Immune Network
http://dx.doi.org/10.4110/in.2015.15.4.191
pISSN 1598-2629 eISSN 2092-6685
REVIEW ARTICLE

Immune-mediated Liver Injury in Hepatitis B Virus Infection

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Hepatitis B virus (HBV) is responsible for approximately 350 million chronic infections worldwide and is a leading cause of broad-spectrum liver diseases such as hepatitis, cirrhosis and liver cancer. Although it has been well established that adaptive immunity plays a critical role in viral clearance, the pathogenetic mechanisms that cause liver damage during acute and chronic HBV infection remain largely known. This review describes our current knowledge of the immune-mediated pathogenesis of HBV infection and the role of immune cells in the liver injury during hepatitis B.

[Immune Network 2015;15(4):191-198]

Keywords: Hepatitis B Virus, Pathogenesis, Immune response

INTRODUCTION

Five hepatitis viruses (hepatitis A to E) are the leading causes of liver disease worldwide. Among them, hepatitis B virus (HBV) is responsible for approximately 350 million chronic infections worldwide and over one million annual deaths, mostly as a result of end-stage liver disease and liver cancer (1). HBV along with the hepatitis C virus (HCV) can cause broad-spectrum diseases such as hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma (HCC) in the setting of chronic infection (1). The natural course of HBV infection is variable, and chronic HBV infection mostly results from vertical transmission from mother to child (2). In comparison, more than 95% of horizontal infections during adulthood only lead to acute infections associated with resolution and lifelong T cell- and antibody-mediated memory responses (3). Although the factors determining the disease outcomes of acute HBV infection are not entirely understood, many studies have demonstrated that they are associated with the immune status of the host.

HBV is noncytopathic for infected hepatocytes (4). Chimpanzee studies have revealed that hepatocytes are infected without any evidence of liver injury prior to the recruitment of HBV-specific T cells into the liver (5,6). Among the multiple cell types that comprise the immune system, cytotoxic T lymphocytes (CTL) have been regarded as the main culprit for liver damage during acute HBV infection (4,7). During acute HBV infection, HBV-specific CTLs can directly attack infected hepatocytes and participate in the pathogenesis of liver disease by orchestrating diverse components of the immune system. However, the exhaustion of HBV-specific CD8 T cells during chronic HBV infection indicate that HBV-specific CTLs are not the major mediator of liver injuries during chronic infections (8). Rather, necroinflammatory liver disease might be caused by secondary recruitment of mononuclear cells.

Received on June 20, 2015. Revised on July 26, 2015. Accepted on August 2, 2015.

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Abbreviations: HBV, hepatitis B virus; cccDNA, covalently closed circular DNA; ISG, inteferon-stimulated gene; ALT, alanine aminotransferase; ADCC, antibody-dependent cell-mediated cytotoxicity

The outcome of HBV infection and the pathogenesis of liver disease are determined by immune-mediated host-virus interactions. However, the detailed pathogenetic mechanisms that cause liver diseases during HBV infection are not fully understood, because experimental approaches to better understand the pathogenesis of HBV infection are difficult due to the lack of small-animal models that are susceptible to HBV infection. This review describes the current knowledge of the immune-mediated pathogenesis of HBV infection and the links between immunological components, which can provide valuable information for the prevention of disease progression and the development of effective therapeutic strategies against chronic HBV infection.

THE NONCYTOPATHIC PECULIARITY OF HBV

The hepatocyte, a parenchymal cell of the liver, is a primary target for HBV infection. When HBV enters hepatocytes via an interaction with a cellular receptor, nucleocapsids transport the genomic HBV DNA into the nucleus where the circular DNA is converted to covalently closed circular DNA (cccDNA) as the template for viral RNAs. Four viral RNAs are exposed to the cytoplasm and used as mRNAs for the translation of HBV proteins such as surface, polymerase, X, and core proteins (7).

After HBV infection into hepatocytes, HBV itself is not directly cytotoxic to the virus-infected cell that has been supported by several lines of evidence. First, HBV persistently replicates in transfected hepatoma cell lines (9,10) or in infected primary human hepatocytes (11) without obvious cellular damage or cell death. Second, HBV transgenic mice with viral replication do not show objective evidence of inflammation in the liver. However, adoptive transfer of HBV-specific CTLs into transgenic mice triggers intrahepatic inflammation (12), suggesting that hepatic inflammation might not be caused by HBV per se, but by HBV infection-induced adaptive immunity. Finally, during the early phase of acute infection before T cell recruitment into the liver, there is no evidence for histological or biochemical liver damage in HBV-infected chimpanzees (5,6). Patients with immune-tolerant phages and high HBV DNA titers in their blood still maintain a normal range of alanine aminotransferase (ALT), which is indicative of T cell-mediated liver injury, further suggesting the noncytopathic characteristics of HBV (13). In addition, in immunocompromised patients or animal models, viral replication occurs at a high level in the liver without liver injury, implying that HBV replicates noncytopathically in the liver and that liver injury might be mediated by HBV-induced immune responses (14-16).

APOPTOSIS MEDIATED BY HBV

Apoptosis of infected cells contributes to antiviral defense by limiting the initial viral spread. Apoptosis, however, may also help the spread of viruses, as evidenced by a measles infection model demonstrating that the measles virus induced Fas-mediated apoptosis of dendritic cells and facilitated the release and spread of viral progenies (17). In order to release infectious progeny, HBV does not require cell destruction. Thus, it remains controversial whether HBV sensitizes hepatocytes to apoptosis for viral spread in the liver (18,19).

A large number of studies have examined the role of HBx proteins in the regulation of apoptotic pathways, but the results have varied depending on the cellular context. Many studies have demonstrated that the overexpression of HBx proteins can render hepatocytes susceptible to apoptosis (19-22), which can be initiated by both extrinsic and intrinsic signaling pathways. Tumor necrosis factor (TNF) protein superfamily members including TNF-α, FasL and TRAIL have been suggested to be important inducers of hepatocyte apoptosis (23). Moreover, generation of reactive oxygen species (ROS) in response to stresses (24), mitochondrial dysfunction (25), ER stress (23) and autophagy (23,26) are also have been suggested to be involved in the induction of apoptosis.

However, there are some reports demonstrating that neither HBV replication nor the expression of HBV proteins resulted in apoptosis of HBV-infected hepatocytes (11,27). Furthermore, HBx proteins were shown to attenuate the apoptotic response by interrupting cellular proteins involved in the FasL- and TGF-β-mediated apoptotic pathways (28-30) or by directly interacting with caspase 3 (31,32) or p53 (33,34). A recent report demonstrated that the apoptosis of HBV-infected hepatocytes prevented the release of infectious viruses (35). Moreover, it has been suggested that HBV has both pro- and anti-apoptotic effects, as demonstrated in cultured cells and transgenic mouse models (36). In addition, previous studies have demonstrated that HBx does not directly induce apoptosis

in certain cells, but rather sensitizes these cells to pro-apoptotic stimuli (37,38).

Taken together, the results from various studies suggest that HBV proteins are capable of inducing an anti- or pro-apoptotic response in various experimental conditions depending on different cellular contexts, suggesting that the ability of HBV to induce or suppress apoptosis may be contradictory during the course of HBV infection. Another possible explanation for this discrepancy is that HBV has different effects in different liver microenvironments depending on the expression of certain transcription factors including NF-κB (38,39).

IMMUNE-MEDIATED PATHOGENESIS OF HBV INFECTION

HBV is a noncytopathic virus; therefore, it is plausible that the associated pathogenesis including liver injury after HBV infection is mainly mediated by immunologically mediated events (4).

The natural killer (NK) cell, an antigen-nonspecific inflammatory cell, is known to participate in the inflammatory process before the recruitment of CTLs into the liver. Subsequently, HBV-specific CTLs are responsible for significant cytotoxicity and play pivotal roles (both directly and indirectly) in viral pathogenesis. Moreover, helper T cells, B cells and antigen-presenting cells are also associated with the pathogenesis of HBV infection. Recent studies have further suggested that HBV-nonspecific inflammatory cells exacerbate CTL-induced immunopathology and that platelets enhance the accumulation of CTLs in the liver (40,41).

The role of NK cells in the pathogenesis of HBV

NK cells account for the majority of innate immune cells in the liver (42), and one of the important functions of NK cells is their ability to destroy infected cells (43). The frequency of intrahepatic NK cells is increased during chronic HBV infection (44-46), and these cells are more active than those in the peripheral blood (45,47). Moreover, NK cells from HBV-infected individuals express higher levels of activating receptors such as NKp30, NKp46 and NKG2C (48) and lower levels of inhibitory receptors such as NKG2A (44).

It is widely believed that NK cells are implicated in the pathogenesis and liver damage of cytopathic hepatotropic viral infection models such as murine cytomegalovirus (MCMV) in mice (49). Many NK cells, NKT cells, and polymorphonuclear leukocytes (PMNs) are found in infected livers, and these cells mediate hepatocellular damage rather than contribute to viral clearance (50). However, there is relatively little evidence that NK cells play a pathogenetic role during HBV infection. NK cells lyse human hepatocytes as well as human stellate cells during HBV infection (51), and the increased cytotoxicity of NK cells, as represented by degranulation and the presence of TRAIL, was observed in patients with chronic HBV infection (45). In addition, during HBV infection, intrahepatic NK cells create inflammatory environments by secreting diverse proinflammatory cytokines and their function is skewed toward cytolytic activities without IFN-γ production, which correlates with liver histological activity (52). This finding implies that the roles of intrahepatic NK cells are polarized toward pathogenesis, with augmented cytotoxicity and reduced antiviral function. In addition, during chronic HBV infection, inflammatory cytokines are decreased in the serum of HBV-infected patients, whereas anti-inflammatory cytokines are increased, (53-55).

However, it remains unclear whether NK cells play crucial roles in liver injury during the early phase of HBV infection before the induction of adaptive immunity. Using genomic array analysis, it was found that interferon-stimulated genes (ISGs) were not expressed in the livers of HBV-infected chimpanzees during the spread of the virus, although NK cells are known to be an abundant source of IFN-γ (5). Accordingly, it is plausible to assume that NK cells are not generally activated during HBV infection. In contrast to HBV, HCV induces many ISGs during the early phase of infection in chimpanzees (56-58), and this difference is attributable to the replication strategy of HBV, which retains transcriptional templates in the nucleus and sequesters its replicating genome within viral capsid particles in the cytoplasm (59). Collectively, because HBV acts as a stealth virus during the early phase of infection, the pathogenetic role of NK cells might be very limited (4).

The role of cellular immunity in the pathogenesis of HBV

CD8 T cells: HBV-specific CD8 T cell responses play a critical role in both viral clearance and the pathogenesis of hepatitis B. Indeed, CD8 T cell responses are vigorous,

polyclonal and multispecific in patients with resolved HBV infection, whereas the CD8 T cell responses are relatively weak and present a narrow spectrum of epitopes in patients with persistent viremia (60-62). The mechanism of viral clearance by CD8 T cells is attributable to not only direct inhibition of viral replication but also apoptosis of infected hepatocytes (63). Genomic analysis has revealed that HBV does not induce immune response-related genes capable of regulating infection during the early phase of acute HBV infection. However, the T cell response facilitates ISG expression during viral clearance, coinciding with elevated levels of alanine aminotransferase (ALT) (64). In addition, a previous report showed that *in vivo* depletion of CD4 or CD8 T cells hampered HBV clearance and clinical recovery (6).

Accumulating evidence suggests that HBV-specific CD8 T cells contribute to viral clearance as well as viral pathogenesis (4). It has been observed that there is a strong correlation between the severity of liver injury and the strength of the HBV-specific CD8 T cell response in patients with acute or chronic HBV infections as well as HBV-infected chimpanzees; patients who cleared an acute HBV infection developed a relatively severe liver injury compared to patients who developed chronic HBV infection (14). In addition, the finding that transgenic mice that express and replicate HBV in hepatocytes do not experience hepatocellular damage until after the adoptive transfer of HBV-specific CD8 T cells demonstrates the importance of CD8 T cells in the pathogenesis of liver disease (12). Finally, liver injury commenced with the recruitment of CD8 T cells into the liver in HBV-infected chimpanzees (5,6).

Considering the mechanisms of immune-mediated pathogenesis by HBV-specific CD8 T cells, these cells cause liver damage through direct as well as indirect mechanisms. HBV-specific CD8 T cells primed by viral antigens can bind infected cells directly and kill them in the liver, inducing destructive changes in hepatocytes much like the apoptotic process. As a result, councilman hyaline bodies accumulate in the liver and can be observed in acute viral hepatitis (65). Moreover, lysis of hepatocytes is induced by interactions with Fas or the perforin-mediated pathway (66,67).

HBV-specific CD8 T cells indirectly affect hepatic pathology by orchestrating the responses of other immune cells. The composition of these immune cells has been demonstrated using transgenic mice, which sustain a high level of HBV replication in the liver (12). Antigen-nonspecific polymorphonuclear or lymphomononuclear inflammatory cells are recruited into the liver in a chemokine-dependent manner and contribute to liver damage (68). Although the mechanisms of liver damage by these recruited inflammatory cells are not fully understood, they are known to amplify the liver damage initiated by HBV-specific CD8 T cells (68). In detail, IFN-γ secreted by HBV-specific CD8 T cells stimulates hepatocytes to produce chemokines such as CXCL9 and CXCL10, which in turn recruit diverse inflammatory cells resulting in liver inflammation. In addition to chemokines, other mechanisms responsible for the recruitment of antigen-nonspecific cells have been revealed. For instance, neutrophils (representative phagocytes) contribute to CTL-induced immunopathology by recruiting antigen-nonspecific inflammatory cells (69). The matrix-degrading metalloproteinases (MMPs) produced by neutrophils mediate the intrahepatic recruitment of inflammatory cells, aggravating liver damage independently of chemokine induction (70). Blocking neutrophils or MMPs via the administration of neutralizing antibodies reduces the accumulation of intrahepatic inflammatory cells and liver damage, but not the antiviral potential of HBV-specific CD8 T cells (69,70).

Interestingly, it has been suggested that platelets actively participate in liver pathogenesis by promoting the intrahepatic accumulation of HBV-specific CD8 T cells and nonspecific inflammatory cells (40). Indeed, platelet depletion or antiplatelet therapy with aspirin and clopidogrel reduced the intrahepatic accumulation of virus-specific CD8 T cells and ameliorated the severity of liver disease (41,71). In a mouse model of LCMV infection, which can induce viral hepatitis, the serotonin released from activated platelets aggravated liver damage by disturbing the liver microcirculation, suggesting another possible mechanism of liver injury after HBV infection (72).

CD4 T cells: Generally, CD4 T cells are required for the induction of effective CD8 T cell and B cell responses. During a viral infection, CD4 T cells contribute to viral clearance by maintaining virus-specific CD8 T cells. Compared to CD8 T cells, the roles of CD4 T cells are less known in chronic HBV infection. However, there are several studies suggesting the roles for CD4 T cells in HBV clearance (73). Like CD8 T cells, HBV-specific CD4 T cells from patients who cleared an acute HCV infection are vigorous and multispecific compared to those from pa-

tients who developed a chronic HCV infection (74). It has also been suggested that CD4 T cells play specific roles in the pathogenesis of HBV infection. For instance, helper T cell clones generated from chronic HBV patient livers showed cytolytic capacity *in vitro* (75). Additionally, in an HBV-transgenic mouse model, CD4 T cells showed effector functions such as cytotoxicity, which directly contributed to disease pathogenesis. Although *in vitro* studies have demonstrated the cytolytic activity of CD4 T cells during HBV infection, the pathogenetic roles of CD4 T cells in real HBV infection remain unclear. Nevertheless, HBV challenge of chimpanzees after depletion of CD4 T cells showed a comparable severity of liver damage compared to chimpanzees without CD4 T cell depletion, and the course of HBV infection was not altered (6). Collectively, these results suggest that HBV-specific CD4 T cells are not likely to mediate direct liver damage during HBV infection, although these cells may help to induce and maintain HBV-specific cytotoxic T lymphocytes.

The role of antibody in the pathogenesis of HBV

Neutralizing antibodies can block viral entry into hepatocytes and also mediate protective immunity (76). Furthermore, these antibodies are suggested to undergo endocytosis by hepatocytes, leading to decreased HBV surface antigen (HBsAg) secretion via an intracellular interaction with HBsAg (77). However, the appearance of neutralizing antibodies occurs relatively late after HBV exposure, which indicates an unlikely role for these antibodies in early viral clearance during acute HBV infection. Neutralizing antibodies play protective roles against HBV and persist for the entire lifetime in patients who recover from an acute HBV infection (78). The role of neutralizing antibody in protective immunity is supported by a report showing that chimpanzees with high titers of neutralizing antibody after acute self-resolution achieved complete immune protection against HBV re-challenge (79).

Virus-specific antibodies can induce antibody-dependent cell-mediated cytotoxicity (ADCC) through the interaction of the Fc portion of antibodies with Fc receptors on phagocytes, which results in liver damage via the induction of apoptosis in HBV-infected hepatocytes (80). Furthermore, neutralizing and non-neutralizing HBV-specific antibodies can promote antiviral and pathogenetic events by activating the complement system responsible for the lysis of virus-infected cells (74,81).

CONCLUDING REMARKS

In recent decades, great progress has been achieved in understating the virological and immunological events during acute and chronic HBV infection, leading to the development of successful preventive vaccines and therapeutic reagents. Although an effective HBV vaccine is available, a better understanding of the immune-mediated pathogenesis of HBV infection is necessary for individuals who already have chronic infections and are at a high risk of developing liver cirrhosis and liver cancer. Because antiviral drugs against chronic HBV infection can suppress but not eradicate HBV infection, further investigation into the immunopathogenesis of HBV may provide valuable information for the prevention of disease progression and the development of effective therapeutic strategies against chronic infections.

ACKNOWLEDGEMENTS

We thank Prof. Eui-Cheol Shin for helpful discussion.

CONFLICTS OF INTEREST

The authors have no financial conflict of interest.

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