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Early protection against foot-and-mouth disease virus in cattle using an inactivated vaccine formulated with Montanide ESSAI IMS D 12802 VG PR adjuvant

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SUMMARY

Foot and mouth disease is an acute disease of cattle with a broad distribution around the world. Due to the fast spread of FMDV infections, control measures must be applied immediately after an outbreak, such as the use of vaccines that induce fast protection. Previously, it was shown that mice vaccinated with FMD inactivated virus (iFMDV) formulated with Montanide™ ESSAI IMS D 12802 VG PR adjuvant (802-iFMDV) were protected when they were challenged 4 and 7 days post-vaccination (dpv) with homologous virus.

In this work, we describe the successful use of this formulation in cattle. In addition, adjuvant Montanide™ IMS 1313 VG NPR was also tested. 802-iFMDV vaccine was able to confer 100% protection against viral challenge at 4 and 7 dpv, while eliciting low antibody levels, at 7 dpv. 1313-iFMDV vaccine induced protection in 60% of cattle.

At 4 dpv, 1313-iFMDV vaccinated animals presented increased levels of IFNγ but not of macrophages. At 4 and 7 dpv, macrophages, IFNγ, nasal IgA and IgG1 antibodies against FMDV, and opsonophagocytosis were increased in animals vaccinated with 802-iFMDV indicating that these phenomena could be involved in protection.It is the first time that total protection against FMDV at early stages post-vaccination is reported using a single dose of the formulation iFMDV plus Montanide™ ESSAI D IMS 12802 VG PR adjuvant.

1. Introduction

Foot and mouth disease (FMD) is a cloven hoofed disease that occurs in most of the world [1]. The economical losses of infection with foot and mouth disease virus (FMDV) in bovine and swine are due to physical and productive deterioration rather than mortality [2]. Nevertheless, for countries exporting animals and animal products, the most relevant economical impact is connected with restrictions on international trade.

Considerable efforts have been applied to the development of vaccines capable of reducing the time between vaccination and the elicitation of a protective immune response. Until now, vaccines formulated with inactive virus plus adjuvant, have shown to require at least 7 days to produce a complete protective response [3].

Montanide ESSAI IMS are aqueous adjuvants containing liquid particles varying in size between 50 and 500 nm and an immunostimulating compound listed as a GRAS substance (Generally Recognized As Safe). Recently, we reported that inactivated FMDV (iFMDV) plus Montanide ESSAI IMS D 12802 VG PR, rises protection against viral challenge in the murine model [4]. Another aqueous adjuvant, Montanide™ IMS 1313 VG NPR, yielded increased protection against viral challenge when it was incorporated into an experimental FMD vaccine, in the same murine model [5]. These results indicate that adjuvants ESSAI IMS D 12802 VG PR and IMS 1313 VG NPR could be adequate for being tested at early times post-vaccination in cattle.

The present work was designed to assess the efficacy of ESSAI IMS D 12802 VG PR and IMS 1313 VG NPR adjuvants in combination with inactivated antigen (iFMDV) in eliciting protection against

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viral challenge and to study the immune response involved in early protection in cattle. Our data suggest that specific antibodies (Abs) and monocytes could be associated with protection. This is the first time that complete protection is reported at early stages post-vaccination by using inactivated antigen plus Montanide™ ESSAI D IMS 12802 VG PR.

2. Materials and methods

2.1. Animals

1–2 year-old bovines ($n = 29$) and 14 regular reared pigs (12 kg ± 2 kg) serologically negative for FMDV, were used. Of the total number of pigs, eight were used as donors for the viral challenge and six as positive unvaccinated controls for infection. Experiments with animals were performed according to INTA Ethics Manual "*Guide for the use and care of experimental animals*".

2.2. Virus

Binary ethylene-imine (BEI)-inactivated and polyethylene glycol (PEG)-concentrated virus, O1 Campos strain, was used for vaccine formulation and ELISA. For challenge and virus neutralization assays, infective virus from the same serotype (kindly donated by the Argentine National Service of Animal Health, SENASA) was used. All the experiments involving infectious virus were performed in the BSL 3A SENASA facilities and BSL 3A boxes at INTA.

2.3. Vaccines

The adjuvants used in this study were Montanide™ ESSAI IMS D 12802 VG PR (named as 802) and Montanide™ IMS 1313 VG NPR (named as 1313) (Seppic, France). Adjuvants were combined with 20 μg/dose of inactivated antigen (iFMDV), defining three vaccines: 802 plus inactivated antigen (802-iFMDV), 1313 plus inactivated antigen (1313-iFMDV) and inactivated antigen formulated in PBS (iFMDV). Formulations were prepared following Seppic's indications. Briefly adjuvant and virus suspension were mixed 1:1 under moderate agitation during 5 min, using a magnetic stirrer. A commercial vaccine (Bioaftogen®) was provided by Biogénesis Bagó (Argentina) and consisted in a water-in-oil single emulsion, containing 4 FMDV strains (A Arg 2000, A Arg 2001, A24 Cruzeiro and O1 Campos). This vaccine has been approved by SENASA with more than 75% of expected percentage of protection against all vaccine strains (OIE guide [6]).

2.4. Immunization

Bovines were vaccinated intramuscularly (i.m.) in the left rear quarter with 2 ml of: 802-iFMDV ($n = 10$), 1313-iFMDV ($n = 10$), iFMDV ($n = 3$) or commercial vaccine ($n = 5$). Five animals in groups 802-iFMDV and 1313-iFMDV were challenged at 4 dpv and the rest at 7 dpv. Animals in group iFMDV and commercial vaccine were challenged at 7 dpv. Control unvaccinated bovine ($n = 1$) and pigs ($n = 6$) were inoculated with phosphate-buffered saline (PBS).

2.5. Infections

Pigs ($n = 8$) were used as the source of virus for aerosol challenge of cattle. Pigs were inoculated intradermally into the heel bulb with 10^6 $TCID_{50}$/ml infective FMDV O1 Campos in 0.5 ml PBS. At 72 h post-inoculation, they displayed clinical signs of FMD and were put in contact with bovines. Vaccinated cattle were divided into the boxes so that there were two infected and two unvaccinated control pigs for every 5–7 vaccinated cattle. Contact was maintained for 4 h and the animals were circulated around the room every 15 min in order to ensure that all animals were exposed to the virus by breathing, contact or both. After challenge, infected donors pigs used to challenge cattle, were euthanized and control unvaccinated pigs were kept separate until the end of the assay. According to OIE guide [6], lesions typical of the disease on tongue and feet were recorded at 7 days post-challenge (dpc). Those bovines which had no vesicles until 11 dpc were considered protected.

2.6. Measurement of anti FMDV antibodies

Serum samples were examined for anti-FMDV neutralizing antibodies as described before [30]. Briefly, serial dilutions of inactivated sera were incubated for 1 h at 37 °C with 100 $TCID_{50}$ of infective FMDV. Then virus-serum mixtures were seeded on BHK-1 monolayers. After 40 min at 37 °C, fresh MEM-D/2% fetal calf serum (FCS) was added to the monolayers, which were incubated at 37 °C, under 5% CO_2. Cytopathic effects were observed after 48 h.

For total Abs measurement, sandwich ELISAs were performed as described before [4] with minor modifications. These modifications include the use of (HRP)-conjugated anti-bovine Ig (KPL, USA) for total Ig measurement and biotin-conjugated anti-bovine IgA or anti-bovine IgG1 (Caltag, USA) followed by (HRP)-conjugated streptavidin, for IgA and IgG1 measurement in nasal swabs. The cut-off was established as the mean A_{490} of the sera at 0 dpv plus two standard deviations (SD).

2.7. RNA extraction and PCR

Viral RNA was extracted using Trizol (Invitrogen) and used as template of a reverse transcription reaction performed with random primers. The resulting cDNA was used to carry out PCR amplification of the FMDV 3D polymerase gene (primers: 1461-GACCCGAAGTTGAGGCTGCC and 1462-GCCGAACTCCGTGCGAAACA) and a host 18S ribosomal RNA as internal control (primers: 18SU-TCAAGAACGAAAGTCGGAGG and 18SD-GGACATCTAAGGGCATCACA). PCR products were separated on a 1.8% agarose gel. Amplicons of 380 pb 480 bp were obtained for 3D viral polymerase and host rRNA, respectively.

2.8. IFNγ measurement

Peripheral blood mononuclear cells (PBMC) were obtained by centrifugation of blood in a Ficoll-Paque™ plus gradient (GE Healthcare) and incubated in culture medium with or without inactivated FMDV, for 48 h at 37 °C 5% CO_2 atmosphere. Culture supernatants were collected and IFNγ was measured using a sandwich ELISA. Briefly, Immulon II plates were coated with mAb anti-IFNγ (kindly donated by Dr. Babiuk) in carbonate–bicarbonate buffer, pH 9.6. Plates were blocked with PBST–0.1% BSA. Dilutions of samples and recombinant IFNγ standard (Serotec, UK) were added. Plates were washed and rabbit polyclonal anti-IFNγ antibodies were added. After incubation, plates were washed and biotin-conjugated antibody anti-rabbit IgG was added. After incubation, alkaline phosphatase-conjugated streptavidine (KPL, USA) was added. Plates were washed, incubated with p-nitrophenyl phosphate as substrate and read at 405 nm. IFNγ concentration was calculated for interpolation of data in the standard curve.

2.9. Flow cytometry

Cell suspensions were blocked with normal bovine serum and incubated with the monoclonal antibody CD14 (VMRD) for 15 min at 4 °C. After washing, the secondary PE-conjugated rat antibody against mouse IgG (Jackson) was added and cells were incubated