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REVIEW

Interplay between hepatitis B virus and the innate immune responses: implications for new therapeutic strategies

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Hepatitis B virus (HBV) infection is still a worldwide health problem; however, the current antiviral therapies for chronic hepatitis B are limited in efficacy. The outcome of HBV infection is thought to be the result of complex interactions between the HBV and the host immune system. While the role of the adaptive immune responses in the resolution of HBV infection has been well characterized, the contribution of innate immune mechanisms remains elusive until recent evidence implicates that HBV appears to activate the innate immune response and this response is important for controlling HBV infection. Here, we review our current understanding of innate immune responses to HBV infection and the multifaceted evasion by the virus and discuss the potential strategies to combat chronic HBV infection via induction and restoration of host innate antiviral responses.

KEYWORDS HBV; innate immunity; viral evasion; interferon; antiviral approaches

INTRODUCTION

Hepatitis B virus (HBV) infection is still a major health problem worldwide. Approximately 350 million people are chronically infected with HBV globally who are therefore at greater risk of liver cirrhosis and hepatocellular carcinoma development. The outcome of HBV infection varies widely among infected patients. Most of the adults with HBV infection will clear the virus spontaneously, but about 5% of individuals infected during adulthood and over 90% of infected infants and young children will develop chronicity. Currently, two subtypes of type I interferons (IFN) and five nucleos(t)ide analogues are approved for treating chronic hepatitis B (CHB), though each of the two classes of agents has obvious limitations, including the side effects, drug resistance, as well as the high cost (Kwon H, et al., 2011). Therefore, novel therapeutic approaches to this major clinical problem are urgently needed. A promising strategy is the development of immunotherapy for viral control.

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It is widely accepted that the adaptive immune responses play major roles in the clearance of HBV infection. However, the role of innate immunity during HBV infection appears not to be well understood, which can be attributed to the fact that the recruitment of patients in the very early, asymptomatic phase of HBV infection is very difficult (Bertoletti A, et al., 2012; Chang J, et al., 2012). With the development of cell culture and animal models, the temporal and spatial immunological changes and the importance of the innate immune responses during HBV infection are being gradually recognized, and meanwhile the numerous strategies employed by HBV to counteract the innate antiviral pathways are being better characterized.

THE ROLE OF INNATE IMMUNITY IN CONTROL OF HBV INFECTION

Viral infection of host cells triggers the antiviral innate immune responses. It is now known that many components of the virus, such as viral nucleic acids and viral proteins, can be initially sensed by the host innate pattern recognition receptors (PRRs), mainly including the toll-like receptors (TLRs) and the RIG-I-like receptors (RLRs). The subsequent activation of the PRR signaling pathways will lead to the induction of type I IFNs, proin-

flammatory cytokines and chemokines, which are not only important in controlling viral replication and spreading very early after infection, but also contribute to the maturation and recruitment of the more specific adaptive immune response.

HBV infects the host cells in an extremely efficient way that even a very small number of HBV virons (<10) are sufficient to establish infection and produce abundant progenies in hepatocytes *in vivo*. It was suggested that HBV can directly enter the hepatocytes or be internalized into liver endothelial cells (LSEC) and other sinusoidal cells through transcytosis, which might facilitate the viral infection by transporting the HBV particles to the host hepatocytes (Protzer U, et al., 2012). It has long been controversial whether HBV induces innate immune responses during the entry and expansion phases. In a prominent study using the chimpanzee model, HBV was described as a 'stealth virus' that did not activate the host innate immune system in the liver (Wieland S, et al., 2004). In agreement with this observation, Dunn et al. showed that circulating type I IFN was barely detectable throughout the early course of infection when analyzing a cohort of 21 acute HBV patients (Dunn C, et al., 2009).

However, the interpretation that HBV efficiently evades the innate immune recognition was challenged by many recent observations. It could be shown that HBV replication in HepaRG cell lines led to early activation of the innate antiviral response, resulting in a suppression of HBV replication. The physiological relevance of this observation remains uncertain, as an overexpression system based on recombinant baculoviruses was used (Lucifora J, et al., 2009). In uPA (urokinase-type plasminogen activator)/SCID (severe combined immunodeficiency) mice harboring human hepatocytes, baseline levels of human ISGs (interferon-stimulated genes) were slightly increased in the liver of HBV-infected mice when compared with uninfected ones (Lutgehetmann M, et al., 2011). Besides the hepatocytes, numerous innate immune cells reside in the livers. HBV was also shown to be recognized by those non-parenchymal cells, primarily Kupffer cells, which displayed an innate immune response that led to the expression of interleukin-6 (IL-6), but not type I IFNs, to restrain viral transcription (Hosel M, et al., 2009). A study in the woodchuck model also supports the hypothesis that HBV does not completely evade the innate immune recognition (Guy C S, et al., 2008). The infection with WHV (woodchuck hepatitis virus) significantly enhanced the intrahepatic transcription of IFN-γ and IL-12 as early as 3 hours post infection and the virus replication was significantly reduced before T cell activation (Guy C S, et al., 2008). Evidence from the clinic showed the early development of NK and NKT cell responses before the onset of adaptive T cell responses in acute hepatitis B, implicating that the innate immune system is able to sense HBV infection *in vivo* (Fisicaro P, et al., 2009). Taken together, these data suggest that some cells among the hepatic cell populations can sense and be activated by HBV infection, which enables the host innate immune system to detect and combat the invading virus. However, the PRRs involved in the innate immune responses during HBV infection and the viral components that trigger antiviral pathways require further investigation.

It has been reported that HBV capsids are recognized by TLR-2, although capsids are generally considered to be shielded by the viral envelope (Cooper A, et al., 2005). A recent study suggested that MDA5 was associated with HBV-specific nucleic acids and played a role in sensing HBV infection (Lu H L, et al., 2013). Another study using mouse models and primary human hepatocytes indicated that endoplasmic reticulum (ER)-associated endogenous antigenic lipids produced from the HBV-expressing hepatocytes could be sensed by the host NKT cells and led to the cell activation (Zeissig S, et al., 2012). Although these observations from cell and mouse models reflect some direct and indirect ways of host detection of HBV infection, the real situation in the virus infected patients needs to be assessed.

HBV EVASION OF THE INNATE IMMUNE RESPONSES

Since the innate immunity contributes to the viral clearance, it is not surprising that HBV has developed mechanisms to evade the innate immune responses (Revill P, et al., 2013).

The replication strategy of HBV is primarily thought to facilitate HBV evasion of innate immunity. After the virus is attached to the cells and internalized, the nucleocapsid is released and then the partially double-stranded viral rcDNA (relaxed circular DNA) in it is delivered to the nucleus, where the cccDNA (covalently closed circular DNA) is formed. The cccDNA serves as the template for the transcription of viral RNAs, which are then be capped and polyadenylated by host machinery. The transcripts are exported to the cytoplasm but the viral RNA-DNA chimeric replicative genome is sequestered within viral capsids (Nguyen D H, et al., 2008). Thus it seems that HBV is almost invisible to the innate sensing machinery during the whole life cycle and is hard to be eradicated due to the persistence of cccDNA in the nucleus of infected hepatocytes.

Meanwhile, increasing evidence suggests that HBV actively counteract the host innate immune responses through the viral encoded proteins, which are capable of engaging with many distinct components of innate immune signaling pathways (Table 1).

Table 1. HBV-encoded proteins that interferes with the innate immune signaling at multiple levels

Viral proteins	Cellular targets	References
HBs	TLR2/JNK/IL-12	(Wang S, et al., 2013)
	TLR4/ERK, NF-κB /IL-18	(Cheng J, et al., 2005)
	TLR9/IFN-α	(Vincent I E, et al., 2011; Xu Y, et al., 2009)
HBe	TLR2/MAL	(Lang T, et al., 2011; Visvanathan K, et al., 2007)
Polymerase	RIG-I, TLR3/TBK1, IKKε, DDX3/IFN-β	(Wang H, et al., 2010; Yu S, et al., 2010)
	MITA/IFN-β	(our unpublished data)
	IFN /JAK-STAT	(Chen J, et al., 2013; Foster G R, et al., 1991)
HBx	RIG-I, MDA5/MAVS/IFN-β	(Kumar M, et al., 2011; Wei C, et al., 2010)
Core/precore	IFN/MxA	(Fernandez M, et al., 2003)

HBsAg and HBeAg, the secretory proteins produced during the HBV replication cycle, are the markers of HBV infection and present in circulation at high levels. A possible explanation for the production of these proteins by the virus could be that they may have some immunomodulatory effects which may help the virus to evade the immune system. Indeed, it was reported that the level of plasma HBsAg correlated with the impaired TLR2 and TLR4 ligands-induced proinflammatory cytokine production in PBMCs of CHB patients (Chen Z, et al., 2008) and further studies showed that HBsAg was able to interfere with TLR-induced ERK, JNK, and NF-κB pathways in monocytes/macrophages (Cheng J, et al., 2005; Muller C, et al., 1990; Wang S, et al., 2013). HBsAg has also been shown to impair myeloid dendritic cell function (Op den Brouw M L, et al., 2009) and abrogate TLR9-mediated IFN-α production in plasmacytoid dendritic cells (pDCs) (Vincent I E, et al., 2011; Xu Y, et al., 2009). Moreover, decreased number and declined activation of the hepatic NK cells have been observed in murine chronic HBsAg carriers, which support the thesis that HBV can alter the activation status of different immune cells by manipulating negative regulatory pathways or suppressive cytokines (Han Q, et al., 2013). Some studies showed that HBsAg could be taken up by the macrophages and DCs; however, the molecular mechanism of the immunomodulatory effect of HBsAg on these cells remains to be determined (Wang Q, et al., 2013). Two studies described the interference of HBV secretory proteins HBeAg with TLR (Toll-like receptor)-mediated innate immune responses (Lang T, et al., 2011; Visvanathan K, et al., 2007). Moreover, TLR-mediated innate immune responses in both of the mouse parenchymal and non-parenchymal liver cells were found to be suppressed not only by the HBV viron particles, but also the HBeAg and subviral particles (Wu J, et al., 2009).

The HBV polymerase, a multifunctional protein with reverse-transcriptase activity, has been shown to inhibit RIG-I- and TLR3-induced IFN-β production via interaction with DDX3, which is thought to be a scaffold protein that can facilitate the activation of TBK1/IKKε and IRF3 (Wang H, et al., 2010; Yu S, et al., 2010). And the HBV nonstructural X protein was reported to interfere with the type I IFN induction by targeting MAVS (virus-induced signaling adaptor, also known as IPS-1 and VISA) (Kumar M, et al., 2011; Wei C, et al., 2010). A recent study showed that HBc also contributed to the early inhibition of IFN response by HBV (Gruffaz M, et al., 2013).

In addition to interfere with the PRR-mediated IFN production, HBV was also found to be able to inhibit IFN signal transduction in hepatoma cells and SCID-uPA mice with chimeric human liver cells (Christen V, et al., 2007; Lutgehetmann M, et al., 2011). The viral polymerase was suggested to be responsible for the HBV suppressed IFN-activated responses (Foster G R, et al., 1991) and directly interact with the protein kinase C-δ and importin-α5 to interfere with the IFN-induced phosphorylation of STAT1 and nuclear transportation of STAT1/STAT2 (Chen J, et al., 2013), while the HBV precore/core proteins were shown to interact with MxA promoter to down-regulate the IFN-induced MxA production (Fernandez M, et al., 2003).

Taken together, these studies demonstrated that the HBV-encoded proteins have various functions in inhibiting innate immune pathways, which may contribute to the establishment and maintenance of viral infection. However, the physiological relevance of most of the above findings should be further determined in more natural infection models.

INNATE IMMUNOMODULATORY STRATEGIES FOR THE TREATMENT OF HBV INFECTION

As discussed previously, a large body of work showing the multifaceted mechanisms used by HBV to suppress the innate immunity has accumulated in the past several years and has highlighted several potential targets for immunotherapeutic approaches in HBV infection (Figure 1).

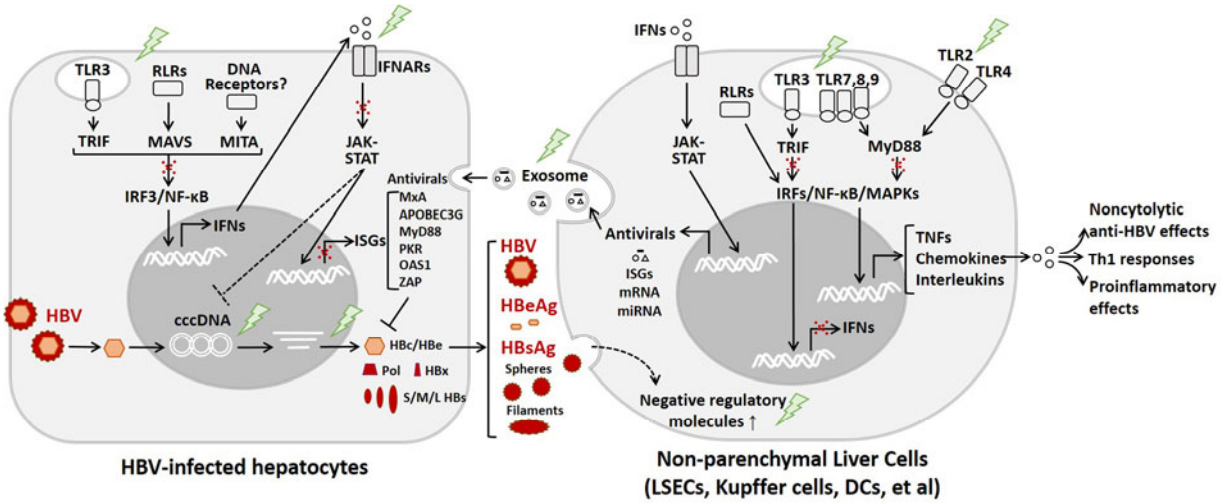

Figure 1. The innate immune signaling pathways manipulated by HBV and the potential targets for developing new therapies.

Overall, activation of the innate immunity using agonists of PRRs, and IFNs-based strategies has been proposed as therapeutic strategies for HBV infection. At the same time, to overcome the inhibitory effect of viral proteins on immune responses, it has been a direction to combine strategies that can efficiently inhibit viral replication and viral proteins expression with immunomodulatory approaches (Table 2).

PRR ligands and adaptors

Though HBV fails to activate or is able to inhibit PRR-mediated innate immune responses, activation of PRRs including TLRs and RLRs can not only induce a host innate immune responses, but also promote the adaptive immunity, and thus could represent a powerful therapeutic strategy for restoration of the suppressed antiviral responses and treatment of chronic HBV infection.

It was reported that intravenous injection of TLR3, 4, 5, 7, 9-ligands to HBV transgenic mice significantly inhibited intrahepatic HBV replication non-cytopathically within 24 h in an IFN-dependent manner (Isogawa M, et al., 2005) and TLR3, 4 agonists-activated innate immune responses of the nonparenchymal liver cells were able to control HBV replication (Wu J, et al., 2007). In addition, TLR2 signaling and its activated innate immune responses resulted in the reduction of HBV/WHV replication in HBV-expressing hepatoma cells and primary woodchuck hepatocytes (Zhang X, et al., 2012). The therapeutic efficacy of the TLR-7 agonist (GS-9620) in chimpanzees and woodchucks has been reported recently. Short-term oral administration of GS-9620 could provide long-term suppression of serum and intrahepatic HBV DNA, lead to the production of IFN-α, proinflammatory cytokines, chemokines and interferon stimulated genes (ISGs) and activate natural killer cells, and certain subsets of lymphocyte (Lanford R E, et al., 2013). Activation of RIG-I and PKR with 5' triphosphorylated RNA was also shown to promote HBV inhibition in cell and mice models (Ebert G, et al., 2011; Han Q, et al., 2011; Han Q, et al., 2011; Lan P, et al., 2013).

Besides the PRRs, some reports have demonstrated that overexpression of the PRRs-related adaptors including myeloid differentiation primary response gene 88 (MyD88), RIG-I/MDA5 adaptor, MAVS, or TIR-domain-containing adaptor-inducing IFN-β (TRIF) could dramatically inhibit HBV replication in human hepatoma cells (Guo H, et al., 2009; Li J, et al., 2010).

Although activation of PRRs appears to be effective to elicit an antiviral response against HBV, it may also lead to acute and chronic inflammation. Therefore, it is necessary to further identify the intracellular signaling and antiviral proteins responsible for control of the viral infection in order to selectively augment the antiviral responses and to limit the harmful inflammatory effects.

IFN-based inhibition of HBV infection

As key components of the innate immune system, IFNs have been demonstrated to restrict HBV replication by affecting multiple steps in the viral life cycle, including HBV RNA synthesis, pgRNA encapsulation, the turnover rate of viral proteins, and the epigenetic modulation of the cccDNA (Belloni L, et al., 2012; Guidotti L G, et al., 2002; Rang A, et al., 1999; Wieland S F, et al., 2000) etc. Considering the low response rate (30-40%) and the unwanted side effects of IFN-α therapy, optimization of the antiviral efficacy of IFNs and reduction of the adverse effects is a goal, which might be achieved through better understanding of the antiviral mechanisms of IFN-α. In fact, several IFN-stimulated proteins have been identified as effectors of IFN action that can specifically inhibit HBV replication. MxA has been shown to inhibit HBV replication through suppression of the

Table 2. New control strategies for HBV infection by activating PRRs or inhibiting viral replication

Strategies		Anti-HBV effects	References
TLR ligands	P2C/P3C (TLR2)	Inhibits viral replication in vitro and *in vivo*	(Zhang X, et al., 2012)
	poly(I:C) (TLR3)	Reduces the levels of viral DNA, HBeAg and HBsAg in vitro and *in vivo*	(Isogawa M, et al., 2005; Wu J, et al., 2007)
	GS-9620 (TLR7)	Induces prolonged suppression of HBV in chronically infected chimpanzees	(Lanford R E, et al., 2013)
RLR ligands	5'-triphosphate RNAs	Controls replication of hepatitis B virus	(Ebert G, et al., 2011; Han Q, et al., 2011; Han Q, et al., 2011; Lan P, et al., 2013)
PRR adaptors	MyD88	Reduces the HBV mRNA and DNA	(Guo H, et al., 2009; Li J, et al., 2010)
	TRIF		
	MAVS		
IFN-related approaches	anti-HBV ISGs	MxA: suppresses the nucleocytoplasmic export of viral mRNA	(Gordien E, et al., 2001)
		APOBEC3G: inhibits viral replication in deaminase activity-dependent and independent manners	(Nguyen D H, et al., 2007; Turelli P, et al., 2004)
		ZAP: down-regulates the viral RNA	(Mao R, et al., 2013)
	Exosomes containing antiviral factors	Exosomes from nonparenchymal liver cells contributes to the IFN-induced antiviral response to HBV and restores the antiviral state in HBV-infected cells	(Li J, et al., 2013)
	Type III IFNs	Induces an intracellular IFN-α/β-like antiviral response through a receptor complex distinct from the IFN-α/β receptor	(Pagliaccetti N E, et al., 2010; Robek M D, et al., 2005)
Sequence-specific silencing	RNAi	Inhibits the viral gene expression	(Chen Y, et al., 2008; Meng Z, et al., 2008)
	ZFP/ZFN	Reduces the transcriptional activity of the viral genome	(Cradick T J, et al., 2010; Zimmerman K A, et al., 2008)
	TALEN	Inactivates the viral replication, cleaves the viral DNA in a site-specific manner	(Chen J, et al., 2013)
Combination Therapies	RIG-I ligands + siRNA	Controls HBV replication more efficiently	(Ebert G, et al., 2011; Han Q, et al., 2011)
	TALEN + IFN	Results in an enhanced antiviral effect *in vitro*	(Chen J, et al., 2013)
	Antivirals + vaccination	Elicits sustained immunological control of chronic hepadnaviral infection *in vivo*	(Kosinska A D, et al., 2013)

nuclear export of viral mRNA via the PRE sequence (Gordien E, et al., 2001). APOBEC3G, an IFN-inducible single-stranded DNA cytidine deaminase (AID), efficiently inhibited HBV replication by both cytidine deamination-dependent and -independent mechanisms (Nguyen D H, et al., 2007; Turelli P, et al., 2004). ZAP is an intrinsic host antiviral factor with activity against HBV through down-regulation of viral RNA (Mao R, et al., 2013). Since it has been long recognized that the resolution of HBV infection depends on both destruction of HBV-infected hepatocytes by cytotoxic T lymphocytes and non-cytopathic process which is probably mediated by IFNs, TNFs and other proinflammatory cytokines (Guidotti L G, et al., 1999). Further investigation on noncytolytic mechanisms involved in control of HBV, particularly the elimination of cccDNA, is extremely necessary.

A very recent study sheds light on the mechanisms of cell-to-cell transmission of IFN-α-induced antiviral immunity (Li J, et al., 2013). IFN-α was found to be able to induce non-parenchymal cells of the liver, such as macrophages, lymphocytes and liver sinusoidal endothelial cells (LSECs) to release exosomes that are loaded with antiviral molecules. These exosomes were able to be internalized by the neighboring hepatocytes, thereby enabling the transmission of viral resistance. As the virus

is hard to evolve different strategies to all of the antiviral packaging in the exosomes, the delivery of antiviral molecules via exosomes could therefore be a safer and more effective treatment option for chronic HBV infection. From this study, it can be speculated that the PRRs-activated intrahepatic antiviral responses are also associated with the non-parenchymal liver cells as well as the exosomes. Hence, targeted delivery of PRR agonists to activate LSECs and Kupffer cells will offer great promise for the treatment of chronic hepatitis B.

In addition to the type I IFNs, IFN-λ is also able to inhibit HBV replication. Besides, it has the potential of reduced adverse effects since it signals through a distinct receptor complex consisting of IL-10R b and IL-28Ra, whose expression is restricted to certain cells, unlike the widely distributed type I IFN receptors (IFNAR1 and IFNAR2) (Pagliaccetti N E, et al., 2010; Robek M D, et al., 2005).

In summary, the better determined antiviral mechanisms and the application of new subtypes of IFNs might ultimately lead to more efficacious and acceptable IFN-based treatments for chronic hepatitis B.

Strategies to control HBV replication and combination therapy

As discussed in the previous sections, HBV can actively suppress the innate immunity through different viral proteins. Therefore, it is also important to develop antiviral strategies that would lead to the restoration of immune responses via the down-regulation of viral genomes and proteins and it could be of advantage to combine antiviral and immunomodulatory approaches in order to increase the anti-HBV efficacy (Zoulim F, 2012).

RNA interference (RNAi)-based technology including *in vitro*-synthesized siRNA, microRNA (miRNA), and endogenously expressed small hairpin RNA (shRNA) to target genes has been suggested to be a potentially rational therapy for HBV infection, although significant challenges including the low delivery efficacy, poor RNA stability, and off-target effects need to be overcome before these can be successfully translated (Chen Y, et al., 2008; Meng Z, et al., 2008). Recently, many techniques have been developed for selectively targeting DNAs. Therefore, there has been a new focus on directly targeting the genomes of DNA virus to eradicate the chronic viral infection. Designed ZFPs that bound to the duck hepatitis B virus (DHBV) DNA resulted in significant reductions in viral RNAs, proteins, and progenies in cell culture (Zimmerman K A, et al., 2008) and designed ZFNs that directly targeted the HBV cccDNA had an activity on site-specific cleavage, which led to a decrease in pregenomic RNA levels (Cradick T J, et al., 2010). Our recent work showed that TALEN, another kind of engineered enzymes that can cleave sequence-specific DNA targets with lower cytotoxic than ZFN, specifically targeted and successfully inactivated the HBV genome (Chen J, et al., 2013). However, these technologies also face the problem of delivery.

Regarding the investigation on the combination therapy, two independent groups showed that 5'-triphosphorylated HBV-specific siRNAs which could on one hand activate RLRs-mediated innate antiviral immune responses and on the other hand directly silence the viral RNA showed higher efficiency in controlling HBV replication than RIG-I agonists alone (Ebert G, et al., 2011; Han Q, et al., 2011). Similarly, TALEN restored HBV suppressed IFN-stimulated response element (ISRE)-directed transcription, which may contribute to the synergistic effect of TALEN and IFN-α on controlling HBV replication (Chen J, et al., 2013). A recent report showed that antiviral treatment plus DNA vaccination led to sustained immunological control of chronic WHV infection in woodchucks (Kosinska A D, et al., 2013). Another report, also based on the woodchuck model, proposed that TLR2-mediated antiviral effects might be enhanced by combination with antiviral treatment since entecavir (ETV) administration restored TLR2 expression in infected cells (Zhang X, et al., 2012). All of these studies may give rise to new promising therapeutic strategies that involve combination treatment.

All in all, we believe that better investigating models of HBV and detailed analyses of clinical samples will continuously deepen our understanding of the interplay between HBV and the host innate immune responses and will further lead to the development of novel innate immunity-based antiviral approaches and optimization of combination therapy regimens of HBV.

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AUTHOR CONTRIBUTIONS

JL Chen drafted the manuscript. ZH Yuan conceived and revised the manuscript.

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