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Use of ENABL® adjuvant to increase the potency of an adenovirus-vectored foot-and-mouth disease virus serotype A subunit vaccine

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ABSTRACT

A foot-and-mouth disease (FMD) recombinant subunit vaccine formulated with a lipid/polymer adjuvant was evaluated in two vaccine efficacy challenge studies in steers. The vaccine active ingredient is a replication-deficient human adenovirus serotype 5 vector encoding the FMD virus (FMDV) A24/Cruzeiro/BRA/55 capsid (AdtA24). In the first study, AdtA24 formulated in ENABL® adjuvant was compared to a fourfold higher dose of AdtA24 without adjuvant. Steers vaccinated with AdtA24 + ENABL® adjuvant developed a significantly higher virus neutralizing test (VNT) antibody titer and an improved clinical response following FMDV A24/Cruzeiro/BRA/55 intradermal lingual challenge at 14 days post-vaccination (dpv) than steers vaccinated with the active ingredient alone. In the second study, vaccination with AdtA24 formulated in ENABL® at the same dose used in the first study, followed by FMDV A24/Cruzeiro/BRA/55 challenge on 7 or 14 dpv, prevented clinical FMD in all steers and conferred 90% protection against viremia. In addition, post-challenge FMDV titers in nasal samples from vaccinated steers compared to unvaccinated steers were significantly reduced. In both studies, none of the AdtA24 vaccinated steers developed antibodies to the FMDV non-structural proteins prior to challenge with FMDV, indicative of the capacity to differentiate infected from vaccinated animals (DIVA). These results demonstrate that administration of AdtA24 formulated in ENABL® adjuvant lowered the protective dose and prevented clinical FMD following exposure of vaccinated steers to virulent FMDV at 7 or 14 dpv.

1. Introduction

Foot-and-mouth disease (FMD) is the most contagious infection in livestock [1,2]. FMD virus (FMDV), an *Aphthovirus* in the *Picornaviridae*, causes significant agro-economic loss throughout many parts of the world [1]. FMDV infects cloven-hoofed animals, including cattle, domestic and feral swine, sheep, goats, and buffalo [2]. There are seven FMDV serotypes, and multiple subtypes within each serotype [2]. FMD is enzootic in Africa and Asia [2], and at least 15 other countries in Asia, northern Africa, South America, and Europe have reported sporadic outbreaks [3]. In FMD-free

Abbreviations: AdtA24, replication-deficient human adenovirus vectored FMDV A24/Cruzeiro/BRA/55 vaccine; APHIS, Animal and Plant Health Inspection Services; CVB, Center for Veterinary Biologics; DIVA, differentiate infected from vaccinated animals; dpc, days post-challenge; dpv, days post-vaccination; FFB, final formulation buffer; FMD, foot-and-mouth disease; FMDV, foot-and-mouth disease virus; GMT, geometric mean VNT titer; NSP, nonstructural protein; PU, particle units; USDA, United States Department of Agriculture; VNT, virus neutralization test.

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countries, recent outbreaks resulted in economic impacts of >$2.8–16 billion [1,4,5].

To prevent FMD outbreaks in enzootic countries, semi-annual vaccination using chemically inactivated FMDV, purified from large in vitro cell culture batches is often practiced [6]. To prepare for an accidental or intentional FMD outbreak in the United States and enable production of FMD vaccine on the mainland without using FMDV, we developed a replication-defective human adenovirus serotype 5 molecular vaccine platform designed to deliver FMDV capsid and capsid processing genes [7–9]. To produce FMD molecular vaccines to match circulating viral strains, the only information required is the nucleic acid sequence of the capsid coding region of the target strain(s), which can be chemically synthesized and inserted into the adenovirus vector (adenovector). Cattle and pigs vaccinated with various adenovectored FMD experimental vaccines were protected from clinical FMD following challenge with the homologous FMDV strain [7,10–13].

One version of the adenovectored vaccine, AdtA24, comprises the FMDV A24/Cruzeiro/BRA/55 P1-2A coding region, plus part of FMDV A12/119/Kent/UK/32's 3B1, and full 3B2, 3B3, and 3C coding regions [8,9,13]. AdtA24 does not replicate in the vaccinated animal [14]. Only the mRNA for the FMDV capsid proteins is expressed and processed, initially in the muscle cells at the injection site, followed by antigen presentation to the immune system in local draining lymph nodes, and eventually in additional lymph nodes, the liver, spleen, and thymus [14].

Previously we demonstrated that AdtA24 administered at relatively high doses without adjuvant prevented clinical FMD and viremia in immunized steers challenged at either 7 or 14 days post-vaccination (dpv) [13]. To support our goal to lower the vaccine cost we formulated AdtA24 with several adjuvants and conducted preliminary cattle serology studies (data not shown). The most promising adjuvant, a lipid/polymer, ENABL®, was formulated with AdtA24 for evaluation of efficacy in cattle challenged via the intradermolingual (IDL) route with FMDV A24/Cruzeiro/BRA/55. Results from our first study demonstrated that addition of this ready-to-use ENABL® adjuvant resulted in protection at a 19-fold lower dose than the predicted 90% effective dose of AdtA24 prepared in buffer [13]. The second of our studies reported herein was designed to meet the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Services (APHIS) Center for Veterinary Biologics (CVB) guidelines to fulfill requirements to obtain a product license.

Our goal is to have effective molecular FMD vaccine formulations that are economically attractive, can be manufactured in United States biosafety level-2 facilities, deployed rapidly in the event of an outbreak, and differentiate infected from vaccinated animals (DIVA) against the highest FMDV global threats.

2. Materials and methods

2.1. Animals

An accredited experimental-livestock provider supplied healthy, 200–250 kg Holstein steers, 9–11½ months old. Prior to vaccination, we allocated steers randomly to treatment groups where they moved freely about their biosafety level-3 room. There was no concomitant medication during the studies or the 5–7 day acclimation period. The Plum Island Animal Disease Center (PIADC) Institutional Biosafety Committee and the Institutional Animal Care and Use Committee approved the protocols.

2.2. Vaccine construction, formulation, and administration to steers

AdtA24 contains the FMDV A24/Cruzeiro/BRA/55 P1-2A coding regions, and FMDV A12/119/Kent/UK/32 3B1, minus the coding regions for the first six amino acids, and the complete 3B2, 3B3, and 3C coding regions. GenVec, Inc. (Gaithersburg, MD) constructed AdtA24, and purified, evaluated for purity and processed capsid by Western blot, and stored AdtA24 at −80 °C [8,13,15]. Adenovector vaccine particle units (PUs) were detected by $Absorbance_{260}$ following anion exchange HPLC [8]. On the day of vaccination, thawed antigens (37 °C) were mixed with final formulation buffer (FFB; Lonza) or ENABL® adjuvant (No. 7010101, VaxLiant) diluted 1:10 with FFB. Steers received a single 2 mL injection containing either the control formulation or formulated vaccine in the cleido-occipitalis muscle.

2.3. Design of efficacy study 1. Comparison of AdtA24 vaccine formulated with or without adjuvant inoculated into steers and challenged at 14 dpv

In the first study, designed to compare efficacy of AdtA24 formulated with and without an adjuvant, the vaccines contained 1. 2×10^{10} PU of AdtA24/dose in FFB (treatment group 1, T1, n = 22 steers) and 3.0×10^{9} PU of AdtA24/dose in ENABL® adjuvant + FF B (T2, n = 10 steers). The difference in the number of steers/group was based on determining whether the AdtA24 vaccine would be licensed by USDA CVB with or without an adjuvant. Five control steers (C1) received FFB. All steers were challenged at 14 dpv (details below).

2.4. Design of efficacy study 2. Evaluation of steers vaccinated with AdtA24 formulated in ENABL® adjuvant and challenged at 7 or 14 dpv

The second study was designed to evaluate a single, adjuvanted vaccine dose to meet the USDA APHIS CVB pivotal immunogenicity study design requirements for vaccine licensure using a 14 dpv challenge model. At 14 days before challenge, AdtA24 was prepared in ENABL® adjuvant + FFB at 2.7×10^{9} PU/dose for T3, n = 34 steers in two rooms, and six steers in C2 in a different room, were sham-immunized (ENABL® + FFB). Although not required by USDA APHIS CVB, T4 (n = 10) received the same dose of AdtA24 vaccine, 7 days prior to challenge, to understand the early onset of protection at the AdtA24 vaccine minimum protective dose. (T4 was limited in size due to available space.)

2.5. Preparation of FMDV challenge

At 7 or 14 dpv (designated per protocol), we challenged sedated steers according to the OIE guidelines via the intradermolingual (IDL) route using FMDV A24/Cruzeiro/BRA/55 stock produced and titrated in bovine tongue [13,16]. The challenge dose was 1×10^{4} bovine infectious dose 50%/0.4 mL, with titers ranging from 5.625 to 6.0 $\log_{10}$ tissue culture infective dose 50% ($TCID_{50}$)/mL after back titration of inocula on LFBK-$\alpha_V\beta_6$ cells, kindly provided by M. LaRocco, USDA Agricultural Research Service, PIADC [17,18]. All samples collected on the day of challenge occurred prior to FMDV inoculations.

Blinding of immunizations, clinical observations (pedal lesions), and laboratory analyses occurred through masked treatment allocation. The primary outcome included the prevention of clinical FMD and lesion development on hooves, assessed on 3, 7, 10, and 14 days post-challenge (dpc) [13].

2.6. Serum virus neutralization test (VNT)

Serum samples from each steer were collected weekly prior to administration of any treatments starting on the day of vaccination. Antibody titers to FMDV A24/Cruzeiro/BRA/55 and to adenovirus serotype 5 (Ad5) were determined by VNT using heat-inactivated serum samples (56 °C, 30 min) [13,16]. FMDV