New concepts in the immunopathogenesis of chronic hepatitis B: the importance of the innate immune response

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Abstract Acute and chronic infection with hepatitis B virus (HBV) is associated with an increased risk of developing liver disease including cirrhosis, decompensated liver disease, and hepatocellular carcinoma. The clinical presentation and natural history of HBV infection is mediated through complex interactions between the virus and the host immune response. HBV is not directly cytopathic to hepatocytes; however, the interaction between the virus and the host immune response plays a central role in the pathogenesis of necroinflammation and liver fibrosis. Emerging data from immunopathogenesis studies in animal models and in vitro studies of liver biopsies from patients with chronic hepatitis B demonstrate a potentially important interaction between hepatitis B e antigen, HBV, and components of the innate immune response including Toll-like receptors, Kupffer cells, natural killer T-cells, and dendritic cells. These findings suggest that the innate immune response has an important role in influencing the outcome of acute and chronic HBV infection. The current knowledge regarding the interaction between HBV and components of the innate immune response during acute and chronic HBV infection is reviewed.

Keywords Immunopathogenesis · Toll-like receptor · Hepatitis B · Dendritic cells · Chronic Hepatitis B

Abbreviations

CCL-3	CC chemokine ligand-3
CMV	Cytomegalovirus
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
IFN	Interferon
IL	Interleukin
MBL	Mannose-binding lectin
MHC	Major histocompatibility complex
NF- κ B	Nuclear factor kappa B
NK	Natural killer
NKT	Natural killer T (cells)
PRR	Pattern recognition receptor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand

Introduction

The outcome following acute hepatitis B virus (HBV) infection is principally determined by the age and immune status of the individual and the route of transmission of infection. Infection in an immunocompetent adult results in a self-limiting illness in which viral clearance occurs in more than 95% of cases. Conversely, vertical transmission from mother to child, or horizontal transmission to young children, is far more likely to result in chronic infection. Such chronic infection results in cirrhosis in 20–30% of cases [1, 2].

The HBV replication cycle is not directly cytotoxic toward hepatocytes. Much of the tissue injury is thought to be a consequence of immune responses directed toward

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viral antigens on the hepatocytes. Successful HBV clearance is associated with a strong, multispecific CD4+ and CD8+ T-cell response coordinated with an effective humoral immune component [3–5]. However, there is emerging evidence that the innate immune system plays a role in influencing both the outcome of acute HBV infection and the pathogenesis of the virus.

The innate immune system

The most primitive component of the host immune response, the innate immune system was once considered as simply providing a nonspecific response, with a minor role confined to containment of microbial infection until the adaptive immune response could be activated. It has now become clear that the innate immune system has a much more important and fundamental role in host defense. This includes pathogen surveillance, direct antimicrobial activity, and, critically, interacting with and influencing adaptive immune responses [6, 7].

Key constituents of this response include phagocytic cells (neutrophils, monocytes, macrophages), cells that release inflammatory mediators (basophils, mast cells, eosinophils), natural killer (NK) cells, molecules such as complement proteins, and inflammatory cytokines such as interferon (IFN)- α , - β , and - γ , tumor necrosis factor (TNF)- α , and interleukin (IL)-12.

Unlike the antigen-specific, somatically generated B- and T-cell receptors of the adaptive immune system, the innate arm uses germ-line encoded and evolutionarily conserved receptors termed pattern recognition receptors (PRRs). They allow the innate immune system to target a few, highly conserved structures present in large groups of microorganisms known as pathogen-associated molecular patterns, rather than having to recognize multiple, specific antigens [8].

The PRRs are grouped into families based on structural characteristics that include Toll-like receptors (TLRs), nucleotide-binding oligomerization domain leucine-rich repeat proteins, peptidoglycan recognition proteins, the caspase recruitment domain-helicase proteins, and mannose-binding lectins (MBLs) [9, 10].

These PRRs differ from antigen-specific receptors in a number of ways. They are expressed on many effector cells of the innate immune system, and their expression is not clonal. Furthermore, once the PRR identifies a pathogen-associated molecular pattern, the effector cell is triggered to perform its functions immediately, rather than after proliferation. This accounts for the rapidity of the innate response [8].

One of the more important and well-characterized PRR groups are the TLRs. The TLRs were initially identified

based on homology with the toll receptor from *Drosophila*. Thus far, 10 TLRs have been identified in humans. While phagocytic cells express the broadest repertoire and highest levels of these molecules, TLRs have been identified on many cell types throughout the body, including intestinal, endothelial, and renal cells [11, 12]. Stimulation by their ligands initiates the activation of complex networks of intracellular signal transduction pathways to coordinate the ensuing inflammatory response (Fig. 1) [13]. These networks include components such as the adaptor protein MyD88, protein kinases (such as IL-1 receptor-associated kinase, p38 mitogen-activated protein kinase, and TNF receptor-associated kinase), and the transcription factor nuclear factor kappa B (NF- κ B). Activation of NF- κ B leads to the expression of a variety of proinflammatory mediators including TNF- α , IL-1, IL-6, and monocyte chemoattractant protein [9, 10, 14, 15]. The signals that become induced upon TLR activation in turn control the activation of the specific immune response. There is evidence that the specific immune system only responds to a pathogen after it has been recognized and processed by the innate immune system.

TLRs 2 through 4 and 7 through 9 have roles in viral sensing by the innate immune system. The host response against viruses involves the induction of type 1 IFNs (IFN- α , - β , and - γ), which leads to the increased expression and activity of IFN-stimulated genes. Induction of IFN-stimulated genes (which include protein kinase receptor, IFNs themselves, and IFN regulatory factors) leads to antiviral, antiproliferative, and immunoregulatory responses. The discovery of TLR-mediated signaling pathways to both NF- κ B and IFN regulatory factor 3, leading to type 1 IFN production, provides an insight into the mechanism of how viral infection leads to the IFN response [16–18].

Acute hepatitis B

After acute HBV infection, HBV DNA is detectable by polymerase chain reaction assay in the circulation within 1 month, although the levels remain relatively low for up to 6 weeks. This initial phase is followed by a logarithmic expansion phase, during which serum levels reach 10^9 to 10^{10} copies/ml and most hepatocytes show evidence of infection. Elevated serum alanine aminotransferase levels or symptoms reflecting the T-cell-mediated response (or both) are not seen until approximately 10–15 weeks after initial infection [19]. Studies in chimpanzees suggest that the HBV DNA level peaks and declines prior to the induction of the T-cell-mediated response. This clearance of HBV DNA from the serum and hepatocytes occurs by noncytolytic, antiviral cytokine activity, probably

Fig. 1 The TLR pathway and interactions with the specific immune response. Used with permission from Inohara and Nunez [13]

mediated largely by the innate and adaptive immune systems [20].

Whether the innate immune system plays a role in the initial lag phase of HBV replication remains unclear. Microarray analysis of serial liver biopsies of experimentally infected chimpanzees found that HBV did not induce any detectable changes in the expression of intrahepatic genes during the first few weeks of infection [21]. This was in contrast to the vigorous intrahepatic type 1 responses induced by hepatitis C in the same animals [22, 23]. This raises the possibility that HBV may possess mechanisms to evade immune recognition at the earliest stage of infection. One also has to take into consideration that acute hepatitis B in chimpanzees follows a milder course than that seen in humans, as well as the possibility that the expression of immune response genes may occur below the level of detection that was carried out in the existing study [24, 25].

Role of hepatocytes in the innate immune response

The lag phase of HBV replication is followed by the release of intrahepatic IFN- α and IFN- β , one of the first immune events in the host response to HBV. These type 1 IFNs are believed to work in both a direct antiviral manner and also in an indirect manner, by recruitment and activation of antigen-presenting cells, including Kupffer cells and dendritic cells. In HBV transgenic mice, this production of IFN- α and IFN- β inhibited the formation of new HBV capsids and caused destabilization of existing capsids; it also caused degradation of preformed HBV RNA

[26–28]. Following these early events, it appears that the hepatocytes then engage the adaptive immune system by up-regulation of major histocompatibility complex (MHC) class I molecules on their cell surface to allow T-cell recognition of foreign antigen [29].

Some investigators have postulated that these type 1 IFNs are secreted by the infected hepatocytes via TLR stimulation. There is evidence for TLR-mediated immune activity by hepatocytes in the non-HBV setting. This includes the discovery that these cells express mRNA for all TLRs and the observance of functional TLR2 and TLR4 activity, in the form of hepatocyte uptake and clearance of endotoxin from the murine circulation [11, 30]. Also, TLR2 and TLR4 ligands have been shown to activate NF- κ B in primary hepatocytes, while in hepatocyte cell lines, TLR3 stimulation with poly(I:C) has resulted in activation of the type 1 IFNs [10, 31, 32].

NK cells and NK T cells

NK cells are a subset of the lymphoid cell population that constitutes a key component of the innate immune system. Involved in the early response against both microbial invasion and neoplastic growth, they are characterized by an ability to spontaneously lyse various cell lines in a non-MHC-restricted manner, as distinct from T and B cells [33].

NK cells appear to play an important role in intrahepatic immune responses. In an experimental murine cytomegalovirus (CMV) model, NK cells were among the earliest cellular responders in the liver, exhibiting a 10-fold

increase in number by day 5 following intraperitoneal CMV injection. This increase was independent of NK T cells (NKT cells), T and B cells, IL-12, and TNF- α [34]. In human livers, NK cells comprise up to one third of all intrahepatic lymphocytes, a proportion that is significantly higher than their 2% contribution to the pool of circulating lymphocytes [35].

Activated NK cells act by two key mechanisms: direct cytotoxicity of infected cells through cell-to-cell contact and production of inflammatory cytokines [29, 33]. The mechanism of NK antiviral cytotoxicity appears to be organ dependent. Receptor-mediated cell death through ligand-receptor pairs belonging to the TNF superfamily likely plays an important role in liver damage. One such pathway is mediated through TNF-related apoptosis-inducing ligand (TRAIL) expressed on infiltrating lymphocytes interacting with TRAIL death-inducing receptors (TRAIL-R1 and TRAIL-R2) on hepatocytes [36]. Cytokines produced by NK cells include those with direct antiviral activity, such as IFN- γ and TNF- α , and those with immunomodulatory activity, such as IL-3, granulocyte-macrophage colony-stimulating factor, and macrophage colony-stimulating factor.

NKT cells are a T-lymphocyte subset expressing classic NK cell markers. They compose up to one third of intrahepatic T cells. They, too, can be induced to produce IFN- γ in response to NK-cell stimulation and IL-12, and, interestingly, they make IL-4 during innate immune responses [33, 37].

NK and NKT cells appear to play a central role in the innate immune response to acute HBV infection. In the HBV transgenic mouse model, a 10- to 12-fold increase in NK numbers in the intrahepatic cellular infiltrate is seen during acute inflammation [38]. Recruitment and activation of NK and NKT cells appear to be mediated by IL-12, IL-18, and chemokine (C-C motif) ligand-3 released by antigen-presenting cells in response to type 1 IFNs [37, 39]. There is also evidence that NK and NKT cells may be activated both directly and effectively by elements such as HBV glycolipids and phospholipids and other stress signals on infected hepatocytes [40, 41].

Animal models suggest that production of IFN- γ and TNF- α by these activated NK and NKT cells leads to significant inhibition of HBV replication and to recruitment of virus-specific and nonspecific cells [39, 40, 42]. In HBV transgenic mice, injection of α -galactosylceramide, a ligand of CD1d (to stimulate NKT cells), led to the secretion of cytokines IFN- α , - β , and - γ and to subsequent control and inhibition of HBV replication without the activation of CD4+ or CD8+ T cells. Suppression of HBV was still detected even in T-cell-depleted mice, which suggested that IFN- γ production is not dependent on CD4+ and CD8+ T-cell activity alone [40]. Similarly, in

chimpanzees that were able to ultimately resolve HBV replication, a rapid drop in virus replication occurred in the presence of intrahepatic IFN- γ prior to the recruitment of T cells [20]. IFN- γ has also been shown to up-regulate MHC class 1 expression on hepatocytes and mediate macrophage and dendritic cell inhibition of viral replication [29].

Studies during the incubation phase of human HBV infection demonstrate increased numbers of circulating NK cells concomitant with the peak of HBV replication. At 2–4 weeks later, HBV-specific CD8+ T cells appear, when viral replication has already dropped [19].

Kupffer cells

Kupffer cells perform a number of roles in their capacity as the resident macrophages of the liver. These include phagocytosis, antigen recognition and presentation, and secretion of proinflammatory mediators. Kupffer cells express TLR2 and TLR4. They have shown involvement in the uptake and excretion of lipopolysaccharide via the latter [43]. IL-12 and IL-18 produced by Kupffer cells can stimulate NK cells to produce IFN- γ [44, 45].

The precise role of Kupffer cells in HBV infection is still emerging. In the HBV transgenic mouse model, Kupffer cells produce the chemokines CXCL9 and CXCL10, which assist in the recruitment of inflammatory cells into the liver [46]. In addition, a direct antiviral role is also possible: when these mice were injected with a liver-specific malaria strain, Kupffer cells produced cytokines, which led to the reduction of both the malaria infection as well as the chronic HBV infection [47].

Dendritic cells

Dendritic cells are the most potent antigen-presenting cells and, indeed, are the only such cells capable of stimulating naïve T cells. In humans, they are classified into two main subtypes: myeloid dendritic cells, which are specialized in the surveillance, uptake, processing, and presentation of antigen, and plasmacytoid dendritic cells, which are specialized in secreting large quantities of type 1 IFNs. Classically regarded as the key translators of innate immune recognition into adaptive immune responses, they also play a role in immunologic tolerance [48, 49].

In acute HBV infection, dendritic cells are likely to play a similar role to that of the other antigen-presenting cells, including intrahepatic recruitment of inflammatory cells, activation of NK cells, and antigen presentation to T cells. In the HBV transgenic mouse model, activation of antigen-presenting cells, including dendritic cells, with CD40 ligand led to profound inhibition of HBV replication [42].

Ultimately, the eventual outcomes of acute HBV infection are associated with distinctly different adaptive

immune response profiles. Self-limited resolution is associated with a vigorous, multispecific antiviral CD4+ and CD8+ response involving adequate anti-envelope antibodies. The development of chronic infection, on the other hand, is characterized by a weak and narrow virus-specific T-cell response [3, 4, 50, 51]. Overall, it appears that the self-limiting illness requires an initial containment of HBV replication and maturation of adaptive immunity that is mediated by the innate immune system through IFN- γ and TNF- α . This is then followed by an adaptive immune response that contributes to the lysis of infected hepatocytes and to long-term control of viral replication. If this overall, multifaceted response is suboptimal, chronic infection ensues [52, 53].

Chronic hepatitis B

Although the most characteristic immunologic feature of chronic HBV infection is a diminished HBV-specific CD8+ and CD4+ T-cell response, there is increasing evidence that the innate immune system is involved in this phase of the illness as well. This evidence includes viral down-regulation of PRRs, a potential role for dendritic cells in inducing HBV-specific T-cell tolerance, and a role for NK cells in hepatocyte damage.

Role of TLRs in chronic HBV infection

In the HBV-transgenic mouse model, administration of specific ligands for TLR 4, 5, 7, and 9 resulted in significant inhibition of viral replication. This occurred within 24 h in an IFN-dependent manner [26, 27]. While this would be consistent with the role postulated for TLRs in antiviral defenses, there is also emerging evidence for viral mechanisms that target PRRs to escape immune surveillance.

Recent studies have shown a down-regulation of TLR2 on hepatocytes, Kupffer cells, and peripheral blood monocytes in patients positive for hepatitis B e antigen (HBeAg), as compared with HBeAg-negative patients and healthy controls. In the patients with HBeAg-negative active hepatitis, there was an up-regulation of TLR2 and related TNF- α secretion [53, 54]. This TLR2 down-regulatory effect was confirmed by in vitro studies using hepatic cell lines and CD14+ monocytes. Another report demonstrated that hepatitis B surface antigen (HBsAg) inhibits lipopolysaccharide-induced expression of cyclooxygenase-2 and also reduces IL-12 and IL-18 production by blocking the extracellular signal-regulated kinase and NF- κ B pathways, in addition to regulating IFN production [55]. These findings all suggest a role for specific HBV antigens targeting TLRs to escape immune recognition.

Mannose-binding lectins

MBL, a calcium-dependent C-type lectin with structural analogy to complement component C1q, also functions as a PRR molecule of the innate immune system by binding through its multiple carbohydrate-recognition domains to repeating arrays of carbohydrate structures on microbial surfaces. MBL then is able to activate the complement system via specific proteases or act as an opsonin to enhance phagocytosis. Serum MBL levels also play a role in the regulation of inflammatory cytokines such as IL-6, IL-1 β , and TNF- α by monocytes in response to pathogens.

Two recent studies have reported on the role of MBL and its gene (*mb12*) polymorphisms in patients with chronic hepatitis B [56, 57]. Both studies found that patients with genotypes associated with low levels of serum MBL were more likely to demonstrate viral persistence, progression of fibrosis, and the development of hepatocellular carcinoma, with an odds ratio ranging from 1.36 to 3.2 for low and extremely low levels of MBL haplotypes, respectively. HBV carriers without progressive liver disease and those who spontaneously recovered show no difference in MBL levels or *mb12* polymorphisms compared with healthy controls. In vitro experiments also demonstrated that MBL could bind HBsAg in a dose-dependent, calcium-dependent, and mannan-inhibitable manner, an interaction that also enhanced C4 deposition [56, 57].

Dendritic cells

Dendritic cells are not only capable of generating and coordinating adaptive immune responses but they also play a role in inducing tolerance to both self and foreign antigens. Because of this, there has been increasing investigation into the possibility that dendritic cells may play a role in the state of relative HBV-specific T-cell tolerance seen in chronic infection. These studies have used both monocyte-derived or ex vivo-derived myeloid dendritic cells and plasmacytoid dendritic cells.

The majority of studies have failed to show a consistent pattern in relation to absolute numbers or frequencies of the peripheral blood dendritic cell subsets in chronic HBV carriers. A number of the studies did identify impaired co-stimulatory molecule expression and reduced IL-12- and IFN- α -secreting capacity by the two subsets, but, again, this functional impairment was not consistent across all the studies, and neither were the results of T-cell stimulatory capacity [58–62]. Studies of intrahepatic dendritic cells from HBV-transgenic mice reveal impaired T-cell proliferative capacity and production of IL-12, IL-6, IFN- γ , and TNF- α [63].

NK cells in chronic HBV infection

Two recent studies suggest an important role for NK cells in the pathogenesis of hepatocyte injury in chronic HBV infection. Both studies using the transgenic mouse model found that the HBsAg mice were much more sensitive to liver injury in response to both polyI:C and concanavalin A. Importantly, this oversensitivity to liver injury was dependent on the accumulation of intrahepatic NK cells and the IFN- γ that they produced [64]. The effect of the concanavalin A appeared to be mediated in part by the stress-inducible expression of NKG2D (an activating killer cell receptor) on hepatocytes, and it was independent of Kupffer cell and IL-12 activity [65].

Conclusion

The two arms of the host immune response against HBV infection need to be considered as a coordinated and dynamic line of attack, rather than as consecutive and independent entities. Both established and potential roles for many components of the innate immune system have been identified. Achieving clearance of HBV appears to require initial viral suppression and recruitment of effector cells by the innate immune system, along with adequate antigen presentation to and activation of the adaptive immune arm. Our increasing understanding of the immunologic mechanisms involved in HBV infection has the potential to identify new therapeutic targets against this challenging virus in the future.

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