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Efficacy of the marker vaccine rAdV-SFV-E2 against classical swine fever in the presence of maternally derived antibodies to rAdV-SFV-E2 or C-strain

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ABSTRACT

Classical swine fever (CSF) is an economically important disease caused by *Classical swine fever virus* (CSFV). In order to eradicate CSF, many marker vaccines that allow differentiation of infected from vaccinated animals (DIVA) have been developed. In our previous studies, a DIVA CSF vaccine rAdV-SFV-E2 has been demonstrated to completely protect pigs against lethal CSFV challenge. In the context of risk assessments for an emergency vaccination scenario, the question has been raised whether preexisting maternally derived antibodies (MDAs) interfere with the efficacy of the vaccine. In this study, six groups of piglets ($n = 5$), with or without anti-C-strain or anti-rAdV-SFV-E2 MDAs, were immunized twice with 10^6 $TCID_{50}$ rAdV-SFV-E2 and challenged with the CSFV Shimen strain. Clinical signs, CSFV-specific antibodies, viremia and pathological and histopathological changes were monitored. The results showed that the vaccinated piglets, either with or without MDAs directed against C-strain (about 67% blocking rate) or rAdV-SFV-E2 (about 50% blocking rate) were completely protected; however, the mock-vaccinated piglets displayed severe CSF-typical clinical symptoms, viremia, pathological/histopathological changes and deaths (5/5). These findings demonstrate that the MDAs to either rAdV-SFV-E2 or C-strain do not interfere with the efficacy of rAdV-SFV-E2, which highlights the great potential of the vaccine for control and eradication of CSF.

1. Introduction

Classical swine fever (CSF) is a highly infectious and often lethal disease of pigs, which is caused by *Classical swine fever virus* (CSFV). The disease leads to a huge socio-economic impact in many countries. CSFV is an enveloped, single-stranded, positive-sense RNA virus and belongs to the genus *Pestivirus* within the family *Flaviviridae* (Pletnev et al., 2011; Lindenbach et al., 2013).

Currently, CSF is endemic in China and prophylactic vaccination with the modified live vaccine (MLV) C-strain has been carried out for decades. However, C-strain does not allow differentiation of infected from vaccinated animals (DIVA) (van Oirschot, 2003). For effective surveillance and eventual eradication of CSF, many DIVA vaccines have been developed (van Gennip et al., 2000; Reimann et al., 2004; Holinka et al., 2009; von Rosen et al., 2014). As one of the efforts, we developed and evaluated a marker CSF vaccine, rAdV-SFV-E2, which is a human adenovirus type 5 (HAdV-5)-vectored alphavirus replicon expressing the E2 protein of CSFV (Sun et al., 2011). The vaccine has been demonstrated to induce sterile immunity comparable to C-strain (Sun et al., 2013).

Maternally derived antibodies (MDAs) originated from the colostrum of mothers are able to protect their offspring against pathogen infections in the early life (Insel et al., 1994; Pravieux et al., 2007). On the other hand, the preexisting MDAs may interfere with the induction of immune responses in newborn animals (Tizard, 2000; Pastoret, 2007; Salmon et al., 2009; Hodgins and Shewen, 2012). For example, the efficacy of the C-strain can be partially or completely inhibited by preexisting anti-CSFV MDAs, depending on the antibody titer at the time of vaccination, which usually leads to vaccine failure (Mierzejewska et al., 1977; Précausta et al., 1978).

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To clarify the influences of MDAs on the efficacy of rAdV-SFV-E2, we performed a vaccination-challenge experiment in piglets with preexisting anti-C-strain or anti-rAdV-SFV-E2 MDAs.

2. Materials and methods

2.1. Vaccines and challenge virus

rAdV-SFV-E2 and C-strain were used for vaccination. Vaccine virus stocks of rAdV-SFV-E2 and C-strain were produced in HEK293 and primary bovine testicle cells, respectively, under good manufacturing practice (GMP) conditions. The highly virulent CSFV Shimen strain (Yu et al., 2001) was used for challenge. All inocula were back-titrated for the true viral titers administered.

2.2. Vaccination-challenge experiment in piglets

Nine pregnant sows from a CSF-free pig farm were tested negative for CSFV and anti-CSFV antibodies. Sows 1–3 and 4–6 were immunized intramuscularly (i.m.) with 4×10^6 $TCID_{50}$ rAdV-SFV-E2 or one dose of C-strain, respectively, at day 60 before farrowing. Sows 7–9 were injected with 1 ml of DMEM serving as controls. All the sows were boosted with the same vaccines 21 days later. The titers of CSFV-specific MDAs were tested in the piglets born to the sows at different time points using a CSFV antibody test kit (IDEXX Laboratories, Shiphol-Rijk, The Netherlands). The piglets were randomly assigned to six groups of 5 each, with Groups A and B, C and D, or E and F born to the rAdV-SFV-E2-immunized, C-strain-immunized, or DMEM-injected sows, respectively. All the piglets of 28-day age in Groups A, C, and E were immunized with 10^6 $TCID_{50}$ rAdV-SFV-E2 and Groups B, D, and F were injected with DMEM serving as mock-vaccinated controls. All the piglets were boosted with the same doses of the vaccine at 3 weeks post-immunization. One week after booster immunization, all the piglets were challenged with 10^6 $TCID_{50}$ CSFV Shimen strain (Table 1). The animal experiments were conducted according to the Guide for the Care and Use of Laboratory Animals of Harbin Veterinary Research Institute.

2.3. Enzyme-linked immunosorbent assay (ELISA)

The serum samples were collected weekly from the sows and at different days post-immunization (DPI) and post-challenge (DPC) from the piglets, and tested for the presence of anti-E2 antibodies with the CSFV antibody test kit (IDEXX Laboratories, Shiphol-Rijk, The Netherlands). The assay is a blocking ELISA, which utilizes microplates coated with the CSFV E2 protein. CSFV-specific antibodies present in the tested samples will inhibit the binding of a horseradish peroxidase-conjugated anti-E2 monoclonal antibody. The bound monoclonal antibody is then detected by a substrate reactive with horseradish peroxidase.

2.4. Serum-virus neutralizing test (SVNT)

In order to determine the titers of the anti-CSFV neutralizing antibodies (NAbs) in the serum samples, SVNT was performed as described previously (Sun et al., 2011).

2.5. Real-time RT-PCR

The CSFV RNA was quantified from the blood samples using CSFV-specific real-time reverse transcription polymerase chain reaction (RT-qPCR) assay as described previously (Zhao et al., 2008).

2.6. Pathological and histopathological examinations

The tissues (tonsils, lymph nodes, spleen, bladder and kidney) from all the piglets were collected for pathological and histopathological examinations (Wang et al., 2015).

2.7. Statistical analysis

Data analysis was performed using the SPSS 14.0 software and GraphPad Prism.

3. Results

3.1. CSFV-specific antibodies in the vaccinated sows

After immunization, all the sows did not show any abnormal symptoms. At 21 DPI, E2-specific antibodies were detectable in all the vaccine-immunized sows, and the mean blocking rates were 67.56% for the rAdV-SFV-E2-immunized sows and 73.66% for the C-strain-immunized ones (the cutoff value of the assay is 40%). At 1 week before farrowing, the mean blocking rates increased to 75.68% and 81.56%, respectively. In contrast, DMEM-injected sows were tested negative for E2-specific antibodies before and after farrowing.

3.2. CSFV-specific antibodies in vaccinated piglets with MDAs

After farrowing, the level of E2-specific antibodies of the piglets from different groups declined over time. The mean blocking rates of Groups A, B, C, and D at 28 days of age were 50.17%, 50.23%, 67.74%, and 65.11%, respectively. No E2-specific antibody was detected in the pigs from Groups E and F (Table 2).

After immunization, the CSFV E2-specific antibodies increased from 21 DPI in Group A and 14 DPI in Group C. The E2-specific antibodies were detected in Group E at 28 DPI with an average blocking rate of 41%.

All piglets were challenged at 28 DPI. After challenge, the titers of anti-E2 antibodies in Groups A, C, and E declined transiently and increased suddenly after 3 DPC and reached to the peak at 12 DPC. Antibody decrease was found in all mock-vaccinated piglets with

Table 1
The vaccination-challenge experiment design for piglets born to immunized sows.

Groups	MDAs against	Vaccination			Challenge
($n = 5$)		Vaccine	Times	Doses	(10^5 $TCID_{50}$)
A	Anti-rAdV-SFV-E2	rAdV-SFV-E2	Double	10^6 $TCID_{50}$	Shimen
B	Anti-rAdV-SFV-E2	DMEM	Double	1 ml	Shimen
C	Anti-C-strain	rAdV-SFV-E2	Double	10^6 $TCID_{50}$	Shimen
D	Anti-C-strain	DMEM	Double	1 ml	Shimen
E	None	rAdV-SFV-E2	Double	10^6 $TCID_{50}$	Shimen
F	None	DMEM	Double	1 ml	Shimen