected T cells), were able to recognize tumor cells expressing HBsAg. Accordingly, HBsAg levels declined and no exacerbation of liver inflammation was observed [68].

Therefore, engineered T cells might present a future method to clear hepatitis B infection, even though high costs and extensive efforts are currently necessary for this treatment.

## Conclusion

The dichotomy of the immune system in establishing viral control on the one hand and causing organ damage by immunopathology on the other hand implies the need of a thoroughly balanced activation of immune responses. This narrow beneficial corridor is difficult to achieve. Many promising substances tested in vitro failed to proof sufficient antiviral effects in vivo. Therefore, combinations of different approaches and personalized therapeutic regimes are most likely required to successfully reach HBV cure.

The struggle developing new antiviral therapies is complicated by the lack of easily accessible animal models, since mice are not susceptible to human HBV and need to be conditioned (e.g., by humanization), and research on chimpanzees

is no longer allowed, thus highlighting the importance of ex vivo studies with human cells and also the need for pre-clinical animal models.

Another important aspect is that sterilizing cure including the eradication of all viral DNA is currently not a realistic option, since most of the abovediscussed therapies fail to degrade cccDNA without inducing an unacceptable amount of liver damage. In order to develop a sterilizing immunotherapeutic cure, much more effort on understanding the interactions of the host's immune system with cccDNA is needed.

Clearly, we need to better understand and further investigate mechanisms of failure of the immune response in chronically infected patients focusing on the key effector cells such as CD8+, CD4+ T cells, and B cells, in order to optimally orchestrate immune stimulation leading to cure of chronic HBV infection.

**Funding Information** Open Access funding provided by Projekt DEAL.

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Ethics Statement** All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

## References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Organization WH (2019) [Available from: <https://www.who.int/news-room/fact-sheets/detail/hepatitis-b>
2. Chen JD, Yang HI, Iloeje UH, You SL, Lu SN, Wang LY, et al. Carriers of inactive hepatitis B virus are still at risk for hepatocellular carcinoma and liver-related death. *Gastroenterology*. 2010;138(5):1747–54.
3. Papatheodoridis GV, Dalekos GN, Yurdaydin C, Buti M, Goulis J, Arends P, et al. Incidence and predictors of hepatocellular carcinoma in Caucasian chronic hepatitis B patients receiving entecavir or tenofovir. *J Hepatol*. 2015;62(2):363–70.
4. Lok AS, Zoulim F, Dusheiko G, Ghany MG. Hepatitis B cure: from discovery to regulatory approval. *Hepatology*. 2017;66(4):1296–313.
5. Hadziyannis SJ. Natural history of chronic hepatitis B in Euro-Mediterranean and African countries. *J Hepatol*. 2011;55(1):183–91.
6. Boettler T, Panther E, Bengsch B, Nazarova N, Spangenberg HC, Blum HE, et al. Expression of the interleukin-7 receptor alpha chain (CD127) on virus-specific CD8+ T cells identifies functionally and phenotypically defined memory T cells during acute resolving hepatitis B virus infection. *J Virol*. 2006;80(7):3532–40.
7. 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(6):1386–96.
8. 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(5):1185–94.
9. Evens AM, Jovanovic BD, Su YC, Raisch DW, Ganger D, Belknap SM, et al. Rituximab-associated hepatitis B virus (HBV) reactivation in lymphoproliferative diseases: meta-analysis and examination of FDA safety reports. *Ann Oncol*. 2011;22(5):1170–80.
10. Thimme R, Wieland S, Steiger C, Ghayeb J, Reimann KA, Purcell RH, et al. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. *J Virol*. 2003;77(1):68–76.
11. Asabe S, Wieland SF, Chattopadhyay PK, Roederer M, Engle RE, Purcell RH, et al. The size of the viral inoculum contributes to the outcome of hepatitis B virus infection. *J Virol*. 2009;83(19):9652–62.
12. Balkow S, Kersten A, Tran TT, Stehle T, Grosse P, Musseteau C, et al. Concerted action of the FasL/Fas and perforin/granzyme A and B pathways is mandatory for the development of early viral hepatitis but not for recovery from viral infection. *J Virol*. 2001;75(18):8781–91.
13. Guidotti LG, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. *Science*. 1999;284(5415):825–9.
14. Xia Y, Stadler D, Lucifora J, Reisinger F, Webb D, Hosel M, et al. Interferon-gamma and tumor necrosis factor-alpha produced by T cells reduce the HBV persistence form, cccDNA, Without Cytolysis. *Gastroenterology*. 2016;150(1):194–205.
15. Hoh A, Heeg M, Ni Y, Schuch A, Binder B, Hennecke N, et al. Hepatitis B virus-infected HepG2hNTCP cells serve as a novel immunological tool to analyze the antiviral efficacy of CD8+ T cells in vitro. *J Virol*. 2015;89(14):7433–8.
16. 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(6176):1221–8.
17. Boni C, Fisicaro P, Valdatta C, Amadei B, Di Vincenzo P, Giuberti T, et al. Characterization of hepatitis B virus (HBV)-specific T-cell dysfunction in chronic HBV infection. *J Virol*. 2007;81(8):4215–25.
18. 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(3):327–36.
19. Kurktschiev PD, Raziorrouh B, Schraut W, Backmund M, Wachtler M, Wendner CM, et al. Dysfunctional CD8+ T cells in hepatitis B and C are characterized by a lack of antigen-specific Tbet induction. *J Exp Med*. 2014;211(10):2047–59.

20. Khan O, Giles JR, McDonald S, Manne S, Ngiow SF, Patel KP, et al. TOX transcriptionally and epigenetically programs CD8(+) T cell exhaustion. *Nature*. 2019;571(7764):211–8.
21. Alfei F, Kanev K, Hofmann M, Wu M, Ghoneim HE, Roelli P, et al. TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. *Nature*. 2019;571(7764):265–9.
22. Yao C, Sun HW, Lacey NE, Ji Y, Moseman EA, Shih HY, et al. Single-cell RNA-seq reveals TOX as a key regulator of CD8(+) T cell persistence in chronic infection. *Nat Immunol*. 2019;20(7):890–901.
23. Wherry EJ. T cell exhaustion. *Nat Immunol*. 2011;12(6):492–9.
24. Schuch A, Salimi Alizei E, Heim K, Wieland D, Kiraithe MM, Kemming J, et al. Phenotypic and functional differences of HBV core-specific versus HBV polymerase-specific CD8+ T cells in chronically HBV-infected patients with low viral load. *Gut*. 2019;68(5):905–15.
25. 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(4):963–73 e9.
26. 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(2):1181–92.
27. Burton AR, Pallett LJ, McCoy LE, Suveizdye K, Amin OE, Swadling L, et al. Circulating and intrahepatic antiviral B cells are defective in hepatitis B. *J Clin Invest*. 2018;128(10):4588–603.
28. Salimzadeh L, Le Bert N, Dutertre CA, 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(10):4573–87.
29. 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(17):6669–74.
30. Menne S, Tumas DB, Liu KH, Thampi L, AlDeghathier D, Baldwin BH, et al. Sustained efficacy and seroconversion with the toll-like receptor 7 agonist GS-9620 in the woodchuck model of chronic hepatitis B. *J Hepatol*. 2015;62(6):1237–45.
31. 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(7):1508–17 17 e1–10.
32. Gane EJ, Lim YS, Gordon SC, Visvanathan K, Sicard E, Fedorak RN, et al. The oral toll-like receptor-7 agonist GS-9620 in patients with chronic hepatitis B virus infection. *J Hepatol*. 2015;63(2):320–8.
33. Janssen HLA, Brunetto MR, Kim YJ, Ferrari C, Massetto B, Nguyen AH, et al. Safety, efficacy and pharmacodynamics of vesatolimod (GS-9620) in virally suppressed patients with chronic hepatitis B. *J Hepatol*. 2018;68(3):431–40.
34. Daffis S, Balsitis S, Chamberlain J, Zheng J, Santos R, Rowe W, et al. Toll-like receptor 8 agonist GS-9688 induces sustained efficacy in the woodchuck model of chronic hepatitis B. *Hepatology*. 2020.
35. Lucifora J, Bonnin M, Aillot L, Fusil F, Maadadi S, Dimier L, et al. Direct antiviral properties of TLR ligands against HBV replication in immune-competent hepatocytes. *Sci Rep*. 2018;8(1):5390.
36. Isogawa M, Robek MD, Furuichi Y, Chisari FV. Toll-like receptor signaling inhibits hepatitis B virus replication in vivo. *J Virol*. 2005;79(11):7269–72.
37. Luangsay S, Ait-Goughoulte M, Michelet M, Floriot O, Bonnin M, Gruffaz M, et al. Expression and functionality of toll- and RIG-like receptors in HepaRG cells. *J Hepatol*. 2015;63(5):1077–85.
38. Wu J, Lu M, Meng Z, Trippler M, Broering R, Szczeponek A, et al. Toll-like receptor-mediated control of HBV replication by nonparenchymal liver cells in mice. *Hepatology*. 2007;46(6):1769–78.
39. Poeck H, Bscheider M, Gross O, Finger K, Roth S, Rebsamen M, et al. Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. *Nat Immunol*. 2010;11(1):63–9.
40. Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, Kato H, et al. 5'-triphosphate RNA is the ligand for RIG-I. *Science*. 2006;314(5801):994–7.
41. Pichlmair A, Schulz O, Tan CP, Naslund TI, Liljestrom P, Weber F, et al. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5'-phosphates. *Science*. 2006;314(5801):997–1001.
42. Suresh M, Korolowicz KE, Balarezo M, Iyer RP, Padmanabhan S, Cleary D, et al. Antiviral efficacy and host immune response induction during sequential treatment with SB 9200 followed by Entecavir in woodchucks. *PLoS One*. 2017;12(1):e0169631.
43. Ebert G, Poeck H, Lucifora J, Baschuk N, Esser K, Esposito I, et al. 5' Triphosphorylated small interfering RNAs control replication of hepatitis B virus and induce an interferon response in human liver cells and mice. *Gastroenterology*. 2011;141(2):696–706 e1–3.
44. Ablasser A, Goldeck M, Cavlar T, Deimling T, Witte G, Rohl I, et al. cGAS produces a 2'-5'-linked cyclic dinucleotide second messenger that activates STING. *Nature*. 2013;498(7454):380–4.
45. Civil F, Deimling T, de Oliveira Mann CC, Ablasser A, Moldt M, Witte G, et al. Structural mechanism of cytosolic DNA sensing by cGAS. *Nature*. 2013;498(7454):332–7.
46. Thomsen MK, Nandakumar R, Stadler D, Malo A, Valls RM, Wang F, et al. Lack of immunological DNA sensing in hepatocytes facilitates hepatitis B virus infection. *Hepatology*. 2016;64(3):746–59.
47. Dansako H, Ueda Y, Okumura N, Satoh S, Sugiyama M, Mizokami M, et al. The cyclic GMP-AMP synthetase-STING signaling pathway is required for both the innate immune response against HBV and the suppression of HBV assembly. *FEBS J*. 2016;283(1):144–56.
48. Guo F, Tang L, Shu S, Sehgal M, Sheraz M, Liu B, et al. Activation of stimulator of interferon genes in hepatocytes suppresses the replication of hepatitis B virus. *Antimicrob Agents Chemother*. 2017;61(10).
49. He J, Hao R, Liu D, Liu X, Wu S, Guo S, et al. Inhibition of hepatitis B virus replication by activation of the cGAS-STING pathway. *J Gen Virol*. 2016;97(12):3368–78.
50. 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(6):1212–9.
51. Fiscaro P, Valdatta C, Massari M, Loggi E, Biasini E, Sacchelli L, et al. Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B. *Gastroenterology*. 2010;138(2):682–93 e1–4.
52. Fiscaro P, Valdatta C, Massari M, Loggi E, Ravanetti L, Urbani S, et al. Combined blockade of programmed death-1 and activation of CD137 increase responses of human liver T cells against HBV, but not HCV. *Gastroenterology*. 2012;143(6):1576–85 e4.
53. Ferrando-Martinez S, Huang K, Bennett AS, Sterba P, Yu L, Suzich JA, et al. HBeAg seroconversion is associated with a more effective PD-L1 blockade during chronic hepatitis B infection. *JHEP Rep*. 2019;1(3):170–8.
54. Liu J, Zhang E, Ma Z, Wu W, Kosinska A, Zhang X, et al. Enhancing virus-specific immunity in vivo by combining therapeutic vaccination and PD-L1 blockade in chronic hepadnaviral infection. *PLoS Pathog*. 2014;10(1):e1003856.
55. Gane E, Verdon DJ, Brooks AE, Gaggar A, Nguyen AH, Subramanian GM, et al. Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: A pilot study. *J Hepatol*. 2019;71(5):900–7 **In this study, two promising drugs were combined in the human**

- setting, therefore providing important information on tolerance and efficacy of these therapies and highlighting the potential of checkpoint inhibitors.**
56. Schurich A, Khanna P, Lopes AR, Han KJ, Peppas 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(5):1494–503.
  57. Lee YB, Lee JH, Kim YJ, Yoon JH, Lee HS. The effect of therapeutic vaccination for the treatment of chronic hepatitis B virus infection. *J Med Virol*. 2015;87(4):575–82.
  58. Al Mahtab M, Akbar SMF, Aguilar JC, Guillen G, Penton E, Tuero A, et al. Treatment of chronic hepatitis B naive patients with a therapeutic vaccine containing HBs and HBc antigens (a randomized, open and treatment controlled phase III clinical trial). *PLoS One*. 2018;13(8):e0201236.
  59. 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(1):227–41 e7.
  60. Zahn T, Akhras S, Spengler C, Murra RO, Holzhauser T, Hildt E. A new approach for therapeutic vaccination against chronic HBV infections. *Vaccine*. 2020;38(15):3105–20.
  61. Ilan Y, Nagler A, Adler R, Naparstek E, Or R, Slavin S, et al. Adoptive transfer of immunity to hepatitis B virus after T cell-depleted allogeneic bone marrow transplantation. *Hepatology*. 1993;18(2):246–52.
  62. Ilan Y, Nagler A, Zeira E, Adler R, Slavin S, Shouval D. Maintenance of immune memory to the hepatitis B envelope protein following adoptive transfer of immunity in bone marrow transplant recipients. *Bone Marrow Transplant*. 2000;26(6):633–8.
  63. Bohne F, Chmielewski M, Ebert G, Wiegmann K, Kurschner T, Schulze A, et al. T cells redirected against hepatitis B virus surface proteins eliminate infected hepatocytes. *Gastroenterology*. 2008;134(1):239–47.
  64. Krebs K, Bottinger N, Huang LR, Chmielewski M, Arzberger S, Gasteiger G, et al. T cells expressing a chimeric antigen receptor that binds hepatitis B virus envelope proteins control virus replication in mice. *Gastroenterology*. 2013;145(2):456–65.
  65. Kruse RL, Shum T, Tashiro H, Barzi M, Yi Z, Whitten-Bauer C, et al. HBsAg-redirection T cells exhibit antiviral activity in HBV-infected human liver chimeric mice. *Cytherapy*. 2018;20(5):697–705 **These studies highlight the potential of engineered virus-specific CD8+ T cells to exert antiviral functions in vivo.**
  66. Koh S, Kah J, Tham CYL, Yang N, Ceccarello E, Chia A, et al. Nonlytic lymphocytes engineered to express virus-specific T-cell receptors limit HBV infection by activating APOBEC3. *Gastroenterology*. 2018;155(1):180–93 e6.
  67. Kah J, Koh S, Volz T, Ceccarello E, Allweiss L, Lutgehetmann M, et al. Lymphocytes transiently expressing virus-specific T cell receptors reduce hepatitis B virus infection. *J Clin Invest*. 2017;127(8):3177–88.
  68. Qasim W, Brunetto M, Gehring AJ, Xue SA, Schurich A, Khakpoor A, et al. Immunotherapy of HCC metastases with autologous T cell receptor redirected T cells, targeting HBsAg in a liver transplant patient. *J Hepatol*. 2015;62(2):486–91.

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.