A DNA vaccine against foot-and-mouth disease elicits an immune response in swine which is enhanced by co-administration with interleukin-2

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Abstract

A plasmid DNA vaccine candidate (pCEIS) encoding two foot-and-mouth disease virus (FMDV) VP1 epitopes (amino acid residues 141–160 and 200–213) has been demonstrated to have the ability to elicit both FMDV-specific T cell proliferation and neutralizing antibody against FMD in swine. In this study, the efficiency of the pCEIS DNA vaccine when administered by intramuscularly injection in swine was confirmed, and the immunogenicity of the pCEIS vaccine candidate was found to be enhanced through co-administration with a newly constructed plasmid (pIL2S) encoding the swine interleukin-2 (IL-2) cDNA. The expression of the pIL2S plasmid was driven by a CMV promoter provided by a pcDNA3.1 vector. Swine IL-2 cDNA was cloned by RT-PCR from swine spleen cells. The pIL2S plasmid was expressed in COS-7 cells after 24 and 96 h of transfection in vitro. In an animal trial, results from T cell proliferation assay indicated that the stimulation index (SI) in response to stimulation of FMDV proteins in the swine groups injected with pCEIS plus pIL2S (SI ranging from 9.9 to 15.5) were significantly higher than that with pCEIS alone (SI ranging from 3.3 to 6.6). However, there was no significant difference in FMDV-neutralizing antibody level detected in these two swine groups. Mouse protection tests (MPTs) showed that the blood sera from immunized swine injected with either pCEIS alone or pCEIS plus pIL2S were able to protect suckling mice from FMDV challenge, with protection levels ranging from 101 to 102 lethal dose 50 (LD50). In a direct FMDV challenge, all swines immunized with either pCEIS plus pIL2S or with pCEIS alone were challenged with 50LD50 (50% lethal dosage in swine) of FMDV. The animals were fully protected (100%) from the FMD viral challenge. These results suggest that co-administration of the plasmids, pCEIS and pIL2S, enhances of the immunogenicity of the pCEIS DNA vaccine candidate, and both intramuscular injection of pCEIS alone and co-administration of the vaccine candidate with pIL2S can protect the swine from direct FMD challenge.

Keywords: DNA vaccine; Foot-and-mouth disease; Interleukin-2; IgG carrier; VP1 epitopes

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious and economically devastating disease of cattle, swine and other cloven-hoofed ruminants. FMD occurs in Europe, Asia, Africa and South America. Although the European community was declared free from FMD in 1992, occasional outbreaks still occur [1]. The recent FMD epidemic in the United Kingdom has had a severe effect on both agriculture and the tourism industry (http://news.bbc.co.uk). The causative agent of FMD is an FMD virus containing a copy of single strand RNA and 60 copies of each of the four structural proteins (VP1–P4). VP1 carries critical epitopes for inducing immune responses to foot-and-mouth disease virus (FMDV) including VP1 (141–160) and VP1 (200–213) [2–4].

Immunization using DNA vaccines is a vaccination approach that is being widely investigated to protect against a large number of infectious diseases including FMD [5–7]. Immunization with plasmid DNA is able to elicit both cell-mediated and humoral immune responses [8–11]. These responses include the generation of antigen-specific cytotoxic T lymphocytes (CTLs) and protective neutralizing antibodies. In an earlier study, it was found that plasmid DNA constructs could elicit CTLs which are always lacking in an immune response elicited by protein vaccines [12]. Since CTLs are considered necessary for a successful defence against intracellular pathogens [13], this particular aspect of the DNA vaccine is one of its great advantages over protein-based vaccines. In addition, it has also been found that the B cell and T helper (Th) cell responses are...
also present in DNA vaccination as antigen genes contained in the plasmids are translated and present for immune surveillance [11].

DNA vaccine-induced immune responses can be enhanced by co-administration of co-stimulatory molecules, pro-inflammatory cytokines, tumor necrosis factors, granulocytes-macrophage colony-stimulating factor (GM-SF) and Th1 and Th2 cytokines [14]. Most cytokines are secreted by Th cells and they can be classified as either the type 1 (Th1) and type 2 (Th2) cytokines, based on their immune functions. Each type positively regulates either the humoral and cellular immune response, while simultaneously negatively regulating the other immune response. Th1 cytokines including IL-12 and interferon-γ (IFN-γ), can enhance cellular immunity by increasing the cytotoxicity of CTLs and the production of MHC-II molecules, and inhibit humoral immunity by inhibiting the proliferation and differentiation of B cells and stimulating the production of MHC-I molecules. Alternatively, the immunological functions of Th2 cytokines including IL-4, IL-5 and IL-10, are opposite to those of Th1 cytokines.

The biological activity of cytokines are difficult to maintain under normal conditions, therefore their applications are limited. DNA vaccine technology facilitates the use of cytokines in vaccination to manipulate the immune responses. Plasmids encoding specific cytokine genes can be conveniently constructed and combined with a specific antigen to achieve targeted immune responses [15–18]. In this study, the particular cytokine we are interested in is the interleukin-2 (IL-2). IL-2 is a 15.5 kDa glycoprotein, which is a growth-promoting factor for T lymphocytes [19], and also acts as an activating factor for B cells, helper and cytotoxic T cells, natural killer (NK) cells, lymphokine-activated killer (LAK) cells, monocytes and macrophages. IL-2 plays an important role in the initiation of antigen-specific immune responses, as well as their maintenance after the responses are established. Our previous work demonstrated the development of a DNA vaccine candidate (pCEIS) against FMDV [6]. The plasmid was administrated with a gene gun system, and it was able to elicit FMDV specific immune responses in both mice and swine. The current study aimed to examine the efficiency of this pCEIS DNA vaccine when it was delivered into swine by intramuscular injection, and also to enhance the immunogenicity of the DNA vaccine candidate by co-delivery with a newly constructed pIL2S plasmid encoding the swine IL-2 gene.

2. Materials and methods

2.1. Virus culture, inactivation and viral protein purification

Serotype O15 HK Type FMDV was supplied by the Hong Kong Agriculture, Fishery, and Conservation Department (China). FMDV was propagated in a baby hamster kidney BHK-21 cell line and the viral protein was purified as previously described [20]. In brief, BHK-21 cells were cultured in a complete RPMI1640 medium (Gibco). Viral infection of cells was performed by incubating BHK-21 cells in FMDV solution for 45 min. The culture was collected for virus protein purification after 75% of the cytopathogenic effect (CPE) of the BHK-21 cells had been reached. The virus was inactivated in 0.01 M binaryethylenimine (BEI, Sigma) at 37°C for 2 h and the viral particles (146S) was obtained by ultra-centrifugation at 40,000 rpm for 2 h. The concentration of extracted viral protein was determined by measuring optical density (OD) at 259 nm [6]. Purified viral protein was verified by Western blot analysis with an anti-FMDV antibody prior to use as a stimulant in the T cell proliferation assay.

2.2. Construction of pCEIS and pIL2S plasmids

Three plasmids, namely, pCEIS, pCIS and pIL2S, were used in this study. The pCEIS plasmid contained two FMDV epitopes (VP1 (141–160) and VP1 (200–213)) and a swine IgG (sIgG) constant region fragment, in which the sIgG was the carrier for the viral epitopes. The pCIS contains only the sIgG constant region fragment. The epitope–sIgG fragment was subcloned into a mammalian expression vector pcDNA3.1, between the restriction sites of EcoRI and XhoI to produce a plasmid (pCEIS). The pCEIS plasmid was transformed into a transformed African green monkey kidney COS-7 cell line (ATCC), and the expression product was analyzed by Western blot analysis.

The pIL2S contains the swine IL-2 cDNA, the expression of which was driven by the CMV promoter provided by pcDNA3.1 vector. Swine IL-2 cDNA was cloned by RT-PCR from swine spleen cells. The spleen cells were cultured in medium containing 5 μg/ml concanavalin (ConA) in order to induce IL-2 expression. Swine IL-2 mRNA was purified with a FastPrep Kit (Gibco). Reverse transcription was carried out with an M-MuLV reverse transcriptase (Gibco) at 37°C for 1 h. The primers used for RT-PCR were designed based on the porcine IL-2 DNA sequence found in Genebank. The sequence of the sense and antisense primers were 5’-ACGAAGCTTGTCATAGGATACACAGTTAAGTAAAACTACAGCT-3’ and 5’-ACGCTCGAGGXhoIATCAAGTCTAGTGGAGT-3’, respectively. The DNA fragment yielded in the RT-PCR was then inserted into the pcDNA3.1 vector between restriction sites of HindIII and XhoI to give rise to the pIL2S plasmid. After construction, the pIL2S plasmid was subjected to restriction endonuclease digestion mapping and sequencing.

2.3. Analysis of the expression of swine IL-2 proteins

The expression of swine IL-2 in mammalian cells was analyzed in the COS-7 cell line transfected with the plasmid pIL2S. The transfection was carried out with Lipofectamine reagent according to the procedure described by...
The plate was incubated at 37 °C for 4 days followed by incubation with 1 μCi/25 μl of (3H) thymidine for 18 h. The cells were harvested and subjected to liquid scintillation counter (Beckman LS6500) for measurement.

2.7. Determination of lethal dosage 50 (ID$_{50}$S) in swine and FMDV challenge in immunized swine

Seronegative swine were intraperitoneally inoculated with 100 μl of nine 10-fold serial diluted FMDV (i.e. 10$^{-4}$ to 10$^{-9}$). Control animals were given 100 μl EMEM medium only. Each dilution of virus was tested in four swines. The concentration of FMDV that could cause death in two of four swine was referred to as 1 ID$_{50}$S. Since FMDV is not always lethal to swine, observation of the appearance of FMD symptoms rather than death was also used as the indicator of FMD infection, as in the mouse MPT assay. Swine were monitored for a period of 10 days after FMDV challenge for the appearance of FMD symptoms, such as an increase in body temperature (above 41 °C) and the appearance of blisters on the mouth or hooves. After the determination of ID$_{50}$S, challenge test was carried out on 25 experimental swine (five swine per group) by intramuscular injection of 1 ml of 50 ID$_{50}$/ml FMDV at the neck region 10 days after the second immunization. All swines were kept in separated open-topped crates within one house. After a 10 day observation, the swine were examined and eventually destroyed.

3. Results

3.1. Cloning of pIL2S and plasmid expression in Cos-7 cells

Cloning of pCEIS and pCIS was carried out as described previously [6]. The expression of F1–sIgG protein in COS-7 cells was analyzed by Western blot at 24 and 96 h after transfection (Fig. 1). Swine IL-2 cDNA, approximately 475 bp in size, was obtained by RT-PCR from the swine lymphocytes (Figs. 2 and 3). The fragment was then inserted into a pcDNA3.1 vector between HindIII and XhoI restriction sites to produce the pIL2S plasmid. Since IL-2 were not detected in neither the cell lysate nor the supernatant of transfected COS-7 cells by Western Blot analysis, using a goat anti-porcine IL-2 monoclonal antibody (data not shown), the expression of IL-2 was analysed with RT-PCR to detect the presence of IL-2 mRNA. A band of the size of IL-2 cDNA was detected by using a T7 promoter primer (Invitrogen), located in the vector but not in the IL-2 fragment, as the 5′ primer. The blank control did not show any bands (Lanes 1 and 2).
The results clearly showed that IL-2 mRNA was present in the transfected cells.

3.2. Immunogenicity assay in mice and swine

None of the blood samples collected after primary administration from any of the immunized swine groups showed a detectable level of immune response. Blood samples collected 10 days after second administration from immunized swine administrated with pCEIS, and pCEIS plus pIL2S, showed a stimulation index (SI) ranging from 9.9 to 15.5 for pCEIS plus pIL2S, and 3.3 to 6.6 for pCEIS alone. The blood sera from both pCEIS and pCEIS plus pIL2S inoculated swine were able to protect suckling mice from FMDV challenge with protection levels ranging from $10^1$ to $10^2$ LD$_{50}$ M. The control group injected with pIL2S, pCIS or PBS, respectively, did not show any protection (Table 1).

3.3. FMD viral challenge test on swine

Twenty-five swine were challenged with 50 ID$_{50}$ S FMDV HK type O and co-housed for 10 days. In the swine groups administrated with pCIS, pIL2S and PBS, blisters appeared on the feet and tongue of all experimental animals and their body temperature increased to 41°C within 4 days. These symptoms indicated that all of them were infected with FMDV. Swine immunized with the pCEIS plasmid or those co-administrated with both pCEIS plus pIL2S exhibited no FMD symptoms during the 10 days observation period (Table 2). These results indicated that both pCEIS alone and pCEIS together with pIL2S could provide full protection against direct FMD challenge.

4. Discussion

In this study, a new DNA vaccination approach has been demonstrated by co-administrating the pCEIS plasmid with a new pIL2S plasmid encoding the swine IL-2 cDNA molecule. This unique plasmid combination was able to elicit both FMDV-specific T cell and B cell responses in mice and swine animal trials. Furthermore, administration with this DNA vaccine candidate was able to fully protect swine from direct FMDV challenge. The pCEIS plasmid has been previously demonstrated to have protective ability...
against FMD in swine by gene gun application [6]. In this study, we demonstrated that this plasmid could be expressed via a mammalian expression system 24 h after injected into swine, and the expression could last for at least 96 h. The animal trial also showed that pCEIS alone could provide a satisfactory level of protection against FMD when administrated intramuscularly. In the mouse protection test (MPT), it was found that the blood sera from swine immunized either with pCEIS or pCEIS plus pIL2S, could provide protection against FMDV challenge in mice (Table 1). Blood sera collected from control groups injected with pIL2S, pCIS, and PBS, respectively, did not show any significant level of T cell proliferation response (SI values ranged from 0.8 to 1.5). In the swine challenge test, the neutralizing antibody level elicited by the pCEIS plasmid alone was as high as 10^2 LD_{50} M, and the range of neutralizing antibody level obtained with the co-administration of the two plasmids (pCEIS plus pIL2S), was very similar to that of pCEIS. This result indicates that the pIL2S does not significantly enhance the activity of pCEIS. Nonetheless, from our previous experience [6], protection in swine immunized with conventional protein vaccine generally requires >10^3 LD_{50} M protection in MPT.

In general, the antibody response induced by the DNA vaccination is lower than that induced by protein vaccine, and could sufficiently provide protection against FMD challenge. Previous studies indicated that protection against FMDV is associated with antibody response [21]. Memory T cells developed from successfully immunized animals can recognize FMDV proteins. Upon recognition, the FMDV-specific memory T cells will divide faster than un-primed T cells, and T cell proliferation assay measures the proliferating ability of T cells from immunized animals to stimulate antigens. Our results showed that immunization with pCEIS and pCEIS plus pIL2S elicited recognizable levels of T cell response. Swine immunized with pCEIS, or pCEIS plus pIL2S, showed a 10–20-fold T cell proliferation response to the stimulation with FMDV protein. As the antigens used to stimulate T cells were purified FMDV proteins, the T cell response was considered FMDV-specific. However, the proliferation assay could not discriminate between T cells of different functions, such as cytototic or helper, therefore it was difficult to tell exactly whether CTL or Th responses were involved in the T cell response observed in the experiment. According to Collen et al. [4] and Piaatti et al. [22], antibody response that provides protection against FMDV is Th cell dependent. The T cell response in swine stimulated by the plasmid pCEIS, or pCEIS and pIL2S, may contribute to the protection against FMDV through either Th or CTL, or both. Considering the fact that the plasmid vaccination elicited a much higher T cell response but much lower antibody response than those elicited by the protein encoded by the plasmid, F1–sIgG [6], CTL might therefore be considered to have a substantial role in enhancing the protection level. In general, the antibody response induced by the DNA vaccination is lower than that induced by protein

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Table 1
B cell and T cell responses in immunized swine

<table>
<thead>
<tr>
<th>Swine groups</th>
<th>Swine 1</th>
<th>Swine 2</th>
<th>Swine 3</th>
<th>Swine 4</th>
<th>Swine 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCEIS + pIL2S injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>T cell proliferation (SI)</td>
<td>12.5</td>
<td>15.5</td>
<td>13.5</td>
<td>13.6</td>
<td>9.9</td>
</tr>
<tr>
<td>Antibody titer (× LD_{50} M)</td>
<td>10^2</td>
<td>10^2</td>
<td>10^2</td>
<td>10^2</td>
<td>10^1</td>
</tr>
<tr>
<td>pCEIS injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cell proliferation (SI)</td>
<td>4.5</td>
<td>6.6</td>
<td>3.3</td>
<td>5.3</td>
<td>N.D.</td>
</tr>
<tr>
<td>Antibody titer (× LD_{50} M)</td>
<td>10^2</td>
<td>10^2</td>
<td>10^2</td>
<td>10^2</td>
<td>N.D.</td>
</tr>
<tr>
<td>pIL2S injection</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cell proliferation (SI)</td>
<td>1.3</td>
<td>1.1</td>
<td>1.3</td>
<td>N.D.</td>
<td>1.2</td>
</tr>
<tr>
<td>Antibody titer (× LD_{50} M)</td>
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<td>0</td>
<td>0</td>
<td>N.D.</td>
<td>0</td>
</tr>
<tr>
<td>pCIS injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cell proliferation (SI)</td>
<td>1.1</td>
<td>0.8</td>
<td>1.5</td>
<td>1.3</td>
<td>1.0</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PBS injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T cell proliferation (SI)</td>
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<td>N.D.</td>
<td>1.1</td>
<td>0.7</td>
<td>0.4</td>
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<tr>
<td>Antibody titer (× LD_{50} M)</td>
<td>0</td>
<td>N.D.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Each group contained five swine. Each swine was injected with 200 μg plasmids (pCEIS, pCIS or pIL2S) solved in PBS. The animals were administered twice within a 4-week interval. Sera were collected for testing 10 days after the second administration. N.D.: not determined (due to blood clotting occurring during collection).

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Table 2
FMD viral challenge in swine

<table>
<thead>
<tr>
<th>Swine group</th>
<th>Number of swine infected/number of swine challenged</th>
<th>Percentage of protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCEIS + pIL2S</td>
<td>0/5</td>
<td>100</td>
</tr>
<tr>
<td>pCEIS</td>
<td>0/5</td>
<td>100</td>
</tr>
<tr>
<td>pIL2S</td>
<td>5/5</td>
<td>0</td>
</tr>
<tr>
<td>pCIS</td>
<td>5/5</td>
<td>0</td>
</tr>
<tr>
<td>PBS</td>
<td>5/5</td>
<td>0</td>
</tr>
</tbody>
</table>
vaccination. Since antibody response is an important element in protection against FMDV, there are several approaches we can try to improve the immunogenicity of the B cell response. These approaches include adding a leading signal sequence to the 5’ of the DNA sequence encoding the antigen, which may facilitate the secretion of the antigen after expression inside the cells, or by giving an additional protein boost administration to generate a protective antibody response.

Studies on the use of cytokines as adjuvants during vaccination have steadily increased, and the most common cytokines, including IL-1 and IL-2, are frequently used in combination with viral antigens to enhance their immunogenicity [9,10,18,23,24]. In our study, co-administration of IL-2 gene together with pCEIS enhanced the T cell proliferation reaction, but had no significant effect on the B cell response. IL-2 has been identified as a strong T cell growth factor and is critical for the proliferation and clonal expansion of antigen-specific T cells [25]. Cytotoxic lymphocytes (CTLs) response was greatly enhanced in the present of IL-2, either from a direct injection of IL-2 molecules [26] or the expression products of IL-2 genes transfected to the cells [27,28]. It was also found that co-administration of plasmids encoding IL-2 with the antigen dramatically increased the Th cells proliferation [18]. This enhancing effect was very strong for Th1 cells and relatively mild for Th2 cells [29].

IL-2 can enhance the proliferation of B cells, thus enhancing the humoral response directly. IL-2 regulates the B cell response mostly through an indirect way. IL-2 activates the proliferation of Th cells, especially Th2 cells, and stimulates the production of Th2 cytokines, including IL-4, IL-6 and so called ‘professional’ B cell growth factors such as IL-5 and IL-10. These Th2 cytokines can increase the growth and differentiation of activated B cells. In a result, they stimulate IgG, IgA, IgM production and enhance IL-4-induced IgE synthesis. Therefore, the Th cells are the important bridge between the IL-2 and B cells. The T cells stimulated by inoculation with pCEIS were mostly CTLs but not Th cells. This may be the reason why there was no detectable enhancement of B cell response in our experiment. Further improvement of the immunogenicity of pCEIS could be made by simultaneous application of combinations of cytokines (i.e. IL-1 and IL-2) [30]. Nevertheless, our results indicate that vaccination by intramuscularly administrating the pCEIS plasmid and co-administrating pCEIS plus plL5 successfully protects animals from FMD infection. The co-administration approach has also been demonstrated to be an important strategy for enhancing immunogenicity during vaccination.

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References


