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A canine adenovirus type 2 vaccine vector confers protection against foot-and-mouth disease in guinea pigs

Annebel R. De Vleeschauwer [a,1], Xiaocui Zhou [b,c,1], David J. Lefebvre [a], Annabelle Garnier [b], Fleur Watier [b], Charly Pignon [d], Sandrine A. Lacour [b], Stephan Zientara [b], Labib Bakkali-Kassimi [b], Kris De Clercq [a], Bernard Klonjkowski [b,*]

[a] Vesicular and Exotic Diseases Unit, Veterinary and Agrochemical Research Centre (CODA-CERVA), Groeselenberg 99, 1180 Brussels, Belgium
[b] UMR Virologie, INRA, ANSES, École Nationale Vétérinaire d'Alfort, Maisons-Alfort, F-94700, France
[c] Laboratory of Zoonoses, China Animal Health and Epidemiology Centre, 369 Nanjing Road, Qingdao, China
[d] Exotics Medicine Service, École Nationale Vétérinaire d'Alfort, Maisons-Alfort, F-94700, France

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ABSTRACT

Vaccination is a key element in the control of foot-and-mouth disease (FMD). The majority of the antigenic sites that induce protective immune responses are localized on the FMD virus (FMDV) capsid that is formed by four virus-encoded structural proteins, VP1 to VP4. In the present study, recombinant canine adenovirus type 2 (CAV2)-based FMD vaccines, Cav-P1/3C R° and Cav-VP1 R°, respectively expressing the structural P1 precursor protein along with the non-structural 3C protein or expressing the structural VP1 protein of the FMDV strain O/FRA/1/2001, were evaluated as novel vaccines against FMD. A strong humoral immune response was elicited in guinea pigs (GP) following immunization with Cav-P1/3C R °, while administration of Cav-VP1 R° did not induce a satisfying antibody response in GP or mice. GP were then used as an experimental model for the determination of the protection afforded by the Cav-P1/3C R° vaccine against challenge with the FMDV strain O_1 Manisa/Turkey/1969. The Cav-P1/3C R° vaccine protected GP from generalized FMD to a similar extent as a high potency double-oil emulsion O_1 Manisa vaccine. The results of the present study show that CAV2-based vector vaccines can express immunogenic FMDV antigens and offer protection against generalized FMD in GP. This suggest that Cav-P1/3C R° FMDV vaccine may protect natural host species from FMD. In combination with an appropriate diagnostic test, the Cav-P1/3C R° FMDV vaccine may also serve as a marker vaccine to differentiate vaccinated from infected animals.

1. Introduction

Foot-and-mouth disease (FMD) is one of the most important infectious diseases of cloven-hoofed livestock and wildlife. The FMD virus (FMDV) belongs to the genus Aphthovirus within the Picornaviridae family. To date, seven different serotypes (O, A, C, Asia 1, (South African territories) SAT1, SAT2 and SAT3) and multiple antigenic subtypes have been identified and new subtypes arise frequently [1]. Control and eradication of FMDV by use of inactivated vaccines has been successful in Europe, South America and parts of Africa and Asia [2,3]. However, shortcomings of classical inactivated vaccines including serotype-dependency, limited antigenic matching between vaccine and outbreak strains, short-term protection after vaccination, antigen instability particularly after vaccine formulation, and a high production cost resulting from the high-containment facilities required for the production of live virus thrive the search for novel vaccine strategies [4]. An alternative vaccine approach relies on the use of recombinant viruses to express FMDV antigens. Adenovirus-based vectors (AdV) represent a promising antigen delivery system. A single parenteral inoculation of a live but replication-defective human adenovirus engineered to express FMDV capsomers induced protection against FMD in pigs and cattle at 7 days post vaccination (dpv) [5,6]. Because veterinary vaccines based on human AdV may for safety reasons be less suitable for mass-vaccination campaigns in food producing animals, non-human adenovirus-based vector vaccines are proposed as an appropriate alternative [7]. Recombinant canine adenovirus type 2 (CAV2) vectors are well characterized and have significant potential for vaccine purposes [8,9].

* Corresponding author at: Equipe « Vaccins et Vecteurs adénoviraux », UMR Virologie, 7 avenue du Général de Gaulle, 94700 Maisons-Alfort, France.
E-mail address: bernard.klonjkowski@vet-alfort.fr (B. Klonjkowski).
[1] These authors contributed equally to this work.

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Here, non-replicative CAV2 vectors expressing either the FMDV capsid polyprotein precursor P1 along with the non-structural 3C protein for its cleavage (Cav-P1/3C R°) or the structural VP1 protein (Cav-VP1 R°) were developed and evaluated *in vivo*.

2. Materials and methods

2.1. CAV2 recombinant FMDV vaccines and cells

Two CAV2 vectors expressing the FMDV capsid polyprotein precursor P1 and the 3C protein for its cleavage (Cav-P1/3C R°) or expressing the VP1 capsid subunit protein (Cav-VP1 R°) using coding sequences of FMDV strain O/FRA/1/2001 (GenBank AJ633821) were developed as previously described [9]. In addition, isogenic vectors expressing either the glycoprotein of rabies virus (Cav-G R°) [10] or the non-structural protein-1 of bluetongue virus (Cav-NS1 R°) [11] were used. All CAV2 vectors were amplified and titrated in dog kidney cells expressing the E1 region of canine adenovirus type 2 (DK-E1) cells [12].

2.2. Immunization of mice

In the first experiment, six-week-old CD1 female mice were randomly assigned to 3 groups. Two groups (n = 3) were immunized with either Cav-P1/3C R° or Cav-NS1 R°. Animals received intramuscularly (IM) a single dose of 2×10^8 $TCID_{50}$ of CAV2-based vaccines in 50 µl of PBS. One unvaccinated mouse served as control. Blood was collected via the retro-orbital sinus at 2 and 4 weeks after vaccination (wpv).

In the second experiment, seven-week-old C57BL/6 female mice were randomly assigned to 5 groups. Groups 1 to 3 (n = 4) received IM 2×10^8 $TCID_{50}$ of Cav-P1/3C R°, Cav-VP1 R° or Cav-NS1 R° vectors, respectively. Four mice in group 4 were inoculated, by the intraperitoneal route, with 2 µg of inactivated O_1 Manisa FMDV antigen (Merial) formulated with an oil adjuvant (Montanide ISA 50 V2, Seppic). All inoculated animals were booster-vaccinated with the same dose 3 weeks later. Two unimmunized mice served as controls (group 5). Blood was collected before each vaccination and 3 weeks after the booster vaccination.

2.3. Antibody responses in mice

Antibody responses against FMDV were assessed by a validated in-house indirect ELISA performed on PrioCHECK FMDV type O kit plates (Prionics AG) with horseradish peroxidase-conjugated rabbit anti-mouse IgG (Millipore) as secondary antibodies.

Antibodies against the CAV2 vectors were detected by ELISA as described previously [8]. Western Blot analysis was performed by resolving lysates from Cav-FMDV construct-transduced CHO-CAR cells in SDS-PAGE 4–12% Bis-Tris gels (Invitrogen). Proteins were transferred from the gel onto 0.45 µm nitrocellulose membranes. Proteins were immunoblotted by sequentially applying the serum samples from immunized animals and alkaline phosphatase-conjugated goat anti-mouse IgG (Promega).

2.4. Vaccination of guinea pigs

Outbred male Dunkin Hartley guinea pigs (GP) (350–450 g) were used. In the first experiment, 9 GP were randomly assigned to 3 groups (n = 3). Groups 1 and 2 were individually IM inoculated with 1×10^9 $TCID_{50}$ of Cav-P1/3C R° or Cav-VP1 R° constructs. Group 3 was inoculated IM with 2 µg of inactivated O_1 Manisa FMDV antigen (Merial) formulated with an oil adjuvant (Montanide ISA 50 V2, Seppic). All animals were immunized with the same dose 3 weeks later. GP were sedated with Xylazine (20 mg/kg) and blood was collected from the jugular vein before each injection and 3 weeks after the second injection. Sera were examined in the in-house indirect ELISA performed on PrioCHECK FMDV type O kit plates (Prionics AG) as described above and in a virus neutralization (VN) assay. In brief, serial dilutions of serum were incubated with 100 $TCID_{50}$ of FMDV O at 37 °C for 1 h in 96-well plate. Then, 4×10^4 IBRS-2 cells/well were added and incubated at 37 °C for 3–4 days. The anti-VP1 MAb D9 and FMDV negative sera were used as positive and negative controls, respectively. Appearance of CPE was used to determine the end-point titers, expressed as the reciprocal of the highest dilution that neutralized 100 $TCID_{50}$ of FMDV type O.

2.5. Vaccination and challenge of guinea pigs

Four groups (n = 4) were vaccinated IM twice with a three-week interval. Per vaccination, group 4 received 1×10^9 $TCID_{50}$ of Cav-P1/3C R°, group 5 received 1×10^9 $TCID_{50}$ of Cav-G R°, group 6 received a full cattle dose (2 ml) of a high potency (>6 PD_{50}) double-oil-emulsion (DOE) vaccine containing the inactivated FMDV strain O_1 Manisa/Turkey/1969 (O_1 Manisa) (MSD Animal Health – Intervet, Köln, Germany) and group 7 was left unvaccinated. Six weeks after the second vaccination, all GP were inoculated in the right hind footpad with 100 50% GP infectious doses of the GP-adapted O_1 Manisa strain and monitored daily as described previously [13]. Samples for analysis of the viral RNA load were collected at 2 days after virus inoculation (dpi) (serum) and at 4 dpi when all animals were euthanized (serum and internal organs). The nucleic acids were extracted with the Nucleospin RNA virus columns (Macherey–Nagel, Duren, Germany) according to the manufacturer's instructions. One-step real-time RT-PCR for the FMDV RNA-dependent RNA-polymerase (FMDV 3D gene) was performed as adapted from the Ref. [14]. The crossing-point (Cp) value refers to the cycle that is used to estimate the quantitative value of the RT-qPCR. Cp-values <40 were considered positive. Blood for serological examination was collected at the time of viral challenge. Sera were examined in an indirect ELISA [13].

Mice originated from Charles River Laboratories and GP from Harlan laboratories. The animal experiments were approved and supervised by the Ethical Committees of the CODA-CERVA (reference number 20130628-01) and ANSES/ENVA/UPEC (reference number 20/12/12-25B).

2.6. Antibody responses in serum of guinea pigs

Antibodies against FMDV were also quantified by a Luminex-based immunoassay by using a Bio-Plex 200® system (Bio-Rad). Briefly, a 1/50 dilution of serum was incubated in 96-well plates with VP1-coupled microspheres (2500 beads/well) at room temperature for 90 min under agitation. After 3 washes with 0.02% PBS using a vacuum manifold, 50 µl volumes of a 1/200 dilution of a rabbit biotinylated anti- GP IgG (Abcam) were dispensed per well and incubated for 45 min at room temperature with agitation. Beads were then washed and incubated for 15 min with 50 µl of a 1/100 dilution of streptavidin-conjugated phycoerythrin (S-PE, Qiagen). After washing to remove the unbound S-PE, beads were analyzed in the Bio-Plex 200® system, which monitored the spectral properties of the beads while simultaneously measuring the amount of fluorescence associated with PE. Data were analyzed using Bio-Plex Manager software version 5.0.

Antibodies against the CAV2 vector were detected by ELISA [8]. Briefly, 96-well Maxisorp plates (Nunc) were coated with 10^5 CAV2 particles in PBS overnight at 4 °C. After a blocking step in PBS-3% Skim Milk, 100 µl of 1/100 dilutions of GP sera, prepared in PBS with 3% Skim Milk, were used with 100 µl of a 1/2500 dilution of peroxidase-conjugated rabbit anti-sheep IgG (DakoCytomation).

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