Virologica Sinica 38 (2023) 851–859

Contents lists available at ScienceDirect

Virologica Sinica

journal homepage: www.keaipublishing.com/en/journals/virologica-sinica
www.virosin.org

Review

Recent advances in understanding T cell activation and exhaustion during HBV infection

Lu Wang [a,b,1], Xiaoqing Zeng [a,1], Zida Wang [a], Ling Fang [c,*], Jia Liu [a,b,*]

[a] Department of Infectious Diseases, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430022, China
[b] Joint International Laboratory of Infection and Immunity, Huazhong University of Science and Technology, Wuhan, 430022, China
[c] Central Sterile Supply Department, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430022, China

ARTICLE INFO

Keywords:
Hepatitis B virus (HBV)
T cell
Immune therapy

ABSTRACT

Chronic hepatitis B virus (HBV) infection remains a major public health concern globally, and T cell responses are widely believed to play a pivotal role in mediating HBV clearance. Accordingly, research on the characteristics of HBV-specific T cell responses, from activation to exhaustion, has advanced rapidly. Here, we summarize recent developments in characterizing T cell immunity in HBV infection by reviewing basic and clinical research published in the last five years. We provide a comprehensive summary of the mechanisms that induce effective anti-HBV T cell immunity, as well as the latest developments in understanding T cell dysfunction in chronic HBV infection. Furthermore, we briefly discuss current novel treatment strategies aimed at restoring anti-HBV T cell responses.

1. Introduction

According to data from the World Health Organization (WHO), the global prevalence of chronic hepatitis B virus (HBV) infection in 2019 was estimated to be 296 million individuals (WHO, 2023). Among them, only 30.4 million people were aware of their hepatitis B status, indicating that a significant proportion of affected individuals remained undiagnosed. Moreover, about 1.5 million people were newly infected with chronic hepatitis B infection and 0.8 million people died from hepatitis B infection-related causes in 2019 (WHO, 2023). HBV is a non-cytopathic, partially double-stranded DNA virus and a prototypical member of the *Hepadnaviridae* family (Iannacone and Guidotti, 2022). HBV is transmitted by contact with infected blood or bodily fluids, exclusively targeting hepatocytes. Exposure to HBV in neonates or early childhood usually leads to viral persistence, whereas more than 95% of infected adults spontaneously clear the virus (Iannacone and Guidotti, 2022). Persistent HBV infection increases the risk of end-stage liver diseases, such as cirrhosis and hepatocellular carcinoma (HCC).

The WHO has set a goal to eliminate viral hepatitis as a major public health threat by 2030, aiming for a 90% reduction in new cases of chronic HBV and hepatitis C virus (HCV) infections. With the current safe and highly effective direct-acting antiviral (DAA) drugs for HCV, a cure for chronic HCV infection is expected in most patients. In contrast, as the currently available treatments for CHB are suboptimal, a cure is difficult to achieve in most patients. Two types of antivirals available for CHB: pegylated interferon alpha 2 (PEG-IFN-α2) and nucleotide/nucleoside analogs (NUCs), such as entecavir and tenofovir. However, both treatments have certain limitations. Side effects, such as flu-like symptoms, mood disorders, and depression, are associated with PEG-IFN-α2 treatment, and only about one-third of patients receiving the treatment achieve long-term virus clearance (Locarnini et al., 2015). Use of NUCs, such as lamivudine, adefovir, selects for resistance mutations and is hampered by frequent episodes of rebounding viremia after the cessation of antiviral therapy (Locarnini et al., 2015). Although novel NUCs including entecavir, tenofovir, and tenofovir alafenamide fumarate have greatly improved drug resistance, improving serological conversion rates remains a challenge (Locarnini et al., 2015). Therefore, alternative strategies for treating chronic HBV infection are urgently needed.

Clearance of HBV relies primarily on the coordinated activation of different immune system components, and T cell responses play a crucial role. Both $CD4^+$ and $CD8^+$ T cells are essential for successful HBV clearance, as demonstrated in studies involving HBV-infected chimpanzees (Thimme et al., 2003). During acute, self-resolving HBV infection, strong HBV-specific $CD8^+$ T cells are detected, which recognize HBV-infected hepatocytes and eliminate the virus through both cytotoxic and non-cytotoxic pathways (Thimme et al., 2003). In contrast, in

* Corresponding authors.
E-mail addresses: 287812165@qq.com (L. Fang), jialiu77@hust.edu.cn (J. Liu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.virs.2023.10.007
Received 4 July 2023; Accepted 18 October 2023
Available online 21 October 2023

chronic HBV infection, severe dysfunction of HBV-specific T cell responses is frequently observed, which is believed to contribute to viral persistence (Bertoletti and Ferrari, 2016). Therefore, modulating the immune response to reconstitute an efficient anti-HBV effector T cell response is considered a promising approach for curing chronic hepatitis B (CHB) (Lumley et al., 2018). Accordingly, numerous studies have sought to characterize the nature of the anti-HBV T cell response and the mechanisms underlying T cell dysfunction and exhaustion during chronic HBV infection (Benechet et al., 2019; Bertoletti and Ferrari, 2016; Fisicaro et al., 2020), making T cell research one of the fastest growing fields in HBV immunology. In this review, we summarize recent developments in the characterization of T cell responses during HBV infection by describing both basic research and clinical studies published in the last five years, with a focus on elucidating the mechanisms that induce effective anti-HBV T cell responses and those that lead to T cell exhaustion (Fig. 1).

2. Mechanisms that induce effective anti-HBV T cell immunity

When adults are exposed to HBV, the virus is typically eliminated spontaneously, and this process induces robust and efficient anti-HBV T cell immunity within the liver. A detailed understanding of the related mechanisms is essential for designing successful functional T cell correction strategies. Investigations on how effective anti-HBV T cell immunity is generated in an HBV-infected individual have long been neglected. However, recent progress has been made in identifying the immune factors that play important roles in mediating anti-HBV T cell responses.

The liver exhibits immune tolerance in some conditions, such as a physiological state or liver transplantation (Lee et al., 2021; Protzer et al., 2012; Ronca et al., 2020). Remarkably, this inherent tolerogenic property of the liver is believed to be involved in the chronicity of HBV infection (Protzer et al., 2012). However, when acute infection of hepatitis A virus (HAV), hepatitis E virus (HEV), or HBV occurs in adults, the liver shows an antigen-specific immune activation, leading to complete clearance of the virus. This suggests the existence of a mechanism of specific immune activation. In HBV infection, liver sinusoidal endothelial cells (LSECs) are involved in T cell activation. We previously demonstrated that under inflammatory conditions, LSECs switch from a tolerogenic state to an immunogenic state and trigger cytotoxic effector $CD8^+$ T cell activation (Huang et al., 2018; Liu et al., 2013). In line with these findings, we recently demonstrated that LSECs also switch from a tolerogenic state to an immunogenic state upon stimulation with HBV e antigen (HBeAg) (Xie et al., 2021, 2022). HBeAg is frequently expressed during both acute and chronic HBV infection (Mutimer et al., 2022), and has been widely regarded as a key immunomodulator for promoting host innate and adaptive immune tolerance during chronic HBV infection via a number of different mechanisms, such as down-regulation of TLR expression, modulating macrophage function and inducing MDSC activation, etc (Xie et al., 2021). These recent findings provide direct evidence to demonstrate that HBeAg could also serve as a stimulator to trigger LSEC maturation and prepare the intrahepatic immune microenvironment to facilitate anti-HBV CTL to execute their effector functions. Activated effector T cells co-cultured with HBeAg-exposed LSECs produce significantly higher amounts of IFN-γ than those co-cultured with control LSECs, and HBeAg assists LSECs as antigen-presenting cells (APCs) to activate T cells in HBV infection. LSECs trigger specific T cell activation by increasing TNFα and IL27 expression in LSECs (Xie et al., 2021). Moreover, *in vivo* TNF-α blockade in an HBV-replicating mice model results in impaired HBV-specific $CD8^+$ T cell immunity and delayed HBV clearance (Xie et al., 2022).

CD100 is a costimulatory molecule that is constitutively expressed on resting T cells and can be cleaved from the cell surface by matrix metalloproteases (MMPs) to generate soluble CD100. We recently reported that during acute HBV infection, the liver produces increased amounts of MMP2, which together with MMP9, mediates CD100 shedding from the surface of T cells and increases serum soluble CD100 (sCD100) levels. CD72, a CD100 receptor, is expressed in most professional APCs. The CD100-CD72 signal promotes T cell activation. By interacting with CD72, sCD100 induces the activation of APCs, including dendritic cells (DCs) and LSECs in the spleen and liver, thus promoting an intrahepatic anti-HBV $CD8^+$ T cell response (Yang et al., 2019).

Meanwhile, cytokines also play an important role in T-cell activation. The classical effector cytokine, IL-2, has recently been shown to play an important role in inducing the intrahepatic effector $CD8^+$ T-cell response against HBV. Bénéchet et al. demonstrated that priming of $CD8^+$ T cells

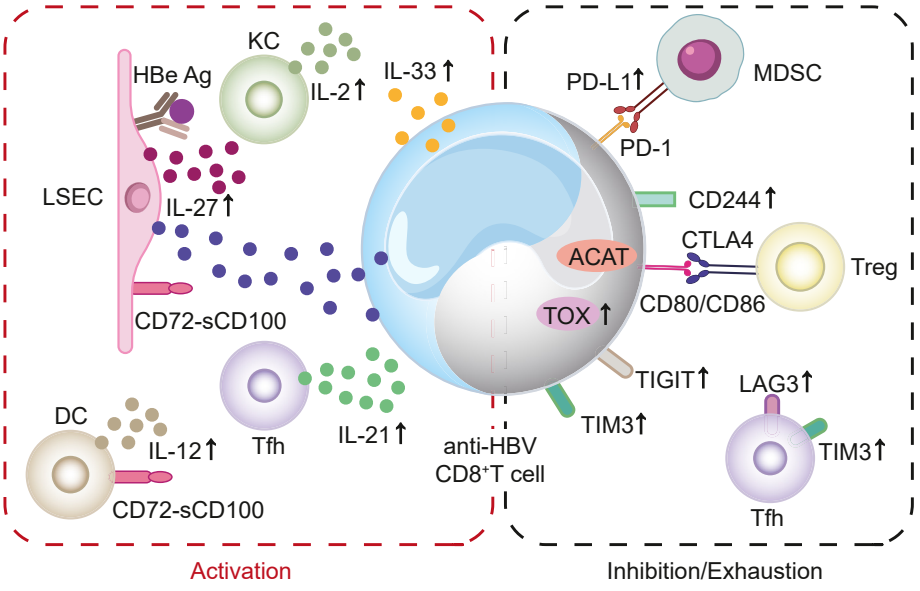

Fig. 1. Schematic diagram of newly identified mechanisms of T cell activation and inhibition/exhaustion in HBV infection. Activation mechanisms: IL-33; IL-2 produced by Kupffer cells; IL-27 produced by HBeAg stimulated liver sinusoidal endothelial cells (LSECs); IL-12 produced by sCD100 stimulated dendritic cells; IL-21 produced by follicular helper T (Tfh) cells. Inhibition/Exhaustion mechanisms: upregulation of PD-L1 by MDSCs; upregulated CD244, TIGIT and TIM3 by CD8 + T cells; upregulation of LAG-3 and TIM-3 by circulating Tfh cells; upregulation of CTLA-4 by Tregs; TOX; ACTA.

by hepatocytes, the natural targets of HBV, leads to local activation and proliferation of these T cells, but does not lead to their differentiation into effector T cells. Instead, an exhaustion-like signature was progressively enriched in the transcriptome of these hepatocyte-primed $CD8^+$ T cells as determined by transcriptomic and chromatin accessibility analyses. Importantly, the effector function of dysfunctional hepatocyte-primed $CD8^+$ T cells could be rescued by treatment with IL-2 but not anti-PD-L1 antibodies (Benechet et al., 2019). The same research group performed a mechanistic analysis and showed that when $CD8^+$ T cells are primed by Kupffer cells (KCs), which are not the usual targets of HBV, they differentiate into effector cells. They determined that by sensing IL-2 and cross-presenting hepatocellular antigens, a subset of KCs (referred to as KC2) overcomes the tolerogenic potential of the hepatic microenvironment and improves the antiviral function of T cells (De Simone et al., 2021). Hepatocellular antigen recognition by effector $CD8^+$ T cells triggers a marked increase in the number of a special intrahepatic subset of lymphocytes, group 1 innate lymphoid cells (ILC1s). ILC1s constrain intrahepatic HBV-specific effector $CD8^+$ T cell proliferation by controlling local IL-2 availability, thus limiting attendant immunopathology (Fumagalli et al., 2022). The effect of IL-2 on the restoration of anti-HBV T cell responses was recently demonstrated in a clinical trial. In a clinical trial, 38 CHB patients who did not respond to IFN-α therapy were treated with or without low-dose IL-2 for 24 weeks. The findings revealed that IL-2 treatment notably enhanced the frequency and function of HBV-specific $CD8^+$ T cells, which resulted in better clinical outcomes for non-responders, including HBeAg seroconversion. Additionally, non-responders exhibited lower levels of regulatory T cells (Tregs) and reduced expression of programmed cell death protein 1 (PD-1) (Wang et al., 2021).

As the third signal, cytokines also exert positive effect to activate T cell function. The lack of cytokine IL-12 secretion contributes to chronic HBV infection, leading to an insufficient HBV-specific adaptive T cell response (Beckebaum et al., 2003; Liu et al., 2022). Consistently, *ex vivo* treatment with IL-12 potently increases the capacity of HBV-specific $CD8^+$ T cells to produce effector cytokines and rescues the antiviral function of exhausted HBV-specific $CD8^+$ T cells (Schurich et al., 2013). Zhang et al. developed a nanovaccine loaded IL-12 expression plasmid adjuvant, resulting in robust HBV-specific $CD8^+$ T and $CD4^+$ T cell responses (Zhao et al., 2021). However, another member of the IL-12 family, IL-35, seems to exert adverse effects by inhibiting the response of HBV-specific CTLs through the JAK1/TYK2/STAT1/STAT4 pathway (Dong et al., 2020). IL-35 exhibits noteworthy immunosuppressive effects against HBV antigen-specific $CD8^+$ T cells through both cytolytic and non-cytolytic mechanisms (Shao et al., 2017). Li et al. demonstrated that exogenous IL-33 treatment on PBMCs of CHB patients could inhibit PD-1 expression on HBV-specific $CD8^+$ T cells, and induce the proliferation and direct cytolytic activity of these cells (Li et al., 2022). Follicular helper T (Tfh) cells are a unique subset of IL-21-producing $CD4^+$ T cells that directly promotes antibody secretion from B cells in germinal centers (GCs) (Ma et al., 2012). According to the study by Hou et al., exogenous IL-21 can restore the function of CXCR5+$CD8^+$ T cells in chronic HBV infection. The study also noted an increase in IFN-γ production from CXCR5+$CD8^+$ T cells in patients with chronic HBV infection (Li et al., 2020). Consistently, mice with prior exposure to IL-21 no longer support HBV persistence owing to increased HBV-specific $CD8^+$ T cell infiltration and activation. Notably, mice lacking the IL-21 receptor exhibit a notable reduction in the frequency of HBV-specific $CD8^+$ T cells, along with elevated levels of hepatitis B surface antigen (HBsAg) in the serum (Shen et al., 2021; Tang et al., 2019). Shen et al. reported that a single injection of an adeno-associated virus (AAV) expressing murine IL-21 efficiently induces HBV clearance from both the serum and liver of mouse models with persistent HBV replication. IL-21-induced clearance is associated with the activation and liver infiltration of $CD8^+$ T cells, and IL-21-cured mice are protected from HBV rechallenge owing to long-term protective T cell memory (Shen et al., 2019). Clinical observations also support an anti-HBV role for IL-21. The frequency of circulating IL-21-producing $CD4^+$ T cells is significantly higher in patients with spontaneously resolved HBV infection than in those with occult HBV infection (OBI) (Zhang et al., 2022). Interestingly, when IL-21 is absent during chronic HBV infection, IL-27 compensates for its function by promoting Tfh-B cell activity. This is crucial for generating a protective antibody response, which may aid in viral clearance (Khanam et al., 2020). However, it is noteworthy that a high frequency of Tfh cells and high serum IL-12/21 levels are also observed in patients with HBV-related acute chronic liver failure (HBV-ACLF). In the presence of HBV-ACLF serum that is rich in IL-12/21, naive $CD4^+$ T cells differentiate into Tfh cells, which is blocked by neutralizing IL-12/21 antibodies (Du B et al., 2021). These results suggest that IL-12/21 may also trigger excessive or improperly regulated immune responses and lead to immunopathological consequences, such as immune-mediated tissue damage, exacerbated inflammatory responses, or other immune-related disorders. Therefore, further studies are needed to fully characterize the mechanisms by which these cytokines induce anti-HBV T cell responses to balance their immune-activating and immunopathological effects.

In addition to $CD8^+$ T cells, the role of $CD4^+$ T cells in mediating HBV clearance should not be neglected. It has long been demonstrated decades ago in the HBV infection chimpanzee model, where the depletion of $CD4^+$ T cells prior to HBV inoculation resulted in persistent HBV infection, suggesting $CD4^+$ T cells are equally important as $CD8^+$ T cells in containing chronic HBV infection (Asabe et al., 2009). In line with this observation, numerous studies have demonstrated that human leukocyte antigen (HLA) polymorphisms, especially those in HLA class II genes, are significantly associated with resolution of HBV infection (systematically reviewed in Xu et al., 2021), which further indicates the association between $CD4^+$ T cell responses and HBV clearance during HBV infection in humans. Recently, Ruben C Hoogeveen et al. analyzed HBV-specific T cell response in 124 HBV-infected individuals. They found that in CHB patients who achieved functional cure, the frequencies of functional HBV-specific $CD4^+$ T cells, but not $CD8^+$ T cells, were significantly increased compared to those with chronic infection (Hoogeveen et al., 2022). Taken together, these results suggest that future immunotherapeutic approaches designed to induce HBV functional cure should also aim to improve $CD4^+$ T cell responses.

Collectively, these findings provide new insights into the development of effective anti-HBV T cell immunity upon HBV exposure (Table 1).

3. Understating T cell inhibition and exhaustion during chronic HBV infection

During acute HBV infection, naive $CD8^+$ T cells undergo robust proliferation and clonal expansion to differentiate into effector $CD8^+$ T cells. These effector cells directly eliminate target cells to control the infection. During the process of effector $CD8^+$ T cell differentiation, the cells undergo transcriptional, epigenetic, and metabolic reprogramming and acquire effector features, such as the ability to generate cytokines and cytotoxic molecules (Kumar et al., 2018). Following antigen clearance and resolution of inflammation, most activated T cells die. However, a subset of T cells persists and differentiates into memory T cells, which survive for long periods (Kumar et al., 2018). In contrast to the events that occur following acute infections, during chronic infections with persistent stimulation, memory T cells fail to efficiently develop and T cells become exhausted (Wherry, 2011). T cells that are exhausted exhibit impaired effector functions, along with amplified and sustained expression of inhibitory receptors. These cells also have altered epigenetic and transcriptional profiles, and display a distinct metabolic lifestyle. Additionally, exhausted T cells lack the ability to transition to the quiescent, antigen-independent characteristics of memory cells (McLane et al., 2019). In chronic HBV infection, virus-specific T cells are deeply dysfunctional, and the severity of their hyporesponsive state depends on multiple factors (Table 2). This hyporesponsive state, known as T cell exhaustion, results from the progressive loss of T cell effector functions because of repeated triggering caused by persistent exposure to high

Table 1
Novel identified mechanisms for inducing anti-HBV effector T cell responses.

Effectors	Mechanism	Cite
MMP2/MMP9 and CD100	MMP2 and MMP9 mediate CD100 shedding from the surface of T cells and increase serum sCD100 levels. By interacting with CD72, sCD100 induces the activation of APCs, such as DCs and LSECs in the spleen and liver, thus promoting the intrahepatic anti-HBV $CD8^+$ T cell response.	Yang et al. 2019
IL-2	(1) The effector function of hepatocyte-primed $CD8^+$ T cells could be rescued by treatment with IL-2, but not by anti-PD-L1 antibodies. (2) By sensing IL-2 and cross-presenting hepatocellular antigens, a subset of KCs (referred to as KC2) overcome the tolerogenic potential of the hepatic microenvironment and improve the antiviral function of T cells. (3) Interactions between ILC1s and effector $CD8^+$ T cells undergoing hepatocellular antigen recognition can constrain $CD8^+$ T cell proliferation by controlling local IL-2 availability.	Benechet et al. 2019 De Simone et al. 2021 Fumagalli et al. 2022
IL-12	(1) IL-12 enhances Tfh cell differentiation. (2) Nanovaccine-loaded IL-12 expression plasmid adjuvant generates robust HBV-specific $CD8^+$ T and $CD4^+$ T cell responses.	Du B et al. 2021 Zhao et al. 2021
IL-21	(1) Exogenous IL-21 could restore the function of CXCR5+CD8+T cells in chronic HBV infection. (2) IL-21 could enhance intrahepatic HBV-specific CD8+T cell infiltration and activation. (3) IL-21 could induce long-term protective T cell memory.	Li et al. 2020 (Shen et al., 2021; Tang et al., 2019) Shen et al. 2019
IL-27	(1) IL-27 could compensate for impaired IL-21 function in chronic HBV infection. (2) LSEC produced IL-27 could revert LSEC induced T cell tolerance.	Khanam et al. 2020 (Xie et al., 2021, 2022)
HBeAg stimulated LSEC	HBeAg stimulation induces LSECs to trigger specific T cell activation by increasing TNFα and IL-27 expression in LSECs.	(Xie et al., 2021, 2022)

Table 2
Advances in understating T cell exhaustion during chronic HBV infection.

Effectors	Mechanism	Cite
PD-1/PD-L1	(1) PD-1 expression on HBV-specific T cells is not only determined by the stage of infection but also by the HBV region targeted by the T cells. PD-1 expressed on HBV-env specific T cells was barely detectable in chronic HBV infection compared to acute infection. (2) HBV-specific $CD8^+$ T cells are not terminally exhausted but rather exhibit a memory-like phenotype in patients with low viral load, and PD-1/PD-L1 blockade could only restore the effector function of HBV-specific $CD8^+$ T cell responses in these patients.	Hoogeveen et al. 2019 (Aliabadi et al., 2022; Ferrando-Martinez et al., 2021; Schuch et al., 2019)
CD244	CD244 and PD-1 are highly co-expressed on virus-specific $CD8^+$ T-cells in chronic HBV infection and blocking CD244 or its ligand CD48 may restore T-cell function in dependent of the PD-1 pathway.	(Liu et al., 2014; Raziorrouh et al., 2010)
TIGIT	(1) High expression of TIGIT on T cells is associated with functional exhaustion during chronic HBV infection. Blocking TIGIT can reverse T cell exhaustion and restore function in patients with chronic HBV infection. (2) PD-1+ TIGIT + $CD8^+$ T-cell populations are associated with accelerated disease progression and poor outcomes in associated hepatocellular carcinoma.	Wei et al. 2022 Liu et al. 2019
TIM-3	TIM-3 is significantly upregulated on $CD8^+$ T cells in patients with active chronic HBV infection compared to inactive chronic HBV group.	Mohammadizad et al. 2019
MDSC	(1) MDSCs affect HBV-specific T-cell response and homing via upregulating PD-L1 in response to IL-1β stimulation. (2) gMDSCs express liver-homing chemokine receptors and accumulate in the liver, and potently inhibit T cells in a partially arginase-dependent manner during chronic HBV infection.	Pal et al. 2022 Pallett et al. 2015
Tfh	Circulating Tfh cells from chronic asymptomatic HBV carriers express higher levels of LAG-3 and TIM-3 in comparison with those of healthy controls.	Cui et al. 2022
Treg	Treg cells suppress the anti-HBV function of Tfh cells via CTLA-4.	Wang et al. 2018
TOX	TOX is necessary and sufficient to induce major features of exhausted $CD8^+$ T cells. TOX expression in HBV-specific $CD8^+$ T cells is linked to chronic antigen stimulation, correlates with viral load, and is associated with the phenotypic characteristics of T cell exhaustion.	(Alfei et al., 2019; Sekine et al., 2020)
ACAT	(1) ACAT inhibition can reduce neutral lipid droplets in T cells by blocking the esterification of cholesterol and divert it to the cell membrane to enhance lipid microdomain formation and TCR signaling, resulting in enhanced functionality. (2) ACAT inhibition can trigger TCR-independent boosting of glycolysis and OXPHOS, thereby optimizing $CD8^+$ T cell bioenergetics to support proliferation and effector function.	Schmidt et al. 2021 Schmidt et al. 2021

antigen concentrations (McLane et al., 2019). Notably, exhausted T cells have distinct transcriptional program and an open chromatin region that distinguishes them from effective cells or memory cells, so exhausted T cells are not just a state of activated effector or memory cells, but probably belong to a distinct cell type (Khan et al., 2019). Exhausted T cells express high levels of inhibitory receptors, including PD-1, cytotoxic T lymphocyte antigen 4 (CTLA-4), T cell immunoglobulin and mucin domain-containing molecule 3 (TIM-3), lymphocyte activation gene 3 (LAG-3), CD244 (2B4), and T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif (ITIM) domain (TIGIT), which suppress the activation signals generated by TCR and costimulatory molecules (Wherry and Kurachi, 2015). However, these cells may maintain some of their effector functions, resulting in a dynamic pathogen-host balance (Wherry, 2011).

PD-1/PD-L1 is one of the most studied inhibitory pathways in T cells during HBV infection. During acute viral infections, PD-1 expression is

upregulated to limit effector functions following T cell activation (Hakim et al., 2020). Following viral clearance, PD-1 expression is down-regulated (Liu et al., 2014). In contrast, PD-1 is continuously expressed in HBV-specific T cells from persistently infected patients and is responsible for the dysfunctional state of HBV-specific T cells in the chronic phase of the disease (Hakim et al., 2020). PD-1 expression in HBV-specific T cells is determined not only by the stage of infection but also by the types of HBV antigens recognized by T cells, including surface or envelope antigens. When detectable, $CD8^+$ T cells targeting the HBV envelope protein have the highest PD-1 expression in acute infection but not in chronic HBV infection, followed by HBV core-specific T cells. HBV polymerase-specific T cells are at the other end of the spectrum, with the lowest PD-1 expression levels (Hoogeveen et al., 2019). Myeloid-derived suppressor cells (MDSCs) are an important subset of suppressive cells that negatively regulate the immune response. Human MDSCs inhibit anti-HBV core-T cell responses via the PD-1/PD-L1 pathway (Pal et al., 2022). In patients with CHB, intrahepatic MDSCs express significantly higher PD-L1 levels than their peripheral counterparts, and blocking PD-L1 on MDSCs restores both IL-2 and IFN-γ secretion by T cells (Pal et al., 2022). Li et al. utilized exogenous IL-33 to inhibit expression of PD-1 to reinvigorate expanding capability and cytokines production of CD8 T cells. This proved that solving exhaustion of $CD8^+$ T cells is meaningful for improving CHB outcomes (Li et al., 2022). However, the blockade of PD-1/PD-L1 enhances the response of HBV-specific $CD8^+$ T cells, but only in patients with lower levels of T cell exhaustion. Conversely, in patients with higher frequencies of exhausted HBV-specific $CD8^+$ T cells, the response to PD-L1 blockade is abrogated (Ferrando-Martinez et al., 2021). This suggests that the efficacy of PD-1 monotherapy and combination strategies with adjuvants to achieve a functional cure for HBV may be limited. We previously demonstrated that combining NUC treatment, therapeutic vaccination, and PD-1/PD-L1 blockade, but not PD-1/PD-L1 blockade alone, rescues the antiviral function of $CD8^+$ T cells and clears the virus in chronic woodchuck hepatitis virus-infected animals (Liu et al., 2014). Other inhibitory molecules also contribute to HBV-specific T cell exhaustion and are potential targets for immunotherapy in chronic HBV infections. In chronic HBV infection, CD244 and PD-1 are highly co-expressed on HBV core-$CD8^+$ T cells, as reported by Raziorrouh et al., and blocking CD244 or its ligand, CD48, may restore T cell function independently of the PD-1 pathway (Raziorrouh et al., 2010). Wei et al. reported that TIGIT expression in T cells is significantly increased in CHB patients, and its expression is associated with functional exhaustion of T cells. Importantly, this study demonstrated that blocking TIGIT reverses T cell exhaustion and restores T cell function in patients (Wei et al., 2022). In line with this observation, Liu et al. demonstrated that patients with advanced stage and progressed HBV-HCC have an elevated population of PD-1+ TIGIT + $CD8^+$ T-cells. These cells have been found to exhibit features of exhausted T-cells, including excessive activation, high expression of other inhibitory receptors, high susceptibility to apoptosis, decreased capacity for cytokine secretion, and patterns of transcription factor expression consistent with exhaustion (Liu et al., 2019). Moreover, circulating $CD4^+$ T cells, especially follicular helper T (Tfh) cells, from chronic asymptomatic HBV carriers, highly express LAG-3 and TIM-3 when compared to the levels in healthy controls (Cui et al., 2022). The number of $CD8^+$ T cells expressing TIM3 is significantly higher in patients with active chronic HBV infection than in patients with inactive chronic HBV infection (Mohammadizad et al., 2019). Recently, Liu et al. observed that circulating NK cells from CHB patients express higher amount of galectin-9 (Gal-9), the natural ligand of TIM-3, and thus interacts with TIM-3+ $CD8^+$ T cells and probably contributes to dysfunction of $CD8^+$ T cells during chronic HBV infection (Liu et al., 2022). Wang et al. reported that Tregs suppress the anti-HBV function of Tfh cells via CTLA-4. Depletion of Treg cells or inhibition of Treg cell function with a CTLA4-blocking antibody restores the Tfh cell response against HBsAg and promotes HBV clearance in mice with persistent HBV infection. Consistently, impaired Tfh cell responses to HBsAg are observed in CHB patients, and depletion of Tregs or blockade of CTLA4 may help to restore responsiveness and improve the immune response against the virus (Wang et al., 2018).

One of the most important developments in characterizing T cell exhaustion during HBV infection is the identification of thymocyte selection-associated HMG BOX (TOX) as a biomarker specific for dysfunctional virus-specific $CD8^+$ T cells. TOX is necessary and sufficient to induce major features of exhausted $CD8^+$ T cells (Tex), including inhibitory receptor expression, expression of transcription factors required for Tex, and decreased features of effector T cells (Tef)(Alfei et al., 2019). Moreover, chromatin accessibility at the TOX locus is higher in Tex cells than in effector $CD8^+$ T cells, and high and sustained TOX was observed only during chronic infection (Khan et al., 2019). TOX is expressed in a subset of $CD8^+$ T cells with increased expression of cytolytic proteins, namely granzyme B (GzmB), perforin, and certain inhibitory receptors such as PD-1, TIGIT, 2B4, and CD39 (Sekine et al., 2020). Transcriptional regulation of HBV-specific $CD8^+$ T cell exhaustion is attracting increasing attention. The expression of TOX in HBV-specific $CD8^+$ T cells is associated with chronic antigen stimulation, and it is correlated with viral load. Furthermore, this expression is linked to the phenotypic features of T cell exhaustion. Hofmann and Thimme found that TOX expression in HBV-specific $CD8^+$ T cells is associated with higher expression of PD-1, CD57, EOMES, and Helios, which is indicative of T cell exhaustion. Interestingly, TOX expression in HBV-specific $CD8^+$ T cells is sustained even after spontaneous or NUC-mediated viral control in chronic HBV infection. However, this expression is not observed in self-limiting acute HBV infection. These findings suggest that chronic stimulation results in a permanent molecular imprint that maintains TOX expression, whereas temporary stimulation does not (Heim et al., 2020).

In addition to the checkpoint molecules associated with T cell exhaustion in HBV, the impact of T cell metabolism on HBV infection has also attracted attention. Bioenergetic pathways play a role in shaping effective immune responses. Schmidt et al. recently showed a significant increase in the percentage of HBV-specific $CD8^+$ T cells among PBMCs collected from patients with HBV upon *ex vivo* acyl-coenzyme A: cholesterol acyltransferase (ACAT) inhibition, similar to the production of IFN-γ by $CD4^+$ and $CD8^+$ T cells (Schmidt et al., 2021). Blocking the esterification of cholesterol by inhibiting ACAT reduces the accumulation of neutral lipid droplets in T-cells, thereby promoting lipid microdomain formation and T cell receptor (TCR) signaling, which leads to enhanced T-cell functionality (Schmidt et al., 2021). ACAT inhibition also triggers TCR-independent boosting of glycolysis and OXPHOS, thereby optimizing $CD8^+$ T cell bioenergetics to support proliferation and effector function. In a clinical cohort, 11 out of 26 patients treated with a combination of ACAT inhibition and PD-1 blockade show the strongest boosting of HBV-specific responses compared to that in the single treatment groups (Schmidt et al., 2021). Therefore, PD-1 blockade and ACAT inhibition act in a complementary manner to optimize efficacy. The proliferative expansion and immune responses of HBV-specific T cells are suppressed by gMDSCs (Pallett et al., 2015). In the local liver milieu, gMDSCs accumulate and they exhibit elevated expression and degranulation of arginase I. Moreover, they are closely associated with T cells in the narrow-lumen sinusoidal vasculature. gMDSCs influence immunotolerance to high levels of HBV replication, and arginase I is a major effector in the mechanism underlying the expansion of the gMDSCs population in CHB. How the metabolism of T cells influences exhaustion in CHB remains to be determined.

T cell exhaustion and dysfunction in patients with CHB have been intensively characterized in recent years. Clinical data collected from 243 patients infected with HBV revealed that the overall lymphocyte population remains unaffected by HBsAg. However, the number of HBs-specific T cells decreases in correlation with the duration of HBsAg exposure, rather than the amount of HBsAg. Additionally, the percentage of HBs-specific T cells among the total HBV-specific T-cell population is significantly higher in patients younger than 30 years, constituting 28.26%, but decreases to 7.14% in patients older than

30 years (Le Bert et al., 2020). HBV-specific T cell response is influenced by patient age but not by HBsAg levels (Aliabadi et al., 2022). In a study of 54 treatment-naive patients with chronic HBV infection aimed to analyze T cell function in CHB, expression of PD-1 in the total $CD4^+$ T cell population is significantly lower in subjects with spontaneous HBsAg seroclearance than in the HBsAg-positive group (Liu et al., 2022). By applying peptide-loaded MHC I tetramer-based enrichment, Thimme et al. detected HBV-specific $CD8^+$ T cell-targeting epitopes in the HBV core and polymerase proteins in most of the 85 tested patients with chronic HBV who had low viral loads. The data showed that in patients with low viral loads, HBV-specific $CD8^+$ T cells are not terminally exhausted, but rather exhibit a memory-like phenotype, which may reflect weak, ongoing cognate antigen recognition (Schuch et al., 2019). This is similar to a study by Bertoletti, which showed HBV-specific $CD8^+$ T cells were commonly found to be present in a CHB patient cohort with low or undetectable viral loads due to antiviral therapy with NUCs (Rivino et al., 2018). Recently, we analyzed the phenotypic profiles of T cells and HBV-specific T cell responses of 172 patients with chronic HBV, including patients who retained (sAg-R) and lost HBsAg (sAg-L), by flow cytometry (Xiong et al., 2021). Compared to patients with sAg-R, patients with sAg-L show upregulation of HLA-DR on both $CD4^+$ and $CD8^+$ T cells and have a potent HBcAg-specific $CD8^+$ T cell response. Expression of HLA-DR, CD95, CD40L, CTLA-4, TIM-3, and CD107a in $CD4^+$ T cells and $CD8^+$ T cells is positively correlated with a decrease in HBsAg levels. There is also a positive correlation between the expression of HLA-DR and CD95 in $CD8^+$ T cells and the magnitude of the HBcAg-specific T cell response in patients with CHB. It is noteworthy that the combined expression of CTLA-4, CD95, and CD107a in $CD4^+$ T cells and HLA-DR and TIM-3 in $CD8^+$ T cells, along with the quantification of HBsAg, could be used as predictive factors for sAg-L in CHB patients.

4. Targeting T cells to cure CHB

Based on the limitations and insufficient virus elimination of current NUC therapy, immunotherapy is currently being explored as a possible direction to achieve a functional cure for HBV. NUCs efficiently block reverse transcription and, together with IFN-α, constitute the current standard of care. However, their effects are not sustained, and NUC treatment must be maintained to avoid HBV reactivation and hepatic flares. Therefore, treatment guidelines have adopted HBsAg loss, with or without the development of hepatitis B surface antibodies (anti-HBs), as a desirable endpoint for anti-HBV therapy (Fanning et al., 2019). In addition to targeting covalently closed circular DNA (cccDNA), an important point for establishing a cure is overcoming the dysfunction of HBV-specific B and/or T cells; therefore, strategies to boost HBV-specific immunity should be further explored.

A functional cure for HBV may be achieved by the activation of antiviral immunity. Theoretically, checkpoint inhibitors, such as anti-PD-1 or anti-CTLA-4 antibodies, as well as therapeutic vaccines, have the potential to enhance HBV-specific immunity. Furthermore, blocking the PD-1-PD-L1 interaction with antibodies can partially restore impaired HBV-specific T and B cell responses (Salimzadeh et al., 2018; Yong et al., 2018). The function of exhausted HBV-specific T cells is partially restored in vitro by blocking anti-PD-1/PD-L1 (Fisicaro et al., 2010). Moreover, the safety and effectiveness of PD-1/PD-L1 blockade in CHB patients were also evaluated in a pilot study, which found that treatment with nivolumab, a PD-1 functional blocking antibody, was well-tolerated in virally suppressed HBeAg-negative CHB patients and led to HBsAg decline in most patients and sustained HBsAg loss in one patient (Gane et al., 2019). In addition to using PD-1 antibodies to accelerate the T cell response, therapeutic vaccination also has positive effects on virus-specific T cells. GS-4774, a vaccine composed of HBV surface antigen, core antigen, and HBeAg with a yeast component, elicits HBV-specific T cell-mediated responses in 88% (52/60) of participants, and two individuals developed low-level (<8.4 IU/mL) anti-HBs (Lok et al., 2016). We have recently explored the potential of Cytomegalovirus (CMV)-based vaccines expressing HBsAg for therapeutic vaccination application. We demonstrated that a combination of CMV-based vaccination and DNA boost vaccination showed promise in persistently infected mice, mediating accelerated HBV clearance and robust HBV-specific $CD8^+$ T-cell responses (Su et al., 2023). Other than CMV based vaccines, the potential of using vaccinia Ankara (MVA)- based vaccine has also been explored by Ulrike Protzer's group in a series study (Cova, 2017; Kosinska et al., 2021; Michler et al., 2020). They have analyzed HBV clearance and HBV-specific B- and T-cell responses in HBV-carrier mice immunized with TherVacB, a therapeutic hepatitis B vaccine that uses a particulate HBV S and a core protein for prime vaccination, and a modified MVA for boost vaccination. TherVacB was more effective in HBeAg-negative mice, leading to the induction of HBV-specific antibodies and the loss of HBsAg with minimal liver damage, highlighting the importance of considering HBeAg status in therapeutic vaccination strategies (Cova, 2017). In line with this finding, they have further demonstrated that reducing HBV antigen expression in the liver could enhance the efficacy of therapeutic vaccines, as knockdown of HBV antigens by siRNAs resulted in improved immune responses, increased numbers and functionality of HBV-specific $CD8^+$ T cells, and viral clearance post TherVacB vaccination (Michler et al., 2020). They also tested various combination of new adjuvants together with TherVacB vaccination and found that those priming a balanced HBV-specific type 1 and 2 T helper response induced robust immune responses, including high-titer anti-HBs antibodies, cytotoxic T-cell responses, and long-term control of HBV. They thus identified CD4 T-cell activation during the priming phase as a crucial factor for vaccine efficacy (Kosinska et al., 2021).

We have previously demonstrated that $CD8^+$ T cells that are exhausted during chronic HBV replication undergo a permanent form of dysfunctional differentiation, and a change to an acute immune environment alone is not enough to restore their antiviral functionality (Tan et al., 2022), suggesting supplying new virus-specific T cells may be essential for overcoming the immune exhaustion induced by chronic HBV infection. It has been observed in previous clinical practices decades ago that transplantation of bone marrow from individuals who spontaneously cleared HBV infection could result in cure of HBV infection in CHB patients (Wang et al., 2018). This observation suggests that adoptive T-cell therapy with HBV-specific T cells represents a promising therapeutic strategy for chronic HBV infection, as it can provide sufficient numbers of functional HBV-specific T cells to patients. In line with this idea, studies have been conducted to examine the feasibility and effectiveness of adoptive transfer of T-cells genetically engineered to target HBV antigens, through either chimeric antigen receptor (CAR) or T-cell receptor (TCR) engineering technology. It has been shown previously that HBsAg-CAR T-cells could eliminate cccDNA from HBV-infected primary hepatocytes *in vitro* (Bohne et al., 2008), and could control HBV replication transiently in a transgenic HBV mouse model (Krebs et al., 2013). Consistently, Robert L Kruse et al. demonstrated that adoptive transfer of HBsAg-CAR T-cells into HBV-infected humanized mice resulted in accumulation of the transplanted T cells within the liver and a significant decrease in plasma HBsAg and HBV-DNA levels in comparison to control mice (Kruse et al., 2018). Recently, Antonio Bertoletti and Fu-sheng Wang reported a trial of eight patients with HBV-HCC who were treated using adoptive transfer of mRNA HBV-TCR T cells, and immune alterations were observed. In each TCR T cell infusion, the frequencies of $CD8^+$ and $CD4^+$ T cells that were activated and proliferating (Ki67+ CD39+/CD8+ or $CD4^+$ T cells) were measured, and patients who showed long-term clinical benefits were found to have activation of the T cell compartment and/or increased levels of serum chemokine (C-X-C motif) ligand (CXCL) 9 and CXCL10 (Tan et al., 2022). Furthermore, Alexandre Klopp et al. included a safeguard system into the adoptive T-cell therapy against CHB by adding a “suicide” trigger into CAR-T and TCR-T cells. They demonstrated very recently that induction of the suicide trigger-inducible caspase 9, in S-CAR T cells led to a strong

and rapid of reduction of transferred T cells and prevented liver toxicity and cytokine release in AAV-HBV-infected immune incompetent mice (Klopp et al., 2021). Nevertheless, despite these advances, an optimized immune modulating strategy for functional cure of HBV remains to be determined.

While T cell therapy holds promise as a potent approach for combating HBV infection, it's crucial to acknowledge and address the potential risks associated with the immune response generated by these therapies. Liver tissue damage can occur as a result of T cell therapy due to several mechanisms: (1) Off-Target Effects: T cells can recognize not only virus-infected cells but also healthy cells that express similar antigens. This phenomenon, known as off-target effects or cross-reactivity, could lead to T cells attacking healthy hepatocytes, thereby causing unintended liver damage (Miao et al., 2021; Upadhyay et al., 2021). (2) Excessive Inflammation: T cell responses, especially when unregulated, can trigger an excessive release of pro-inflammatory cytokines. This cytokine storm can cause collateral damage to liver tissues, leading to inflammation, tissue necrosis, and potentially contributing to liver dysfunction (Jarczak and Nierhaus, 2022; Schubert et al., 2021). (3) Autoimmune Reactions: T cell therapy has the potential to disrupt immune tolerance, leading to the development of autoimmune reactions where the immune system mistakenly attacks its own cells. In the context of HBV, this could lead to immune-mediated liver damage and exacerbate the disease. T cell therapy-induced immune responses could potentially tip the balance toward immunopathology, harming liver tissues in the process (Shan et al., 2022).

5. Conclusions

In conclusion, this review describes the recent advances in understanding the unique T cell immune response in CHB infection. New immune therapies have been explored both on the bench and in clinical studies. Although these studies provide a strong scientific rationale, their efficacy in clinical settings remains to be determined. A new strategy for T cell immune therapy, including but not limited to immune checkpoint antibodies, therapeutic vaccination, and TCR/CAR-T cell adoption, should be optimized for clinical applications. Such options might provide safer immunotherapeutic approaches for CHB treatment.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (82172256, 92169105, 81861138044, and M-0060), the National Scientific and Technological Major Project of China (2017ZX10202203), and the Sino-German Virtual Institute for Viral Immunology.

References

Alfei, F., Kanev, K., Hofmann, M., Wu, M., Ghoneim, H.E., Roelli, P., Utzschneider, D.T., von Hoesslin, M., Cullen, J.G., Fan, Y., Eisenberg, V., Wohlleber, D., Steiger, K., Merkler, D., Delorenzi, M., Knolle, P.A., Cohen, C.J., Thimme, R., Youngblood, B., Zehn, D., 2019. TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. Nature 571, 265–269.

Aliabadi, E., Urbanek-Quaing, M., Maasoumy, B., Bremer, B., Grasshoff, M., Li, Y., Niehaus, C.E., Wedemeyer, H., Kraft, A., Cornberg, M., 2022. Impact of HBsAg and HBcrAg levels on phenotype and function of HBV-specific T cells in patients with chronic hepatitis B virus infection. Gut 71, 2300–2312.

Asabe, S., Wieland, S.F., Chattopadhyay, P.K., Roederer, M., Engle, R.E., Purcell, R.H., Chisari, F.V., 2009. The size of the viral inoculum contributes to the outcome of hepatitis B virus infection. J. Virol. 83, 9652–9662.

Beckebaum, S., Cicinnati, V.R., Zhang, X., Ferencik, S., Frilling, A., Grosse-Wilde, H., Broelsch, C.E., Gerken, G., 2003. Hepatitis B virus-induced defect of monocyte-derived dendritic cells leads to impaired T helper type 1 response in vitro: mechanisms for viral immune escape. Immunology 109, 487–495.

Benechet, A.P., De Simone, G., Di Lucia, P., Cilenti, F., Barbiera, G., Le Bert, N., Fumagalli, V., Lusito, E., Moalli, F., Bianchessi, V., Andreata, F., Zordan, P., Bono, E., Giustini, L., Bonilla, W.V., Bleriot, C., Kunasegaran, K., Gonzalez-Aseguinolaza, G., Pinschewer, D.D., Kennedy, P., Naldini, L., Kuka, M., Ginhoux, F., Cantore, A., Bertoletti, A., Ostuni, R., Guidotti, L.G., Iannacone, M., 2019. Dynamics and genomic landscape of CD8(+) T cells undergoing hepatic priming. Nature 574, 200–205.

Bertoletti, A., Ferrari, C., 2016. Adaptive immunity in HBV infection. J. Hepatol. 64, S71–S83.

Bohne, F., Chmielewski, M., Ebert, G., Wiegmann, K., Kurschner, T., Schulze, A., Urban, S., Kronke, M., Abken, H., Protzer, U., 2008. T cells redirected against hepatitis B virus surface proteins eliminate infected hepatocytes. Gastroenterology 134, 239–247.

Cova, L., 2017. Present and future DNA vaccines for chronic hepatitis B treatment. Expet Opin. Biol. Ther. 17, 185–195.

Cui, D., Jiang, D., Yan, C., Liu, X., Lv, Y., Xie, J., Chen, Y., 2022. Immune Checkpoint Molecules Expressed on CD4(+) T Cell Subsets in Chronic Asymptomatic Hepatitis B Virus Carriers With Hepatitis B e Antigen-Negative. Front. Microbiol. 13, 887408.

De Simone, G., Andreata, F., Bleriot, C., Fumagalli, V., Laura, C., Garcia-Manteiga, J.M., Di Lucia, P., Gilotto, S., Ficht, X., De Ponti, F.F., Bono, E.B., Giustini, L., Ambrosi, G., Mainetti, M., Zordan, P., Benechet, A.P., Rava, M., Chakarov, S., Moalli, F., Bajenoff, M., Guidotti, L.G., Ginhoux, F., Iannacone, M., 2021. Identification of a Kupffer cell subset capable of reverting the T cell dysfunction induced by hepatocellular priming. Immunity 54, 2089–2100.e8.

Dong, Y., Li, X., Yu, Y., Lv, F., Chen, Y., 2020. JAK/STAT signaling is involved in IL-35-induced inhibition of hepatitis B virus antigen-specific cytotoxic T cell exhaustion in chronic hepatitis B. Life Sci. 252, 117663.

Du, B., Teng, J., Yin, R., Tian, Y., Jiang, T., Du, Y., Cai, W., 2021. Increased circulating T follicular helper cells induced via IL-12/21 in patients with acute on chronic hepatitis B liver failure. Front. Immunol. 12, 641362.

Fanning, G.C., Zoulim, F., Hou, J., Bertoletti, A., 2019. Therapeutic strategies for hepatitis B virus infection: towards a cure. Nat. Rev. Drug Discov. 18, 827–844.

Ferrando-Martinez, S., Snell, B.A., Lino, E., Gehring, A.J., Feld, J., Janssen, H., Robbins, S.H., 2021. Functional exhaustion of HBV-specific CD8 T cells impedes PD-L1 blockade efficacy in chronic HBV infection. Front. Immunol. 12, 648420.

Fisicaro, P., Barili, V., Rossi, M., Montali, I., Vecchi, A., Acerbi, G., Laccabue, D., Zecca, A., Penna, A., Missale, G., Ferrari, C., Boni, C., 2020. Pathogenetic mechanisms of T cell dysfunction in chronic HBV infection and related therapeutic approaches. Front. Immunol. 11, 849.

Fisicaro, P., Valdatta, C., Massari, M., Loggi, E., Biasini, E., Sacchelli, L., Cavallo, M.C., Silini, E.M., Andreone, P., Missale, G., Ferrari, C., 2010. Antiviral intrahepatic T-cell responses can be restored by blocking programmed death-1 pathway in chronic hepatitis B. Gastroenterology 138, 682–693, 693.e1-4.

Fumagalli, V., Venzin, V., Di Lucia, P., Moalli, F., Ficht, X., Ambrosi, G., Giustini, L., Andreata, F., Grillo, M., Magini, D., Rava, M., Friedrich, C., Fontenot, J.D., Bousso, P., Gilmore, S.A., Khan, S., Baca, M., Vivier, E., Gasteiger, G., Kuka, M., Guidotti, L.G., Iannacone, M., 2022. Group 1 ILCs regulate T cell-mediated liver immunopathology by controlling local IL-2 availability. Sci Immunol 7, eabi6112.

Gane, E., Verdon, D.J., Brooks, A.E., Gaggar, A., Nguyen, A.H., Subramanian, G.M., Schwabe, C., Dunbar, P.R., 2019. Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: a pilot study. J. Hepatol. 71, 900–907.

Hakim, M.S., Rahmadika, N., Jariah, R., 2020. Expressions of inhibitory checkpoint molecules in acute and chronic HBV and HCV infections: implications for therapeutic monitoring and personalized therapy. Rev. Med. Virol. 30, e2094.

Heim, K., Binder, B., Sagar, Wieland, D., Hensel, N., Llewellyn-Lacey, S., Gostick, E., Price, D.A., Emmerich, F., Vingerhoet, H., Kraft, A., Cornberg, M., Boettler, T., Neumann-Haefelin, C., Zehn, D., Bengsch, B., Hofmann, M., Thimme, R., 2020. TOX defines the degree of CD8+ T cell dysfunction in distinct phases of chronic HBV infection. Gut 0, 1–11.

Hoogeveen, R.C., Dijkstra, S., Bartsch, L.M., Drescher, H.K., Aneja, J., Robidoux, M.P., Cheney, J.A., Timm, J., Gehring, A., de Sousa, P., Ximenez, L., Peliganga, L.B., Pitts, A., Evans, F.B., Boonstra, A., Kim, A.Y., Lewis-Ximenez, L.L., Lauer, G.M., 2022. Hepatitis B virus-specific CD4 T cell responses differentiate functional cure from chronic surface antigen(+) infection. J. Hepatol. 77, 1276–1286.

Hoogeveen, R.C., Robidoux, M.P., Schwarz, T., Heydmann, L., Cheney, J.A., Kvistad, D., Aneja, J., Melgaco, J.G., Fernandes, C.A., Chung, R.T., Boonstra, A., Kim, A.Y., Baumert, T.F., Timm, J., Lewis-Ximenez, L.L., Tonnerre, P., Lauer, G.M., 2019. Phenotype and function of HBV-specific T cells is determined by the targeted epitope in addition to the stage of infection. Gut 68, 893–904.

Huang, S., Zou, S., Chen, M., Gao, X., Chen, L., Yang, X., Yu, Q., Zhao, X., Du, Y., Yang, X., Lin, Y., Wang, B., Lu, Y., Liu, J., Zheng, X., Gong, F., Lu, M., Yang, D., Wu, J., 2018. Local stimulation of liver sinusoidal endothelial cells with a NOD1 agonist activates T cells and suppresses hepatitis B virus replication in mice. J. Immunol. 200, 3170–3179.

Iannacone, M., Guidotti, L.G., 2022. Immunobiology and pathogenesis of hepatitis B virus infection. Nat. Rev. Immunol. 22, 19–32.

Jarczak, D., Nierhaus, A., 2022. Cytokine storm-definition, causes, and implications. Int. J. Mol. Sci. 23, 11740.

Khan, O., Giles, J.R., McDonald, S., Manne, S., Ngiow, S.F., Patel, K.P., Werner, M.T., Huang, A.C., Alexander, K.A., Wu, J.E., Attanasio, J., Yan, P., George, S.M., Bengsch, B., Staupe, R.P., Donahue, G., Xu, W., Amaravadi, R.K., Xu, X., Karakousis, G.C., Mitchell, T.C., Schuchter, L.M., Kaye, J., Berger, S.L., Wherry, E.J.,

2019. TOX transcriptionally and epigenetically programs CD8(+) T cell exhaustion. Nature 571, 211–218.
Khanam, A., Ayithan, N., Tang, L., Poonia, B., Kottilil, S., 2020. IL-21-Deficient T follicular helper cells support B cell responses through IL-27 in patients with chronic hepatitis B. Front. Immunol. 11, 599648.
Klopp, A., Schreiber, S., Kosinska, A.D., Pule, M., Protzer, U., Wisskirchen, K., 2021. Depletion of T cells via inducible caspase 9 increases safety of adoptive T-cell therapy against chronic hepatitis B. Front. Immunol. 12, 734246.
Kosinska, A.D., Festag, J., Muck-Hausl, M., Festag, M.M., Asen, T., Protzer, U., 2021. Immunogenicity and antiviral response of therapeutic hepatitis B vaccination in a mouse model of HBeAg-negative. In: Persistent HBV Infection, 9. Vaccines, Basel.
Krebs, K., Bottinger, N., Huang, L.R., Chmielewski, M., Arzberger, S., Gasteiger, G., Jager, C., Schmitt, E., Bohne, F., Aichler, M., Uckert, W., Abken, H., Heikenwalder, M., Knolle, P., Protzer, U., 2013. T cells expressing a chimeric antigen receptor that binds hepatitis B virus envelope proteins control virus replication in mice. Gastroenterology 145, 456–465.
Kruse, R.L., Shum, T., Tashiro, H., Barzi, M., Yi, Z., Whitten-Bauer, C., Legras, X., Bissig-Choisat, B., Garaigorta, U., Gottschalk, S., Bissig, K.D., 2018. HBsAg-redirected T cells exhibit antiviral activity in HBV-infected human liver chimeric mice. Cytotherapy 20, 697–705.
Kumar, B.V., Connors, T.J., Farber, D.L., 2018. Human T cell development, localization, and function throughout life. Immunity 48, 202–213.
Le Bert, N., Gill, U.S., Hong, M., Kunasegaran, K., Tan, D., Ahmad, R., Cheng, Y., Dutertre, C.A., Heinecke, A., Rivino, L., Tan, A., Hansi, N.K., Zhang, M., Xi, S., Chong, Y., Pflanz, S., Newell, E.W., Kennedy, P., Bertoletti, A., 2020. Effects of hepatitis B surface antigen on virus-specific and global T cells in patients with chronic hepatitis B virus infection. Gastroenterology 159, 652–664.
Lee, B.T., Fiel, M.I., Schiano, T.D., 2021. Antibody-mediated rejection of the liver allograft: an update and a clinico-pathological perspective. J. Hepatol. 75, 1203–1216.
Li, C., Yu, T., Shi, X., Yu, J., 2022. Interleukin-33 reinvigorates antiviral function of viral-specific CD8(+) T cells in chronic hepatitis B virus infection. Viral Immunol. 35, 41–49.
Li, Y., Tang, L., Guo, L., Chen, C., Gu, S., Zhou, Y., Ye, G., Li, X., Wang, W., Liao, X., Wang, Y., Peng, X., Liu, G., Zhang, X., Sun, J., Peng, J., Hou, J., 2020. CXCL13-mediated recruitment of intrahepatic CXCR5(+)CD8(+) T cells favors viral control in chronic HBV infection. J. Hepatol. 72, 420–430.
Liu, F., Zhang, S., Wong, D.K., Huang, F.Y., Cheung, K.S., Mak, L.Y., Fung, J., Yuen, M.F., Seto, W.K., 2022. Phenotypic changes of PD-1 and GITR in T cells are associated with hepatitis B surface antigen seroclearance. J. Clin. Gastroenterol. 56, e31–e37.
Liu, J., Jiang, M., Ma, Z., Dietze, K.K., Zelinskyy, G., Yang, D., Dittmer, U., Schlaak, J.F., Roggendorf, M., Lu, M., 2013. TLR1/2 ligand-stimulated mouse liver endothelial cells secrete IL-12 and trigger CD8+ T cell immunity in vitro. J. Immunol. 191, 6178–6190.
Liu, J., Zhang, E., Ma, Z., Wu, W., Kosinska, A., Zhang, X., Moller, I., Seiz, P., Glebe, D., Wang, B., Yang, D., Lu, M., Roggendorf, M., 2014. Enhancing virus-specific immunity in vivo by combining therapeutic vaccination and PD-L1 blockade in chronic hepadnaviral infection. PLoS Pathog. 10, e1003856.
Liu, S., Xu, C., Yang, F., Zong, L., Qin, Y., Gao, Y., Su, Q., Li, T., Li, Y., Xu, Y., Zheng, M., 2022. Natural killer cells induce CD8(+) T cell dysfunction via galectin-9/TIM-3 in chronic hepatitis B virus infection. Front. Immunol. 13, 884290.
Liu, X., Li, M., Wang, X., Dang, Z., Jiang, Y., Wang, X., Kong, Y., Yang, Z., 2019. PD-1(+) TIGIT(+) CD8(+) T cells are associated with pathogenesis and progression of patients with hepatitis B virus-related hepatocellular carcinoma. Cancer Immunol. Immunother. 68, 2041–2054.
Liu, Y., Meng, X., Wang, C., Zhang, Y., Hua, W.W., Wang, Z., 2022. Interleukin-18 plays a positive feedback activity to natural killer-like B cells in hepatitis B virus-associated acute-on-chronic liver failure. Viral Immunol. 35, 50–59.
Locarnini, S., Hatzakis, A., Chen, D.S., Lok, A., 2015. Strategies to control hepatitis B: public policy, epidemiology, vaccine and drugs. J. Hepatol. 62, S76–S86.
Lok, A.S., Pan, C.Q., Han, S.H., Trinh, H.N., Fessel, W.J., Rodell, T., Massetto, B., Lin, L., Gaggar, A., Subramanian, G.M., McHutchison, J.G., Ferrari, C., Lee, H., Gordon, S.C., Gane, E.J., 2016. Randomized phase II study of GS-4774 as a therapeutic vaccine in virally suppressed patients with chronic hepatitis B. J. Hepatol. 65, 509–516.
Lumley, S.F., McNaughton, A.L., Klenerman, P., Lythgoe, K.A., Matthews, P.C., 2018. Hepatitis B virus adaptation to the CD8+ T cell response: consequences for host and pathogen. Front. Immunol. 9, 1561.
Ma, C.S., Deenick, E.K., Batten, M., Tangye, S.G., 2012. The origins, function, and regulation of T follicular helper cells. J. Exp. Med. 209, 1241–1253.
McLane, L.M., Abdel-Hakeem, M.S., Wherry, E.J., 2019. CD8 T cell exhaustion during chronic viral infection and cancer. Annu. Rev. Immunol. 37, 457–495.
Miao, L., Zhang, Z., Ren, Z., Li, Y., 2021. Reactions related to CAR-T cell therapy. Front. Immunol. 12, 663201.
Michler, T., Kosinska, A.D., Festag, J., Bunse, T., Su, J., Ringelhan, M., Imhof, H., Grimm, D., Steiger, K., Mogler, C., Heikenwalder, M., Michel, M.L., Guzman, C.A., Milstein, S., Sepp-Lorenzino, L., Knolle, P., Protzer, U., 2020. Knockdown of virus antigen expression increases therapeutic vaccine efficacy in high-titer hepatitis B virus carrier mice. Gastroenterology 158, 1762–1775.e9.
Mohammadizad, H., Shahbazi, M., Hasanjani, R.M., Soltanzadeh-Yamchi, M., Mohammadnia-Afrouzi, M., 2019. TIM-3 as a marker of exhaustion in CD8(+) T cells of active chronic hepatitis B patients. Microb. Pathog. 128, 323–328.
Mutimer, D., Elsharkawy, A., Hathorn, E., Arunkumar, S., 2022. Hepatitis B e antigen and e antibody in a multi-ethnic cohort of adult chronic hepatitis B virus patients followed at a single liver unit for a period of 20 years. J. Viral Hepat. 29, 879–889.
Pal, S., Dey, D., Chakraborty, B.C., Nandi, M., Khatun, M., Banerjee, S., Santra, A., Ghosh, R., Ahammed, S.M., Chowdhury, A., Datta, S., 2022. Diverse facets of MDSC in different phases of chronic HBV infection: impact on HBV-specific T-cell response and homing. Hepatology 76, 759–774.
Pallett, L.J., Gill, U.S., Quaglia, A., Sinclair, L.V., Jover-Cobos, M., Schurich, A., Singh, K.P., Thomas, N., Das, A., Chen, A., Fusai, G., Bertoletti, A., Cantrell, D.A., Kennedy, P.T., Davies, N.A., Haniffa, M., Maini, M.K., 2015. Metabolic regulation of hepatitis B immunopathology by myeloid-derived suppressor cells. Nat. Med. 21, 591–600.
Protzer, U., Maini, M.K., Knolle, P.A., 2012. Living in the liver: hepatic infections. Nat. Rev. Immunol. 12, 201–213.
Raziorrouh, B., Schraut, W., Gerlach, T., Nowack, D., Gruner, N.H., Ulsenheimer, A., Zachoval, R., Wachtler, M., Spannagl, M., Haas, J., Diepolder, H.M., Jung, M.C., 2010. The immunoregulatory role of CD244 in chronic hepatitis B infection and its inhibitory potential on virus-specific CD8+ T-cell function. Hepatology 52, 1934–1947.
Rivino, L., Le Bert, N., Gill, U.S., Kunasegaran, K., Cheng, Y., Tan, D.Z., Becht, E., Hansi, N.K., Foster, G.R., Su, T.H., Tseng, T.C., Lim, S.G., Kao, J.H., Newell, E.W., Kennedy, P.T., Bertoletti, A., 2018. Hepatitis B virus-specific T cells associate with viral control upon nucleos(t)ide-analogue therapy discontinuation. J. Clin. Invest. 128, 668–681.
Ronca, V., Wootton, G., Milani, C., Cain, O., 2020. The immunological basis of liver allograft rejection. Front. Immunol. 11, 2155.
Salimzadeh, L., Le Bert, N., Dutertre, C.A., Gill, U.S., Newell, E.W., Frey, C., Hung, M., Novikov, N., Fletcher, S., Kennedy, P.T., Bertoletti, A., 2018. PD-1 blockade partially recovers dysfunctional virus-specific B cells in chronic hepatitis B infection. J. Clin. Invest. 128, 4573–4587.
Schmidt, N.M., Wing, P., Diniz, M.O., Pallett, L.J., Swadling, L., Harris, J.M., Burton, A.R., Jeffery-Smith, A., Zakeri, N., Amin, O.E., Kucykowicz, S., Heemskerk, M.H., Davidson, B., Meyer, T., Grove, J., Stauss, H.J., Pineda-Torra, I., Jolly, C., Jury, E.C., McKeating, J.A., Maini, M.K., 2021. Targeting human Acyl-CoA:cholesterol acyltransferase as a dual viral and T cell metabolic checkpoint. Nat. Commun. 12, 2814.
Schubert, M.L., Schmitt, M., Wang, L., Ramos, C.A., Jordan, K., Muller-Tidow, C., Dreger, P., 2021. Side-effect management of chimeric antigen receptor (CAR) T-cell therapy. Ann. Oncol. 32, 34–48.
Schuch, A., Salimi, A.E., Heim, K., Wieland, D., Kiraithe, M.M., Kemming, J., Llewellyn-Lacey, S., Sogukpinar, O., Ni, Y., Urban, S., Zimmermann, P., Nassal, M., Emmerich, F., Price, D.A., Bengsch, B., Luxenburger, H., Neumann-Haefelin, C., Hofmann, M., Thimme, R., 2019. 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 68, 905–915.
Schurich, A., Pallett, L.J., Lubowiecki, M., Singh, H.D., Gill, U.S., Kennedy, P.T., Nastouli, E., Tanwar, S., Rosenberg, W., Maini, M.K., 2013. The third signal cytokine IL-12 rescues the anti-viral function of exhausted HBV-specific CD8 T cells. PLoS Pathog. 9, e1003208.
Sekine, T., Perez-Potti, A., Nguyen, S., Gorin, J.B., Wu, V.H., Gostick, E., Llewellyn-Lacey, S., Hammer, Q., Falck-Jones, S., Vangeti, S., Yu, M., Smed-Sorensen, A., Gaballa, A., Uhlin, M., Sandberg, J.K., Brander, C., Nowak, P., Goepfert, P.A., Price, D.A., Betts, M.R., Buggert, M., 2020. TOX is expressed by exhausted and polyfunctional human effector memory CD8(+) T cells. Sci Immunol 5, eaba7918.
Shan, F., Somasundaram, A., Bruno, T.C., Workman, C.J., Vignali, D., 2022. Therapeutic targeting of regulatory T cells in cancer. Trends Cancer 8, 944–961.
Shao, X., Ma, J., Jia, S., Yang, L., Wang, W., Jin, Z., 2017. Interleukin-35 suppresses antiviral immune response in chronic hepatitis B virus infection. Front. Cell. Infect. Microbiol. 7, 472.
Shen, Z., Gao, Z., Gu, C., Wu, J., Wang, J., Zhang, J., Xie, Y., Liu, J., 2021. Prior transient exposure to interleukin-21 delivered by recombinant adeno-associated virus vector protects mice from hepatitis B virus persistence. Antivir. Res. 190, 105076.
Shen, Z., Liu, J., Wu, J., Zhu, Y., Li, G., Wang, J., Luo, M., Deng, Q., Zhang, J., Xie, Y., 2019. IL-21-based therapies induce clearance of hepatitis B virus persistence in mouse models. Theranostics 9, 3798–3811.
Su, J., Brunner, L., Ates, O.E., Sacherl, J., Frank, G., Kerth, H.A., Thiele, F., Wiegand, M., Mogler, C., Aguilar, J.C., Knolle, P.A., Collin, N., Kosinska, A.D., Protzer, U., 2023. Activation of CD4 T cells during prime immunization determines the success of a therapeutic hepatitis B vaccine in HBV-carrier mouse models. J. Hepatol. 78, 717–730.
Tan, A.T., Meng, F., Jin, J., Zhang, J.Y., Wang, S.Y., Shi, L., Shi, M., Li, Y., Xie, Y., Liu, L.M., Zhou, C.B., Chua, A., Ho, Z.Z., Luan, J., Zhao, J., Li, J., Wai, L.E., Koh, S., Wang, T., Bertoletti, A., Wang, F.S., 2022. Immunological alterations after immunotherapy with short lived HBV-TCR T cells associates with long-term treatment response in HBV-HCC. Hepatol Commun 6, 841–854.
Tang, L., Chen, C., Gao, X., Zhang, W., Yan, X., Zhou, Y., Guo, L., Zheng, X., Wang, W., Yang, F., Liu, G., Sun, J., Hou, J., Li, Y., 2019. Interleukin 21 reinvigorates the antiviral activity of hepatitis B virus (HBV)-Specific CD8+ T cells in chronic HBV infection. J. Infect. Dis. 219, 750–759.
Thimme, R., Wieland, S., Steiger, C., Ghrayeb, J., Reimann, K.A., Purcell, R.H., Chisari, F.V., 2003. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J. Virol. 77, 68–76.
Upadhyay, R., Boiarsky, J.A., Pantsulaia, G., Svensson-Arvelund, J., Lin, M.J., Wroblewska, A., Bhalla, S., Scholler, N., Bot, A., Rossi, J.M., Sadek, N., Parekh, S., Lagana, A., Baccarini, A., Merad, M., Brown, B.D., Brody, J.D., 2021. A critical role for fas-mediated off-target tumor killing in T-cell immunotherapy. Cancer Discov. 11, 599–613.
Wang, D., Fu, B., Shen, X., Guo, C., Liu, Y., Zhang, J., Sun, R., Ye, Y., Li, J., Tian, Z., Wei, H., 2021. Restoration of HBV-specific CD8(+) T-cell responses by sequential low-dose IL-2 treatment in non-responder patients after IFN-alpha therapy. Signal Transduct. Targeted Ther. 6, 376.

Wang, Q., Pan, W., Liu, Y., Luo, J., Zhu, D., Lu, Y., Feng, X., Yang, X., Dittmer, U., Lu, M., Yang, D., Liu, J., 2018. Hepatitis B virus-specific CD8+ T cells maintain functional exhaustion after antigen reexposure in an acute activation immune environment. Front. Immunol. 9, 219.

Wang, X., Dong, Q., Li, Q., Li, Y., Zhao, D., Sun, J., Fu, J., Meng, F., Lin, H., Luan, J., Liu, B., Wang, M., Wang, F.S., He, F., Tang, L., 2018. Dysregulated response of follicular helper T cells to hepatitis B surface antigen promotes HBV persistence in mice and associates with outcomes of patients. Gastroenterology 154, 2222–2236.

Wei, Y.Y., Fan, J., Shan, M.X., Yin, D.D., Wang, L.L., Ye, W., Zhao, W., 2022. TIGIT marks exhausted T cells and serves as a target for immune restoration in patients with chronic HBV infection. Am J Transl Res 14, 942–954.

Wherry, E.J., 2011. T cell exhaustion. Nat. Immunol. 12, 492–499.

Wherry, E.J., Kurachi, M., 2015. Molecular and cellular insights into T cell exhaustion. Nat. Rev. Immunol. 15, 486–499.

WHO, 2023. In: World Health Statistics 2023: Monitoring Health for the SDGs, Sustainable Development Goals, 4. World Health Organization, Geneva, https://www.who.int/publications/i/item/9789240074323. (Accessed June 4 2023)

Xie, X., Luo, J., Broering, R., Zhu, D., Zhou, W., Lu, M., Zheng, X., Dittmer, U., Yang, D., Liu, J., 2021. HBeAg induces liver sinusoidal endothelial cell activation to promote intrahepatic CD8 T cell immunity and HBV clearance. Cell. Mol. Immunol. 18, 2572–2574.

Xie, X., Luo, J., Zhu, D., Zhou, W., Yang, X., Feng, X., Lu, M., Zheng, X., Dittmer, U., Yang, D., Liu, J., 2022. HBeAg is indispensable for inducing liver sinusoidal endothelial cell activation by hepatitis B virus. Front. Cell. Infect. Microbiol. 12, 797915.

Xiong, S., Zhu, D., Liang, B., Li, M., Pan, W., He, J., Wang, H., Sutter, K., Dittmer, U., Lu, M., Liu, D., Yang, D., Liu, J., Zheng, X., 2021. Longitudinal characterization of phenotypic profile of T cells in chronic hepatitis B identifies immune markers associated with HBsAg loss. EBioMedicine 69, 103464.

Xu, J., Zhan, Q., Fan, Y., Yu, Y., Zeng, Z., 2021. Human genetic susceptibility to hepatitis B virus infection. Infect. Genet. Evol. 87, 104663.

Yang, S., Wang, L., Pan, W., Bayer, W., Thoens, C., Heim, K., Dittmer, U., Timm, J., Wang, Q., Yu, Q., Luo, J., Liu, Y., Hofmann, M., Thimme, R., Zhang, X., Chen, H., Wang, H., Feng, X., Yang, X., Lu, Y., Lu, M., Yang, D., Liu, J., 2019. MMP2/MMP9-mediated CD100 shedding is crucial for inducing intrahepatic anti-HBV CD8 T cell responses and HBV clearance. J. Hepatol. 71, 685–698.

Yong, Y.K., Saeidi, A., Tan, H.Y., Rosmawati, M., Enstrom, P.F., Batran, R.A., Vasuki, V., Chattopadhyay, I., Murugesan, A., Vignesh, R., Kamarulzaman, A., Rajarajeswaran, J., Ansari, A.W., Vadivelu, J., Ussher, J.E., Velu, V., Larsson, M., Shankar, E.M., 2018. Hyper-expression of PD-1 is associated with the levels of exhausted and dysfunctional phenotypes of circulating CD161(++)TCR iValpha7.2(+) mucosal-associated invariant T cells in chronic hepatitis B virus infection. Front. Immunol. 9, 472.

Zhang, W., Luo, S., Li, T., Wang, M., Huang, J., Liao, Q., Liu, B., Rong, X., Li, L., Allain, J.P., Fu, Y., Li, C., 2022. Hepatitis B virus-specific cellular immunity contributes to the outcome of occult hepatitis B virus infection. Front. Microbiol. 13, 850665.

Zhao, H., Wang, H., Hu, Y., Xu, D., Yin, C., Han, Q., Zhang, J., 2021. Chitosan nanovaccines as efficient carrier adjuvant system for IL-12 with enhanced protection against HBV. Int. J. Nanomed. 16, 4913–4928.