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Original Article

Recombinant VP1 Protein of FMD Virus Type O/IRN/2010 as an Immunogenic Peptide Expression System

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Abstract

Background and Aims: Foot and Mouth Disease (FMD) is a highly contagious disease among cloven-hoofed animals. FMD virus has structural and non-structural proteins. Vp1 is the most immunogenic structural peptides of FMD virus, applied for major vaccine studies. The aim of this study was construction of Pet28-VP1 cassette for FMD virus type O/IRN/2010 and expression VP1 peptide as the most immunogenic antigen was the aim of this study.

Materials and Methods: FMD virus type O/IRN/2010 was isolated from cattle in Qom, Iran and propagated on $IBRS_2$ and BHK_{21} cell lines. The VP1 gene was amplified using the specific primer pair and RT-PCR technique. The purified PCR product was sub-cloned into the unique BamHI and Xho1 cloning sites to construct the PTZ57R/T -VP1 cassette. The $DH_{5\alpha}$ strain was transformed with this cassette. The digested VP1 gene was cloned in the digested Pet28 and confirmed using double digestion. Then the Pet28-VP1 construction was transformed in BL21 strain.

Results: Expression of VP1 peptide was evaluated by IPTG induction and SDS-PAGE and confirmed using Guinea pig specific polyclonal antibody against FMD virus type O and conjugated rabbit anti Guinea pig antibody- HRP. Also neutralizing antisera titre was protective for vaccinated animals by recombinant VP1 protein.

Conclusion: Since the isolation of new FMD virus strains in different geographical locations expression of VP1 peptide can be used in emergency and control settings as a recombinant vaccine in the same area. Therefore the Pet28-VP1 cassette which was constructed in this study may be a good candidate for preparation of peptide vaccine against FMD virus type O/IRN/2010 in future.

Keywords: Foot and Mouth Disease Virus; VP1 protein; Peptide vaccine; Immune Response

Introduction

Foot and Mouth Disease is a highly contagious disease among cloven-hoofed animals. This virus is a member of Picornaviridea family, Aphtovirus Genus and includes 7 serotypes; A, O, C, Asia1 and three type of south Africa territory, namely SAT1, ST2, SAT3. Each virion includes a single stranded positive sense RNA genomic, an icosahedral capsid structure, and 60 copy of structural proteins vp1-vp4 with seven non-structural proteins. The VP1 peptide is the most important structural protein and the major immunogenic antigen with critical epitopes due to its high immunogenicity properties (1).

FMD virus is to blame for huge economic losses of livestock and is one of the major concern regarding animal health-care, raising livestocks and livestock products. The conventional vaccine against FMD virus is a chemically inactivated whole virus preparation with adjuvant which has some problems include: short lasting protection; no discrimination between infected and vaccinated animal; high cost of containment facilities with permanent risk of virus escape and sometimes insufficient chemical inactivation (2). In the recent years, one of the most important recombinant vaccines is protein vaccine, allowing a safe and efficient alternative to conventional vaccination. Finally, the aim of this research is to construct of Pet28-VP1 cassette for FMDV type O/IRN/2010 and expression VP1 peptide as the most immunogenic antigen for using in emergency and control settings in Iran and other neighboring infected countries.

Methods

Virus:

The FMD virus type O/IRN/2010 isolate was collected from epithelial cells of infected cattle in Qom-Iran in 2010, and then propagated on $IBRS_2$ cell line in Razi Vaccine and Serum Research Institute of Karaj, Iran. The virus was multiplied in BHK21 cells and serotyped using polyclonal antibodies against the seven serotypes using ELISA technique, and then total RNA was extracted directly from the supernatant of infected cells using RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions (3, 4).

Primer design and RT-PCR

The primer was designed for amplification and cloning of the VP1 gene based on FMDV O/IRN/2007 nucleotide sequence (Accession number: JF288761) for sense primer and antisense primer NK61 was based on aligned sequences of the 2B gene of types O, A, C and SAT 2 (5, 6). The sequences of forward and reverse primers were designed by the AlleleID6 software package and included: F-5'-GATCGGATCC**ACCATGGTTGACGCTC GCACGCAG-3**' and R-**5**'-TCGACTCGAG**CTATTA**GACATGTCCTCT GCATCTG-**3**'. The pair primer was made by Metabion Company. There are *BamHI* and *XhoI* sequences and four overhanging nucleotides at the start of forward and reverse primers, respectively. The forward primer contains the kozak consensus sequence and start codon. The reverse primer contains two stop codons. The extracted RNA was reverse transcribed and amplified using the VP1 gene, the AccuPower one-step RT-PCR kit (Bioneer) and the specific primer pair with suitable annealing temperature (63°C). The PCR product was about 700 bp length and purified by a DNA gel extraction kit (Fermentas #k0513).

Coloning and sequencing

The PCR product about 700 bp in size was cloned into the unique *BamHI* and *XhoI* cloning sites of PTZ57R/T vector (Fermentas) and the cloned VP1 gene was confirmed by restriction enzyme digestion (*BamHI* and *XhoI*). The confirmed clone was sequenced using PTZ57R/T universal primer and the nucleotide sequence data was deposited in Gen Bank database (7). The VP1 gene was digested from PTZ57R/T vector by *BamHI* and *XhoI* enzymes and cloned in Pet28. The Pet28-VP1

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cassette was transformed into BL21 strain of *E. coli* and protein expression was induced by IPTG (0.4 mM) in order to express VP1 peptide as the most immunogenic antigen against FMD virus type O/IRN/2010 (8).

Expression of FMDV type O/IRN/2010 VP_1 protein in BL21 strain of *E. coli* and purification of the protein

One hundred milliliter of the transformed BL21 strain with Pet28-VP1 cassette was induced by IPTG (Isopropyl-Beta- D-thiogalactopyranosid- 0.4 mM), centrifuged 5000 ×g at 4 °C for 15 min and the bacteria pellet was harvested by lysis buffer with PMSF (phenyl methyl sulfonyl flourid- protease inhibitor). The harvested cells were freeze thawed several times in liquid nitrogen then centrifuged at 12000×g at 4°C for 15 min, and the supernatant was used for SDS-PAGE and Western blot analysis. The specific band of the VP1 protein was detected by Guinea pig specific polyclonal antibody against FMD virus type O and conjugated rabbit anti Guinea pig antibody with HRP as the second antibody using western blotting technique [8 and 9]. The expressed protein was purified by nickel affinity column (Sigma) according to manufacturer's protocol, concentrated by PEG (poly ethylene glycol) and dialyzed against PBS. The concentration of the purified protein was measured by a Nanodrop ND-1000 spectrophotometer.

Immune response of purified VP1 protein on guinea pig

Twelve guinea pigs (250-300 g weight) were divided into four groups (each group included three animals) for evaluation of immune response. Three groups were vaccinated by 50, 100 and 150 µg recombinant VP1 protein, subcutaneously and two booster doses, in 2 weeks intervals, and the last group as negative control. After the last vaccination all of the animals were bled and the sera were used to measure the neutralizing antibody by SNT method.

Results

The serotype of FMD virus isolate was determined by ELISA technique as type O. The VP1 nucleotide sequence data of FMDV type O/IRN/2010 was deposited in Gen Bank database under the accession number JN 676146. FMD virus type O/IRN/1/2010 VP1 gene caused a DNA band about 700 bp after RT-PCR and digestion of PTZ57R/T - VP1 cassette by BamHI and XhoI enzymes (Figure 1).

Ligation of VP1 gene into the digested plasmid of Pet28 made a cassette of Pet28-VP1. Double digestion of the cassette by BamHI and XhoI enzymes made two bands with 5300 bp (digested Pet28) and 700 bp (digested VP1) in Figure 2A. Also the result of PCR reaction by a pair specific primer for VP1 was made a band with 700 bp which was VP1 gene amplification (Figure 2B). Both of these reactions confirmed the ligation of VP1 in Pet28 vector.

To evaluate VP1 protein expression, the lysate of transfected BL21 strain of *E. coli* with the Pet28-VP1 cassette after 48 h were analyzed using SDS-PAGE and western blotting and observed the specific protein band, about 25 kDa (Figures 3 and 4).

The result of immune response

Antibody titres were expressed as the logarithm of the reciprocal of the last serum dilution in the virus/serum mixture to

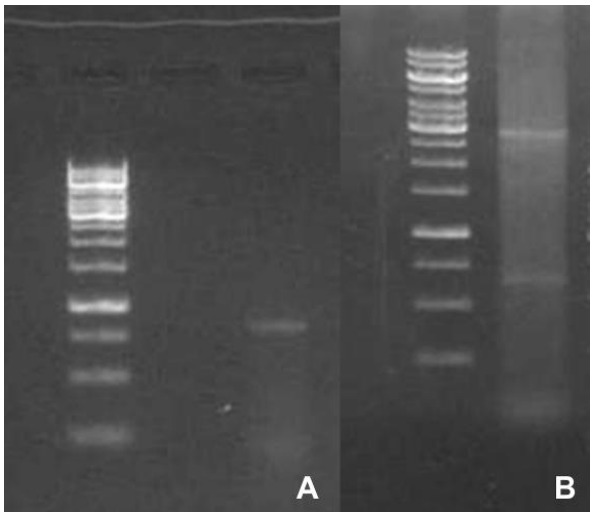

Fig. 1. (A) Lane 2: VP1 band after RT-PCR (B) Lane 2: Double digestion of PTZ57R/T VP1 cassette.

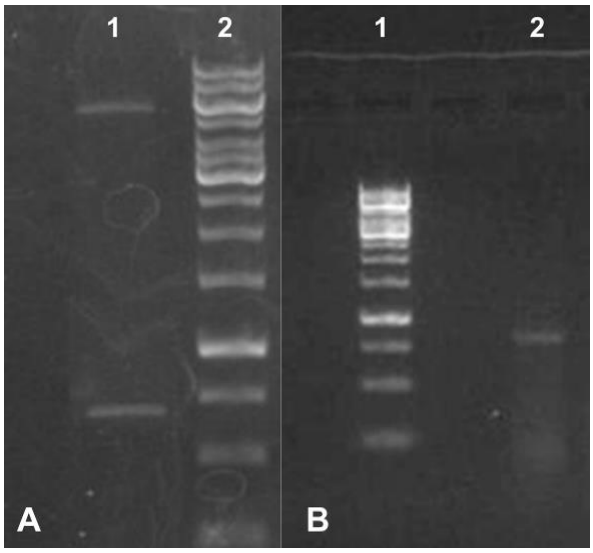

Fig. 2. (A) Lane 1: Double digestion of Pet28-VP1 by BamHI and XhoI enzymes (B) Lane 2: PCR product VP1 gen.

neutralize 100 $TCID_{50}$ of homologous FMD virus antigen at the 50% endpoint (Table 1). The neutralizing antibody titres were calculated from immunized guinea pigs using three different concentration of recombinant protein and showed significant differences in comparison to negative control ($P<0.05$). Titres of neutralizing sera of vaccinated groups by protein concentration 100 and 150 µg were protective (more than 1.2).

Discussion

FMD virus is still an important pathogen worldwide and it frequently escapes from endemic to non-endemic areas. The development of a new recombinant vaccines is still an objective of immense practical importance (10, 11). FMDV type O has five antigenic sites which three of them are located in Vp1 peptide; therefore VP1 is the most immunogenic peptide for FMD virus. Considering this fact, VP1 gene of FMD type O/IRN/1/2010 was used for preparation subunit vaccine.

To treat FMD, inactivated vaccines are used, but vaccine strains must be carefully matched to prevailing field virus strains to induce a satisfactory level of protection; vaccination must cover a level of at least 80% effectiveness

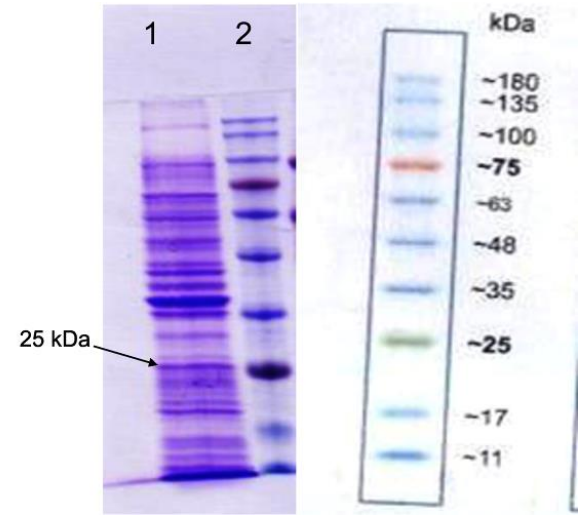

Fig. 3. SDS-PAGE electrophoresis analysis of transfected BL21 cell lyses. Lane 1: lyses of the transfected cell with Pet28-VP1 vector; Lane 2: Protein Marker (fermentase, SM0441).

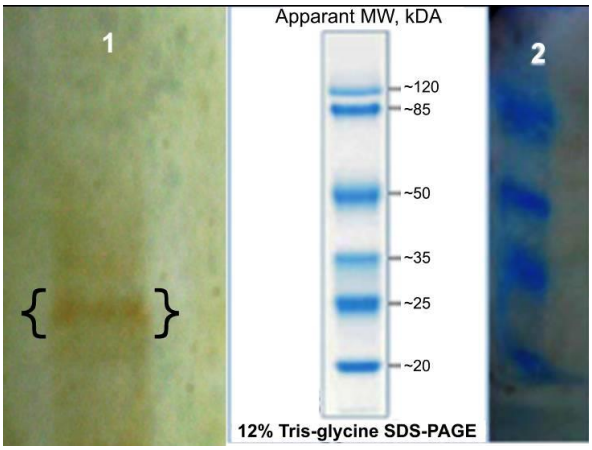

Fig. 4. The specific band of FMDV type O/IRN/2010 VP1protein in western blotting analysis is about 25 kDa (Lane 1), Protein Marker, fermentase, SM0441 (Lane 2).

Table 1: The anti FMDV type O/IRN/1/2010 sera titration 10 days after last vaccination

Vaccinated groups	Mean of Antiserum Titration ±SD
Recombinant protein 50 µg	0.9 ± 0.065
Recombinant protein 100µg	1.5 ± 0.086
Recombinant protein 150 µg	1.95 ± 0.00
Negative control	≤0.6±0.0745

(12). Due to the multiple serotypes of FMDV in circulation, identification of the serotype affecting any one region is required in order to select the most appropriate antigens for inclusion in the vaccine preparation. The most important immunogenic site of FMDV is the VP1 surface antigen encoded by the 1D region (1). The G-H loop structure of the VP1 capsid protein is a surface-exposed immuno-dominant site and the target for neutralizing antibodies (13).

Subunit vaccine is a viral antigen free of viral nucleic acid by chemical extraction or bio-expressing. It is less likely to cause adverse reactions than a vaccine containing the whole virion. With increased knowledge concerning viral capsid structure, researchers have determined that VP1, one of the FMDV capsid proteins, had a prominent surface exposure (9, 14, 15). Some strategies were designed to develop subunit vaccines as alternatives to conventional inactivated vaccine. Kupper and et. al cloned the VP1 gene, and transferred the recombinant plasmid into *E. coli* (16). Results indicated that VP1 produced in *E. coli* could protect both swine and cattle from virus challenge (17). Peptide-based vaccines would have numerous advantages as an alternative to traditional vaccines such as high security and there is no involvement of infectious FMDV in vaccine production. In addition, peptides are stable at room temperature for long periods of time, a characteristic necessary for production and storage of vaccine reserves for use in outbreak response. Further, peptide vaccines can be designed to induce protection against multiple FMDV serotypes. Finally, with peptide vaccines, there is no possibility of induction of antibody reactivity with non-structural proteins of FMDV, the primary targets of recently developed diagnostic tests that distinguish FMDV-vaccinated from infected animals (18, 19).

Also the current vaccines are effective in preventing the disease but the antigenic variability of the virus demands that they must be closely matched with the circulating virus (13). In this study one expression cassette of FMD type O/IRN/1/2010 based on VP1 peptide was designed using Pet28 expression system, since the Iranian isolate of FMDV type O/IRN/2010 caused outbreak in Iran, its neighborhood countries and Middle East, therefore the immunogenic peptide (VP1) expression system can be a good vaccine candidate against FMD virus type O/IRN/1/2010 and can use in this area at next future.

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