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A DNA vaccination regime including protein boost and electroporation protects cattle against foot-and-mouth disease

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ABSTRACT

Protection against foot-and-mouth disease (FMD) using DNA technology has been documented for sheep and pigs but not for the highly susceptible species of cattle.

Twenty-five Holstein Friesian cross-bred cattle were vaccinated twice, 21 days apart, with a DNA vaccine containing the capsid coding region (P1) along with the non-structural proteins 2A, 3C and 3D (pcDNA3.1/P1-2A3C3D) of O_1 Kaufbeuren alone or coated onto PLG (D,L-lactide-co-glycolide) microparticles. In some pcDNA3.1/P1-2A3C3D was also combined with an adjuvant plasmid expressing bovine granulocyte macrophage colony stimulating factor (GM-CSF). DNA vaccinations were administered intramuscularly with, or without, the use of electroporation and at 42 days post primary vaccination cattle received a protein boost of 146S FMD virus (FMDV) antigen and non-structural protein 3D. For comparison, four cattle were vaccinated with a conventional FMD vaccine and two more included as unvaccinated controls. Apart from those immunised with PLG microparticles all cattle were challenged with 10^5 $TCID_{50}$ cattle adapted O_1 Lausanne FMDV virus at day 93 post primary vaccination.

All DNA vaccinated cattle regardless of regime developed good humoral and cell mediated responses prior to challenge. The best overall virus neutralising antibody, IFN-γ and clinical protection (75%) were seen in the cattle whereby the DNA was delivered by electroporation. In contrast, only 25% of cattle vaccinated with the DNA vaccine without electroporation were clinically protected. The addition of GM-CSF in combination with electroporation further improved the efficacy of the vaccine, as demonstrated from the reduction of clinical disease and virus excretions in nasal swabs.

We thus demonstrate for the first time that cattle can be clinically protected against FMDV challenge following a DNA prime-protein boost strategy, and particularly when DNA vaccine is combined with GM-CSF and delivered by electroporation.

1. Introduction

Foot-and-mouth disease (FMD) is an acute systemic disease of cloven-hoofed animals of which the causal agent is foot-and-mouth disease virus (FMDV). The disease is ranked first in the L'Office International des Epizooties (OIE, World Organisation for Animal Health) list of notifiable diseases, which by definition, means that it has the potential for rapid and extensive spread within and between countries. In addition, the virus can cause significant weight loss and a decrease in milk production in cattle (Alexandersen et al., 2003) and is thus one of the most economically important diseases of livestock worldwide.

European Union (EU) policy on FMD control recognises vaccination as a principal measure, and strategic vaccine reserves have been set up by the EU and many member countries for emergency purpose. Indeed, vaccination has moved more to the forefront of EU control policy, making it important to improve on the current chemically inactivated virus antigen vaccines which, although effective at preventing clinical signs of the disease, have several significant limitations (Doel, 2003). These include the requirement of high disease secure containment facilities for manufacture, poor thermostability and thus a relatively short shelf life, lack of induction of sterile immunity resulting in the occurrence of carrier animals and limited antigenic spectrum of protection.

A vaccine, or vaccination regime, which addresses many of these shortcomings would provide a more powerful prophylactic for the control of this disease. DNA vaccines have potential for use in very young animals and offer the vaccine manufacturers negligible risk

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of contamination from adventitious agents of animal origin in the final product. Other practical advantages of this approach include (i) a considerable reduction in the cost of production by negating the need for high containment facilities to produce this non infectious vaccine product, (ii) good thermo-stability properties reducing the need for a cold-chain and (iii) the ease of manipulation (Babiuk et al., 2000; Gurunathan et al., 2000; Cichutek, 2000) allowing incorporation of marker genes, the co-expression of multiple antigens, and ability to rapidly cover newly emerging field isolates. Underlining this, such new generation vaccines are beginning to find a niche in the veterinary products portfolio with the recent granting of licences for DNA based products including West Nile virus in horses (West Nile Innovator, Fort Dodge) (Davidson et al., 2005), haematopoietic necrosis virus in salmon (Apex-IHN, Novartis) (Garver, 2005), melanoma in dogs (Canine Melanoma Vaccine, Merial) (Bergman et al., 2006) and growth hormone releasing hormone (GHRH) (LifeTide SW5, VGX Animal Health) (Thacker et al., 2006).

It has already been shown that a DNA plasmid (pCDNA3.1) encoding the precursor (P1) of empty capsid and non-structural proteins 2A, 3C and 3D of FMDV in combination with an adjuvant plasmid expressing granulocyte macrophage colony stimulating factor (GM-CSF) induced strong immune responses to FMDV and protected pigs against live virus challenge (Cedillo-Barron et al., 2001).

Further optimisation of this vaccine strategy dramatically improved the neutralising antibody response and induced an early cellular immune response (Li et al., 2006). It has also been demonstrated that the same DNA (Cedillo-Barron et al., 2001) coated onto poly-D,L-lactide-co-glycolide (PLG) particles promoted specific antibody and cellular immune responses that fully protected sheep against viraemia and local virus replication in the oropharynx at, or before, 28 days post challenge (Niborski et al., 2006). However, even more dramatic improvement in the immune responses has been reported when such vaccines are used in combination with FMD inactivated homologous virus antigen and 3D recombinant protein as a final protein boost in pigs (Li et al., 2008).

Cattle are economically and agriculturally important, have very high susceptibility to FMDV, and can be long-term viral carriers after infection. However, reports on the efficacy of DNA vaccines in cattle are limited. Indeed, there is only one key paper cited, involving a two dose DNA prime-protein boost regime, or vice versa, which was sufficient to stimulate enhanced immunity against FMDV in cattle (Jin et al., 2005). In contrast to other studies however, this experiment involved DNA constructs and a protein boost that only represented part of the virus and the ability of this vaccination regime to protect against challenge was not evaluated.

Utilising the FMD DNA vaccine pcDNA3.1/P1-2A3C3D combined with an adjuvant plasmid expressing GM-CSF we evaluated different DNA prime and protein boost vaccination regimes in cattle, and based on the previous observations in pigs and sheep, examined how effective these varying immunisation regimes are at eliciting protective immunity and inhibiting FMDV replication up to 14 days post infection in this target.

2. Materials and methods

2.1. Plasmid DNA

Plasmids pcDNA3.1/P1-2A3C3D containing FMDV O_1 Kaufbeuren precursor P1 with self cleaving peptide 2A and viral protease 3C, to assemble icosahedral particles, and non-structural protein 3D, to provide additional T-cell epitopes, were prepared as previously described (Li et al., 2008). In order to insert the bovine GM-CSF gene into the same backbone plasmid as the FMDV DNA, pC1-neobGMCSF (provided by Dr. Geraldine Taylor, Compton Laboratory, Institute for Animal Health) was restriction enzyme digested with *Xho I* and *Not I* to release the GM-CSF gene that was then subsequently cloned into the multiple cloning region of pcDNA3.1 using the same restriction enzyme sites (pcDNA3.1/bGM-CSF). Endotoxin free plasmid DNA's were prepared and supplied commercially by Aldevron (USA). Recombinant 3D protein was prepared as per Li et al. (2008).

2.2. Preparation of PLG microparticles

PLG microparticles were prepared as (Lawlor et al., 2011) with the following modifications. The PLGA polymer (D,L-lactide-co-glycolide) was RG503 (Boehringer-Ingelheim, Germany), which has a copolymer ratio of 50/50 and a molecular mass of 34 kDa (manufacturer's data). Briefly, the 50 mg of microparticles were prepared by dissolving PLGA polymers in 2 ml dichloromethane by probe sonication for 8 s at 4 W creating the oil phase. One millilitre of 2.5% poly(vinyl) alcohol was added to the oil phase creating water in oil primary emulsion. The water in oil (W/O) phase was then remotely sonicated for 16 s at 4 W. The W/O emulsion was added in a drop wise manner to 20 ml of a 1% solution of cetyltrimethylammonium bromide (CTAB) and homogenised at 6500 rpm. Once all the W/O emulsion was added the speed of the homogeniser was increased to 17,500 rpm for 2 min. Following homogenisation, the solution was stirred overnight at 200 rpm in a fume hood to allow the DCM to evaporate and the spherical particles to form. Particles were then washed twice in deionised water and centrifuged at 8000 rpm for 8 min. Particles were then sized using a Malvern Mastersizer 200 and the electrokinetical potential was determined by zetasizer. The suspension was then flash frozen in liquid nitrogen before being freeze dried for 24 h.

DNA (pcDNA3.1/P1-2A3C3D) was adsorbed onto the microparticles by adding 10 ml of 5.1 mg/ml DNA to 90 ml TE (Tris/EDTA) buffer and incubated with 600 mg (2% w/w) cationic microparticles at 4 °C for 16 h. The microparticles were then separated by centrifugation at 8000 rpm, the pellet was washed three times with Tris–EDTA buffer, and the microparticles freeze-dried. The wash fluid was quantified using a nanodrop to determine DNA loss. PLGA DNA microparticles were re-suspended to 4 mg/ml.

2.3. Vaccination and sampling of cattle pre-challenge

Thirty-two Holstein Friesian cross-bred cattle of 6–7 months of age were housed separately in six groups of five (vaccinated animals) and one group of two (un-vaccinated controls) within the isolation units at the Institute for Animal Health, Pirbright. The six groups of vaccinates were immunised as follows:

Group 1: 2 mg PLG-pcDNA3.1/P12A3C3D + 0.4 mg pcDNA3.1/bGM-CSF (DNA + PLG + GMCSF)
Group 2: 2 mg PLG-pcDNA3.1/P12A3C3D + 0.4 mg pcDNA3.1/bGM-CSF + electroporation (DNA + PLG + GMCSF + elec)
Group 3: 2 mg pcDNA3.1/P12A3C3D + 0.4 mg pcDNA3.1/bGM-CSF (DNA + GMCSF)
Group 4: 2 mg pcDNA3.1/P12A3C3D + 0.4 mg pcDNA3.1/bGM-CSF + electroporation (DNA + GMCSF + elec)
Group 5: 2 mg pcDNA3.1/P12A3C3D + electroporation (DNA + elec)
Group 6: Conventional vaccine (7.5 μg, BEI-inactivated O_1 Lausanne, sucrose density gradient purified 146S FMDV antigen with Montanide ISA 2006 (Seppic) as the adjuvant)

Cattle in groups 1–5 received a repeat of the appropriate DNA boost at 21 days post first vaccination which was followed by a protein boost, consisting of 7.5 μg, BEI-inactivated, sucrose density