Antiviral Research 96 (2012) 405–413

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Review

The innate immune response to hepatitis B virus infection: Implications for pathogenesis and therapy

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ARTICLE INFO

Article history:
Received 12 August 2012
Revised 2 October 2012
Accepted 5 October 2012
Available online 13 October 2012

Keywords:
Hepatitis B virus
Pattern recognition receptors
Toll-like receptors
Interferons
TLR7 agonists

ABSTRACT

Pattern recognition receptor (PRR)-mediated innate immune responses play an essential role in defending the host from viral infections. Intriguingly, hepatitis B virus (HBV) has been shown to induce negligible innate immune responses during the early phase of infection. Whether this is due to the failure of the virus to activate PRRs or suppression of PRR signaling pathways by the virus remains controversial. However, a plethora of evidence suggests that HBV is sensitive to PRR ligand-induced antiviral responses. This review summarizes current understanding of the interaction between HBV and PRR-mediated host innate immunity, antiviral mechanisms of PRR responses against HBV and strategies to combat chronic HBV infection *via* induction of host innate antiviral responses.

Contents

1. Introduction

The outcome of hepatitis B virus (HBV) infection, as well as the severity of HBV-induced liver diseases, varies widely among infected individuals. While most adulthood HBV infections are transient, approximately 5% of infected adults and over 90% of infected neonates fail to resolve the infection and evolve to chronicity (McMahon, 2005). Patients with chronic hepatitis B are at increased risk of developing severe liver diseases, including cirrhosis and hepatocellular carcinoma (Lee, 1997; Lok, 2004). Other than the occasional appearance of ground-glass hepatocytes in chronic HBV carriers, which is presumably due to the accumulation of large envelope protein in the endoplasmic reticulum, HBV infection of hepatocytes induces negligible cytopathic effect (Wang et al., 2003). It is, therefore, generally believed that the outcome of HBV infection and the severity of associated liver diseases are

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http://dx.doi.org/10.1016/j.antiviral.2012.10.001

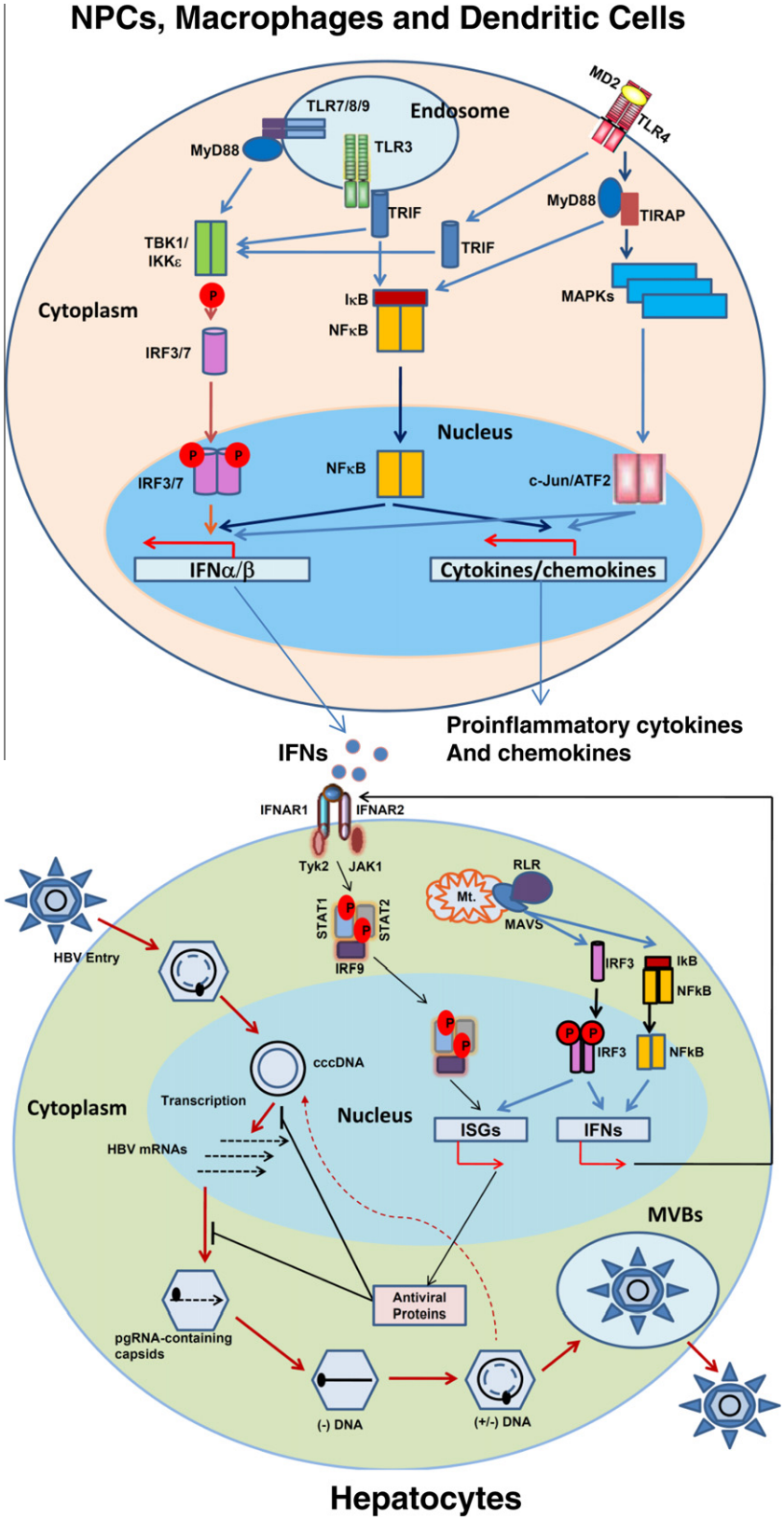

Fig. 1. Molecular pathways of TLR- and RLR-mediated innate immunity against HBV. Activation of TLRs in macrophages, dendritic cells or liver nonparenchymal cells (NPCs) by their cognate ligands induces the production of type I IFNs, proinflammatory cytokines and chemokines. Type I IFNs bind to their receptors to trigger the JAK–STAT signaling pathway, inducing the expression of ISGs, which limit HBV replication *via* inhibition of cccDNA transcription and encapsidation of HBV pgRNA. Activation of RLRs in hepatocytes induces IFNs and proinflammatory cytokines, and also activates intracellular antiviral pathways *via* NFκB and IRF3, inducing antiviral proteins to disrupt HBV replication by targeting multiple steps of the viral life cycle. Mt: mitochondria. See text for other abbreviations.

determined by the nature and strength of host immune responses against the virus (Chisari and Ferrari, 1995; Guidotti and Chisari, 2006).

Viral infections are initially sensed by host innate pattern recognition receptors (PRRs), including toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors, C-type lectins and others. As illustrated in Fig. 1, activation of PRRs by viral components, such as viral nucleic acids, oligomers of envelope proteins and nucleocapsids induces cellular responses leading to production of type I interferons (IFN), proinflammatory cytokines and chemokines. These cytokines and chemokines control viral replication and spreading before the onset of more specific and powerful adaptive immune responses (Takeuchi and Akira, 2010). The PRR-mediated early innate immune response is also required for orchestrating the activation and development of the adaptive immune response, which ultimately resolves viral infections and provides long-term protection against subsequent infection (Watts et al., 2010).

In the case of HBV infection, compelling evidence suggests that humoral and cell-mediated immune responses are required for efficient and persistent control of viral infection, and that they also serve as the driving force for liver injury and disease progression (Bertoletti and Ferrari, 2011; Guidotti and Chisari, 2006). However, due to the lack of cell culture and animal models supporting efficient HBV infection, the nature and role of PRR-mediated innate immune responses in HBV infection and pathogenesis remain to be understood (Bertoletti et al., 2010).

2. Lack of evidence for PRR-mediated innate immune responses during the early phase of HBV infection

Due to the prompt recognition of viral components by host cellular PRRs, induction of type I interferon and other inflammatory cytokines is a hallmark of viral infections. Accordingly, up-regulation of IFN-stimulated genes (ISGs) in cultured cells and virally infected tissues *in vivo* has been demonstrated by microarray studies to be one of the most common transcriptome signatures of many distinct viral infections (Katze et al., 2008; Su et al., 2002). However, HBV infection appears to be an exception. Using an acute HBV-infected chimpanzee model, Chisari and colleagues have reported that HBV fails to induce transcription of any cellular genes that relate to the entry and expansion of the virus, implicating the lack of PRR-mediated innate cytokine response during the early phase of HBV infection (Wieland et al., 2004). In agreement with this observation, a clinical study of the early phase of acute HBV infection failed to detect the induction of either type I or type III interferons in the sera of patients (Dunn et al., 2009; Fisicaro et al., 2009).

However, results obtained from studies performed with recently developed HBV cell culture and animal models challenge these early observations. For instance, Zoulim and colleagues reported that transduction of HepaRG cells with a baculovirus vector expressing HBV pregenomes resulted in massive activation of the innate antiviral response, leading to a significant inhibition of HBV DNA synthesis (Lucifora et al., 2010). Moreover, HBV infection of uPA-SCID mice induced ISG expression in human hepatocytes, which apparently limited HBV spread during the early phase of the infection (3 weeks postinfection). However, HBV ultimately breaks the containment and spreads into almost every human hepatocyte by approximately 6 weeks postinfection (Volz et al., 2011). Beside hepatocytes, upon infection of primary human liver cells, HBV is recognized by nonparenchymal cells, primarily Kupffer cells, which mount an innate immune response leading to production of IL-6 and other inflammatory cytokines, but not type I IFNs (Hosel et al., 2009). However, in all the above experimental conditions, the viral components as well as the PRRs involved in the activation of innate immune responses remain to be determined.

Taken together, the evidence obtained thus far suggests that HBV does not efficiently induce a PRR-mediated innate cytokine response in its natural hosts during the early phase of infection. However, the possibility that the virus activates PRR pathways in infected hepatocytes and nonparenchymal liver cells under certain circumstances to limit viral replication could not be ruled out. In addition, it is worth noting that HBV does activate other branches of innate immune responses, such as natural killer (NK) cells and NKT cells, although it is compromised by the virus-induced immunosuppressive cytokine IL-10, which is also a member of the interferon superfamily and is produced by dendritic cells and/or activated CD4 T lymphocytes (Dunn et al., 2009; Zeissig et al., 2012; Zhang et al., 2011).

3. Evidence suggesting that HBV inhibits PRR and IFN signal transduction

Failure to detect the induction of PRR-mediated innate immune response in HBV infected livers could be due to either the inability of HBV to activate PRRs or to active inhibition of PRR signaling pathways by the virus. An example of the latter scenario is illustrated in a recent study showing that human immunodeficiency virus-1 (HIV-1) can be recognized by PRRs within host cells to trigger a robust innate immune response, and this response is only unmasked in the absence of the viral protein vpu, which targets IRF3 for degradation (Doehle et al., 2012). In fact, several lines of evidence suggest that HBV proteins are capable of engaging with many distinct components of innate immune response pathways. First, two independent studies suggest that over-expression of the HBV polymerase in human hepatoma cells efficiently inhibits IRF3 activation by interacting with host RNA helicase DDX3, which is essential for the activation of TBK1/IKKε, the kinases that phosphorylate IRF3 (Wang and Ryu, 2010; Yu et al., 2010). Second, expression of the HBx protein in human hepatoma cells inhibited dsRNA-, dsDNA- and virus-induced IFN-β gene expression by promoting the decay of mitochondrial antiviral signaling protein (MAVS) , the adaptor of RIG-I and MDA5 receptors (Fig. 1) (Kumar et al., 2011; Wei et al., 2010). Third, although the exact molecular targets were not identified, Wu et al. reported that HBV virion particles, subviral particles and soluble HBeAg could efficiently inhibit TLR3 agonist-induced antiviral responses, which correlated with suppression of IFN-β production and the subsequent induction of ISGs, as well as suppressed activation of IRF-3, NF-κB and ERK1/2 (Wu et al., 2009). Fourth, a clinical study suggested that expression of TLR2 on hepatocytes and Kupffer cells was reduced in HBeAg-positive chronic hepatitis B patients, in comparison with HBeAg-negative patients and healthy controls. Those authors also observed that the presence of HBeAg significantly inhibited the induction of TNF-α by hepatoma cells transduced with baculovirus vector-expressing HBV (Visvanathan et al., 2007). Along this line, it was also reported that HBV and HBsAg preferentially abrogated TLR9, but not TLR7, agonist-induced IFN-α production in plasmacytoid dendritic cells (Vincent et al., 2011; Woltman et al., 2011). Finally, in addition to inhibiting IFN production, HBV has been shown in cultured hepatoma cells and in the livers of SCID-uPA mice to inhibit IFN signal transduction by either preventing STAT1 nuclear import or accelerating the dephosphorylation of nuclear STAT1 (Christen et al., 2007a,b; Lutgehetmann et al., 2011).

While the studies described above suggest that multiple HBV-encoded proteins are capable of inhibiting distinct innate immune pathways, most of the experiments were performed either under the conditions of overexpression of a single virus protein or in cell types that are not normally infected by HBV. Hence, it is important to determine whether or not HBV inhibits PRR and IFN responses in the context of viral replication in human hepatocytes during a

natural infection. Despite the failure to mount an efficient immune response to clear HBV infection, the majority of HBV carriers do not manifest increased susceptibility to infection by other microorganisms. Accordingly, the pathobiological significance of the observed inhibition of HBV on the function of dendritic cells needs to be interpreted with caution.

4. Activation of PRR-mediated innate immune responses potently inhibits HBV replication

Regardless of the fact that HBV fails to activate or inhibits PRR-mediated innate immune responses, there is overwhelming evidence that HBV replication can be potently inhibited through experimental activation of multiple PRRs. For example, it was shown that HBV transgenic mice receiving a single-dose intravenous injection of ligand(s) specific for TLR2, TLR3, TLR4, TLR5, TLR7 or TLR9 noncytolytically inhibited HBV replication in a type-I IFN receptor-dependent manner (Isogawa et al., 2005). However, treatment of an immortalized murine hepatocyte cell line derived from HBV transgenic mice (HBV-Met) with the TLR ligands did not inhibit HBV replication. The latter observation was consistent with the finding that the murine hepatocytes did not express sufficient amounts of TLRs and were thus nonresponsive to TLR ligands (Isogawa et al., 2005). Accordingly, the observed antiviral effects of TLR agonists in transgenic mice *in vivo* are most likely due to the activation of antiviral cytokine production in liver nonparenchymal cells or extrahepatic cells that express high levels of TLRs. In fact, incubation of HBV-Met cells with conditioned medium harvested either from TLR3 or TLR4 ligand-treated murine Kupffer cells or from TLR3 ligand-treated liver sinusoidal endothelial cells (LSECs) efficiently inhibited HBV replication. Antiviral activity was completely neutralized by an antibody against IFN-β (Wu et al., 2007). While this study suggests that liver-resident KCs and LSECs may mediate the antiviral effects of TLR3 and/or TLR4 ligands, the cell types that mediate the antiviral effects of other TLR ligands remain unknown. Interestingly, our recent studies indicate that treatment of an immortalized murine hepatocyte cell line supporting HBV replication (AML12HBV10) with conditioned media from the murine macrophage cell line, RAW264.7, treated with the ligands specific to TLR1/2, TLR3, TLR4, TLR5, TLR7 or TLR9, inhibits HBV replication (Xu et al., 2010) (Chang et al., unpublished results). As illustrated in Fig. 1, these observations imply that activation of multiple TLRs in either liver-resident nonparenchymal cells or extra-hepatic macrophages and dendritic cells can efficiently inhibit HBV replication in hepatocytes through secretion of type I IFNs, and probably also other cytokines.

Besides TLRs, activation of RLRs also induces a strong antiviral response against HBV, as highlighted by two recent studies (Ebert et al., 2011; Han et al., 2011). Both groups demonstrated that 5′-triphosphorylated siRNA targeting HBV mRNA controlled HBV replication more efficiently and for longer periods of time than 5′-triphosphorylated siRNA without silencing capacity or siRNA that targeted identical HBV sequences, but did not contain 5′-triphosphate, in human hepatoma cells and in HBV-transgenic mice *in vivo*. These observations imply that HBV-specific 5′-triphosphorylated siRNAs activate a RIG-I-mediated innate immune response as well as directly silencing HBV mRNAs to inhibit viral replication.

5. How does the PRR-mediated innate response inhibit HBV infection?

5.1. IFN-induced antiviral response targets multiple steps of HBV replication

Type I IFNs, represented by IFN-αβ, are produced by all types of nucleated cells in response to virus infection, *via* activation of PRRs. IFN-γ is the sole Type II IFN and is produced by activated T lymphocytes and NK cells. Resolution of HBV and other animal hepadnavirus infections *in vivo* depends on both killing of infected hepatocytes by viral antigen-specific cytotoxic T lymphocytes and noncytolytic suppression of viral replication, which is most likely mediated by IFNs and other proinflammatory cytokines, such as TNF-α (Guidotti et al., 1994, 1996, 1999; Guo et al., 2000; Jilbert et al., 1992; Kajino et al., 1994; Summers et al., 2003).

As illustrated in Fig. 1, IFNs elicit an antiviral response by binding to their cognate receptors, which trigger a signaling cascade (the JAK–STAT signaling pathway) leading to expression of IFN-stimulated genes (ISGs), whose products exhibit antiviral effects (Jiang et al., 2008; Sadler and Williams, 2008; Stark et al., 1998). However, studies with mice deficient in IFN regulatory factor-1 (IRF-1), the double-stranded RNA-activated protein kinase (PKR), or RNase L demonstrated that the three well-characterized ISGs did not mediate the antiviral response of IFNs against HBV (Guidotti et al., 2002). In order to identify ISGs that mediate the post-transcriptional inhibition of HBV replication by IFN-α and IFN-γ, we systematically tested 37 ISGs, that are highly induced by the cytokines in hepatocytes, for their ability to inhibit HBV replication in human hepatoma cells. We found that indoleamine 2, 3-dioxygenase (IDO), an IFN-γ-induced enzyme catalyzing tryptophan degradation, efficiently reduced the level of intracellular HBV DNA, without altering the steady state level of viral RNA (Mao et al., 2011). In addition, expression of the apolipoprotein B mRNA-editing enzyme catalytic polypeptide 3 protein G (APOBEC3G), an IFN-inducible cytidine deaminase specific for single-stranded DNA, in HepG2 cells also efficiently inhibited viral DNA replication (Rosler et al., 2005). The antiviral function of APOBEC3G depends on its incorporation into replication-competent HBV nucleocapsids, in a fashion that is dependent on both the viral reverse transcriptase (RT) and viral RNA packaging signal epsilon, a stem-loop structure situated near the 5′ end of the HBV pregenomic (pg) RNA (Nguyen and Hu, 2008). While the encapsidated APOBEC3G deaminates dC to dU in nascent minus-strand viral DNA, resulting in G-to-A hypermutation in the plus-strand DNA (Baumert et al., 2007), it also interacts with RT to directly inhibit viral DNA replication in a deaminase activity-independent manner (Nguyen et al., 2007). A recent clinical study demonstrated that up to 35% of HBV genomes in cirrhotic livers were edited by APOBEC3 family enzymes, suggesting they play a restrictive role in HBV infection (Vartanian et al., 2010).

While the complete abolition of IFN-γ-induced inhibition of HBV replication by tryptophan supplementation suggests that IDO is a primary mediator of the IFN-γ induced antiviral response against HBV (Mao et al., 2011), suppression of APOBEC3B, APOBEC3F and APOBEC3G expression by RNA interference and HIV virion infectivity factor (vif)-mediated degradation do not abrogate the inhibitory effect of IFNs on HBV (Jost et al., 2007). Moreover, the known antiviral activities of IDO and APOBEC3 family members cannot explain the observed inhibition of IFNs on cccDNA transcription (Belloni et al., 2012) and interference with pgRNA-containing nucleocapsid formation and stability (Guo et al., 2003; Schultz et al., 1999; Wieland et al., 2005; Xu et al., 2010). While two independent studies showed that IFN-induced reduction of pgRNA-containing nucleocapsids required proteasome activity (Robek et al., 2002; Xu et al., 2010), a direct role of IFN-inducible proteasome subunits LMP2 and LMP7 in the antiviral response could not be confirmed (Robek et al., 2007). Therefore, further investigation on IFN-induced cellular pathways responsible for the inhibition of cccDNA transcription, pgRNA encapsidation and nucleocapsid decay is warranted.

5.2. TNF-a disrupts HBV nucleocapsid formation

In addition to type I IFNs, other proinflammatory cytokines and chemokines play essential roles in controlling HBV infection.

Particularly, studies using HBV transgenic mice, WHV-infected woodchucks and HBV-infected chimpanzees consistently demonstrated that intrahepatic expression of TNF-α is associated with noncytolytic inhibition of hepadnavirus replication and resolution of a transient infection (Guidotti et al., 1996, 1999; Guo et al., 2000). Moreover, recent clinical studies suggest that anti-TNF-α therapies for patients with rheumatic, digestive, and dermatologic autoimmune diseases have been associated with frequent reactivation of HBV replication in inactive carriers and liver damage in patients with chronic HBV infection (Cassano et al., 2011). Studies in HBV genome-transfected HepG2 cells demonstrated that TNF-α interfered with HBV nucleocapsid formation and stability *via* activation of the NF-κB pathway (Biermer et al., 2003; Puro and Schneider, 2007). A recent study further revealed that TNF-α induced the expression of cellular inhibitor of apoptosis protein 2 (cIAP2), which in turn accelerated the ubiquitin-proteosome-mediated degradation of the HBV polymerase and thus prevented the encapsidation of pregenomic RNA (Wang et al., 2011).

5.3. Activation of PRRs induces intracellular antiviral pathways

In addition to inducing IFNs and proinflammatory cytokines, activation of TLRs and RLRs also stimulates the expression of many other cellular genes, including genes with antiviral functions. It is, therefore, possible that activation of PRRs in HBV-infected hepatocytes may induce intracellular antiviral responses that inhibit viral replication in a cytokine-independent manner. In fact, we have demonstrated that activation of the PRR response *via* over-expression of TLR adaptors, myeloid differentiation primary response gene 88 (MyD88), TIR-domain-containing adaptor-inducing interferon-β (TRIF), or RIG-I/MDA5 adaptor, MAVS, in human hepatoma cells dramatically reduced the levels of HBV mRNA and DNA (Guo et al., 2009). However, HBV replication was not significantly affected by treatment of HBV genome-transfected cells with culture media harvested from cells transfected with each of the 3 adaptors. This observation indicates that the adaptor-induced antiviral response is predominantly mediated by intracellular factors, rather than secreted cytokines. While activation of NFκB is essential for the adaptors to elicit an antiviral response against HBV, the intracellular antiviral proteins that post-transcriptionally down-regulate HBV mRNA remain to be identified.

6. Activation of innate immune response as therapeutic approaches for chronic hepatitis B

Viral infection only becomes clinically relevant when the viral offense overcomes the defense of host immunity, often through immune evasion mechanisms that suppress the activation of innate and adaptive immune responses. Hence, restoration of functional host innate and adaptive antiviral immunity should ultimately resolve persistent viral infections. Accordingly, activation of PRR-mediated innate immunity as well as subsequent induction of adaptive antiviral responses against HBV has been considered to be potentially curative approaches for chronic hepatitis B. Currently, IFN-α, the primary mediator of PRR-mediated antiviral innate responses, and PRR agonist therapies are the two major approaches toward restoration of host innate immunity and rejuvenation of adaptive immune responses against HBV infection. To overcome the side effects of indiscriminate activation of a broad-spectrum inflammatory response induced by PRR agonists, selective induction of specific antiviral pathways and intrahepatic delivery of PRR agonists are new directions of innate immunity activation therapy against chronic hepatitis B.

6.1. Interferon therapy

As one of the primary antiviral cytokines mediating innate immune control of HBV infection, IFN-α is the only approved therapy for chronic hepatitis B with immunomodulatory as well as viral inhibitory properties (Perrillo, 2009). Compared to nucleoside analogues, which inhibit the HBV DNA polymerase, the advantages of IFN-α therapy include the lack of emergence of drug-resistant viruses, a finite and defined treatment course, and a higher likelihood of HBsAg clearance. A standard 48-week course of pegylated IFN-α therapy induced HBeAg seroconversion in about one-third of HBeAg-positive patients and a lasting biochemical and virological response in approximately 40% of HBeAg-negative patients (Lau et al., 2005; Marcellin et al., 2009). Moreover, long-term follow-up of virological responders has demonstrated a progressive increase in the rate of HBsAg clearance, particularly in patients who were initially HBeAg-positive (Buster et al., 2008). Hence, despite the side effects which make it a challenging therapeutic option, pegylated IFN-α is still recommended as a first-line therapy for chronic hepatitis B patients who have higher baseline ALT levels and lower baseline HBV DNA concentrations (Perrillo, 2009).

Although combination therapy with IFN-α and ribavirin significantly increases the rate of sustained virologic response in chronic hepatitis C, ribavirin does not improve the therapeutic efficacy of IFN-α against HBV (Liu et al., 2006; Rijckborst et al., 2010). Considering the curative potential of IFN-α therapy, the development of drugs that enhance the antiviral efficacy and/or reduce the adverse effects of IFN-α in chronic hepatitis B patients ought to be an important direction. Thus far, there is no reported research effort toward this goal. However, better understanding the antiviral mechanism of the cytokine *in vivo* will provide clues on the potential targets of drugs with the expected pharmacological activity.

In addition to the type I IFNs, IFN-λ, or type III IFNs, are also produced upon activation of multiple PRRs (Onoguchi et al., 2007). However, IFN-λ signals through a distinct receptor complex consisting of IL-10Rβ and IL-28Rα, rather than IFNAR1 and IFNAR2 used by type I IFNs (Onoguchi et al., 2007; Osterlund et al., 2007). Interestingly, although neither the cytokines nor the receptors display significant sequence similarity, type I and type III IFNs share the same post-receptor signaling components. Not surprisingly, type III IFNs trigger a type I IFN-like ISG expression profile and inhibit a variety of viruses, including HBV, in cultured cells and *in vivo* (Alexopoulou et al., 2001; Ank et al., 2006; Bartlett et al., 2005; Marcello et al., 2006; Pagliaccetti et al., 2010; Robek et al., 2005). Recently, it was also demonstrated in multicenter clinical studies that polymorphisms near the IFN-λ3 (IL28B) gene were independently associated with HBeAg seroconversion at the end of pegylated IFN-α therapy and HBsAg clearance during long-term follow-up in patients with chronic hepatitis B, suggesting that IFN-λ3 could play a role in HBV pathobiology and the therapeutic response to exogenous IFN-α (Lampertico et al., 2012; Sonneveld et al., 2012).

Because pegylated IFN-α therapy is frequently associated with adverse effects, including flu-like symptoms, fatigue, depression, anxiety, and bone marrow suppression, which results in anemia, neutropenia, and thrombocytopenia (Perrillo, 2009), an IFN with potent antiviral effects and a more favorable side-effect profile would dramatically improve the treatment of chronic hepatitis B. Unlike the widely distributed type I IFN receptor, expression of the type III receptor is restricted to epithelial cells, but is either not expressed or expressed at low level in fibroblasts, microvascular endothelial cells, adipocytes, primary central nervous system cells or hematopoietic cells, with the exception of B lymphocytes (Donnelly and Kotenko, 2010; Kelly et al., 2011). The limited distribution of the type III IFN receptor suggests that IFN-λ-based therapy has a potential for reduced adverse effects along with

preservation of the antiviral effect against HBV. In supporting this prediction, recent clinical studies revealed that pegylated IFN-λ1 therapy indeed had a reduced frequency and severity of IFN-α-type side effects, but significantly reduced the viral load across a broad range of doses in patients with chronic HCV infection (Muir et al., 2010). Comparison of antiviral efficacy and safety profiles between pegylated IFN-α and IFN-λ1 in patients with chronic hepatitis B is currently under way.

In summary, the application of new types of IFNs and the development of drugs that enhance the antiviral efficacy and/or improve the safety profiles of these cytokines may eventually lead to more efficacious and tolerable IFN therapy for chronic hepatitis B.

6.2. TLR and RLR agonists

It is now well documented that activation of TLRs and RLRs not only induces the innate antiviral response to limit viral replication and spread, but also modulates the activation of adaptive immune response by the viruses. Not surprisingly, a significant amount of effort has been devoted to discovering and developing TLR and RLR agonists as antiviral agents, as well as adjuvants for preventive and therapeutic vaccination against viral diseases (Kanzler et al., 2007). The potential of TLR and RLR agonists to induce a host antiviral response that not only inhibits HBV replication, but also restores the adaptive antiviral immune response to ultimately control HBV infection, is especially attractive. As mentioned in previous sections, treatment of HBV-transgenic mice with agonists of multiple TLRs, as well as RLRs, potently inhibited HBV replication. However, only TLR3 and TLR7 agonists have thus far been explored as potential therapeutic agents against HBV.

Synthetic double-stranded RNA, polyribosinic:polyribocytidic acid (poly I:C), was initially identified by Field and colleagues as a strong IFN inducer (Field et al., 1967). Two modified versions of polyI:C were developed in the 1970s to reduce toxicity and improve biological profiles, which included a mismatched double-stranded RNA, poly I:C_{12}U or Ampligen (Carter et al., 1972), and a polylysine and carboxymethylcellulose-modified polyriboinosinic: polyribocytidylic acid (polyICLC) (Levy et al., 1975). When TLRs were discovered in the late 1990s, it was soon recognized that poly I:C was an agonist of TLR3 (Alexopoulou et al., 2001) and RLRs (Kato et al., 2008). However, Ampligen appeared to have exquisite specificity for TLR3 (Nicodemus and Berek, 2010). Early studies showed that treatment of chronically HBV-infected chimpanzees with polyICLC transiently reduced the levels of serum viral DNA, HBsAg and HBeAg (Purcell et al., 1976). Transient suppression of DHBV in ducks by Ampligen was also observed in two independent studies (Ijichi et al., 1994; Niu et al., 1993). Consistent with the proposed antiviral mechanism, IFN-like activity was detected in the sera of Ampligen-treated ducks.

GS-9620, a potent and selective oral small-molecule agonist of TLR7, is currently being developed for the treatment of chronic hepatitis B and C by Gilead Sciences, Inc. Four-week treatment with GS-9620 of woodchucks chronically infected with WHV resulted in a greater than 4-log reduction of viral load in all treated animals. Intriguingly, the suppressive effect was sustained after cessation of treatment, and antibody against the surface antigen of WHV became detectable in a subset of woodchucks (Menne et al., 2011). Furthermore, GS-9620 treatment for 8 weeks of chimpanzees chronically infected with HBV also reduced viral load by more than 2 logs and resulted in a 50–61% reduction in HBsAg and 58–93% decrease in HBeAg serum levels. In agreement with an immune response-mediated suppression of HBV infection, viral load reduction was accompanied by cytokine and chemokine responses and lymphocyte infiltration in the liver (Lanford et al., 2011).

Although the antiviral efficacy of TLR- and RLR agonists has been observed in HBV-transgenic mice, as well as in animals infected with HBV, WHV or DHBV, systemic administration of the PRR agonists in doses necessary to achieve antiviral effects is usually associated with significant adverse effects, due to the activation of a wide spectrum of cellular responses and production of proinflammatory cytokines (Damm et al., 2012; Fidock et al., 2011; Horsmans et al., 2005; Pockros et al., 2007). In fact, Aldara (5% imiquimod cream), a small molecule TLR7 agonist, is thus far the only FDA-approved PRR agonist used for treatment of a viral disease, papilloma virus-induced genital warts, as a topical drug. A few TLR3, TLR7 and TLR9 agonists reached phase I or II clinical trials for HCV or HIV infection and achieved limited therapeutic efficacy (Kanzler et al., 2007).

The lessons learned from the previous studies suggest that clinically useful PRR agonists for the treatment of chronic hepatitis B should induce a strong antiviral response to resolve the viral infection, but not a systemic cytokine response that may cause tissue damage and adverse effects. Based on the known properties of TLRs and RLRs, these goals will most likely be achieved *via* intrahepatic activation of TLR3 or RIG-I. Although hepatocytes express a low level of TLR3, the receptor is highly expressed on Kupffer cells and LSECs. As discussed in the previous sections, poly I:C activation of the two cell types strongly inhibited HBV replication in hepatocytes. In addition, compared with other TLRs, TLR3 activates cellular responses primarily *via* the adaptor molecule TRIF, but not MyD88 (Kawai and Akira, 2010). Because MyD88 mainly mediates the induction of inflammatory cytokines by TLR4 and other TLRs, it is conceivable that activation of TLR3, but not TLR4, especially within the liver, might induce a limited inflammatory response. Hence, targeted delivery of TLR3 agonists *via* liposomes or nanoparticles to activate Kupffer cells and LSECs holds great promise for the treatment of chronic hepatitis B, and thus warrants preclinical evaluation in hepadnavirus-infected animals.

6.3. PRR signaling modulators and activators of specific antiviral pathways

Induction of type I IFNs and other pro-inflammatory cytokines upon activation of TLRs and RLRs is controlled by multiple overlapping, but distinct signal transduction pathways (reviewed in (O'Neill and Bowie, 2010)). As illustrated in Fig. 1, while activation of NFκB and distinct mitogen-activated protein kinase (MAPK) pathways is critical for the production of many proinflammatory cytokines and chemokines, only activation of the IRF3 (or IRF7) pathway is required for induction of type I IFNs, as well as a group of antiviral proteins, such as IFIT1, guanylate binding protein 1 and zinc finger antiviral protein (Elco et al., 2005; Geiss et al., 2001; Wang et al., 2010). In addition, although the three MAP kinases, p38, ERK and JNK, can be activated by TLR and RLR agonists and by viral infection (Ermolaeva et al., 2008; Schrofelbauer et al., 2009), each of the three MAPKs has been demonstrated to play a distinct role in regulating the expression of type I IFN and other proinflammatory genes (Makela et al., 2009; Steer et al., 2006; Stewart et al., 2006). For instance, it has been shown recently that ERK activation is required for TLR3-induced chemokine production in murine dendritic cells, whereas JNK activation had a negative regulatory effect on chemokine production (Mitchell and Olive, 2010). It is therefore possible to pharmacologically modulate the virus- and/or PRR-agonist-induced innate immune response by targeting distinct signal transduction pathways, to selectively enhance antiviral responses, but alleviate detrimental inflammatory responses. It is conceivable that such a therapy should be effective against a broad spectrum of viral infections, either alone or in combination with PRR agonists. In an effort to identify compounds that enhance TLR3 agonist-induced antiviral, but not inflammatory

responses, we recently discovered a compound, RO 90-7501 ('2'-(4-Aminophenyl)-[2,5′-bi-1H-benzimidazol]-5-amine), that significantly promoted both TLR3 and RIG-I ligand-induced IFN-β gene expression and antiviral responses, most likely by modulating the activation of the p38 MAPK pathway, but not the NFκB or IRF3 pathway (Guo et al., 2012).

Alternatively, direct activation of intracellular signal transduction pathways to induce the expression of antiviral proteins could be another option to avoid adverse effects induced by TLR/RLR agonists. Along this line, we recently obtained encouraging results indicating that treatment of human or murine hepatocytes with KIN101 (Bedard et al., 2012), a small molecule activator of IRF3, inhibited HBV replication *via* the post-transcriptional inhibition of HBV replication (Chang et al., unpublished results).

7. Conclusions and future directions

Due to the lack of convenient HBV cell culture infections and animal models, our understanding of the interaction between HBV and PRR-mediated host innate immunity and its role in pathogenesis remains incomplete. It is our hope that creation of better experimental systems and detailed molecular analyses of clinical samples from patients will help advance our knowledge of PRR-mediated innate immunity against HBV infection. Moreover, preclinical and clinical studies have demonstrated that activation of PRR-mediated innate immune responses by PRR agonists or antiviral cytokines (IFN-α and IFN-λ) not only significantly inhibited HBV replication, but also efficiently reduced antigenemia and induced HBsAg and HBeAg seroconversions. Intrahepatic delivery of TLR3 and RIG-I agonists and the development of novel drugs that enhance PRR agonist- and IFN-induced antiviral responses and/or limit their inflammatory effects should further improve the clinical benefits of innate immune activation therapy against chronic hepatitis B.

Acknowledgements

Preparation of this manuscript was supported in part by The Hepatitis B Foundation and The Commonwealth of Pennsylvania. We thank Dr. Pamela Norton for critical reading of the manuscript.

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