Engineering Foot-and-Mouth Disease Virus with Improved Properties for the Development of Effective Vaccine

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Introduction:
To improve properties of the candidate vaccine strain of foot-and-mouth disease viruses for the development of effective vaccine and marker vaccine, such as growth properties, increasing the biological safety, longer duration of protection, faster onset of protection, better discrimination of vaccinated animals that go on to become infected, safer to make and easier to administer, and so on, using reverse genetics technology, we finished the fellow works, the following are progress reports of presentation.

Materials and methods:
Construction of the chimeric candidate vaccine strain by a novel plasmid-based reverse genetics system. Evaluation of the growth properties of the chimeric strain by TCID$_{50}$ and the growth curve. Evaluation of immune responses in cattle after vaccination by liquid-phase ELISA (lpELISA) and 3ABC-ELISA. Evaluation of the vaccine matching by virus neutralization test (VNT) and liquid-phase ELISA (lpELISA). Evaluation of cattle protection experiment by challenging with the field strain.

Results:
Successfully developed some novel reverse genetic systems (RGS) for FMDV. One of these systems is a plasmid expressing infective FMDV in vivo, the results shown that we exploited reverse genetics is efficient for various virus rescue. Importantly, the virus can be recovered from model and host animal directly injected with the plasmid, which implied to fit to rescue the viruses with lack of a suitable cell culture system. Used this RGS, we finished the fellow works:

1. Engineering Foot-and-Mouth Disease Viruses with Improved Growth and Protective Potency for Vaccine

   No licensed vaccine is currently available against serotype A foot-and-
mouth disease (FMD) in China, despite the isolation of A/WH/CHA/09 in 2009, partly because this strain does not replicate well in baby hamster kidney (BHK) cells. A novel plasmid-based reverse genetics system was used to construct a chimeric strain (rA/P1-FMDV) by replacing the P1 gene in the vaccine strain O/CHA/99 with that from the epidemic strain A/WH/CHA/09. The chimeric virus displayed growth kinetics similar to those of O/CHA/99 and was selected for use as a candidate vaccine strain after 12 passages in BHK cells. Cattle were vaccinated with the inactivated vaccine and humoral immune responses were induced in most of the animals on day 7. A challenge infection with A/WH/CHA/09 on day 28 indicated that the group given a 2.6-µg dose was fully protected and neither developed viremia nor seroconverted to a 3ABC antigen. These results suggest that reverse genetics technology is a useful tool for engineering vaccines for the prevention and control of FMD.

**Cross-Protective Efficacy of Engineering Serotype A Foot-and-Mouth Disease Virus Vaccine against the Two Pandemic Strains in Swine**

The FMD virus (A/GDMM/CHA/2013) from China’s Guangdong province (2013) was responsible for this new incursion that has been characterized as belonging to the Sea-97 genotype of ASIA topotype, which has lower amino acid identity (93.9%) in VP1 with the epidemic strain A/WH/CHA/09 from Wuhan, China in 2009. Therefore, the application of a new vaccine strain with cross-protective efficacy is of fundamental importance to control the spread of the two described pandemic strains. A chimeric strain rA/P1-FMDV which was constructed by our lab previously through replacing the P1 gene in the vaccine strain O/CHA/99 with that from the epidemic strain A/WH/CHA/09, has been demonstrated to exhibit better growth characteristics in culture, and the rA/P1-FMDV inactivated vaccine can provide greater protection against epidemic strain A/WH/CHA/09 in cattle described above. However, it is still unclear whether the vaccine produces efficient protection against the new pandemic strain (A/GDMM/CHA/2013). Here, vaccine matching and pig 50% protective dose (PD$_{50}$) tests were performed to assess the potency of the vaccine. The vaccine matching showed cross-reactivity of sera from chimera-vaccinated pigs with A/WH/CHA/09 and A/GDMM/CHA/2013, with r values of 1.0 and 0.7, which indicates that the rA/P1-FMDV vaccine is likely to confer higher cross-protection against the two isolates. When challenged with two pandemic isolates, the vaccine achieved 13.8 PD$_{50}$ (challenged with
A/WH/CHA/09 strain) and 10.81 PD50 (challenged with A/GDMM/CHA/2013 strain) per dose (2.8μg). The results indicated that the rA/P1-FMDV inactivated vaccine could protect pigs against both A/WH/CHA/09 and A/GDMM/CHA/2013 pandemic isolates.

The recombinant vaccine has been authorized to manufacture by Ministry of Agriculture in China.

2. Engineering no pathogenic Foot-and-Mouth Disease SAP-mutant virus with higher level of biosecurity is potential vaccine candidate strain

Construction of the Foot-and-Mouth Disease SAP-mutant virus strain by a novel plasmid-based reverse genetics system. Evaluation of the growth properties of the strains in BHK-21, SK6 and BTY cells. Evaluation of the Pathogenic characteristics of the strains in pigs and cattle. Immune responses in cattle after vaccination by liquid-phase ELISA (lpELISA) and 3ABC-ELISA. Evaluation of the vaccine matching by virus neutralization test (VNT) and liquid-phase ELISA (lpELISA). Evaluation of pig and cattle protection experiment (PD50 test) by challenging with the field strain. The vaccine induces early immune responses and protection against disease.

3. Developing marker vaccine to distinguish infection and vaccination (DIVI)

Based on the Foot-and-Mouth Disease SAP-mutant virus strain, construction of vaccine strain bearing negative marker (The antigen site of the recombinant vaccine strain will be changed into negative site that the strain do not produce the mAb against the antigen site , however all field strains can produce the mAb in host ). Evaluation of the growth properties of the strains in BHK-21, SK6 and BTY cells. Evaluation of the Pathogenic characteristics of the strains in pigs and cattle. Immune responses in cattle after vaccination by liquid-phase ELISA (lpELISA) and 3ABC-ELISA. Evaluation of the vaccine matching by virus neutralization test (VNT) and liquid-phase ELISA (lpELISA). Evaluation of cattle protection experiment by challenging with the field strain. Established the ELISA to distinguish infection and vaccination based on the mAb

4. The development of effective vaccine against A/Iran-05,
O/MESA/PanAsia-2 and Asia1GVII trains

A/Iran-05, O/MESA/PanAsia-2 and Asia1 GVII trains new threatened strains are widespread circulation, and several reports in 2011 associated with poor laboratory vaccine matching this is being closely monitored by OIE FMD reference laboratory at Pirbright. Construction of the A/Iran-05, O/MESA/PanAsia-2 and Asia1GVII chimeric strain by a novel plasmid-based reverse genetics system. Evaluation of the growth properties of the chimeric strains. Immune responses in cattle after vaccination by liquid-phase ELISA (lpELISA) and 3ABC-ELISA. Evaluation of the vaccine matching by virus neutralization test (VNT) and liquid-phase ELISA (lpELISA). Evaluation of pig and cattle protection experiment by challenging with the field strain.

Discussion:
we have gotten some engineering foot-and-mouth disease candidate vaccine strain with improved properties for the development of effective vaccine, these results shown that reverse genetics technology is a useful tool to engineer a vaccine candidate for FMD prevention and control.
**Biography** Haixue Zheng, PhD, professor,

Dr. Haixue Zheng is the head of Viral Gene Engineering Research Group, National FMD reference laboratory, State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (CAAS).

September 2004 – June 2007, he studied in the postgraduate school, Chinese Academy of Agriculture Science (CAAS), He obtained PhD on “Development in reverse genetics system for recovery of animal RNA viruses” in 2007.

May 2008 to February 2009, as a visiting scientist, he worked for Pirbright Laboratory, Institute for Animal Health. Main research was focused on molecular pathogenesis of foot-and-mouth disease virus.

May 2009 to present, he is the head of Viral Gene Engineering Research Group, National FMD reference laboratory, State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, CAAS. Main research was focused on revealing the rule of phenotypic variation and its molecular basis of foot-and-mouth disease virus (FMDV), engineering foot-and-mouth disease viruses with improved properties for vaccine and marked vaccine development, clarifying molecular basis underlying host tropism variation of FMDV and mechanisms of innate immune. In recent five years, obtained 6 national patents for invention, published 10 SCI papers and more than 40 chinese papers, participated to edit 3 new books, obtained 3 items of new veterinary drug licenses.