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A novel dendritic-cell-targeting DNA vaccine for hepatitis B induces T cell and humoral immune responses and potentiates the antivirus activity in HBV transgenic mice

Debin Yu[a], Hong Liu[b], Shuai Shi[b], Liwei Dong[a], Hongge Wang[a], Nuoting Wu[a], Hui Gao[b], Zhaojun Cheng[b], Qun Zheng[b], Jiaojiao Cai[b], Libo Zou[b,**], Zhihua Zou[a,*]

[a] Key Laboratory for Molecular Enzymology and Engineering of the Ministry of Education, National Engineering Laboratory for AIDS Vaccine, School of Life Science, Jilin University, Changchun 130012, China
[b] Zhejiang Normal University—Jinhua People's Hospital Joint Center for Biomedical Research, Jinhua, China

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ABSTRACT

Strategies for inducing an effective immune response following vaccination have focused on targeting antigens to dendritic cells (DCs) through the DC-specific surface molecule DEC-205. The immunogenicity and efficacy of DNA vaccination can also be enhanced by fusing the encoded antigen to single-chain antibodies directed against DEC-205. Here, we investigated this promising approach for its enhancement of hepatitis B virus (HBV)-specific cellular and humoral immune responses and its antiviral effects in HBV transgenic mice. A plasmid DNA vaccine encoding mouse DEC-205 single-chain fragment variable (mDEC-205-scFv) linked with the hepatitis B surface antigen (HBsAg) was constructed. Vaccination with this fusion DNA vaccine in HBV transgenic mice induced robust antiviral T cell and antibody immunity against HBsAg. The levels of serum-circulating HBsAg and the HBV DNA copy number were downregulated by the induction of a higher HBsAg-specific response. Thus, in this study, we demonstrated the therapeutic efficacy of the novel mDEC-205-scFv-fused DNA vaccine in a mouse model of immune-tolerant, chronic HBV infection.

1. Introduction

Hepatitis B virus (HBV) infection is a major global health problem. An estimated 2 billion people worldwide have been infected with the virus, and approximately 350 million have chronic infections that may lead to liver cirrhosis and hepatocellular carcinoma, causing 600,000 deaths per year [1]. To date, the treatment for chronic hepatitis B infection is limited, including type-1 interferons and nucleoside analogs, which are only used in about 10% of chronic HBV carriers since the drugs are costly, exhibit limited efficacy, and have considerable side effects [2,3]. Therefore, it is necessary to develop an alternative effective therapeutic approach for the chronically infected patients.

DNA immunization can induce both arms of immunity against a specific antigen, and has shown great potential in prophylactic and therapeutic vaccinations against viral diseases [4,5]. DNA vaccines specific for HBV antigens induced strong humoral and cellular immunity in various animal models [6–8]. In phase I clinical trials, antigen-specific antibody responses and CD8+ T cells were induced in healthy volunteers after HBV DNA vaccination [9]; and in chronic HBV carriers, interferon-gamma (IFN-γ)-producing T cells specific for the preS2 or S antigen were detectable in 50% and 100% of the patients, respectively, and serum HBV DNA levels decreased after vaccination with the HBV preS2/S DNA vaccine [10]. Despite its potentiality, DNA vaccine application in large animals is limited because of its low potency. Therefore, a significant step is highly demanded to improve the immunogenicity of DNA vaccines in humans.

DEC-205, which is an endocytic receptor belonging to the mannose receptor family, is highly expressed in dendritic cells (DCs) [11,12]. An antigen coupled to an anti-DEC-205 monoclonal anti-

* Corresponding author at: Key Laboratory for Molecular Enzymology and Engineering of the Ministry of Education, National Engineering Laboratory for AIDS Vaccine, School of Life Sciences, Jilin University, Changchun 102208, China.
** Corresponding author at: Zhejiang Normal University—Jinhua People's Hospital Joint Center for Biomedical Research, Zhejiang Normal University, Jinhua 321004,China.
E-mail addresses: zoulibo1969@163.com (L. Zou), Jobzou@163.com (Z. Zou).

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body induced an antigen-specific immune response that eliminated metastatic melanoma in a mouse model [13]. The fusion of antigens to mouse DEC-205 single-chain fragment variable (mDEC-205-scFv) has been proven as a potential approach to enhancing the immunogenicity of DNA vaccines, even in large animals [14]. Cao et al. demonstrated that a fusion DNA vaccine containing mDEC-205-scFv was able to induce both a robust antibody response and T cell response [15]. The strategy was quickly applied to elicit several antitumor and antiviral immune responses and showed great promise in the prevention and control of viral diseases and tumors.

The hepatitis B surface antigen (HBsAg) is a main component of HBV envelope proteins, and is known to be tolerant in the specific immune response in patients with chronic HBV infection as well as in HBV transgenic mice [16,17]. It is generally acknowledged that the antibody response contributes to the clearance of circulating virus particles and the prevention of viral spread within the host [18]. HBsAg is also an important candidate antigen for a DNA vaccine to induce robust specific antibody and T cell responses [19]. In this study, we constructed a fusion plasmid by linking mDEC-205-scFv to HBsAg, and evaluated its enhancement of the HBsAg-specific immune responses and antiviral effects in HBV transgenic mice.

2. Materials and methods

2.1. Plasmid construction and preparation

The eukaryotic expression vector pVAX1 (Invitrogen, USA) was used to construct the DNA vaccine plasmids. The full-length HBsAg DNA fragment (GenBank Accession No. E02156.1), which contains the DNA sequence encoding the immunoglobulin-kappa (Igκ)-chain leader sequence at the 5′ terminal and the glycosyl phosphatidyl inositol (GPI; GSGTTSGTTRLLSGMTCFTLTGLLGTLVTMGLLT) sequence at the 3′ terminal, was synthesized by Genewiz Inc. (South Plainfield, NJ, USA) and then cloned into pVAX1 to generate the recombinant plasmid, pVAX1-HBsAg. The DNA fragment encoding mDEC-205-scFv [20] was synthesized by Genewiz Inc. To construct the mDEC-205-scFv-HBsAg fusion plasmid, the full-length DNA fragment of HBsAg was placed downstream of the mDEC-205-scFv gene and joined by a (G4S) 3 flexible linker. The fusion DNA sequence of mDEC-205-scFv-HBsAg was then inserted into pVAX1 to generate pVAX1-mDEC-205-scFv-HBsAg. All nucleotide sequences introduced into the vectors were verified by DNA sequencing.

2.2. Transfection, flow cytometry, and immunofluorescence assays

The 293T cell line was cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. The purified plasmids, pVAX1, pVAX1-HBsAg, and pVAX1-mDEC-205-scFv-HBsAg, were transfected into 293T cells with Lipofectamine™ 2000 (Invitrogen, USA), respectively. Forty-eight hours after transfection, the cells were harvested. The expression levels of HBsAg and mDEC-205-scFv-HBsAg in the cells were detected by flow cytometry and immunofluorescence staining.

2.3. In vivo *targeting assay*

A 100 μg aliquot of either pVAX1-HBsAg or pVAX1-mDEC-205-scFv-HBsAg in 100 μL of PBS was injected into the upper leg muscle of the left hind limb of mice followed by *in vivo* electroporation. Then 60 h later, the spleen was harvested from the injected mice and single-cell suspensions were prepared and stained with the fluorescein–isothiocyanate-conjugated anti-CD11c antibody and phycoerythrin-conjugated anti-HBsAg antibody for 30 min. The red fluorescence in the CD11c-positive DCs was analyzed by flow cytometry.

2.4. Mice and DNA immunization

Female HBV transgenic mice on a BALB/c background (6–8 weeks old) were purchased from Transgenic Engineering Research Laboratory, Infectious Disease Center of PLA (Guangzhou, China). The transgene in the mice consists of 1.3 copies of the HBV complete genome (subtype ayw). These mice stably express HBsAg and have detectable HBV DNA in their sera, and strongly express HBsAg and hepatitis B core antigen in their liver [21]. The mice were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, Revised 1996). Experimental procedures conformed to international guidelines for the care and use of laboratory animals and were approved by the Animal Ethics Committee of Jilin University. For a higher efficiency of gene transfer, the quadricep muscles of the BALB/c mice were immunized with 100 μg of pVAX1, pVAX1-HBsAg, or pVAX1-mDEC-205-scFv-HBsAg *via* intramuscular injection and electric pulsing. The mice were then boosted twice at intervals of 10 days, respectively.

2.5. Assays for serum HBsAg and anti-HBsAg

At different time points before and after immunization, blood was collected from the mice by retrobulbar puncture with heparinized glass pipettes, and the sera obtained by centrifugation were stored at −80 °C for further analysis. The HBsAg was assayed by solid-phase radioimmunoassay using a commercial kit (Boson Biotech, Xiamen, China). Anti-HBsAg titers were quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit (AUSAB; Abbott, Wiesbaden, Germany). The IgG subclasses were determined by ELISA. Briefly, the ELISA plates were coated with 10 μg/mL of recombinant HBsAg (rHBsAg; provided by Beijing Wantai Bio-Pharmaceutical Co., Ltd., China) in bicarbonate buffer. After blocking with 5% powdered milk in PBS containing Tween-20, the serially diluted mice sera were added. After incubation at 37 °C for 2 h, rat anti-mouse IgG1 or rat anti-mouse IgG2a coupled to biotin (BD Pharmingen, CA, USA) was added. The plates were then washed before 100 μL of horseradish–peroxidase-conjugated streptavidin (PharMingen) was added, following which the plates were incubated at 37 °C for 1 h. After three washes with PBS, the color was developed with tetramethylbenzidine as the substrate, and the reaction was stopped with the addition of 2 M H_2SO_4. The absorbance at 450 nm was measured using an ELISA plate reader. The cutoff value was set as two times over negative controls. The antibody titers were expressed as the reciprocal of the highest dilutions that were regarded as positive.

2.6. IFN-γ enzyme-linked immunospot assay

HBV transgenic mice were sacrificed 4 weeks after the third immunization. Splenocytes were harvested from individual mice. The IFN-γ enzyme-linked immunospot (ELISPOT) assay was performed according to the protocol supplied by the manufacturer. In brief, 96-well polyvinylidene fluoride plates (Dakewe Biotech Ltd., Shenzhen, China) were precoated with the coating antibody overnight at 4 °C and blocked for 1 h at 37 °C. The purified splenocytes (4×10^5 cells/well) together with HBsAg, or control protein BSA (10 mM) as negative control, or 5 μg/mL of ConA as positive control, were added to the well and incubated at 37 °C for 48 h. Each test condition was performed in triplicate. The spots were counted and analyzed with the ELISPOT Reader (AID, Germany).

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