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Modulators of innate immunity as novel therapeutics for treatment of chronic hepatitis B

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Abstract

The first line defense mechanisms against viral infection are mediated by the innate immune system. Viral components are detected by infected cells and/or innate immune cells that express different sensory receptors. They in turn mediate induction of direct antiviral mechanisms and further modulation of innate and adaptive immune responses. For evading the innate system, most viruses have evolved efficient mechanisms to block sensing and/or antiviral functions of the innate response. Interestingly, hepatitis B virus (HBV) seems to act like a stealth virus that escapes cell intrinsic antiviral mechanisms through avoiding recognition by the innate system rather than blocking its effector functions. In line with this concept, agonistic activation of innate immunity has emerged as a promising novel anti-HBV therapy approach with several compounds having advanced to the clinical stage.

Introduction

The innate immune system represents the first line defense of a host cell to viral infection. It consists of cell surface and intracellular pattern recognition receptors (PRR) such as cytoplasmic retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and DNA sensors, or membrane-bound toll-like receptors (TLRs) that recognize pathogen-specific structures called pathogen-associated molecular patterns (PAMPs). Incoming viruses can be sensed at the cell surface by TLRs typically recognizing PAMPs exposed on virions (TLR2, TLR4) [1,2]. Intracellularly, endosomal TLRs recognize double stranded (ds)RNA (TLR3), single stranded (ss)RNA (TLR7/8), or CpG motif-containing unmethylated DNA (TLR9) derived from incoming or replicating viruses [2]. Cytoplasmic RLRs (e.g., RIG-I, MDA5) detect virus derived dsRNA or ssRNA, while DNA sensors (e.g. cGAS, IFI16) recognize viral

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DNA [1]. PAMP recognition by PRRs typically results in rapid induction of type I/III interferons (IFNs) and/or other inflammatory cytokines in the infected cell [1–4]. Secreted IFNs signal to surrounding cells and induce expression of a large number of interferon-stimulated genes (ISGs) that inhibit viral replication and protect naïve cells from *de novo* infection [5]. Besides infected cells, also innate immune cells can respond to viral infection upon either directly sensing virus-infected cells (e.g. natural killer (NK) cells [6]), or engulfing viral components (e.g. dendritic cells (especially plasmacytoid (p)DCs) [7] and macrophages [8]). In the liver, also liver sinusoidal endothelial cells (LSEC) and hepatic stellate cells (HSC) contribute to innate immune responses [9]. Not surprisingly, most viruses evolved to counteract cellular innate immune responses by often actively suppressing PAMP recognition, IFN induction, IFN signaling and/or ISG activity [10,11]. Here we briefly summarize the current understanding of the interplay between HBV and the different aspects of the innate immune system and how this knowledge is exploited for the development of novel therapies to combat chronic HBV infection.

Lack of innate response signature during HBV infection

Consistent with observations for many different viruses, innate immune induction is also evident for infections with hepatotropic viruses including hepatitis A virus [12], hepatitis C virus (HCV) [13], hepatitis D virus (HDV) [14] and hepatitis E virus [15] all of which are associated with an intrahepatic IFN/ISG response [12–15]. Surprisingly however, HBV infection does not induce an intrahepatic IFN/ISG response in chimpanzees [16]. Likewise, no intrahepatic IFN/ISG response was associated with early-acute infection in the woodchuck model [17] and only minimally or not at all evident in HBV-infected human hepatocyte chimeric mice [14,18,19]. Similarly, virtually no cytokine induction was detected in the serum of human patients with acute HBV infection [20,21]. These results suggest that HBV acts like a stealth virus efficiently spreading throughout the liver without alerting the innate immune system [16,22].

Although early studies in *in vitro* model systems suggested that HBV could transiently induce an innate response in the infected cell, these results might have been hampered by the lack of optimal model systems at the time [23,24]. Indeed, recent studies by Cheng et al. [18] and Mutz et al. (Mutz et al, personal communication) using newly developed efficient *in vitro* HBV infection systems revealed that also *in vitro* HBV infection does not trigger a cell intrinsic innate immune response. Taken together, the stealth characteristic of HBV seems to enable the avoidance of innate recognition in its host organ and cell.

Innate response modulation by HBV

Many viruses have evolved to actively counteract PAMP recognition, IFN induction/signaling and/or ISG function in the infected cells. Thus, it is possible that the lack of innate responses to HBV infection is due to a very robust and effective suppression of those responses by HBV. Indeed, it has recently been suggested that intrahepatic innate immune response pathways could be downregulated in chronic hepatitis B (CHB) [25]. This however, is contradictory to similar studies in patients [20,21], chimpanzees [16], and other HBV model systems [14,18,26] where no suppression of these pathways was observed.

Furthermore, innate responses can not only be easily induced in the presence of HBV but in turn also efficiently inhibit HBV replication. For example, it has been recognized early on that poly(I/C) mediated IFN- α/β induction in HBV-infected chimpanzees resulted in a reduction in many viral parameters [27]. Likewise, HBV replication in a transgenic mouse model is strongly inhibited by TLR activation [28,29]. Furthermore, and as will be discussed below, agonistic activation of TLR7 induces prolonged suppression of HBV in different CHB models [30,31]. Also, HBV superinfection of chronically HCV-infected chimpanzees is strongly attenuated [32], suggesting that HBV is also sensitive to the HCV-triggered innate responses. Finally, HDV superinfection triggers an innate response inhibiting preexisting HBV replication *in vitro* [33].

Nevertheless, several studies suggested that HBV proteins and replicative intermediates can interfere with innate immune responses in different *in vitro* HBV systems (reviewed in [34]). It has to be noted however, that most of these studies involved over-expression of viral components which might not reflect the situation during natural infection. Indeed, new studies utilizing recently developed efficient *in vitro* HBV infection models unequivocally demonstrate that HBV does not interfere with PRR mediated induction of innate responses in the HBV infected cell ([18] and Mutz et al., personal communication). Furthermore, using *ex vivo* cultured human liver biopsies, we could show that PRR-mediated IFN and ISG induction is not suppressed in the HBV-infected hepatocytes in the liver of CHB patients [35]. In summary, the data from *in vivo* studies and state of the art *in vitro* models would suggest that HBV, besides not inducing innate responses, does also not interfere with innate immunity mechanisms in the infected cell.

HBV and innate immune cells

Apart from cell intrinsic innate immune responses, viruses can also trigger innate responses in cells they do not infect such as innate immune cells. In the liver, these include a number of non-parenchymal cells (NPCs) such as pDCs, NK cells and liver resident macrophages (Kupffer cells). Indeed, *in vitro* exposure of human liver derived NPCs [36] or primary monocyte-derived macrophages [18] to high doses of HBV induces cytokine production, suggesting that Kupffer cells might sense HBV in the liver. While Kupffer cell activation might therefore contribute to HBV clearance [36], studies in mice suggest that macrophage activation by HBV might also promote HBV persistence through negative modulation of HBV-specific adaptive immune responses [37–39]. In addition, HBV proteins have also been implicated in downregulation of TLRs on Kupffer cells (and other cells) in CHB, possibly contributing to a proviral environment [40,41].

NK cells

Although NK cells are highly enriched in the human liver [42], data on the interplay of HBV with NK cells in patients is mostly derived from analysis of blood-derived NK cells [20,43–45]. Although early activation of NK cells was observed in acute HBV infection [20,44], in some cases their non-cytolytic antiviral functions seem to be suppressed at the peak of viremia [20] and also during chronic infection [43,45]. Since NK cells can kill HBV-specific

T-cells [46] and exacerbate HBV-related liver damage [47,48], it appears that antiviral and host damaging effects of NK cells need to be carefully balanced to achieve HBV clearance.

pDCs

pDCs are specialized in IFN production upon recognition of captured viruses or viral-derived nucleic acids and therefore are indispensable for mounting a robust immune response during many viral infections (reviewed in [7]). Interestingly however, exposure of pDCs to HBV does not trigger IFN- α production, although it interferes with their capacity to respond to TLR9 stimulation [49,50]. Considering that pDC frequencies and functions otherwise are not altered in HBV-infected patients (reviewed in [51]), it remains to be determined what role pDCs might play during HBV infection.

Vertical HBV transmission

It is noteworthy that most studies on the interaction of HBV with different aspects of the innate immune system are focused on chronic or acute HBV infection in adults. In contrast, comparatively little is known about these interactions in newborns during vertical HBV transmission even though this is the more common route of HBV infection today. Nonetheless, experiments in animal models have suggested that HBV might induce an immunotolerant state in newborns and thereby establish persistent HBV infection [37,52,53]. While these studies provided an attractive explanation for the high rate of chronicity of vertically transmitted HBV infection, they are at odds with the observation that infants born to HBV-positive mothers respond well to HBV vaccination [52]. Furthermore, a recent study showed that HBV exposure in utero can even induce a state of trained immunity both for innate and adaptive immune functions [54]. Taken together, these results show that the virus host interactions during vertical transmission of HBV are complex and warrant further investigation.

Innate immune activation in HBV therapy

Since chronic HBV infection does not trigger a cell intrinsic innate immunity within liver, compounds capable of activating an intrahepatic, anti-HBV specific innate response are evaluated as novel therapeutic interventions of CHB [34,55–60]. Agonistic acting immunomodulators that have already advanced to the clinical stage are discussed (Fig. 1).

IFN- α

Conventional and pegylated IFNs are the only immunomodulators currently approved for treatment of CHB. Compared to standard treatment with nucleos(t)ide analogs (NAs), a higher, although still modest, rate of functional cure is attained with IFN- α in patients during a finite treatment course [60,61]. Unlike the oral availability and safety of NAs [62,63], IFN- α treatment includes parenteral delivery and substantial systemic effects, making it impractical to administer this cytokine to all patients.

Despite clinical use for over two decades, and due to its pleiotropic effects, the mechanistic understanding of IFN- α interference with HBV is still limited [64]. IFN- α displays

immunomodulatory [65–68] and direct antiviral effects [69–74]. A study in woodchucks addressed the mechanisms of IFN- α activity after subcutaneous administration for 15 weeks [75]. Comparable to patients [76], treatment reduced replication of the HBV-like woodchuck hepatitis virus (WHV) at varying degree in responder, partial responder, and non-responder animals, with maximum mean declines in WHV DNA and WHsAg of 3.2 and 2.0 logs, respectively. Most woodchucks experienced viral rebound after the end of treatment, although viremia and antigenemia stayed suppressed in responder animals, with one achieving a functional cure. The differential treatment response to IFN- α in individual woodchucks, however, did not correlate with the intrahepatic induction of most ISGs. Instead, induction of NK and T-cell markers, elevation in liver inflammation, consistent with migration and/or proliferation of these cells, and increase in IFN- γ expression were observed, indicating that cytolytic and non-cytolytic mechanisms play a pivotal role in the IFN- α treatment response. As also described for patients [65,66], the antiviral response mediated by IFN- α in woodchucks closely correlates with its immunomodulatory effects. Altogether, these results suggest that immunomodulators, which activate antiviral immune cells in the liver like IFN- α , but are without its limitations, are promising compounds for the treatment of CHB.

TLR agonists

Selective activation of TLRs within pDCs by agonists for the local induction of IFNs is increasingly investigated. The oral TLR7 agonist GS-9620 reduced HBV DNA in chimpanzees by 2.2 logs on average after two rounds of 4-week treatment [30]. Durable suppression of viremia persisted at least for 2–4 months and was associated with a 50% reduction in HBsAg and HBeAg levels. In woodchucks, one of the GS-9620 treatment regimens produced a functional cure in all woodchucks [31]. GS-9620 was initially administered for 2 weeks, halted due to thrombocytopenia in some animals, and then reinitiated with half of the original dose for an additional 2 weeks. Short-term treatment resulted in a rapid decline in WHV DNA of 6.2 logs on average. Viremia stayed suppressed for more than 7 months and was accompanied by undetectable WHsAg in all woodchucks, and by anti-WHs seroconversion in a subset of animals. The antiviral effect mediated by GS-9620 in liver of chimpanzees and woodchucks following induction of IFN- α and antiviral ISGs was facilitated, at least in part, by the cytolytic activity of CD8⁺ T-cells and/or NK cells. In the subsequent phase Ib clinical trial, GS-9620 administered to treatment-naïve and NA-suppressed patients once or twice was well tolerated, but did not result in significant changes in HBV markers [77]. Since induction of hepatic flares or autoimmunity are main concerns in any immunomodulatory therapy, the maximum dose applied to patients was approximately 15–45-fold lower than those tested in animals. Phase II clinical trials of GS-9620, testing longer treatment duration and higher doses, alone or in combination with tenofovir, are ongoing (NCT02166047 and NCT02579382). RG7854 [78] is another oral TLR7 agonist, which induced functional cure in woodchucks (Menne et al., unpublished). The compound is currently evaluated in a phase I clinical trial (NCT02956850). The oral TLR7 agonist AL-034 showed efficacy against HBV in a mouse model [79], and is also in phase I clinical development (NCT03285620).

Agonists of TLR8 are considered important activators of innate immunity since they induce the secretion of IL-12 and IL-18 from monocytes within human liver, which in turn leads to the production of IFN- γ by intrahepatic innate immune cells such as mucosal-associated invariant T cells (MAIT) and NK cells [80]. IFN- γ has been shown to mediate HBV clearance in chimpanzees [81] and to suppress viral replication in HBV transgenic mice, woodchucks and CHB patients following treatment with IL-12 or IL-18 [82–85]. IL-12 can further rescue the antiviral function of exhausted HBV-specific CD8⁺ T cells *in vitro* [86].

GS-9688, an oral TLR8 agonist, was tested in woodchucks [87]. Treatment for 8 weeks reduced WHV DNA by 5 logs in most animals. Three woodchucks with sustained suppression in viremia had undetectable WHsAg, and developed anti-WHs antibody and WHV-specific T-cell responses. Comparable to patients [88], the latter suggested that the T-cell dysfunction present in CHB is reversible. A phase Ib clinical trial of GS-9688 is initiated (ACTRN12617000235303).

CpG oligodeoxynucleotides are TLR9 agonists that induce IFN- α following stimulation of pDCs and B-cells [89]. CpGs have been tested during HBV vaccine development [90,91]. Subcutaneous treatment of woodchucks with CpG 21798 in combination with entecavir (ETV) reduced WHV DNA and especially WHsAg to undetectable levels much earlier than ETV monotherapy, and delayed viral relapse [92]. CpG monotherapy, however, was only modestly efficacious.

AIC649, an inactivated parapoxvirus ovis particle preparation, targets antigen presenting cells *via* TLR9 and leads to cytokine release, including IFN- α [93,94]. The antiviral efficacy of AIC649 was evaluated in woodchucks during 36 weeks of intravenous/intramuscular administration, alone or in combination with ETV during the initial 12 weeks [95]. The AIC649 dose was equal to the high dose administered to patients in an ongoing phase I clinical trial (AiCuris website). The previously observed bi-phasic response pattern [96] was confirmed during AIC649 monotherapy. Combination treatment with AIC649 and ETV produced maximum mean reductions in WHV DNA and WHsAg of 7.6 and 4.1 logs, respectively, and WHsAg became undetectable in a subset of woodchucks. WHV suppression was quite durable, but viral rebound eventually occurred, although viremia and antigenemia stayed below pretreatment level at the end of the study. The treatment response was further associated with WHV-specific T-cell responses and anti-WHs antibodies in most woodchucks. The overall results from these studies indicate that TLR9 agonists can exhibit additive effects, when administered in combination with NAs.

Intracellular PRR agonists

Since the above TLRs are mainly found within pDCs, but are barely expressed in other cells, PRRs that are located within the cytoplasm of HBV-replicating hepatocytes are another attractive target for stimulating antiviral innate immunity. Activation of RIG-I induces not only IFNs but has also been suggested to block HBV through sensing of the epsilon-structure within pre-genomic RNA [19]. SB 9200, a selective, oral prodrug of the dinucleotide SB 9000 has been shown in woodchucks to activate RIG-I expression in liver and to increase its protein level in hepatocytes [97]. RLR upregulation further induced the

intrahepatic expression of key innate immune response molecules, including STING and IRF3, and was consistent with the induction of IFN- α/β and antiviral ISGs. Induction of innate immunity, likely in addition to the direct antiviral effect mediated by activated RIG-I, then contributed to the dose-dependent antiviral response observed with SB 9200 in woodchucks. In this context, it is noteworthy that the murine STING agonist DMXAA induced an IFN- α/β mediated cytokine release in macrophages, which suppressed HBV replication in mouse hepatocytes and liver [98]. In another woodchuck study, short-term ETV treatment was sequentially applied before or after SB 9200 treatment for 12 weeks [99]. Activation of innate immunity by SB 9200 followed by ETV resulted in maximum mean reductions in WHV DNA and WHsAg of 6.4 and 3.3 logs, respectively. The antiviral effect was transient, but WHV markers stayed suppressed until the end of the study. These results suggested that activation of intrahepatic innate immunity in CHB patients prior to initiation of standard treatment may be beneficial for augmenting the antiviral effect. This concept is investigated in a phase IIa clinical trial of SB 9200 (NCT027519968). Treatment-naïve CHB patients were pretreated with SB 9200 for 12 weeks, then switched to tenofovir, and followed for additional 12 weeks [100]. SB 9200 treatment resulted in a mean reduction in HBV DNA of 0.6 logs, and 5 of 16 patients experienced a greater than 0.5 log decline in HBsAg. The antiviral efficacy observed in patients is noteworthy considering that the applied SB 9200 dose is approximately 80-fold lower than the woodchuck dose. Following the switch to tenofovir, an enhancement of the antiviral effect was noted in HBeAg-positive patients, suggesting an added benefit of immunomodulation followed by standard treatment.

Challenges for therapeutic use of agonistic acting immunomodulators

Although the preclinical results overall are promising, targeting innate immunity by agonists comprises challenges that need to be addressed before application in CHB patients. While clinical trials with the above agonists have not concluded, it has been suggested that the utilized animal models, in contrast to patients, may be more sensitive to this kind of therapy or may tolerate doses exceeding those considered to be physiological and safe in patients. This proposition is based on the rather disappointing outcome of monotherapy with GS-9620 in patients so far [77] using doses and dosing frequencies significantly different to the treatment applied to chimpanzees and woodchucks [30,31]. Since monotherapy with agonistic activators of innate immunity sometimes failed to induce pronounced antiviral effects in animal models as well as patients and, unlike standard drugs, cannot be administered indefinitely, combination treatment with NAs will be required for achieving sustained suppression of HBV. Such treatment regimens (sequential or concomitant) may further allow reducing the dose of an agonist and shortening the duration of immunomodulation in patients, whereby limiting the risk that the activated adaptive immune response could target most or all HBV-infected hepatocytes and result in fatal liver damage.

Conclusions

Considering the different models used for studying the interplay between HBV and the innate immune system, it emerges that HBV is neither sensed nor interferes with the innate immune response of the cell it infects, thus acting like a stealth virus in this regard. On the other hand, the consequences of the interplay between HBV and different cells of the innate

immune system seem to substantially influence whether an HBV infection will be resolved or become persistent. The encouraging results from the above preclinical and clinical studies indicate that agonistic activation of innate immunity, most likely in combination with NAs, holds great promise for treatment of CHB.

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*of special interest

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Highlights

- The current understanding of the interplay between HBV and different aspects of the innate immune system are discussed
- HBV apparently neither induces nor interferes with cell intrinsic innate responses acting as a stealth virus in this regard
- The interplay between HBV and different cells of the innate immune system significantly contributes to the outcome of HBV infection.
- Agonistic acting immunomodulators that have already advanced to the clinical stage are discussed
- Agonistic activation of innate immunity, most likely in combination with standard treatment, holds great promise for treatment of chronic HBV infection

Figure 1.

Activation of antiviral innate immunity in CHB. Activators can be separated into modulators that systemically stimulate innate immunity (e.g., IFNs and other recombinant cytokines) and modulators that act specifically and locally (e.g., agonists of cytoplasmic PRRs, IFN signaling pathway molecules, and endosomal TLRs). Available IFN- α preparations include Intron A (IFN- α 2b; Merck) and Pegasys (pegylated IFN- α 2a; Genentech). SB 9200 (Spring Bank Pharmaceuticals) is an agonist of RIG-I. GS-9620 (Gilead Sciences), RG7854 or RO7020531 (Hoffmann-La Roche), and AL-034 (Janssen) are agonists of TLR7. GS-9688 (Gilead Sciences) is an agonist of TLR8. CpG 21798 (Pfizer) and AIC649 (AiCuris) are agonists of TLR9. Upon stimulation of PRRs or TLRs by their respective ligands, downstream signaling pathways are activated, including key molecules such as STING or MyD88, leading to the nuclear translocation of the transcription factors NF- κ B, IRF3 and IRF7, and subsequent transcription of IFNs, pro-inflammatory cytokines, and ISGs. Note that the figure only indicates the main cell subsets and cellular compartments that are targeted by IFN- α and agonists but does not depict any systemically induced innate immunity by these modulators as it is known for IFN- α due to the pleiotropic nature of this cytokine.

IFNAR, interferon- α/β receptor; STING; stimulator of interferon genes; IKK, Kinase involved in the upstream NF- κ B signal transduction cascade; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B-cells; IRF; interferon regulatory factor; IRSE, interferon-responsive sequence element; and MyD88, myeloid differentiation primary response gene 88.