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Immunogenicity of a recombinant Sendai virus expressing the capsid precursor polypeptide of foot-and-mouth disease virus

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ABSTRACT

In this study, SeV was used as a vector to express capsid precursor polypeptide (P1) of Foot-and-mouth disease virus (FMDV) by using reverse genetics. The rescue recombinant SeV (rSeV-P1) can efficiently propagate and express P1 protein by Western blot and IFA analysis. To evaluate the immunogenicity of rSeV-P1, BALB/c mice were divided into several groups and immunized intramuscularly with various doses of rSeV-P1, rSeV-eGFP, PBS and commercial FMD vaccine, respectively, and then challenged with an intraperitoneal injection of 1×10^6 $TCID_{50}$ of virulent serotype O FMDV O/ES/2001 strain 4 weeks after booster immunization. Mice vaccinated with rSeV-P1 acquired FMDV-specific ELISA antibodies, neutralizing antibodies as well as cellular immune response. Meantime, mice immunized with rSeV-P1 (dose-dependent) had the ability to inhibit the replication of FMDV in the sera after FMDV challenge. Our results indicated that the recombinant SeV-P1 virus could be utilized as an alternative strategy to develop a new generation of safety and efficacious vaccine against FMDV infection.

1. Introduction

Foot-and-mouth disease (FMD) is one of the most contagious and economically important diseases of a wide variety of cloven-hoofed animals (Alexandersen and Mowat, 2005; Jamal and Belsham, 2013). The characteristic clinical signs of FMD are high fever, anorexia and vesicles lesions on the tongue, nose and feet (Alexandersen et al., 2003; Pacheco et al., 2010). Foot and mouth disease virus (FMDV), a member of the family *Picornaviridae*, is the causative agent of FMD. There are seven serotypes of FMDV (A, O, C, Asia 1, SAT1, SAT2 and SAT3) and a large number of subtypes (Domingo et al., 2002; Knowles and Samuel, 2003; Carrillo et al., 2005; Domingo et al., 2005). The virion contains a positive single-stranded RNA genome encoding a polyprotein which is processed into structural and nonstructural proteins (Mason et al., 2003). The structural proteins, including VP1, VP2, VP3 and VP4, are the secondary cleavage products of the capsid precursor polypeptide (P1-2A) by the 3C protease. P1 protein contains T/B cell epitopes and is the main antigens responsible for inducing protective responses and make it is a candidate immune antigen for the development of novel vaccine (Balamurugan et al., 2003; Li et al., 2008a, 2008b).

Effective vaccines have played an important role in control campaigns and eradication of FMD (Sutmoller et al., 2003). Inactivated FMDV vaccines have been proved to be effective tools for the prevention of this disease in most countries. However, there still exist some dangerously potent factors, such as little or no cross-protection, posing hindrance in differentiation of infected from vaccinated animals and incomplete inactivation (Rodriguez and Gay, 2011). FMD outbreak due to improper inactivation and leakage of virus were reported (Barteling and Vreeswijk, 1991; Enserink, 2007). In order to overcome those problems, several new experimental FMDV vaccine platforms have been developed to produce effective and safe vaccine (Ludi and Rodriguez, 2013). Meamwhile, FMDV is highly sensitive to interferon and double-stranded RNA-dependent protein kinase and 2′-5′A synthetase/RNase L were involved in the inhibition of FMDV replication (Chinsangaram et al., 2001). Interferons have been used to control FMDV replication and as an adjuvant in the development of novel vaccines. Several experiments showed that adenovirus expressing Alpha interferon or Alpha and Gamma interferons could rapidly protects swine from Foot-and-Mouth Disease (Chinsangaram et al., 2003; Summerfield et al., 2009; Su et al., 2013; Kim et al., 2015).

Sendai virus (SeV) is an enveloped virus with a non-segmented negative strand RNA virus (NNSV). SeV belongs to the family *Paramyxoviridae* including human parainfluenza viruses type 1 and 3 (hPIV-1 and 3), and bovine parainfluenza virus type 3 (Karron and Collins, 2013). SeV associated disease (influenza-like disease) has a worldwide distribution and has been found in mice, hamsters, rats,

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guinea pigs, as well as men and pigs (Faisca and Desmecht, 2007). With the development of reverse genetics technology, the application of SeV as a recombinant viral vector has been investigated in recent years because of several special features including cytoplasmic gene expression, strong immunogenicity and wide host cell specificity. Nowadays, SeV vectors are widely used for gene therapy and vaccine (Griesenbach et al., 2005; Bukreyev et al., 2006; Nakanishi and Otsu, 2012; Hikono et al., 2012).

In this study, a recombinant rSeV-P1 expressing the capsid precursor polypeptide (P1) of FMDV was generated. The efficiency of rSeV-P1 to express the capsid precursor protein was examined and the immunogenicity was investigated in a mouse model. The results showed that direct immunization with rSeV-P1 induced a higher level of FMDV-ELISA antibody, neutralizing antibody and gamma interferon. Most importantly, the immunization with rSeV-P1 offered partial protection in a mouse model of FMDV infection. This study demonstrates the great potential of SeV as a novel vaccination vector that offers substantial flexibility for FMDV infection.

2. Materials and methods

2.1. Viruses and cells

FMDV O/ES/2001 strain (serotype O) was propagated in baby hamster kidney (BHK-21, ATCC) cells, and the supernatants of infected cells were clarified and stored at −80 °C. Wild type Sendai virus (wt SeV) was kindly provided by Professor Shaobo Xiao (Huazhong Agricultural University) and propagated in BHK-21 cells. Recombinant vaccinia virus (rVV-T7) expressing T7 phage RNA polymerase was used for rescue recovery experiments. BHK-21 and BSR cells (a BHK-21 cell stable expressing the phage T7 RNA polymerase, kindly provided by Professor Zhenfang Fu, Huazhong Agricultural University) were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA), supplemented with 10% fetal bovine serum (FBS; Gibco, New Zealand) and 100 U/ml penicillin (Invitrogen) and 100 μg/ml of streptomycin (Invitrogen).

2.2. Plasmids and FMD vaccine

The plasmid pMD-P1, containing the coding regions of capsid precursor (P1) of type O FMDV O/ES/2001 strain, was constructed previously (Li et al., 2008b). pSeV, containing a cDNA coding for the full length antigenome of SeV deleting the envelope fusion protein gene (F), pGEM-N, pGEM-P and pGEM-L, containing a cDNA coding region for the N, P and L sequence of SeV according to the sequence of SeV (GeneBank accession number DQ219803) were synthesized according to Touzelet O's method (Sangon Biotech, China) (Touzelet et al., 2009). eGFP gene was cloned from the plasmid of peGFP-C1 (Invitrogen). Commercial inactivated FMD vaccine was used as a positive control for animal experiment (China Agricultural Vet. Bio. Science and Technology Co., Ltd. Lanzhou, China).

2.3. Construction of recombinant virus rSev-P1

All primers used in this study are summarized in Table 1. The 2208 bp of P1 coding sequences of FMDV type O was sub-cloned into the pSeV *Bam*HI site between N and P, yielding plasmids pSeV-P1 (Fig. 1A). pSeV-eGFP was also gained by inserting a 750 bp eGFP fragment from peGFP-C1 (Clontech) into pSeV with the same method. Co-transfection was carried out using Lipofectine 2000 (Invitrogen, USA) method according to the manufacturer's instructions to rescue recombinant SeV-P1 (rSev-P1) and SeV-eGFP viruses. Briefly, BHK-21 cells were infected with 0.5 MOI rVV-T7 for 1 h at 37 °C, and then co-transfected with helper plasmids pGEM-N (5 μg), pGEM-P (5 μg), pGEM-L (1.5 μg) and 5 μg of pSeV-P1 or pSeV-eGFP, and then incubated at 33 °C for 5 h. The transfection mixtures were then replaced with DMEM (1% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml of streptomycin) for a further 48 h. The transfected monolayers were harvested by 2 cycles of freezing and thawing, and 200 ul of the suspension was injected into the allantoic cavity of 9-day-old embryonated chicken eggs. After 3 days the allantoic fluids were harvested and identified by Western blot and immunofluorescence assay (IFA).

Table 1
Primers and probe used in this study.

Primer	Sequence 5′ → 3’
FMDVpF (Forward)[a]	TTT*ACGCGT*ATGGGAGCCGGACAATC
FMDVpR (Reverse)[a]	TTT*ACGCGT*TTACTGCTTTACAGGTGC
FMDVvpF[b]	GTTGAGAACTACGGTGGCGAG
FMDVvpR[b]	TTCTGCTTGTGTCTGGCGTC
eGFP-F[c]	TTT*ACGCGT*GCAGAAGAACGGCATCAAGG
eGFP-R[c]	TTT*ACGCGT*GTGCTCAGGTAGTGGTTGTC
IFN-γ-F[d]	TGGCATAGATGTGGAAGAA
IFN-γ-R[d]	GTGTGATTCAATGACGCTTA
2B-F[e]	ACAAAACACGGACCCGACTT
2B-R[e]	CTTTTACTCCTATGGCCAGTTCCT
GAPDH-F[f]	GCCCAAGATGCCCTTCAGT
GAPDH-R[f]	CCTTCCGTGTTCCTACCCC
Probe[g]	AACCGACTGGTGTCCGCGTTT

[a] Primers used to clone the FMDV P1 gene.
[b] Primers used for the identification of recombinant virus rSev-P1.
[c] Primers used to the clone eGFP gene.
[d] The special primers for analysis of IFN-γ mRNA expression through real-time RT-PCR.
[e] Primers used for the quantification of FMDV targeted at 2B gene by real-time RT-PCR.
[f] Primers used for the control of real-time RT-PCR.
[g] The specific probe used for the quantification of FMDV targeted at 2B gene by real-time RT-PCR.

2.4. Western blot and indirect immunity fluorescence analysis

The protein expression was evaluated by Western blot and IFA. 9-day-old embryonated chicken eggs were injected with rSeV-P1 and allantoic fluids were harvested, centrifuged and were subjected to Western blot analysis three days later. Samples were separated using 10% SDS-polyacrylamide gel electrophoresis and then transferred to nitrocellulose. The membrane was blocked with 5% nonfat milk in TBS and incubated with rabbit anti-FMDV serum (diluted 1:500, kindly provided by Doctor Keshan Zhang, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China) for 1 h at 37 °C followed by three washes of 5 min each with PBST. The blots were then reacted with HRP-conjugated goat anti-rabbit IgGs (dilution 1:5000, Sigma) for 1 h at 37 °C. At last, the bands were visualized by the ECL method with Chemi-DOCTM system (Bio-Rad).

For IFA, BHK-21 cells were seeded into 24-well plates and infected 1 with rSeV-P1 and wtSeV at a MOI. At 36 h post-infection, the cells were fixed with methanol/acetone (1:1) for 30 min at −20 °C and then with 1% bovine serum albumin (Sigma). The cells were subsequently incubated with rabbit anti-FMDV serum (diluted 1:100, kindly provided by Doctor Keshan Zhang, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China). The fixed cells were incubated with CY3-conjugated goat anti-rabbit antibody (1:100 dilution, Sigma). The cells were examined under a laser scanning confocal microscope (LSM 510, Zeiss, USA).

2.5. Growth properties of rSeV-P1

To analyze the genetic stability of the foreign gene in the recombinant rSeV-P1 virus, the virus was sequentially grown on CEFs for 20 passages and viral RNAs were extracted and analyzed using VP1-specific primers. The expression of P1 protein was also determined by Western blot analysis with rabbit anti-FMDV serum (diluted 1:100, kindly provided by Doctor Keshan Zhang, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China). One step