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Research paper

A promising multiple-epitope recombinant vaccine against classical swine fever virus

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ABSTRACT

Classical swine fever (CSF) is a highly contagious and often fatal disease of swine. It is caused by classical swine fever virus (CSFV), one of the members of the genus *Pestivirus* of the *Flaviviridae* family. The development of a safe and effective vaccine against the CSF is critical to pandemic control, this article shows a tandem-repeat multiple-epitope recombinant vaccine can protect pigs from CSFV challenge. That was composed as following: two copies each of glycoprotein E2 residues 693–707, 241–276 and 770–781, and two copies amino acid residues 1446–1460 of the non-structural protein NS2-3. In the challenge test, all of the swine vaccinated with Chinese vaccine strain (C-strain) were fully protected from a challenge with CSFV. However, after three successive vaccinations with the multiple-epitope recombinant vaccine, three out of five pigs were protected from challenge with CSFV (in terms of both clinical signs and viremia). These results demonstrate that multiple-epitope recombinant vaccine which carrying the major CSFV epitopes can induce a high level of epitope-specific antibodies and exhibit a protective capability that parallels induced by C-strain to a certain extent.

1. Introduction

Classical swine fever (CSF) is an extremely infectious and often deadly swine disease caused by classical swine fever virus (CSFV), a *Flaviviridae* family member belonging to the genus *Pestivirus* (Becher et al., 1994). CSFV infection in swine results in a highly contagious and severe disease which is characterized by fever and hemorrhages with an acute or chronic course (Lindenbach and Rice, 2003). The genome of CSFV is a positive-stranded RNA that contains a single long open reading frame (ORF) coding for a polyprotein encompassing all viral proteins (Rümenapf et al., 1991).

The structural proteins comprise the nucleocapsid protein C and three envelope glycoproteins: Erns, E1, and E2. Erns and E2, located at the surface of infected cells (Weiland et al., 1999), induce virus-neutralizing antibodies and mount protective immunity in the natural host (Bouma et al., 2000; König et al., 1995; Weiland et al., 1992). E2 is essential for virus replication and infection, and also is the major immunogenic protein that is responsible for inducing neutralizing antibodies to elicit protective immunity against CSFV (Elbers et al., 1996; Kimman et al., 1993). Previous studies have shown that E2 envelope protein contains conserved epitopes which induce CSFV-neutralizing antibodies (Wensvoort et al., 1989; Dong and Chen, 2006). However, in most cases they failed to confer complete clinical protection upon viral challenge. Still, little is known

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about the effect of these peptide vaccines on the levels of viremia and virus shedding (Dong and Chen, 2006).

Epitope-based peptide vaccines have shown to induce a specific immune response (Deres et al., 1989) that protects the host against disease (Bittle et al., 1982; Menne et al., 1997). Thus, for the development of a synthetic peptide vaccine, characterization of the porcine immune response against CSFV is necessary, and much experimental work has been carried out to characterize the cellular immune response to CSFV (Tian et al., 2012; Kimman et al., 1993). The viral proteins are responsible for the induction of the virus-specific T lymphocyte response, and a viral T cell epitope from the NS4A (non-structural 4A) protein was identified (Pauly et al., 1995).

This work describes a new potential vaccine produced in *Escherichia coli* (*E. coli*). The antigen, based on B, T cell epitopes of CSFV, was highly expressed and purified from the supernatant of recombinant *E. coli* with a single affinity chromatography step. Our study shows that epitope-based vaccine can protect the pig from the attack of CSFV in some extent.

2. Materials and methods

2.1. Materials

E. coli BL21 (DE3) and restriction enzymes were purchased from TaKaRa (Dalian, CHN), and T4 DNA ligase and pEGX-6p-1 vectors were obtained from Promega (Madison, USA). Glutathione Sepharose 4B and columns were purchased from GE Healthcare. Horseradish peroxidase (HRP) conjugated rat anti-pig IgG were purchased from Sigma (St. Louis, USA). Immobilon-P Transfer Membrane was obtained from Millipore (Millipore, USA). CSFV Shimen, C-strain Swine anti-CSFV positive and negative serum were preserved at the Virology department of Lanzhou Veterinary Research Institute, Gansu of PR China.

2.2. Design and artificial synthesis of the CSFV multi-epitope gene

In this paper, the multi-epitope contained two copies of E2 residues 693–707, 241–276 and 770–781, and coupled with two copies of amino acid residues 1446–1460 of the CSFV non-structural protein NS2-3. A linker sequence, GGSSGG, was used to separate adjacent epitopes, the length of multi-epitope gene was 507 bp. Which was synthesized and cloned into the pMD18-T vector (Dalian, CHN) to produce plasmid pMD-T500. The T500 DNA fragment was digested by *Eco*RI and *Bam*HI and cloned into the vector plasmid pGEX-6p-1 (Darmstadt, GER) to produce pGEX-BT500 (here after abbreviated as GST-BT500).

2.3. Identification of the reactivity of the fusion protein by Western blot

And then cloning, expression and purification of GST-BT500 (data not shown), western blotting were used to analysis the immunoreactivity of the fusion protein. The experiment went like this: purified GST-BT500 protein was subjected to 12% SDS–PAGE and electrophoretically transferred onto a Polyvinylidene Fluoride (PVDF) membrane with a transfer apparatus (Bio-Rad). After blotting, the membrane was immersed in blocking buffer (5% skim milk in PBS) overnight at 4 °C, then immunoblotted with primary antibody (Pig anti-CSFV positive serum at a 1:40 dilution) for 1.5 h at 37 °C. The membrane underwent three 5 min washes with PBST (0.3%Tris, 0.8% NaCl, 0.02% KCl, 0.1% Tween-20), followed by 1 h incubation at room temperature with horseradish peroxidase (HRP) conjugated secondary antibody (rabbit anti-pig IgG) diluted 1:10,000. The color was develop with a solution containing 3,3′-diaminobenzidine tetrahydrochloride (DAB) as the HRP substrate.

2.4. Immunogen

Eleven swine weighing 20–30 kg and free of antibodies against of CSFV were chosen for experiments, and randomly assigned them to epitope vaccine group ($n = 5$), sentinel group (blank control) ($n = 3$) and a positive control group (C-strain vaccinated) ($n = 3$). The control group was separated from the vaccinated pigs. All of pigs were acclimatized for 1 week, during which their environmental and body temperatures were measured daily; the average temperature of pig's rectum was 39.0 ± 0.5 °C, and a temperature exceeding 41 °C was considered to be a fever. The BT-500 group was inoculated with 600 μg recombinant multi-epitope protein in Freund's complete adjuvant for the first immunization; this was followed by second and third inoculations of recombinant multi-epitope protein in Freund's incomplete adjuvant, with a two-week interval between immunizations. The sentinel group was immunized with a commercial C-strain vaccine (Guangdong Yongshun, CHN) as positive control, while the negative control group was immunized with PBS (Phosphate Buffer Solution).

2.5. Challenge experiments

Three weeks after the third dose, all animals were challenged with 100 ID_{50} of homologous CSFV "Shimen" strain (GenBank accession number: AF092448; obtained from the Virology department of Lanzhou Veterinary Research Institute, Gansu of PR China) by intramuscular injection.

2.6. Detection of anti-CSFV antibodies in vaccinated animals

Serum samples for anti-CSFV antibodies were taken at week 1 after the first immunization as well as each subsequent week until challenge. The sera were tested with the use of the IDXX CSFV Antibody ELISA Kit according to manufacturer's instructions (IDXX, USA).

2.7. Virus quantification in blood to assay for viremia

Heparinized blood samples were collected on post-challenge day 0, 2, 4, 6, 8, 10 and 12. The viral RNA of all samples were extracted by using QIAamp Viral RNA Mini Kit (Qiagen, GER) and quantified using Brilliant® II Fast