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Immune responses in mice vaccinated with a suicidal DNA vaccine expressing the hemagglutinin glycoprotein from the peste des petits ruminants virus

Yong Wang[a,d], Guangqing Liu[c], Lijun Shi[a], Wenchao Li[a], Chuanfeng Li[c], Zongyan Chen[c], Hongyan Jin[a], Binrui Xu[b,*], Gang Li[a,*]

[a] State Key Laboratory of Animal Nutrition, Beijing Institute of Animal Science and Veterinary Medicine, Chinese Academy of Agricultural Sciences, Beijing 100193, China
[b] Key Laboratory of Animal Epidemiology and Zoonosis, Ministry of Agriculture, College of Veterinary Medicine, China Agricultural University, 2 Yuanmingyuan Road West, Beijing 100193, China
[c] Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, No. 518 Ziyue Road, Shanghai 200241, China
[d] College of Animal Science and Technology, Anhui Agricultural University, 130 Changjiang West Road, Hefei 230036, China

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A B S T R A C T

Peste des petits ruminants (PPR), an acute and highly contagious disease, affects sheep, goats, and some small ruminants. The hemagglutinin (H) glycoprotein of the PPR virus (PPRV) is considered important for inducing protective immune responses. In this study, a suicidal DNA vaccine based on the *Semliki Forest virus* (SFV) replicon was constructed and tested for its ability to induce immunogenicity in a mouse model. For this, the H gene of PPRV was cloned and inserted into pSCA1, an SFV replicon vector. The resultant plasmid named pSCA1-H was then transfected into BHK-21 cells following which the antigenicity of the expressed protein was confirmed by Western blotting and immunofluorescence. The pSCA1-H plasmid was then injected intramuscularly into BALB/c mice thrice at 2-week intervals. To evaluate the immunogenicity of pSCA1-H, specific antibodies and neutralizing antibodies against PPRV-H were measured using an indirect enzyme-linked immunosorbent assay and a microneutralization test, respectively. Cell-mediated immune responses were also examined using a lymphocyte proliferation assay. The results showed that pSCA1-H could express the H protein in BHK-21 cells. Specific antibodies, neutralizing antibodies, and lymphocyte proliferation responses were all induced in mice. Thus, this suicidal DNA vaccine could be a promising new approach for vaccine development against PPR.

1. Introduction

Peste des petits ruminants (PPR) is an infectious and fast-spreading disease that affects wild and domestic small ruminants and is caused by the peste des petits ruminants virus (PPRV). It is characterized by high fever, erosive stomatitis, enteritis, pneumonia and diarrhea. PPR outbreaks can cause severe economic losses because they often result in severe morbidity and high mortality. The mortality rate can reach about 80% and the animals often die within 12 d of infection (Diallo et al., 2007). PPR was first reported in West Africa in 1942; however, it is seen presently in West and Central Africa, Turkey, India, Southwest and Central Asia (Nanda et al., 1996; Taylor and Abegunde, 1979; Taylor et al., 2002; Wambura, 2000; Wang et al., 2009; Zahur et al., 2008).

PPRV is a non-segmented, negative-stranded virus, which belongs to the family *Paramyxoviridae*, genus *Morbillivirus* (Banyard et al., 2010; Gibbs et al., 1979). Expression of the hemagglutinin (H) protein, which is an external glycoprotein on the surface of the virus, is critical for viral attachment and penetration into the host cell (Berhe et al., 2003; Das et al., 2000). The H protein is also a major antigen that can stimulate a protective immune response (Chen et al., 2010; Diallo et al., 2002; Qin et al., 2012).

PPR has been controlled for many years by the use of a rinderpest virus (RPV) tissue culture-adapted vaccine. However, this vaccine was later banned within rinderpest-free zones because of the Global Rinderpest Eradication Program (Anderson and McKay, 1994). A homologous live-attenuated vaccine, Nigeria 75/1 (Nig

Abbreviations: PPR, peste des petits ruminants; PPRV, peste des petits ruminants virus; Nig 75/1, Nigeria 75/1; SFV, *Semliki Forest virus*; H, hemagglutinin; Vero cell, African green monkey kidney cells; DIVA, differentiating infected from vaccinated animals; BHK-21, baby hamster kidney; DMEM, Dulbecco's Modified Eagle's Medium; HRP, horseradish peroxidase; DAB, 3,3′-Diaminobenzidine tetrahydrochloride; HRP, horseradish peroxidase; TMB, tetramethylbenzidine; OIE, World Organization for Animal Health; $TCID_{50}$, 50% tissue culture infectious dose; CPE, cytopathic effect; VNA, virus-neutralizing antibody.

* Corresponding authors. Tel.: +86 10 62813876; fax: +86 10 62818775.
E-mail addresses: brxu@yahoo.cn (B. Xu), gli358@gmail.com (G. Li).

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75/1), was developed by continuous passage of the PPRV in African green monkey kidney (Vero) cells (Diallo et al., 1989). Although it shows better efficacy and safety in the field than RPV, Nig 75/1 cannot be used easily for differentiating infected from vaccinated animals (DIVA) (Diallo et al., 2007). The heat sensitivity of Nig 75/1 is also a concern for its use in hot climates (Diallo, 2003; Sarkar et al., 2003). Therefore, to facilitate serosurveillance and seromonitoring, a DIVA, heat-stable, and efficient vaccine against PPR is required.

Recently, alphavirus replicon-based expression vector systems (also called suicidal DNA vaccines), and, in particular, the *Semliki Forest virus* (SFV), have emerged as an important strategy for various applications in cancer gene therapy and vaccine development (Schlesinger, 2001). Suicidal DNA vaccines can induce high levels of humoral and cell-mediated immunity against various antigens (Berglund et al., 1998; Deshpande et al., 2002; Kirman et al., 2003; Li et al., 2007; Sun et al., 2010; Tian et al., 2012). In addition, it has been proven that suicidal DNA vaccines can break immunological tolerance by activating innate antiviral pathways and that efficient immune responses can be obtained with a small amount of plasmid (Leitner et al., 2003; Zhou et al., 1994).

In the present study, a suicidal DNA vaccine expressing the H glycoprotein of PPRV was designed and constructed. Its immunogenicity was then evaluated in a mouse model in order to assess its potential as a PPR vaccine.

2. Material and methods

2.1. Virus and cells

Live attenuated PPRV vaccine strain Nig 75/1 was obtained from the China Institute of Veterinary Drug Control. African green monkey kidney (Vero) and baby hamster kidney (BHK-21) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Carlsbad, CA, USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Invitrogen, USA), 100 U/ml penicillin, 100 mg/ml streptomycin, and 25 mM HEPES (Sigma–Aldrich, St. Louis, MO, USA) at 37 °C in 5% CO_2. PPRV was propagated and titrated in Vero cells in DMEM containing 2% FBS. BHK-21 cells were used for the plasmid transfections.

2.2. Construction of the suicidal DNA vaccine

PPRV total RNA was extracted from a viral suspension by using RNeasy (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The cDNA was synthesized by reverse transcriptase polymerase chain reaction with M-MLV reverse transcriptase (Promega, Madison, WI, USA) and then used as a PCR template. The open reading frame of the H gene was obtained by PCR using LA Taq high-fidelity polymerase (Takara, Dalian, China). The primers were designed using gene sequences from the Nig 75/1 strain (GenBank accession no. X74443.2) and *BamHI* restriction enzyme sites introduced at the respective 5′-termini (underlined): forward primer 5′-CG*GGATCC***GCCACC**ATGTCCGCACAAAGGGAAAG-3′ and reverse primer 5′-CG*GGATCC*TCAGACTGGATTACATGTTACCTC-3′. To optimize the expression of the target foreign gene, a Kozak sequence (in bold) was added to the forward primer. The resulting PCR product was purified by gel electrophoresis and subcloned into the pMD19-T vector (TaKaRa) for sequencing. The H gene was released with *BamHI* digestion from pMD19-T-H and ligated to a *BamHI* digested, dephosphorylated vector, pSCA1. The pSCA1 plasmid is an SFV replicon-based vector that was provided by Dr. Guangqing Liu, Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences. The resultant plasmid was named pSCA1-H and confirmed by *BamHI* digestion and sequence analysis.

The recombinant plasmid was transformed into competent *Escherichia coli* (*E. coli*) DH5α cells. Large-scale plasmid preparations were then performed using the Wizard Plus maxiprep DNA purification system (Promega) according to the manufacturer's instructions. The plasmid obtained was used for *in vitro* transfection and mouse immunization.

2.3. In vitro plasmid transfection

Recombinant pSCA1-H was transfected into BHK-21 cells in 6-well tissue culture plates (Costar, Corning, New York, NY, USA) by using Lipofectamine 2000 reagent (Invitrogen). Briefly, 4 μg of pSCA1-H plasmid was diluted in 250 μl of OPTI-MEM medium (Invitrogen) and mixed gently. At the same time, 10 μl of Lipofectamine 2000 was mixed with 250 μl OPTI-MEM medium at room temperature for 5 min. After 5-min incubation, the diluted DNA was combined with diluted Lipofectamine 2000 and incubated for 20 min. The DNA-liposome complexes were added slowly to monolayer cells that had been prewashed twice with OPTI-MEM. After a 4-h incubation, the complexes were removed, and 2 ml of fresh complete growth medium containing 10% FBS was added to each well. The plates were then incubated at 37 °C in 5% CO_2.

2.4. Western blotting and immunofluorescence analyses

The expression of the H protein was confirmed by Western blotting. Briefly, 48 h after transfection, the cells were washed twice with PBS (pH 7.4) and lysed in lysis buffer. The proteins expressed were separated by 10% SDS-PAGE and transferred onto a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA) by electroblotting. The membrane was blocked with 10% skimmed milk in PBST (0.05% Tween-20 in PBS) overnight at 4 °C. After washing thrice with PBST, mouse anti-PPRV polyclonal antiserum (1:1000) was added, and the membrane was incubated at 37 °C for 1 h. The membrane was then incubated with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG secondary antibodies (Zhongshanjinqiao, Beijing, China) according to the manufacturer's recommended protocol. Finally, protein bands were visualized using 3,3′-diaminobenzidine tetrahydrochloride (DAB, Tiangen, Beijing, China) staining.

For the indirect immunofluorescence assay, a monolayer of BHK-21 cells was fixed with cold 100% acetone at −20 °C for 30 min at 48 h after transfection. The cells were then incubated in a humid box with mouse anti-PPRV polyclonal serum at 37 °C for 30 min followed by incubation with an fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG (Zhongshanjinqiao) antibody at 37 °C for 30 min. After the cells were rinsed 3 times with PBS, they were analyzed immediately for fluorescence under a Leica fluorescence microscope (Olympus, Tokyo, Japan).

2.5. Immunization of mice

Forty 6-week-old female BALB/c mice were purchased from Weitonglihua (Beijing, China) and divided randomly into 4 groups (10 mice in each group). Two groups of mice were vaccinated intramuscularly in the quadriceps muscle with 100 μg of pSCA1-H or pSCA1. The purified PPRV Nig 75/1 strain was inoculated subcutaneously as an immune control group (10^3 $TCID_{50}$ for each mouse). The remaining group was injected with PBS as the negative control. The booster dose was given twice at 2-week intervals. Serum samples were collected from the retro-orbital plexus at 0 (before the first immunization), 2, 4 and 6 weeks post-immunization. Sera were separated and stored at −20 °C until use. All animal experiments were carried out in accordance with the requirements of the Regulations of Experimental Animal Administration of China.

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