Construction of Recombinant Porcine Reproductive and Respiratory Syndrome Virus Expressing CSFV E2 Glycoprotein

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Abstract

Classical Swine Fever (CSF) and Porcine Reproductive and Respiratory Syndrome (PRRS) are two highly contagious infectious diseases caused by CSF virus (CSFV) and PRRSV virus (PRRSV), respectively. Recombinant PRRSV expressing CSFV E2 glycoprotein could be used for the development of bivalent vaccine, antiviral drug or antibody screening assays against PRRSV and CSFV. In this study, a recombinant PRRSV expressing CSFV E2 glycoprotein (P129-CSFV-E2) was constructed. The E2 gene from CSF V C-strain vaccine was cloned and inserted between ORF1b and ORF2 gene of the PRRSV P129 strain. An additional transcriptional regulatory sequence 6 (TRS6) was inserted following the CSFV E2 for driving the transcription of ORF2. The construct efficiently produced progeny viruses and the expressed CSFV E2 protein was detected by immune staining of infected MARC-145 cells. The growth ability of the p129-CSFV-E2 virus is comparable to the parental p129 virus. The genetic stability and stable expression of CSFV E2 of P129-CSFV-E2 virus could reach 11 passages in cell culture. The results showed that CSFV E2 glycoprotein could be expressed as a separated subgenomic unit in the PRRSV genome. The recombinant P129-CSFV-E2 virus can be used for the development of novel vaccines, cell-based high throughput antiviral drug and antibody screening system against PRRSV and CSFV.

Keywords: CSFV; PRRSV; P129; E2 Glycoprotein;

Introduction

Classical swine fever (CSF) is a highly contagious viral disease of swine, including wild (feral) pigs [1]. E2 glycoprotein is the major antigenic protein of CSFV, which can induce neutralizing antibodies and confer protective immunity in pigs [2]. Our previous studies showed that CSFV E2 from hog cholera lapinized virus C-strain (HCLV, Genotype 1.1) can protect pigs from sublethal CSF infection and severe reproductive failures in sows [5]. Recently, the development of PRRSV infectious cDNA clones and engineering of the PRRSV genome made possible PRRSV serving as a vaccine vector [6-10]. In this study, we successfully constructed a PRRSV North American (NA) strain P129 based recombinant PRRSV expressing HCLV E2 glycoprotein. Humoral immune responses to PRRSV and CSFV E2 after P129-CSFV-E2 recombinant virus vaccination were tested in pigs.

Material and Methods

An infectious cDNA clone of pCMV-P129 with two unique restriction sites (AflII and Mlu I) and a copy of the transcription regulatory sequence of ORF6 (TRS6) between ORFs 1b and 2a was used as the backbone. CSFV E2 gene was amplified from hog cholera lapinized virus C-strain (HCLV, Genotype 1.1) using AccuPrime™ PfX Super Mix (Invitrogen, MA, USA) with primers 5’-GAATTCGAGTTTAGAGTGTGGATGATTTTGCGGCTGACAAGT-3’ (the AflII site is underlined) and 5’-CGGAACACGCGTCAACGATGATCATTCCTCCATCATCTGTTGTACAAAGT-3’ (the Mlu I site is underlined). The amplified product was cloned into AflII/Mlu I-cut pCMV-P129 vector [6-10]. In this study, we successfully constructed a PRRSV North American (NA) strain P129 based recombinant PRRSV expressing HCLV E2 glycoprotein. Humoral immune responses to PRRSV and CSFV E2 after P129-CSFV-E2 recombinant virus vaccination were tested in pigs.
randomly allotted into 2 groups (n=5/group). On Day 0, one group was immunized intramuscularly with a single dose of $5 \times 10^5$ TCID$_{50}$ of P129-CSFV-E2 and the other group was mock vaccinated with PBS as control. Pigs were monitored daily for clinical signs (respiratory changes, lethargy, anorexia, and rectal temperature and weight gains). Serum samples were collected every 3 days until 21 days post vaccination (DPV). Animal care and use protocols were approved by Institutional Animal Care and Use Committee (IACUC) at Kansas State University. PRRSV-specific antibody titers were tested by using IDEXX HerdChek ELISA kits as described previously [12]. Measurement of anti-CSFV E2 antibody titers in the sera were performed as described previously [3].

**Results**

The CSFV E2 gene was inserted between the non-structural and structural genes of the p129 genome using reverse genetic manipulation. The transcription of CSFV E2 gene was controlled by TRS2. An additional TRS6 was inserted following the CSFVE2 for driving the transcription of ORF2 (Figure 1A). As shown in Figure 1B-D, infectious clone P129-CSFV-E2 efficiently produced progeny viruses with successful expression of CSFV E2 protein in MARC-145 cells. P129-CSFV-E2 virus induced CPE, characterized by cellular rounding and clumping, was visible 24 hours post infection (HPI), and 80-100% of cells exhibited extensive CPE at 4 DPI tested. Expression of CSFV E2 protein could be detected at 12 (HPI) in some cells by IFA testing. By 4 DPI, almost all the cells were positive for expression of CSFV E2. To examine the growth characters and genetic stability of the recombinant P129-CSFV-E2 virus, the P129-CSFV-E2 virus and its parental virus were inoculated into and passaged in MARC-145 cells. The results showed that the growth ability of the P129-CSFV-E2 virus was comparable to the parental p129 virus (Figure 2A). The genetic stability of CSFV E2 gene insertion was confirmed by RT-PCR and sequencing. Revealing that no mutation was introduced during passages. The stability of CSFVE2 expression was tested by IFA. Indicating the stable expression of CSFVE2 through 11 passages in cell culture. The rescued recombinant virus was stable for 11 passages. However, after 11 passages the virus begins losing ability to express HCV E2 protein when tested by IFA.

From 5 DPV to 12 DPV, vaccinated pigs exhibited transient fever (of 40°C-40.7°C). Clinical symptoms diminished after 12 DPV and pigs returned to normal. The PRRSV-associated syndrome, aural cyanosis, commonly known as “blue ear” was not detected at any time point during the experimental period. No clinical signs were observed in any pigs in the control groups. In vaccinated group, PRRSV specific antibodies appeared 1 week after vaccination (Figure 2B). But no CSFV glycoprotein E2 specific antibodies and CSFV neutralizing antibodies appeared during the experiment (data not shown). In control group, PRRSV specific antibodies were not detected during the experiment.

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**Figure 1:** Construction of PRRSV P129 expressing CSFV E2 glycoprotein

(A) Schematic of infectious PRRSV cDNA clone, P129-CSFV-E2.

(B) MARC-145 cell control (40X).

(C) Infection of P129-CSFV-E2 virus (11th passage of the rescued viruses, P11) in MARC-145 cells, 4 DPI (40X).

(D) IFA test the expression of CSFV E2 in P129-CSFV-E2 virus (P11) infected MARC-145 cells; 4 DPI (200X).
Figure 2: Growth curves of P129-CSFV-E2 recombinant virus and serological response against PRRSV after the recombinant P129-CSFV-E2 virus vaccination. (A) Multistep growth curves. A multiplicity of infection (MOI) of 0.01 for the parental (p129 virus) and recombinant P129-CSFV-E2 virus (P11) was used to infect fresh MARC-145 cells. Supernatants were harvested at 12, 24, 36, 48, 60, 72, 84, 96, 108, and 120 hpi. Viral titers were determined by the TCID₅₀ and expressed as LogTCID₅₀/ml. (B) ELISA (S/P ratio) analysis for humoral immune response against PRRSV specific antibodies of pigs after P129-CSFV-E2 recombinant virus vaccination.

Discussion

Over the past decade, many efforts have been made to develop PRRSV reverse genetic systems with different PRRSV strains and to construct recombinant viruses using PRRSV as a vector to express foreign genes [6-10, 13-17]. Several reports have highlighted the importance of the insertion position of exogenous genes and insertion size, which could affect genetic stability of foreign gene insertion [7-10, 13-16]. In this study, to obtain a genetically stable recombinant virus that could stably express CSFV E2 glycoprotein, the HCLV E2 gene (1,086 bp) was inserted between ORF1b and ORF2 of P129 genome under the regulation of authentic TRS2, while ORF2 expression was regulated by the extra TRS6 copy. Our results showed that the HCLV E2 gene can be expressed from the extra subgenomic mRNA. The rescued recombinant virus was stable for 11 passages, which confirmed that the region between ORF1b and ORF2 is a suitable site for foreign gene insertion for PRRSV [9, 14]. However, after 11 passages the virus begins losing its ability to express HCLV E2 protein when tested by IFA. Previous reports have indicated that the PRRSV vector has a limited capacity for incorporation of exogenous genes and perhaps the large size of the HCLV E2 gene limits its stability in further passages [9, 14, 16].

Single-shot intramuscular immunization of P129-CSFV-E2 virus into pigs induced PRRSV-specific antibodies. However, no CSFV E2 specific antibody appeared during the experiment. This phenomenon indicates that the B- and T-cell responses against CSFV E2 glycoprotein were suppressed by P129-CSFV-E2, which is consistent with other reports that PRRSV possesses the capacity to inhibit B- and T-cell responses [18-21]. Although the results indicate that the recombinant P129-CSFV-E2 virus developed in this study may not be a good candidate for bivalent vaccine, it could be used to develop cell-based high throughput antiviral drug and antibody screening system against PRRSV and CSFV and in studying virus-host interactions.

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References


