Host Factor-Targeted Hepatitis B Virus Therapies

Adam J. Gehring, Antonio Bertoletti, John E. Tavis

Department of Molecular Microbiology and Immunology, Saint Louis University School of Medicine, St. Louis, Mo. USA; Emerging Infectious Diseases (EID) Program, Duke-NUS Graduate Medical School, Singapore Institute for Clinical Sciences, Agency of Science Technology and Research (A*STAR), Singapore, Singapore

Key Words
Hepatitis B virus · Therapy · Immunotherapy

Abstract
In this review we will focus on host factors known to impact hepatitis B virus (HBV) replication as current or potential targets for therapeutic intervention. Some immunotherapeutic strategies will be discussed because they have the potential to activate interferon-mediated clearance of HBV, but attention will also be paid to host machinery and proteins that silence covalently closed circular DNA, destabilize viral RNA, or disrupt entry and trafficking of HBV virions. Many of these are in the early stages of development, but may represent novel avenues to reduce HBV burden when combined with nucleos(t)ide analogues.

Introduction
Current therapy with nucleos(t)ide analogue antiviral drugs can reduce hepatitis B virus (HBV) replication below clinically detectable levels, reduce liver inflammation, reverse fibrosis, and partially restore immune responses capable of controlling HBV [1–3]. However, nucleos(t)ide analogues fail to achieve what would be considered a cure in more than 95% of patients – disappearance of HBV DNA and HBV surface antigen (HBsAg) from the circulation and emergence of anti-HBs antibodies [4–6]. What usually occurs upon therapy termination is a sharp rebound in HBV DNA levels and liver inflammation.

Nucleos(t)ide analogues inhibit the reverse transcriptase of HBV, which is required for the conversion of HBV pregenomic RNA (pgRNA) to the double-stranded DNA in mature viral particles. However, this therapy does not directly target the viral covalently closed circular DNA (cccDNA) in the infected cell’s nucleus. cccDNA is the key molecule in HBV’s persistence because of its unusually long half-life in cells. It serves as the template for transcription of viral pgRNA and all viral mRNAs. Since nucleos(t)ide analog therapy does not interfere with cccDNA activity, it does not routinely suppress viral protein production. Thus, eliminating or interfering with the function of cccDNA is the key to effective long-term control of HBV.

This goal has been extremely difficult to achieve therapeutically. During successful resolution of an acute HBV infection, the immune response clears cccDNA from a majority of infected hepatocytes through an interferon (IFN)-γ-mediated noncytolytic mechanism [7]. The exact process is unclear, but is controlled by host factors induced by the antiviral immune response. This immune response is impaired in chronic HBV patients and hence there is little immune pressure on the infected hepatocytes. Identifying host factors that can interfere with the viral replication cy-
Blocking Key Steps in the HBV Replication Cycle

Long-term suppression of HBV replication coupled with hepatocyte proliferation can reduce the intrahepatic cccDNA content via dilution [8, 9]. Blocking infection or preventing reinfection of cleared hepatocytes may help accelerate and maintain progress made by antiviral therapy. Currently, hepatitis B immune globulin is given to help minimize infection following liver transplants in HBV-positive recipients. Hepatitis B immune globulin is purified from the plasma of vaccinated individuals and prevents reinfection in 90% of transplant patients, which demonstrates that blocking infection of new cells is possible – just impractical for routine use [10]. Synthetic molecules capable of blocking HBV infection have not been available until recently. However, Urban and colleagues [11–13] have developed myristoylated peptides derived from the HBV preS1 protein responsible for receptor binding that can block natural HBV infection. The ability to make large batches of quality controlled synthetic reagents may help increase their use in patients as a standard therapy.

Inside the infected hepatocyte, additional host factors have been identified or exploited to target specific steps in HBV replication. Conversion of the viral pgRNA to DNA has been the primary focus of antiviral therapy because the reverse transcriptase is a proven target. However, pgRNA encapsidation and DNA replication are also targets for intrinsically antiviral mechanisms because of their unique nature. Mao et al. [14] recently demonstrated that the human zinc finger antiviral protein (ZAP) binds to the terminal redundant region of the HBV pgRNA and leads to its decay. The ZAP enzymes were induced by IFN-α and upregulated in the liver of patients with chronic active hepatitis. The APOBEC3 family of proteins has also been studied for its role in suppressing HBV DNA replication [15–20]. However, the mechanism by which these proteins inhibit HBV is unclear and their physiological role during infection remains to be determined. However, it was recently demonstrated that IFN-α and lymphotixin-β up-regulate APOBEC3A&B. Through their interaction with the HBV core protein, APOBEC3A&B associated with nuclear cccDNA leading to cytidine-deamination and degradation [21]. The molecular chaperone heat shock protein 90 (HSP90) is exploited by HBV to promote reverse transcription [22, 23]. HSP90 facilitates docking of the HBV reverse transcriptase onto the pgRNA. HSP90 also plays a significant role in multiple human cancers, and inhibitors are being tested in clinical trials that could also interfere with HBV reverse transcription [24]. Finally, conversion of the viral DNA from its gapped form in virions to the nuclear cccDNA by cellular proteins has been targeted in a recent inhibitor screen [25].

Reducing viral replication usually does not significantly reduce antigen load in chronic HBV patients. The persistent antigenemia is believed to be primarily responsible for the exhaustion of HBV-specific T cells. Identifying mechanisms to reduce the antigen load could permit restoration of HBV-specific immunity and lead to long-term control of HBV infection. One mechanism characterized within the IFN-γ-mediated antiviral response is indoleamine 2,3-dioxygenase (IDO). IFN-γ-mediated upregulation of IDO in hepatocyte cell lines reduced the intracellular HBV DNA content. This inhibition of HBV replication was mediated through enzymatic degradation of tryptophan, which preferentially reduced viral protein translation [26]. Another approach that would therapeutically target HBV antigen production, as well as replication, is RNA interference. While the small interferingRNAs (siRNA) themselves are synthetic molecules, their effect is mediated by host machinery, the RNA-induced silencing complex, which degrades homologous viral RNAs within the cell. Using siRNAs that target conserved regions of the HBV genome can significantly reduce both viral load and circulating viral antigens [27]. Continuing research has been focused on strategies to achieve specific, nontoxic, delivery of siRNA to the liver to reduce viral and antigen load [28, 29].

Antiviral Activity of IFN-α

Antiviral Activity

IFN-α is the original host factor used to treat HBV infection. It can achieve sustained reduction of viremia and antigenemia in chronic HBV patients with a defined treatment duration, but it also induces severe side effects and rarely leads to HBV clearance [30]. Due to its pleo-
tropic antiviral effects, it has never been clear how IFN-α reduces the viral load, i.e. whether it is an antiviral effect or an immunomodulatory effect. Elucidating the primary antiviral mediators of IFN-α therapy could permit a more targeted approach and avoid the side effects of systemic IFN-α administration.

Identifying the antiviral mediators induced by IFN-α has been difficult due to the lack of good infection systems. However, the development of the uPA-SCID mouse repopulated with human hepatocytes and cell culture systems has shed light on the regulation of cccDNA transcriptional activity by IFN-α. Recent studies have demonstrated that IFN-α alone, without the involvement of the immune system, can reduce HBV antigenemia in the uPA-SCID mouse model [31]. An IFN-α-induced antiviral state leads to epigenetic regulation of host-derived histones associated with HBV cccDNA and reduces transcriptional activity [32, 33]. The exact mechanisms are still being investigated, but epigenetic drugs have been clinically approved and are currently being tested in clinical trials in cancer patients. Given the ability of HBV to directly interfere with IFN-α signaling in infected hepatocytes [34], using drugs to induce silencing of the cccDNA could be a viable option to reduce the functional cccDNA pool to bring down antigenemia. In addition to epigenetic silencing, data from Liu et al. [32] also suggested that IFN-α accelerates cccDNA decay, supporting the data on IFN-α-induced APOBEC3 mediated cccDNA degradation mentioned above.

**Immunomodulation by IFN-α**

Similar to the antiviral effects, the immune modulatory effects of IFN-α remain to be clearly defined. Two studies have recently investigated the effects of IFN-α on innate immunity. In particular, these studies showed that IFN-α activates NK cells and enhances their ability to produce IFN-γ [35, 36]. This is particularly significant because NK cells comprise 30% of the intrahepatic lymphocytes and the ability to localize IFN-γ production in the liver could reduce cccDNA levels. A possible approach to facilitate liver-specific IFN-α delivery could be achieved by targeting receptor complexes presented on infected hepatocytes. Using antibodies conjugated with IFN-α that recognize HBV-peptide MHC-I on the surface of infected hepatocytes, we could preferentially induce IFN-α-associated genes in hepatocytes expressing HBV antigens [37]. Specific delivery could help reduce systemic side effects of IFN-α and lead to local activation of NK cells. However, despite the ability of IFN-α to activate NK cells, its administration does not simultaneously correspond to a precipitous drop in viral load [35] and IFN-α fails to achieve viral clearance in a majority of patients. This is likely associated with the observations that IFN-α therapy does not enhance the virus-specific T cell response, which is key to controlling HBV infection [35–38].

### Immune Mediators of the Host Anti-HBV Response

**Triggering Liver-Specific Innate Immunity**

In contrast to systemic administration of IFN-α, localized production of antiviral cytokines in the HBV-infected liver may be far more effective at mediating the host antiviral response [39–41]. Recently developed strategies to induce this effect have centered on the activation of Toll-like receptors (TLRs). TLRs are pattern recognition receptors that, when activated by conserved bacterial and viral motifs, stimulate the production of inflammatory and antiviral cytokines. Given orally, synthetic TLR agonists are believed to preferentially activate immune cells in the liver and induce local cytokine production. TLR-7 agonists, currently in human clinical trials, stimulate production of IFN-α from plasmacytoid dendritic cells and have shown promising results in reducing viral load and antigenemia in chronically infected chimpanzees [42]. However, IFN-α produced in response to the TLR-7 agonist was not specific to the liver, and activated immune cells in the blood to a similar level. The primary effect of this strategy may be activation of intrahepatic innate lymphocytes to produce other antiviral cytokines such as IFN-γ. Supporting this idea, we have observed that TLR-8 agonists preferentially activate innate lymphocytes in the human liver (NK cells, mucosal associated invariant T cells and gamma/delta T cells) to produce IFN-γ [Antonio Bertoletti, pers. commun.]. These cells comprise up to 50% of the intrahepatic lymphocyte pool [43]. Such robust production of IFN-γ could stimulate the noncytolytic mechanisms responsible for HBV clearance in acute HBV infection in adults.

**Boosting Antiviral T Cell Immunity**

The HBV-specific T cell response plays a key role in controlling HBV infection. Attempts to boost antiviral T cells have ranged from therapeutic vaccines to gene therapy [44]. Gene therapy is currently limited by practical considerations. Therapeutic vaccines have shown some promise with aggressive prime-boost vaccination strategies [45]. However, therapeutic vaccines are complicated by the obstacles of patient and viral diversity [46]. HBV genotypes differ by 8% at the amino acid level [47]. Therefore, selecting a single recombinant antigen for a vaccine could mis-
direct the immune response due to sequence differences between the vaccine antigen and the infecting virus. This problem could be overcome by the fact that monocytes in the circulation of chronic HBV patients internalize and retain a depot of viral antigen. Activation of monocytes led to cross-presentation of the intracellular antigen depot and expansion of autologous virus-specific T cells [48]. Since monocytes are the dominant professional antigen-presenting cells in the circulation, effectively targeting these cells with cytokines or adjuvant alone could provide a personalized vaccine capable of efficiently activating HBV-specific T cells. Three recent studies alluded to this possibility. Administration of the adjuvant alone (Alum) to chronic HBV patients achieved a response rate similar to the vaccine in a phase III clinical trial with HBsAg immune complexes [49]. In the second study, administration of the CD40 ligand alone activated intrahepatic dendritic cells and resulted in efficient T cell priming in HBV transgenic mice [50]. In the third, administration of a TLR-9 agonist induced inflammatory monocyte-dependent intrahepatic T cell localization and proliferation [51]. If successful, this strategy could exploit in situ components to personalize the vaccine antigen for each patient and overcome the issues of viral diversity for vaccines.

Conclusions

Many of the host-targeted factors discussed fall into the IFN-α-inducible antiviral pathway. Identifying specific factors could allow for more precisely targeted therapy and avoid systemic effects. We also know that IFN-γ is critical for the noncytolytic clearance of HBV during acute infection. Identification of host factors mediating the IFN-γ antiviral response might control HBV replication more effectively but, as yet, very few host factors involved in the IFN-γ pathway have been described. Therefore, inducing its production through synthetic TLR agonists or boosting antiviral T cells using circulating viral antigen and monocytes might control HBV.

Many of the cellular proteins discussed as antiviral mediators target intermediate steps in the HBV replication cycle, similar to antiviral drugs. Suppression of HBV reverse transcription alone may be beneficial and achieve viral clearance from the blood, but this could require an extremely long duration of therapy [52]. A greater understanding of cccDNA maintenance and enzymes that lead to its active decay might ultimately provide the best targets to exploit for therapy.

With our current experience, sterilizing immunity is being questioned as a definition of ‘cure’ in chronic HBV patients [53]. The ability of cccDNA to persist in hepatocytes at virtually undetectable levels suggests that restoring some level of HBV-specific adaptive immunity will be necessary to achieve stable long-term control without routine administration of antivirals. Ultimately, targeting nonredundant aspects of HBV infection to reduce antigen and viral load, prevent reinfection, and clear infected hepatocytes from the liver would be the ideal situation. This would minimize the emergence of resistant mutants and hopefully allow endogenous virus-specific immunity to gain the upper hand.

References


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