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Immune Response in Hepatitis B Virus Infection

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Hepatitis B virus (HBV) can replicate within hepatocytes without causing direct cell damage. The host immune response is, therefore, not only essential to control the spread of virus infection, but it is also responsible for the inflammatory events causing liver pathologies. In this review, we discuss how HBV deals with host immunity and how we can harness it to achieve virus control and suppress liver damage.

Innate and adaptive immunity have evolved different tasks to control infections. Through recognition of viral nucleic acids, viral proteins or tissue-damage innate immunity is triggered during the early phases of viral infections. Activation of different families of cellular receptors (toll-like receptors [TLRs], RIG-1) leads to rapid production of antiviral cytokines, such as interferon (IFN)-α, and, in concert with activation of natural killer (NK) cells, limits the initial spread of hepatitis B virus (HBV). The activation of innate immunity is also necessary for the efficient recruitment of the adaptive immune system (Akira et al. 2006) which acts through functional maturation and expansion of distinct B- and T-cell clones that specifically recognize and kill infected hepatocytes. This process eventually leads to the control of an infection and generates a memory response, which protects the host from subsequent infections with the same pathogen.

As different pathogens target different organs and cause a variety of clinical conditions, they also evolved distinct strategies to escape host immunity. HBV infection of hepatocytes is characterized by several unique features (Bertoletti et al. 2010). Although many virus infections are characterized by an initial logarithmic phase of virus production, HBV infections show delayed virus amplification and spread through the liver. Similarly, febrile symptoms occur immediately in many acute viral infections, whereas acute HBV infections are mostly asymptomatic. Finally, although low viral load and protein expression is characteristic of most chronic viral infections (e.g., hepatitis C virus [HCV], human cytomegalovirus), HBV persistence is often associated with the production of large amounts of viral proteins, such as the hepatitis B surface (HBsAg) and e (HBeAg) antigens, respectively (Bertoletti and Ferrari 2003; Wieland and Chisari 2005).

Another peculiarity of HBV infections is that the immune system often cannot completely resolve infections. Although "recovered" patients maintain protective immunity for the re-

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mainder of their lives, trace amounts of HBV DNA can still be detected sporadically (Rehermann 1996). These trace amounts of HBV DNA are infectious and stimulate HBV-specific B- and T-cell responses, which, in turn, control viremia (Rehermann 1996). The basis for the apparent persistence is the covalently closed circular DNA (cccDNA) that persists in infected hepatocytes in the form of a minichromosome (Seeger and Mason 2000). Thus, successful HBV immunity must often be considered protective rather than sterilizing.

In this review, we will summarize the role of different components that contribute to anti-HBV immunity, and discuss how we can exploit recent knowledge gained about the immune response to achieve control of chronic HBV infections in the future.

INNATE IMMUNITY DURING HBV INFECTION: RECOGNITION DEFECT OR ACTIVE INHIBITION?

Technical limitations restrict our knowledge of innate host response against HBV. Data obtained during acute natural infection are limited by the difficulty in recruiting patients at the earliest presymptomatic stages (reviewed in Bertoletti et al. 2010). In addition, despite the recent discovery of the putative receptor of HBV, we still lack a robust HBV in vitro infection system. In vitro HBV infection efficiency is often poor and the level of HBV replication is low (Gripon et al. 2002; Hantz et al. 2009). Equally, animal models of hepadnavirus infections are plagued by ethical issues and high costs (chimpanzees), the scarcity of reagents to analyze immunological events (woodchucks, ducks) (Roggendorf and Tolle 1995; Guy et al. 2008), or technical difficulties involved with the production of human livers in chimeric mice (Dandri et al. 2001; Jo et al. 2013).

Despite these limits, information gained from animal studies and human liver specimen established a scenario of weak activation of innate immunity as the hallmark of acute HBV infection in adults. Proinflammatory cytokines are low or undetectable within the first 30 days of HBV infection, their production is of lower magnitude, and the kinetics were also delayed compared with HCV- and HIV-infected patients (Dunn et al. 2009; Stacey et al. 2009). These observations are consistent with results obtained in chimpanzees, in which a limited induction of IFN-related genes was observed after HBV infection, in contrast to the rapid up-regulation observed in HCV (Wieland et al. 2004). Lack of induction of known IFN-α-stimulated genes was not only observed during acute HBV infection, but also during chronic reactivation and in the livers of woodchucks chronically infected with woodchuck hepatitis virus (WHV) (Fletcher et al. 2012).

The reason for the apparent lack of a robust IFN-α-mediated innate response during HBV infections is still controversial. One possibility is that HBV escapes innate recognition by sequestering cccDNA to the cell nucleus, and replicative RNA and DNA intermediates to cytoplasmic core particles and, hence, preventing their recognition by host-sensing receptors (Wieland and Chisari 2005). Recent reports have, however, challenged this view, claiming that HBV is sensed by the innate immune system, but that it actively suppresses its activation (Durantel and Zoulim 2009). For example, HBV replication in HepaRG cells, which are physiologically closer to normal hepatocytes than established hepatoma-derived cell lines, activates IFN-β and other IFN-stimulated genes (ISGs) (Lucifora et al. 2010). In addition, acute infection of woodchucks with high doses of WHV can induce ISGs immediately after infection (Guy et al. 2008). Also, Kupffer cells, despite not replicating the virus, seem to be able to sense HBV with up-regulation of interleukin (IL)-6 production (Hösel et al. 2009). The physiological relevance of these observations needs to be confirmed in natural infections that occur with much lower doses of virus (Unterholzner and Bowie 2011). Thus, overexpression of HBV in HepaRG cells (Lucifora et al. 2010) and the exceedingly high dose of WHV used to infect woodchucks (Guy et al. 2008) might have triggered the observed stimulation of the innate system. Indeed, the quantity of initial viral inoculum is known to be an important parameter, which can influence the outcome of HBV infec-

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tions (Michalak et al. 1994; Coffin and Michalak 1999; Asabe et al. 2009).

However, the ability of the innate immunity to sense and react to HBV was recently supported by the demonstration of a transient activation of ISGs in human hepatocytes infected with HBV in chimeric mice (Lütgehetmann et al. 2011). The viral and cellular factors involved in ISG activation have not yet been elucidated. Possible cellular candidates might belong to the growing family of pathogen-recognition receptors (PRRs) (Sharma and Fitzgerald 2011). A similar lack of information is apparent concerning the mechanism(s) that suppress IFN-α/β production in HBV-infected cells. For example, evidence has been obtained that the HBV polymerase can interfere with IRF-3 and IRF-7 signaling by binding to the RNA helicase DDX3 (Fig. 1) (Foster et al. 1991; Christen et al. 2007; Wu et al. 2007; Wang and Ryu 2010; Yu et al. 2010).

The HBV X protein (Hbx) has also been implicated in the inhibition of intracellular innate immunity by interfering with signaling, mediated by cytosolic sensory molecules, such as RIG-I (Wang et al. 2010; Wei et al. 2010; Kumar et al. 2011). Nevertheless, similar to previous work, intracellular IFN-β production was activated by heterologous inducers (poly dAT:dAT, poly I:C, or vesicular stomatitis virus), and HBx, RIG-I, and IPS-1 were overexpressed in HepG2 cells. Thus, these studies are important to reveal potential clues on the interplay between HBV and innate immunity, but need to be confirmed with more natural HBV infection systems.

HOST IMMUNITY AND CONTROL OF HBV REPLICATION

Another important aspect of antiviral immunity concerns the mechanisms that reduce and control HBV replication. Although killing of infected hepatocytes by NK or cytotoxic T lymphocytes (CTLs) will reduce HBV load, noncytolytic mechanisms are also believed to play a critical role in the control of HBV replication (Guidotti 1999). IFN-α can inhibit HBV replication in cell lines and human hepatocytes in chimeric mice by several mechanisms, including induction of epigenetic changes in histones of the cccDNA minichromosome (Belloni et al.

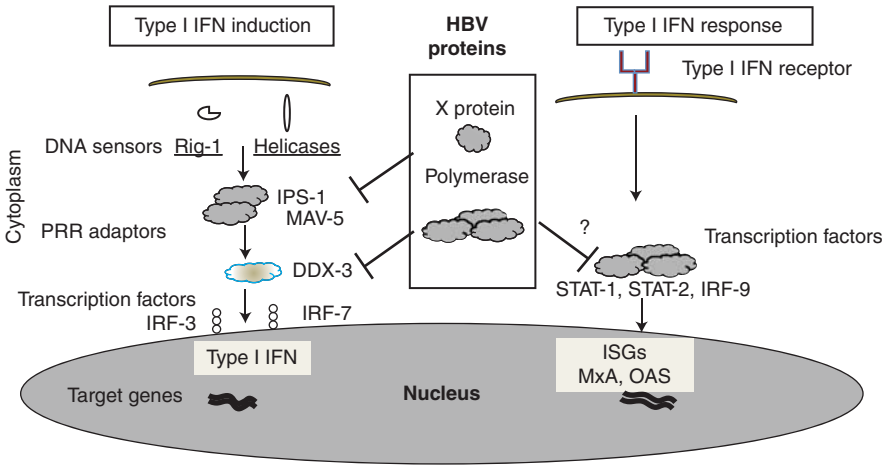

Figure 1. Proposed mechanisms for the suppression of the host innate immune response by different hepatitis B virus (HBV) proteins. Induction of interferon (IFN)-α/β (type I IFN) production might be suppressed by HBV polymerase through binding to DDX-3 (Wang and Ryu 2010; Yu et al. 2010) or by HBx through down-regulation of the mitochondrial antiviral-signaling protein MAV-5 (Wang et al. 2010; Wei et al. 2010; Kumar et al. 2011). IFN-α-induced response can also be blocked by the HBV polymerase as a result of a block of signal transducer and activator of transcription (STAT)-1 nuclear translocation (Foster et al. 1991; Christen et al. 2007; Wu et al. 2007). PRR, Pathogen-recognition receptor.

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2012) and accelerated decay of replication-competent HBV nucleocapsids (Xu et al. 2010). At very high doses, IFN-α has also been shown to induce direct degradation of cccDNA through activation of APOBEC3A cytidine deaminase (Lucifora et al. 2014). However, the antiviral activity exerted by IFN-α during natural HBV infections is weak and lower than observed in HCV infections. In HCV-infected patients, IFN-α-based therapies result in a sharp decrease in viremia within the first 48 h (Neumann et al. 1998), whereas in patients with chronic hepatitis B (CHB), HBV titers begin to drop only 3–4 wk after the start of therapy. Therefore, it is likely that therapeutic effect of IFN-α in CHB is indirect through activation of other components of the innate immune system, such as NK cells.

The limited direct antiviral efficacy of IFN-α has been investigated in vitro and in vivo. In human hepatocytes in chimeric mice, HBV prevents IFN-α-mediated signaling by inhibiting nuclear translocation of signal transducer and activator of transcription (STAT)-1 and, thus, interfering with transcription of ISGs (Lütgehetmann et al. 2011). Interestingly, IFN-α has also been shown to promote HBV infection in HBV transgenic mice or mice inoculated with HBV DNA by hydrodynamic transfection. This activity was detectable only when the viral load was low (Tian et al. 2011) and might explain why IFN-α-based therapies are generally not very effective against HBV. In this model, IFN-α activated STAT-3, which, in turn, stimulated HBV gene expression and replication. A possible explanation of why the pro-HBV effect can be detected only when HBV replicates at low level might be that, at higher levels of HBV replication, the viral polymerase and X proteins are produced at levels sufficient to inhibit STAT methylation (Christen et al. 2007) and other IFN-α-mediated cellular responses (Foster et al. 1991; Wu et al. 2007).

Suppression of HBV replication can also be mediated by IFN-γ and tumor necrosis factor (TNF)-α (Cavanaugh et al. 1998; Nakamoto et al. 1998; Guidotti 1999). Production of these cytokines has been associated mainly with an efficient HBV-specific T-cell response in transgenic mice and chimpanzees (Guidotti et al. 1996; Guidotti 1999; Phillips et al. 2010). However, IFN-γ and TNF-α are also secreted by NK and natural killer T (NKT) cells at levels sufficient for inhibition of HBV. This was, for example, not only shown with HBV transgenic mice (Kimura et al. 2002), but also with HBV-infected chimpanzees treated with TLR agonists, which can stimulate not only IFN-α, but also an IFN-γ-dependent antiviral response (Lanford et al. 2013). More important, the human intrahepatic environment is enriched for NK^{bright} cells and a type of NKT cells called mucosal-associated invariant T (MAIT) cells, which can produce large quantities of IFN-γ after activation with IL-12 and IL-18 (Tu et al. 2008; Jo et al. 2014; Ussher et al. 2014).

Innate immune pathways induced by the lymphotoxin-β receptor (LT-βR) might also play a role in the suppression of HBV replication through activation of nuclear deaminases, which target cccDNA (Lucifora et al. 2014). The physiological ligands of LT-βR are two members of the TNF-superfamily ligands, expressed on subsets of activated T, B, and NK cells. One is the heterotrimeric LT-α1β2 formed by a single molecule of LT-α and two of LT-β. The heterotrimer activates LT-βR signaling by inducing dimerization of the LT-βRs expressed on hepatocytes (Sudhamsu et al. 2013). The other LT-βR ligand, termed LIGHT (homologous to lymphotoxin [LT]-inducible expression, and compete with herpes simplex virus [HSV] glycoprotein for herpes simplex entry mediator, expressed by T lymphocytes), is expressed on immature dendritic cells and activated T cells, can bind to LT-βR in a soluble form, and has been shown to play a role in maturation of the adaptive immune system and also in hepatitis (Anand et al. 2006). Despite the potential importance of the LT-βR pathway in the control of HBV, information about the role of this pathway during natural HBV infections is limited to studies in CHB infections, in which expression levels of LT-α/β were shown to be up-regulated and associated with hepatocellular carcinoma (HCC) development (Haybaeck et al. 2009).

Indeed, a note of caution about the antiviral effect of cytokines in HBV infections is nec-

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essary. Most of the experiments performed to measure the ability of cytokines to inhibit HBV replication have been performed in experimental systems devoid of chronic inflammatory events. However, intrahepatic levels of SOCS3, a negative regulator of cytokine signaling, are known to be increased in patients and woodchucks with chronic hepadnavirus infections (Koeberlein et al. 2010; Fletcher et al. 2012). SOCS3, a predictor of poor IFN-α responses in HCV-infected patients (Kim et al. 2009), may also attenuate the antiviral efficacy of IFN-α and other cytokines in patients with chronic HBV infections. IL-10 (Das et al. 2012), TGF-β (Sun et al. 2012), and arginase (Das et al. 2008), all factors that impair T and NK functions, are also elevated in chronic HBV infections (Peppa et al. 2010). It is, therefore, important to keep in mind that the impact of the activation of different components of innate and adaptive immunity might be modulated in a liver microenvironment characterized by chronic inflammatory events.

NK AND NKT CELLS IN HBV INFECTION

NK cells recognize and kill virus-infected cells. Loss of major histocompatibility complex (MHC) class I on the surface of virally infected cells, along with up-regulation of host or pathogen-encoded ligands that signal cell stress, optimize NK cell recognition. NK cells can also be directly activated by cytokines induced in viral infections, such as type 1 interferons, IL-12, and IL-18 (Biron and Brossay 2001). The cytokine-mediated pathway of NK activation can be particularly important in the liver where NK^{bright} cells, which are highly responsive to cytokine-mediated activation, are preferentially compartmentalized (Tu et al. 2008). Other cells at the crossroads between innate and adaptive immunity, which are extremely abundant in the liver, are invariant natural killer T (iNKT) and MAIT cells. Classical iNKT cells, a lymphocyte population that is activated after recognition of lipid antigen associated with MHC class I–like molecule CD-1, are abundant in mouse liver, and elegant work has shown their ability to be directly activated by hepatocytes overexpressing HBV antigens (Zeissig et al. 2012). These data suggested that direct activation of NKT cells by HBV-infected hepatocytes represent the first step of innate immune activation during HBV primary infection. However, the impact of such innate immune cells during natural HBV infections in humans is controversial because such CD-1-restricted NKT cells are abundant in mouse, but extremely rare in human livers. Instead, in human livers, different types of NKT cells, such as MAIT cells, which do not recognize antigens presented by CD-1 cells, are abundant (Tang et al. 2013). However, the role of MAIT cells, lymphocytes that are known to play a major role in antibacterial immunity, during acute or chronic HBV infection, is not known.

The role of classical NK cells in HBV infection has been investigated in more detail. Studies of patients around the time of first detection of HBsAg and HBV DNA revealed an increase in the number of circulating NK cells (Webster et al. 2000; Fisicaro et al. 2009), but their activation and effector function was suppressed as viral load increased and peaked only once viremia had resolved (Dunn et al. 2009). The importance of NK cells in the immediate early response to infection was also shown in woodchucks infected with high doses (10^{11}) of WHV, which displayed an activation of a gene related to NK cell activation immediately after infection (8–12 h) (Guy et al. 2008). Moreover, the initial IFN-γ production detected in acutely infected chimpanzees was suggested to be sustained by NK cell activation (Guidotti 1999).

NK cells also play a role in chronic HBV infection (Dunn et al. 2007; Oliviero et al. 2009; Peppa et al. 2010). Their functionality seems often suppressed by the presence of different immunomodulatory cytokines, such as IL-10 or TGF-β (Peppa et al. 2010), whereas other data have suggested an increased cytotoxic ability and involvement in liver damage (Zhang et al. 2011b). One other interesting possibility is that NK cells, during chronic HBV infection, act as a rheostat of HBV-specific T cells. Intrahepatic NK cells were shown to induce apoptosis of HBV-specific T cells with up-regulated PD-1 expression (Peppa et al. 2013).

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NK cell activation has also been associated with IFN-α treatment efficacy. NK cell proliferation and activation is detectable immediately after IFN-α therapy (Tan et al. 2014). HBV inhibition during IFN-α treatment coincided with an increase in the frequency of circulating $CD56^{bright}$ NK cells, increased expression of the activating receptor NKp46, and the cytotoxic receptor TNF-related apoptosis-inducing ligand (TRAIL) by NK cells and recovery of their IFN-γ production (Micco et al. 2013).

ADAPTIVE IMMUNITY AGAINST HBV

The adaptive immunity has generally been recognized as a crucial player in the clearance of HBV infection; it comprises a complex web of effector cell types. CD4 T cells are robust producers of cytokines and required for the efficient development of effector CD8 CTLs and B-cell antibody production. CD8 T cells clear HBV-infected hepatocytes through cytolytic and noncytolytic mechanisms, reducing the levels of circulating virus (Chisari 1997), whereas B-cell antibody production neutralizes free viral particles and can prevent reinfection (Fig. 2) (Alberti et al. 1978). This antiviral immune response is induced in adults after acute HBV infection and leads to HBV control. In contrast, chronic HBV patients fail to mount such an efficient antiviral response.

Although little is known about the induction and kinetics of the B-cell response in acute HBV, HBV-specific CD4 (helper) and CD8 (cytotoxic)-mediated responses become generally detectable at the time of exponential increase in HBV replication, which follows an initial phase of negative or weakly positive HBV DNA levels lasting for ~4–7 wk after infection (Webster et al. 2000; Fisicaro et al. 2009). CD4 T helper cells recognize preferentially epitopes of the capsid protein, whereas CD8 T cells typically recognize epitopes located within different HBV proteins. HBV-specific T cells are Th1

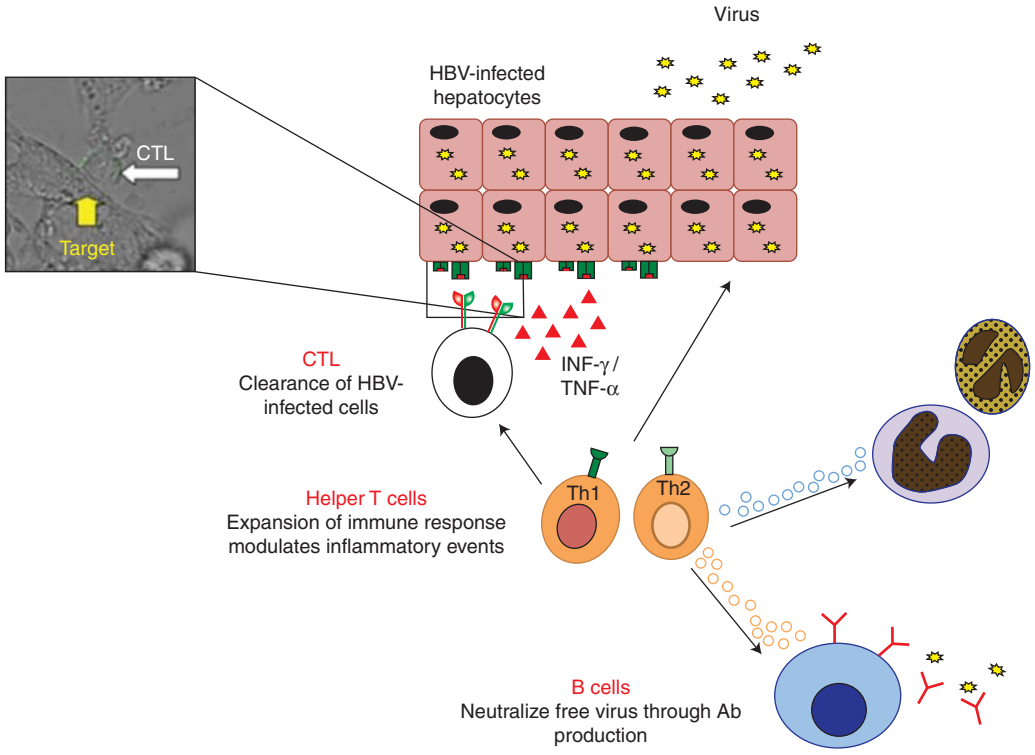

Figure 2. Antiviral adaptive immune response during hepatitis B virus (HBV infection). CTL, Cytotoxic T lymphocyte; INF, interferon; TNF, tumor necrosis factor.

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oriented and much stronger in self-limited than in chronic infection (Bertoletti and Ferrari 2003; Rehermann and Nascimbeni 2005; Wieland and Chisari 2005; Bertoletti et al. 2010).

In self-limited infections, HBV DNA declines by >90% within 2–3 wk after the peak of viral replication and before detection of liver damage, indicating that a large quantity of virus is eliminated by noncytopathic mechanisms controlled by IFN-γ and TNF-α, secreted by CD8 T cells (Guidotti 1999; Guidotti and Chisari 2006). Intrahepatic recruitment of HBV-specific CTLs, which is facilitated by the secretion of chemokines (i.e., CXCL-10) and platelet activation (Iannacone et al. 2005, 2007; Sitia et al. 2007, 2004), also leads to killing of infected hepatocytes with subsequent recruitment of antigen-nonspecific cells that amplify hepatocellular damage.

When infection is successfully controlled, maturation of T-cell memory occurs efficiently (Wherry and Ahmed 2004; Wherry et al. 2004). This stage is, however, preceded by a functional HBV-specific CD8 T-cell impairment, which is detectable at the peak of disease, when the majority of HBV-specific $CD8^+$ T cells are activated but poorly able to proliferate and are functionally exhausted (Dunn et al. 2009; Sandalova et al. 2010). This functional decline has been reported to be associated with a peak of IL-10 production (Dunn et al. 2009), but could also be caused by the increased levels of arginase released by dying hepatocytes (Chisari 1978). By depleting the essential amino acid L-arginine, arginase contributes to the down-regulation of the CD3ζ chain on T cells (Das et al. 2008). These mechanisms of acute phase suppression can represent a homeostatic process common to many virus infections to avoid excessive immunopathology and favor T-cell contraction (Marshall et al. 2011).

In patients with chronic HBV infection, the HBV-specific T-cell response is extremely weak. Irrespective of the primary causes of HBV chronicity (infection at birth, dose of antigen, HLA class I and II profile), the prolonged expression of high doses of HBV antigens in hepatocytes can delete or cause functional inactivation of HBV-specific T cells that express inhibitory molecules like PD-1, CTLA-4, TIM-3, and are defective in proliferation, cytokine production (Boni et al. 2007; Fisicaro et al. 2010; Raziorrouh et al. 2010; Schurich et al. 2011), and are prone to apoptosis caused by Bim up-regulation (Lopes et al. 2008). The HBV-specific T-cell defects present in chronic patients are inversely correlated to viremia levels (Maini et al. 2000a; Webster et al. 2004; Boni et al. 2007), with suppression of HBV-specific T-cell responses more profound in highly viremic patients and T cells more dysfunctional within the liver than in the periphery (Fisicaro et al. 2010).

One other important factor influencing the residual HBV-specific T-cell function in CHB patients is the length of the chronic infection (Crispe et al. 2000; Bertolino et al. 2001; Mueller and Ahmed 2009). Indeed, young CHB patients show a less-profound defect of HBV-specific T cells in comparison with adults (Kennedy et al. 2012). These experimental data suggest that the length of persistent infection affects HBV-specific T-cell presence, and challenged the popular notion that the initial phase of HBV chronic infection is characterized by a state of immunotolerance. So far, such a definition of immune tolerance has only been supported by clinical observation, indicating the absence of serological markers of liver inflammation. However, it is now well established that the quantity of liver enzymes is not directly proportional to the quantity of HBV-specific T cells. Antiviral specific T-cell responses within the liver can be present without any elevation of alanine amino transferase (ALT) levels. Furthermore, quantity and function of HBV-specific T cells correlate with viral control and not with the extent of liver damage (Maini et al. 2000b; Stabenow et al. 2010). As such, we think that the definition of the "immunotolerant" state in HBV infections requires a better immunological definition and should cover only those subjects who are unable to mount an HBV-specific T-cell response (Bertoletti and Kennedy 2014). Studies with HBV transgenic mice suggested that HBV exposure in the early stages of development can block the proper induction of an HBV-specific T-cell response (Publicover et al. 2013). However, such mouse data contrast with the scenario

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detected in young CHB patients who have a better HBV-specific T-cell response than adults (Kennedy et al. 2012). Analysis of immune-response profiles in children vertically infected with HBV is eagerly awaited to better understand the influence of this mode of transmission on antiviral immunity.

THE EXTENDED T-CELL FAMILY: Treg, Th17, AND Th22 IN HBV INFECTION

Analysis of HBV-specific T cells in acute and CHB has been mainly focused on T cells producing IFN-γ, TNF-α, and IL-2 (so-called Th1/Th0 cytokine profiles) in relation to their association with antiviral property. However, an extended family of T cells with regulatory or inflammatory functions can play a role in HBV pathogenesis (O'Shea and Paul 2010). Treg and T cells producing IL-10 or TGF-β can have immunoregulatory roles, IL-17- or IL-8-producing T cells can be proinflammatory, whereas Th2 and T cells producing IL-13 have profibrotic effects (Chiaramonte et al. 1999), and cells producing IL-22 have been reported to have hepatoprotective effects (Zenewicz et al. 2007).

Many studies have shown that Treg, Th17, and Th22 cell frequencies are higher in patients with chronic hepatitis than in healthy subjects (Xu et al. 2006; Yang et al. 2007; Zhang et al. 2010). These cells are often enriched in the intrahepatic environment and express CD161 and CXCR-6 receptors (Billerbeck et al. 2010). These correlations do not, however, clarify their role in HBV pathogenesis. Treg cells can suppress, in vitro, HBV-specific T-cell functions (Stoop et al. 2005; Xu et al. 2006), but such an effect is also observed in patients who are perfectly able to control the virus (Franzese et al. 2005). Furthermore, because Treg frequency is correlated with ALT levels (Xu et al. 2006), Treg might have an anti-inflammatory effect and not play any role in HBV persistence.

Th17 cells are detectable at higher frequency in CHB patients with severe liver damage (Zhang et al. 2010), but they were also reported, in HCV infection, to be associated with mild hepatitis (Billerbeck et al. 2010). Such controversial data can be explained by the inherent plasticity of T-cell cytokine production. Th17-producing cells can coexpress IL-22, a cytokine that should have a prominent hepatoprotective role (Zenewicz et al. 2007), although, in HBV-transgenic mice, it was shown that IL-22 has a proinflammatory effect (Zhang et al. 2011a). The necessary stimulus to trigger T-cell production of IL-17 and IL-22 during HBV infection has been analyzed, and a report has proposed that HBsAg, through stimulation of IL-23 from hepatic macrophages and dendritic cells, might directly activate Th17 cells (Wang et al. 2013). Such data are, however, difficult to reconcile with the fact that HBsAg levels are not proportional to liver damage or fibrosis, and many subjects with high levels of HBsAg are actually protected from hepatic fibrosis (Seto et al. 2012; Martinot-Peignoux et al. 2013). Furthermore, although HBV-specific IL-17-producing cells were initially reported (Zhang et al. 2010), more recent data failed to detect IL-17-producing HBV-specific T cells in acute and chronic HBV patients, both in the periphery and the intrahepatic environment (Gehring et al. 2011a). HBV-specific T cells maturing in the intrahepatic inflammatory environment can, instead, produce CXCL-8 (IL-8) (Gehring et al. 2011a), a cytokine that has a proinflammatory effect (Zimmermann et al. 2011), which can contribute to the development of liver pathology through the recruitment of granulocytes (Sitia et al. 2002). Thus, with the exception of some interesting correlative analysis, we are quite far from a clear understanding of the impact that T cells with regulatory, proinflammatory, or hepatoprotective effects have on HBV infection.

IMMUNOMODULATORY ROLES OF HBV ANTIGENS

A hallmark of HBV infection is the persistent production of the soluble form of HBsAg and e antigen derived from the core protein in excessive amounts over whole virions. Persistent exposure to circulating HBsAg has been suggested to impair the frequency and function of myeloid (van der Molen et al. 2004; Op den Brouw et al. 2009), plasmacytoid (Xu et al. 2009; Woltman et al. 2011; Shi et al. 2012), and mono-

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cyte-derived dendritic cells (Beckebaum et al. 2003) by modulating TLR-2 surface expression (Visvanathan et al. 2007) and interfering with TLR-mediated cytokine production (Wu et al. 2009). It is believed that soluble viral antigens can inhibit antigen-presenting function, altering their ability to produce cytokines, and inhibit the induction of HBV-specific T cells (Martinet et al. 2012). However, it is somehow difficult to understand why these defects are limited to HBV infection. In fact, we would expect that CHB patients are susceptible to bacterial and other opportunistic infections. However, to our knowledge, there have been no reports of increased incidences of bacterial infections or vaccine unresponsiveness in $HBsAg^+$ children. In contrast, reports have shown that, in patients with malaria, HBsAg positivity is associated with lower parasitemia (Andrade et al. 2011), or episodes of cerebral malaria, that is, a pathological manifestation indicative of a heightened Th1 response against the parasite (Oakley et al. 2013). To add to the confusion, a recent report has suggested that HBsAg, instead of having suppressive role, might directly induce a heightened Th17 response through activation of IL-23 on macrophages (Wang et al. 2013).

A caveat of the studies that have suggested an immunomodulatory role of HBV antigens, is that they have been often performed in vitro with proteins expressed in *Escherichia coli* or yeast, or purified from the sera of CHB patients. Despite the high level of purity of these preparations, contaminants from bacteria or enzymes cannot be ruled out. For example, the phenomena of lipopolysaccharide (LPS)-induced tolerance of antigen-presenting cells following stimulation with TLR agonists may have influenced the outcome of some experiments (Rodrick et al. 1992; Granowitz et al. 1993). Moreover, it is important to consider that, in CHB patients with chronic liver disease, the presence of high doses of circulating antigens is often linked with immunosuppressive cytokines (IL-10) (Das et al. 2012) or liver enzymes (i.e., arginase), known to alter the function of different components of cellular immunity (Das et al. 2008; Sandalova et al. 2012). In a study performed with CHB patients with mild or absent liver inflammation but high HBsAg levels, the frequency and T-cell stimulatory activity of circulating professional antigen-presenting cells (monocytes, dendritic cells, and B cells) were not altered (Gehring et al. 2013). In contrast, another study reported alteration of dendritic cell (DC) function ex vivo in CHB infection corresponding with HBsAg and HBeAg levels, but also with high levels of CXCL-10, a chemokine associated with liver-inflammatory events causing increased arginase/IL-10 levels (Martinet et al. 2012). It is, therefore, plausible that these different results are caused by the difference in suppressive cytokines or enzymes in the circulation of patients with liver-inflammatory diseases, and not by differences in HBsAg levels.

The hypothesis that circulating HBsAg and HBeAg can suppress the T-cell response to viral proteins is also difficult to envisage. Such a hypothesis would be plausible if antigen-specific T-cell responses against the HBV polymerase, a HBV protein produced at very low levels, would remain intact in CHB. This is not the case because HBV-specific T-cell responses against the HBV polymerase are rarely detected ex vivo in patients with CHB (Webster et al. 2004; Boni et al. 2007), and, overall, the magnitude of HBV-specific T-cell response in CHB patients is inversely correlated with levels of HBV replication (HBV DNA) (Webster et al. 2004; Boni et al. 2007), and not with the quantity of circulating antigens. It is, therefore, likely that the persistent viral antigen presentation in the infected liver is the cause of the exhaustion or deletion of HBV-specific T cells. This notion is supported by studies demonstrating that intrahepatic recognition of viral antigen triggers T-cell dysfunction, which is associated with the inability to produce antiviral cytokines and the up-regulation of inhibitory receptors (Fisicaro et al. 2012).

IMMUNOLOGICAL-BASED TREATMENTS OF CHRONIC HBV INFECTION

How can we use our knowledge of HBV immunity to develop better therapeutic strategies against HBV? The limited activation of the innate immune system during primary

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and chronic HBV infections has stimulated therapeutic strategies to specifically target this system. Because IFN-α can clear HBV-infected hepatocytes at high doses (Ji et al. 2012), increasing intrahepatic IFN-α levels could be clinically beneficial. In addition, exogenous TLR-mediated activation can suppress HBV replication in HBV-transgenic mice (Isogawa et al. 2005) and in HBV-transfected HepG2 and Huh7 cells (Guo et al. 2009). Production of intrahepatic antiviral cytokines (IFN-α, IFN-γ), through oral administration of TLR agonists, has also shown efficacy in HBV-infected woodchucks (Menne et al. 2011) and chimpanzees (Lanford et al. 2013). Thus, to maximize intrahepatic innate immune function, strategies to specifically deliver antiviral cytokines to the infected liver or to target activation of intrahepatic Kupffer and NK cells have been proposed. The discovery of peptides able to specifically bind to hepatocytes (Petersen et al. 2008) and the production of antibodies with HBV-infected cell specificity (Sastry et al. 2011) could be used to target cytokines and/or TLR agonist to the HBV-infected liver.

The clear dichotomy between the immune response present in acute, resolved versus chronic persistently HBV-infected patients leads to therapeutic strategies designed to boost HBV-specific immunity in CHB patients. Because antigen persistence in the liver seems to be a major factor driving the HBV-specific CD4 and CD8 T-cell defects, the suppression of HBV antigen production can lead to a functional reconstitution of antiviral T-cell responses (Wherry and Ahmed 2004; Wherry et al. 2005). Unfortunately, T cells chronically exposed to antigen carry permanent changes in their differentiation program as a permanent "epigenetic signature." Results obtained with mice infected with lymphochoriomeningitis virus (LCMV) showed that adoptive transfer of dysfunctional virus-specific CD8 cells from a chronically infected to a naïve uninfected MHC-compatible animal is not sufficient to restore T-cell memory maturation (Wherry et al. 2004). These experimental data were confirmed by recent studies in patients who control HBV after treatment in whom T cells, even after complete control of virus replication, never fully recover their T-cell functionality (Boni et al. 2012).

If antigen reduction is not sufficient to obtain a robust functional recovery, additional strategies, such as blocking inhibitory pathways, new aggressive vaccination regimens, and experimental gene therapy strategies, have been proposed (Fig. 3). Blocking inhibitory pathways associated with T-cell exhaustion has shown therapeutic efficacy in cancer patients (Armand et al. 2013). Interfering with these pathways achieves partial functional recovery of HBV-specific T cells from CHB patients in vitro, but we still lack in vivo data evaluating the efficacy of this approach in CHB patients (Maini and Schurich 2010).

Vaccine therapy aims, instead, to induce functionally efficient HBV-specific T cells on the background of virus-specific T-cell exhaustion. Several strategies have been tested in clinical trials with disappointing results. Often, vaccine therapy did not induce HBV-specific T-cell response or, when such response was boosted, it did not have a therapeutic effect (Mancini-Bourgine et al. 2004, 2006). Most of these data were derived, however, from trials in which classical HBsAg-based prophylactic vaccines were used (Couillin et al. 1999; Pol et al. 2001; Yalcin et al. 2003). Alternative strategies or refinements of current vaccine therapies have, therefore, been tested. A proper design of antigens for therapeutic vaccination might be important. The T-cell response against HBcAg is crucial for the resolution of an infection, but therapeutic HBV vaccines designed to date have mostly relied on HBV envelope proteins. The use of core or polymerase antigen might be advisable because core-specific T-cell responses were induced in chronically HBV-infected chimpanzees successfully treated with a therapeutic vaccine (Sallberg et al. 1998).

A further step toward a refinement of vaccine therapy is supported by the demonstration that a DNA prime-adenovirus boost vaccine with HBcAg in combination with antiviral treatment can stimulate a robust T-cell response in the woodchuck model of CHB (Kosinska et al. 2013). New data are indeed emerging that reduction of viral replication can induce im-

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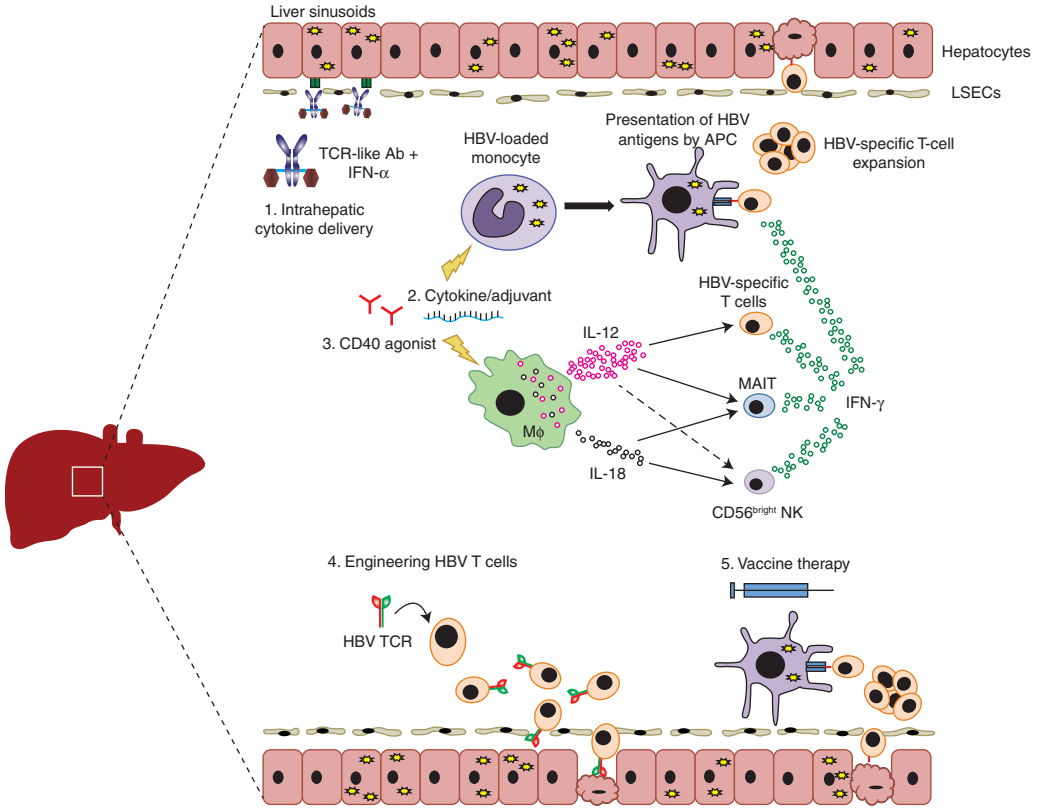

Figure 3. Different immune-based therapeutic strategies that aim to increase hepatitis B virus (HBV) control: (1) T-cell receptor-like antibodies conjugated with interferon (IFN)-α, which specifically target HBV-infected hepatocytes, can increase intrahepatic IFN-α delivery. (2) Toll-like receptors (TLRs) or anti-CD40 agonists mature HBV-loaded monocytes into monocyte-derived dendritic cells (moDCs) that might stimulate intrahepatic HBV-specific T cells. (3) Activation of monocytes/macrophages producing interleukin (IL)-12 and IL-18 through cytokines and/or TLR agonists can stimulate intrahepatic HBV-specific T cells, mucosal-associated invariant T (MAIT) cells, or $CD56^{bright}$ natural killer (NK) cells to produce large quantities of IFN-γ, which can suppress HBV replication. (4) New HBV-specific T cells can be engineered through transfer of HBV-specific T-cell receptors to reconstitute functional HBV-specific immunity. (5) Vaccine therapy performed in combination with antiviral treatment and/or combining with immunomodulation methods, such as PD-1/PD-L1 blockade, might induce antiviral T-cell responses. APC, antigen-presenting cell; LSECs, liver sinusoidal endothelial cells; TCR, T-cell receptor.

munological alterations in CHB patients (Tan et al. 2014), which goes beyond the transient reconstitution of HBV-specific T-cell response detected early after nucleoside analog therapy (Boni et al. 2003) and alter, instead, the inflammatory microenvironment of the liver. Further strategies have associated the use of vaccines with modulators of T-cell fitness. A triple combination therapy with antiviral treatment, therapeutic DNA vaccination, and PD-L1 antibody treatment potently suppressed viral replication and led to production of anti-WHsAg antibodies in woodchucks (Liu et al. 2014).

An alternative strategy of vaccine therapy in CHB could be to directly stimulate the patient's antigen-presenting cells to efficiently present the circulating HBV antigens to T cells. Monocytes present in the circulation of CHB patients internalize HBV antigens and can stimulate expansion of autologous HBV-specific T cells fol-

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lowing maturation with inflammatory stimuli (Gehring et al. 2013). These data support the possibility that multiple injections of adjuvants alone could induce an inflammatory environment capable of activating intrahepatic HBV-specific T cells. This hypothesis is supported by studies in mice demonstrating that intrahepatic stimulation of myeloid cells with a TLR agonist results in expansion of virus-specific CTLs (Huang et al. 2013). Moreover, agonistic activation of myeloid dendritic cells with CD40L can rescue naïve CD8 T cells, primed in the liver by recognition of HBV antigens expressed in hepatocytes and suppressed by PD-1 signaling (Isogawa et al. 2013). The possibility that vaccine therapy for CHB could be performed using adjuvants alone is also supported by the virological results obtained in a trial performed with HBsAg-anti-HB immunogenic complex (IC) vaccine in CHB patients. Here, multiple injections of alum alone were sufficient to trigger a significant virological response in 21% of the treated patients (Xu et al. 2013).

However, more radical approaches could be needed to circumvent HBV-specific T-cell deletion in patients with high viral loads, in which HBV-specific T cells are often not only functionally altered, but completely undetectable. Engineering HBV-specific T cells through transfer of HBV-specific T-cell receptors (Gehring et al. 2011b; Koh et al. 2013) or HBV-specific chimeric antigen receptors (CARs) (Krebs et al. 2013) showed encouraging results in vitro and in animal models. The concept of adoptively transferring a functionally efficient HBV-specific immune system is not new in HBV. CHB patients receiving bone marrow transplants from HBV-immune donors were cured. Likewise, transplantation of an HBV-infected liver into a recipient who previously recovered from HBV infection resulted in viral control (Loggi et al. 2009). Thus, gene therapy approaches might have great potential, but safety concerns, cost, and ethical issues related to viral vector use need to be addressed.

A radically different perspective in CHB is to consider it a necroinflammatory disease rather than a viral disease. Recent data in HBV transgenic mice clearly indicate that suppressing intrahepatic CTL activity in the liver using antiplatelet therapy can prevent HCC, the real life-threatening complication of CHB infection. Platelets promote the accumulation of CD8 T cells in the liver and antiplatelet therapy blocks this process, reducing hepatocellular injury and fibrosis (Sitia et al. 2012).

In conclusion, characterization of the immunological profiles present during acute and chronic HBV infections support the rationale to boost antiviral immunity to achieve control of HBV replication. However, further understanding of the relationship of HBV with host immunity is necessary to clearly understand whether HBV therapy should focus toward virus or inflammation control.

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