Chapter 4
Hepatitis B Virus Immunopathogenesis

Matteo Iannacone and Luca G. Guidotti

Introduction

Hepatitis B virus (HBV) is the prototype member of the Hepadnaviridae family and it is a small, enveloped DNA virus whose host range is restricted to man and chimpanzees and whose tropism is restricted to the parenchymal cell of the liver, i.e., the hepatocyte [1]. Over the last 30 years, fundamental principles of HBV replication and gene expression have been uncovered, infectious viral genomes have been cloned and sequenced, and all of the viral gene products have been basically characterized [1]. Essential aspects of HBV pathogenesis have been also elucidated during this time, namely that HBV replicates noncytopathically in the hepatocyte and that most of the clinical syndromes associated with this infection reflect the immune response [1]. The innate immune response appears not to contribute significantly to the pathogenesis of liver disease or viral clearance, while the adaptive immune response, especially the virus-specific effector CD8+ T cell response, contributes to both [1–3]. Although effector CD8+ T cells are central to HBV pathogenesis, several other liver resident (including Kupffer cells and stellate cells) and nonresident

M. Iannacone (✉)
Division of Immunology, Transplantation and Infectious Diseases,
IRCCS San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy

Vita-Salute San Raffaele University, Milan 20132, Italy
e-mail: iannacone.matteo@hsr.it

L.G. Guidotti (✉)
Division of Immunology, Transplantation and Infectious Diseases,
IRCCS San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy

Department of Immunology and Microbial Sciences, The Scripps Research Institute,
La Jolla, CA 92037, USA
e-mail: guidotti.luca@hsr.it

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(including platelets and polymorphonuclear or mononuclear antigen-nonspecific inflammatory cells) cells play distinctive roles in it, indicating that the host response to this infection is a highly complex but coordinated process [1, 4].

Despite this large body of information, further improvement in our understanding of the molecular and cellular mechanisms that are ultimately responsible for viral clearance and liver disease is required; however, if we are to develop better treatments for chronic HBV infection and its complications. Indeed, our limited capacity to specifically treat liver fibrosis/cirrhosis and HCC makes extremely important to eliminate their most important trigger, i.e., the chronic liver injury associated with persistent HBV infection.

Termination of chronic HBV infection by available antiviral therapies has been associated with significantly reduced occurrence of liver fibrosis/cirrhosis and HCC development [5]. Unfortunately, a large fraction of chronically infected patients do not respond to these therapies with permanent elimination of HBV; while in the case of first-generation antivirals this was primarily due to dose-limiting side effects and, especially, the emergence of drug-resistant mutants [6], in the case of last-generation antivirals (which are safer and seldom confer resistance) this mostly depends on the impossibility to sustain the cost of treatments that, albeit efficacious at inhibiting HBV replication, cannot be discontinued [7]. Since HBV can be naturally (and permanently) controlled by the immune system, there is a general consensus in the scientific community that new immune therapeutic strategies should be explored for the treatment of chronic HBV infection. These strategies comprise the use of therapeutic T cell vaccines and the infusion of virus-specific effector CD8+ T cells previously expanded ex vivo. Undoubtedly, the implementation of these strategies will greatly benefit from a clearer understanding of the mechanisms by which T cells exert their effector functions in vivo. That our current knowledge on HBV pathogenesis needs to be improved is further suggested by the few applications of therapeutic vaccines attempted thus far [8]. These applications—based on either prophylactic surface antigens or T cell vaccines containing different viral peptides/polypeptides—showed some evidence of T cell response restoration in the blood of chronically infected patients, but it remains poorly understood why such responses failed to induce sustained virological or clinical benefits.

HBV Infection and the Innate Immune Response

The recent identification of sodium taurocholate cotransporting polypeptide (NTCP) as relevant entry receptor [9] has started to shed some light onto the mechanisms whereby HBV gains access to hepatocytes. As discussed at length in other chapters of this book, following viral entry, HBV nucleocapsids are released into the cytoplasm and transported to the nucleus, whereupon the relaxed circular viral DNA genome is repaired by cellular enzymes into an episomal "minichromosome" termed covalently closed circular (ccc) DNA [1, 10–12]. The cccDNA molecule

represents the viral transcriptional template and encodes four capped and polyadenylated RNAs producing structural and nonstructural viral proteins [1, 10–12]. The largest HBV transcript is a 3.5 kb greater-than-genome length RNA, which is translated to produce the viral core and polymerase proteins and also serves as a pregenomic RNA [1, 10–12]. Once encapsidated in the cytoplasm, the pregenomic RNA is reverse transcribed to produce a single-strand DNA copy that serves as the template for second-strand DNA synthesis, producing a circular double-stranded DNA genome [1, 10–12]. Viral capsids containing double-stranded DNA traffic either back to the nucleus to amplify the viral cccDNA genome or to the endoplasmic reticulum where they engage the viral envelope proteins, bud into the lumen, and exit the cell as virions that can infect other cells [1, 10–12].

Of note, the double-stranded HBV DNA genome is completely sequestered within cytoplasmic capsid particles [1, 10–12]. This feature renders the virus potentially invisible to the innate sensing machinery of the host and, in fact, HBV appears not to induce early innate defense mechanisms. In many other viral infections these mechanisms include the induction of apoptosis by the virus [13], the production of antiviral cytokines such as IFNαβ by the infected cells [14], and the triggering of effector functions (e.g. the destruction of infected cells and/or the production of antiviral cytokines such as IFN-γ by NK or NKT cells [15]. Most of what is known about innate defense mechanisms during the early phase of HBV infection has been indirectly inferred from the longitudinal analysis of liver biopsies in experimentally infected chimpanzees. Thanks to these studies, it is apparent that HBV replicates and spreads throughout the liver noncytopathically, and that early innate defense mechanisms significantly contribute neither to clear the infection nor to promote liver injury. Indeed, during the initial spread of HBV infection in the chimp liver (i.e., before T cells enter the organ) there is little or no evidence of hepatocyte damage [16]. This notion goes along with the observations that high hepatic levels of HBV replication in patients and transgenic mice are not associated with overt pathological consequences (including the induction of hepatocellular apoptosis) when cellular immune responses are pharmacologically suppressed [1, 10, 17] or deficient [18]. Global gene expression profiling performed on chimp liver RNA samples at multiple time points after infection further indicates that HBV acts like a stealth virus, remaining largely undetected by infected hepatocytes (which do not show signs of IFNαβ production) or NK and NKT cells (which do not show signs of IFN-γ production) until the onset of the adaptive immune response several weeks after exposure [19]. Whether the lack of relevant innate immune cell activation also involves active suppression of NK or NKT cells (as suggested by the temporal association between the inhibition of NK activity ex vivo and the levels of IL-10 observed in the blood of patients undergoing longitudinal studies [20]) remains to be fully determined. Like in the case of NK cells, little is known about the role of NKT cells during the early phase of HBV infection. Studies in human and mouse hepatocytes showed that hepatocellular HBV gene expression has the potential to induce the production of lysophospholipids capable of activating NKT cells [21], and studies in transgenic mice showed that activated NKT cells have the potential

to perform antiviral activities (mostly dependent on the capacity of these cells to produce IFN-γ [21, 22]. These latter results suggest that NKT activation, although not strongly apparent during the initial phase of infection, may be explored therapeutically. The same is true for the activation of NK cells, which are highly abundant in the human liver (representing 30–40 % of the total intrahepatic lymphocytes [23]) and also capable of producing high levels of IFN-γ [22].

Priming and Arrival of the Adaptive Immune Response

Whatever the role of innate defense mechanisms, the initial evidences of viral clearance and liver pathology associated with HBV infection in chimpanzees occur concomitantly with the arrival of effector CD8+ T cells into the liver [16]. In keeping with this, both liver pathology and viral clearance do not occur as a consequence of CD8+ T cell depletion in these animals [24].

For viruses like HBV that seem not to infect professional antigen presenting cells (APC), tissue-derived dendritic cells capable of processing antigen are likely to migrate to regional lymph nodes [25–27]. Within lymph nodes, dendritic cells present antigens to naïve CD4+ and CD8+ T cells, which in turn become activated and differentiate into populations of effector cells. Although very little is known as per when and where T cell priming occurs during HBV infection, it is widely accepted that T cell priming occurring within the liver would likely induce T cell inactivation, tolerance or apoptosis (thus predisposing the host to viral persistence). At any rate, functional effector T cells that have expanded in secondary lymphoid organs need to eventually migrate to infection sites in order to perform antiviral and pathogenic activities. In most circumstances this is made possible because activation-dependent signals program T cells to express a range of homing molecules that are required to enter specific nonlymphoid tissues [28–30]. For instance, effector T cells migrating to the skin, mucosal tissues or the central nervous system express distinct arrays of selectins, integrins, or chemokine receptors [28–30]. While the nature of these and other tissue-specific T cell homing signals has been elucidated in the last few years [30], the in vivo requirements regulating T cell trafficking into the HBV-replicating liver have been addressed just very recently.

Few observations related to leukocytes other than T cells suggest that the liver may be an exception to the classic multi-step leukocyte migration paradigm involving rolling, adhesion and extravasation in and from post-capillary venules [28, 31]. For example, neutrophil or monocyte adhesion is not restricted to the endothelium of post-capillary venules, but it also occurs in sinusoids [31]. Further, neutrophil or monocyte adhesion to liver sinusoidal endothelial cells (LSEC) often occurs independently of any notable rolling [32]. Notably, LSEC lack both tight junctions between cells as well as a basal membrane [33]. This is in sharp contrast to most vascular beds in other tissues and organs, where a continuous endothelial cell layer and a basement membrane physically separate parenchymal cells from circulating leukocytes [33].

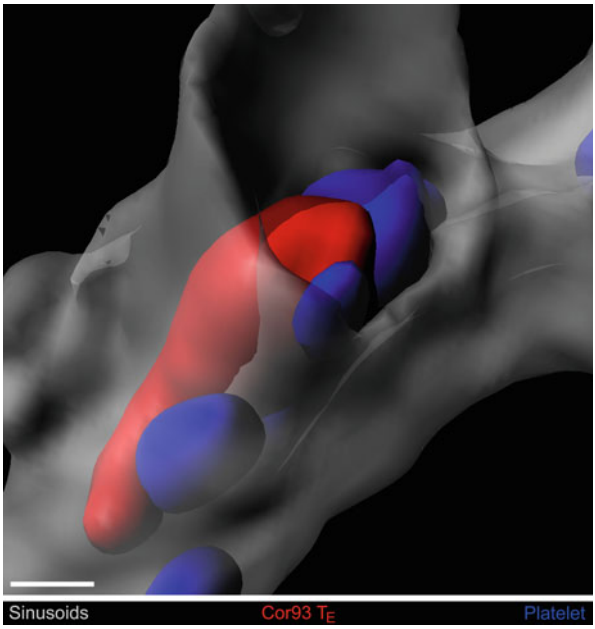

Fig. 4.1 Hepatic effector CD8+ T cell accumulation requires platelets that have adhered to liver sinusoids. Confocal micrographs of the liver of a HBV replication-competent transgenic mouse that was injected 2 h earlier with effector CD8+ T cells specific for HBV core antigen (Cor93 CD8 T_E, *red*). Platelets are shown in *blue* and sinusoids in *gray*. To allow visualization of intravascular event and to enhance image clarity, the transparency of the sinusoidal rendering was set to 45 %. Scale bar represents 2 μm

Recent data indicate that selectins, β2- and α4-integrins, platelet endothelial cell adhesion molecule (PECAM)-1, vascular adhesion protein (VAP)-1, and Gαi-coupled chemokine receptors (all previously thought to be variably relevant for leukocyte trafficking in other organs) are not required for the hepatic homing of effector CD8+ T cells in mouse models of HBV immunopathogenesis [34]. Studies in similar mouse models have instead demonstrated that the intrahepatic recruitment of virus-specific effector CD8+ T cells critically depends on platelets [34–36]. Indeed, the first of those reports showed that platelet depletion is associated with a significant reduction in the intrahepatic accumulation of effector CD8+ T cells and a proportional reduction in liver disease [35]. Both phenotypes are restored upon reconstitution with normal platelets, but not upon reconstitution with platelets that are treated in advance with inhibitors of platelet activation [35]. In vitro findings also indicate that, under the low shear flow conditions likely occurring in the venous circulation of the liver, effector CD8+ T cells tightly interact with platelets [35]. The concept of physical platelet-T cell interaction (Fig. 4.1) leading to hepatic accumulation of the latter cells has been more recently corroborated by in vivo experiments utilizing intravital microscopy; there, it has been shown that the initial sinusoidal arrest of circulating effector CD8+ T cells depends on their capacity to dock onto platelets that have previously adhered to sinusoidal hyaluronan via CD44 [34].

Antiviral Functions of the Adaptive Immune Response

Thanks to additional intravital microscopy studies it was also recently shown that after the initial platelet-dependent arrest, effector CD8+ T cells actively crawl along liver sinusoids (at an average speed of about 10 μm/min) and extend cellular protrusions through sinusoidal endothelial fenestrate to probe underlying hepatocytes for the presence of antigen [34]. Unexpectedly, hepatocellular recognition of HBV antigens leading to cytokine production and hepatocyte killing occurs in a diapedesis-independent manner (Figs. 4.2 and 4.3), i.e., when effector CD8+ T cells are still intravascular and before they extravasate into the parenchyma [34]. Notably, CD8+ T cell antigen recognition and effector functions are inhibited by sinusoidal defenestration and capillarization—two pathological conditions that typify liver fibrosis (see below)—suggesting that the process of liver fibrosis might reduce CD8+ T cell immune surveillance towards infected or transformed hepatocytes [34]. Altogether, the abovementioned studies highlight the notion that rather peculiar mechanisms regulate the ways by which HBV-specific CD8+ T cells recognize hepatocellular antigens and perform effector functions aimed at viral clearance.

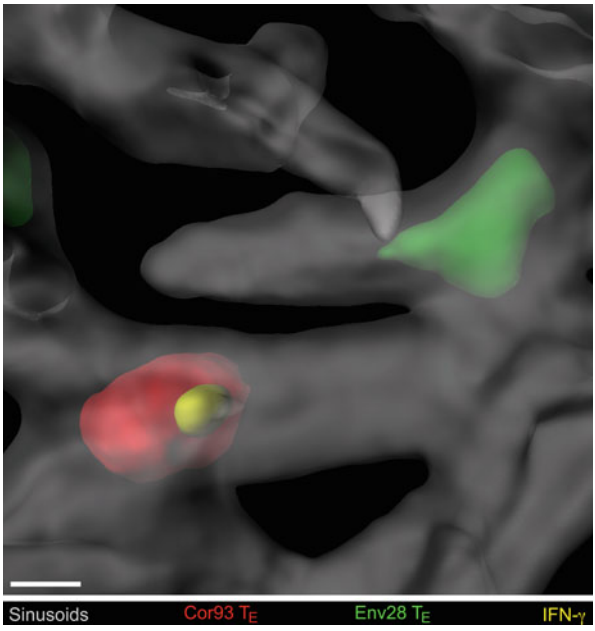

Fig. 4.2 Effector CD8+ T cells recognize hepatocellular antigens and produce antiviral cytokines in a diapedesis-independent manner. Confocal micrograph showing an intravascular HBV-specific effector CD8+ T cell (Cor93 CD8 T_E, *red*) that produces IFN-γ (*yellow*) upon recognition of hepatocellular antigen within the liver of HBV replication-competent transgenic mice. Note that a nearby intravascular MHC-mismatched HBV-specific effector CD8+ T cells (Env28 CD8 T_E, *green*) does not produce IFN-γ. To allow visualization of intravascular events and to enhance image clarity, the transparency of the sinusoidal rendering (*grey*) was set to 70 % and that of the T cell to 60 %. Scale bar represents 4 μm

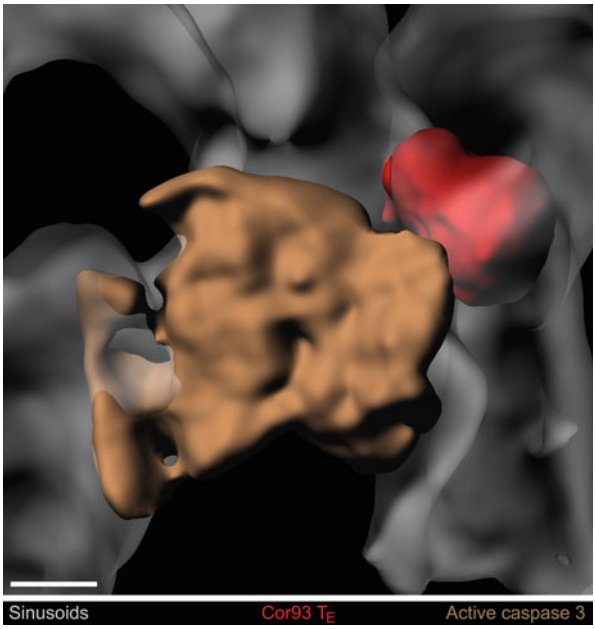

Fig. 4.3 Effector CD8+ T cells kill HBV-expressing hepatocytes in a diapedesis-independent manner. Confocal micrograph showing an intravascular effector CD8+ T cell specific for HBV core antigen (Cor93 CD8 T_E, *red*) juxtaposed to an apoptotic HBV-expressing hepatocyte (*brown*). To allow visualization of intravascular events and to enhance image clarity, the transparency of the sinusoidal rendering (*grey*) was set to 50 %. Scale bar represents 4 μm

There is little doubt that target cell killing by effector CD8+ T cells represents a highly relevant means by which effector CD8+ T cells contribute to HBV clearance. Target cell killing, however, is an intrinsically inefficient process, requiring physical contact between the infected hepatocyte and the T cell. As such, it may not be possible for the effector CD8+ T cells to reach and kill all infected hepatocytes, particularly if one considers that (a) all of the hepatocytes (~10^{11} cells) are routinely infected during HBV infection in chimpanzees and (b) relatively few virus-specific effector CD8+ T cells circulate in the bloodstream of these animals [24]. Thus, viral clearance may require more efficient T cell functions than killing. Important insights into such functions have spawned from studies in HBV replication-competent transgenic mice. There, it was demonstrated that rapid inhibition of HBV replication by effector CD8+ T cells is mostly mediated by noncytolytic mechanisms involving the local production of IFN-γ by these cells [37]. Indeed, IFN-γ, largely via its ability to induce nitric oxide in the liver [38], was shown to prevent the hepatocellular assembly of replication-competent HBV RNA-containing capsids in a proteasome- and kinase-dependent manner [39, 40]. During this process, the levels of viral nucleocapsids in the cytoplasm of hepatocytes rapidly decline, and viral RNAs are destabilized in the hepatocellular nucleus by an SSB/La-dependent mechanism [41–43].

Antibody blocking and knockout experiments in the HBV transgenic mouse model further demonstrated that the cytolytic and antiviral functions of effector CD8+ T cells are completely independent of each other [37]. Thus, effector CD8+ T cells have the potential to inhibit viral gene expression and replication noncytopathically. Similar antiviral activities were recently shown to extend, via a gradient of IFN-γ, more than 80 μm beyond the site of antigen presentation, promoting pathogen clearance in the absence of immunological synapse formation [44].

Of note, additional work in the HBV transgenic mouse model also indicates that, upon entry into the liver, effector CD8+ T cells rapidly lose the capacity of secreting IFN-γ (i.e., the IFN-γ-producing phenotype is maintained only for the few days during which HBV antigens are cleared from the liver), and this is followed by the intrahepatic expansion of IFN-γ-non-producing virus-specific effector CD8+ T cells with unaltered cytotoxic capabilities [45]. These results suggest that sustained antigen stimulation, as occurs during chronic infection, may create an environment in which antiviral (i.e., production of IFN-γ) but not pathogenic (i.e., killing of hepatocytes) functions of intrahepatic virus-specific effector CD8+ T cells are relatively impaired (see below).

Pathogenic Functions of the Adaptive Immune Response During Acute Hepatitis

Even when the adaptive immune response effectively clears a virus, immune-mediated mechanisms can cause significant injury to host tissues. Besides, viruses like HBV can persist in the presence of an active adaptive immune response, predisposing the host to chronic tissue damage (see below). Thus, the balance between the protective and the harmful effects of immunity in some cases clearly shifts to immunity being the primary cause of tissue pathology. In these cases, virus-induced tissue damage is referred to as immunopathology.

Virus-specific T cells can promote immunopathology by directly killing infected cells, by releasing cytokines or other soluble mediators with intrinsic cytotoxic properties and, also, by recruiting antigen-nonspecific inflammatory cells that have the potential to amplify tissue damage. Studies in HBV-infected patients have indeed shown that hepatic infiltrates contain a large antigen-nonspecific component, whose extent correlates with the degree of liver damage [46]. This observation goes along with data obtained in mouse models of HBV immunopathogenesis, where it was shown that the initial apoptotic process triggered by passively transferred virus-specific effector CD8+ T cells involves only a relatively small number of hepatocytes. Using these same models, it was also shown that Kupffer cells (the resident macrophages of the liver) rapidly remove apoptotic hepatocytes in a manner largely dependent on scavenger receptors [47]. As time progresses, though, apoptotic hepatocytes not readily removed by Kupffer cells become secondarily necrotic and release damage-associated molecular pattern molecules (DAMPs) such as the high-mobility group box 1 (HMGB1) protein [48]. HMGB1 is an

abundant nuclear protein acting as an architectural chromatin-binding factor that can be passively released by necrotic, but not apoptotic, cells [49]. Once discharged by necrotic hepatocytes, HMGB1 chemo-attracts mainly polymorphonuclear cells (e.g., neutrophils), the first antigen-nonspecific inflammatory cells arriving at the site of disease [48]. Neutrophil activation leading to production of matrix metalloproteinases (MMPs) rapidly degrades matrix components (e.g., collagen, laminin, fibronectin, and proteoglycans) that are deposited de novo by stellate cells, myofibroblasts and fibroblasts during the process of liver repair [50]. In turn, these matrix-degrading events favor the intrahepatic arrival of numerous antigen-nonspecific mononuclear cells (e.g., antigen-nonspecific CD8+ and CD4 T cells, B cells, monocytes), which respond to their own chemoattractants (mostly chemokines such as CXCL9 and CXCL10 produced locally by parenchymal and non-parenchymal liver cells [51]) and exacerbate disease severity [50]. The pathogenic mechanisms whereby antigen-nonspecific mononuclear cells thus recruited induce organ damage are not well understood and may involve the local production of pro-inflammatory and cytotoxic mediators (including TNF-α, perforin, hydrogen peroxide, superoxide anion, and nitric oxide) by these cells. Moreover, antigen-nonspecific mononuclear cells (in particular NK cells, NKT cells, and T-helper cells) and platelets express Fas-L, a glycoprotein that triggers hepatocellular apoptosis by ligating Fas on the hepatocyte membrane [1].

Observations in acutely infected chimpanzees depleted of CD4+ T cells at the peak of acute HBV infection indicate that the liver disease in this animal is comparable to that detected in immunologically unmanipulated controls [24]. Thus, CD4+ T helper cells may contribute to HBV pathogenesis mainly by facilitating the induction and maintenance of virus-specific effector CD8+ T cells, as has been suggested for other viruses such HCV [52]. In keeping with this, relatively vigorous HBV-specific T helper responses are always associated with quantitatively and qualitatively significant effector CD8+ T cell responses in humans and chimpanzees that resolve HBV infection [1].

Viral and Host Factors Contributing to Viral Persistence

Based on the studies abovementioned, it is apparent that the adaptive immune response to HBV, particularly the CD8+ T cell response, plays key roles in viral clearance and liver disease. Thus, it is reasonable to assume that HBV persistence demands that such response must be either not induced or deficient, or if present it must be overwhelmed, counteracted or evaded.

Notably, both viral and host factors can be involved in the establishment of chronicity. Among the former, it has been suggested that circulating hepatitis B e-antigen (HBeAg) functions as a tolerogenic protein that induces anergy of HBcAg/HBeAg cross-reactive T cells [53, 54]. The capacity of circulating HBeAg to functionally suppress HBcAg/HBeAg-specific T cell responses may explain clinical observations whereby HBeAg-negative variants are frequently cleared following neonatal

exposure and they are usually associated with more severe courses of liver disease in adults [55]. Through its capacity to function as high dose tolerogen, circulating hepatitis B surface antigen (HBsAg) is also considered a viral factor that retains immune suppressive potential [55]. Indeed, the extremely high-serum HBsAg titers observed in certain chronically infected patients are often associated with absence of peripheral HBsAg-specific T cell responses [55]. Mutational inactivation of HBV-derived B cell or T cell epitopes is also thought to facilitate viral persistence [55], albeit this process is likely to play a more prominent role during infection with viruses (such as HCV) that intrinsically possess a much higher mutation rate. Nonetheless, mutations involving epitope residues that anergize or antagonize recognition by the T cell receptor have been reported to arise during HBV infections evolving towards a chronic phase [56].

Among the latter, the notion that immune tolerance is likely responsible for viral persistence in most neonatal HBV infections coupled with the fact that a vigorous, multispecific, and polyclonal cellular immune response is associated with viral clearance in immunocompetent adults strongly suggest that host factors significantly determine infection outcome [1]. Why T cell responses are quantitatively weak and qualitatively inadequate to terminate infection in some adult onset infections remains to be fully determined. An increasing body of studies in HBV infected patients or surrogate animal models suggests that several nonexclusive mechanisms favor viral persistence; they include the inhibition of functional T cell priming as a results of antigen presentation by the hepatocyte [57] or the induction of anergy and exhaustion of initially vigorous T cell responses as a result of (a) antigen overload and excessive T cell stimulation [1], (b) action of regulatory T cells [1], and (c) activation of negative regulatory pathways in T cells (such as those promoted by programmed cell death protein 1 [PD1], cytotoxic T-lymphocyte antigen-4 [CTLA-4], or T-cell immunoglobulin and mucin 3 (Tim-3) [1, 55, 58, 59]. Additional factors contributing to suppression of pre-existing T-cell responses during chronic HBV infection may relate to the relative intrahepatic abundance of selected cytokines (e.g., IL-10 or TGF-β) or enzymes (e.g., arginase) possessing immunosuppressive potential [60–62]. All together, these results indicate that both primary and secondary immunological unresponsiveness to HBV presumably occurs, and this likely contributes to the establishment of persistent infection.

Pathogenic Functions of the Adaptive Immune Response During Chronic Hepatitis

As mentioned above, HBV has the capacity to persist in face of an active, albeit functionally inefficient, adaptive immune response. Indeed, chronic HBV infection could be characterized by a dysfunctional virus-specific CD8+ T cell response that fails to eliminate HBV from the liver but maintains continuous cycles of low-level hepatocellular injury, promoting the development of liver fibrosis/cirrhosis and, ultimately, HCC.

Of note, liver fibrosis and cirrhosis are pathological conditions characterized by an imbalance between fibrogenesis and fibrolysis, resulting in the excessive intrahepatic deposition by stellate cells, myofibroblasts, and fibroblasts of extracellular matrix (ECM) that is qualitatively different in its composition and organization from that of normal liver repair [63]. As a result of this process, a dense, reticulated ECM is initially deposited around the portal areas of the liver and, as a function of time, the fibrosis progressively expands into the lobules with the formation of septa that can eventually connect portal and central veins [63]. Liver cirrhosis represents the final stage of fibrosis in which fibrous septa surround nodules of regenerating hepatocytes, causing profound architectural distortion of the liver, functional insufficiency and diversion of venous blood containing intestinal toxins into the systemic circulation [63]. As mentioned above, liver fibrosis and cirrhosis are also associated with a reduction in number and size of sinusoidal fenestrae (a process often described as "defenestration" of the hepatic sinusoids) and the formation of a basal membrane separating hepatocytes from sinusoidal blood (a process often described as "capillarization" of the hepatic sinusoids) [64]. These events alter the normal exchange of soluble factors between blood and hepatocytes [64] and worsen HBV morbidity (possibly, as stated earlier, because of reduced immune surveillance). The severity and duration of chronic liver disease positively influence liver fibrosis/cirrhosis, and the same is true for HCC where almost all cases take place after many years (usually several decades) of a chronic hepatitis characterized by a sustained liver disease with associated hepatocellular regeneration (i.e., cellular DNA synthesis) and inflammation (i.e., the production of mutagens) [1]. Chronic liver cell injury, therefore, also appears to be a premalignant state promoting cellular processes, like enhanced cellular DNA synthesis and production of inflammatory mutagens, which are oncogenic. Persistence of these events for a sufficiently long period of time results in the random/multiple genetic and chromosomal alterations that contribute to HCC development [1]. Consistent with this, it has been shown in mouse models of immune-mediated chronic HBV infection that the maintenance of low-level liver cell destruction caused by a dysfunctional and detrimental virus-specific CD8+ T cell response is sufficient to cause the development of liver fibrosis/cirrhosis and HCC, and this occurs in the absence of cofactors (e.g., viral integration, HBV X gene expression, or genotoxic agents) that have been proposed to contribute to the development of hepatocellular carcinoma in humans [65, 66].

The notion that a virus-specific CD8+ T cell response, although inefficient and essentially harmful, remains detectable in the liver of patients chronically infected with HBV can be exploited therapeutically. One reasonable approach is to restore the functionality of such response to the levels that are observed in patients undergoing self-limited acute infection. There, a number of different hurdles must be overcome and, in particular, the severe exhaustion that typifies T cells chronically exposed to large amounts of antigens (with the hope that these cells do not carry dysfunctional signatures that are permanent) [67]. Another approach, conceptually different, is to further reduce the capacity of T cells to induce chronic liver damage with the idea that—in so doing—the onset of liver fibrosis/cirrhosis might be prevented or delayed. In keeping with this and building on the observation that platelets are instrumental to

intrahepatic effector CD8+ T cell homing, a recent mouse study demonstrated that clinically achievable doses of the antiplatelet drugs aspirin and clopidogrel, when administered continuously after the onset of liver disease, can prevent the development of advanced fibrosis and HCC, greatly improving overall survival [66]. These outcomes were preceded by and associated with reduced hepatic accumulation of pathogenic virus-specific effector CD8+ T cells and pathogenic virus-nonspecific inflammatory cells, and reduced hepatocellular injury and hepatocellular proliferation [66]. Irrespective of antiplatelet treatment, intrahepatic virus-specific effector CD8+ T cells analyzed at multiple times during chronic liver injury were found to express virtually no IFN-γ [66]; this is consistent with the abovementioned observation that HBV-specific effector CD8+ T cells rapidly abandon the ability to produce this antiviral cytokine after intrahepatic antigen recognition in mice [45] and that IFN-γ-nonproducing HBV-specific CD8+ T cells are commonly present in the liver of chronically infected patients [68]. Altogether, the abovementioned results indicate that the antiplatelet drugs aspirin and clopidogrel effectively prevent or delay the onset of severe liver fibrosis HCC and improve survival, supporting the concept that platelets promote CD8+ T cell-induced liver immunopathology. The results also reinforce the notion that a detrimental CD8+ T cell response is both necessary and sufficient to induce the complications of chronic viral hepatitis and they suggest that future drugs targeting platelet function or other functions linked to disease severity may be a therapeutic option in patients with chronic HBV infection.

Conclusive Remarks

Our comprehension of the immunopathogenesis of HBV infection has significantly advanced over the last 30 years. Regardless of this, however, we are still far away from the clinical application of immune therapeutic approaches capable of terminating chronic HBV infection. As largely discussed in other chapters of this book, last generation antivirals have proven potent efficacy in the absence of significant side effects. Unfortunately, the relative incapacity of these molecules to completely eliminate the virus renders the likelihood of viral rebounds following treatment withdrawal quite high. Future work intended to expand our current knowledge of the complex host-virus relations that determine the immunopathogenesis of HBV infection may guide us to the design of new strategies that, alone or in combination with existing or forthcoming antivirals, will direct the immune system to terminate chronic HBV infection and/or its life-threatening complications.

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