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Short communication

Preserved immunogenicity of an inactivated vaccine based on foot-and-mouth disease virus particles with improved stability

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ABSTRACT

Foot-and-mouth disease virus (FMDV) is the etiological agent of a highly contagious disease that affects important livestock species. Vaccines based on inactivated FMDV virions provide a useful tool for the control of this pathogen. However, long term storage at 4 °C (the temperature for vaccine storage) or ruptures of the cold chain, provoke the dissociation of virions, reducing the immunogenicity of the vaccine. An FMDV mutant carrying amino acid replacements VP1 N17D and VP2 H145Y isolated previously rendered virions with increased resistance to dissociation at 4 °C. We have evaluated the immunogenicity in swine (a natural FMDV host) of a chemically inactivated vaccine based on this mutant. The presence of these amino acid substitutions did not compromise the immunological potential, including its ability to elicit neutralizing antibodies. These results support the feasibility of this kind of mutants with increased capsid stability as suitable viruses for producing improved FMDV vaccines.

1. Introduction

The picornavirus foot-and-mouth disease virus (FMDV) is the etiological agent of a highly contagious disease that affects cloven-hoofed animals, including important livestock species such as swine, cattle, sheep and goat (Sobrino and Domingo, 2017). Nowadays, vaccines based on chemically inactivated FMDV virions are the choice for FMD control (Cao et al., 2016; Diaz-San Segundo et al., 2016; Rodriguez and Gay, 2014). These vaccines are mainly produced by infection of cultured cells and inactivation of the virus with binary ethylenimine (BEI) (Bahnemann, 1975). FMDV virions are composed of 60 copies of each of the four structural proteins (VP1-VP4) arranged into 12 pentameric subunits. Remarkably, FMDV virions are extremely labile to environmental factors such as acidic pH and temperature. Thus, acidification, long term storage (at low temperature, 4 °C) or rupture of this cold chain provoke dissociation of FMDV virions reducing the immunogenicity of the vaccine (Harmsen et al., 2011, 2015; Rao et al., 1994; Wild and Brown, 1968). Moreover, the concurrency of climate and socioeconomic factors in many areas where FMDV is circulating often results in ruptures of the cold chain and failures of the vaccine to confer protection. Therefore, improving the stability of FMDV particles used for vaccine formulation is of crucial interest to develop better FMDV vaccines (Kotecha et al., 2015; Porta et al., 2013; Rincon et al., 2014).

We have previously reported the isolation of a mutant FMDV (VP1 N17D + VP2 H145Y) that produced virions with increased resistance to acidic pH that were also more resistant to dissociation at 4 °C, the temperature for vaccine storage (Vazquez-Calvo et al., 2014). Interestingly, these results obtained with type C FMDV, have been independently extended by other authors to other serotypes (Liang et al., 2014; Park et al., 2016; Wang et al., 2014). Considering that virions carrying these amino acid substitutions provide a unique opportunity for the stabilization of multiple FMDVs, we have explored the suitability of mutant VP1 N17D + VP2 H145Y as seeding virus for the production of an improved chemically-inactivated vaccine.

2. Results and discussion

The antigenicity of mutant VP1 N17D + VP2 H145Y was investigated performing neutralization assays with different monoclonal antibodies (MAbs SD6 and 5C4) directed against two representative epitopes located at two major antigenic sites (sites A and D, respec-

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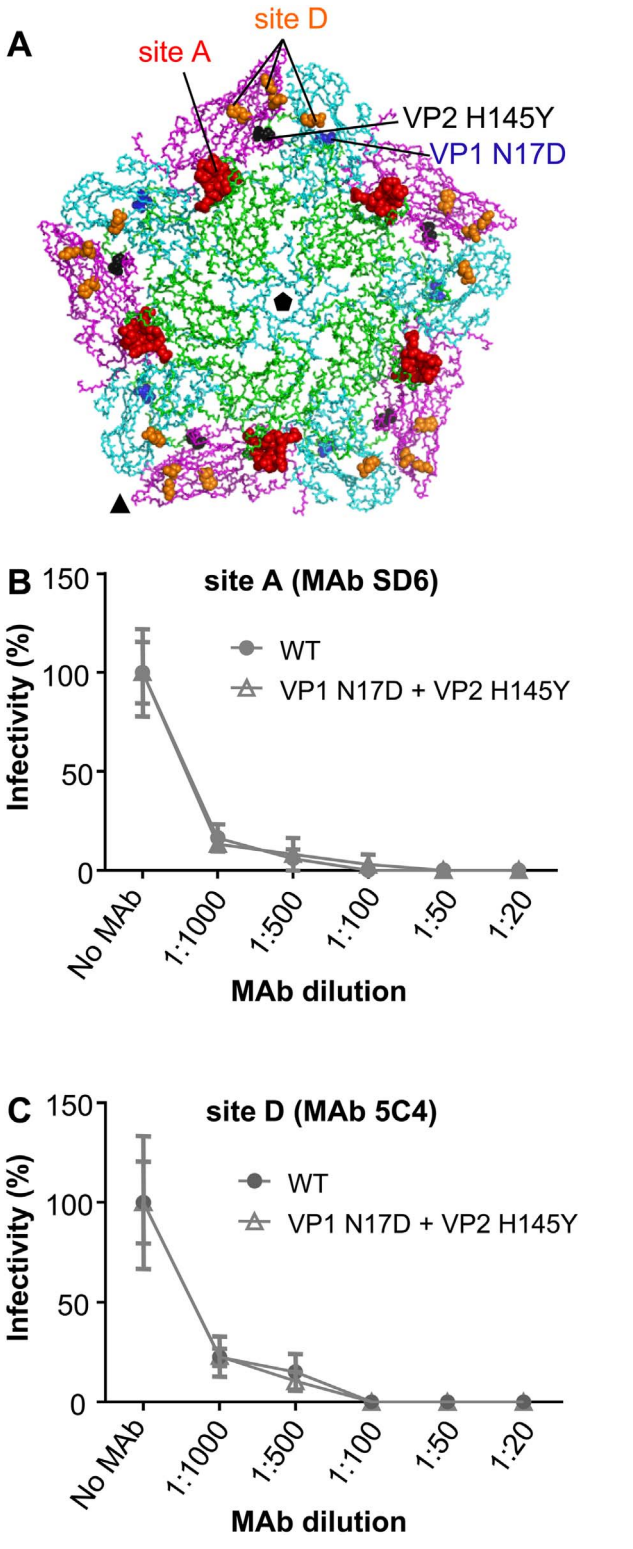
tively) identified in the parental virus C-S8c1 (WT) (Fig. 1A) (Lea et al., 1994). In these experiments, mutant VP1 N17D + VP2 H145Y was neutralized in a manner similar to that of the WT (Fig. 1B and C). These results suggest that although the capsid stabilizing mutations map near these antigenic sites, they do not disturb the antigenicity of the virion.

Next, the immunogenicity of FMDV particles carrying amino acid replacements VP1 N17D + VP2 H145Y was evaluated in swine, an important natural host for FMDV. Animal experimentation was performed at the animal facilities of the Institute of Virology and Immunology (Mittelhausern, Switzerland) in compliance with the Swiss animal protection law and approved by the animal welfare committee of the canton of Berne, Switzerland (Authorization Number BE89/11). Ten pigs were randomly separated into two groups of 5 animals each, which were immunized intramuscularly with a 2.5 ml of a vaccine containing 10 μg of either BEI-inactivated purified WT or mutant VP1 N17D + VP2 H145Y virions emulsified with 15% Montanide ISA 25VG (kindly donated by Seppic, Puteaux, France). A scheme of the immunization schedule, sample collection and the performed analyses is shown in Fig. 2A. Procedures for virus production in BHK-21 cells and its inactivation with BEI and purification by sucrose gradient centrifugation have been previously described (Bahnemann, 1975; Martin-Acebes et al., 2011). Animals were boosted with the same vaccine dose at 28 days post-immunization and blood samples were collected at 7 days intervals throughout the experiment. High levels of circulating neutralizing antibodies correlate well with the protection elicited by FMDV vaccines in swine (Doel, 2005; Sobrino and Domingo, 2017). Thus, neutralizing antibody titers were determined by a micro-neutralization assay in BHK-21 cells in accordance to the standardized protocol recommended by the OIE (OIE, 2012) (Fig. 2B). Most of the animals inoculated with each of the vaccines elicited undetectable titers of neutralizing antibodies 7 days after the first immunization. Within the next three weeks (days 14, 21 and 28 post-immunization) all the animals reached detectable levels of neutralizing antibodies. After the second dose of vaccine (day 28 post-immunization), neutralizing antibody titers increased in all pigs, ranged for most of the animals between 64 and 128 (35 and 41 days post-immunization) and were maintained until the end of the experiment (70 days post-immunization). Interestingly, no statistically significant differences between the groups immunized with WT or mutant virions were found.

To further characterize the humoral response induced in vaccinated animals, the levels of specific anti-FMDV antibodies elicited after one (day 28 post-immunization) or two vaccine doses (day 70 post-immunization) were investigated. Total antibody titers were determined by end-point titration using an in-house developed sandwich ELISA and BEI-inactivated FMDV as viral antigen (Borrego et al., 2013). Total antibody levels were similar between both groups of vaccines, with most of the animals showing titers above 1000 after the second vaccine dose (Fig. 3A). Additionally, IgM were analyzed at 7, 14, 21 and 28 days post-vaccination (Fig. 3B). The only statistically significant differences were found at 14 days post-vaccination, being the levels of IgM induced by mutant virions lower than those of animals vaccinated with WT virions. Sera were also analyzed by means of a modified indirect double sandwich ELISA specific for the detection of IgG1 and IgG2 isotypes (Salt et al., 1996). The results obtained for IgG1 (Fig. 3C) were very similar to those observed for total antibodies (Fig. 3A). All the animals elicited detectable levels of IgG1 after the first vaccination that increased after the second dose administration. Regarding IgG2, most of the animals of the two groups were negative at 28 days post-immunization whereas all became positive after the second dose (70 days post-immunization), being the titers similar among animals vaccinated with either WT or mutant virions (Fig. 3D). Since mucosal immunity is important for protection against FMDV (Sobrino and Domingo, 2017), the presence of specific IgA in sera was also investigated. Whereas all the animals were negative for IgA after the first dose of the vaccine, most of the animals seroconverted after the second dose (Fig. 3E) and the differences observed between animals vaccinated with mutant virions or WT virions were not statistically significant. Overall, these results support that the introduction of amino acid replacements VP1 N17D + VP2 H145Y in the seeding virus utilized for vaccine production does not provoke major effects on the immunogenicity of a BEI-inactivated vaccine.

Considering the potential advantages of viruses with increased capsid stability for vaccine production, our results support that utilization of mutant such as VP1 N17D + VP2 H145Y could improve vaccine



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