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Vesicular Stomatitis Virus glycoprotein G carrying a tandem dimer of Foot and Mouth Disease Virus antigenic site A can be used as DNA and peptide vaccine for cattle

Alejandra V. Capozzo [a,*], Maximiliano Wilda [a], Danilo Bucafusco [b], María de los Ángeles Lavoria [a], Olga L. Franco-Mahecha [a], Florencia C. Mansilla [a], Daniel M. Pérez-Filgueira [b], Pablo R. Grigera [a]

[a] Instituto de Ciencia y Tecnología Dr. César Milstein, CONICET, Saladillo 2468, 1440 Ciudad de Buenos Aires, Argentina
[b] Instituto de Virología, INTA, Los Reseros y Las Cabañas, Hurlingham, Ciudad de Buenos Aires, Argentina

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ABSTRACT

Effective Foot and Mouth Disease Virus (FMDV) peptide vaccines for cattle have two major constraints: resemblance of one or more of the multiple conformations of the major VP1 antigenic sites to induce neutralizing antibodies, and stimulation of T cells despite the variable bovine-MHC polymorphism. To overcome these limitations, a chimeric antigen was developed, using Vesicular Stomatitis Virus glycoprotein (VSV-G) as carrier protein of an in tandem-dimer of FMDV antigenic site A (ASA), the major epitope on the VP1 capsid protein (aa 139–149, FMDV-C3 serotype). The G-ASA construct was expressed in the Baculovirus system to produce a recombinant protein (DEL BAC) (cloned in pCDNA 3.1 plasmid) (Invitrogen Corporation, Carlsbad, CA) and was also prepared as a DNA vaccine (pC DEL). Calves vaccinated with both immunogens elicited antibodies that recognized the ASA in whole virion and were able to neutralize FMDV infectivity *in vitro*. After two vaccine doses, DEL BAC induced serum neutralizing titers compatible with an "expected percentage of protection" above 90%. Plasmid pC DEL stimulated FMDV specific humoral responses earlier than DEL BAC, though IgG1 to IgG2 ratios were lower than those induced by both DEL BAC and inactivated FMDV-C3 after the second dose. DEL BAC induced FMDV-specific secretion of IFN-γ in peripheral blood mononuclear cells of outbred cattle immunized with commercial FMDV vaccine, suggesting its capacity to recall anamnestic responses mediated by functional T cell epitopes. The results show that exposing FMDV-VP1 major neutralizing antigenic site in the context of N-terminal sequences of the VSV G protein can overcome the immunological limitations of FMDV-VP1 peptides as effective protein and DNA vaccines for cattle.

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1. Introduction

Foot-and-mouth disease (FMD) is one of the most economically and socially devastating diseases affecting animal agriculture throughout the world (Rodriguez and Gay, 2011). FMD is highly contagious and affects wild and domestic cloven-hoofed animals. The causative agent is Foot-and-Mouth Disease Virus (FMDV) which belongs to the genus *Aphthovirus* in the family *Picornaviridae* (Grubman and Baxt, 2004). Due to the devastating economical consequences that could arise from an FMD outbreak, this disease is considered a potential bioterrorism weapon (Hietela and Ardans, 2003).

Vaccination against FMDV is a major strategy to control the disease during an outbreak and in endemic areas. Current FMDV vaccines are serotype-specific and consist of inactivated virus formulated in oil or aluminum hydroxide adjuvants (Doel, 2003). Although these vaccines can induce strong humoral protective immunity, there are major drawbacks associated with their use, namely, the requirement of propagating virulent virus in containment facilities (Barteling and Vreeswijk, 1991) and the associated risk of escape from manufacturing sites (Strohmaier et al., 1982), the limited shelf life and the relatively short-term nature of protection (Grubman and Baxt, 2004; Rodriguez and Gay, 2011).

Much effort has been made to develop alternative vaccines that are both efficient and safe based on either recombinant proteins, peptides, replicating vectors or plasmid DNA (Grubman, 2005). DNA vaccines are particularly attractive for field application, mainly because pure DNA is stable and immune-stimulating, does not need a cold chain or adjuvant (Carvalho et al., 2009). Plasmids encoding large fragments of an FMDV genome-like P1-2A3C3D construct (Cedillo-Barrón et al., 2001; Li et al., 2006), VP1 (Li et al., 2007; Park et al., 2006) or FMDV B and T-cell epitopes (Borrego et al., 2006; Cedillo-Barrón et al., 2003; Dory et al.,

* Corresponding author. Tel./fax: +54 11 4687 6735x2318.
E-mail address: acapozzocevan@centromilstein.org.ar (A.V. Capozzo).

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2009a,b; Fan et al., 2007) have been tested in mice and swine. Considerable progress has been made to enhance DNA vaccination efficacy but at least two to three doses of plasmids are needed to achieve immunity, even in mouse models (Borrego et al., 2006).

Attempts to develop peptide FMD vaccines have been based on the B-cell target antigenic site A (ASA), spanning amino acids 139 through 149 of the capsid protein VP1 (G-H loop). ASA is exposed on the surface of the viral particle (Acharya et al., 1989; Bittle et al., 1982; DiMarchi et al., 1986; Pfaff et al., 1982), and neutralizing antibodies are mostly induced and targeted to the ASA of VP1 capsid protein. This sequence contains an arginine–glycine–aspartic acid (RGD) motif, highly conserved among the different FMDV serotypes and involved in the integrin-mediated attachment of FMDV virions to the cellular membrane (Baxt and Becker, 1990; Fox et al., 1989; Mason et al., 1994).

Crystalographic data demonstrated that the serotype C3-ASA sequence in solution has no unique but rather multiple conformations, each of which displays a particular immunogenicity (Harrison, 1989; Mateu et al., 1990). In fact, the limited capacity of linear synthetic peptides spanning the GH loop of VP1 to induce an effective antiviral response in cattle (Taboga et al., 1997) could be at least partially attributed to their inability to mimic one or more of the multiple conformations of the ASA present in the surface of the virion.

Efforts to improve and broaden VP1 G-H loop peptide immunogenicity entailed the association of ASA sequences to T helper (Th) epitopes able to amplify the antiviral response. A dendrimeric peptide that integrates ASA repeats, and Th epitopes enhanced the effectiveness of presentation to the porcine immune system of viral antigenic sites capable of stimulating B- and T-cell-specific lymphocytes (Cubillos et al., 2008). Earlier studies demonstrated that the inclusion of consensus residues into the hypervariable positions (“UBI peptide”) resulted in high level of protection in swine following FMDV 01 Taiwan challenge (Wang et al., 2002) but was unable to protect cattle at 3 weeks post-vaccination (Rodriguez et al., 2003). Failures to induce protection in cattle have been related to lower activation of naïve T cells due to the highly variable Bovine Major Histocompatibility Complex (MHC/BoLA) (Baxter et al., 2009; Leach et al., 2010).

In this study, a chimeric construct containing the first 193 aa of the ectodomain of the Vesicular Stomatitis Virus glycoprotein (VSV-G) harboring an in tandem dimer insertion of the FMDV-C3 ASA sequence was evaluated as peptide and DNA vaccine in cattle. ASA tandem dimer was inserted within the non-linear epitope IV of the VSV N-terminal region, a major disulfide bond-stabilized loop exposed in the surface of the protein (Grigera et al., 1992). Conformation of ASA in this particular display was found to mimic one or several of the alternative conformations of ASA in the virions, critical to induce infection blocking antibodies in mice (Grigera et al., 1996). This study shows that the association of ASA to N-terminal sequences of the VSV glycoprotein, a powerful inducer of neutralizing antibodies and protection in cattle (Glass, 2004; Grigera et al., 1996), can provide appropriate conformation and circumvent poor responses induced by ASA peptides themselves.

2. Materials and methods

2.1. Cells and viruses

Growth of COS and BHK cells in monolayer cultures have been described previously (Capozzo et al., 1997; Grigera et al., 1996, 1992). Conditions for infection, cloning, and harvesting of recombinant baculovirus grown in *Spodoptera frugiperda* (Sf9) cells were carried out as described before (Grigera et al., 1996). Purified VSV-G New Jersey (NJ) serotype Glycoprotein was kindly provided by Grigera et al. (1992).

FMDV-C3/Arg/85 whole particles used as target antigen for the enzyme-linked immunosorbent assay (ELISA), functional or vaccination experiments were purified from a vaccine Manufacturer’s inactivated antigen produced by the Frenkel or BHK method. Whole particles (140 S) were purified by a standard sucrose density gradient centrifugation method and selected fractions were ultra-centrifuged and pellet suspended in NET (sodium–EDTA–Tris buffer: 0.1 M NaCl, 0.004 M EDTA, 0.5 M Tris–HCl, pH 8) buffer to 1 μg/ml (Grubman et al., 1985).

2.2. Polyclonal and monoclonal antibodies

Anti-FMDV serotype C3/Arg/85 and anti-VSV-G NJ serotype polyclonal sera were prepared in rabbits (Grigera et al., 1996, 1992). Preparation and characterization of monoclonal antibody (MAb) directed to epitope IV of the VSV-NJ G protein (MAb 9) as well as Mabs used in Liquid Phase Blocking ELISA (LPBE) (Capozzo et al., 1997; Robiolo et al., 2010), have been described in detail elsewhere (Bricker et al., 1987; Freer et al., 1994). MAb against serotype C3-VP1 (MAb 43) used for Western blot was produced in our institute (Seki et al., 2009). Anti-bovine isotypes IgGl and IgG2 MAbs were purchased to VMRD (Veterinary Medical Research & Development, Pullman, WA) and Sigma (St. Louis, MO), respectively.

2.3. Chimeric constructs

Detailed construction of pC G (from VSV-NJ) and the construct containing a tandem dimer of the ASA endecapeptide ARRGDLAHLAT of the VP1 protein of the FMDV C3 serotype (comprises sequences of more than ten C3 isolates) inserted between G-gene codons 160 and 161 of truncated VSV-G (aa 193) have already been published (Grigera et al., 1996). This chimeric construct was subcloned in pC DNA 3.1 (Invitrogen, Gaithersburg, MD) between Eco RI y XbaI sites to produce pC DEL plasmid. Plasmids were purified using plasmid Mega kit (Qiagen, Hilden, Germany) columns according to the Manufacturer’s instructions, and prepared in sterile PBS to a final concentration of 1.5 mg/ml for cattle vaccination.

DEL BAC was produced in Sf21 cells grown in spinner flasks, infected (in serum-free medium) at a high MOI (5 PFU/cell) with recombinant BV (Grigera et al., 1996) and harvested 2–3 days later. Centrifuged-sonicated pellet was kept as the antigen source, from which chimeric proteins were purified by elution from anti-VSV G antibody–Sepharose columns as described previously (Grigera et al., 1996). DEL BAC was quantified by using a commercial protein assay method (Micro BCA, Pierce, Rockford, USA). Production yielded 32 ± 0.98 μg per 1×10^6 cells.

2.4. Transient expression in COS cells

Experimental conditions for expression of the plasmids in COS cells were identical to those already described (Grigera et al., 1992). Briefly, COS monolayers were cultured to 80% confluence and lipotransfected with 2 μg of plasmid pC DEL, pC G and pC DNA for 3 h using lipofectamine-plus transfection reagent (Invitrogen) according to the Manufacturer’s instructions. The cells were further incubated for another 6 h in the presence of medium containing 10% fetal calf serum and then labeled with [^{35}S] methionine (150 mCi/ml) for 1 h in methionine-free medium. After the labeling period, cells were washed with PBS, lysed in radio-immunoprecipitation assay buffer, and processed for immuno-precipitation (Grigera et al., 1992).

Immunofluorescent staining of transfected cells was performed on fixed COS cells following standard procedures and revealing the

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