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# The immune response of rhesus macaques to novel vaccines comprising hepatitis B virus S, PreS1, and Core antigens

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ABSTRACT

Therapeutic vaccines represent a unique approach to hepatitis B virus (HBV) treatment and have the potential to induce long-term control of infection. This study explored the immune responses of rhesus macaques to novel vaccines comprising the S, PreS1, and Core antigens of the HBV that showed promise as prophylactic and therapeutic approaches in a mouse model. The tested vaccines included two DNA vaccines (pVRC-SS1, pVRC-CS1), an HBV particle subunit (HBSS1) vaccine and the recombinant vaccinia virus- (RVJ-) based vaccines (RVJSS1 and RVJCS1) in which SS1 containing S (1–223 aa) and PreS1 (21–47 aa), CS1 containing Core (1–144 aa) and PreS1 (1–42 aa). The humoral immunity and cell-mediated immunity (CMI) induced by vaccines comprising the S, PreS1, and Core antigens of HBV were investigated in a longitudinal study that continued up to 98 weeks after the first vaccination. In rhesus macaques, anti-PreS1 antibody was induced more rapidly than anti-S or anti-Core antibody after DNA vaccination. The antibody and cell-mediated immune responses against S, PreS1, and C were significantly enhanced in macaques boosted with RVJSS1 and RVJCS1, whereas the cell-mediated response to C was most robust and durable. The immune response to S, PreS1, and C was restored by HBSS1 boosting and detected in macaques until weeks 74 and 98 after the first vaccination. Additionally, robust neutralizing activity was detected at week 52. In conclusion, novel HBV vaccine candidates, especially those used for therapeutic applications should incorporate the PreS1 and Core antigens.



## 1. Introduction

Although a safe and effective prophylactic vaccine has been available for almost three decades, hepatitis B virus (HBV) infection continues to be major public health problem; indeed, more than 240 million individuals worldwide are positive for the hepatitis B surface antigen (S) [1,2]. Individuals with chronic hepatitis B (CHB) infection are not only at high risk of developing severe liver diseases, such as cirrhosis and hepatocellular carcinoma, but are also the reservoir for viral spread [3]. Although much effort has focused on controlling viral replication and preventing progressive liver damage and the resulting complications, there is still no cure for CHB. Pegylated interferon-alpha (peg-IFN-α) and nucleoside/nucleotide analogs (Lamivudine, Adefovir, Telbivudine, Entecavir, and Tenofovir) are widely used and have contributed to the progress in CHB treatment [4]; however, their undesirable side effects and the cost of long-term therapy limit their use and significant gaps in our understanding of the correlates of immune protection hamper rational design of HBV immunotherapies [4]. Therefore, there is a pressing need to develop novel immune-based interventions to achieve a functional cure of HBV and reduce the HBV-related disease burden [5,6].

The ability of vaccine components to induce humoral and cell-mediated immune responses is important for the development of therapeutic HBV vaccines [5–8]. Various S-based vaccines (some containing only S and others S with the PreS1 and PreS2 antigens), S in combination with antiviral agents or Th1-promoting adju-

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vants, and antigen-antibody complex-based vaccines (S complexed with human anti-S) have been assessed in clinical trials [5], but none fully controlled HBV. This is most likely because recombinant protein vaccines with alum-based adjuvants induce antibody but not cytotoxic T-cell responses [5,6]; the latter are required for therapeutic efficacy [7–10]. The lack of efficacy may be explained by the choice of antigen: vaccination with an envelope antigen (S or PreS) alone does not induce strong multi-specific immunity (especially T-cell responses) [5,6,11].

Incorporation of the more immunogenic HBV pre-S and core antigen domains into vaccine formulations as well as use of a core antigen may induce more potent B- and T-cell responses [5,6,12–15]. The PreS1 region not only mediates viral entry by interacting with the HBV functional receptor (sodium-taurocholate cotransporting polypeptide [NTCP]) on the surface of hepatocytes but also expresses abundant T- and B-cell epitopes [16–19]. The PreS1-polypeptide can break immune tolerance to S and induce robust antibody and cell-mediated immune responses in HBV carrier mice, making it a candidate for inclusion in a HBV therapeutic vaccine [20,21]. We previously developed several HBV vaccine candidates containing the HBV S (1–223 aa) and PreS1 (21–47 aa) fusion antigens, such as the recombinant protein subunit vaccine, HBSS1, expressed in Chinese hamster ovary (CHO) cells [22,23]; a recombinant vaccinia virus (Tiantan) vaccine, RVJSS1 [23]; and a recombinant replication-defective adenovirus serotype 5 vector expressing the fusion protein SS1 vaccine (rAdSS1) [24]. Such vaccines containing the S+PreS1 fusion (SS1) antigen elicit HBV S- and PreS1-specific humoral immunity and cell-mediated immunity (CMI) in mice and rhesus macaques and therefore should be subjected to clinical trials against HBV infection [10,22–25]. In addition to the HBV S- and PreS1-specific CMI responses, hepatitis B core antigen (Core) contains multiple Th and cytotoxic T lymphocyte (CTL) epitopes [26], and Core-specific CTL is a critical regulator of HBV replication and clearance in the liver [5–9]. Core enhances the priming of T cells by activating B cells and enabling them to function as potent primary antigen-presenting cells [26]. Core also activates Toll-like receptor signaling, which facilitates generation of HBV-specific T cells and T cells specific for heterologous antigens [26,27]. Additionally, a highly immunogenic super structure is formed when Core is mixed with S, which breaks tolerance in HBV transgenic mice and has been shown to be safe in clinical trials [14]. Patients with chronic hepatitis B who can repress HBV replication have significantly higher levels of Core-specific CTLs than those who fail to control HBV replication and liver damage [9]. Thus, several studies have evaluated Core-based candidate therapeutic vaccines, which were found to enhance humoral immunity and CMI in animal models and CHB patient [5,6,12–14]. An example is the candidate vaccine TG1050, which consists of a non-replicative adenovirus 5 vector encoding multiple HBV genotype D antigens (S, Core, and polymerase) [28]. So, it might be a good candidate for the development of novel HBV therapeutic vaccine.

We previously developed a DNA HBV vaccine pVRC-HBCS1 comprising the Core (1–144 aa) and PreS1 (1–42 aa) antigens and pVRC-HBSS1 comprising the HBV S (1–223 aa) and PreS1 (21–47 aa) fusion antigens. Administration of this two DNA vaccines together by intradermal injection plus electroporation (i.d.-EP) produced the strongest PreS1-, S-, and Core-specific cell-mediated immune response and the highest anti-S and -PreS1 antibody levels [29]. And we also reported previously that combinations of subunit and recombinant vaccinia (Tiantan) vector-based vaccines containing an SS1 fusion protein elicited S- and PreS1-specific anti-HBV humoral and CMI responses in rhesus macaques [25]. However, the immune responses in macaques by these novel vaccines comprising S, PreS1, and Core antigens of HBV have not been evaluated. In this study, we developed a novel recombinant vaccinia virus, RVJCS1, and evaluated the immune responses of rhesus macaques administered the above novel HBV vaccines using a prime-boost regimen. Our results suggest that the DNA/RVJSS1+RVJCS1/HBSS1 vaccination regimen induces strong and long-lasting humoral and cellular immune responses to HBV and that the PreS1 and Core antigens should be incorporated in novel HBV vaccines, particularly those for therapeutic applications.

## 2. Materials and methods

### 2.1. Ethics statement

This study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH; Bethesda, MD). Rhesus macaque housing and experimental protocols were approved by the Institutional Care and Use Committee of the National Institute for Viral Disease Control and Prevention (NIVDC) of China (#A2010-028).

### 2.2. Vaccine candidates

HBSS1 particle subunit vaccine was prepared as described in previous reports [22,23]. Aluminum hydroxide (alum) was used as the adjuvant and was kindly provided by the North China Pharmaceutical Group Corporation GeneTech Biotechnology Development Company. Poly (I:C) (Sigma, St. Louis, MO) in saline was also used as an adjuvant.

Two DNA vaccines, pVRC-SS1 and pVRC-CS1, were used in this study [29]. Both plasmids were prepared using the EndoFree Plasmid Giga kit (Qiagen, Hilden, Germany) and dissolved in phosphate-buffered saline (PBS) (Gibco, Invitrogen, Paisley, United Kingdom).

Two recombinant vaccinia (Tiantan strain) virus-based HBV vaccines (RVJSS1 and RVJCS1) were used in this study. RVJSS1 expressing the HBV S (aa 1–223) and PreS1 (aa 21–47) fusion protein was produced as described previously [23]. RVJCS1 expressing the HBV core (aa 1–144) and PreS1 (aa 1–42) fusion proteins was developed by homologous recombination in chick embryo fibroblast (CEF) cells with the shuttle plasmid pJSA1175-CS1 and the parent vaccinia virus (Tiantan stain) as described previously [23,30,31]. Briefly, the fusion gene fragment CS1 was cloned into pJSA1175, an original vaccinia Tiantan strain (TTV) and the dual-promoter insertion vector; next, the recombinant plasmid pJSA1175-CS1 was transfected into CEF cells infected with TTV. The recombinant vaccinia virus RVJSS1 was then purified and propagated in CEF cells. The resulting RVJCS1 was demonstrated to be stable via expansion in CEF cells at a multiplicity of infection (MOI) of 0.01–0.1 and purified as described previously [23,30,31].

To confirm expression of the HBV antigens, CEF cells infected with RVJCS1 were maintained for 24 h at 37 °C with 5% $CO_2$ for 48 h, the levels of the fusion proteins in CEF cell lysates were determined by Western blotting with rabbit antiserum against PreS1 or Core.

### 2.3. Immunization of rhesus macaques [25,31]

Four healthy rhesus macaques aged 5–6 years were used in this study. Animals were housed at the Laboratory Animal Center in the Academy of the Military Medical Sciences (AMMS) of the People's Liberation Army (PLA). The macaques were free of known infectious or immunological diseases and had no previous contact with HBV-related pathogens, and they keep good health, without significant weight loss, liver and kidney damage, and other diseases through the experiment. Enzyme-linked immunosorbent assay (ELISA) and IFN-γ enzyme-linked immunosorbent spot (ELISpot)

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