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Insights From Antiviral Therapy into Immune Responses to HBV and HCV Infection

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Abstract

There are 257 million persons worldwide with chronic hepatitis B virus (HBV) infection, a leading causes of liver cancer. Almost all adults with acute HBV infection have a rapid immune response to the virus resulting in life-long immunity, but there is no cure for individuals with chronic HBV infection, which they acquire during early life. The mechanisms that drive the progression of hepatitis B through distinct clinical phases to to end-stage liver disease are poorly understood. Likewise, it is not clear whether and how immune responses can be modulated to allow control and/or clearance of intrahepatic HBV DNA. We review the innate and adaptive immune responses to acute and chronic HBV infections and responses to antiviral therapy. Comparisons with hepatitis C virus infection provide insights into the reversibility of innate inflammatory responses and the potential for successful therapy to recover virus-specific memory immune responses.

Keywords

Hepatitis B virus; hepatitis C virus; immune memory; innate

Studies of the immune response to hepatitis B virus (HBV) antigens began >50 years ago, when Baruch Blumberg discovered HBV surface antigen (HBsAg), originally called Australia antigen, in sera from Australian aborigines [1]. One important advance in our understanding of the immune response to HBV infection came with the observation that seroconversion from $HBsAg^+$ to anti-HBs^+ was a biomarker for immune control of the infection and protective immunity. Researchers began testing recombinant HBsAg in preventative vaccines, and countries that adopted universal immunization with HBsAg

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reported a decreased incidence of de novo infections,[2] providing evidence for protective immunity.

Although we understand many features of acute self-limited hepatitis B and natural and vaccine-induced immunity, our understanding on the immune response to chronic HBV infection is lacking. Almost all cases of chronic HBV infection are established from perinatal or early childhood transmission. The geographic distribution and young age of the patients and the asymptomatic nature of early childhood infection have made it difficult to determine how infection during early life results in chronic infection. Due to the unpredictable timing of disease phases and disease activity flares, there have been few prospective studies of the immune response during chronic infection. Little is known about the mechanisms of immune activation and intrahepatic inflammation with increasing age.

We review the adaptive immune responses to HBV infection, highlighting similarities to and differences from hepatitis C virus (HCV) infection and identify areas for future research (Table 1). As the focus of drug development has moved from treatment of HCV infection to HBV infection, new immune cell targets and biomarkers for treatment are needed.

Interestingly, there are shared features of the innate and adaptive immune responses to both infections. Chronic HBV and HCV infections are each associated with exhaustion and reduced antiviral function of virus-specific T cells, thought to result from chronic T-cell receptor stimulation by persisting viral antigens [3]. Chronic HBV and HCV infections also share inflammation-induced changes in natural killer (NK) cell functions, such as increased cytotoxicity and decreased production of antiviral cytokines [4], which contribute to the regulation and deletion of T cells during HBV infection [5, 6]. Interferon (IFN)-based and direct-activating antiviral (DAA) therapies have been used to treat patients with HBV or HCV infection. The intense study of HCV infection over the past decade has provided novel information on immune responses that may be applicable to and should be examined in the context of research on HBV infection. We review these parallels, and discuss the added complexities of the HBV lifecycle, immune response, and development of hepatitis, which make HBV infection more difficult to cure than HCV infection.

The Liver Immune System

HBV and HCV each infect cells of the liver. Based on its location and anatomical features, the liver's immune system induces tolerance to antigens, which are delivered via the portal vein and sampled by sinusoidal cells and liver-resident macrophages [7]. The liver's immune tolerance is well known in the field of transplantation, because livers for transplantation do not require HLA matching with the recipient [8].

The liver contains specialized immune cell populations, such as memory T cells and NK cells that are not found in the blood [9–11] and innate-like T cells, such as mucosal-activated invariant T (MAIT) cells, which are shared by gut and liver [12, 13]. Much of this immune system was only recently discovered and its role in the pathogenesis of viral hepatitis and use as a target for immunomodulator therapies has not been recognized. Likewise, the spatial organization of intrahepatic immune cell clusters is an area of active investigation. Some

structures, such as B cell follicles, [14] are permanent whereas others, such as intrahepatic myeloid aggregates [15], form transiently to promote optimal proliferation of antiviral effector cells.

HBV and HCV each establish acute self-limited and chronic infection of the liver. However, they have different structures of genome organization and replication strategies, so it is not surprising that they use unique strategies to persist (Table 2). HBV, a double-stranded partially open DNA virus with multiple overlapping open reading frames, establishes a mini-chromosome as its transcriptional template and also integrates randomly into the host genome. It replicates within its own nucleocapsids in the cytoplasm of infected hepatocytes and does not induce IFN-mediated responses [16]. In contrast, HCV is a single-stranded RNA virus with a single open reading frame. Its replication in the cytoplasm of infected cells is readily detected by intracellular pattern recognition receptors and results in the release of type I and III IFNs, which HCV escapes [reviewed in [17]].

The Immune Response During Acute, Self-limited Infection

Individuals infected with HBV or HCV can mount a success antiviral immune response. HBV is cleared by more than 95% of adults and HCV is cleared by about 30% of adults. Although takes several weeks for HBV to reach high viral titers in the blood, HCV viremia reaches high levels within days after infection (Fig. 1A, B). HBV persists as a stealth virus in infected cells [16, 18, 19], whereas HCV immediately induces a large number of IFN-stimulated genes (ISGs) and persists via elaborate strategies to escape the innate immune response (reviewed in [17]).

Virus-specific T cells can be detected in the blood about 8–10 weeks after HBV or HCV infection. This means that T cells respond when HBV viremia reaches high levels, but trail the appearance of HCV viremia by several weeks. In both infections, viremia starts to decrease shortly after antiviral T cells become detectable in the blood and the size of the $CD8^+$ T cell infiltrate increases in the liver [20, 21]. The ability to mount effective anti-virus immune responses is determined partly by genetic factors and is increased in individuals with specific HLA alleles. For example, HLA-B27 presents dominant epitopes from many viruses to $CD8^+$ T cells, including HCV [22, 23].

In individuals with acute HBV infection, the decrease in viremia precedes the peak level of alanine aminotransferase (ALT), [20] indicating that antiviral functions of HBV-specific T cells occur before the initiation of severe liver damage. These antiviral functions are mediated by cytokines such as IFN gamma (IFNG) and tumor necrosis factor (TNF) [24, 25]. This process is more efficient than perforin- and granzyme-mediated killing of infected cells, which requires direct interactions between each immune cell and its target cell [20].

Depletion of $CD8^+$ T cells from chimpanzees interferes with clearance of HBV [26] and HCV [27]. Depletion of CD4+ T cells prevents the effective of induction of $CD8^+$ T cell-mediated responses and also prevents clearance of acute HBV [28] and HCV infection [29]. HBV viremia typically decreases to undetectable levels with the onset of virus-specific T-cell responses. However, HCV viremia often is only temporarily controlled, and rebounds multiple times,

due to the selection of viral quasispecies with mutations that allow them to escape detection by T cells [30], ultimately establishing chronic infection.

Humoral immune responses are detectable during acute HBV and HCV infections. During HBV infection, the seroconversion from $HBeAg^+$ to anti-HBe^+, and from HBsAg+ to anti-HBs^+, are used as biomarkers on the road to recovery. Standard immunoassays for these serological markers can be used to monitor clearance of HCV infection, but the transient appearance of strain-specific antibodies has been associated with HCV clearance [31]. Interestingly, HBV-specific antibodies persist for life, whereas HCV-specific antibodies disappear 10–20 years after recovery from acute hep atitis [32]. This might result from continued antigen stimulation after resolution of acute HBV infection vs the complete antigen clearance after resolution of acute HCV infection.

Resolution of acute HBV and HCV infection therefore results in different outcomes. Self-limited acute HBV infection is effectively controlled by the immune response, resulting in the appearance of a cure, but the virus is never completely eliminated—small amounts of covalently closed circular DNA (cccDNA) and integrated HBV DNA persist. Trace amounts of HBV DNA below the limit of detection by quantitative commercial assays might sporadically induce responses by virus-specific T cells and antibodies [33]. In contrast, self-limited acute HCV infection results in complete clearance of all viral RNA and viral antigens. This is consistent with the observation that HBV-specific memory T cells maintain an activated phenotype after resolution of acute HBV infection—they can be depleted from peripheral blood mononuclear cells with antibodies against the activation marker HLA-DR [33]. In contrast, HCV-specific memory T cells have a resting phenotype [32].

This distinction may also have implications for protective immunity upon re-infection. Whereas spontaneous recovery from acute HBV infection results in life-long protective immunity, due to persistence of virus-specific T cells and antibodies, protective immunity after recovery from HCV infection is not always maintained. Complete immune protection with rapid clearance of a re-infecting virus has been observed in some but not all chimpanzees upon re-challenge [27, 34–36], and injection drug users who cleared HCV infection often develop hepatitis upon re-infection, [37] despite evidence for some limited strain-specific immune responses from prior exposure[38]. This concept is also relevant for strategies to develop a protective vaccine against HCV—the use of replicating viral vectors that are not completely cleared may be better suited to induce long-lasting T cell-based immunity than the use of protein vaccines[39,40].

Establishment and Course of Chronic Infection

One of the keys to understanding chronic HBV infection lies in the immune response to of early childhood infection. After comparing cord blood from neonates of HBV-infected and uninfected mothers, Hong et al proposed that in utero exposure to HBV trains the immune response to tolerate the virus, resulting increased innate immune cell maturation and Th1 cell differentiation (trained immunity, a form of innate immune memory) [41]. This was associated with an increased ability of cord blood immune cells to respond to bacterial infections in vitro. Perinatally acquired HBV has therefore been proposed to be a symbiont

that confers advantages to the host. This observation is supported by the recent discovery that the entire hepadnaviral lineage, to which HBV belongs, co-evolved with mammals over at least 400 million years [42]. Perinatal transmission is a perfect result of this co-evolution because it achieves a very high (>95%) rate of persistent infection without any immediate detriment to the host.

Several studies in mice have identified factors that may contribute to the age-dependent differences in immune responses against HBV. Although mice cannot become infected with HBV, because they do not express its entry receptor, it is possible to introduce a transgene that encodes replicating HBV. This can be done in *Rag1*$^{-/-}$ mice, which then secrete intact and infectious HBV in the absence of T and B cells [43]. Adoptive transfer of naïve immune cells into adult transgenic mice results in T- and B-cell priming and seroconversion from HBsAg^{+} to anti-HBs^{+}, whereas adoptive transfer of immune cells to young transgenic mice does not [44]. This differential outcome has been attributed to age-dependent expression of costimulatory molecules on hepatic antigen-presenting cells [43]. This was also associated with impaired responses of T-follicular helper cells, which are required for the optimal generation of virus-specific responses of CD8^{+} T cells and antibodies[45]. Young mice also have lower hepatic levels of CXCL13, a chemokine that is required for optimal induction of virus-specific B cells [44]. Age-dependent changes in the gut microbiota might contribute to this differential immune response, because sterilization of gut microbiota in nontransgenic immunocompetent adult mice decreases HBV-specific immune responses in a model of acute hepatitis B [46].

In contrast to the chronic outcome of early childhood HBV infection, early childhood HCV infection is frequently cleared [47]. Virus-specific factors are likely to be responsible for this difference in outcomes. In utero exposure to the secreted HBeAg has been implicated in the attenuation of HBe- and HBeAg-specific immune responses in neonates [48]. Tian et al tried to model this prenatal exposure to HBV antigens by crossing female hemizygous HBV transgenic mice to naïve male mice. Offspring of these mice were exposed to HBeAg in utero, but are HBV negative after birth [49]. When adult offspring of HBV-positive mothers is challenged by hydrodynamic injection of an HBV-encoding plasmid, their HBV-specific T-cell responses were impaired compared to those of adult offspring of HBV-negative mothers. This supports the concept of tolerance where abundant HBV antigens such as HBeAg and HBsAg functionally impair or delete T-cell clones [50]. Accordingly, HBV-infected neonates and children typically have a non-inflammatory phase, with barely detectable HBV-specific T cells and normal levels of liver enzymes, despite high levels of circulating HBV DNA. Meanwhile, HBV integrates into the host genome, resulting in clonal expansion of hepatocytes and increased risk for cancer [51]. The establishment of cccDNA as the template for HBV replication results in long-term HBV persistence.

Not only are the virus life cycle and immune response more complex for HBV than for HCV, but but so is the course of chronic infection. Although viremia and liver enzyme levels are relatively constant in patients with chronic HCV infection, there are wide variations in HBV titers and liver disease activity in patients with chronic infection (Fig. 1C). The non-inflammatory, immune-tolerant phase eventually transitions into an inflammatory phase with more severe liver disease and fluctuations in HBV DNA titer and ALT activity [52].

Thereafter, many patients enter a phase of low virus replication, with loss of HBeAg and seroconversion to antiHBe$^+$, reduced inflammation, and low HBV titers. Patients who enter this phase after the age of 40 years have a higher risk of developing cirrhosis and liver cancer, compared to patients who enter it earlier [53]. Finally, a subset of patients loses HBeAg expression due to viral mutations. These patients develop active liver disease with incrased, fluctuating levels of liver enzymes, but lower levels of HBV DNA than during the HBeAg$^+$ inflammatory phase. In general, HBeAg– patients with active live r inflammation progress more rapidly to fibrosis and cirrhosis than HBeAg$^+$ patients [52].

Innate Immune Responses During Chronic HBV or HCV Infection

One of the most interesting immunological difference between HBV and HCV infection is the difference in interferon-mediated responses. Acute HBV infection does not induce a significant ISG-mediated response in liver [54]; liver biopsies from patients with HBV infection do not have higher levels of ISG expression than those from patients without HBV infection [55]. When liver biopsies of HBV-infected patients were exposed to toll-like receptor ligands, IFN signaling was not suppressed. This was corroborated by the observations that HBV does not affect the magnitude or breadth of ISG expression by other inducers of IFN and ISGs, such as poly(I:C), Sendai virus, or IFN alpha, [18] and that it does not protect HCV from the antiviral effects of IFN [19].

This differs from HCV, which induces strong IFN- and ISG-mediated responses [56, 57] (regulated by allelic variants near the *IFNL4* gene [58]) yet persists for decades despite the expression of hundreds of ISGs. Several mechanisms of viral interference with the hepatic IFN system have been identified and are reviewed elsewhere [59]. Their relative roles require elucidation.

If HBV cannot be detected by the innate, IFN-mediated response, what leads to the inflammatory response in livers of patients with chronic infection? Co-culture experiments showed that macrophages were capable of sensing HBV if exposed to high HBV titers [18]. When activated, the macrophages produced inflammatory cytokines, including IL1B, IL6, TNF, and CXCL10 [18]. These cytokines have direct antiviral effects [60] and activate other immune cells, such as NK [61], T, and B cells [62, 63]. In mice with acute HBV infection, depletion of macrophages reduced recruitment of inflammatory cells and decreased liver injury [62].

To identify immune cells that promote the progression of chronic hepatitis B to liver cirrhosis, Vanwolleghem et al studied transcriptomes of peripheral blood samples from patients [64]. Compared to blood mononuclear cells from patients in the noninflammatory disease phase, with high viral antigen levels and no liver disease, blood mononuclear cells from patients in the immunoactive phase highly upregulated expression of immunoglobulin genes. Overall, the results showed pronounced activity of B cells during the transition from the immunetolerant to the immunoactive phase, but no significant changes in gene expression patterns among T cells [64]. This is consistent with a report from Park et al, who did not find differences in HBV-specific responses of T cells in patients during different phases of chronic HBV infection [65]. Vanwolleghem et al reported induction of genes related

to NK-cell activation during the immune active phase and HBeAg$^-$ hepatitis phase in patients [64]. This is of interest because during chronic HBV and HCV infections, NK cells have increased cytotoxicity and decreased production of antiviral cytokines (reviewed in [4]).

What Has IFN-based Therapy Taught Us About Innate Immune Responses?

IFN was part of the first treatment regimens for HBV and for HCV infection. IFN has direct antiviral effects against HBV. High doses of IFN alpha induce epigenetic changes in cccDNA-bound histones in cultured hepatoma cells, which inihibit HBV replication and reduce transcription of pregenomic RNA and subgenomic RNA from cccDNA [66, 67]. IFN alpha also induces degradation of HBV cccDNA by upregulating expression of the deaminase APOBEC3A[25] and inhibits HBV nucleocapsid formation [68]. Whether it also accelerates the decay of nucleocapsids with pregenomic HBV RNA is unclear, as effects were shown by Xu et al [69] but not by Wieland et al [68]. IFN's direct antiviral effect is sufficient to reduce viral load and antigen levels in HBV-infected uPA/SCID mice with humanized livers, which lack immune cells [70].

Based on HBV's susceptibility to IFN and the lack of endogenous IFN responses in HBV-infected patients, we would expect IFN-based therapies to be effective. However, for still unknown reasons, this is not the case. In contrast to the sharp 1–2 $\log_{10}$ decrease in HCV viremia within the first 48 hrs after initiation of pegylated IFN-based therapy [71], a decrease in HBV viremia is observed not earlier than 3–4 weeks into therapy [72, 73] and takes several months to reach its maximum [74]. Less than 10% of HBV-infected patients convert to anti-HBs and most do so long after the end of therapy. Of note, IFN-based therapy does not increase the HBV-specific immune response. To the contrary, IFN treatment reduces numbers of CD8$^+$ T cells (particularly late effector CD8$^+$ T cells)[74], whereas expression of immune inhibitory cell-surface markers (such as programmed cell death 1 [PDCD1 or PD2], LAG3, and CTLA4) [75] is maintained, resulting in impaired immune function[74].

Loss of HBeAg and HBsAg and seroconversion to anti-HBe$^+$ and anti-HBs+ is associated with reduced virus replication and long-term normalization of ALT levels [52]. Loss of HBeAg and HBsAg and seroconversion can be associated with increased serum levels of IL12, IFNG, and IL2 [76] and a temporary flares in levels of ALT, which suggests that the loss of HBeAg and HBsAg isimmune immune-mediated. Cytotoxic CD8$^+$ T cells can be more readily expanded from blood of treatment responders than nonresponders [77]. Changes in the inflammatory environment in the liver, which may take a long time to develop, might be a pre-requisite for off-treatment immune responses. This would be consistent with the observation that long-term (> 4 years) nucleos(t)ide analog therapy increases responses of HBeAg-negative patients to pegylated IFN add-on therapy [78]. Strategies to enhance this response are an important area for further investigation.

Studies of patients with HCV infection receiving IFN-based therapy have provided unique insights into determinants of innate immune responsiveness [79]. Pre-treatment levels of ISG expression associate with response to IFN alpha-based therapy; patients with high baseline levels of ISG expression cannot increase these levels with IFN-based therapy. Induction of negative feedback mechanisms, such as suppressors of cytokine signalling, likely contributes

to resistance [80]. In addition, genetic determinants of innate immune responses associate with the response to IFN alpha-based therapy. Rs1297960 and rRs368234815 are in linkage disequilibrium but rs368234815 has a higher predictive value in African Americans [81]. Rs12979860 is located in the first intron of the *IFNL4* gene [81] and rs368234815 regulates the transcription of the *IFNL4* gene. The rs368234815 [DG] allele creates an open reading frame for expression of the IFNL4 protein, which induces expression of ISGs. In contrast, the rs368234815 [ΔG] haplotype results in a frameshift so that IFNL4 protein is not produced. This haplotype is associated with HCV clearance after acute infection and response to IFN-based therapy [82–84].

The negative correlation between pre-treatment levels of ISG expression and on-treatment increases in expression of ISGs is recapitulated in the response of innate immune cell populations that are sensitive to IFN alpha. For example, pre-treatment expression levels of the activating NK cell receptors NKp30, NKp46 and DNAM1 correlate inversely with the increase in theeexpression levels of these receptors during IFN-based treatment and the subsequent virologic response to treatment [85]. NK cells from patients with a rapid first-phase decrease in HCV RNA have maximal responsiveness to type I IFN, based on high levels of phosphorylated STAT1 in NK cells, compared to that of patients with slow decrease in HCV RNA [86]. This is consistent with greater NK cell cytotoxicity in treatment responders than nonresponders [87].

Although a virologic response to IFN-based therapy is durable in more than 97% of patients [88] it takes a long time until HCV is completely cleared. Small traces of HCV RNA can sporadically re-appear in the circulation within the first years after treatment along with a transient increase in HCV-specific T-cell responses [89]. The persisting HCV RNA is replication-competent and infectious because it can transmit infection to chimpanzees [90] and on rare occasions result in relapse in the treated patients [91].

T-cell responses in Patients With Chronic HBV or HCV infection

Patients with HBV or HCV infection have low numbers of virus-specific T cells, compared to patients with other chronic infections, such as CMV or HIV[3]—may be related to the tolerogenic environment of the liver (for review, see [92]). HBV-specific $CD8^+$ and $CD4^+$ T cells are more difficult to detect and expand than HCV-specific T cells [93]. This could be due to reduced induction of HBV-specific T cells during perinatal infection or increased T-cell exhaustion. Increased T-cell exhaustion may be due to fact that HBV infection typically occurs earlier in life than HCV. This results in a longer period of antigen-specific stimulation followed by exhaustion and ultimately depletion of virus-specific T cells. HBV also has a lower rate of mutation than HCV (Table 2), and the HBV genome encodes several overlapping open reading frames, which reduces the likelihood of T-cell escape variants being compatible with viral fitness. This may result in a somewhat lower prevalence of viral escape variants in patients with HBV [94, 95] vs HCV infection [96, 97].

The ex vivo detectability of HBV-specific $CD8^+$ T cells appears to be limited to individuals with low viral load and those, who have also cleared HBeAg [98–104]. T-cell responses against HBV polymerase are the easiest to detect in recall assays whereas T-cell responses against

HBsAg are almost completely absent [102, 105]. This recapitulates the differential abundance of these proteins: HBsAg is expressed in subviral particles, which outnumber complete polymerase-containing virions by a factor of $100{,}000^4$.

In HBV and HCV infection, most virus-specific $CD8^+$ T cells express multiple inhibitory receptors and are functionally impaired [102, 106–110]. Dysregulated expression of transcription factors [104] and mitochondrial alterations have been identified as additional factors associated with the impaired function of HBV-specific T cells [111]. Increased expression of the apoptosis-activating molecule Bim is thought to contribute to ultimate clonal deletion [112]. However, studies from mice indicate that T-cell responses to individual viral epitopes can be polyclonal [113]—not all virus-specific T cells are terminally exhausted. Likewise, HCV-specific $CD8^+$ T cells are not a homogeneous population [114]. Terminally exhausted $CD127^-$ $PD1^{hi}$ $CD8^+$ T cells co-exist with $CD127^+PD1^+$ $CD8^+$ T cells that target the same HCV epitope without viral escape mutation (Fig. 2). The $CD127^+PD1^+$ $CD8^+$ T-cell subset has memory-like characteristics due to the expression of the memory transcription factor TCF1 and the expression of the IL7 receptor CD127, which allows them to maintain their capacity for self-renewal and act as a continuous source of effector cells that then undergo terminal exhaustion (Fig. 2B) [115]. Memory-like cells can persist in the absence of antigen, shown by transfer of these cells from mice with chronic lymphocytic choriomeningitis virus infection to uninfected mice, [116] and give rise to a rapid, memory-like recall response. In this mouse model of chronic lymphocytic choriomeningitis virus infection, $TCF1^+$ T cells have provide the proliferative burst after blockade of the inhibitory receptor PD1 [117]. Blockade of inhibitory receptors can increase the functional response of some HBV- and HCV-specific $CD8^+$ T cells in vitro [65, 102, 109], so memory-like $CD8^+$ T cells might be targeted to revive exhausted $CD8^+$ T-cell populations in patients with viral hepatitis.

Does Antiviral Therapy Restore Protective Immune Responses?

DAAs and nucleos(t)-ide analogues effectively inhibit viral replication in patients with HCV or HBV infection. Antiviral therapy decreases viral titers in blood and liver and reduces inflammatory liver disease in patients with HCV or HBV infection. Differences exist, however, in response kinetics. Whereas a sustained virologic response can be reached within 8 weeks for most patients treated for HCV infection and viral antigens are eventually complete cleared, HBV typically requires life-long therapy, and viral antigens remain despite suppression of viral replication. Studying immune responses to HCV and HBV can elucidate the relative roles of viral replication and persisting antigen on innate and adaptive immunity.

DAA-mediated clearance of HCV is accompanied by rapid downregulation of ISGs in the liver and blood, regardless of treatment outcome. Analysis of paired pre-treatment and end of treatment samples revealed that viral clearance is accompanied by decreased expression of type II and III IFNs, but surprisingly by increased expression of type I IFN (IFNA2) at the end of treatment [118]. Restoration of type I IFN levels in the liver might therefore contribute to DAA-mediated HCV eradication and prevention of HCV re-infection. This is consistent with findings from Alao et al, who observed higher baseline expression of ISGs in patients who respond to DAA therapy compared to those with viral breakthrough [119]. Successful

DAA therapy also normalizes NK cell function and to restores their responsiveness to IFN alpha [120, 121], so innate immunity might contribute to HCV clearance during DAA therapy by preventing viral breakthrough. Nonetheless, even after HCV is cleared, the diversity of the NK cell repertoire remains altered. [122]

DAA therapy does result in restoration of all parts of the innate immune response, as shown for cytokines and MAIT cells. MAIT cells are a population of innate-like T cells that is enriched at barrier sites such as liver and gut and activated by antigen (vitamin B metabolites displayed on non-classical MHC molecules) and by inflammatory cytokines (IL12 and IL18). Monocyte-derived IL18 is increased in the HCV-infected liver, resulting in MAIT cell activation and reduction of peripheral and intrahepatic MAIT cells [123]. Effective DAA therapy decreases intrahepatic activation of monocytes and plasma levels of IL18, followed by a decrease in MAIT cell activation and an increase in intrahepatic MAIT cell frequency [123]. The frequency of MAIT cells in peripheral blood is reduced for many months after the end of treatment [124] and their antigen-dependent effector function is not restored [123 124]. Likewise, several cytokines do not normalize during the follow-up period [125]. Collectively, these findings indicate that decades of HCV infection have lasting effects on parts of the innate immune system, even after treatment-induced viral clearance.

At the level of adaptive immune responses, a partial recovery of virus-specific T-cell responses, especially their proliferative function, has been reported in patients with HBV or HCV infection. In patients treated with nucleos(t)-ide analogues for HBV infection, this recovery was observed in assays that depend on in vitro stimulation [99, 126] and has been proposed to be transient [127]. This could be the reason it is impossible to stop nucleos(t)-ide analogues treatment for most patients with chronic HBV infection. There is an association between the quantity and function of HBV-core and -pol specific $CD8^+$ T cells and the ability to control HBV replication after nucleos(t)-ide analogue withdrawal [128]. Functional HBV-specific $CD8^+$ T cells were selectively enriched in the $PD1^+$ population of T cells, supporting the existence of a persistent, memory-like population. The strongest recovery of HBV-specific $CD8^+$ T-cell responses has been reported in patients with HBsAg seroconversion during nucleos(t)-ide analogue therapy. This includes the recovery of T-cell responses against HBsAg, which is typically not observed in patients with HBV chronic infection [103]. As in patients with acute self-limited hepatitis, HBsAg clearance indicates effective immune control and is a prognostic marker for a good outcome of liver disease [129, 130]. It is therefore tempting to speculate that viremia and the level of virus antigen contribute to HBV-specific T-cell suppression during chronic infection.

We have learned much about the fate of memory-like T cells in patients with HCV infection treated with DAAs. During and after DAA therapy, memory-like $CD8^+$ T cells persist, whereas terminally exhausted T cells are rapidly lost (Fig. 3). This is similar to the immune contraction phase after acute viral infection, in which memory T-cell populations are maintained after successful antigen elimination while terminally differentiated effector T cells disappear. Memory-like HCV-specific $CD8^+$ T cells therefore appear to be capable of antigen-independent survival, whereas terminally differentiated effector cells depend on continuous antigen stimulation.

Memory T cells are reactivated upon re-exposure viral antigen. However, even though HCV-specific T cells from patients who are successfully treated have increased proliferation and function, compared to exhausted HCV-specific effector cells, they do not reach the functional capacities of conventional memory T cells in in vitro assays [131]. In a patient with a virologic relapse after DAA therapy, recall responses of HCV-specific $CD8^+$ T cells were observed at the time of virological relapse and antigen re-exposure, but these did not mediate viral clearance and ultimately acquired a terminally exhausted phenotype [114]. This finding is in agreement with results from a study in chimpanzees, which reported the persistence of HCV-specific $CD8^+$ T cells after DAA-mediated HCV elimination but no protection from re-infection [132]. Likewise, chronic infections are frequently observed in re-infected injection drug users. Antiviral therapy does not, therefore, fully restore immunity. The mechanisms of this effect are not well understood and could include quantitative and qualitative differences in the small population of memory-like T cells, a permanent and irreversible epigenetic imprinting of most virus-specific $CD8^+$ T cells, [133] or indirect effects of cytokines or regulatory T cells[134].

Future Directions and Opportunities

Research into HBV infection should aim to provide a better understanding of chronic disease and immune control. Host, viral, and environmental factors that affect the transition from non-inflammatory to inflammatory phases, and from $HBeAg^+$ to anti-HBe^+ phases, need to be identified and studied in order for us to understand mechanisms of pathogenesis and for identification of biomarkers. Patients with chronic HBV infection who spontaneously seroconvert to anti-HBs should be identified and assessed, as they represent a functional cure—the goal of treatment goal for chronic HBV infection. An ideal antiviral therapy would not just decrease HBV replication but also effectively reduce levels of HBsAg and eliminate or at least control cccDNA—it might need to synergize with the adaptive immune response to achieve this [135]. Decrease in HBV replication, reduction of HBsAg levels and control of cccDNA might be achieved by directly increasing the immune response (such as with agonists of the toll like receptor, a therapeutic vaccine, or adoptive T-cell transfer) or through indirect activation (inhibition of HBsAg production).

Altough antiviral therapies can now completely eradicate HCV, even with reduction in treatment duration and costs, these regimens will likely not be affordable in countries where the prevalence of HCV and the incidence of new infections are highest. The development of a prophylactic vaccine that induces protective immunity is therefore necessary. A better understanding of the basic mechanisms of immune failure and its possible restoration by DAA therapy will be important to guide this attempt.

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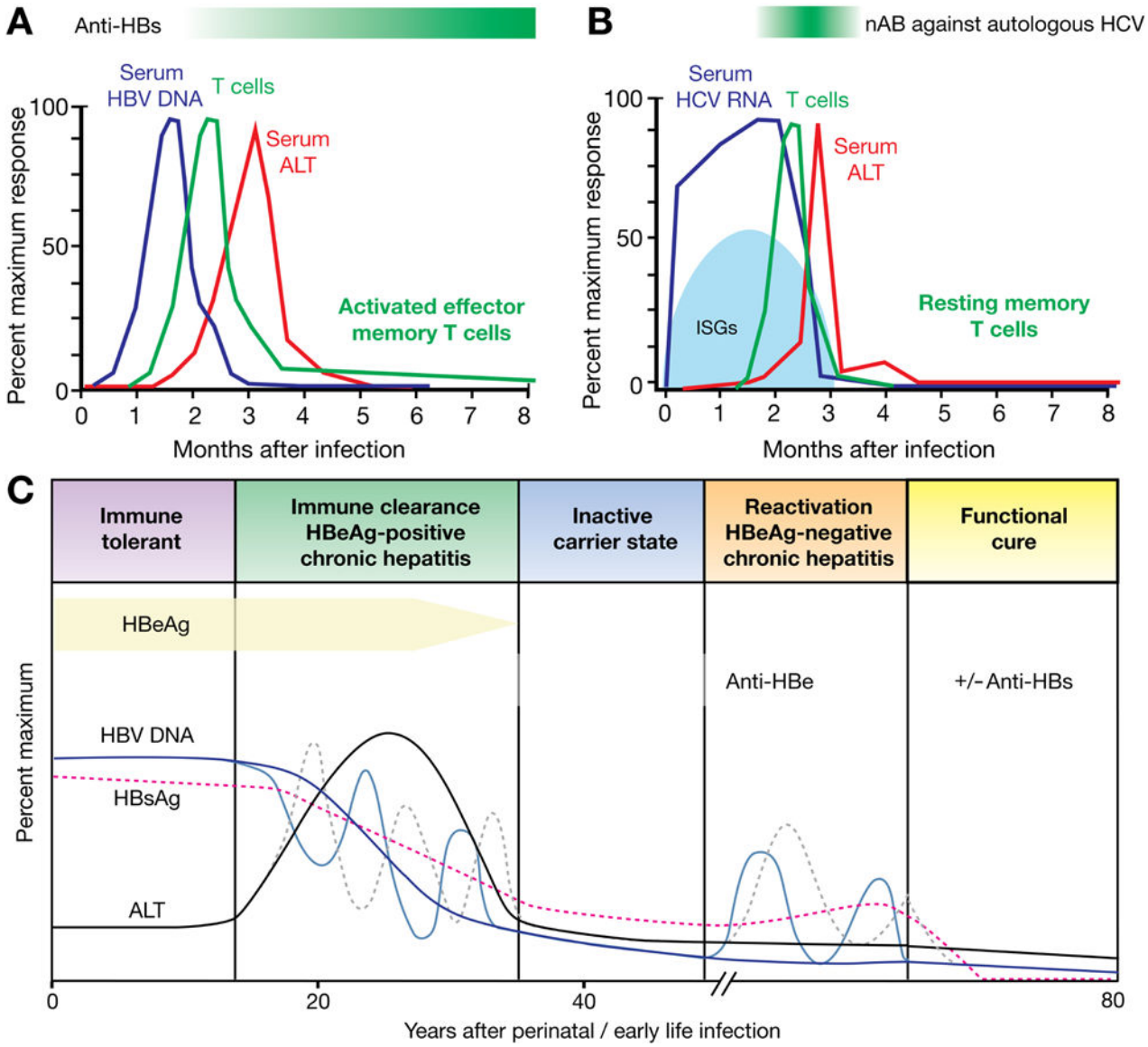

Figure 1. Acute, Self-limited HBV and HCV infection and Chronic HBV Infection

(A, B) Studies of acute, self-limited HBV and HCV infection provide information about successful immune responses. (A) HBV DNA is detectable within the first 2 months of infection and its peak precedes the onset of T-cell responses and the increase in serum level of ALT. (B) HCV RNA becomes detectable within a few weeks after infection and is associated with the induction of ISGs. T-cell responses occur after 8–12 weeks in most patients concomitant with a surge in serum ALT levels. Neutralizing antibodies (nAb) against HCV become detectable a few weeks later. (C) Phases of chronic HBV infection: the immune-tolerant phase is characterized by high levels of HBV DNA and HBsAg and a normal level of ALT. In the immune-clearance phase and the $HBeAg^+$ chronic hepatitis phase, levels of HBV DNA and ALT decrease and the level of ALT increases, indicating liver injury. The transition between the first 2 phases of disease can be dynamic and associates with repeated increases and decreases in level of HBV DNA and activity of ALT

(indicated by the dashed lines). This phase can ultimately lead to HBeAg seroconversion. The subsequent inactive carrier state is characterized by low levels of HBV and HBsAg and a normal level of ALT, but reactivation in the HBeAg-negative chronic hepatitis phase is associated with flares in levels of ALT and ongoing liver disease.

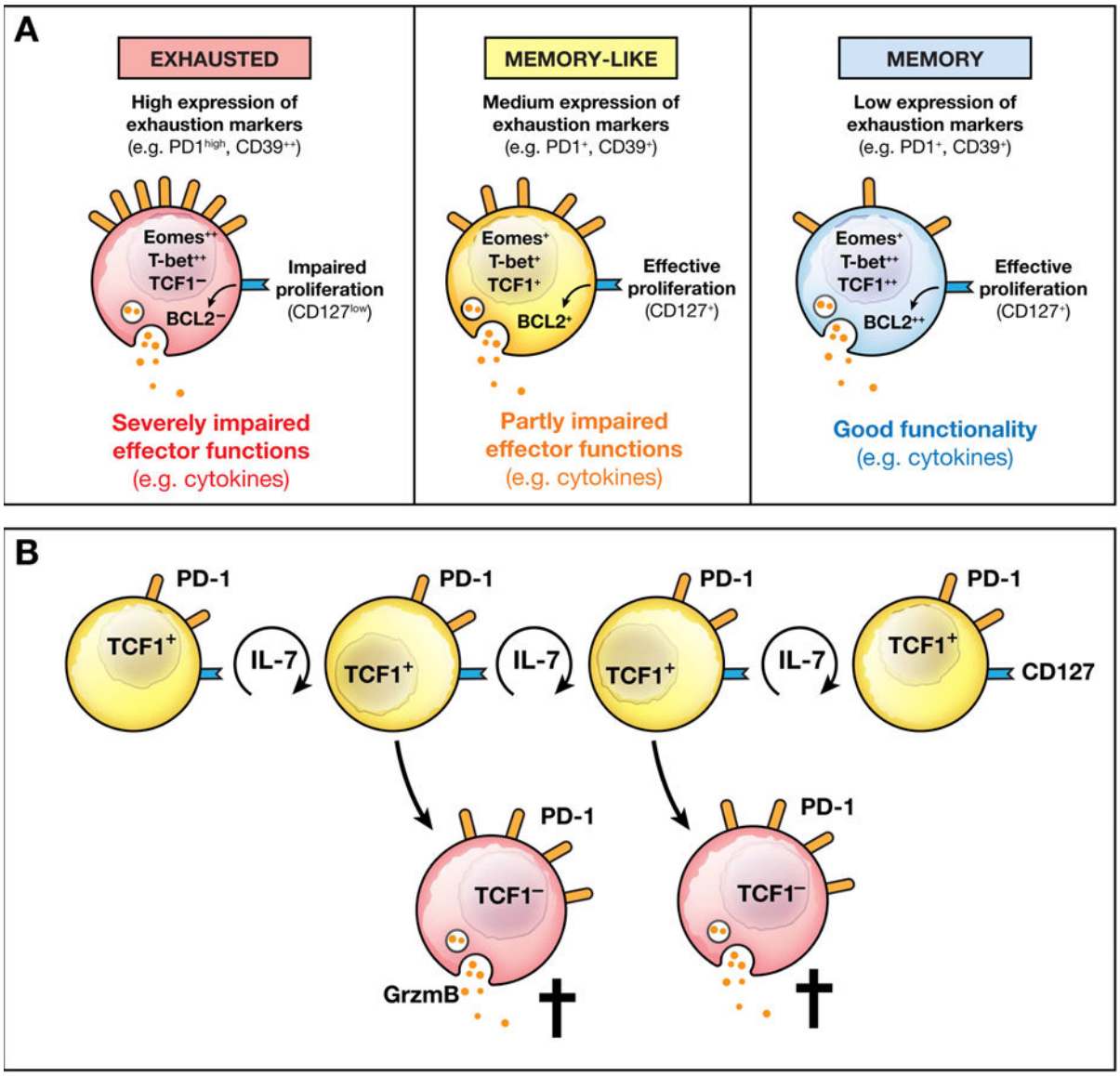

Figure 2. Phenotypic, Transcriptional, and Tunctional Properties of Virus-specific CD8+ T Cells

(A) Exhausted $CD8^+$ T cells are characterized by high expression of exhaustion markers and the transcription factor eomesodermin and an impaired capacity to produce cytokines or proliferate. Memory-like $CD8^+$ T cells have features of exhausted cells (PD1 expression) and memory cells (expression of the IL7 receptor CD127 and the transcription factor TCF1). Their functional properties are increased in comparison to exhausted cells but impaired in comparison to memory cells. Memory $CD8^+$ T cells express low levels of exhaustion markers, have recall effector functions, and express the transcription factors T-box 21 (TBX21 or TBET) and HNF1 homeobox A (HNF1A or TCF1).(B) Virus-specific memory-like $CD8^+$ T cells sustain the T-cell response during chronic infection (modified from [115] with permission).

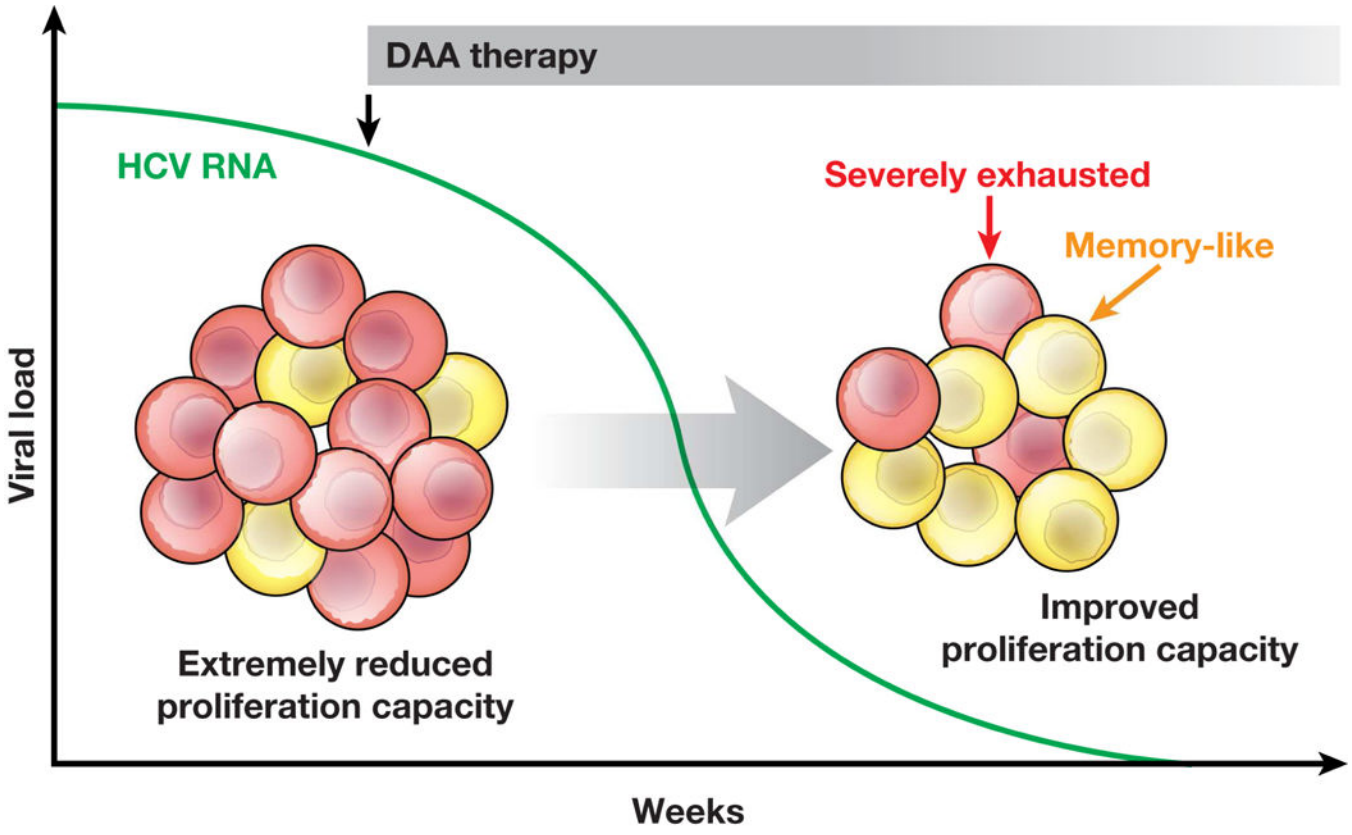

Figure 3. Changes in HCV-specific T-cell Subsets During and After DAA Therapy

During chronic HCV infection, the 2 subsets of $PD1^+$ HCV-specific $CD8^+$ T cells are severely exhausted T cells and memory-like T cells. After successful DAA therapy, higher numbers of memory-like $CD8^+$ T cells are maintained due to their antigen-independent proliferative capacity while severely exhausted, antigen-dependent $CD8^+$ T cells decrease in number. This is reflected by an improved proliferative capacity of the overall HCV-specific $CD8^+$ T-cell population.

Table 1.

Current Knowledge and Knowledge Gaps related to innate and adaptive immune responses in HBV and HCV infection.

	What we know		What we do not know	
	HBV	**HCV**	**HBV**	**HCV**
Innate Response	– no ISG induction "stealth virus"	– strong ISG induction	– mechanisms circumventing ISG-induction	– relative role of ISGs in HCC and HCV-mediated interference with hepatic IFN system
	– functional and phenotypical alterations of NK cells – IFN therapy leads to activation of immunity	– DAA therapy leads to rapid downregulation of ISGs and normalization of NK cell phenotype	– Responsible pathways and reversibility of NK cell alteration	
Adaptive Response	– viral clearance associated with multi-specific CD4+ and CD8+ T cell responses		– relative role of cytolytic- versus non-cytolytic effector mechanisms	
	– enrichment of T cells at the site of infection		– impact of liver environment on function and phenotype of immune cells – nature of immune responses that drive disease progression	
	– early loss of CD4+ T cell help leads to viral persistence		– mechanisms responsible for early CD4+ T cell failure	
	– T cell dysfunction and viral escape contribute to T cell failure		– relative role of different mechanisms of T cell failure	
	– antiviral therapy leads at least to partial recovery of virus-specific T cell recovery		– extent to which antiviral therapy and viral control lead to restoration of protective immunity	
	– humoral immune responses detectable		– role of antibodies and B cells in different disease phases	– contribution of antibodies to viral control

ISG, interferon stimulated gene; HCC, hepatocellular carcinoma

Table 2.

Comparison of HBV and HCV virology, immunology and treatment

	HBV	HCV
Prevalence worldwide	257 million people infected	71 million people infected
Virology		
Virus	42 nm; enveloped nucleocapsid; partially double-stranded DNA genome	50 nm; enveloped nucleocapsid; positive stranded RNA genome
Family	Hepadnaviridae	Flaviviridae; Hepacivirus Genus
Genotypes	8 genotypes	6 major genotypes; more than 50 subtypes; quasispecies in each infected patient
Mutation rate	low	high
Virus half-life	2–3 days	3 hours
Virus production	10^{10} –10^{12} virions / day	10^{12} virions / day
Acute Infection		
Outcome	>90% self-limited infection	<30% self-limited infection
Immunity	Longterm protective immuity	?
Chronic Infection		
Cause	Mostly from vertical/perinatal transmission:mother-to-neonate, early childhood infection	Mostly from horizontal transmission injection drug use, parenteral, sexual, nosocomial
Course	Distinct phases defined by ALT and viremia level HBeAg / antibody status	Stable ALT levels and viremia
Spont resolution	About 1% per year (HBsAg loss)	No
Treatment		
IFN-based	PegIFN; 25% HBeAg seroconversion 3–7% HBsAg loss	PegIFN/ribavirin
Antiviral	Inhibition of transcriptase activity of viral polymerase; suppression of viral replication without elimination of cccDNA	Inhibition of viral protease and polymerase; >95% cure
Cure	functional cure (cccDNA persists, reactivation possible)	virological cure (virus completely cleared)