Veterinary Microbiology 193 (2016) 106–115

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

DNA vaccine (P1-2A-3C-pCDNA) co-administered with Bovine IL-18 gives protective immune response against Foot and Mouth Disease in cattle

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ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form 2 July 2016
Accepted 6 July 2016

Keywords:
Foot and mouth disease
DNA vaccine
P1-2A-3CpCDNA
bIL-18 pCDNA
Humoral response
Cell mediated immune response
Protective immune response

ABSTRACT

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals causing considerable economic loss in the affected countries. Presently used tissue culture inactivated vaccine protects the vaccinated animals for a short duration. DNA vaccines along with appropriate adjutants is one of the approach for the development of alternative vaccine. In the present study, we constructed P1-2A-3CpCDNA (containing P1-2A-3C coding sequences of FMDV Asia-1 Ind 63/72) and bovine IL-18 pCDNA plasmids and evaluated in cattle. Four groups of calves each group containing six calves were vaccinated with 200 μg of plasmid DNA vaccine P1-2A-3CpCDNA, P1-2A-3CpCDNA+ bIL-18pCDNA and inactivated vaccine respectively where as fourth group was unvaccinated. P1-2A-3CpCDNA + bIL-18pCDNA vaccinated animals have shown higher levels of neutralizing antibodies and specific T-cell proliferation responses. Higher levels of $CD4^+$ and $CD8^+$ cells were observed in these animals. Similarly, IL-18 adjuvanted group has shown increased Th1 and Th2 cytokine responses. All the vaccinated animals were challenged with cattle adapted FMD homologous Asia1 virus two weeks after the booster dose. IL18 co administered DNA vaccine construct has protected four out of six animals challenged with homologous virus.

1. Introduction

Foot-and-mouth disease (FMD) caused by foot-and mouth disease virus (FMDV) is an infectious disease affecting cloven-hoofed animals, and poses a serious threat for animal health and exacts an economic toll on the livestock industry. In countries where disease eradication has not been achieved, vaccination played a crucial role in its control. Although inactivated virus vaccines effectively prevent FMD, they have residual potency problems, including incomplete viral inactivation or virus escape from vaccine producing facilities (Sutmoller et al., 2003). As a result, alternative approaches are being investigated, including the construction of modified live virus, subunit vaccines, synthetic peptides, naked DNA plasmids (Doel, 2003; Mason et al., 1997; Grubman et al., 1993). Several reports have shown the efficiency of DNA vaccination to induce protective immunity in the mouse model (Yang et al., 2005). However, major drawback with DNA vaccination is its poor immunogenicity in target species (Van Drunen Littel-van den Hurk et al., 2004). Even multiple doses of DNA vaccine failed induce protective immune responses large animals (Schrijver et al., 1997; Nobiron et al., 2003). FMD viral genome is a positive-sense single stranded RNA of approximately 8.5 kb. It is translated as a single polypeptide precursor that is subsequently processed by virus-encoded proteases 2A and 3C to produce the structural and non-structural proteins required for virus assembly and replication. Studies have identified linear and conformational epitopes that are present on both empty capsids and virions, and antiserum raised against either form has the same serological specificity (Doel and Chong, 1982). Thus, sequences coding for the structural protein precursor, P1-2A, and the 3C protease of FMDV will be able to produce product with the required antigenic epitopes to induce good immune responses.

Cytokines play an important role both in the development of a functional immune system as well as in the responses of the organism to infections (Schijns, 2000). Interleukin-18 (IL18) is a potent interferon γ (IFNγ) inducing factor (IGIF), enhances Th1 immune responses. It was also shown that IL-18 also promotes the Th-2 type responses and increases dendrite number in lymph

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http://dx.doi.org/10.1016/j.vetmic.2016.07.007
0378-1135/

nodes in mice (Pollock et al., 2003). In addition, IL-18 has been used as an adjuvant to DNA vaccines for classical swine fever virus, pseudo rabies virus, porcine reproductive and respiratory syndrome virus (Daniel et al., 2005; Shen et al., 2007). IL-18, co expressed along with FMDV VP1 in Pichia as fusion protein, enhanced humoral responses and marginally the cell mediated immune responses (CMI) in mice (Shi et al., 2007). Recombinant fowl pox co-expressing FMDV P1-2A-3C and IL18 enhanced the immune responses and gave higher protection in swine. In our earlier report we have shown that P1-2A-3CpCDNA plasmid when co inoculated with bIL-18 pCDNA plasmid was effective in giving protection to guinea pigs challenged with FMD virus (Siva Reddy et al., 2010). In the present report we evaluated the immune responses induced by P1-2A-3C pCDNA construct in cattle when Co-administered with bIL-18 pCDNA.

2. Materials & methods

2.1. Reagents and antigens DNA constructs

pCDNA3.1 vector, Trizol and cDNA synthesis kits were purchased from Invitrogen, Taq DNA polymerase and all restriction enzymes from Fermentas, SYBR green kit purchased from Applied Biosystems and anti bovine- CD3, antibovine-CD4, anti-bovine -CD8, anti bovine-IFNγ, anti-bovine IL-4 antibodies were purchased from Serotech, horseradish peroxidase (HRP)-conjugated goat anti-bovine, IgG1 and IgG2, MTT and ODD, OPD were purchased from Sigma.

2.2. Vaccine constructs

P1-2A-3C -pCDNA (pCDNA vector carrying P1-2A-3C of FMDV serotype "Asia1 Ind 63/72") and bIL-18 pCDNA (pCDNA vector carrying bovine (Bos indicus IL-18 coding sequence) cloning strategy were described in previously (Siva Reddy et al., 2010). These plasmid were available in FMD research laboratory, ICAR-Indian Veterinary Research institute (IVRI), Bangalore, India, and were used for these study.

2.3. Cell lines and virus strains

The cell lines and virus strain used in this study were maintained and available at the Indian Veterinary Research Institute (IVRI), Bangalore, India.Baby Hamster Kidney (BHK)-21 clone 13 (Glasgow) cell line was grown at 37 °C under 5% CO_2 in Dulbecco's modified minimum essential medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), 25 mM HEPES (Sigma).

Vaccine virus strain "FMDV Asia/Ind/63/72" grown in BHK-21 cells was used for inactivated antigen preparation, cloning for P1-2A-3C and virus neutralization assays. Bovine adapted "FMDV Asia/Ind/63/72" available as 10% homogenate of tongue lesion material in PBS was used for challenge virus preparation.

2.4. Experimental cattle

2.4.1. Vaccination

Animal experimentation was carried out at Animal Isolation unit, Indian veterinary Research Institute, Bangalore, India in accordance with the guidelines of the committee for purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Environments and forests, Government of India. Twenty-four male calves of Hallikar breed (Bos indicus) aged between 6 months to one year were used for these experiment. The animals were dewormed and screened for anti-FMDV neutralizing antibodies prior to use in experiment. The four groups of three vaccinated animals and one group un vaccinated controls. The groups of un vaccinated and vaccinated were immunized as follows:

Group 1: Nil

Group 2: 200 μg P1-2A-3CpCDNA

Group 3: 200 μg P1-2A-3CpCDNA + 200 μg bIL-18pCDNA

Group 4: Inactivated vaccine (20 μg, BEI inactivated "Asia-1" antigen, sucrose denesity gradient purified 146S FMDV antigen with Montanide ISA 2006 (Seppic, France). Above mentioned doses were per animal. All the vaccinations were performed by deep intramuscular injection into deltoid muscle. Cattle in groups 2–4 groups were given booster dose once at 21 st day after first immunization.

2.4.2. Sample collection and processing

Heparinised and clotted blood samples were collected on 0, 14, 21, 28 dpv and 21 days post challenge (dpc) using Vacutainer. The heparinised blood samples were transported to the laboratory at ambient temperature and used for lymphocyte proliferation assay, cytokine profile quantified by Real time PCR, CD3 $CD4^+$ and CD3 $CD8^+$ profile, IFNγ, IL-4 levels were analyzed by flow cytometry. The clotted blood samples were processed serum separation and the sera were heat inactivated at 56 °C for 30 min in water bath for use for SNT and ELISA to evaluate neutralization antibodies, anti-FMDV antibodies, and non structural proteins.

2.5. Quantification of Anti FMDV antibodies

Sera samples collected form the vaccinated animals were subjected to ELISA. The plates were coated with anti-rabbit Asia-1 virus146S antibodies in carbonate and bicarbonate buffer pH 9.6 and incubated at 37 °C for 1 h and later transferred to 4 °C overnight. After incubation, the wells were washed three times with washing buffer and the unquenched areas of the wells were blocked by incubating plates with blocking buffer (5%BSA, 0.1% Tween 20 in PBS) for 1hr. The plates were washed three times with washing buffer and added 50 μl/well of blocking buffer containing FMD viral antigen at a concentration of 2 μg/ml and further incubated for 1hr. Following washings serial dilution of serum samples were added to the wells were charged with sera samples with serial doubling dilutions (starting from1:8 to 1:512 in blocking buffer) and incubated at 37 °C for 1 h followed by three washings. The wells were added with 50 μl/well of 1:4000 diluted rabbit anti-bovine HRPO conjugate (Sigma) in blocking buffer and incubated for 1hr at 37 °C. The unbound conjugate was discarded and the wells washed three times with wash buffer, OPD containing 0.015% H2O2 substrate solution was added absorbance at 492 nm was documented. The cut off was established as the mean of the negative sera plus and two standard error.

2.6. Assay for neutralizing antibodies

Heat inactivated test sera samples were serially diluted two fold from starting 1:8 to 1:256 (in triplicate) in Eagle's MEM in Corning, 96-Well Flat Bottom Tissue Culture Polystyrene plates (50 μl/well). Titrated reference virus was diluted in Eagle's MEM to contain 100 $TCID_{50}$/50 μl (FMDV Asia-1). This virus was added to each of the wells at the rate of 50ul/well. Appropriate controls with respect to test serum, virus, cell control were put up. The plates were incubated at 37 °C in a humidified chamber with 5% CO_2 tension for 1 h. Then 50 μl of BHK-21 cell suspension each well were added. The plates were agitated gently for even distribution of the cells and returned to the incubator for 48 h. The 50% serum neutralization end-point was calculated by the method of Reed and Munch method and hence the titres of the serum samples were calculated.