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Current progress in the development of therapeutic vaccines for chronic hepatitis B virus infection

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ABSTRACT

Chronic hepatitis B is still a major public health issue despite the successful prophylactic vaccination attempts. Chronicity of hepatitis B virus (HBV) is mainly due to its ability to debilitate host's immune system. Therefore, major measures have been taken to stop this process and help patients with chronic hepatitis B infection recover from their illness. While satisfactory results have been achieved using preventive HBV vaccines, a reliable and effective therapeutic treatment is still in need of extensive studies. Current treatments for chronic hepatitis B include direct antiviral agents and nucleoside/nucleotide analogs, which are not always effective and are also costly. In addition, due to the fact that chronic HBV is responsible for debilitation of the immune system, studies have focused on developing therapeutic vaccines to help host's immune system recover and limit the infection. Several approaches including but not restricted to recombinant peptide-based, DNA-based, viral vector-based, and cell-based approaches are currently in use to develop therapeutic vaccines against the chronic form of HBV infection. In the current review, the authors will first discuss the role of the immune system in chronic hepatitis B infection and will then focus on latest advancements in therapeutic vaccination of HBV especially the clinical trials that have been carried out so far.

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Introduction

Hepatitis B virus (HBV) is responsible for a significant viral infectious disease. Despite the availability of an effective vaccine against the hepatitis B virus, more than 350 million developed chronic infection (1). Chronic infection occurs due to the persistence of HBV and is commonly assessed based on the presence of HBV surface antigens (HBsAg) in serum samples of individuals for more than six months. HBV viral antigens and HBV DNA in the liver are shown in HBV chronic infection as well. Cirrhosis, liver failure, and hepatocellular carcinoma are potential complications of chronic HBV infection which develop in 15–40% of cases of those who are chronic carriers of HBV infection (2). HBV-related chronic liver diseases are responsible for over a million deaths annually (3).

Modes of transmission have important clinical implications, as individuals are more likely to develop chronic HBV infection if they are infected perinatal or in the preschool age (4).

HBV prophylactic vaccination is the most important prevention measure that has dramatically decreased the incidence rate of HBV infection in many countries (5).

Chronic hepatitis B virus infection (CHB) is mainly treated using direct antiviral agents, such as lamivudine, adefovir, telbivudine, and entecavir. Even though these drugs successfully decrease serum viral load to an undetectable level, they cannot completely eradicate HBV infection due to the fact that covalently closed circular DNA (CCCDNA) of HBV persists in hepatocytes and this may then result in the emergence of resistant virus particles (6,7). Moreover, long-term treatment with these agents is expensive and is associated with adverse side effects as well as the development of drug resistance especially during long-term treatment (8). Antiviral resistance is the most important factor in treatment failure. To overcome this problem in CHB patients, therapeutic vaccination has been introduced as a potentially efficient method based on the

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observation that strong adaptive and innate immune responses are necessary to clear HBV chronic infection and that HBV infection impairs such vital responses (9). Thus therapeutic vaccination aims to stimulate patient's immune responses to eliminate HBV infection.

Virology

HBV belongs to the *Hepadnaviridae* family, the small enveloped animal viruses that contain DNA (10). HBV is a 42–44 nm spherical virus and its surface antigen (HBsAg) envelops the nucleocapsid that contains the core protein and HBV genome. HBV genome is a partially double-stranded relaxed circular DNA (rcDNA) containing around 3200 base pairs and is linked to the terminal part of the viral polymerase (11). Four overlapping open reading frames (ORFs) exist in HBV rcDNA: (I) The preS/S ORF encodes three hepatitis B surface antigens (HBsAg), named large (L), middle (M), and small (S) based on their size ranges. However, they all share the same C-terminal domain. (II) The precore/core ORF encodes the core protein (HBcAg) and a non-structural protein called precore or secreted e antigen (HBeAg), which is not essential for viral replication but contributes to viral persistence due to immune-modulating properties. (III) The pol ORF encodes the viral polymerase that has reverse transcriptase, RNase H, and DNA polymerase activities. (IV) As shown *in vivo*, the X ORF encodes a small regulatory protein called x protein that has a vital role in viral replication (12-14).

Natural immune responses to HBV

Innate immunity

During the first weeks of HBV infection, no changes in the expression of intrahepatic genes are observed (15). Therefore, it seems that HBV cannot induce strong innate immune responses such as induction of type I interferon and production of proinflammatory cytokines early in the course of infection (15, 16). However, *in vitro* studies indicated that innate immunity of hepatocytes may sense and limit the HBV infection (17-19).

Considering all aspects, innate immunity still acts earlier than adaptive immunity. It was shown in a study of HBV-infected chimpanzees that most HBV DNA can be cleared from the serum and livers of the animals before an adaptive immune response was detected (20).

Important effectors of innate immunity defensive lines are type I Interferons (IFN-α and IFN-β) and proinflammatory cytokines such as interleukins. Toll-like receptors (TLRs), a pathogen recognition receptor, mediate the production of type I IFNs (19), which in turn stimulates antigen-presenting cells (APCs) such as dendritic cells and Kupffer cells that could lead to the production of interleukin-8 (IL-8), IL-12, IL-18 and other cytokines (21).

Cellular immunity

A fundamental role of T-cell responses in HBV clearance was shown in a chimpanzee model showing that *in vivo* depletion of CD4+ or CD8+ T cells prevents HBV clearance (22). The strength of specific T cell responses determines the outcome of HBV infection. Previous studies suggested that strong, polyclonal, and multispecific CD8+ and CD4+ T cell responses are correlated with acute self-limited HBV infection (reviewed in (23)) whereas weak and limited focused T cell responses are observed more often in chronic HBV infection (24).

HBeAg induces a T-helper 2 (Th2) immune response whereas HBcAg stimulates a Th1 response. Additionally, polymerase and X antigens can also induce CD4+ T cell responses (25). The Th2 response to HBeAg outperforms the Th1 response to the HBcAg. Therefore, the HBcAg-specific T cells have been shown to be depleted *in vivo* (26). Interestingly, it seems that different doses of virus generate different responses. A Th1-mediated response is produced by low doses of the virus while a Th2-mediated response is produced by high doses of the virus (26).

CD8+ T cells recognize HBV epitopes, especially HBcAg epitopes that are presented on the surface of infected liver cells through HLA class I molecules. Upon recognition of infected cells, cytotoxic T lymphocytes begin a direct cell killing process along with secretion of IFN-γ and TNF-α (tumor necrosis factor). These two cytokines induce non-cytolytic downregulation of HBV replication through multiple mechanisms. Even though it is produced by HBV-specific CD8+ T cells, IFN-γ produced by macrophages, NKT cells, and HBV-non-specific T cells in response to other pathogens such as the choriomeningitis virus can also downregulate HBV replication (27, 28).

Humoral immunity

Acute HBV infection recovery results in a lifelong protective immunity. HBsAg-specific antibodies, as well as HBV-specific CD4+ and CD8+ T cells, are responsible for this protection. HBV DNA disappearance from blood and liver is followed by maximal CD4+ and CD8+ T cell responses in the liver and blood, maximum alanine aminotransferase (ALT) levels, and the presence of HBeAg and HBsAg-specific antibodies in the blood (15, 22).

In addition to the role of humoral immunity in HBV protection, HBV-specific antibodies could help understand different stages of the disease. HBcAg-specific IgM is the first antibody detected in the course of infection, whereas antibodies against HBeAg and HBsAg appear late and imply a better prognosis. The appearance of HBsAg-specific antibodies is correlated with protective immunity. Both HBsAg-specific antibodies and HBcAg-specific IgG persist lifelong after clinical recovery (29).

Even after clinical recovery of acute hepatitis B infection and mediation of lifelong protective immunity, minimal amounts of HBV persist in the blood and are controlled by adaptive immune responses. Accordingly, clinically recovered patients with protective HBsAg and HBeAg-specific antibodies may experience reactivation of HBV after immunosuppression due to cancer chemotherapy (30). Moreover, organ transplantation from HBsAg-specific antibody positive donors may transmit HBV to immunosuppressed transplant recipients (31). Some studies suggest that booster vaccinations are required for maintenance of vaccine-induced HBV-specific humoral and cellular responses. However, this is still a controversy because others consider the antigen-specific B and T cells' immunological memory to be sufficient for efficient responses against later infections, even when vaccine-induced antibodies is reduced to undetectable levels (32).

Challenges in developing HBV therapeutic vaccines

HBV genetic variability

HBV has been changing gradually over a long period of time, resulting in a large amount of genetic diversity. Nowadays, there are 8 distinct genotypes of HBV A to H and each genotype may have different subtypes that are different in terms of disease severity, geographical distribution and response to therapy (33).

This genetic diversity creates a challenge in the development of effective HBV vaccines as a vaccine against a specific genotype of the virus may not be protective against other genotypes of the virus and thus cannot offer effective immunity.

Hepatitis B persistence and immune evasion

Acute HBV infection leads to chronic infection in many cases. Many factors contribute to the persistence of HBV and identifying them may provide us with clues in combating the persistence mechanisms. Chronic hepatitis B occurs mainly in vertical transmission of the virus from HBsAg and HBeAg positive mothers to neonates.

Another factor that contributes to the persistence of HBV infection is survival of the cccDNA inside hepatocytes and establishment of a virus-host interaction network that manipulates the cellular machinery to replicate and produce HBV particles while evading the antiviral response of the host (34).

Chronic HBV infection is characterized by innate and adaptive immunity dysfunction (34). HBV interferes with the innate immune system of the hepatocytes, thus evading the associated immune responses (35).

Several mechanisms contribute to the dysfunction of HBV-specific T-cell immunity in chronic hepatitis B. This includes high levels of viral antigens and the tolerogenic microenvironment of the liver (36).

Liver, being the major site of HBV infection, is found to be a more tolerant environment. Murine Kupffer cells express IL-10 (a main anti-inflamatory cytokine (37)) and transforming growth factor β (TGF-β). These two immunosuppressive cytokines generate an environment that induces tolerance in nearby lymphocytes (38). Notably, IL-10 is negatively associated with hepatitis B outcome (38).

The main source of IL-10 and TGF-β is the regulatory CD4+ T cell. Interestingly, CD4+ T cells protect the liver from tissue damage, but also contribute to the failure of CD8+ T cells (39, 40). Chronically HBV-infected patients have higher levels of regulatory CD4+ T cells in their blood which inhibit proliferation of HBV-specific CD8+ T cells and lead to CD8+ T cell failure (41-43).

Several studies suggest a significant correlation between HBV clearance and strong multi-epitope-specific CD8+ T-cell responses (44, 45). However, CD8+ T cell failure is a major component of chronic HBV infection (46).

Dysfunctional CD8+ T cells have impaired proliferative capacity and cannot secrete antiviral cytokines such as IL-2 and IFN-γ (47). Persistence and high viral loads and impaired CD8+ T-cell functions may finally result in the depletion of these T cells (48-50).

Many factors that contribute to dysfunction of CD8+ T cells and apoptosis are upregulated in chronic HBV infection including the pro-apoptotic molecule Bim (51, 52), inhibitory receptor of programmed death-1 (PD-1) (53, 54), inhibitory molecule 2B4 (55), inhibitory molecule cytotoxic T-lymphocyte antigen 4 (CTLA-4) (56), and inhibitory receptor T cell immunoglobulin mucin-3 (Tim-3) (57).

Viral evolution was shown to be important in the immune evasion of chronic hepatitis B as it was demonstrated that PC/BCP region sequence diversity is higher in HBeAg patients with seroconversion compared with patients not showing seroconversion even before HBeAg seroconversion occurs (58). Development of escape mutants seems to have a more important role in escaping from vaccine-induced humoral immune responses than cellular immune responses (59-61).

Lack of appropriate models

Another obstacle in the development of HBV therapeutic vaccine is the lack of convenient *in vitro* infection systems and animal models. The first animals found to be susceptible to HBV infection were chimpanzees (62). These primates are the only known species that develop HBV-specific cellular immune responses similar to those in humans (63). The chimpanzee model has contributed a lot to the development of safe HBV vaccines and therapeutic agents. However, their large size, high costs associated with keeping and raising, and the

associated ethical issues greatly restrict their use for experimental purposes (64).

Another animal model used for the study of HBV infection is the tree shrew species Tupaia belangeri. The ability of these animals to elucidate HBV infection as well as their adaptability to laboratory environments makes them appropriate for studies both *in vivo* and *in vitro* (65, 66). Although a major setback of this model is that inoculation with HBV only results in a transient infection (64).

Due to the limited host range of HBV and limitations to work with the above-mentioned models of natural HBV infection, other viruses from *Hepadnaviridae* family that showed similarity to HBV were identified and studied over the last decades. These HBV-related *Hepadnaviridae* viruses include, woolly monkey hepatitis B virus (WM-HBV) woodchuck hepatitis virus (WHV) (110), ground squirrel hepatitis virus (GSHV), arctic squirrel hepatitis virus (ASHV), and avian hepatitis viruses such as duck hepatitis B virus (DHBV) and heron hepatitis B virus (HHBV) (all reviewed in (64)).

An animal system containing HBV-permissive human hepatocytes would permit more practical testing of antivirals and study of HBV pathogenesis especially the early steps of HBV infection. Therefore, various mouse strains carrying HBV transgenes such as transgenic mice (reviewed in (67)) and chimeric mice (reviewed in (68)) were developed.

HBV vaccines

Current vaccines

Prophylactic vaccines against HBV contain S envelope protein and are produced by processing of HBsAg obtained from plasma of HBV carriers or from the yeast *Saccharomyces cerevisiae* containing a recombinant DNA plasmid expressing S. Another type of prophylactic vaccine contains both S and M envelope proteins obtained from genetically engineered Chinese ovary cell lines.

The efficacy of HBV prophylactic vaccines has been demonstrated in large clinical studies of high-risk populations including infants born to HBsAg-positive mothers, men who have sex with men (MSM) and healthcare workers. Anti-HBs antibody titers of over 10 IU per ml ensure protection. However, the anti-HBs antibody titer may decrease to undetectable levels several years after vaccination but protective immunity continues, suggesting the existence of an immunologic memory. Accordingly, a booster dose is not recommended in healthy individuals in whom the anti-HBs antibody titers have declined to undetectable levels but are not constantly exposed to HBV infection.

The vaccine is highly immunogenic and induces a protective anti-HBs antibody titer in more than 95% of vaccinated children or young adults. About 5% fail to respond to the vaccine and could not develop protective antibodies. Several factors seem to be responsible, such as over 40 age, genetic factors, high body mass index, and immunosuppression (69, 70).

Vaccine escape mutants

Vaccine escape mutants, reported in follow-up studies of individuals receiving HBsAg vaccines containing the S protein, is a very rare phenomenon and different from the 5% who do not respond to the vaccination. In this phenomenon, a vaccinated individual becomes seropositive for HBsAg and develops chronic HBV infection despite the presence of vaccine-induced antibodies to the “a” determinant of HBsAg. This can be explained by the presence of mutated HBsAg in the serum of these patients that lack the group specific “a” determinant, generally as a result of missense mutations in the region of S that encodes amino acids 124 to 147 (71). The increase of these mutant viruses may be of concern due to their risk for vaccinated individuals (72). However, another study suggested that vaccine escape mutants may remain rare despite the universal coverage of HBV vaccination programs (73).

HBV therapeutic vaccines

Based on the fact that strong adaptive and innate immune responses are required to successfully clear HBV chronic infection and the ability of HBV to debilitate these responses, therapeutic vaccination was introduced to further enhance the efficacy of current antiviral therapy regimes. Many studies have been published on the development of therapeutic vaccination against the hepatitis B virus. Here, the authors would try to summarize some of these studies to further enhance our understanding of HBV therapeutic vaccination. (Table 1)

Recombinant peptide- based vaccines

First attempts to develop a therapeutic vaccine against HBV were peptide-based vaccines. Peptide-based vaccines are easy to produce and induce high titers of HBV-specific antibodies. However, they induce weak cellular immunity responses and require adjuvant and repeated administrations for better efficacy (74).

Pol *et al* conducted the first HBV therapeutic vaccination trial including 32 patients with active chronic HBV infection. Patients were given three doses of GenHevac B vaccine (GenHevac B®, Pasteur Mérieux Connaught, 1993) containing HBsAg and Pre-S2 protein with aluminum hydroxide adjuvant, administered at 1-month intervals. Vaccination resulted in significantly decreased serum HBV DNA levels in 44% of subjects (75). Another study using GenHevacB vaccine in 118 patients demonstrated the capability of this vaccine to decrease HBV replication. However, serum HBsAg did not

significantly reduce in any of the subjects (75). This study provided evidence that a therapeutic HBV vaccine may activate T-cell responses, reduce HBV viral load, and accelerate the rate of HBeAg seroconversion in chronic HBV patients (76).

Another peptide-based vaccine approach involves the combination of recombinant HBV core particles (HBcAg) with HBsAg. HBcAg is an immunogenic target that acts as a Th1 adjuvant for HBsAg (77). Safety and efficacy of this vaccination method were demonstrated in a phase I trial in which the combined antigens were administered through intranasal route to volunteers (78). A phase I–II trial in 18 chronic HBV patients in Bangladesh was conducted using this combined vaccine (100 µg of HBsAg and 100 µg of HBcAg) (79). All patients had persistently normalized ALT levels and half of them had sustained HBV DNA negativity after ten vaccinations at 2 weeks interval (80). Initial results of an ongoing phase III trial in chronic hepatitis B patients in Bangladesh suggested that a 20-week course of treatment with this therapeutic vaccine was almost as efficient in controlling HBV viral as a 48-week course of treatment with pegylated IFN-α (ClinicalTrials.gov: NCT01374308).

An interesting approach to the development of therapeutic HBV vaccination was combining recombinant HBsAg with anti-HBs antibodies to form an antigen-antibody immune complex (IC). These immune complexes increased the chance of the HBsAg being captured by antigen presenting cells (APCs), thereby enhancing the immunogenic effects of the vaccine and inducing more potent HBs-specific T cell responses (81, 82). A yeast derived HBs antigen-antibody immune complex vaccine (YIC) which was previously found to be safe and efficient (83) was evaluated in a phase IIb study (84). 242 HBeAg-positive chronically infected HBV patients were enrolled, at the end of the follow-up, the rate of HBeAg seroconversion was 21.8% in the vaccinated patients compared to a seroconversion rate of 9% in the placebo group. Following the results of this trial, a phase III study evaluating the YIC vaccine in 450 chronic hepatitis B patients was conducted. In contrast to the phase IIb trial where six doses of vaccine were used, in the phase III study, 12 doses were administered that resulted in decreased efficacy of the vaccine probably due to immune fatigue in hosts (85). An interesting finding of these trials was that repetitive administrations of adjuvant alone could result in an inflammatory environment that could stimulate pre-existing T cells in the liver. Therefore, it was suggested that some of the T cell responses detected upon therapeutic vaccination may be due to the effect of the vaccine adjuvant on pre-existing T cells. Considering these observations, it was suggested that patients with active liver inflammation may benefit more from therapeutic vaccination.

DNA-based vaccines

In contrast to peptide-based vaccines that mainly induce humoral immune responses, DNA-based vaccines induce both humoral and cellular immune responses including CD8+ and CD4+ T cell responses (86). The immune response generated upon immunization with the DNA-based vaccine is similar to that observed in individuals with cleared HBV infection (87).

Encouraging results have been acquired using DNA vaccination in mice and chimpanzees (88, 89).

A clinical trial was conducted in 2004 to assess HBV DNA vaccination in 10 patients with chronic hepatitis B who were non-responder to antiviral treatments. Patients were given 4 injections of a DNA vaccine encoding HBV envelope proteins. Immunization was well tolerated and two patient's demonstrated proliferative responses to HBsAg, HBV-specific IFN-γ-secreting T cells were significantly increased in all subjects. HBV DNA levels were decreased in the serum of 5 patients, and one patient completely cleared the infection. This vaccine showed promising results and induced effective but transient T cell responses (90).

Viral vector-based vaccines

Vaccines based on live attenuated viruses are able to stimulate broad and sustained immune responses. Thus, recombinant viral vectors have been investigated in the development of an efficient vaccine against chronic HBVinfection as they have been investigated in many other diseases (91, 92). A recombinant retroviral vector expressing HBcAg was administered, HBeAg seroconversion and HBV clearance, as well as significant ALT elevation, were seen in one chimpanzee. ALT was elevated due to the restoration of HBV-specific cytotoxic T cell responses. Anti-HBe antibody titers were increased in the other two chimpanzees and HBcAg-specific cytotoxic T cell responses were developed in one of them (93).

Cell-based vaccines

Based on the pivotal role of innate immunity in HBV infection, a vaccine strategy was developed to stimulate pathogen recognition receptors PRR. GlobeImmune, Inc. created a novel vaccine platform using heat-killed recombinant yeasts expressing one or more disease-associated antigens. This method stimulates Toll-like receptors (TLRs) and other dendritic cells (DCs) receptors leading to maturation of DCs that would, in turn, stimulate specific CD4+ and CD8+ T cells *in vivo* and *in vitro* (94). A phase Ia trial was carried out in 60 healthy adults in a single center in the United States to assess the safety,

tolerability, and immunogenicity of a novel vaccine, GI-13020 (GS-4774); a heat-killed recombinant *S. cerevisiae* expressing HBV X antigen and large surface protein and core antigens. HBV-specific T-cell responses were induced in subjects, and the vaccine was shown to be well tolerated and safe (95). Based on this trial, a phase II trial is ongoing to evaluate the safety and efficacy of GS-4774 in 175 chronically HBV-infected patients (ClinicalTrials.gov Identifier: NCT01943799).

Dendritic cells (DCs) are antigen-presenting cells (APCs) distributed throughout the body. The weak antiviral responses seen in some chronic HBV infected patients have been attributed to functional defects in APCs especially dendritic cells (96, 97). In a study on HBV transgenic mice, the ability of endogenous DCs pulsed with HBsAg to induce antibodies against HBsAg was shown (98, 99). Based on this, in a clinical trial five chronic HBV-infected patients were administered HBsAg-pulsed dendritic cells intradermally one to three times. The vaccine was safe and induced higher levels of IL-12 and IFN-γ in comparison to unpulsed DCs. Furthermore, two patients exhibited anti-HBs antibodies and one patient demonstrated HBsAg-specific cellular immunity (100).

Another clinical trial on 380 chronic hepatitis B patients was conducted and patients were administered autologous DCs primed with HBV core and Pre S2 antigens. The vaccine was shown to be safe. HBV DNA levels decreased to an undetectable level in 46.36% of HBeAg-negative patients and 3.13% of HBeAg-positive patients. Moreover, alanine aminotransferase (ALT) levels were significantly normalized in both HBeAg negative and positive patients (101).

Other strategies

Prime-boost strategy

Viral vector-based vaccination can also be used in heterologous prime/boost vaccination regime to further enhance the immunogenicity of DNA-based vaccines (102). A DNA prime/poxviral boost vaccination, using a DNA prime encoding S protein followed by a canarypox boost vaccination encoding S and preS1/2 proteins, was studied in chimpanzees. This treatment led to HBVDNA loss for more than three years (103). Based on these promising results, a DNA/MVA prime/boost vaccination was investigated in a clinical trial on chronic HBV-infected patients in Gambia. Different doses and application routes were tested with or without lamivudine. Although the treatment was well tolerated, it did not enhance the effects compared to standard antiviral treatment (104). This may be due to the fact that vaccination with surface antigen alone is insufficient to generate multi-specific and strong T-cell responses. Moreover, most of the subjects were infected with HBV around birth or during early childhood and thus adapted extreme immune tolerance (105).

Combination therapy

CD8+ T cells are exhausted in chronic HBV patients with high viral loads (>10^7 copy/ml) (101) and express inhibitory molecules as discussed before (PD-1, CTLA-4 and Tim-3). The absence of CD4+ T cells amplifies the CD8+ T cells exhaustion, ultimately resulting in the physical deletion of CD8+ T cells (106). This explains the transient activation of HBV-specific T cell responses by the therapeutic recombinant vaccines. Preliminary studies have shown that recombinant vaccines are more beneficial in patients with a low viral load at the beginning of the treatment (107). Lamivudine, an antiviral drug, has been reported to suppress viral loads by restoration of specific immune responses (108, 109). This phenomenon resulted in combination of antiviral agents with therapeutic recombinant protein vaccines to enhance the restoration of T-cell responses (110-112).

A randomized, controlled clinical trial including 180 patients was conducted to assess the efficacy of combination therapy of a recombinant peptide vaccine containing preS1, preS2 and HBs antigens with lamivudine and alum as an adjuvant. Subjects were categorized into three groups: (I) Vaccine monotherapy, (II) lamivudine monotherapy, and (III) combination therapy. Enhanced virological responses were observed in the combination therapy group compared to the vaccine and lamivudine monotherapy groups. However, these effects were not continuous. Interestingly, half of the patients in the combination therapy group expressed anti-HBs antibodies but only 5% cleared HBsAg. This suggests that the vaccine-induced antibodies were not efficient enough to neutralize the viral particles in the serum (110). Another clinical trial evaluated the efficacy of combination therapy in 72 chronic HBV infected patients. Subjects received either lamivudine alone or in combination with a recombinant HBsAg peptide vaccine for 12 months. At the end of the treatment, serum HBV DNA became negative in all members of the combination therapy
group, whereas 48% of the subjects receiving lamivudine monotherapy were negative for serum HBV DNA. Moreover, the rate of HBeAg seroconversion was significantly higher in patients receiving combination therapy (56% in combination therapy versus 16% in lamivudine monotherapy) (111). This study suggested a higher efficacy for combination therapy compared to lamivudine monotherapy. However, another randomized controlled study in 195 HBeAg-positive chronic HBV infected patients achieved contradictory results. Despite 12 injections of HBsAg with AS02B adjuvant candidate vaccine over a 1-year period, HBe seroconversion rate and HBV DNA levels did not improve significantly compared to treatment with lamivudine alone (112).

Table 1. Clinical trials for therapeutic vaccination in chronic hepatitis B

Vaccine type	Authors (Year)	Administered regime	Adjuvant	Tested in	Results summary	Reference
Recombinant peptide vaccine	Pol *et al* (1994)	Recombinant HBsAg and Pre-S2 protein (GenHevacB)	Alum	32 CHB patients	decreased serum HBV DNA	75
	Pol *et al* (2001)	Recombinant HBsAg and Pre-S2 protein (GenHevacB)	Alum	118 treatment naïve CHB patients	decreased HBV replication, however, serum HBsAg did not disappear	116
	Al-Mahtab *et al* (2013)	recombinant HBV core particles (HBcAg) with HBsAg	-	18 CHB patients	normalized ALT levels in all subjects and sustained HBV DNA negativity in half of the subjects	80
	Xu *et al* (2008)	recombinant HBsAg with anti-HBs antibodies	Alum	242 HBeAg-positive CHB patients	21.8% HBeAg seroconversion (6 doses were administered)	84
	Xu *et al* (2013)	recombinant HBsAg with anti-HBs antibodies	Alum	450 HBeAg-positive CHB patients	14.0% HBeAg seroconversion (12 doses were administered)	85
	Le Hoa *et al* (2009)	recombinant preS1, preS2, and HBs antigens with or without lamivudine	Alum	180 CHB patients	A transient enhanced virological response in the combination therapy group compared to the vaccine and lamivudine monotherapy groups	110
	Horiike *et al* (2005)	recombinant HBsAg peptide with lamivudine or lamivudine alone	-	72 CHB patients	Serum HBV DNA became negative in all of the combination therapy groups, compared to 48% of the subjects receiving lamivudine monotherapy	111
	Vandepapeliére *et al* (2007)	recombinant HBsAg peptide with lamivudine or lamivudine alone	AS02B	195 HBeAg-positive CHB patients	HBe seroconversion rate and HBV DNA levels did not improve significantly compared to treatment with lamivudine alone	112
DNA vaccines	Mancini-Bourgine *et al* (2004)	DNA vaccine encoding HBV envelope proteins	-	10 CHB patients	HBV-specific IFN-γ-secreting T cells were significantly increased, HBV DNA levels were decreased in the serums of 5 patients, and one patient completely cleared the infection	90
	Yang *et al* (2006)	DNA-vaccine encoding S, preS1/S2, core, polymerase, X proteins and human IL-12 (HB-100) with lamivudine	-	12 Caucasian CHB patients	Therapy induced a multi-specific T-cell response, reduced HBV DNA levels and led to HBeAg seroconversion in 50% of patients	113

	Kim *et al* (2008)	DNA-vaccine encoding S, preS1/S2, core, polymerase, X proteins, and human IL-12 (HB-110) with adefovir	-	27 Asian CHB patients	efficacy of combination therapy with HB-110 DNA vaccine and adefovir was lower in Asian patients, compared to Caucasian patients in HB-100 study	115
Viral vector-based vaccines	Sållberg *et al* (1998)	A recombinant retroviral vector expressing HBcAg	-	3 chronically HBV-infected chimpanzees	Increased anti-HBe antibodies and restoration of HBV-specific cytotoxic T cell responses. Also, HBeAg seroconversion and HBV clearance were seen in one chimpanzee	93
	Cavenaugh *et al* (2011)	DNA/MVA prime/boost vaccine with or without lamivudine	-	12 HBeAg negative and 12 HBeAg-positive CHB patients	the treatment was well tolerated but it did not add any beneficial effects to standard antiviral treatment	104
Cell-based vaccines	Gaggar *et al* (2014)	heat-killed recombinant *Saccharomyces cerevisiae* expressing HBV X and large surface protein and core antigens (GS-4774)	-	60 healthy adults	Phase Ia trial : vaccine was well tolerated and safe	95 (phase II : ClinicalTrials.gov Identifier: NCT01943799)
	Akbar (2011)	HBsAg-pulsed dendritic cells	-	5 CHB patients	Vaccine-induced high levels of IL-12 and IFN-γ and 2 patients showed anti-HBs antibodies and 1 patient exhibited HBsAg-specific cellular immunity	79
	Luo *et al* (2010)	autologous DCs primed with HBV core and Pre S2 antigens	-	380 CHB patients	HBV DNA levels decreased to an undetectable level in 46.36% of HBeAg-negative patients and 3.13% of HBeAg-positive patients and alanine aminotransferase levels were significantly normalized in all patients	101

CHB: Chronic hepatitis B virus infection, HBV: Hepatitis B virus, HBsAg: Hepatitis B virus surface antigen, HBcAg: Hepatitis B virus core antigen, HBeAg: Hepatitis B virus E antigen

Efficacy of therapeutic DNA vaccination was enhanced by combining a DNA vaccine encoding S, preS1/S2, core, polymerase, X proteins and human IL-12 (HB-100), and lamivudine (113). Combination therapy induced a multi-specific T-cell response. Also, it reduced HBV DNA levels and led to HBeAg seroconversion in 50% of patients. Notably, 15% of patients cleared HBV DNA and sustained the acquired clearance for 3 years after the therapy was ceased (114).

After obtaining encouraging results from HB-100 DNA vaccine, another trial was carried out to test the potential efficacy of the improved version of this vaccine (HB-110) in patients chronically infected with HBV in Korea (115). Twenty-seven patients received 12 injections of HB-110 DNA vaccine every other week, from week 0 to week 22 and adefovir from week 10 to week 48 while members of control groups only received adefovir. Results indicated that the efficacy of combination therapy with HB-110

DNA vaccine and adefovir was lower in Asian patients compared to Caucasian patients in the HB-100 study [113]. This inconsistency might be explained by the fact that the mean HBV DNA viral load at the start of the trial was 100-fold higher in Korean subjects than in Caucasians. Also, Korean subjects were mainly infected at birth or during early childhood whereas Caucasian patients were mainly infected during adolescence via horizontal routes (112).

A phase I/II clinical trial was conducted to investigate the efficacy of envelope DNA vaccination on HBeAg-negative chronic HBV-infected patients who were treated efficiently with nucleoside/-nucleotide analogs. Patients with undetectable HBV DNA levels after at least 3 years of treatment with nucleoside/nucleotide analogs were randomized into 2 groups, one receiving DNA vaccine and the other without undergoing vaccination. Both stopped taking nucleoside/-nucleotide analogs after 52 weeks, 2 weeks after the last DNA injection (113). Number, diversity, and functionality of the HBsAg-specific T-cell responses were maintained after the treatment was stopped, but this treatment was unable to prevent HBV reactivation (114). However, the viremia after ceasing nucleoside/nucleotide analogs treatment was better controlled in patients with lower HBsAg levels at the start of the study (115, 116).

Conclusion

HBV is a major viral infectious disease and more than 2 billion people are infected worldwide from which more than 350 million developed chronic infection**.** A current prophylactic vaccine against HBV contains S envelope protein of the virus, which is mainly produced in yeast *S. cerevisiae* containing a recombinant DNA plasmid expressing S antigen. The vaccine is not effective in clearing HBV from chronically HBV-infected individuals. Current antiviral drugs are expensive with adverse side effects and are not able to eliminate HBV in chronically infected patients. Drug resistance, especially in long-term treatment, is observed in these patients as well. Therefore, there is a need to develop new therapeutic vaccines in order to eliminate HBV chronic infections and cure the patients.

Currently studied HBV therapeutic vaccines include recombinant peptide-based vaccines, DNA-based vaccines, viral vector-based vaccines, and cell-based vaccines. The challenge for the development of an efficient vaccine with relatively low side effects is still in progress.

While focusing on using several approaches to develop more effective HBV therapeutic vaccines with lower side-effects is indeed precious, it is also critical to pay extra attention for probable widespread problems such as the accelerated evolution of HBV genetic content in the population which may, in turn, lead to the creation of enhanced-resistance HBV species in the population. In worst cases, this means the normal preventive HBV vaccine would no longer be as effective as previous vaccines. Considering using a wide range of approaches that are currently enrolled and the improved understanding of the complex immune interactions involved in the host immunity, efficient and reliable therapeutic vaccines against HBV infection are expected to be available in the near future.

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Conflicts of interest

The authors have nothing to disclose.

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