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Short communication

Construction of stabilized and tagged foot-and-mouth disease virus

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Foot-and-mouth disease (FMD) is a highly contagious and economically devastating disease that affects cloven-hoofed animals worldwide. Construction and purification of stable antigen for vaccine are necessary but technically difficult and laborious. Here, we have tried to investigate an alternative method by inserting a hexa-histidine tag (6xHIS) in the VP1 C-terminal for easy purification and replacing two amino acids of VP1/VP2 to enhance the stability of the capsid of the FMD virus (FMDV) Asia1/MOG/05. In addition, infectious 6xHIS-tagged stable (S/T) FMDVs were maintained under acidic conditions (pH 6.0) and were readily purified from small-scale cultures using a commercial metal-affinity column. The groups vaccinated with the S/T FMDV antigen showed complete protection comparing to low survival rate in the group vaccinated with non-S/T FMDV against lethal challenge with Asia1 Shamir in mice. Therefore, the present findings indicate that the stabilized and tagged antigen offers an alternative to using the current methods for antigen purification and enhancement of stability and has potential for the development of a new FMD vaccine.

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Foot-and-mouth disease (FMD) is a highly contagious disease that affects cloven-hoofed animals, such as cattle, sheep, deer, and pigs. FMD can result in economically devastating losses to farms that breed susceptible animals (Alexandersen et al., 2003). The FMD virus (FMDV) can be classified serologically into seven serotypes: O, A, C, Asia1, South-African Territories (SAT)1, SAT2, and SAT3. Its viral genome consists of structural proteins (VP1, VP2, VP3, and VP4) and nonstructural proteins (L, 2A, 2B, 2C, 3A, 3B, 3C, and 3D) (Longjam et al., 2011; Ryan et al., 1989). VP4 is located inside the capsid, whereas VP1, VP2, and VP3 are present on the surface of the capsid (Logan et al., 1993). The FMDV capsid is made up of 60 copies of the four viral structural proteins (Acharya et al., 1989). The structural proteins form a single protomer, and five promoters form one pentamer; finally, 12 pentamers assemble to form the capsid structure of a complete icosahedral lattice that contains the RNA (Grubman and Baxt, 2004). This viral capsid particle is the most effective antigen known that can be used as a vaccine. However, the viral particle is easily degraded at a pH of 6.8 or lower, and therefore, a stable vaccine manufacturing process and antigens are required (Park, 2013).

The hexa-histidine (6xHIS) tag is being studied in various areas as an efficient tool for general protein purification (Arnau et al., 2006). Immobilized metal-affinity chromatography for the 6xHIS tag can be used to easily and quickly purify recombinant proteins and antigenic peptides (Crowe et al., 1995). Furthermore, the expression of recombinant proteins can be verified with a commercial specific anti-His antibody (Schmitt et al., 1993). The 6xHIS-tagging is a versatile method that can be used to establish a vaccine for distinguishing between infected and vaccinated animals using ELISA (Enzyme-linked immunosorbent assay) (Bhat et al., 2013; Mezencio et al., 1998; van Oirschot et al., 1986).

We produced a backbone of recombinant FMDV that is stable in acidic conditions by replacing amino acids in VP1 (N17D) and VP2 (H145Y) and inserting a 6xHIS tag at the VP1/2A cleavage site. Resistance to acidic conditions was induced in the candidate vaccine viruses that reacted to the anti-6x His antibody by the replacement of a single amino acid of VP1 and VP2. Laboratory animals were vaccinated with the purified recombinant protein to ensure protection, and an experiment was conducted to investigate its potential as a new vaccine that is easy to handle at the processing stage.

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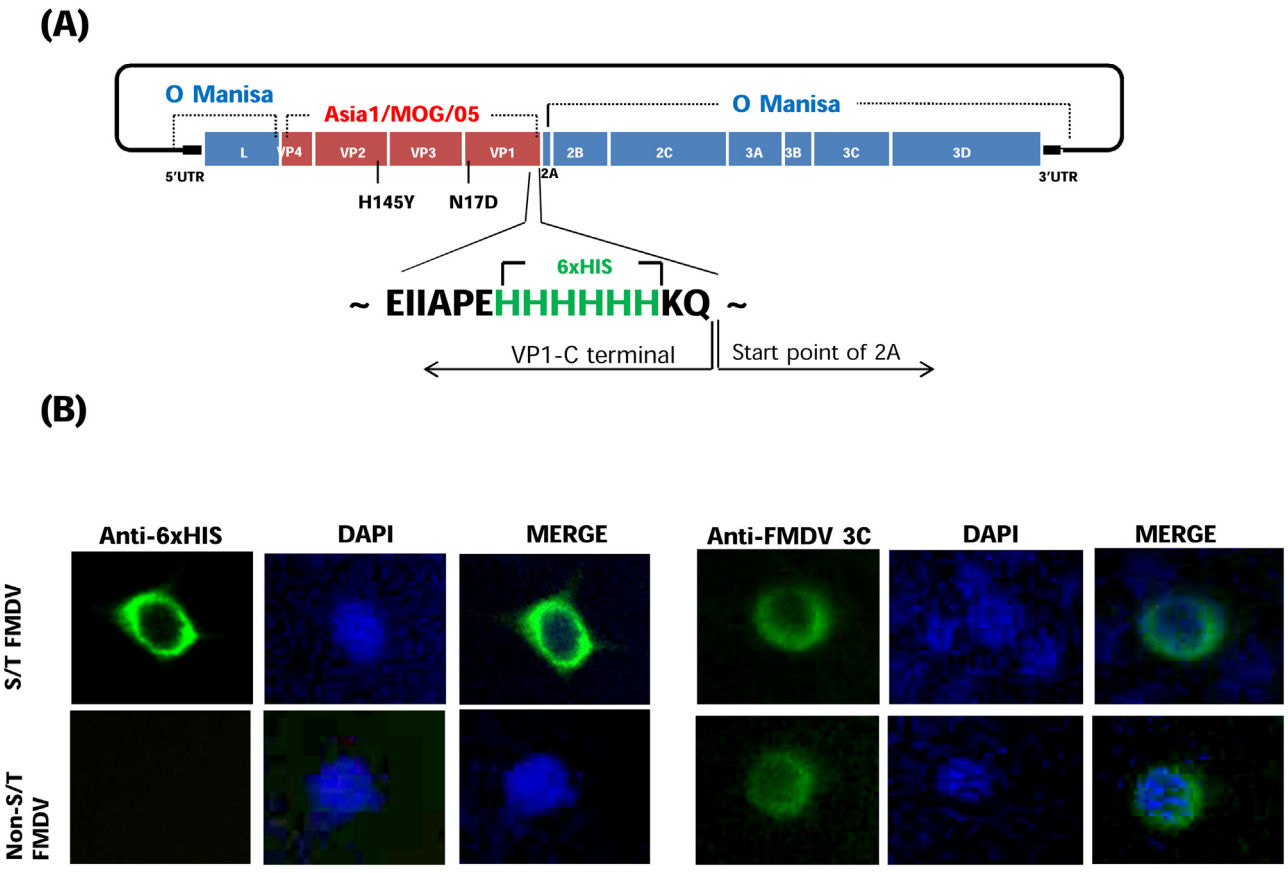

Fig. 1. Schematic representation of the stable/tagged-FMDV clone and expression of the 6xHIS tag on the FMDV. (A) Strategy for the replacement or insertion of Asia1/MOG/05 P1 (red) and the 6xHIS tag into O Manisa (blue) in the pBluescript vector. The inserted amino acid residues (hexa-histidine tag) were located within the VP1 site of the FMDV genome. (B) Detection of the stable/tagged (S/T) or O1m_AsM P1 (non-S/T) FMDV in infected ZZ-R cells by indirect immunofluorescence (IF) assay. Left panel: reaction with the anti-HIS antibody, Right panel: reaction with the anti-FMDV-3C antibody. Nuclei were stained with DAPI (blue) and observed under a fluorescence microscope. Abbreviations: FMDV, foot-and-mouth disease virus; MOG, Mongolia; 6xHIS, hexa-histidine tag; ZZ-R, ZZ-R 127 (fetal goat tongue epithelial) cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The full genome of the O/Manisa/Turkey/69 (O Manisa) strain (GenBank No. AY593823) was cloned into a plasmid vector (Kim et al., 2015), and the P1 gene of O/Manisa/Turkey/69 replace with the corresponding region of Asia1/MOG/2005 (GenBank EF614458) through PCR using Phusion® High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Vantaa, Finland) according to the manufacturer's instructions (Fig. 1) based on the sequence of the Asia1/MOG/05 (AsM) strain (GenBank No. EF614458). The sequence encoded 6xHIS tag in the 3′ terminal of VP1 was inserted into the infectious clone using a mutagenesis kit (Takara, Tokyo, Japan). The plasmid vectors, O1m_AsM P1_VP1 N17D, VP2 H145Y_6H (Stable/Tagged (S/T) FMDV), O1m_AsM P1_VP1 N17D, VP2 H145Y (Stable (S) FMDV) or O1m_AsM P1 (non-S/T FMDV) was transfected into BHK/T9-7 cells expressing T7-RNA polymerase (Ito et al., 2003) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

To detect the 6xHIS tag on FMDV, ZZ-R 127 cells (fetal goat tongue epithelial cell, ZZ-R) were infected with S/T FMDV or S FMDV at a multiplicity of infection (MOI) of 0.5. After 5 h, the cells were fixed in 4% formaldehyde, permeabilized in 0.1% Triton X-100 in phosphate-buffered saline (PBS), washed with PBS, and blocked with 3% bovine serum albumin in PBS. The cells were reacted with a 1:500 dilution of monoclonal anti-6xHIS (Abcam, Cambridge, UK) or rabbit anti-FMDV 3C antibodies and a 1:1000 dilution of FITC-conjugated goat anti-mouse (or rabbit) antibody (KPL, Gaithersburg, MD, USA). Nuclei were then stained with DAPI and observed under a fluorescence microscope.

To determine the stability of FMDV in response to acid treatment, a previously reported protocol was followed (Martin-Acebes et al., 2011). Briefly, the virus was incubated with 30 vols of PBS containing 50 mM $NaPO_4$ and 140 mM NaCl (pH 5.5, pH 6.0, and pH 7.4) for 30 min at room temperature. Two volumes of Tris solution (1 M, pH 7.6) of the virus was then added for neutralization.

To purify a virus particle antigen, a 175-cm^2 T-flask of ZZ-R cells was infected with FMDVs used in this study at an MOI of 0.02. After 24 h, cytopathic effect was observed, and the cells were freeze-thawed and centrifuged to remove cell debris. The viruses (S/T FMDV, S FMDV and non-S/T FMDV) were inactivated with 0.003N of binary ethyleneimine for 24 h, and concentrated with polyethylene glycol-6000 and after centrifugation, sedimented through 15–45% sucrose gradient in an ultracentrifuge (Park et al., 2014a) or separated by the PrepEase His-Tagged Protein Purification Midi kit (Affymetrix, Santa Clara, CA, USA). The antigens were washed three times with the TN buffer (50 mM Tris [pH 7.6], 100 mM NaCl) using Amicon ultracentrifugation filter devices (Millipore, Darmstadt, Germany). The proteins were loaded on a 12% bis-Tris Gel (Novex, San Diego, CA, USA) and transferred onto a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, USA) by a Trans-Blot Turbo machine (Bio-Rad). The membrane was blocked with 5% skimmed milk in Tris-buffered saline (50 mM Tris and 150 mM NaCl [pH 7.6]) containing 0.1% Tween 20 and then incubated with 1:500 dilutions of rabbit anti-FMDV Asia1-VP1 polyclonal antibody and mouse anti-6xHIS antibody (Abcam, Cambridge, UK). The bound primary antibodies were detected by the 1:1000 dilution of appro-

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