REVIEW
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Diseases & Research

Maintenance Mechanism of Chronic HBV Infection and the Strategy for Functional Cure

Ziyuan Ma[1], Lili Wu[2], Fengmin Lu[3,#]

[1] School of Medical Science, The University of Sydney, Sydney 2050, Australia
[2] Department of Infectious Diseases, The Third Affiliated Hospital of Sun Yat-sen University, GuangZhou 510630, China
[3] Department of Microbiology & Infectious Disease Center, School of Basic Medical Science, Peking University Health Science Center, Beijing 100191, China

Abstract: Chronic Hepatitis B virus (HBV) infection is a leading cause of chronic hepatitis, liver cirrhosis (LC), liver failure (LF) and hepatocellular carcinoma (HCC) in China. In addition to a 3.2 kb partially double-stranded relaxed circular DNA (rcDNA) genome, the HBV DNA exists in two other forms, covalently closed circular DNA (cccDNA) serves as the source of HBV replication and its persistence sustains chronic HBV infection, while integrated HBV DNA (iDNA) can continuously express surface antigen (HBsAg) independently of the viral life cycle. Following cccDNA clearance or transcriptional silencing, iDNAs becomes the primary source of serum HBsAg. Thus, in the future, there is an urgent need to develop drugs that can more effectively inhibit viral replication, accelerate the depletion/silencing of cccDNA as well as iDNA, and specifically enhance the host immunity against HBV with fewer side effects to achieve the functional cure of CHB. This review analyzes the mechanism sustaining chronic HBV infection through virus-immune interaction and examines recent clinical studies on CHB treatment and potential cure strategies.

Keywords: Chronic HBV infection; Functional cure; Integrated HBV DNA; Host immune interaction.

Introduction

Hepatitis B virus (HBV) is the most prevalent type of hepatitis virus. At present, approximately 254 million people chronically infected by HBV worldwide, result in an estimated 800,000 deaths annually, making HBV a significant global public health issue. Fortunately, it is a vaccine-preventable disease. In 1992, the World Health Organization (WHO) recommended hepatitis B vaccination for newborns, which has substantially reduced the incidence of chronic hepatitis B (CHB) infections; however, vaccination coverage rates vary significantly across regions. The African Region accounts for 63% of new hepatitis B infections, with only 18% of newborns vaccinated against hepatitis B, in contrast to 80% in the Western Pacific[1]. In China, neonatal hepatitis B vaccination coverage has reached 95%. However, due to the high number of pre-existing infections, approximately 80 million individuals with chronic HBV infection in China, and hepatitis B-related deaths and primary liver cancer cases in China account for over 45% of the global total[2]. The ultimate goal of treatment is achieving a functional cure, characterized by sustained suppression of serum HBV DNA below detectable levels following a limited course of antiviral therapy, hepatitis B e antigen (HBeAg) negativity or seroconversion, and hepatitis B surface antigen (HBsAg) negativity with or without anti-HBs presence, and more importantly, such responses should be maintained after drug withdrawal[3]. To achieve functional cure is related to cccDNA and iDNA. The existing antiviral treatment drugs, whether nucleos (t) ide analogues (NAs) or pegylated interferon-α (Peg-IFN-α), cannot directly target the elimination of covalently closed circular DNA (cccDNA) present in the nucleus of infected hepatocytes. Therefore, the urgent need to develop drugs that can more effectively inhibit viral replication, accelerate the depletion/silencing of both cccDNA and iDNA, and examines recent clinical studies on CHB treatment and potential cure strategies.

The basic process of HBV infection replication

In viral taxonomy, HBV belongs to the hepadnaviridae family and processes a 3.2 kb partially double-stranded relaxed circular DNA (rcDNA) genome[4]. Upon entry, rcDNA is repaired in the nucleus to cccDNA, which serves as a template for the transcription of pregenomic RNA (pgRNA) and subgenomic RNA. Subsequently, HBV RNA is translated to produce HBeAg, core protein, P protein, L-HBsAg, M-HBsAg, S-HBsAg and HBx proteins. The viral P protein, translated from pgRNA, is synthesized by reverse transcription using pgRNA as template within the nucleocapsid in the cytoplasm. During the HBV DNA unique reverse transcription, the compact nucleocapsid, formed by core

Correspondence: Fengmin Lu. E-mail: lu.fengmin@hsc.pku.edu.cn. Address: Department of Microbiology & Infectious Disease Center, School of Basic Medical Science, Peking University Health Science Center, No.38 Xueyuan Road, Haidian District, Beijing 100083, China.
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proteins, sequester newly synthesized HBV DNA from pattern recognition receptors in the cytoplasm. Compared with other viruses, this "Stealthy" self-replication strategy not only avoids the degradation of viral DNA by DNase, but also avoids the destruction of host liver cells due to the activation of innate immunity. Additionally, during reverse transcription, pgRNA is degraded by RNaseH, leaving an RNA fragment of less than 20 nucleotides at the 5' end. The DR1 sequence in this fragment acts as a primer for the synthesis of the viral plus strand DNA. If the primer jumps and binds to the DR2 region of the newly synthesized minus-strand DNA, it forms an open-strand loop of rcDNA (90%). If plus-strand synthesis initiates directly in situ without the jump, it results in double-strand linear DNA (dslDNA, 10%, Fig. 1). Double-terminal free dslDNA can integrate into the host cell's chromosomal DNA via microhomology-mediated end joining or non-homologous end joining. iDNA affects chromosome stability and forms fusion viral proteins, which may play a pathogenic role in promoting hepatocyte malignancy transformation and hepatocellular carcinoma.

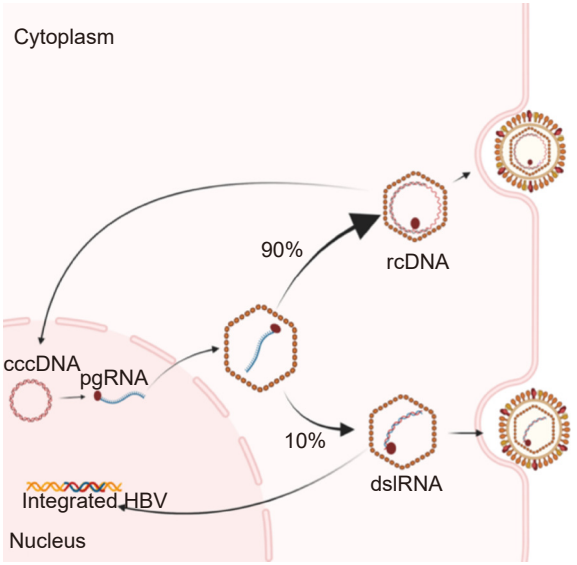

Fig. 1. The two sources of intracellular viral reservoirs: ab initio infection and replenishment from neonatal rcDNA/dslDNA synthesized in nucleocapsid in cytoplasm, during HBV DNA replication process[5].

Mechanism relevant to the chronic HBV infection maintenance

Although the replication mode of HBV DNA is relatively covert, it cannot fully evade detection by the immune system. To avoid the potential immune response against HBV, the virus secretes non-structural HBeAg and excess HBsAg, which aid in maintaining chronic infection by suppressing the host's specific immune response[6]. In innate immunity, dendritic cells (DCs) are crucial for initiating immune responses during HBV infection and play a significant role in determining the outcome of chronic infection. HBeAg can induce naive DCs to differentiate into subsets of regulatory DCs and inhibit their antigen-presenting function, which makes subsequent cell-specific immune responses unable to be initiated normally and weakens the innate immune defens[7]. In addition, natural killer (NK) cells, as another line of defense of innate immunity, are also inhibited. Plasmacytoid DCs activate NK cells through OX40L and IFNα, which in turn activate T lymphocytes and initiate effective immune responses. The mechanism may be related to the impaired ability of plasmacytoid DCs to secrete IFN-α, since HBsAg can down-regulate the expression of OX40L on the surface of DCs and weaken the killing activity of NK cells. Moreover, it induces monocytes to secrete cytokines such as TNF-α and IL-10, down-regulates TLR9 expression on plasmacytoid DCs, and inhibits their IFN-α secretion[8].

Mother-to-child transmission (MTCT) is the main transmission route of HBV infection in China. Studies in transgenic mice indicate that HBeAg can cross the placenta and mediate immune tolerance in newborns[9]. Due to the immature immune system of newborns and infants, it is easy to establish persistent chronic infection after exposure to maternal blood and body fluids containing high concentrations of hepatitis B virus during the perinatal period and early infancy[10], and the chronic infection rate is as high as 95%[11]. Hepatitis B vaccination is the most effective way to prevent HBV infection in neonates. Effective and safe prophylactic hepatitis B vaccines have been available after three generations of development[12] (Table 1). However, despite vaccination, approximately 5-10% of infants born to HBsAg-positive mothers with high HBV DNA levels still contract HBV[3]. Epidemiological investigation shows that the HBsAg-positive rate among childbearing women in China is approximately 6.3%, compared to 14.3% in Africa, where hepatitis B prevalence is higher[11]. Therefore, blocking MTCT is still an important task in Africa, for the prevention and control of CHB.

Immune escape against HBe/HBcAg

It is well known that most patients will became HBeAg negative during the natural history of chronic hepatitis B infection. Le Bert et al.[13] found that HBe/HBcAg-specific cellular immunity persists in chronic HBV infection, especially the active cytotoxic T lymphocyte (CTL) immune response. Under the pressure of immunity against HBe/HBcAg, A1762T/G1764A double site mutation in basic core promoter region (BCP) and G1896A mutation in preC region (PC) or combined mutation (BCP/PC) in chronic HBV infection can inhibit HBeAg expression or even the loss of HBeAg expression due to the G1896A premature mutation, which makes the mutant HBV gain a selective advantage due to immune escape[14].

Mechanistically, in vitro experiments have shown that HBcAg/HBeAg cannot be distinguished in terms of CTL drive and target recognition[15], because HBcAg and HBeAg have similar sequences. Therefore, HBcAg-positive hepatocytes in the liver are greatly reduced under active CTL immune response, after HBeAg disappearance and/or seroconversion. HBcAg is wrapped in nucleocapsids and less exposed to the outside of hepatocytes, and does not contain glycosylation or linked lipoprotein sites, it is difficult for macrophages or DCs to recognize and then to present such circulating HBcAg antigen[16], which limits the clearance of HBV-infected hepatocytes by CTL targeting HBe/HBcAg after HBeAg negative conversion. During the active phase of HBV infection, the core protein appears simultaneously with HBV DNA,

Table 1. The development of HBV vaccines[12].

Generation	Vaccine	Research or Development Department
First Generation: Plasma-derived Vaccines (Purification of plasma HBsAg)	Heptavax-B	Merck & Co., Inc
	Hevac-B 7571	Institute Pasteur Hepatology Institute of PKU
Second Generation: Recombinant Vaccines Expression of HBsAg in yeast cells	Recombivax-HB	Merck & Co., Inc
	Engerix-B High dose (60 ug)	GlaxoSmithKline (GSK) BioKangtai
Third Generation: Adjuvanted with CpG Enhanced with pre-S1/pre-S2	Sci-B-Vac	SciVac Israel Ltd
	PreHevbrio	VBI vaccines Inc
	Heplisav-B	Dynavax

killing HBcAg-specific CTL may mediate the progression of the disease in HBeAg-negative population, and the interaction between core protein and immunity under the repeated fluctuation of HBV DNA promotes the progression of inflammation and fibrosis. In line with this, HBcAg was found binding to TLR2 and heparin sulfate of monocytes through its arginine-rich carboxyl terminal to mediate its entry and interact with heparan sulfate proteoglycans on the surface of macrophages to activate nuclear factor-κB and MAPK, thereby promoting the secretion of pro-inflammatory factors by macrophages[17]. The relatively strong specific immunity against core protein and the accompanying non-specific inflammation also indirectly mediates the clearance of part of infected hepatocytes where transcription active cccDNA present. And in this process, the number of hepatocytes containing iDNA with the advantage of clonal expansion is further increased, which hinders the subsequent functional cure.

HBsAg specific immunity is defective to some extent

To evade immune responses, HBV secretes an excess of HBsAg, resulting in specific immune deficiencies. A recent study has demonstrated a decrease in Pol/HBsAg specific T cells in patients with chronic HBV infection, indicating a link between chronic HBV infection and the exhaustion of HBsAg specific cells[18]. The persistent expression of HBsAg induces the accumulation of regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSC), leading to the loss of effector function of lymphocytes[19]. Granulocytic myeloid-derived suppressor cells (gMDSCs) accumulated in the liver, would express liver-homing chemokine receptors, which might potently inhibit T cell's function through a partially arginase-dependent mechanism during chronic HBV infection[20]. Follicular helper T (Tfh) cells secrete cytokines such as IL-21, promoting differentiation of B cells into plasma cells that produce IgG antibodies[21]. However, due to the inhibitory effect of Treg cells, the function of Tfh cells is restricted, impairing the production of protective antibody IgG.

In the process of HBeAg clearance, cccDNA derived HBsAg decreased significantly, as reflected by the decline of serum HBsAg level. In contrast, HBsAg-positive hepatocytes only showed when revealed by immunohistochemical staining in the liver tissue of patients[22]. The reason for this sustained HBsAg expression is related to the integration of HBV DNA mentioned above. Although the HBsAg secretion efficiency of iDNA is lower than that of cccDNA, it retains some secretion function, impeding the recovery of HBsAg-specific immune function and functional cure[23] (Fig. 2).

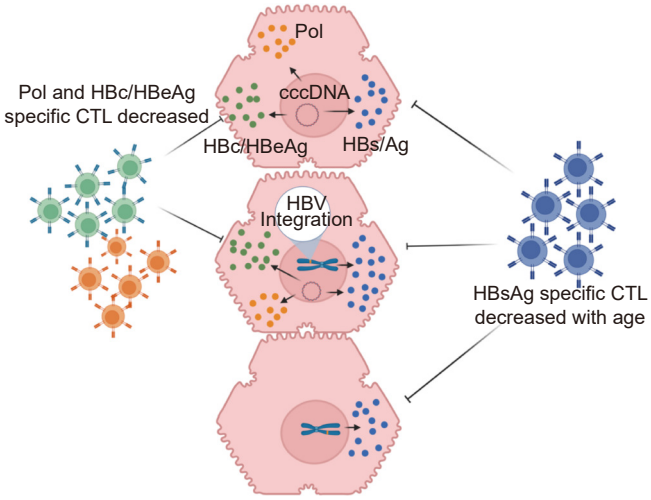

Fig. 2. Immunodeficiency and depletion of specific CTL cells would lead to the persistent of chronic HBV infection[5].

Functional cure strategies of CHB

HBsAg clearance is essential for achieving functional cure. Current therapeutic drugs aim to reduce cccDNA load or its transcriptional activity as the source of HBV replication, but there are no specific inhibitors that directly target the cccDNA or promote its degradation. Gene therapy CRISPR/Cas9 can directly target and destroy cccDNA and even the iDNAs, to inhibit viral replication and HBsAg expression, but its clinical application is constrained by limited delivery safety and efficiency, as well as off-target effects[24,25]. In addition, Hepatitis B Core-Related Antigen (HBcrAg) is a virological indicator of the transcriptional activity of cccDNA. Scholars from Taiwan China[26] found that HBV DNA, HBeAg and HBcrAg with cccDNA transcriptional activity turned negative much earlier than HBsAg loss. This indicating that merely eliminating or silencing cccDNA is insufficient for achieving functional cure, and strategies targeting iDNA are still needed.

Summarizing the available treatment strategies shows that have low baseline serum HBsAg levels are consistently low in patients who achieve functional cure (Table 2). The inhibitors of programmed cell death protein 1 (PD-1) / programmed cell death ligand 1 (PD-L1) exhibits limited immunomodulatory effect regarding to the cause of serum HBsAg loss only in individuals

Table 2. Characteristics of those patients who achieved functional cure under the current treatment strategies or new medicine under development.

Project/Research	Mechanism	Characteristics of Those Patients Who Achieved Functional Cure
Immune Checkpoint Inhibitor (ICI)[27] PD-1 : Nivolumab, Pembrolizumab PD-L1: Durvalumab, Atezolizumab	Blocking PD-1/PD-L1 interaction with antibodies to partially restore impaired HBV-specific T and B cell responses	Baseline HBsAg ≤ 100 IU/mL
Everest Project[28] NAs PEG-IFN	Directly targeting HBV transcription and replication while modulating host immunity	Baseline HBsAg ≤ 1500 IU/mL
GSK[29] Bepirovirsen (ASO)	Inhibiting viral protein expression at the mRNA level and moderately activating non-specific immune responses	Baseline HBsAg < 3000 IU/mL
Drug Withdrawal[30,31]	Trace expressed viral protein induce the activation of specific immune response and HBsAg negative conversion	Asian: HBsAg < 100 IU/mL Caucasian: HBsAg < 1000 IU/mL

with serum HBeAg negative and very low serum HBsAg levels (HBsAg ≤ 100 IU/mL)[27]. In the Everest Project, patients who already on NAs treatment were treated with sequential/combination PEG-IFN. After grouped analysis the results, it was found that the highest HBsAg clearance rate was 56.1% at the low baseline (HBsAg ≤ 100 IU/mL)[28]. Bepirovirsen has therapeutic effect on patients with negative serum HBeAg and high serum HBsAg value, but there is a risk of recurrence, and its immunomodulatory effect needs to be further elucidated[29]. In addition, non-cirrhotic patients with virologic suppression, negative serum HBeAg and low serum HBsAg levels at the time of drug withdrawal are more likely to achieve serum HBsAg clearance[30,31]. This may be because the small amount of viral protein expressed after drug withdrawal act as an "autovaccine immunity", effectively activating specific immunity and promoting serum HBsAg clearance. In conclusion, low serum HBsAg levels are a favorable factor for achieving functional cure with existing drug treatment; However new drugs that regulate host specific immunity with stronger ability to inhibit viral replication and fewer side effects are needed.

In addition to direct antiviral and immune modulation treatments, therapeutic vaccines also could be a promising therapeutic strategy to effectively combat HBV and ultimately cure the disease by enhancing the body's immune response specifically against HBV. The existing therapeutic hepatitis B vaccines are mainly divided into four categories, namely recombinant protein vaccines, DNA vaccines, lipopeptide vaccines and mRNA vaccines (Table 3). Most of them are in the research and development stage, there are still disadvantages such as vaccine degradation and inability to produce neutralizing antibodies[32]. Secondly, sequential/combination therapies are an emerging trend in CHB treatment. A recent study by Buti et al.[33] used sequential therapy that with Bepirovisen followed by PEG-IFN in HBsAg-positive CHB patients on stable NAs treatment. This sequential therapy achieved functional cure in 42% of patients, significantly minimized clinical relapse and viral rebound after drug withdrawal, compared to bepirovirsen therapy alone. However, sequential/combination therapies only achieve functional cure in some patients, the therapeutic regiment still need to be improved.

Table 3. Current development of therapeutic vaccines.

Vaccines	Mechanism	Research Progress	Research or Development Department
Recombinant Protein-Based Vaccine - BRII-179[34]	Recombinant HBV antigens (expression of Pre-S1, Pre-S2 and S), to enhance immune responses	In Phase 2 trials, it is being tested alongside siRNA and PEG-IFNα for increased efficacy.	VBI vaccine
DNA-based vaccine - TherVacB[35]	Recombinant HBsAg, HBcAg and boosting immunization with modified vaccinia virus Ankara vector (MVA) expressing HBV antigen were adopted	Clinical trials started in early 2024. To evaluate the safety and immunogenicity of TherVacB in healthy volunteers (aged 18 to 65 years)	Helmholtz Munich in collaboration with European partners
Lipopeptide-Based Vaccine - HB-400[36]	Use an arenavirus vector to deliver HBV-specific lipopeptides, aiming to stimulate immune response	In phase I trials	HOOKIPA Pharma in partnership with Gilead Sciences
mRNA-Based Vaccine - YS-HBV-002[37]	Stimulate immune responses against hepatitis B virus (HBV) antigens to break immune tolerance.	The Phase I trial is approved in the Philippines, starting mid-2024	YS Biopharma

Conclusion

In conclusion, HBV is a weak activator of innate immunity. First, HBV DNA is encapsulated in nucleocapsid, preventing its detection by cytoplasmic DNA sensors and thus escaping the intracellular innate immune response. Secondly, during chronic infection, HBeAg induces immune tolerance. Meanwhile, prolonged high levels of HBsAg contribute to the exhaustion of host-specific immunity. Regarding to the achievement of functional cure for chronic HBV infection, it will require direct-acting antiviral drugs to inhibit viral replication, deplete cccDNA, and allow residual cccDNA and iDNA, if any, to undergo transcriptional silencing through epigenetic modifications. Simultaneously, serum HBsAg reducing would result in the restoration of exhausted HBsAg-specific CTL function. Additionally, subsequent immunomodulation may enhance host immunity to target and eliminate those hepatocytes with transcriptionally active cccDNA and/or iDNA to achieve functional cure. In the future, the most effective treatment strategy to ultimately achieve functional cure should meet two conditions. First, using of direct antiviral drugs with strong antiviral activity to block virus replication and reduce or eliminate viral antigen load. Then sequenced/combined with immunomodulatory agents that restore host immunity, especially anti-HBV specific immunity, to clear residual HBV-infected hepatocytes and integrated cell colonies. However, developing new drugs with border functions and fewer side effects is still essential.

Abbreviations

BCP, Basic Core Promoter; cccDNA, Covalently Closed Circular DNA; CHB, Chronic Hepatitis B; CRISPR-Cas9, Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-related protein 9; CTLs, Cytotoxic T Lymphocytes; ICI, Immune Checkpoint Inhibitor; DC, Dendritic Cell; dslDNA, Double-Stranded Linear DNA; gMDSC, Granulocytic Myeloid-derived Suppressor cell; HBcAg, Hepatitis B Core Antigen; HBcrAg, Hepatitis B Core-Related Antigen; HBeAg, Hepatitis B e-antigen; HBsAg, Hepatitis B Surface Antigen; HBV, Hepatitis B Virus; HCC, Hepatocellular Carcinoma; iDNA, Integrated HBV DNA; LF, Liver Failure; MDSC, myeloid-derived suppressor cells; MTCT, Mother-to-child Transmission; MVA, Modified Vaccinia Virus Ankara Vector; NAs, Nucleos(t)ide Analogues; NK, Natural Killer; PC, preC; PD-1, Programmed Cell Death Protein 1; PD-L1, Programmed Cell Death 1 Ligand 1; PEG-IFN α, Pegylated Interferon-α; pgRNA, Pre-genomic RNA; rcDNA, Relaxed Circular DNA; siRNA, Small Interfering RNA; Tfh, Follicular Helper T Cell; Treg, Regulatory T cells; WHO, World Health Organization.

Authors' contributions

These authors were involved with this manuscript: LLW and ZYM draft of the manuscript. FML and ZYM revise the article critically for important intellectual content. All authors read and approved the manuscript.

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