Therapeutic Vaccine for Hepatitis B Virus
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Abstract
Although prophylactic hepatitis B virus (HBV) vaccination in children has dramatically reduced HBV incidence, but hepatitis B infection remains a global health problem. Chronic HBV carriers, among the large population infected by HBV, are facing the risk of progressing to liver cirrhosis and liver cancer due to the insufficiency and noticeable side effects of current antiviral treatments. The host immune response is integral to determining the outcome of HBV infection. HBV is non-cytopathic. However, repeated attempts by the host immune response to clear the virus can cause the observed liver damage. While HBV can induce immune tolerance to establish a state of chronic infection, potent and specific immunotherapy to break immune tolerance has the potential to facilitate immunemediated viral clearance and host recovery. Thus, HBV vaccine has been researched as a therapeutic approach. Although some levels of success have been achieved, more research is necessary to examine the effectiveness and safety of such vaccines. A new approach, dendritic cell-based vaccine, has shown promise for delivering HIV therapeutic vaccine, but remains to be thoroughly investigated. In this review, HBV infection epidemiology and pathogenesis are discussed, and the strategies and challenges of HBV therapeutic vaccine development, including dendritic cell-based vaccines, are summarized.

Keywords: Hepatitis; Vaccination; Adjuvant

Introduction
Hepatitis viruses, type A to G, belong to different families of viruses that cause human liver infection [1]. Hepatitis A, B and C viruses lead to over 95% of liver infection which often progress to cirrhosis and liver carcinoma, whereas the clinical significance of Hepatitis D, E, F and G viruses is minor. Hepatitis A virus causes an acute, self-limiting infection, while hepatitis B and C viruses cause chronic infections which lead to severe liver damage such as cirrhosis and liver cancer. About 90% of healthy adults who recover from acute HBV infection develop protective antibodies against future HBV infection, while a remaining 5-10% may develop chronic infection to become HBV carriers. Globally, there are two billion people infected with HBV, including more than 370 million chronic HBV carriers, of whom 1/3 are Chinese [2]. These carriers constitute the reservoir for continued spread of the disease. The risk of HBV infection is greatest in developing regions, where the population density is the highest, and medication and vaccination are the most deficient, such as in Southeast Asia, China, sub Saharan Africa and the Amazon [3]. In contrast, HBV incidence is much lower, in the range of 0.5-2%, within developed countries in North America, Northern and Western Europe and Australia, where the health care standard is considerably higher [1].

Prophylactic HBV vaccination and HBIG immune therapy administered to babies of hepatitis B surface antigen (HBsAg) and/ or HBeAg positive mothers, is an effective strategy for the prevention of HBV. The first HBV vaccine, containing HBsAg, was licensed in the USA in 1981 [4]. It was first introduced to infants born to HBsAg positive mothers in 1984 in the universal vaccination program in Taiwan, and later used for all infants and youth [5,6]. A new recombinant DNA vaccine was approved in the USA in 1986, and has been used in the Taiwan vaccination program since 1991. By 2010, 179 countries had incorporated the infant immunization program into their national health plans, in response to results from several pilot programs that had demonstrated significant reduction in HBsAg prevalence, among vaccinated populations [7].

For chronic HBV carriers, treatment options are still limited. Current treatments include recombinant subcutaneous interferon-α (IFN-α), and oral nucleoside analogues such as lamivudine and adefovir. More recently, tenofovir and entecavir have been approved and recommended as first-line oral antiviral medications in place of adefovir, because they reduce viral replication and rarely cause viral resistance. They are successful in suppressing HBV replication and remising liver diseases, but there’s still a need for alternative therapeutics since these drugs have notable shortcomings. Lamivudine is economical but drug-resistant mutants may develop [8]. IFN and nucleoside analogs are expensive and have considerable side effects including Fanconi syndrome, renal insufficiency, as well as osteomalacia and decreases in bone density [9]. Furthermore, therapeutic responses to these drugs can vary widely depending on the virological and immunological status of patients. Therefore, research on new therapeutics is needed.

Understanding the natural history of chronic HBV infection is critical to developing new therapeutics. There are generally three stages involved in chronic HBV infection:

1) an immune tolerance phase characterized by minimal liver damage, despite the presence of active viral replication,
2) an immune clearance phase with chronic active hepatitis and,
3) an inactive HBV non-replicative phase accompanied by the development of liver cirrhosis [10].

Chronic HBV carriers exhibit a prolonged immune tolerance phase during which, due to unresponsiveness of the immune system, they do not develop symptoms of liver disease, despite having high levels of HBV replication. The mechanism of this tolerance is not completely understood, but experiments in mice demonstrated that transplacental transfer of maternal HBeAg might induce a specific unresponsiveness of T helper cells to hepatitis B envelope antigen (HBsAg) in neonates born to HBV carrier mothers [11]. This
unresponsiveness could be reversed by depleting suppressor T cells, suggesting that the unresponsiveness was due to malfunction of T helper cells [4]. It was also suspected that since HBcAg and hepatitis B core antigen (HBcAg) were highly cross-reactive at the T-cell level, a reduction of T helper cell response to HBcAg might impair the cytotoxic T-cell response to HBcAg, which is required for the lysis of infected hepatocytes [12]. The anti-HBe antibodies appear in the viral clearance phase. During this phase, viral replication fluctuates, and patients may demonstrate symptoms which are commonly observed in acute hepatitis B. Also in this phase, infected hepatocytes are more likely to be attacked by cytotoxic T lymphocytes due to the increased expression of histocompatibility class I antigen [13]. In the final stage, HBV DNA has been incorporated into the host genome and viral replication stops. At this point, viral replication is very low, while HBcAg is still being produced by hepatocytes which have the viral DNA [14].

Among the large HBV-infected population, some individuals progress to become chronic carriers, while others completely recover soon after acute infection. Some chronic carriers never develop notable illness while others suffer progressive liver damage. Reasons behind the phenomenon are not completely clear. Numerous factors determine the natural history and outcome of HBV infection, including age at infection, gender, and HBV genotype. It has been demonstrated that there is an inverse relationship between the risk of chronic infection and age [15]. Clinical and experimental evidence also suggests that the status of host immune response at the time of HBV infection is a key factor in determining infection outcomes [1,16]. Since chronic HBV patients were observed to have suppressed immune responses relative to acute HBV patients [17,18], it is rational to hypothesize that if immune responses against HBV in chronic carriers could be made strong and specific enough, clearance of HBV would be achieved.

Unlike HBV prophylactic vaccines, therapeutic vaccines provide both preventive and therapeutic effects against HBV infection. Prophylactic vaccination stimulates the production of antibodies against pathogens. In contrast, therapeutic vaccination induces potent immune responses, which break the barrier of immune tolerance and suppress the replication of pathogens. Pol et al. first observed the therapeutic function of a prophylactic vaccine in chronic HBV carriers in 1994. This is considered the first clinical trial to evaluate vaccine therapy in chronic HBV carriers [19]. The principal mechanism of HBV therapeutic vaccine is the recognition of HBV specific antigen in the context of human leukocyte antigen (HLA) class I - restricted cytotoxic T-lymphocytes (CTLs). Vaccine developers have been focusing on an HBV-specific immune response which, unlike a nonspecific response, has antiviral effects without causing liver damage in chronic carriers [20]. An HBV-specific immune response relies on the production of anti-viral cytokines such as IFN and TNF by humoral and cell-mediated immunity. Experimental evidence in a chimpanzee model has shown that IFN and TNF are highly effective at inhibiting viral replication without severe destruction of hepatocytes [21]. The ultimate goal in developing HBV immunotherapy is to break tolerance in chronic HBV carriers, and induce a vigorous immune response against HBV while avoiding damage of hepatocytes. Table 1 is a summary of five types of HBV therapeutic vaccines, that are either approaching or currently in clinical trials.

### Peptide vaccines

Peptide vaccines use specific peptide antigens to induce CD4+ and CD8+ T cell immune responses. Among many of the HBV epitopes that were identified, HBV epitopes on the surface protein, core protein and polymerase are commonly used [11,13,14,22]. An oligopeptide, consisting of HBcAg 18-27 linked to both the tetanus toxoid T helper cell epitope and the PreS2 18-24 sequence of B cell epitope was constructed, and shown to induce CTL response in human peripheral blood mononuclear cells in vitro and in transgenic mice in vivo [23]. The main component of peptide HBV therapeutic vaccines is the core antigen 18-27 peptide [12,22,24], which is predominant in different genotypes of HBV, and universally induces CTL responses in chronic hepatitis B patients with different HBV genotypes. Wu’s group, in Chongqing, China, found that rearrangements and combinations of a set of B cell, T helper and CTL epitopes induced effective CTL responses in vivo, and the responses to the B and T helper epitopes assisted CTL function [25]. Peptide vaccines with lipids have also been investigated. Shih et al. [12] demonstrated that palm-p18, an HBcAg CTL epitope covalently linked to palmitic acid,

Table 1: Therapeutic HB vaccines in development.

<table>
<thead>
<tr>
<th>Type of Vaccine</th>
<th>Vaccine Antigen</th>
<th>Adjuvant</th>
<th>Host Cell Target</th>
<th>Current Status</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptide</td>
<td>(a) Palmitolylated TT830-843 HTL epitope covalently linked to the HBcAg 18–27 CTL epitope</td>
<td>N/A</td>
<td>Cytotoxic T Lymphocyte</td>
<td>Phase II clinical trial</td>
<td>Livingston et al., 1999 [22]</td>
</tr>
<tr>
<td></td>
<td>(b) HBV core antigen 18-27 peptide + tetanus toxoid peptide 830 843+palmitic acid molecules</td>
<td>N/A</td>
<td>Cytotoxic T Lymphocyte</td>
<td>Phase I</td>
<td>Vitiello et al., 1995 [23]</td>
</tr>
<tr>
<td>Protein</td>
<td>HBsAg/anti-HBsAg</td>
<td>Alum</td>
<td>Cytotoxic T Lymphocyte</td>
<td>Phase II clinical trial</td>
<td>Wen et al., 2003, Xu et al., 2008 [28]</td>
</tr>
<tr>
<td>DNA</td>
<td>Small(S) and middle (preS2/S) proteins of the HBV envelope (HBsAg)</td>
<td>N/A</td>
<td>CD8+ T Lymphocyte</td>
<td>Phase I clinical trial</td>
<td>Mancini et al., 2004</td>
</tr>
<tr>
<td>Vector-based</td>
<td>Modified prHBV1.3/HBV core protein</td>
<td>N/A</td>
<td>CD4+ and CD8+ T Lymphocyte</td>
<td>Pre-clinical</td>
<td>Deng et al., 2009 [8]</td>
</tr>
<tr>
<td>Cell-based</td>
<td>DCs pulsed with HBs28-39 peptide or recombinant preS2/S particles</td>
<td>N/A</td>
<td>Cytotoxic T Lymphocyte</td>
<td>Pre-clinical</td>
<td>Akbar et al., 2004 [36]</td>
</tr>
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primed moderate CTL response in mice, suggesting that palm-p18 is a potential candidate for use in therapeutic peptide vaccines against viral hepatitis B [26].

**Protein vaccine**

Protein vaccine is the most widely used type of HBV vaccine and has provided effective prevention against HBV infection worldwide. Most protein vaccines contain different amounts of HBsAg, or HBsAg plus preS1 and/or preS2 recombinant proteins with alum adjuvant. These vaccines usually induce cytotoxic T cell responses and type 1 T helper cell (Th1) response. Recombinant HBsAg can also be combined with anti-HBs antibodies and delivered as antigen–antibody complexes. Both approaches have shown encouraging results. Mancini et al. were the first to demonstrate, using transgenic mice which constitutively expressed HBsAg in the liver from before birth, that immunization with recombinant HBsAg induced a detectable immune response without causing damage to hepatocytes, suggesting that immune tolerance in chronic carriers could be overcome by immunization [9]. Based on this observation, clinical trials were conducted and demonstrated that anti-HBV vaccination significantly reduced HBV replication in 50% of chronic carrier subjects [19].

Vaccination with antigen-antibody complexes has also been investigated as a method of improving existing protein-based vaccines. In 2005, a group led by Wen in Shanghai, China evaluated a hepatitis B immunogenic complex vaccine composed of yeast derived recombinant HBsAg combined with human anti-HBs immunoglobulin (YIC), for safety and immune response in phase I and IIa clinical trials. The study showed that anti-HBV IgG, IFN-γ and lymphocyte proliferation were elicited in all the human volunteers studied, while IL-2 levels were elicited in most human volunteer studied, and no significant difference was observed in levels of IL-4, IL-6 and IL-10. While these results demonstrated that the vaccine generated a potent immune response and was safe, its therapeutic efficacy still requires further investigation [27]. More recently, Xu et al. [28] conducted a randomized controlled phase Ib clinical trial of the same vaccine, in which 242 chronic HBV carriers were divided into three groups and immunized with two doses of either 30 μg YIC, 60 μg of YIC or alum adjuvant as placebo. During immunization and for 24 weeks after, levels of HBV markers and HBV DNA were carefully monitored. At the end of the study, it was found that there was a significant difference in HBeAg seroconversion between the 60 μg YIC and placebo groups, with no significant difference in HBeAg loss or HBV DNA suppression.

**DNA vaccine**

DNA vaccines induce immune responses against in vivo synthesized antigens, after introducing the DNA encoding antigen sequences directly into the host. This approach of vaccination has been investigated to improve the existing protein and peptide based vaccines. In transgenic mice which constitutively express the HBsAg in the liver, vaccination with plasmids DNA which encodes HBV envelope protein generates a decrease in HBV mRNA and an increase in anti-HBV antibodies, which promote the clearance of circulating HBsAg [29]. Alternatively, Thermet et al. [30] used Pekin duck as a model to show that immunizing healthy uninfected ducks with a vaccine encoding a large duck HBV envelope protein induced strong and long-lasting anti-preS response. In 1996, after successfully immunizing transgenic mice, Davis et al. [21] conducted an immunization study using the chimpanzee animal model. They vaccinated two chimpanzees with plasmid DNA encoding the major and middle HBV envelope proteins in order to induce production of group, subtype and preS2-specific antibodies. The first chimpanzee immunized with the vaccine at a high dose, demonstrated a potent immune response. In the second chimpanzee, a first injection at a lower dose generated a weak response, which was augmented somewhat by a second immunization, but the antibody titer was still relatively low even after a third boost [21,31]. Collectively, the above results suggest that DNA vaccines may be potentially useful as therapeutic approaches against HBV infection in humans.

**Vector-based vaccine**

Vector-based vaccines utilize a vector (a virus in most cases) to deliver the desired antigen to the subjects. In 2009, Deng et al. [8] designed a recombinant HBV (rHBV) containing a modified HBV core gene, that specifically delivered a foreign antigenic polypeptide to the liver. The foreign antigen stimulated a vigorous T cell response without causing significant destruction of hepatocytes, meanwhile, the expression of HBsAg from the rHBV vector was markedly suppressed. Further studies using this model are warranted.

**Cell-based vaccine**

Dendritic cells (DCs) are professional antigen-presenting cells which recognize, internalize, process and present antigens. They are crucial to the maintenance and induction of antigen–specific immune responses. Antigen-pulsed DC based vaccines have been primarily investigated to treat cancer. In experimental models of cancer, peptide or protein-pulsed DCs have demonstrated the ability to induce strong CD8+ CTL mediated antitumor responses in vivo [32]. Studies conducted in murine models showed that the implantation of activated DCs can induce specific CTL response [33,34]. In HBV transgenic mice, the function of DCs has been impaired and therefore, cannot induce CTL-specific immune response. It may be possible to load an HBsAg vaccine with activated DCs to improve the effectiveness of the vaccine. In one study, bone marrow-derived DCs pulsed with HBs28-39 peptide or recombinant preS2/S particles were used to immunize HBV transgenic mice in order to break tolerance of HBsAg at the B and T cell levels. Results showed that DNA vaccination alone only stimulated an antibody response, but infusion of activated DCs derived from transgenic or non-transgenic mice also stimulated a splenic CTL response in all three lineages of HBV transgenic mice [35]. In 2004, Akbar et al. [36] prepared a DC-based therapeutic vaccine and evaluated its therapeutic potential in HBV transgenic mice. They cultured murine spleen DCs and HBsAg together to produce HBsAg-pulsed DCs. A small dose of pulsed DCs induces a potent CTL-specific response, which could not be induced by using unpulsed DCs. Administration of pulsed DCs to HBV transgenic mice also induced potent responses. These results demonstrated that antigen pulsed DCs may be very promising for treating chronic HBV infection.

Directing antigens to DCs in order to induce optimal immune responses, could potentially employ alternative genetic methods. You et al. [37] immunized mice intramuscularly with a DNA vaccine, encoding a fusion protein comprising HBeAg and an IgG Fc fragment. The fusion protein was expressed and captured by DCs via Fcγ receptor [37]. More recently, Li et al. [16] reported that targeting antigens to human DCs via DC-asialoglycoprotein receptor (DC-ASGPR) favored the generation of antigen–specific suppressive CD4+
T cells that produced IL-10. These approaches were applicable to both self and foreign antigens, and were capable of stimulating both memory and naive CD4+ T cells. They further demonstrated that antigen-specific CD4+ T cells producing IL-10 in vivo were generated after immunizing nonhuman primates with antigens fused to anti-DC-ASGPR. Thus, there appears to be great potential for targeting antigen to DCs for the improvement of therapeutic HBV vaccines.

Adjuvant choice

Since many prospective antigens are not highly immunogenic or are unstable in vivo, adjuvants are added to vaccine to enhance the immunogenicity of the antigens in vivo. Adjuvants can achieve this goal by either localizing the antigen, enhancing the capability of antigen presentation or directly provoking immune response [38]. Currently, alum-based adjuvants including various aluminum salts: aluminum phosphate, aluminum hydroxide and aluminum potassium sulfate, are the only ones that are licensed in the United States. Adding aluminum to vaccines can help to achieve the same level of immune response using fewer antigens per dose or a smaller amount of the vaccine. Although different types of vaccine adjuvants have been identified and found to be more potent than alum in animal models, they are toxic to the human body and have yet been modified for clinical use [39]. Other adjuvants are in use outside the United States. For example, AS04, an adjuvant that contains alum and monophosphoryl lipid A, is part of a vaccine licensed in Europe by GlaxoSmithKline, to prevent infection from hepatitis B for specific high-risk patient groups [40].

Summary

As chronic HBV carriers are the primary population responsible for perpetuating the spread of HBV, developing low-cost but effective therapeutic vaccines against HBV will certainly improve the status of HBV infection globally. Based on the understanding of HBV pathology and immunology, it is clear that developing a therapeutic vaccine for chronic HBV carriers will require careful design and optimal selection of antigen and adjuvant. But unlike developing preventive vaccines, in order to clear the HBV from chronic HBV carriers, stronger CD4 and CD8 responses must be induced, and the barrier of HBV tolerance in chronic HBV carriers must be broken. The problem of hepatocytes destruction must also be addressed in the effort to develop a truly efficacious and safe vaccine. While research results have been mostly encouraging, further investigation is clearly needed to examine and ensure the safety and effectiveness of these and upcoming vaccines.

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