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Application of mouse model for effective evaluation of foot-and-mouth disease vaccine ☆

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ABSTRACT

Efficacy evaluation of foot-and-mouth disease (FMD) vaccines has been conducted in target animals such as cows and pigs. In particular, handling FMD virus requires a high level of biosafety management and facilities to contain the virulent viruses. The lack of a laboratory animal model has resulted in inconvenience when it comes to using target animals for vaccine evaluation, bringing about increased cost, time and labor for the experiments. The FMD mouse model has been studied, but most FMD virus (FMDV) strains are not known to cause disease in adult mice. In the present study, we created a series of challenge viruses that are lethal to adult C57BL/6 mice. FMDV types O, A, and Asia1, which are related to frequent FMD outbreaks, were adapted for mice and the pathogenesis of each virus was evaluated in the mouse model. Challenge experiments after vaccination using in-house and commercial vaccines demonstrated vaccine-mediated protection in a dose-dependent manner. In conclusion, we propose that FMD vaccine evaluation should be carried out using mouse-adapted challenge viruses as a swift, effective efficacy test of experimental or commercial vaccines.

1. Introduction

Foot-and-mouth disease virus (FMDV) is a member of the family Picornaviridae, genus *Aphthovirus*. The virus is highly contagious and infects cloven-hoofed animals all over the world [1]. FMDV outbreak causes enormous economic damage by infecting a broad range of farm animals [2–5]. There are seven serotypes of FMDV, namely serotypes A, O, C, Asia1, South African Territories (SAT) 1, SAT2, and SAT3. The seven serotypes of FMDV do not have cross-protection against one another. In addition, each serotypes has numerous subtypes [6]. Hence, in countries where FMD occurs in various districts, it is difficult to select FMD vaccines that will be effective across these regions [7,8].

Mice are often employed as an animal model instead of natural hosts for various virus studies such as those related to Ebola virus, hanta virus, Dengue virus, and herpes simplex virus [9–12]. Vaccine evaluation in a mouse model is advantageous for cutting cost and time by measuring the immune responses induced by vaccines before testing them in host animals. The pathogenesis of FMDV in mice depends on the animal strain, the route of virus challenge, and the features of the virus [13]. According to recent studies [13], C57BL/6 mice are sensitive to artificial FMDV infection and only a few FMDVs, such as C1 C-S8c1, SAT1, Asia1 Shamir, and A/Arg/01, can cause 100% fatality in the mice.

The use of laboratory animal models to evaluate vaccine candidates can save cost and time, although the use of small laboratory animals to assess FMD vaccines could raise concerns on a relation of result between target and laboratory animals. Thus, there is a need to improve the evaluation in a convenient and effective way. It is crucial to continue performing such experiments to prove and develop evaluation approaches. In FMD endemic countries or countries at risk of FMD outbreak, swift evaluation of FMD vaccines against existing or potential field viruses is critical to control the disease. The aim of this study is to develop pathogenic FMDV O, A, Asia1 serotypes in C57BL/6 mice and demonstrate the vaccine evaluation technique used to assess the response of challenge viruses after immunization.

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2. Materials and methods

2.1. Cells, viruses, and animals

The fetal goat tongue epithelial ZZ-R 127 (ZZ-R) cell line was maintained in Dulbecco's modified Eagle's medium F-12 (Corning) with 10% fetal bovine serum (FBS) and supplemented with 1% antibiotics [14]. Cells were grown at 37 °C under 5% CO_2 in humidified conditions. Viruses like O/Jincheon/SKR/2014 (GenBank KX162590, Mya-98 lineage of the SEA topotype) of FMDV serotype O, which was prevalent in Republic of Korea in 2014; A Malaysia 97 (GenBank KJ933864, Sea-97 lineage of the ASIA topotype) used frequently in Southeast Asia, which is considered to have a medium priority of vaccine recommendation from the World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD, Pirbright, UK) [15]; and Asia1/MOG/05 (GenBank EF614458, geno-group V in the Asia region), which was prevalent in East Asia in 2005–2009, were used. O Jincheon/SKR/2014 was isolated in Jincheon, Chungbuk province in 2014 during the FMD outbreak in Korea. A Malaysia 97 was provided by the WRLFMD. Finally, the Asia1 MOG/2005 virus was kindly provided by the Mongolian government. Eight-week-old female C57BL/6 mice were purchased from KOSA BIO (Korea); animals were kept in a biosafety level 3 (BSL3) facility. All mouse experiments were performed in accordance with the ethical guidelines of the animal welfare committee of the Animal and Plant Quarantine Agency.

2.2. In vivo and in vitro passage of FMDVs

The O Jincheon/SKR/2014, A Malaysia 97, and Asia1 MOG/2005 viruses were passaged three times in ZZ-R cells, and $10^{5.0}$ $TCID_{50}$/0.1 ml of the viruses were inoculated intraperitoneally (IP) in 8-week-old C57BL/6 mice ($n = 2$). The serum was collected at 2 days post infection (dpi) and filtered through a 0.2 μm syringe filter (Pall Corporation, USA). The filtered serum was inoculated back to ZZ-R cells for two passages. A cycle of *in vivo* and *in vitro* passages was repeated five times (Fig. 1). After the final passage in ZZ-R cells, the viruses were collected and labeled O Jincheon-M5Z10, A Malaysia97-M5Z10, and Asia1 MOG/05-M5Z10.

2.3. Comparison of virus replication

The ZZ-R cells were prepared in 12-well plates at 2×10^5/well; this was done 16 h prior to the virus infection. ZZ-R cells were washed twice with phosphate-buffered saline (PBS), and inoculated with each virus, namely O Jincheon/SKR/2014, O Jincheon-M5Z10, A Malaysia97, A Malaysia97-M5Z10, Asia1 MOG/05, and Asia1 MOG/05-M5Z10, at a multiplicity of infection (MOI) of 0.1. After 1 h of adsorption in a 37 °C incubator, cells were washed with PBS, and incubated in DMEM F-12 medium (Corning; with 2% FBS and supplemented with 1% antibiotics) for 24 h. The supernatants were taken at 0, 2, 4, 6, 8, 10, 12, and 24 h and stored at −70 °C. Virus RNA was harvested from the supernatant using the MagNA pure 96 instrument (Roche, Switzerland) and the virus RNA copy number was determined using a real-time reverse transcription polymerase chain reaction (RT-PCR) kit (Bioneer, Korea) and Bio Rad CFX96 Real-Time PCR Machine (Bio Rad, USA). Primers targeting the 3D region of FMDV genome were forward 5′-GGAA CYGGGTTTTAYAAACCTGTRAT-3′ and reverse 5′-CCTCTCCTTTGCAC GCCGTGGGA-3′. The probe was 5′-CCCADCGCAGGTAAAGY GATCTGTA-3′ and its 5′ end or 3′ end was labeled with 6-FAM or TAMRA [16].

2.4. Pathogenesis in mice and sequence analyses of mouse-adapted viruses

Groups of 8-week-old C57BL/6 mice ($n = 4$) were challenged IP with $10^{1.0}$–$10^{5.0}$ $TCID_{50}$/0.1 ml of each mouse-adapted virus to observe the pathogenesis of viruses. After challenge with serial-diluted viruses, the survival rate of the mice were monitored daily for 8 days, and the 100 median lethal dose (LD_{50}) of each mouse-adapted virus was determined from the 8-day survival data. Viral RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). Viral RNA amplification was performed using the Qiagen OneStep RT-PCR Kit (Qiagen, Valencia, CA, USA) to amplify the coding sequence of viruses; sequencing was carried out at Macrogen (Korea) based on known sequence information (type O, GenBank KX162590; type A, GenBank KJ933864; type Asia1, GenBank EF614458). Nucleotide sequences encoding structural and non-structural proteins of the adapted viruses were compared to those of their parental viruses.

2.5. Efficacy evaluation of experimental vaccines

To create a chimeric virus with powerful antigenicity for a broad spectrum sub- or serotypes of FMDV, P1 or VP1 of O1 Manisa was replaced with that of other sub- or serotypes of FMDV (Supplementary Fig. 1). Moreover, O1 maAD was used as an antigen for type O (only the VP1 sequence of O1 Manisa was replaced with that of O/Andong/SKR/2010); O1 maPoc-A22 (the VP4, VP2, and VP3 sequences of O1 Manisa were replaced with those of A/Pocheon/SKR/2010; only the VP1 sequence of O1 Manisa was replaced with that of A22 Iraq) was used for type A; and O1 maASM-Shamir (the VP4, VP2, and VP3 sequences of O1 Manisa were replaced with those of Asia1 MOG/05; only the VP1 sequence of O1 Manisa was replaced with that of Asia1 Shamir) was used for type Asia1. The target genes were amplified through PCR amplification from the RNA of target viruses (type O, AY593823, KC503937; type A, KC588943, AY593762; type Asia1, EF614458, JF739177) and re-cloned into infectious cDNA clones through ligation of the vector and insert amplified using PCR [17].

The inactivated antigens of three viruses were produced as experimental vaccines after mixing with ISA 201 adjuvant as described previously [18]. Groups of 8-week-old C57BL/6 mice ($n = 8$) were inoculated intramuscularly with 0.5 μg/0.1 ml of inactivated vaccines (type O, A, Asia1). Three groups of mice ($n = 4$) were used as the non-vaccinated controls. Four weeks after vaccination, the vaccinated and non-vaccinated groups were challenged IP with 100 LD_{50} of mouse-adapted challenge viruses, namely O Jincheon-M5Z10, Malaysia97-M5Z10, and Asia1 MOG/05-M5Z10. After the virus challenge, mice were examined daily for survival and body weight was measured for 8 days. The sera were collected at 0, 3, and 7 dpi and tested for type-specific antibody response (the value presents percent inhibition) to type O, A, Asia1 using PrioCHECK Kit (Prionics AG, Switzerland) in accordance with the manufacturer's protocol. The percentage inhibition (PI) of the reference sera and the test sera were calculated according to suggested formula. The cutoff percentage of PI for positive reaction is 50 or higher. Virus genome in the blood was detected using real-time RT-PCR from the sera collected on 3 dpi, as previously described [16].

2.6. Efficacy evaluation of commercial vaccines

Groups of 8-week-old C57BL/6 mice ($n = 5$) were vaccinated intramuscularly with commercial monovalent vaccines, specifically O1 Manisa and O 3039 (Aftopor, Merial, UK). The 1/20 (0.1 ml of the recommended dose [2 ml] for cattle) or 1/200 (0.01 ml) vaccine for administered to mice via the intramuscular

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