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**Innate immune recognition of hepatitis B virus**

Liu HY *et al.* Innate immune recognition of hepatitis B virus

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**Abstract**

Hepatitis B virus (HBV) is a hepatotropic DNA virus and its infection results in acute or chronic hepatitis. It is reported that the host innate immune system contributes to viral control and liver pathology, while whether and how HBV can trigger the components of innate immunity remains controversial. In recent years, the data accumulated from HBV-infected patients, cellular and animal models have challenged the concept of a stealth virus for HBV infection. This editorial focuses on the current findings about the innate immune recognition to HBV. Such evaluation could help us to understand HBV immunopathogenesis and develop novel immune therapeutic strategies to combat HBV infection.

**Key words:** Hepatitis B virus; Hepatocytes; Innate immunity; Pathogen-recognition receptor; Interferon

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**Core tip:** Hepatitis B virus (HBV) infection is prevalent worldwide as a major public health problem and the leading cause of severe liver diseases.  A plethora of evidence suggests that innate immune pathways are involved in the cross-talk between HBV components and host immune cells. Many type of cells, including hepatocytes, kupffer cells and circulating monocytes, could sense and be activated by HBV infection through specific pathogen recognition receptors, resulting in the production of pro-inflammatory cytokines and interferons. Understanding of the nature of innate immunity induced by HBV will aid to characterize the immunopathogenesis of HBV infection and to further design novel immune-based therapeutic strategies for HBV infection.

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**INTRODUCTION**

Hepatitis B virus (HBV) is a hepatotropic DNA virus belonging to the *Hepadnaviridae* family and causes different outcomes of liver disease in humans, such as acute or chronic hepatitis, liver cirrhosis and hepatocellular carcinoma[[1](#_ENREF_1)]. Patients with chronic HBV infection are mostly asymptomatic but at risk of developing life-threating complications. Despite the availability of effective prophylactic vaccines, HBC infection is highly epidemic in developing countries and about 1 million people die from HBV-associated severe liver diseases annually[[2](#_ENREF_2)]. Generally, the pathogenesis and outcomes of HBV infection are mainly determined by the magnitude of host antiviral immune response[[3](#_ENREF_3)]. It has been experimentally proved that the CD8+ T cells –mediated adaptive immune response is necessary for controlling of HBV infection, and exogenous activation of host innate immune system is able to inhibit HBV replication and gene expression[[4](#_ENREF_4)]. However, during the occurrence of HBV infection, whether and how HBV trigger the components of innate immunity remains controversial. This review will summarize and evaluate the current findings, some of which are still contradictory, regarding the induction of innate immunity by HBV infection and how innate immune sensors are able to recognize HBV components.

**HBV INFECTION ACTIVATES HOST INNATE IMMUNITY**

During the early phase of viral infections, the production of pro-inflammatory cytokines and interferons (IFNs), and the activation of natural killer (NK) cells is frequently observed. Previously, HBV was considered as a stealth virus that could establish persistent infection in liver by evading the host innate immune system[[5](#_ENREF_5)]. Using an experimentally infected chimpanzee model, Wieland *et al*[[6](#_ENREF_6)] had reported that HBV was unable to interfere host cellular gene transcription significantly and to induce interferon (IFN)-stimulated genes (ISGs) expression in the liver. However, by quantification of serum cytokines, a study, which was enrolled 21 HBV-infected patients during the pre-symptomatic phase, indicted that HBV infection was unable to elicit a strong production of IFNs and interleukin (IL)-15, but did induce the production of anti-inflammatory cytokine IL-10[[7](#_ENREF_7)]. In addition to this observation, another study suggested that many cytokines were weakly induced during acute HBV infection. After initiation of viral expansion and before the peak of viremia, IFN-α, TNF-α, IL-15, IL-10, IL-6 and IL-1β levels were detectable in serum samples from about half of HBV patients[[8](#_ENREF_8)]. Interestingly, a longitudinal study performed in woodchuck model demonstrated that NK and NKT cell responses were activated within hours after inoculation with high dose of woodchuck hepatitis virus (WHV)[[9](#_ENREF_9)]. This result was consistent with the observation in two blood donors developing HBV infection without elevation of ALT at very early stage of infection[[10](#_ENREF_10)]. Recently, Hong *et al*[[11](#_ENREF_11)] revealed that HBV exposure in utero induced innate immune cell maturation and Th1 response development, which in turn enhanced the responses of cord blood immune cells to bacterial infection *in vitro*. Therefore, rather than being silent, HBV may be efficient in inducing anti-/pro-inflammatory cytokines, but less potent to activate IFN response in patients.

In agreement with the findings above, HBV was shown to be sensed by different types of liver cells with *in vivo* and *in vitr*o models. In the chimeric uPA-SCID mice harboring human hepatocytes, a weak activation of ISGs was detected in HBV-infected human hepatocytes, but not in mouse hepatocytes without HBV infection[[12](#_ENREF_12)]. Further, transduction of liver progenitor cell line HepaRG cells with a baculovirus vector expressing HBV resulted in significant activation of IFN-β and ISGs expression[[13](#_ENREF_13)]. The possible explanation for activation of IFN pathway in HepaRG cells is that the exceedingly high dose of HBV baculovirus inoculum is able to induce different intracellular pathways. However, when using cultured primary human hepatocytes and non-parenchymal liver cells, it was shown that HBV was recognized by kupffer cells. This recognition led to NF-κB pathway activation and IL-6 production, while no induction of type-I IFNs[[14](#_ENREF_14)]. Moreover, circulating monocytes were shown to respond to HBsAg *in vitro*, resulting in strong production of pro-inflammatory cytokines TNF-α and IL-6[[15](#_ENREF_15)].

Taken together, these data obtained from recent studies suggested that liver cell populations, as well as circulating innate immune cells, could sense and respond to HBV infection, which enables the innate immune system to detect and restrict the invading virus. Then, it is necessary to explore the receptors and the signaling pathways responsible for sensing HBV within the infected hepatocytes or other immune cells.

**INNATE IMMUNE RECEPTORS INVOLVED IN RECOGNITION OF HBV**

In general, various pattern-recognition receptors (PRRs) which recognize specific structures and components of pathogens by cells are responsible for activation of host innate immune system. The main PRRs sensing viral infection consist of Toll-like receptors (TLRs), NOD-like receptors, retinoic acid inducible gene I (RIG-I)-like receptors including RIG-I and melanoma differentiation associated gene 5 (MDA5). Viral envelope proteins, nucleocapsids and nucleic acids are able to activate special intracellular signaling pathways and induce the production of IFNs, pro-inflammatory cytokines and chemokines[[16](#_ENREF_16)]. In the case of HBV infection, several PRRs in different cell types were identified to be involved in recognition of HBV. For example, Cooper *et al*[[17](#_ENREF_17)] demonstrated that HBV nucleocapsids could activate TLR2-mediated signaling pathway in human THP-1 macrophages to induce pro-inflammatory cytokines production. In HBV replicating hepatocytes, Lu *et al*[18] reported the expression of MDA5 was up-regulated in Huh7 cells transfected with the HBV genotype D replicative plasmid and in the livers of plasmid hydrodynamically injected mice. Further, they found that MDA5, but not RIG-I, was able to associate with HBV-specific nucleic acids, suggesting that MDA5 may sense HBV[[18](#_ENREF_18)]. In contrast, a recent study suggested that RIG-I was the most important innate immune sensor of HBV in hepatocytes. They demonstrated that IFN-λ but not type-I IFNs is predominantly induced in HBV infected primary human hepatocytes and hepatoma cell lines. Moreover, the induction of IFN-λ is dependent on the RIG-I-mediated sensing the 5′-ε region of HBV pregenomic RNA[[19](#_ENREF_19)]. These contradictory results might mainly arise from the usage of different genotype of HBV plasmid and cellular models. In addition, the results also clarified that two previously reported cytosolic DNA sensors, including cyclic GMP-AMP synthase and IFN-γ-inducible protein 16, were not involved in HBV recognition in hepatocytes[[19](#_ENREF_19)]. In addition, it is worth noting that HBV-induced IFN responses in hepatocytes is relatively weak, as compared with other virus infection, which is consistent with the observations from the studies obtained in chimpanzee[[6](#_ENREF_6)] and mouse models[[14](#_ENREF_14)].

**PRR ACTIVATION CONTROLS HBV INFECTION**

Although the PRR-mediated innate immunity is weakly activated by HBV infection, numerous studies have clarified that HBV replication and gene expression can be inhibited by different PRR agonist stimulation in *vitro*and in *vivo*[[4](#_ENREF_4)]. For example, Isogawa*et al*[20] firstly reported that intravenous injection of TLR3, TLR4, TLR5, TLR7 or TLR9 ligands resulted in HBV inhibition by type I IFN induction in HBV transgenic mice model. This finding was consistent with *in vitro* observation that the culture medium derived from TLR3-activated murine kupffer cells or liver sinusoidal endothelial cells could inhibit HBV replication indirectly by IFN-β induction in immortalized murine hepatocytes[[21](#_ENREF_21)]. Moreover, in primary woodchuck hepatocytes or hepatoma cell lines, it had been shown that TLR2 or TLR4 ligands were able to inhibit HBV and WHV replication through activation of MAPK-ERK and PI3K-Akt pathways directly[[22](#_ENREF_22),[23](#_ENREF_23)]. Besides TLRs, activation of RIG-I in hepatocytes by 5’-triphosphorylated siRNA or HBV 5′-ε region derived RNA also induced a vigorous IFN response against HBV in hepatocytes[[19](#_ENREF_19),[24](#_ENREF_24)]. These studies mentioned above indicated that the PRR-induced anti-HBV response was dependent on the secreted cytokines from immune cells and the intracellular signaling pathways of hepatocytes. It is worth mentioning that recent preclinical studies revealed that oral administration of a TLR7 agonist GS-9620, which was capable of stimulating robust IFN-α responses in plasmacytoid dendritic cells and triggering ISGs expression in PBMCs and liver, resulted in HBV suppression in chronically infected chimpanzees[[25](#_ENREF_25)] and woodchuck models[[26](#_ENREF_26)]. Of note, this antiviral activity is also associated with activation of intrahepatic T, NK, and NKT cell responses that produce IFN-γ[[25](#_ENREF_25)]. Therefore, TLR7 agonist might be a promising drug candidate for immune modulation therapy of chronic HBV infection due to its dual effect on host innate and adaptive immune system[[27](#_ENREF_27)].

**CONCLUSION**

The accumulated data highlight that HBV is recognized by host PRRs and thus induces innate immune responses that restrict virus replication and expansion. However, the specific PRRs and intracellular signaling pathways involved in the HBV recognition and inhibition still require further investigation. An in-depth understanding of immune mechanisms induced by distinct components of HBV will provide the opportunity to characterize the immunopathogenesis of HBV infection and develop immune-based therapeutic strategies for HBV infection. Considering the suppressive effect of different viral proteins on innate immune system may contribute to viral persistence in chronic HBV infection[[23](#_ENREF_23)], the activation of host innate immune system by specific PRR agonists to overcome the immune suppressive effect of HBV, like TLR7 ligand GS-9620[[27](#_ENREF_27)], may be helpful in clearing HBV infection.

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