Immune response in mice inoculated with plasmid DNAs containing multiple-epitopes of foot-and-mouth disease virus

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Abstract

This paper focuses on the development of candidate DNA vaccine encoding antigenic epitopes of type O foot-and-mouth disease virus (FMDV). A series of plasmids encoding different combinations of B cell epitopes and a T cell epitope were constructed and characterized by inoculating BALB/c mice. The specific antibodies were only detectable in the mice inoculated with plasmids encoding the T cell epitope and B cell epitopes from sites 5 and 1, within which site 5 includes residues 135–167 of VP1 and site 1 includes 141–160 region (G–H loop) and carboxyl terminus of VP1. Stronger cellular immune responses were also observed in these mice using T cell proliferation assay.

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The foot-and-mouth disease virus (FMDV) is a member of the picornavirus family. The virus particle contains 60 copies of each of four structural proteins designated VP1, 2, 3 and 4, respectively [1]. Five sites containing B cell epitopes were defined on these proteins through monoclonal antibody escape mutant studies [2,3]. Site 1 is the predominant site encoding two VP1 epitopes (residues 141–160 and 200–213 of VP1), which was able to protect animals from viral attack [4]. Site 3, encoding residues 40–60 of VP1, has been shown to modulate the function of epitopes encoded by site 1 [5]. Moreover, site 2 (encoding residues 70–78 and 131–134 of VP2), site 4 (encoding residues 56–58 on VP3) and site 5 (encoding residue 149) function independently [2,6]. However, livestock were not fully protected from virus challenge [7]. Many scientists have focused their interests on DNA vaccine against foot-and-mouth disease (FMD) [8–11]. But to date, none of the recombinant plasmid DNA encoding these antigenic sites were comparable with inactivated vaccines [12], and it is far necessary to find a better combination of these sites. Site 1 is linear, whereas all the other identified sites are conformational, or less conformationally dependent [2]. So it is easy to be mimicked for site 1 than other four sites. The aim of this work was to mimic the epitopes from the known sites in the form of ‘nude DNA’, and to find a better combination of these sites.

For assessing the different combination of these sites, a multiple-epitopes gene (SG) was synthesized according to the sequence of the five sites and the sequence encoding a single T cell epitope (T site) of VP1 [13]. Linkers, comprised of glycine and serine, were used to avoid the interference resulting from the direct ligation between epitopes or subsequent generation of new epitopes. The residue 135–167 in VP1 was retained as a flanking sequence of site 5 (Fig. 1a).

Five plasmids were constructed in frame with Glutathione S-transferase (GST) in order to evaluate the different combinations (Fig. 1a–e) in a GST-fusion expression system [14]. Those fusion proteins, GST-SG (45 kDa), GST-N4 (44 kDa), GST-T51 (40 kDa), GST-N51 (35 kDa), and GST-51 (36 kDa) compared with GST alone (26 kDa) (Fig. 2b, lanes 7–12) were induced with 1 mM IPTG (iso-propyl β-d-thiogalactoside) in E. coli k802 strain and then separated by SDS-PAGE. The separated proteins were either electrophoretically transferred onto nitrocellulose membrane for western blotting analysis (Fig. 2a) or stained with 0.05% Coommassie Brilliant Blue (Fig. 2b). As shown in Fig. 2a, all the fusion proteins, instead of GST, positively reacted with polyclonal antibodies against FMDV type O (purchased from Lanzhou Veterinary Institute, China). The band corresponding to SG or N4 gene presented a stronger reaction with positive serum (Fig. 2a, lanes 2 and 4) than bands corresponding to other combinations (Fig. 2a, lanes...
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1, 3 and 6). The results showed that these denatured recombinant proteins were specifically recognized by positive serum. However, it was not enough in reflection of the naturally conformational characteristics of those epitopes.

To evaluate the immunogenicity of these five combinations in mice, the five genes were cloned into pVAX1 vector (Invitrogen) under the promoter of cytomegalovirus. The vector pVAX1 has almost the same backbone with its mother vector, pcDNA3.1, except the replacement resistant gene from ampicillin to kanamycin. And the expression of recombinant proteins from pVAX1 was comparable to those achieved with pcDNA3.1[15,16]. The five constructions were pVAX-SG (Fig. 1a), pVAX-N4 (Fig. 1b), pVAX-N51 (Fig. 1c), pVAX-T51 (Fig. 1d) and pVAX-51 (Fig. 1e). Each was confirmed by sequencing. All plasmids were prepared according to the instruction of the kit and dissolved in phosphate-buffered saline (PBS) at a concentration of 2 mg ml\(^{-1}\) (Qiagen, Chatsworth, California).

Forty-two BALB/c mice (female, 4–15 weeks old) were randomly divided into seven groups. Groups 1 and 2 are negative controls and were inoculated with PBS and pVAX1 vector, respectively. Animals from groups 3 to 7 were inoculated with pVAX-SG, pVAX-N4, pVAX-N51 (Fig. 1c), pVAX-T51 (Fig. 1d) and pVAX-51 (Fig. 1e). Each was confirmed by sequencing. All plasmids were prepared according to the instruction of the kit and dissolved in phosphate-buffered saline (PBS) at a concentration of 2 mg ml\(^{-1}\) (Qiagen, Chatsworth, California).

In this experiment, the reliability of plasmid DNAs to experimental animals depended on two parts: pyrogen level (including endotoxin) in extracted plasmid DNA was lower than 20 EU mg\(^{-1}\), and there was no protein detectable by argent-dyeing SDS-PAGE. Throughout the experiment, all the immunized mice remained healthy. There was no adverse reaction after immunization including lethargy, weight loss, etc. (data not shown). Results were restrictively compared between every two groups using Student’s t-test.

All sera were collected weekly and kept at \(-20^\circ\text{C}\) for detection of specific anti-virus antibody. IgG antibody responses to FMDV type O were measured in sera samples by an indirect enzyme-linked immunosorbent assay (ELISA) as previously described [17]. The titres were calculated...
as $A_{492}$ at a 1:50 dilution of samples. Specific antibody (IgG) were detectable in mice immunized with plasmids pVAX-SG, pVAX-N4, and pVAX-T51, but not in those immunized with plasmids pVAX-N51 and pVAX-51 and not in the control mice. The antibodies of group pVAX-SG or pVAX-N4 were higher than that of group pVAX-T51. Specific antibody increased 1 week after the first immunization (WAFI) with three plasmids (pVAX-SG, pVAX-N4, or pVAX-T51) and rose quickly after booster. The antibody peaked at 4 WAFI (Fig. 3a). The virus neutralization antibody (VNA) anti-homologous virus (Table 1) was also determined in a micro-neutralization test according to the method of Crowther et al. [2]. The sera of six mice in one group were pooled together. The end-point titres were calculated as the reciprocal of the last serum dilution to neutralize 50 TCID$_{50}$ of serotype O1K HK type FMDV in 50% of the wells (Table 1). The VNA titres confirmed the results shown in Fig. 2. The VNA was only detectable in groups pVAX-SG, pVAX-N4, and pVAX-T51, and the VNA titre of group pVAX-T51 was lower than that of group pVAX-SG or pVAX-N4 (Table 1). The similar antibody level between group SG and N4 implied no effect of site 4 in inducing humoral immunity, which maybe due to the difficulty in producing specific antibody against site 4 [2]. Site 3 has been shown to modulate the function of epitopes encoded by site 1 [5]. And sites 3 and 2 elicited specific antibody independently [2,6]. So both sites 3 and 2 possibly contributed to the higher responses of SG/N4 versus T51 clones. More experiments remains further studies to determine which site or both

Table 1

<table>
<thead>
<tr>
<th>Test samples</th>
<th>Number of animals</th>
<th>Week after first immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td>pVAX-T51</td>
<td>6</td>
<td>0.67 1.15 0.85 0.75</td>
</tr>
<tr>
<td>pVAX-N4</td>
<td>6</td>
<td>0.6 1.15 0.85 0.75</td>
</tr>
<tr>
<td>pVAX-T51</td>
<td>6</td>
<td>0.3 0.6 0.55 0.5</td>
</tr>
<tr>
<td>pVAX-N51</td>
<td>6</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>pVAX-S1</td>
<td>6</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>pVAX</td>
<td>6</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>PBS</td>
<td>6</td>
<td>0 0 0 0</td>
</tr>
</tbody>
</table>

The sera of six mice in one group were pooled together. 1 Log$_{10}$ reciprocal antibody titre to neutralize 50 TCID$_{50}$ of homologous FMDV in 50% of the wells.
contribute to the elevation of responses of SG/N4 clones. Site 5 possible was also involved in the elevation of antibody level of pVAX-SG/N4 clones through the interaction of G-H loop region with residues within VP2 and VP3 [2].

The cellular immune response was assayed by the proliferative ability of immunized splenocytes in vitro as described elsewhere [18]. In brief, all mice were killed 8 days post-vaccination, and the splenocytes of six mice in one group were pooled together. The splenocytes (1 × 10^7 ml^{-1}) were incubated with 10 μg ml^{-1} of homologous type O viral whole protein (purchased from Lanzhou Veterinary Institute) in triplicate wells of flat bottom plates (Nunc DenMark). Cells cultivated in medium alone served as negative controls. The plates were incubated in a 5% CO₂ humidified incubator at 37°C for 3 days, and OD_{570} was measured after 3 days by a standard 3-(4,5-dimethylthiazol-2-yI)-2,5-diphenyltetrazolium bromide (MTT) method. The results are expressed as the value of stimulation index (SI). SI value was calculated as the ratio of absorbent value at 570 nm of pools incubated with viral whole protein to that of pools incubated with medium only.

There was a significant proliferation of splenocytes in experimental mice but not in control ones (Fig. 3B). Plasmid pVAX-SG, pVAX-N4 or pVAX-T5I stimulated a higher level of proliferation of splenocytes in mice with SI value between 7.09 and 7.25 than pVAX-N51 or pVAX-51 did (with SI value between 3.17 and 3.23) (P < 0.05). These results indicated that the different rates of proliferation between each group were closely related to sites 5, 1 and the T cell epitope. T cell immune response was important not only in helping antibody eliciting [19], but also in possibly neutralizing epitopes from type O foot-and-mouth disease virus. This work was supported by a grant from the National Foundation of Science (2001AA213111).

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References


