Therapeutic strategies for a functional cure of chronic hepatitis B virus infection

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Abstract  Treatment of chronic hepatitis B virus (HBV) infection with the viral DNA polymerase inhibitors or pegylated alpha-interferon has led to a significant retardation in HBV-related disease progression and reduction in mortality related to chronic hepatitis B associated liver decompensation and hepatocellular carcinoma. However, chronic HBV infection remains not cured. The reasons for the failure to eradicate HBV infection by long-term antiviral therapy are not completely understood. However, clinical studies suggest that the intrinsic stability of the nuclear form of viral genome, the covalently closed circular (ccc) DNA, sustained low level viral replication under antiviral therapy and homeostatic proliferation of hepatocytes are the critical virological and pathophysiological factors that affect the persistence and therapeutic outcomes of HBV infection. More importantly, despite potent suppression of HBV replication in livers of the treated patients, the dysfunction of HBV-specific antiviral immunity persists. The inability of the immune system to recognize cells harboring HBV infection and to cure or eliminate cells actively producing virus is the biggest challenge to finding a cure. Unraveling the complex virus–host interactions that lead to persistent infection should facilitate the rational design of antivirals and immunotherapeutics to cure chronic HBV infection.

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1. Introduction

Hepatitis B virus (HBV) infection is one of the major public health challenges worldwide. While the availability of a vaccine has reduced the number of new HBV infections, it does not benefit the 350 million people already chronically infected by the virus. In fact, approximately one-third of these chronically infected individuals will die from serious liver diseases, such as cirrhosis, hepatocellular carcinoma (HCC) and liver failure, if left untreated. The therapeutic goal of chronic HBV infection is thus to decrease the risk of liver disease progression and prevent its detrimental clinical sequelae, which can be achieved by sustained suppression of the virus replication, or ideally by curing the virus infection.

Currently, seven drugs have been approved by the Food and Drug Administration of USA for the treatment of chronic hepatitis B, which include two formulations of alpha-interferon (standard and pegylated) that enhance the host antiviral immune response and five nucleos(t)ide analogs (lamivudine, adefovir, entecavir, telbivudine, and tenofovir) that inhibit HBV DNA polymerase with varying potencies and barriers to resistance. At present, the preferred first-line treatment choices are pegylated-interferon alpha-2a (pegIFN-α), entecavir and tenofovir, based on their superior antiviral efficacy and/or high resistance barrier. However, even with the first-line treatment options, pegIFN-α is effective in achieving sustained virological response in only 30% of HBeAg-positive and 40% of HBeAg-negative cases, and usually associated with severe side-effects. On the other hand, the nucleos(t)ide analogs are well tolerated and potently suppress HBV replication in the vast majority of treated patients. However, even the most potent nucleos(t)ide analogs rarely induce HBV surface antigen (HBsAg) seroconversion, the hallmark of a successful immunologic response to HBV with complete and durable control of infection, or a "functional cure." Hence, long-term, and possibly life-long, nucleos(t)ide analog treatment is required to continuously suppress HBV replication, which may be associated with significant cost burden and limited by drug-associated toxicity. It is, therefore, a pressing need for the introduction of therapeutic regimens that are safer and effective in achieving a functional cure. Apparently, unraveling the complex virus-host interactions that lead to persistent infection and better understanding of the obstacles to a cure are essential for the development of curative therapeutics for chronic HBV infection.

2. Pathobiological features of HBV infection

HBV is the prototype virus of the hepadnaviridae family and contains a relaxed circular (rc) partially double stranded DNA (3.2 kb in length) genome. A hallmark of HBV genomic DNA replication is protein-prime reverse transcription of a RNA intermediate called pre-genomic (pg) RNA. However, unlike that of classic retroviruses, integration of viral genomic DNA into host cellular chromosomes is not an obligatory step in the HBV life cycle. Briefly, as illustrated in Fig. 1, HBV virion binds to its cellular receptor, sodium taurocholate cotransporting polypeptide (NTCP), on the surface of hepatocyte and is subsequently internalized by macropinocytosis. Viral envelope fuses with endosomal membrane to release nucleoacapsid into the cytoplasm, where genomic rcDNA is deproteinized and delivered into the nucleus to be converted into covalently closed circular (ccc) DNA by host cellular DNA repair machinery. The cccDNA exists as an episomal minichromosome and serves as the template for the transcription of viral RNAs. The viral pregenomic (pg) RNA is translated to produce both the core protein and DNA polymerase. The DNA polymerase binds to the epsilon sequence within the 5' portion of pgRNA to prime viral DNA synthesis and initiate nucleocapsid assembly. The encapsidated pgRNA is then reverse transcribed into minus strand viral DNA, which serves as a template for the subsequent synthesis of plus strand DNA by viral polymerase. The nucleocapsid matures as rcDNA is formed and can be either enveloped and secreted out of the cell as a virion particle, or delivered into the nucleus to amplify the nuclear cccDNA pool.

The outcome of HBV infection as well as the severity of HBV-induced liver diseases varies widely among the infected individuals. While over 95% percent of adult-acquired infections are spontaneously cleared within 6 months, more than 90% of exposed neonates and approximately 30% of children aged 1–5 years will develop chronic infection. Individuals infected during infancy represent the majority of the global reservoir of chronic carriers. Other than the occasional appearance of ground-glass hepatocytes in chronic HBV carriers, which is presumably due to accumulation of large envelope protein in the endoplasmic reticulum, HBV infection of hepatocytes induces negligible cytopathic effect. It is, therefore, generally believed that the outcome of HBV infection and severity of its associated liver diseases are determined by the nature and magnitude of host immune response against the virus.

Studies in HBV infected people and chimpanzees show that unlike other viruses that promptly induce an inflammatory cytokine response in the early phase of infection, HBV infection is stealthy and flies under the radar of the host immune sensors for a few weeks before the activation of humoral and T lymphocyte-mediated cellular immune responses. While a polyclonal and vigorous HBV-specific T-cell response resolves HBV infection through coordinated kill and cure of infected hepatocytes, a weaker or barely detectable HBV-specific T-cell response fails to eliminate the virus and results in chronic infection. Unfortunately, despite extensive research efforts, the precise mechanism by which HBV fails to induce a vigorous immune response in the chronic carriers remains elusive.

3. Virologic goal of chronic hepatitis B therapies

In principle, cure of HBV infection means eradication of the virus from an infected individual, which requires elimination of all extracellular virions and kill or cure of every virally-infected cell in the body. Because either extracellular virions or virally-infected cells dynamically turnover with kinetics regulated by host pathophysiological cues, such as antiviral immune response and proliferation of host cells, cure of HBV infection can, in theory, be achieved by the inhibition of viral replication for a period of time that allows for complete decay of extracellular infectious virions and the most stable viral replication intermediate, presumably the cccDNA, in infected cells. However, if there are long-living cells infected by HBV, inhibition of viral replication alone may not be possible to eradicate the virus infection. Hence, suppression of HBV gene expression as well as constant immune surveillance of the residual infected cells might be essential for a durable off-therapy control of HBV infection. In fact, it is evident that the cccDNA is not completely eradicated from the liver following resolution of an acute infection, but appears to be controlled at extremely low levels by host antiviral
This residual infection becomes clinically relevant only under the condition of systematic immunosuppression. Therefore, a realistic virologic goal of anti-HBV therapy may not necessarily be the eradication of HBV or an absolute cure, but the suppression of viral replication as well as the restoration of host antiviral immune response to sustainably control HBV infection to a condition that is equivalent to that achieved by natural resolution of an acute infection, i.e., a functional cure.

However, achievement of the functional cure is currently hampered by the following conditions.

4. Obstacles to a functional cure

4.1. The intrinsic stability of HBV cccDNA

Treatment of chronic hepatitis B patients with nucleoside analogs for more than a year reduces HBV load in plasma by more than 4 logs. However, analyses of intrahepatic viral DNA indicate that the antiviral therapies only reduce the cytoplasmic HBV core DNA and nuclear HBV cccDNA by approximately 2 and 1 log, respectively. These observations are well corroborated with
the inability of nucleoside analogs to significantly reduce the level of HBsAg antigenemia, which is quantitatively correlated with the level of cccDNA. Similarly, extensive analyses of intrahepatic woodchuck hepatitis virus (WHV) and duck hepatitis B virus (DHBV) DNA intermediates under nucleoside analog therapy clearly demonstrated that cccDNA become the predominant form of viral DNA replication intermediates upon long-term suppression of viral DNA replication. These findings strongly suggest that cccDNA is the most stable HBV replication intermediate and elimination of cccDNA is the key to cure HBV infection.

As stated above and illustrated in Fig. 1, cccDNA is initially synthesized from the rcDNA from the incoming viral nucleocapsids during HBV infection of a hepatocyte. In the early phase of infection, additional cccDNA are produced from newly synthesized cytoplasmic rcDNA through an intracellular amplification pathway. These two pathways culminate in the formation of a regulated steady-state population of 5–50 cccDNA molecules per infected hepatocyte. Obviously, persistent infection of hepadnaviruses relies on the stable maintenance and proper function of the cccDNA pool in the nuclei of infected hepatocytes as the source of viral RNAs and therefore, cure of HBV infected hepatocytes requires elimination of cccDNA.

Because cccDNA cannot amplify itself via semiconservative DNA replication, complete inhibition of cytoplasmic rcDNA synthesis by viral polymerase inhibitors should preclude de novo cccDNA synthesis. Hence, the cure of HBV infected hepatocytes by nucleoside analogs relies on the decay of pre-existing cccDNA. Accordingly, extensive efforts have been made to determine the half-lives of cccDNA under the variety of condition and obtained apparently contradictory results (Table 1), implying that the rate of cccDNA metabolism varies under different pathobiological conditions. Concerning the potential mechanisms of cccDNA decay, recent studies suggest that inflammatory cytokines, such as IFN-α and lymphotixin-β, induce intrinsic cellular response to promote the decay of cccDNA through APOBEC3 family enzyme-catalyzed cytidine deamination and subsequent DNA repair processes.

In addition, cccDNA can be diluted during cell division and cccDNA-free cells could arise through multiple rounds of cell division and unequal partitioning of cccDNA molecules into daughter cells. Furthermore, studies with integrated WHV DNA as a genetic maker of virally infected hepatocytes during chronic and transient WHV infection of woodchucks unambiguously demonstrated that virus-free hepatocytes can be derived from infected cells. In another word, WHV-infected hepatocytes are indeed curable in vivo. However, it is not yet clear if the division of infected hepatocytes is required for the host immune response to purge cccDNA in vivo.

Based on the mechanistic analyses of cccDNA metabolism, failure of long-term antiviral therapies with viral DNA polymerase inhibitors to eliminate cccDNA is most likely due to either incomplete suppression of HBV rcDNA synthesis, which allows for continuous replenishment of cccDNA pool via intracellular amplification pathway, or slow turnover of at least a subpopulation of HBV infected cells that serve as reservoirs of the virus.

### Table 1. Half-lives of hepadnaviral cccDNA.

| Experimental condition                                      | cccDNA half-life (days) | Reference |
|-------------------------------------------------------------|-------------------------|-----------|
| HepG2 cells transduced by HBV-expressing baculovirus vector | 3                       | 46        |
| HBV infected chimpanzees under nucleoside analog therapy    | 9–14                    | 47        |
| HBV infected chimpanzees during the early phase of clearance| 3                       | 48        |
| WHV infected primary woodchuck hepatocytes                  | >42                     | 45        |
| WHV infected woodchucks under nucleoside analog therapy     | 33–50                   | 40        |
| Primary hepatocytes from DHBV congenitally infected ducks   | 3–5                     | 49        |
| DHBV infected woodchucks under nucleoside analog therapy    | 35–57                   | 50        |

### 4.2. Incomplete suppression of viral replication

Although clinical studies on the antiviral efficacy of nucleos(t)ide analogs under the variety of clinical conditions demonstrate striking reduction of viral load in peripheral blood, intrahepatic HBV core DNA and cccDNA are still detectable after long-term antiviral therapy. Moreover, sequential accumulation of drug resistance mutations during apparently effective nucleos(t)ide analog therapy provides additional evidence suggesting that residual HBV replication and de novo cccDNA synthesis occur under long-term DNA polymerase inhibitor therapy. Interestingly, analyses of viral DNA replication intermediates and core antigen-positive hepatocytes in the livers of WHV-infected woodchucks under the therapy of clevudine demonstrated that after more than 30 weeks of therapy, the predominant WHV DNA species in the liver is cccDNA. However, core-associated viral DNA replication intermediates, such as partial single-stranded DNA, are also clearly detectable. Intriguingly, while the vast majority of hepatocytes become core antigen-negative, a small fraction of hepatocytes expresses core antigen at the level similar to that in the pre-treated hepatocytes. This observation indicates that while majority of infected hepatocytes have been cured after long-term nucleoside analog therapy, the residual viral DNA replication and cccDNA synthesis occur in discrete hepatocytes. In another word, the failure to cure HBV infection is most likely due to a fraction of HBV infected cells that are refractory to nucleoside analog therapies.

Why should this be? Because the nucleoside analogs are prodrugs that require activation by host cellular nucleoside kinases in virally infected cells, it is thus possible that the cells refractory to the therapy are incapable of activating the nucleoside analogs. Alternatively, considering the important role of cell division in elimination of pre-existing cccDNA, it is also possible that the refractory cells are long-live cells and have not divided during the therapy. Nevertheless, further understanding the biological feature of the refractory cell population is important for the treatment of chronic HBV infection.

### 4.3. Turnover of HBV host cells

The rate of infected cell turnover is one of the key parameters of HBV infection dynamics in vivo. Hepatocyte death, initiated...
through attack by antiviral cytotoxic T-lymphocytes (CTL), and compensatory hepatocyte proliferation, are both believed to be major contributing factors in the loss of virus DNA during immune resolution of transient infections. Although non-cytolytic cure of infected hepatocytes have been approved to occur, it is estimated that a minimum of 0.7–1 and approximately 2 complete random turnovers of the hepatocyte population of the liver occurs during the resolution of WHV infection in woodchucks\textsuperscript{50} and HBV infection in chimpanzees\textsuperscript{48}, respectively. Hepatocyte turnover also plays an important role in viral pathogenesis and immune selection of hepatocytes infected with mutant strains of HBV and in the emergence of hepatocytes that appear refractory to HBV infection through clonal expansion. Under the condition of therapeutic inhibition of ongoing HBV DNA replication, the rate of HBV infected cell turnover is a critical determinant of cccDNA decay kinetics\textsuperscript{41,58}. Accordingly, hepatocyte turnover has been investigated on the variety of pathobiological conditions by either directly measuring hepatocyte proliferation activity from liver biopsies or mathemetic modeling of viral dynamics. These studies from multiple laboratories reveal that while the half-life of hepatocytes in the healthy adult liver is approximately half a year, the median half-lives of infected hepatocytes in patients with chronic hepatitis B are 257 h (\(=10.7\) days) \(n=9\), range 112–762 h)\textsuperscript{59} and 7 days in patients with chronic hepatitis B under lamivudine treatment\textsuperscript{60}. The results thus imply the overall rate of hepatocyte turnover is significantly accelerated in patients with chronic hepatitis B, which should favor the eradication of cccDNA with viral replication inhibitor therapies.

### 4.4. HBV reservoirs or extrahepatic infection

While it is possible that a fraction of infected hepatocytes with slower rates of turnover may serve as reservoirs for HBV after immune resolution of transient HBV infection or long-term antiviral therapy, the existence of extrahepatic reservoirs also cannot be ruled out. In fact, although there are reports claiming the existence of HBV DNA or antigens in peripheral lymphocytes and other tissues, productive HBV infection of cell types other than hepatocytes has not yet been convincingly approved\textsuperscript{1,62}. However, DHBV and WHV have been demonstrated to have an unanticipated broad cell tropism in vivo. For instance, WHV DNA replication intermediates and/or mRNA can be detected in lymphoid cells of spleens, peripheral T and B lymphocytes upon activation\textsuperscript{63}. In addition to liver, DHBV antigen expression, DNA replication intermediates and/or mRNA can also be detected in the brain, lung, heart, intestine, kidney, pancreas and spleen\textsuperscript{64}. In situ hybridization showed evidence of viral replication in the lung epithelium, germinal center of spleen, acinar cell of pancreas and tubular epithelium of kidney\textsuperscript{65}. Moreover, DHBV infects both pancreatic \(\alpha\) and \(\beta\) endocrine cells and impairs the arginine-stimulated insulin response\textsuperscript{66}. Intriguingly, treatment of DHBV congenitally infected ducks with the guanosine analog, ganciclovir, efficiently reduced intrahepatic viral core DNA and reduced core antigen-positive hepatocytes. However, the treatment did not affect viral antigen expression in the bile duct epithelial cells, putative oval cells and DHBV-infected cells in extrahepatic sites such as the pancreas, kidney and spleen\textsuperscript{67}. The studies thus showed that cure of HBV infection requires combination therapies targeting all types of infected cells, but not only hepatocytes. However, due to the intrinsic stability of cccDNA in non-dividing cells, control of HBV replication in the long-lived cell types may require elimination of the “reservoir” cells or silence the viral gene expression and replication by host immune response.

### 4.5. Dysfunction of antiviral immune response

Due to the failure of nucleos(t)ide analog therapy to completely eliminate HBV infected cells, viral infection can be restored within a few weeks to several months after the cessation of antiviral therapy. Hence, cure or durable control of a chronic infection by HBV replication inhibitor therapy may not be possible in a host that lacks an antiviral immune response capable of producing antibodies to neutralize residual viruses and cellular immune response that eliminate infected cells and keeps the reservoirs under immune surveillance.

Although failed to resolve HBV infection, specific humoral and cellular immune responses against HBV antigens are readily detectable in chronic HBV carriers, which is distinct from the immune responses observed in patients who resolve transient HBV infection\textsuperscript{26,59}. For instance, although antibodies against core proteins, HBeAg, polymerase and X protein can be detected in all or a portion of chronic HBV carriers, antibodies against three envelope proteins are not produced or exist in forms of immuno-complexes. Concerning T cell response, HBV persistence is always associated with defective HBV-specific CD4 and CD8 T cell functions\textsuperscript{68}. Despite the phenotypes of the altered immune response in HBV chronic carriers in the different clinical stages have been extensively characterized, the mechanism of HBV infection to induce the dysfunctional immune responses leading to a persistent infection remains elusive\textsuperscript{69}. However, it is generally believed that the defective T cell function is maintained primarily by the effect of the prolonged exposure of T cells to high quantities of viral antigens and by the tolerogenic features of hepatocytes and liver resident cells\textsuperscript{69,70}. These two combined mechanisms can result in the deletion of HBV-specific T cells or in their functional inactivation (exhaustion), which is characterized by an increased expression of negative co-stimulatory molecules and a dysregulation of co-stimulatory pathways, such as PD-1/PDL1, affect the quality and intensity of the antiviral T cell response\textsuperscript{71}.

Considering the critical role of the prolonged exposure of T cells to high quantities of viral antigens in the maintenance of HBV-specific T cell dysfunction, it is anticipated that reduction of HBV antigen load through long-term antiviral therapy to significantly reduce cccDNA should help improve T cell function, which might, in turn, lead to sustained suppression or functional cure of HBV infection. However, although as expected that long-term nucleoside analog therapy at least partially restores the function of HBV specific T cells, a durable control of HBV infection could still not be achieved in the majority of the treated patients\textsuperscript{72–74}. Hence, although reduction of HBV antigen load is helpful, restoration of functional T cell immune response requires the correction of additional defects in the priming, expansion and differentiation of HBV specific T cells.

### 5. Strategies toward a functional cure

Obviously, achievement of a functional cure of chronic HBV infection needs to remove one or multiple obstacles discussed above. Strategically, the following four therapeutic approaches, alone or in combination, have the potential to achieve this goal.
5.1. Combination therapy with antivirals targeting multiple distinct steps of HBV replication

The key to the success of antiviral therapies against the infection of hepatitis C virus and human immunodeficiency virus is the combination therapy with antivirals targeting multiple viral and/or host functions. Such a therapeutic approach improves antiviral efficacy and prevents emergence of drug-resistant viruses. However, treatment of chronic hepatitis B with combination therapies of two different nucleoside analogs or a nucleoside analog and pegIFN-α of chronic hepatitis B with combination therapies of two different nucleoside analogs or a nucleoside analog and pegIFN-α does not demonstrate significantly improved clinical benefits\(^7\). Currently, compounds that inhibit HBV entry into hepatocytes\(^9\), RNase H activity\(^7\) and assembly of nucleocapsids\(^78,81\) are under preclinical and clinical development (Fig. 1 and Table 2). It is anticipated that combination therapies with nucleoside analogs and one or multiple of the novel antivirals should more potently suppress HBV replication and de novo cccDNA synthesis in all HBV infected cells, which could potentially improve the rate of functional cure. In absence of a functional cure, it is also expected that the combination therapies may improve the control of HBV replication in the minority of patients for whom nucleoside analog monotherapy is inadequate to suppress viral titers below the clinical detection limit\(^91\). In addition, the combination therapy may make the antiviral drugs with low genetic barriers to resistance, such as lamivudine, retain effectiveness, which is particularly valuable in developing countries\(^92\).

5.2. Elimination or functional suppression of HBV cccDNA

Although it is generally believed that noncytolytic eradication of cccDNA from virally infected cells or permanent silence of its transcription is essential for a cure or durable control of HBV infection, promising approaches and molecular targets for therapeutic elimination and/or silence of cccDNA have not been identified. Despite studies with cccDNA sequence-specific endonucleozaes or zinc-finger proteins to cleave cccDNA molecules or inhibit its transcription have been successfully demonstrated in cultured hepatoma cells\(^51,52,85\), efficient and targeted delivery of the genes that express those antiviral proteins in all HBV infected cells in vivo is the biggest challenge toward their clinical application\(^85\).

It is obvious that further understanding the molecular mechanism of cccDNA biosynthesis, maintenance and functional regulation as well as developing convenient cell-based assays for investigation of cccDNA biology and search of cccDNA-targeting compounds are essential for development of antiviral drugs to eliminate or silence cccDNA\(^1\).

For instance, the recent discovery that IFN-α and lymphotoxin-β are capable of inducing non-cytolytic degradation of cccDNA through cytidine deamination invokes possibility to cure HBV infected cells via pharmacological activation or argumentation of the host intrinsic antiviral pathway\(^87\). Moreover, studying the molecular mechanism of IFN-α suppression of hepadnavirus cccDNA transcription reveals that the cytokine induces a distinct silence network to shut off cccDNA transcription. Hence, identification of host and viral proteins that are recruited to cccDNA minichromosomes and suppress its function in response to IFN-α may reveal host and viral targets for rationale development of antiviral drugs to eliminate or transcriptionally silence cccDNA\(^51,85\).

5.3. Stimulation of intrahepatic innate immune response

Innate immune response plays an essential role in defending the host from viral infections. Particularly, the cytokine response elicited by activation of host pattern recognition receptors (PRRs) not only contains viral replication and spreading during the early phase of infection, but also orchestrates the activation and development of the adaptive immune response, which ultimately resolves viral infections\(^96\). Intriguingly, HBV infection has been shown to induce a negligible proinflammatory response during the early phase of infection. Whether this is due to the failure of the virus to activate the PRRs or suppression of the PRR signaling pathways by the virus remains controversial\(^73\). However, a plethora of evidence suggests that artificial activation of intrahepatic Toll-like receptors (TLR)\(^98,99\) and RIG-I-like receptors (RLR)\(^100,101\) induces robust cytokine responses in HBV-replicating mice and efficiently suppresses the virus replication. It is also worth noting that HBV activates other branches of innate immune responses, such as NK cells and NKT cells, although it is compromised by virus-induced immunosuppressive cytokine IL-10 or increase of the inhibitory receptor NKG2A on NK cells\(^102,103\). Accordingly, as illustrated in

| Table 2 | Antiviral agents currently in clinical use or under development. | Representative drugs or compounds | Reference |
|---|---|---|---|
| 1. Activation of TLR7 | TLR7 agonists | 82 |
| 2. Activation of TLR3 | TLR3 agonists | 83 |
| 3. Inhibition of virus entry | Myrcludex-B | 76 |
| 4. Inhibition of cccDNA formation | Disubstituted sulfonamide | 84 |
| 5. Elimination and/or silence of cccDNA | IFN-α | 51,52,85 |
| 6. Inhibition of nucleocapsid formation | Lyphotoxin-β | 51 |
| | Heteroaryldihydropyrimidines | 78 |
| | Phenylpropenamides | 80,86 |
| | Sulfamoylbenzamides | 81 |
| 7. Inhibition of DNA synthesis | Nucleos(t)ide analogs | 87 |
| DNA polymerase inhibitors | RNase H inhibitors | 77 |
| 8. Inhibition of virion assembly/secrection | Iminosugars | 88 |
| | Tetrahydro-tetrazolo-pyrimidine | 89 |
| 9. Therapeutic vaccinations | Extensively reviewed in | 90 |
| 10. Promotion of functional CTL differentiation and maturation | Extensively reviewed in | 29 |

\(^a\)The numbers are consistent with that illustrated in Fig. 1.
Fig. 1, pharmacological activation of intrahepatic innate immune response in liver non-parenchymal cells, such as Kupffer cells and dendritic cells, has been considered as a therapeutic approach that not only suppresses HBV replication in hepatocytes via induction of antiviral cytokines and activation of NK cells, but also facilitate the priming and development of a successful HBV-specific adaptive immune response. Indeed, the great potential of TLR agonists for treatment of chronic hepatitis B has been demonstrated by the promising results obtained with TLR7 small molecular agonists. For instance, four week treatment of woodchucks chronically infected with WHV with GS-9620, a potent and orally available TLR7 agonist, resulted in a greater than 4 log reduction of viral load in all treated animals. Intriguingly, the suppressive effect on the virus 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, EASL 46th Annual Meeting, Berlin, Germany). Similarly, oral administration of GS-9620 to HBV chronically infected chimpanzees for eight weeks reduced viral load by more than 2 logs and more than ten-fold reduction of viral load persisted for several months after the cessation of treatment. In addition, serum levels of HBsAg, HBeAg and numbers of HBV antigen-positive hepatocytes, were reduced as hepatocyte apoptosis increased. In consistence with the activation of TLR7, GS-9620 administration induced production of interferon-α and other cytokines and chemokines, and activated interferon-stimulated genes.

In addition to activate intrahepatic cytokine response, recent studies in a HBV-replicating mice model also showed that blockade of the natural killer cell inhibitory receptor NKG2A increases activity of NK cells and clears HBV infection. This result suggests that interruption of the interaction between NKG2A and its ligand HLA-E might be an ideal therapeutic approach to treat CHB infection in humans.

5.4. Restoration of HBV specific adaptive immune response

Current, it is not yet known at what extent the dysfunction of viral specific adaptive immunity in chronic HBV carriers is reversible. Phenotypic analyses of HBV-specific T lymphocytes in chronic carriers suggest that the dysfunctional adaptive immune response is, at least in part, due to the exhaustion of cytolytic T cells induced by the prolonged exposure to high quantities of viral antigens. The fact that reduction of HBV antigenemia and viral load in patients receiving long-term effective antiviral therapy partially improves the HBV-specific adaptive immune response seems to support this notion. However, it remains to be demonstrated whether more profound and faster reduction of HBsAg antigenemia through inhibition of viral protein expression and replication by hepatocyte-targeting siRNA, such as ARC-520 (http://www.arrowheadresearch.com/press-releases/arrowhead-receives-regulatory-approval-begin-phase-2a-chronic-hepatitis-b), or HBsAg secretion by small molecules or nucleic acid-based polymers (REP9AC) (http://replicor.com/antiviral-technologies/hepatitis-b) could further improve the antiviral immune response and result in a functional cure.

Interestingly, the observation that adoptive transfer of dysfunctional virus-specific CD8 cells from a chronically infected to a naïve uninfected MHC compatible mouse is not sufficient to restore the T cell memory maturation and differentiation process suggests that in addition to reduction of viral antigen load, direct target cellular pathways mediating the immunopathology of HBV infection is essential for the reconstitution of a fully functional immune response against the virus. The differentiation and maturation of functional T-cells are regulated by numerous receptor and ligand interactions in separate cellular compartments at different phases of the immune response. Recent studies suggest that multiple inhibitory receptors, including PD1, CTLA-4, TIM-3 and LAG-3, play important roles in T-cell exhaustion during persistent HBV infection. Hence, strategies to directly target the negative co-stimulatory pathways involved in the pathogenesis of T cell exhaustion as well as manipulate the liver microenvironment that regulates T cell function, such as production of immunomodulatory cytokines and regulatory T cells, have been extensively investigated in HBV replicating mice models, WHV infected woodchucks and humans in clinic. Data obtained from these studies suggest that the function of the different immune regulatory pathways is not redundant in the pathogenesis of T cell exhaustion and combinational interruption of multiple key regulatory pathways might be required to reverse the dysfunctional adaptive immune response.

Another immunotherapeutic strategy is to induce functional anti-HBV immune responses in chronic HBV carriers by vaccination. During last two decades, vaccination strategies using conventional HBsAg vaccine, immunocomplexes of HBsAg and human anti-HBs, apoptotic cells that express HBV antigens, DNA vaccines or viral vectors expressing HBV proteins have been evaluated in animal models and clinical trials, alone or in combination with antiviral therapy, expression of immune stimulatory cytokines or modulators of T cell function. Although functional antiviral immune response could indeed be induced under selected experimental conditions, vaccination strategies for induction of immune response that can resolve or durably control chronic HBV infection remains to be defined. It can be speculated that a successful therapeutic vaccination strategy should, at least, be able to reduce viral load, break the HBV antigen-specific immune tolerance and modulate T cell differentiation and maturation to prevent their exhaustion.

6. Concluding remarks

Achievement of a functional cure of chronic HBV infection relies on eradication of cccDNA from the vast majority of virally infected cells as well as sustained immune control of HBV replication in a small number of residual HBV-infected cells. Hence, keys to the success are development of drugs targeting distinct viral and/or host functions to potently suppress HBV replication as well as therapeutics to restore or induce functional antiviral adaptive immune response. However, lack of biologically relevant animal models of chronic HBV infection and efficient HBV infectious cell culture system has hampered our investigation toward understanding of the HBV immunopathogenesis and molecular mechanism of cccDNA metabolism/functional regulation as well as search for immunotherapeutic regimes and cccDNA-targeting antivirals. Research to resolve these important issues should ultimately lead to the rational design of antiviral and immunotherapeutic strategies to cure chronic hepatitis B.

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