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Piglets with maternally derived antibodies from sows immunized with rAdV-SFV-E2 were completely protected against lethal CSFV challenge

Shui-Li Xia[1], Mingliang Du[1], Jian-Lin Lei, Yan Liu, Yimin Wang, Shengwei Ji, Guang-Tao Xiang, Lian-Feng Li, Xin Cong, Yuzi Luo, Lina Shao, Hua-Ji Qiu*, Yuan Sun*

State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, 427 Maduan Street, Harbin 150001, Heilongjiang, China

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ABSTRACT

Classical swine fever (CSF) is an economically important infectious disease of pigs caused by *Classical swine fever virus* (CSFV). To facilitate the eradication of CSF in endemic areas, a marker vaccine enabling differentiation of infected from vaccinated animals (DIVA) is urgently needed. Previously, we have demonstrated that the DIVA vaccine rAdV-SFV-E2, an adenovirus-vectored Semliki Forest virus replicon expressing the E2 glycoprotein of CSFV, induces complete protection from lethal CSFV challenge. The aim of this study was to investigate whether maternally derived antibodies (MDAs) from sows immunized with rAdV-SFV-E2 can effectively protect piglets against lethal CSFV challenge. Three groups of five-week-old piglets ($n = 4$), with or without MDAs, were challenged with the highly virulent CSFV Shimen strain. Clinical signs, CSFV-specific antibodies, viremia and pathological and histopathological changes were monitored. The results showed that the piglets with MDAs from the sow immunized with rAdV-SFV-E2 were protected clinically, virologically and pathologically, while the piglets with undetectable MDAs from the rAdV-SFV-E2-immunized sow were partially protected (2/4 survival), in contrast with the piglets from the non-vaccinated sow, which displayed CSF-typical clinical signs, viremia, deaths (4/4) and pathological/histopathological lesions. These results indicate that MDAs from the sow immunized with rAdV-SFV-E2 are able to confer full passive immunity to newborn piglets.

1. Introduction

Classical swine fever (CSF), which is caused by *Classical swine fever virus* (CSFV), is a highly contagious often fatal disease of pigs notifiable to the World Organization for Animal Health (OIE). The disease causes significant losses to the swine industry in many countries including China. CSFV belongs to the genus *Pestivirus* of the family *Flaviviridae* (Pletnev et al., 2011; Lindenbach et al., 2013). At present, modified live vaccines (MLVs), such as C-strain, are the most efficient vaccines available against CSF (de Smit et al., 2000). Although MLVs have played a critical role in the control or eradiation of global CSF, they have some disadvantages, one of which is that they do not allow differentiation of infected from vaccinated animals (DIVA) (van Oirschot, 2003), which leads to restriction on the export of live pigs or pork. Therefore, there is a need for development of efficacious and safe CSF marker vaccines to overcome this problem.

During the past years, many CSF marker vaccine candidates have been developed by several groups (van Gennip et al., 2000; Reimann et al., 2004; Wang et al., 2013). Our group has developed a chimeric vector-based marker vaccine rAdV-SFV-E2, which is able to induce sterile immunity comparable to C-strain. Moreover, its efficacy is not interfered by anti-CSFV antibodies, anti-BVDV antibodies or co-administered MLVs against other swine diseases (Sun et al., 2011, 2013).

Passive immunity is the primary protection against infections in early life for animals including pigs (Pravieux et al., 2007; Brinkhof et al., 2013; Elbers et al., 2014). Lactogenic transfer of maternally derived antibodies (MDAs) from immune sows is considered as an effective and economic way to provide passive protection of their piglets from CSFV infection from birth to acquisition of active immunity. For this reason, this study was designed to investigate whether the MDAs derived from rAdV-SFV-E2-immunized sows can protect their piglets against lethal CSFV challenge.

* Corresponding authors.
E-mail addresses: huajiqiu@hvri.ac.cn, qiuhuaji@163.com (H.-J. Qiu), sunyuan@hvri.ac.cn (Y. Sun).
[1] These authors contributed equally to this paper.

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2. Materials and methods

2.1. Vaccine and challenge virus

rAdV-SFV-E2, an adenovirus-vectored Semliki Forest virus replicon expressing the E2 glycoprotein of CSFV (Sun et al., 2011), was used for vaccination. Vaccine virus stock was produced in bioreactors with HEK293 cells under Good Manufacturing Practice (GMP) conditions in Weike Biotech Co., Harbin, China. The GMP-produced rAdV-SFV-E2 vaccine had a virus titer of 10^6 median tissue culture infective doses ($TCID_{50}$)/ml. Challenge was performed with the highly virulent CSFV Shimen strain (Yu et al., 2001). All inocula were back-titrated to verify the true viral titers administered.

2.2. Animals and experimental design

The animal experiments were conducted according to the Guide for the Care and Use of Laboratory Animals of Harbin Veterinary Research Institute (HVRI), Chinese Academy of Agricultural Sciences, China. Three pregnant sows were purchased from a CSF-free pig farm. These sows were tested and proven to be free of CSFV by serum-virus neutralization test (SVNT), enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). All the sows were maintained in the animal facility at HVRI.

The sows were synchronized for estrus and gave birth within a few days. Sows 1 and 2 were vaccinated intramuscularly (i.m.) with 4×10^6 and 2×10^6 $TCID_{50}$ rAdV-SFV-E2 at day 60 of gestation, respectively. Sow 3 was injected 2 ml of DMEM serving as a negative control. Three weeks later, all the sows were boosted with the same vaccine and dose. After the piglets were born, the CSFV-specific MDAs titers were monitored using SVNT and an IDEXX HerdChek® CSFV antibody test kit (IDEXX Laboratories, Shiphol-Rijk, The Netherlands). Four piglets were selected randomly from the piglets born from one particular sow and assigned to the matching group. The piglets in Group A (born from the sow immunized with 4×10^6 $TCID_{50}$ rAdV-SFV-E2) but not those in Group B (from the sow immunized with 2×10^6 $TCID_{50}$ rAdV-SFV-E2) or Group C (from the sow injected with DMEM) had detectable MDAs. When the titers of MDAs in Group A decreased to about 1:50 for SVNT (about 50% blocking rate for ELISA) (around 5-week-old age), all the piglets were challenged with 10^6 $TCID_{50}$ CSFV Shimen strain. Following challenge, clinical signs, rectal temperature, viremia and CSFV-specific antibodies were monitored throughout the experiment. At 15 days post-challenge (DPC), all surviving piglets were euthanized. Various tissues from the piglets died from CSFV challenge and euthanized at 15 DPC were collected and subjected to pathological and histopathological examinations.

2.3. Blocking ELISA

The serum samples of the piglets were collected at −7, 0, 3, 6, 9 and 12 DPC and tested for the presence of E2-specific antibodies by using the IDEXX Herdchek® CSFV antibody test kit (IDEXX Laboratories, Shiphol-Rijk, The Netherlands).

2.4. Serum-virus neutralizing test (SVNT)

The serum samples were also tested by SVNT as described previously (Buonavoglia et al., 1989). The diluted sera were mixed with an equal volume of 200 $TCID_{50}$ CSFV Shimen strain and incubated for 60 min at 37 °C. The serum–virus mixtures were inoculated to confluent PK-15 cells cultured in 96-well plates and incubated for 60 min at 37 °C. The inoculated cells were then incubated for 72 h at 37 °C. Indirect immunofluorescence assay (IFA) was performed as described previously (Sun et al., 2011). The cells were examined under a fluorescence microscope, and the titers of CSFV-specific NAbs were determined and expressed as the reciprocal of the highest dilution at which infection of the PK-15 cells was inhibited in 50% of the culture wells.

2.5. Real-time RT-PCR

The anticoagulated blood samples treated with EDTA were collected at −7, 0, 3, 6, 9 and 12 DPC and CSFV RNA was extracted using an RNA isolation kit (Qiagen, USA) and quantified by a real-time RT-PCR with a CSFV-specific probe (5′-FAM-AGG ACT AGC AAA CGG AGG GAC TAG CCG-TAMRA-3′) and a primer pair (5′-GAA CTG GGC TAG CCA TG-3′ and 5′-ACT GTC CTG TAC TCA GGA C-3′) (Zhao et al., 2008).

2.6. Pathological and histopathological examinations

The piglets died from CSFV challenge and euthanized at 15 DPC were subjected to pathological examinations. The tissues from all the piglets were subjected to pathological and histopathological examinations as described previously (Peng et al., 2008; Sun et al., 2011; Wang et al., 2015). Various tissues (tonsils, bladder, spleen, lymph nodes and kidney) were collected from the challenged piglets, fixed with buffered 4% formalin and subsequently embedded in paraffin wax. Tissue sections (around 4-μm thick) were prepared and stained with haematoxylin and eosin for histopathological examinations.

3. Results

3.1. CSFV-specific antibodies in sows close to farrowing

The E2-specific antibodies in Sow 1 were detected at one week after the booster, with the level of 68% (112 for SVNT), and then the antibody blocking rates rose to about 75% (289 for SVNT) at one week before farrowing. No E2-specific antibodies were detected in Sows 2 and 3 before farrowing.

3.2. CSFV-specific antibodies in piglets

In Group A, the CSFV E2-specific antibodies were declining over time, with blocking rates of 49% (−7 DPC) and 43% (0 DPC), respectively. After lethal CSFV challenge, E2-specific antibodies were detected only in one of four piglets in Group A, with the mean antibody blocking rate of 48% at 3 DPC. The absence of CSFV E2-specific antibodies was confirmed in Groups B and C throughout experiment (Table 1).

Decaying anti-CSFV NAbs were detected in Group A from −7 to 12 DPC, with 1:88 (−7 DPC), 1:49 (0 DPC), 1:26 (3 DPC) and around 1:20 thereafter. No neutralizing titers were detectable in Groups B and C (Table 2).

3.3. Protection of piglets with MDAs from lethal CSFV challenge

All the piglets in Group A displayed no clinical symptoms after lethal CSFV challenge and survived at the end of experiment. All the piglets in Group B exhibited fever from 4 DPC, two piglets displayed slight CSF-specific clinical signs (depression, depression and constipation from 7 DPC) and returned to normal at 11 and 13 DPC, but the others remained febrile and displayed severe signs (chill, conjunctivitis, inappetence from 6 DPC followed by prostration, ataxia) until death (10 and 14 DPC). All the piglets in Group C displayed typical CSF clinical signs (inappetence, apathy, chill, prostration, incoordination, constipation at 3 DPC followed by diarrhea, locomotor ataxia and posterior paresis) with high fever (40.5–42 °C) from 1 DPC until death (13–14 DPC). The