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Protection to homologous and heterologous challenge in pigs immunized with vaccine against foot-and-mouth disease type O caused an epidemic in East Asia during 2010/2011

Jeong-Nam Park[a,1], Seo-Yong Lee[a,1], Jia-Qi Chu[a,b], Yeo-Joo Lee[a], Rae-Hyung Kim[a], Kwang-Nyeong Lee[a], Su-Mi Kim[a], Dong-Seob Tark[a], Byounghan Kim[a], Jong-Hyeon Park[a,*]

[a] Animal and Plant Quarantine Agency, 175 Anyang-ro, Manangu, Anyang city 430-757, Gyeonggi-do, Republic of Korea
[b] Clinical Research Center of the Affiliated Hospital of Guangdong Medical College, No. 57 South Renmin Avenue, Zhanjiang 524001, China

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ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious infectious disease, and the use of vaccines is known to be effective for its prevention. In 2010/2011, there was an epidemic of the South East Asia (SEA) topotype in East Asian countries. We adapted the SEA topotype virus isolated in November 2010 in Korea in cells to analyze the characteristics of the virus and evaluate its possibility as a vaccine. After cell culture adaptation, the FMD virus particle 146S was purified to develop an inactivated oil vaccine for SEA or other topotypes. To measure its immunogenicity, pigs were inoculated with the experimental vaccine at different concentrations of the antigen. The results indicated that the groups immunized with at least 7.5 μg antigen were protected from homologous challenge. The immunized pigs were also protected against heterologous virus (ME–SA topotype) challenge. The genetic variations between the two field isolates and the adapted vaccine strains were identified in six amino acids by complete genome sequencing.

1. Introduction

Foot-and-mouth disease (FMD) is an economically important disease because it is highly contagious, infects many cloven-hoofed animals (such as cattle, sheep, and pigs), and there is no treatment method; thus, stamping out policies are implemented in most countries once animals have been infected. The pathogenic FMD viruses (FMDVs) are classified into small icosahedral viruses in the *Aphthovirus* genus in the Picornaviridae family [1]. There are seven serotypes of FMDVs (O, A, C, SAT1, SAT2, SAT3, and Asia1). The O and A serotypes frequently occur globally, but the Asia1 serotype occurs restrictedly in Asian countries. Among these, the O type is the most widespread. Infection with one serotype does not confer immunity against another.

Animals may have different protective abilities for different topotypes even if they belong to the same serotype of FMD. Therefore, various types of appropriate vaccines should be developed [2,3]. Among the seven serotypes known thus far, the O types are most widespread throughout the world. Serotype O is divided into eight topotypes: ME–SA (Middle East–South Asia), EURO–SA (Europe–South America), CHY (Cathay), SEA (South East Asia), ISA (Indonesia)-I, ISA-2, EA (East Africa), and WA (West Africa) [4]. Among topotypes, ME–SA, SEA, and Cathay topotypes are virus types that prevail in the Asian region. The SEA topotype occurred in FMD-susceptible animals, such as cattle and swine, in the East Asian region in 2010–2011. Most animals show severe typical symptoms, regardless of the species, and this disease has had a grave effect in many Asian countries, such as Korea, Japan, and North Korea. In this outbreak, about 3.5 million swine and cattle were culled, and the direct economic losses alone amounted to 3 billion US$ [5]. FMD spread in Korean livestock rapidly in late 2010, but nationwide vaccinations prevented its further expansion. The decline in the frequency of FMD occurrences was observed in pigs 3 weeks after the vaccinations and in cattle 2 weeks after vaccination [6]. In order to gain the same neutralizing antibody level through vaccination, pigs require a longer period of immunity than cattle [7]. The immunological relationship between O Manisa vaccine and field strains was relatively low or moderate (r value of approximately 0.3). Effective vaccination using O Manisa during the 2010–2011 outbreaks in Korea was controversial. The development of a new vaccine using field isolates caused large-scale

* Corresponding author. Tel.: +82 31 467 1719; fax: +82 31 463 4516.
E-mail addresses: parkjhvet@korea.kr, parkjhvet@nate.com (J.-H. Park).
[1] These authors contributed equally to this study.

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outbreaks was required. In particular, since more viruses are discharged from pigs than from cattle in general, vaccines should be developed that can be effectively used for pigs.

However, despite these large-scale FMD occurrences, a vaccine targeted to the SEA topotype has not yet been commercialized. We therefore tried to develop vaccines using O–SEA-topotype viruses isolated in Korea since effective vaccines are required that can accurately protect against this topotype or others when it occurs. The virus antigens were purified to analyze the properties of the vaccine strains, and immunogenicity and challenge tests were conducted to evaluate efficacy of the experimental vaccines.

2. Materials and methods

2.1. Viruses and cells

We used baby hamster kidney (BHK) 21, swine kidney (IBRS-2), and fetal goat tongue epithelial (ZZ-R) cell lines maintained at the Animal, Plant, and Quarantine Agency (QIA). We received kindly bovine calf kidney (LF-BK) cells from the Plum Island Animal Disease Center (USA). BHK21, IBRS-2, and LF-BK cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM; Corning, USA) with 5% fetal bovine serum (FBS; Gibco, USA). ZZ-R cells were sustained in DMEM F-12 (Corning) with 10% FBS. Cells were grown at 37 °C with 5% CO_2 in a humidified atmosphere.

Seed viruses for the production of virus antigens used in the vaccines were obtained from FMD viruses isolated in swine vesicles of two regions (Andong in Gyeongbuk province, Yangju in Gyeonggi province) in Korea in November and December 2010. To adapt the viruses to in vitro culture and increase the viral titers, the virus was subjected to serial passages in suckling mice and ZZ-R cells and was subsequently adapted to BHK21 cells. Infected cells were frozen within 24 h and subjected to three cycles of freezing–thawing steps to release the viral particles. The virus was harvested by centrifugation at 13,500 rpm for 5 min at 4 °C, and the supernatant was stored at −70 °C until used.

FMDV strains O/Andong/SKR/2010 and O/YJ/SKR/2010 for the SEA topotype, O Manisa (O1/Manisa/Turkey/69) and O/SKR/2002 for the ME–SA topotype, and O/ASP/Cathay (phenotype of Genbank accession HQ412603) for the Cathay topotype were used for the cross-virus neutralization test (VNT) or challenge test.

2.2. Virus adaptation and purification of virus particles (146S)

The virus isolate O/Andong/SKR/2010 from Andong was subjected to serial passages in suckling mice five times, in ZZ-R cells three times, and in BHK21 cells 15 times, for a total of 23 serial passages (named as AD-P, M5Z3B15); the virus O/YJ/SKR/2010 from Yangju was subjected to serial passages in mice seven times, in ZZ-R cells three times, and in BHK21 cells 14 times, for a total of 24 serial passages (named as YJ-P, M7Z3B14).

To produce FMDV antigens, BHK21 cells were inoculated with the viruses when the cells formed a monolayer in a 175-cm^2 T-flask. When a 100% cytopathic effect (CPE) was formed 24 h later, the viruses were kept in a freezer at −70 °C. The viruses were harvested, binary ethyleneimine-inactivated, concentrated using polyethylene glycol (PEG)-6000, and then purified with sucrose density gradient centrifugation in an ultracentrifuge [8]. The viruses were concentrated using Amicon Ultracentrifuge filters (100 kDa) and were exchanged with TN buffer (50 mM Tris [pH 7.6], 100 mM NaCl). The 146S virus particles were quantified at 259 nm using a spectrophotometer [9].

2.3. Immunization of pigs by experimental vaccines

The purified 146S antigen was mixed with Montanide ISA 206 VG (Seppic, Paris, France), an oil adjuvant, at a mixing ratio of 1:1 to make vaccines in the form of water in oil in water, and the vaccines were kept refrigerated at 4 °C for 1 day. All experimental animals were cared for according to the animal management guidelines of the QIA. The two or five of 3-month-old pigs with FMD antibody-free per group were vaccinated intramuscularly in the neck with an experimental vaccine containing the AD-P strain or the commercial vaccine (Merial Co. Ltd, Pirbright, UK) containing the O Manisa strain. For the negative control, PBS mixed with the adjuvant through the same method was used. We designed experiment 1 (2, 5, 20 μg per dose) for the immunity level (Supplement Table 1) and experiment 2 (7.5, 10, 15 μg per dose) for immunity and challenge (Table 1). The virus challenge for the second group was conducted at 30 days after the first inoculation. The neutralizing antibody level for the cross VNT was tested using the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Animal Health Organization.

2.4. Challenge test of immunized pigs

The pigs of experiment 2 (7.5, 10, 15 μg per dose) were immunized with the experimental (AD-P) vaccine for 4 weeks. All pigs of the experiment were challenged with the virus O/Andong/SKR/2010 ($10^{5.0}$ $TCID_{50}$/0.1 ml) on each footpad (0.1 ml/animal) to identify protective effects with clinical signs (Table 1 and Fig. 1) at 30 days post vaccination (DPV). To identify protective effects to the heterologous virus, the 10-μg group of the experiment was challenged with virus O/SKR/2002 ($10^{5.0}$ $TCID_{50}$/0.1 ml) of the ME–SA topotype on each footpad (0.1 ml/animal) at 49 DPV (Table 2 and Fig. 2). The pigs' clinical scores were based on the sum of each FMD lesion or sign (maximum score =16) according to the following parameters. Clinical score was determined by the addition of points distributed by the method of Alves et al. [10] as follows: an elevated body temperature of 40 °C (1 point), 40.5 (2 points), or 41 (3 points); reduced appetite (1 point), or no food intake and food left over from the day before (2 points); lameness (1 point) or reluctance to stand (2 points); presence of heat and pain after palpation of the coronary band (1 point), or not standing on the affected foot (2 points); vesicles on the feet, dependent on the number of feet affected and with a maximum of 4 points; visible mouth lesions on the tongue (1 point), gums, or lips (1 point) or snout (1 point), with a maximum of 3 points. The scores of each pig were recorded daily. After the challenge inoculation, nasal discharges and serum were monitored for 10–13 days by collecting them at one-day intervals; viruses were detected using real-time RT-PCR [11].

PrioCHECK FMDV NSP (Prionics AG, Schlieren-Zurich, Switzerland), an ELISA for the detection of FMD virus non-structural protein (NSP) antibodies in serum samples of pigs, was employed to detect NSP antibodies.

2.5. Sequence analysis

Viral RNA extraction was performed using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). cDNA was synthesized with SuperScript III™ (Invitrogen, Karlsruhe, Germany) and amplified by a Phusion™ Hot Start (Thermo) kit. The complete genome was sequenced by a sequencing service company (Macrogen Corporation, Seoul, Korea) using sequencing primers based on GenBank