Virology 518 (2018) 143–151

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

# Systemic antibodies administered by passive immunization prevent generalization of the infection by foot-and-mouth disease virus in cattle after oronasal challenge

Florencia Barrionuevo[a,b], Sebastián Di Giacomo[a], Danilo Bucafusco[a,b], Andrea Ayude[a], Juan Schammas[a], M. Cruz Miraglia[a,b], Alejandra Capozzo[a,b], Manuel V. Borca[c], Mariano Perez-Filgueira[a,b,*]

[a] Instituto de Virología, CICVyA, INTA, N Repetto y De Los Reseros s/n, Hurlingham (1686), Buenos Aires, Argentina
[b] CONICET, Godoy Cruz 2290 (C1425FQB), Buenos Aires, Argentina
[c] Plum Island Animal Disease Center, ARS, USDA, Greenport, NY, USA

ARTICLE INFO

*Keywords:*
Foot-and-mouth disease
Cattle
Protective immunity
Vaccines
Aerogenous infection

ABSTRACT

The role of passively transferred sera in the protection against aerogenous foot-and-mouth disease (FMD) virus infection in cattle was evaluated using vaccine-induced immune serum preparations obtained at 7 and 26 days post-vaccination (dpv). We showed that circulating antibodies were sufficient to prevent disease generalization after oronasal infection in animals passively transferred with 26-dpv serum but not with the 7-dpv serum. Conversely, conventional FMD vaccination provided clinical protection at 7 dpv, promoting fast and robust antibody responses upon challenge and even though antibody titers were similar to those found in animals passively immunized with 7-dpv serum. These results demonstrate that presence of antigen-specific antibodies is critical to prevent the dissemination of the virus within the animal. Conventional FMD vaccination additionally promoted the deployment of rapid, high titer and isotype-switched antibody responses at systemic and mucosal levels after infection, thus conferring protection even in the presence of low pre-challenge antibody titers.

## 1. Introduction

Foot and mouth disease (FMD) remains a major threat for livestock production and derived industries, affecting biungulate species worldwide. The devastating effects associated with the presence of FMD may be verified at different stages of the production chain: from smallholders, directly affected by the reduced productivity (Knight-Jones et al., 2017), to whole-country economies impacted by the domestic control measures and the severe restrictions imposed on international trade (Thompson et al., 2002). FMD's etiological agent is a small non-enveloped positive-sense single-stranded RNA virus (FMDV) belonging to the *Picornaviridae* family, genus *Aphthovirus*. The FMDV possesses the ability to infect a wide range of domestic and wildlife species (Alexandersen and Mowat, 2005) causing an acute, febrile and vesicular disease with an extremely high morbidity and a variable mortality rate usually restricted to young individuals (Gulbahar et al., 2007). The virus combines its high antigenic variability (Domingo et al., 2002) with an efficient transmission, making it highly contagious among susceptible individuals even from different species (Alexandersen et al., 2003).

Conventional vaccines comprising inactivated whole-FMDV particles as antigens and formulated in aqueous or oil vehicles containing different adjuvants (Doel, 2003) have been successfully used to control the disease in different regions of the world, including Europe (Sutmoller et al., 2003) and large areas of South-America (Mattion et al., 2004; Saraiva and Darsie, 2004). Yet, and in spite of being the first viral disease identified in animals (Brown, 2003), a number of FMD outbreaks have been reported worldwide in the last few years (Brito

*Abbreviations:* ANOVA, analysis of variance; ASC, antibody secretory cells; AUC, area under the curve; dpi, days post-infection; dpv, days post-vaccination; FMD, foot and mouth disease; FMDV, foot and mouth disease virus; HRP, horseradish peroxidase; LPBE, liquid-phase blocking enzyme-linked immunosorbent assay; MNC, mononuclear cell; MRL, medial retropharyngeal lymph nodes; Nab, neutralizing antibodies; OD, optical density; OIE, World Organisation for Animal Health; PBS, phosphate-buffered saline; PFU, plaque-forming units; RT, room temperature; SD, standard desviation; TBL, tracheobronchial lymph nodes; VNT, virus neutralization

* Corresponding author at: Instituto de Virología, CICVyA, INTA, N Repetto y De Los Reseros s/n, Hurlingham (1686), Buenos Aires, Argentina.
*E-mail addresses:* barrionuevo.f@inta.gob.ar (F. Barrionuevo), digiacomo.sebastian@inta.gob.ar (S. Di Giacomo), bucafusco.danilo@inta.gob.ar (D. Bucafusco), ayude.andrea@inta.gob.ar (A. Ayude), schammas.juan@inta.gob.ar (J. Schammas), miraglia.maria@inta.gob.ar (M.C. Miraglia), capozzo.alejandra@inta.gob.ar (A. Capozzo), Manuel.Borca@ars.usda.gov (M.V. Borca), perez.mariano@inta.gob.ar (M. Perez-Filgueira).

https://doi.org/10.1016/j.virol.2018.02.012
Received 8 January 2018; Received in revised form 8 February 2018; Accepted 12 February 2018

et al., 2017), not only in endemic settings but also in countries and regions which have been free of the disease for long periods of time (FAO, 2017). This reinforces the need for improved vaccines and control strategies fit-for-purpose according to the particular epidemiological scenarios (Robinson et al., 2016). However, the development of such new prophylactic tools requires a clear understanding of the immune mechanisms involved in protection in susceptible species.

We have previously described the onset of the mucosal and systemic immunity after FMDV aerosol infection in cattle (Pega et al., 2013). We found that mucosal responses begin as soon as 4 days post-infection (dpi), with systemic responses following a similar time-course and isotype profile pattern as the local ones. As a result of these robust and fast responses —mainly mediated by IgM antibodies— FMDV was cleared out of the blood circulation by day 5 after experimental aerosol infection. We also studied the development of mucosal responses in FMD-vaccinated cattle, before and after oronasal infection with a homologous virus strain (Pega et al., 2015). We found that besides systemic antibody responses, FMDV-specific antibody-secreting cells (ASC) could also be detected at lymph nodes draining the respiratory tract as early as 7 days after systemic vaccination by the intramuscular route. Aerosol challenge performed 30 days after vaccination elicited a boosted antibody reaction particularly evident at the local level, resulting in complete protection of the infected animals. These results emphasized the idea that systemic and mucosal responses occur in parallel in both FMDV-infected and vaccinated bovines, regardless of the initial route of contact with viral antigen. However, the relative importance in the generation of protective immunity of the different immune mechanisms involved could not be discerned in those experiments.

Here we initially evaluated the efficacy of passively transferred vaccine-induced circulating anti-FMDV antibodies in preventing the development of FMD in cattle after infection through the oronasal route. We used two different preparations of FMDV-specific immune serum, obtained at 7 and 26 days post-vaccination (dpv), differing in the immunoglobulin isotype composition and in total antibody titers. We compared these results with those of animals vaccinated and challenged at the same times post-vaccination and thus carrying similar titers and isotype profiles as those of the passively immunized steers.

Challenge results demonstrate that circulating antibodies are sufficient to prevent generalization of the disease after oronasal infection in animals passively transferred with 26-dpv serum but not with the 7-dpv serum. Conversely, conventional FMD vaccination afforded clinical protection even at 7 dpv, promoting the generation of fast and robust antibody responses upon aerogenous virus challenge. Thus, the absence of the antigenic priming induced by vaccination might explain the lack of protection observed in animals passively transferred with 7-dpv serum even though their circulating antibody titers were similar to those found in animals at day 7 post-vaccination. These results demonstrate that presence of antigen-specific antibodies is critical to prevent the dissemination of the virus within the animal. Further characterization of the post-challenge responses at both systemic and local levels also revealed that systemic immunization with conventional FMD vaccines promoted the deployment of rapid, high titer and isotype-switched protective antibody responses at systemic and mucosal levels after infection, which might be compatible with the generation of early anti-FMDV memory B-cells as discussed herein.

## 2. Materials and methods

### 2.1. Experimental animals

Ten calves (180–220 kg each, 6- to 8-months old) and eight Hereford steers (350–400 kg each, 24-months old) were purchased from a livestock breeder located in the province of Chubut (Patagonia Argentina), an FMDV-free region without vaccination. All animals were checked by liquid-phase blocking enzyme-linked immunosorbent assay (LPBE) for the absence of FMDV-specific antibodies upon their arrival to the experimental field of CICVyA-INTA. Experiments not including infected bovines were carried out at the CICVyA-INTA experimental field while those involving infected animals were performed at the BSL-4 OIE animal boxes facilities also located at the CICVyA. All assays were completed by following biosecurity and animal welfare internal and federal regulations and according to protocol 71/2015 approved by the Institutional Committee for Use and Care of Experimental Animals (CICUAE), CICVyA-INTA.

### 2.2. Vaccines and vaccinations

All vaccinated animals received one dose of a single-oil-emulsion monovalent vaccine produced by Biogénesis Bagó (Argentina) according to good manufacturing practices ($PD_{50}$ > 6) using inactivated FMDV O1/Campos/Brazil/58 (O1 Campos). Vaccine was controlled and approved by SENASA (Argentine Animal Health Authority) for safety, purity, and potency following local, OIE, and European Pharmacopeia standards.

### 2.3. Passive immunization

Blood (between 4.5 and 5 L/animal) from steers vaccinated with the monovalent FMD vaccine was collected at 7 dpv (n = 4) and 26 dpv (n = 4) using sterile bags containing anticoagulant solution with citrate, phosphate and dextrose (0.3% citric acid anhydrous, 2.63% sodium citrate dehydrate, 0.22% monosodium phosphate, 3.19% dextrose monohydrate and 0.027% adenine). Sterile serum fractions from each bag were obtained by centrifugation (1600 × *g* for 13 min). Sera from blood taken at 7 or 26 dpv were grouped in separate pools and parenterally transferred (~ 3 L/animal) through an intravenous catheter with an appropriate blood filter to naïve calves (n = 3 for each pool) previously sedated using xylazine 2% (0.075 mg/kg intramuscularly) to reduce stress and aid in catheter placement. The sera was maintained at ~ 37 °C using a warm water bath to avoid hypothermia and the initial rate of transference was ~ 3 mL/kg/h for the first 20 min and then up to 15 mL/kg/h (Balcomb and Foster, 2014). Experimental infections were performed approximately 16 h after passive immunization.

### 2.4. Aerosol infections and clinical assessment in cattle

The OIE FMD Reference Laboratory at SENASA provided virulent FMDV O1 Campos strain. Experimental infections through the oronasal route were performed with a jet nebulizer attached to an aerosol delivery system ($10^7$ 50% tissue culture infective doses [$TCID_{50}$] in a 2 mL volume per animal) according to the protocols previously described (Pacheco et al., 2010). After the infection, animals were daily monitored for clinical signs of FMD up to 7 dpi. Symptoms included vesiculation in mouth, tongue and feet, lameness, increased salivation, fever (rectal temperature above 39 °C) and loss of appetite. Clinical scores were determined by assigning a score of 0.5 for fever between 39.1 °C and 40.0 °C, 1 for fever > 40.0 °C, 1 for lesions in the oral (dental pad, tongue, gingiva or lips) and nasal cavities and 1 for each foot that developed vesicles, with a maximum clinical score of 6.

### 2.5. Inactivated FMDV antigens

Concentrated suspensions of inactivated FMDV O1 Campos were provided by Biogénesis-Bagó S.A and 140 S viral particles for *in vitro* experiments were purified using a sucrose density gradient centrifugation method as previously described (Pega et al., 2013).

### 2.6. Experimental design and sampling

Passively immunized calves were infected 16 h after serum transfer. In addition, vaccinated calves were infected at 7 (n = 1) and 26 dpv