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Efficacy and immunogenicity of recombinant swinepox virus expressing the A epitope of the TGEV S protein

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ABSTRACT

To explore the possibility of developing a vaccine against transmissible gastroenteritis virus (TGEV) infection, a recombinant swinepox virus (rSPV-SA) expressing a TGEV protective antigen has been constructed. Immune responses and protection efficacy of the vaccination vector were assessed in both mice and pig models. An indirect ELISA assay suggested that when mice were vaccinated with rSPV-SA, the level of IgG against TGEV was enhanced dramatically. The cytokine assays were employed and the results indicated that both the Th1-type and Th2-type cytokine levels raised after vaccination with rSPV-SA in mice models. Results from the passive immunity protection test of new born piglets demonstrated that the recombinant live-vector vaccine, rSPV-SA, could 100% protect piglets from the SPV infection, and there was no significant clinical symptom in the rSPV-SA treatment group during this experiment. The data suggest that the novel recombinant swinepox virus is a potential vaccine against TGEV infection.

1. Introduction

Transmissible gastroenteritis virus (TGEV) is a member of Coronaviridae, which is the etiological agent of transmissible gastroenteritis. Although the virus is capable of infecting swine of all ages, suckling piglets are the most susceptible and have a mortality rate up to 100% [1]. TGEV is a pleomorphic enveloped virus containing a positive-stranded RNA genome and four structural proteins: the spike (S) protein, the integral membrane (M) protein, the minor envelope (E) protein, and the nucleocapsid (N) protein. Among which, the spike (s) protein, one of the key structural membrane proteins of coronaviruses, is an attractive target for generating neutralizing antibodies against the virus due to the critical role it plays in the host cell invasion [2,3]. Precisely, the S protein mediates the attachment of virus particles to targets via binding of itself to the specific receptors. At the N terminus of the S protein, there are four antigenic sites, A, B, C, and D, which have been shown to be involved in the stimulation of neutralizing antibodies (Fig. 1) [4]. Previous studies have determined that the A site (which is fully dependent on glycosylation for proper folding) is predominantly responsible for stimulating neutralizing host antibodies [5–10].

SPV is a natural mild attenuated virus and has been widely applied as a vaccine. Given that Poxvirus-vectors can prevent a great deal of important diseases in both humans and animals it is not surprising that many of these vectors been licensed and used extensively [11–13]. Additionally, SPV is a safe vaccine vector as there is no risk of cross-species infection[14]. Therefore, both for biological and clinical practicality, SPV is regarded as an appropriate and promising veterinary vaccine for swine, owing to its ability to effectively express foreign genes, its large packaging capacity for recombinant DNA, its low cost of delivery and its specific host restriction [15]. The potential value of SPV as a live vector vaccine is being studied extensively. Because SPV is able to pack large amounts of recombinant DNA and to induce appropriate immune responses in vivo, it is a promising candidate for the development of a recombinant vaccine [16,17]. As of yet, pigs are the only known hosts of swinepox virus and therefore may be useful in developing a safe vaccine for clinical application [18,19].

In this study, we constructed a recombinant swinepox virus expressing S-A (A epitope of the S protein) of TGEV and characterized recombinant virus replication and expression of the S protein in PK-15 cells. We further investigated the potential of this approach for use in the vaccination of pigs against TGE.

2. Materials and methods

2.1. Cells and viruses

Wild type swinepox virus (wtSPV, Kasza strain, ATCC: VR363), swine testicle cells (ST, ATCC: CRL174), and porcine kidney cells (PK-15, ATCC: CCL-33) were purchased from the American

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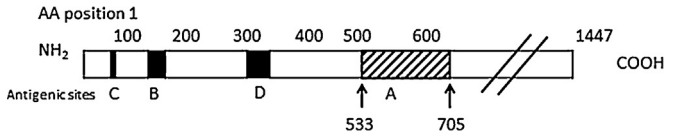

Fig. 1. Schematic representation of the four antigenic sites in the S protein of TGEV. The antigenic sites (C, B, D and A from left to right) of the TGEV S protein is shown using the Madrid nomenclature.

Type Culture Collection. Swine transmissible gastroenteritis virus (TGEV, China strain, SHXB) was purchased from the Jiangsu Academy of Agricultural Sciences, and the titer was determined as 1×10^8 PFU/ml ST cell. TGEV convalescent positive serum was purchased from the Jiangsu Academy of Agricultural Sciences, and the neutralizing antibodies were used at a dilution of 1:10,000.

2.2. Construction and identification of the rSPV-SA plasmid

The pUSZ11 swinepox virus vector was generated previously [20]. Two primers, SA1 (5′-GCGTCGACATGGGTCTTGGTATGA-AGCGTAG-3′) and SA2 (5′-CGGGATCCTTA TAGCGTCCTGT-TAGTTTGTC-3′) were used to amplify the S-A gene (516 bp, KM980444) from the TGEV genome, which was inserted into the pUSZ11 plasmid to construct pUSZ11-SA subsequently. The recombinant swinepox virus, rSPV-SA, was generated by homologous recombination of wtSPV with pUSZ11-S-A as previously described [21]. PCR and indirect immunofluorescence were employed to analyze the S-A gene expression and the expression of S protein. The replication capacity and genetic stability of rSPV-SA were also evaluated by.

2.3. Generation and screening of rSPV-SA

The generation and screening of the recombinant swinepox virus assays were performed as described previously [20]. A subconfluent culture of PK-15 cells was infected with wtSPV (0.02 moi) for 2 h, and subsequently transfected with 10 μg of the pUSZ11-SA plasmid using Exfect™ Transfection Reagent (Vazyme Biotech Co., Ltd). After 72 h, PK-15 cells were harvested and lysed by five rounds of freezing and thawing. Subsequently, the lysate was used to infect PK-15 cells grown in a 12-well plate for further purification of recombinant viruses. 1.5 ml of medium with 1% LMP agarose (DingGuo, beijing, china) was added to each well and incubation was continued for five days until plaques became visible under a light microscope. After 1–2 days, a second overlay medium containing X-gal was added. The plaques were resuspended in 0.3 ml of medium with 2% FBS. Plaque isolation was repeated for 5–6 rounds until all plaques in a given well were stained blue. The recombinant SPV bearing S-A of TGEV was designated as rSPV-SA.

2.4. PCR analysis of the recombinant swinepox virus

The rSPV-SA genomic DNA from the PK-15 cells infected with rSPV-SA was extracted by SDS-protease K-phenol. We utilized wtSPV genomic DNA from PK15 cells infected with wtSPV as a negative control. PCR was performed for 5 min at 94 °C; followed by 32 cycles of 1 min at 94 °C, 1 min at 50 °C, and 1 min at 72 °C. Amplifications were performed with DNA polymerase (Promega, Shanghai, China) using primers SA1 (5′-GCGTCGACATGGGTCTTGGTATGAAGCGTAG-3′) and SA2 (5′-CGGGATCCTTATAGCGTCCTGTTAGTTTGTC-3′).

2.5. Immunofluorescence assays

Indirect immunofluorescence assays (IFA) were performed as described previously [21]. PK-15 cells were grown on a 24-well plate and infected with the wtSPV and rSPV-SA at 1×10^8 PFU/ml per well. PBS-treated cells were used as a negative control. At 60 h post-infection, cells were washed three times in PBST and fixed with cold methanol for 10 min at −20 °C. Cells were then washed three times with PBST and blocked by PBST with 10% BSA. Preparations were incubated for 1 h at 37 °C with TGEV convalescent positive serum (1:1000 in dilution buffer, PBST with 1% BSA). After three washes with PBST, cells were treated with the rhodamine-conjugated secondary antibody (Staphylococcal protein A-Rhod, Boshide, Wuhan, China) at a 1:5000 dilution (diluted in PBS) for 30 min at 37 °C. After a final wash with PBS, all wells were examined by fluorescence microscopy (Zeiss, Germany).

2.6. Immunization of mice and swine

Nine six-week-old BALB/C mice were randomly divided into three groups (3 mice per group), and immunized three times at 0, 14, and 28 days with rSPV-SA (1×10^8 PFU/ml in 0.2 ml of PBS) or wtSPV (1×10^8 PFU/ml in 0.2 ml of PBS), the control group injected with PBS.

Eight one-month-old swine (Large White) were randomly divided into four groups (2 pigs per group) and were immunized twice at 0 and 28 days with infectious rSPV-SA (1×10^8 PFU/ml in 2 ml of PBS), inactivated-TGEV (1×10^8 PFU/ml in 2 ml of PBS), wtSPV (1×10^8 PFU/ml in 2 ml of PBS) or PBS, each time via three routes: oral, nasal, and intraperitoneal. Serum was collected 14 days after the last immunization.

Twelve one-day-old pigs were randomly divided into four groups for passive immunization experiments (3 pigs per group). High titers of antibodies were collected from piglets following the first immunization. Mice and swine serum were incubated at 56 °C 30 min to complement inactivated.

All experimental protocols involving mice or swine were approved by the Laboratory Animal Monitoring Committee of Jiangsu Province.

2.7. Western blot assays

PK-15 cell monolayers were infected with wtSPV and rSPV-SA (moi of 5) and incubated for 72 h at 37 °C. Extracts, representing approximately 1×10^5 cells, were electrophoresed through an SDS-12% polyacrylamide gel and the separated proteins were transferred onto a PVDF membrane. After a 2 h transfer, the membrane was blocked with 5% skim milk in phosphate buffered saline with 0.05% Tween-20 (PBST) overnight at 4 °C. The membrane was incubated with swine convalescent serum (1:1000 dilution) containing TGEV for 2 h at 37 °C and washed three times with PBST. Immunodetection was performed with staphy-lococcal protein A-HRP at 37 °C. Following the secondary antibody probing, the membrane was washed four times with PBST. The membrane was then developed with 3,3′-diaminoben-zidine substrate until optimal color development was observed.

2.8. Indirect ELISA

Serum was collected from mice and pigs, and detected the TGEV-specific antibodies by indirect ELISA. The purified TGEV was resuspended in 100 μl PBS (pH 7.2), and used the best titer of virus for coating 96-well plates, which was determined by titration. Samples were then incubated overnight at 4 °C. This incubation was followed by three PBST washes, and blocking with 5% skim milk (in PBST) at 37 °C for 2 h. Serum samples were serially diluted and