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REVIEW

T-cell responses in hepatitis B and C virus infection: similarities and differences

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Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are global health problems affecting 600 million people worldwide. Indeed, HBV and HCV are hepatotropic viruses that can cause acute and chronic liver disease progressing to liver cirrhosis and even hepatocellular carcinoma. Furthermore, co-infections of HBV and HCV with HIV are emerging worldwide. These co-infections are even more likely to develop persistent infection and are difficult to treat. There is growing evidence that virus-specific $CD4^+$ and $CD8^+$ T-cell responses play a central role in the outcome and pathogenesis of HBV and HCV infection. While virus-specific T-cell responses are able to successfully clear the virus in a subpopulation of patients, failure of these T-cell responses is associated with the development of viral persistence. In this review article, we will discuss similarities and differences in HBV- and HCV-specific T-cell responses that are central in determining viral clearance, persistence and liver disease.
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INTRODUCTION

Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are serious health care problems affecting about 600 million individuals worldwide. Both viruses are hepatotropic and can lead to the development of severe liver diseases, such as liver cirrhosis and hepatocellular carcinoma. HBV and HCV are responsible for about 1 million deaths per year and represent a major indication for liver transplantation. More than 90% of newborns exposed to HBV at birth become persistently infected; however, adult-onset HBV infections are typically self-limited and cleared in about 95% of patients. In contrast, only a minority (about 30%) of HCV-infected adults is able to clear the virus spontaneously. Innate and adaptive host immune responses play an important role in the successful elimination of both viruses. Innate immunity against HBV and HCV includes the release of antiviral cytokines such as type I interferon (IFN-α and IFN-β) and the activation of innate immune cells such as natural killer cells. Even though it has been shown that innate immune responses contribute to the outcome of infection, the hallmark of successful viral elimination is a sustained adaptive immune response. Specifically, $CD4^+$ and $CD8^+$ T-cell responses have been shown to play a central role in the outcome of infection.[1,2] Also, the emerging co-infections of HBV and HCV with HIV have become a further striking health care problem. This is mainly due the high likelihood of co-infections to develop persistent infection.

While there has been an effective vaccine against HBV infection available for the last 30 years, no protective vaccine against HCV infection is available yet. Additionally, treatment options of chronic HBV and HCV infection are limited. Indeed, while several drugs, such as nucleoside/nucleotide analogues (NUCs) and pegylated IFN-α (PegIFN-α) are available for the treatment of HBV infection, sustained virological responses and viral eradication are rarely achieved.[3] The treatment of HCV is based on a novel combination of viral protease inhibitors, PegIFN-α and ribavirin, leading to sustained virological response rates of up to 70%.[4] However, considerable side effects and high costs constrain antiviral therapy to a relatively small group of patients. In order to develop novel immunological prophylactic and therapeutic treatment options for HBV and HCV infection, a better understanding of virus–host interactions, as well as the underlying mechanisms of immune failure and viral persistence is of major interest. In this review, we will discuss the components of adaptive immunity that have been described to contribute to successful immune responses against HBV and HCV and assemble the current knowledge about possible mechanisms leading to the failure of the immune response.

VIROLOGY AND NATURAL HISTORY OF HBV AND HCV INFECTION

HBV is a double-stranded DNA virus, while HCV is a single-stranded RNA virus. To date, eight HBV genotypes (A–H) and six major HCV genotypes (1–6) have been identified.[5–7] It is important to note that humans are the only known natural host for both viruses; however, for experimental purposes both can be transmitted to chimpanzees.[8] Although HBV and HCV both infect the same type of cell, the hepatocyte, viral entry and viral life cycle differ significantly. The viral entry mechanisms of HBV into the hepatocytes are yet hardly understood. Importantly, however, the transmembrane transporter sodium taurocholate cotransporting polypeptide has recently been suggested to play an important role for cellular attachment and entry.[9] After binding of the HBV particle to the cell surface, the nucleocapsid is released into the cytoplasm and transported to the nucleus. In the nucleus the covalently closed circular DNA (cccDNA) may then be formed by covalent ligation.[10,11] The newly formed cccDNA remains in the

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nucleus of infected cells, while capped and polyadenylated viral mRNA is produced. Transcription and nucleocapsid assembly take place in the cytosol of infected cells and newly produced nucleocapsids can either be reimported in the nucleus for the amplification of cccDNA or they can be enveloped for secretion by the endoplasmic reticulum.[11,12] The cccDNA plays a key role in the life cycle of HBV, since it remains in the nucleus as a template for further RNA synthesis and thus represents the basis for the persistence of HBV infection. Importantly, even in patients with serological evidence of viral clearance (e.g. presence of HBV surface antigen (HBsAg)-specific antibodies), cccDNA may persist in the nucleus of hepatocytes.[13]

In contrast, entry mechanisms of HCV into the hepatocytes are better understood. Indeed, cellular attachment and entry of HCV is mediated by several surface molecules such as the tetraspanin CD81 and the two tight junction proteins occludin and claudin-1.[14–16] In contrast to HBV, the HCV genome is not transported into the nucleus. Instead, replication proceeds in association with membranous structures of the endoplasmic reticulum in the cytosol of infected hepatocytes.[17,18] The whole viral RNA genome is translated as one open reading frame encoding a polyprotein. This HCV polyprotein is processed during and after translation by host and viral proteases. Importantly, very little is known about the later steps of the HCV viral life cycle, such as assembly and viral particle release.

The natural history of both viruses differs significantly. Indeed, the majority of acutely HBV-infected patients are able to clear the virus. Only about one-third of adults develop jaundice and hepatitis and less than 1% show a fulminant course.[19] Within 6–8 weeks, HBV DNA reaches its peak titer in the plasma and HBV e antigen (HBeAg) and HBsAg are secreted. In the majority of acutely infected adults, a successful HBV-specific immune response leads to the eradication of HBeAg followed by the elimination of HBsAg as indicated by the emergence of HBeAg- and HBsAg-specific antibodies. Therefore, lifelong protective immunity is maintained.[8] In contrast, a course of chronic HBV infection can clinically be assumed when HBsAg persists for more than 6 months. As a result of cccDNA persistence, reactivation can occur in these patients even after several years. Thereby, either wild-type HBV or, more commonly, HBV variants are reactivated.[20,21]

Similar to HBV infection, acute HCV infection is often asymptomatic and therefore clinically not recognized in the majority of patients.[17] Irrespective of the outcome of HCV infection, HCV RNA becomes detectable within 1–2 weeks after exposure to HCV and reaches peak levels of up to 10^7 genome equivalents/mL after 6–10 weeks.[22] Acute resolving infection is characterized by a rapid decline of viral replication after this peak. In contrast, in cases of acute persisting infection that occurs in the majority of acutely infected patients, viral titers fall to some degree indicating partial control. However, in the next few weeks viral titers rebound and reach steady state titers. In these chronically infected patients, alanine transaminase (ALT) levels may be elevated. However, the level of ALT elevation and consequently the degree of liver inflammation and damage can vary from mild to severe. Thus, like in HBV infection, the outcome of HCV infection is determined within the first 6 months after exposure to the virus. However, in contrast to HBV infection, HCV establishes persistent infection in the majority of patients, suggesting that this viral infection is an even more challenging combatant for the host's immune system to fight. Key characteristics of both viruses are summarized in Table 1.

SUCCESSFUL ADAPTIVE IMMUNE RESPONSES IN HBV AND HCV INFECTION

The adaptive immune response can be divided into the humoral and cellular arm. It has been shown that humoral responses such as virus-specific neutralizing antibodies exist in both infections. However, they do not seem to significantly contribute to viral clearance during acute HBV or HCV infection.[23,24] In contrast, various studies have shown that $CD4^+$ helper T-cell- and $CD8^+$ cytotoxic T-cell-mediated immune responses determine the outcome of HBV and HCV infection. Thus, spontaneous viral clearance of HBV and HCV infection is characterized by vigorous and sustained multi-epitope-specific $CD4^+$ and $CD8^+$ T-cell responses during the acute phase of infection. In contrast, chronic infection with both viruses is correlated with late, transient, week or narrowly focused $CD4^+$ and $CD8^+$ T-cell responses.[25–32] However, it is important to note that the effects of $CD4^+$ and $CD8^+$ T-cell responses are not only important for viral control but also critical for liver injury and the establishment of liver diseases in both viral infections.[33,34]

In the following, we will focus on the current knowledge about HBV- and HCV-specific $CD4^+$ and $CD8^+$ T-cell responses and their antiviral effector functions, as well as possible mechanisms of T-cell

Table 1 Important characteristics of hepatitis B and hepatitis C virus

Characteristics	Hepatitis B virus	Hepatitis C virus
Virological features		
Genome (size)	dsDNA (3.2 kb)	ssRNA (9.6 kb)
Family	*Hepadnaviridae*	*Flaviviridae*
Host cell	Hepatocyte	Hepatocyte
Viral entry factors	Sodium taurocholate cotransporting polypeptide	Low density lipoprotein receptor, scavenger receptor class B type I, CD81, occludin, claudin-1
Clinical features		
People infected worldwide	More than 240 million individuals	About 150 million individuals
Transmission routes	Vertical transmission: mother to neonatal or during childhood	Vertical transmission: rare
	Horizontal transmission: parenteral, intravenous drug abuse, sexual	Horizontal transmission: parenteral, intravenous drug abuse, sexual
Outcome	Persistence in more than 90% of newborns exposed to HBV at birth Persistence in about 5% of adult-onset HBV infection	Persistence in about 70% of adult-onset infection
Complications	Fulminant hepatitis (1%), liver cirrhosis, HCC	Liver cirrhosis, HCC
Deaths per year	About 600 000 individuals	More than 350 000 individuals
Vaccine	Yes	No
Therapy	Nucleoside/nucleotide analogues and pegylated IFN-α	Viral protease inhibitors, pegylated IFN-α and ribavirin

Abbreviations: dsDNA, double-stranded DNA; HCC, hepatocelullar carcinoma; ssRNA, single-stranded RNA.

Table 2 Key points of T-cell responses in HBV and HCV infection

- Vigorous multi-epitope-specific $CD4^+$ and $CD8^+$ T-cell responses are essential for viral clearance in both infections.
- $CD4^+$ T-cell depletion leads to viral persistence in both infections, suggesting a central role of $CD4^+$ T-cell help for the elimination of HBV and HCV.
- $CD8^+$ T-cell depletion is associated with high viral loads in both infections, suggesting that $CD8^+$ T cells are the major immune cells contributing to clearance of HBV and HCV.
- $CD8^+$ T-cell failure in HCV infections is caused by T-cell exhaustion, deletion and viral escape.
- In contrast, $CD8^+$ T-cell failure in HBV infection is mainly due to T-cell exhaustion and deletion.

failure. Key characteristics of $CD4^+$ and $CD8^+$ T cell in HBV and HCV infection are also summarized in Table 2.

$CD4^+$ AND $CD8^+$ T CELLS AND THEIR ANTIVIRAL EFFECTOR FUNCTIONS

In patients with acute HBV infection, HBV core antigen-specific $CD4^+$ T-cell responses are detectable within a few weeks after infection.[35] In patients with a self-limiting acute HBV infection, a vigorous and multispecific $CD4^+$ T-cell response that targets primarily HBV core antigen epitopes and produces Th1-type cytokines such as IFN-γ and tumor-necrosis factor α (TNF-α) has been described.[29,36] The important role of $CD4^+$ T-cell help during acute HBV infection has been elegantly demonstrated in the chimpanzee model. Indeed, a depletion of $CD4^+$ T cells prior to HBV infection lead to the development of persistent infection in the absence of a detectable $CD8^+$ T-cell response.[37] However, there was no effect on viral clearance or liver disease when $CD4^+$ T cells were depleted at the peak of infection when virus-specific $CD8^+$ T cells were readily detectable.[37] Similarly, in HCV infection, depletion of $CD4^+$ T cells prior to re-infection of chimpanzees that had previously cleared HCV infection resulted in viral persistence via the abrogation of protective $CD8^+$ T-cell mediated immunity due to the emergence of viral escape mutations.[28] Thus, in both infections $CD4^+$ T cells seem to be responsible for the induction and maintenance of successful $CD8^+$ T-cell responses. Accordingly, the absence of $CD4^+$ T-cell help leads to viral persistence. A recent study in acutely HCV-infected patients has supported the important role of $CD4^+$ T cells showing that multi-epitope-specific $CD4^+$ T-cell responses are primed during acute HCV infection, but disappear rapidly when viral persistence is established.[38] These data are in line with previous studies demonstrating very weak or even absent and functionally impaired $CD4^+$ T-cell responses in chronic HCV infection.[32,39] However, due to the very low frequency of circulating HBV- and HCV-specific $CD4^+$ T cells, very little is known about the exact differentiation and function of these cells. Taken together, HBV- and HCV-specific $CD4^+$ T cells do not seem to primarily mediate direct antiviral effects, but play an important role for the clearance of the virus by enhancing the effector responses of virus-specific $CD8^+$ T cells.[23,40,41]

A strong correlation between vigorous, sustained and multi-epitope-specific $CD8^+$ T-cell responses and HBV or HCV clearance has been demonstrated in several studies.[25,26,29,30,42,43] The important role of HBV- and HCV-specific $CD8^+$ T cells for the elimination of both viruses is further supported by the following findings. First, depletion studies in chimpanzees clearly showed that virus-specific $CD8^+$ T cells are the main effector cells driving disease progression and pathogenesis during acute infection.[27,44] Second, there is a strong temporal correlation between the appearance of virus-specific $CD8^+$ T cells and viral clearance.[25,26,44] Indeed, in experimentally infected chimpanzees viral elimination generally coincides with the intrahepatic occurrence of HBV- and HCV-specific $CD8^+$ T cells.[43,44] Third, a strong association between certain human leukocyte antigen (HLA) class I alleles and spontaneous elimination of HCV exists. Indeed, the HLA class I alleles HLA-A*03, HLA-B*27 and HLA-B*57 have a protective effect that can be linked to immunodominant HCV-specific $CD8^+$ T-cell epitopes.[45–49] However, the exact $CD8^+$ T-cell effector functions that mediate viral control are not completely defined. In HBV, studies in chimpanzees and the HBV-transgenic mice model have suggested that non-cytolytic effector mechanisms mediated by virus-specific IFN-γ and TNF-α producing $CD8^+$ T cells can contribute to viral control during acute infection.[44,50,51] However, since viral control is coinciding with liver disease and since cell death plays an essential role for the elimination of the cccDNA in hepatocytes, direct cytolytic effector functions of virus-specific $CD8^+$ T cells might also contribute to viral clearance.[12,44,52] At this point, it is important to note that in contrast to adult-onset HBV infection more than 90% of newborns exposed to HBV at birth become persistently infected. However, a study performed by Kennedy *et al.*[53] has suggested that chronic HBV infection in children is not associated with a compromised or tolerogenic T-cell profile suggesting that other mechanisms may contribute to the elevated rate of persisting HBV infection in children.

The exact mechanisms by which HCV-specific $CD8^+$ T cells control viral replication are not completely understood due to the absence of a small animal model such as transgenic mice. However, studies in chimpanzees have shown that viral clearance can also occur in the absence of significant liver disease.[43] Furthermore, studies in acutely infected patients that ultimately clear the virus have shown that virus-specific $CD8^+$ T cells present during the early infection are impaired in their effector functions. This status of HCV-specific $CD8^+$ T cells, referred to as 'stunned' phenotype, is reversed in a later stage of the acute phase.[25,26] Then HCV-specific $CD8^+$ T cells start to produce IFN-γ. This is synchronized with a rapid decrease in virus load and subsequently viral elimination. The important role of non-cytolytic antiviral effector mechanisms such as IFN-γ production for the inhibition of HCV replication could be further supported by a recent study using a cell culture model.[54] Additionally, killing of infected cells and uninfected bystander cells by HCV-specific $CD8^+$ T cells via perforin, Fas/Fas ligand and TNF pathways has been suggested.[55,56] However, in both, HBV and HCV infection, virus-specific $CD8^+$ T cells may fail to clear the virus and thus $CD8^+$ T cell failures represent a hallmark of chronic infection. Chronic infections with both viruses can be treated with PegIFN-α, mostly in combination with other drugs. In the case of chronic HBV infection, using PegIFN-α treatment a sustained virological response and viral eradication are rarely achieved. Importantly, a recent study could show that during treatment with PegIFN-α HBV-specific $CD8^+$ T cells remain at low levels and no restoration of T-cell effector functions can be achieved.[57] In contrast, a study analyzing the effect of PegIFN-α therapy on HCV-specific $CD8^+$ T cells has shown that at least an early therapeutic intervention can restore HCV-specific $CD8^+$ T-cell survival and function.[58] Thus, the differential restoration of $CD8^+$ T-cell effector functions during PegIFN-α therapy in both infections may contribute to the differing responses to treatment during HBV versus HCV infection. However, considering only one drug and one subset of cells in isolation cannot cope with the complex interplay between several drugs such as PegIFN-α and NUCs for treating HBV infection or PegIFN-α, ribavirin and protease inhibitors

for treating HCV infection and the complete immune system including both innate and adaptive immunity. For example in contrast to PegIFN-α therapy, treatment with NUCs is able to restore HBV-specific CD8$^+$ T-cell functions[59] whereby a recovery of the innate immune effector cells (natural killer cells) cannot be achieved.[60] In contrast, PegIFN-α therapy drives proliferation and activation of natural killer cells.[57] Clearly, additional studies will be needed in order to understand this complex interplay of host, virus and drugs.

FAILURE OF ADAPTIVE IMMUNE RESPONSE IN HBV AND HCV INFECTION

It is important to note that there are several mechanisms that may contribute to the failure of HBV- and HCV-specific CD8$^+$ T-cell responses[61,62] (Figure 1). For example, CD4$^+$ T-cell help is required for the induction of sustained CD8$^+$ T-cell effector functions, therefore the lack of CD4$^+$ T-cell help is a possible cause for CD8$^+$ T-cell failure.[28,63] Indeed, as discussed above, a lack of proper maturation of functional and sustained HBV- and HCV-specific CD8$^+$ T-cell responses due to the absence of CD4$^+$ T-cell help seems to be associated with the onset of persistent HBV and HCV infection. However, the main factors driving CD8$^+$ T-cell failure in chronic viral hepatitis are not fully understood, but growing evidence indicates that T-cell dysfunction, as well as viral escape, contributes to this failure.

MECHANISMS OF HBV- AND HCV-SPECIFIC CD8$^+$ T-CELL FAILURE: T-CELL DYSFUNCTION

Dysfunction of CD8$^+$ T cells is mainly characterized by an impaired proliferative capacity and the loss of antiviral effector functions such as the secretion of IL-2 and IFN-γ.[64] Virus-specific CD8$^+$ T-cell dysfunction is typically observed during chronic infections with high levels of replicating virus, such as lymphocytic choriomeningitis virus in the mouse or during chronic infection with HIV, HBV and HCV in humans. Indeed, even though HCV-specific CD8$^+$ T cells can be detected in the blood and liver of some chronically infected patients, they are often functionally impaired.[31,65] Similarly, persistence of HBV could be associated with impaired CD8$^+$ T-cell functions, finally resulting in the deletion of these virus-specific CD8$^+$ T cells.[33,66,67] Interestingly, HBV-specific CD8$^+$ T cells are hardly detectable in patients with viral loads above 10^7 copies/mL. Again, this may be due to the very high and sustained exposure to viral antigens.[67,68] The exact mechanisms leading to the dysfunctional phenotype of virus-specific CD8$^+$ T cells under sustained antigen exposure in chronic HBV and HCV infection are not completely understood, but most likely intrinsic as well as extrinsic pathways are involved.

As an example for an intrinsic effect, the pro-apoptotic molecule Bim is specifically upregulated in HBV- and HCV-specific CD8$^+$ T cells obtained from chronically infected patients.[69–71] Additionally, the expression of the inhibitory receptor programmed death-1 (PD-1) has been shown to be increased on HBV- and HCV-specific CD8$^+$ T cells obtained from chronically infected patients and may thereby contribute to CD8$^+$ T-cell dysfunction and apoptosis.[66,71–76] High levels of the ligand for PD-1 (PD-L1) have also been reported on intrahepatic cells in patients with chronically inflamed liver diseases, such as HBV and HCV. However, it is possible that this upregulated expression is rather linked to the degree of liver inflammation than to a specific viral infection.[77] Nevertheless, blocking the PD-1/PD-L1 interaction may serve as a potential target for immunotherapy in chronic hepatitis. Indeed, in the lymphocytic choriomeningitis virus mouse model the treatment with antibodies to PD-L1 lead to the restoration of CD8$^+$ T-cell functions and the reduction of viral replication.[78] Moreover, blockade experiments of this inhibitory pathway have demonstrated relevance for HBV infection in the HBV mouse model. For example, HBV-transgenic mice were treated with blocking antibodies for PD-L1 that resulted in an increased number of

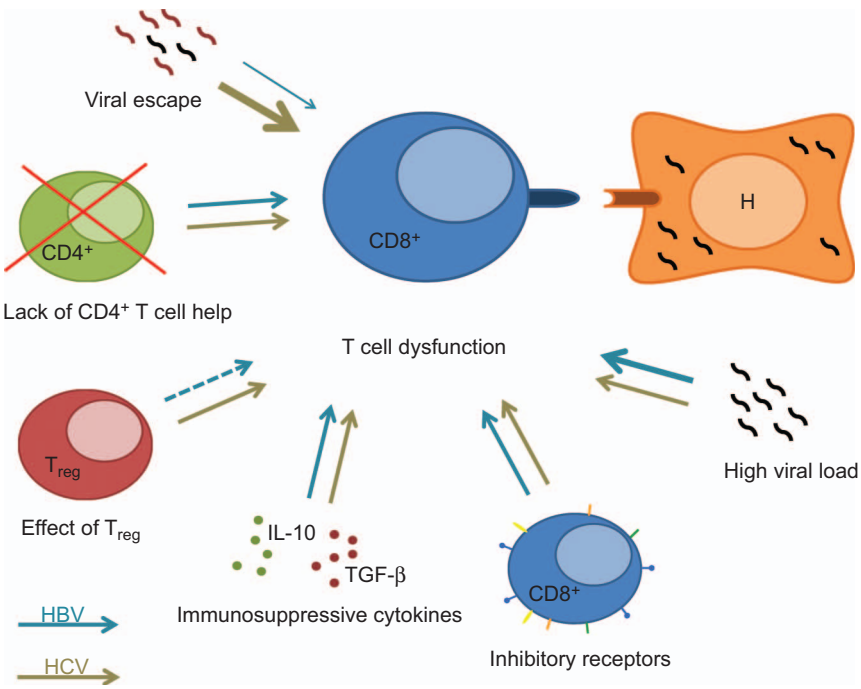

Figure 1 Mechanisms involved in the failure of HBV- and HCV-specific CD8$^+$ T-cell responses. Several mechanisms have been proposed to contribute to HBV- and HCV-specific CD8$^+$ T-cell failure. However, some of these effects are more prominent in HBV infection or HCV infection, respectively. Predescribed mechanisms driving CD8$^+$ T-cell failure are indicated by colored arrows (HBV, green and HCV, khaki); the thickness of the arrows in dicates the relative importance of the respective mechanisms for the failure of CD8$^+$ T cells in HBV versus HCV infection. Dashed arrow lines indicate conflicting data sets present for the respective mechanism.

IFN-γ producing CD8$^+$ T cells in the liver.[79] Similarly, a restoration of HBV- and HCV-specific CD8$^+$ T cells isolated from chronically HBV or HCV-infected patients, respectively, could be achieved *in vitro* by blocking PD-1/PD-L1 interaction.[72,73] Furthermore, the blockade of this inhibitory pathway is particularly promising, since PD-1 blockade seems to be well tolerated in cancer immunotherapy trials.[80] However, several other co-inhibitory as well as co-stimulatory molecules seem to play a role in HBV- and HCV-specific CD8$^+$ T-cell dysfunction. First, the inhibitory molecule 2B4 is highly co-expressed with PD-1 on HBV- and HCV-specific CD8$^+$ T cells in chronically infected patients.[68,81] Second, in chronic HBV infection the inhibitory molecule cytotoxic T-lymphocyte antigen 4 (CTLA-4) is highly expressed on HBV-specific CD8$^+$ T cells that show high levels of Bim. However, in this case CTLA-4 and PD-1 pathways seems to be nonredundant.[82] In contrast, in chronic HCV infection both PD-1 and CTLA-4 pathways seem to contribute to HCV-specific CD8$^+$ T-cell dysfunction by a redundant mechanism that requires combined PD-1/CTLA-4 blockade in order to restore T-cell dysfunction.[83] Third, a recent study suggested that the combination of the blockade of the co-inhibitory molecule PD-1 and the stimulation of the costimulatory molecule CD137 can increase the responsiveness of intrahepatic HBV-specific CD8$^+$ T cells but not of HCV-specific CD8$^+$ T cells.[84] Finally, the pathway of the inhibitory receptor T-cell immunoglobulin and mucin domain-containing molecule 3 (Tim-3) seems to be upregulated in chronically HBV-infected patients.[85] Similarly, Tim-3 has been shown to be highly co-expressed with PD-1 on HCV-specific CD8$^+$ T cells and could be associated with viral persistence.[86] Importantly, in both infections blockade of Tim-3 could restore virus-specific CD8$^+$ T-cell dysfunction and this effect was even enhanced by a combined Tim-3/PD-1 blockade.[85–87] Thus, the consideration of a combined modulation of several co-inhibitory and costimulatory pathways might be beneficial. However, based on the differing redundancy and synergy of the multiple pathways in HBV- and HCV-specific CD8$^+$ T cells, carefully compiled approaches for the combined modulation of these pathways need to be adapted independently for HBV and HCV immunotherapy.

Extrinsic pathways that may contribute to CD8$^+$ T-cell dysfunction in chronic HBV and HCV infection include immunosuppressive cytokines and regulatory T cells. In general, the liver, as the site of HBV and HCV infection is known to be a tolerogenic environment. For example, murine Kupffer cells constitutively express the immunosuppressive cytokines interleukin-10 (IL-10) and transforming growth factor β (TGF-β) that are involved in the generation of a unique cytokine environment mainly inducing tolerance of liver-infiltrating lymphocytes.[88] In this context, it is important to note that IL-10 is negatively associated with the outcome of HBV and HCV infection.[89–94] For example, during acute HCV infection, high levels of IL-10 are associated with progression to chronic infection.[90] In addition, intrahepatic IL10 producing CD8$^+$ T cells were found in chronically HCV-infected patients suggesting that they may contribute to the regulation of HCV-specific CD8$^+$ T-cell responses.[95] Additionally, in both HBV and HCV infection, specific polymorphisms of IL-10 have been found to correlate with increased susceptibility to chronic HCV infection and an increased severity of chronic HBV infection, respectively.[96–98] TGF-β also has negative effects on virus-specific CD8$^+$ T-cell function. Indeed, blockade of TGF-β secretion resulted in an enhanced production of IFN-γ by HCV-specific CD8$^+$ T cells.[99] Importantly, the major sources of the immunosuppressive cytokines IL-10 and TGF-β are regulatory CD4$^+$ T cells. These cells are characterized by the expression of forkhead box P3 and CD25 and play a central role in immunological self-tolerance, homeostasis and protection from overwhelming immune responses, e.g. mediated by CD8$^+$ T cells that if not controlled, may lead to severe tissue damage. Importantly, in both HBV and HCV infection, regulatory CD4$^+$ T cells have been suggested to contribute to the protection from overwhelming liver tissue damage, but also to the failure of CD8$^+$ T cells.[100–102] Indeed, the impact of regulatory CD4$^+$ T cells on HBV-specific CD8$^+$ T cells and the outcome of HBV infection has been analyzed in several studies, although the results of those are sometimes contradictory. On the one hand, it has been shown that patients with chronic HBV infection have elevated frequencies of regulatory CD4$^+$ T cells in the blood that inhibit the proliferation of HBV-specific CD8$^+$ T cells.[103,104] On the other hand, others have not found a higher frequency or suppressive capacity of regulatory CD4$^+$ T cells in chronic HBV infection.[105] Similarly, conflicting data were obtained with respect to the correlation of the frequency of regulatory CD4$^+$ T cells and viral load.[103,106] However, a more recent study has shown that chronically HBV-infected patients with high viral loads have higher frequencies of regulatory CD4$^+$ T cells in the liver, but not in the blood. Thus, these results suggest that a higher proportion of intrahepatic regulatory CD4$^+$ T cells observed in these patients may contribute to CD8$^+$ T-cell failure and accordingly explain the lack of control of viral replication.[107] In contrast to HBV infection, the role of regulatory CD4$^+$ T cells in HCV infection is better understood. Indeed, regulatory CD4$^+$ T cells have been found at a higher frequency in the blood of chronically infected patients compared to patients with resolved HCV infection or healthy controls. These cells are able to suppress HCV-specific CD8$^+$ T-cell proliferation and IFN-γ secretion.[108–110] Furthermore, regulatory CD4$^+$ T cells accumulate in the liver of patients with chronic HCV infection.[111] Finally, the finding that patients with normal ALT levels and decreased liver inflammation have an increased frequency of functional regulatory CD4$^+$ T cells compared to patients with elevated ALT levels further supports the biological role of regulatory CD4$^+$ T cells.[112] Taken together, these combined data suggest that regulatory CD4$^+$ T cells, as well as their cytokines IL-10 and TGF-β, play a role in the inhibition of HBV- and HCV-specific T-cell function.

MECHANISMS OF HBV- AND HCV-SPECIFIC CD8$^+$ T-CELL FAILURE: VIRAL ESCAPE

An important mechanism of T-cell failure is the emergence of escape mutations within viral sequences targeted by virus-specific CD8$^+$ cells. CD8$^+$ T-cell responses drive viral sequence variations that may result in viral persistence by affecting the epitope processing and presentation or abrogating major histocompatibility complex (MHC) molecule binding, as well as T-cell receptor recognition.

In HBV infection, controversial data have been published regarding the contribution of viral escape mutations to viral persistence. It was shown that CD8$^+$ T-cell immune escape variants can arise during acute HBV infection; however, they do not occur frequently and do seem not to have a strong impact on progression to chronicity.[113] The lack of sequence variants can be explained by the typically ineffective and weak HBV-specific CD8$^+$ T-cell responses observed in HBV-infected patients.[114] However, one study suggested the emergence of viral escape mutations in patients with strong CD8$^+$ T-cell responses targeting immunodominant epitopes.[115] Taken together, these data mainly show that the strength of CD8$^+$ T-cell responses may determine the extent of selective pressure on the virus and thereby on the evolution of sequence variants. By analyzing the relationship between HBV sequence variations and the HLA-allele types of chronically HBV-infected patients a recent study found HLA-associated

polymorphisms within known CD8$^+$ T-cell epitopes with matching HLA restrictions. Thus, this study strongly supports the hypothesis that HBV may be under immune pressure in chronic HBV infection.[116] However, whether the identified sequence variations represent complete viral escape mutations that effectively abrogate CD8$^+$ T-cell recognition has not been analyzed yet. In contrast to HBV infection, a strong correlation between the emergence of viral escape mutations within targeted CD8$^+$ T-cell epitopes and viral persistence has been described in HCV infection.[117–120] HCV is a RNA virus with an enormous replication rate and because of its error-prone polymerase several different viral variants can emerge rapidly in a single person. Viral escape mutations are present in about 50% of all epitopes targeted by HCV-specific CD8$^+$ T cells in chronically HCV-infected patients.[121–123] Indeed, studies analyzing viral evolution in patients infected with a known viral source or during the early infection phase have demonstrated the emergence of escape mutations within epitopes restricted by HLA class I alleles. Of note, the emergence of viral escape mutations was associated with the development of persistent infection[120,124,125] and these escape mutations were not detectable in patients who were subsequently able to resolve the infection.[120,125] Importantly, in chronically infected patients the HCV sequence can harbor several mutations within one single epitope. This may have different underlying mechanisms. First, reduced viral fitness caused by a mutation within an epitope can be reverted by subsequent mutations referred to as compensatory mutations.[49] Second, clustered mutations may be necessary to completely abrogate CD8$^+$ T-cell recognition.[126] Thus, two or more mutations may be required in order to balance viral escape and viral fitness cost or to achieve complete viral escape and may hence give the host's immune response the chance to clear the virus beforehand. Indeed, epitopes for which this phenomenon have been described are restricted by protective HLA class I alleles, such as HLA-B*27 and HLA-A*03. Finally, it is important to note that the development of escape mutations is only rarely detectable within HCV-specific CD4$^+$ T-cell epitopes[127,128] suggesting that even though viral escape plays an essential role for CD8$^+$ T-cell failure, other mechanisms may contribute to the failure of CD4$^+$ T cells.

Taken together, in contrast to HBV infection, viral escape mutations play an essential role for viral persistence in HCV infection. However, more distinctive studies of viral sequence variations need to be performed in HBV infection before an effect on viral pathogenesis can be excluded with certainty.

CONCLUDING REMARKS AND OUTLOOK

During the last decades, host adaptive immune responses and especially the effects of antiviral CD8$^+$ T cells have been shown to play an essential role in the elimination or control of the most important human viral infections, such as HBV, HCV and HIV infection. Thus, CD4$^+$ T-cell help is central to induce and maintain a virus-specific CD8$^+$ T-cell response that can clear the virus by cytolytic and non-cytolytic effector mechanisms. Importantly, however, these viral infections can lead to viral persistence. Compelling progress has been achieved in understanding mechanisms of T-cell effector function and failure. These findings are important for our comprehension of immunological factors of virus–host interactions, as well as the development of antiviral vaccine strategies against HCV and immunotherapies that help to cure chronic HBV and HCV infections. Nevertheless, there are still several open questions that need to be addressed in order to completely understand T-cell failure in viral hepatitis. Indeed, it is important to note that while the effector functions of HBV- and HCV-specific CD8$^+$ T cells are mainly elucidated, other aspects of the immune response of CD8$^+$ T cells are less understood. For example, the exact site and process of virus-specific CD8$^+$ T-cell encounter with its specific antigen (CD8$^+$ T-cell priming) and the precise mechanisms of liver homing of virus-specific T cells are still unknown (Figure 2). Thus, next to the elucidation of exact factors of CD8$^+$ T-cell failure, the understanding of virus-specific CD8$^+$ T-cell priming and liver homing may help to catch on the underlying mechanisms of T cells failure and thus chronic disease progression.

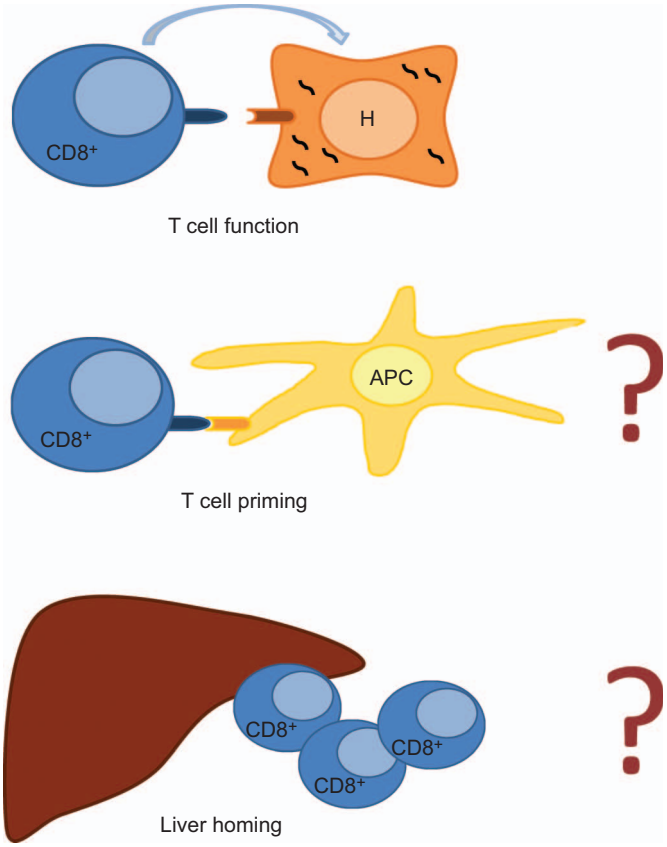

Figure 2 Open questions about the immune responses of HBV- and HCV-specific CD8$^+$ T cells. In contrast to the effector functions of HBV- and HCV-specific CD8$^+$ T cells, that are mainly understood, the exact site and process of virus-specific CD8$^+$ T-cell encounter with its specific antigen (T-cell priming) and the precise mechanisms of liver infiltration (liver homing) of virus-specific T cells are still unknown.

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