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Vaccination of mice with plasmids expressing processed capsid protein of foot-and-mouth disease virus—Importance of dominant and subdominant epitopes for antigenicity and protection

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Abstract

The capsid of foot-and-mouth disease virus (FMDV) displays several independent B cell epitopes, which stimulate the production of neutralising antibodies. Some of these epitopes are highly variable between virus strains, but dominate the immune response. The site A on VP1 is the most prominent example of a dominant and variable site. This variability is a problem when designing vaccines against this disease, because it necessitates a close match between vaccine strain and virus in an outbreak. We have introduced a series of mutations into viral capsid proteins with the aim of selectively silencing two dominant and highly variable epitopes and thereby divert immune responses toward less dominant but more conserved, protective epitopes. When mice were immunized with modified antigens, the resulting immune responses showed a higher degree of cross-reactivity towards heterologous virus as compared to mice vaccinated with wild type epitopes. Most of the modifications did not adversely affect the ability of the plasmids to induce complete protection of mice against homologous challenge.

Keywords: Foot-and-mouth disease; FMDV; B-cell epitopes; Protection; Cross-reactivity

1. Introduction

Foot-and-mouth disease virus (FMDV) remains a serious threat to livestock production. Although eradicated in large parts of the world, introduction of virus from infected areas is a risk to be taken very seriously, as recently demonstrated by outbreaks in the United Kingdom and elsewhere. Outbreaks in previously FMDV free areas are usually dealt with by culling and subsequent destruction of all animals on infected premises. However, this is a very costly control strategy, and therefore only possible in the more economically fortunate countries. Furthermore, large scale culling and destruction of potential sources of food is not only an economic, but also an ethical and environmental issue. For these reasons, vaccination is now more likely to be employed as a control strategy than just a few years ago.

There are several problems when contemplating vaccination against FMDV. One of these is the presence of extensive antigenic variation between different FMDV isolates. In order to be prepared for the introduction of the virus, vaccine must be pre-fabricated and stocked before an outbreak. Thus, the choice of vaccine strain is crucial. Seven serotypes of FMDV (types A, O, C, Asia1 and SAT-1, -2, -3) have been identified. There is considerable variation not only between serotypes,

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but also within serotypes. In the case of an outbreak of FMD, these variations currently necessitate the use of vaccines matched with the current strain of virus.

The present strategy is to select vaccine strains based on knowledge of currently circulating virus strains in different parts of the world, assuming that an introduction will come from such a 'hot spot'. Even though this may be the case, the availability of a more serotype cross-reactive vaccine would certainly increase the applicability of a strategic vaccine reserve.

The three-dimensional as well as the antigenic structure of FMDV particles are well described for several different serotypes of FMDV [1–3]. Several independent neutralising epitopes have been defined [4–8]. These and other studies have revealed large antigenic variability in some of these neutralising epitopes. This variability allows virus variants to evade immune control [9]. A strong antibody response against the highly exposed and highly variable G–H loop within VP1 (1D) is known to be protective against the virus. This G–H loop specific antibody response is known to play a major role in protection induced by the current FMDV vaccines [10]. However, due to high sequence variation in this loop within and between serotypes, it could be more advantageous to direct the immune response towards other protective epitopes in which the variability is less pronounced. The existence of such epitopes, and their protective capacity has been demonstrated previously by others [4,5]. Thus, by selectively deleting the variable epitopes, we aim at directing the immune response to more conservative and cross-reactive epitopes.

We present here a series of experiments to elaborate further on this hypothesis. We have studied a serotype C virus and have shown previosly, that DNA vaccination with plasmids, which express a P1 + 3C cassette, induces an immune response in mice, which confers protection to challenge by homologous virus (Frimann et al., submitted for publication). We have now selectively modified two dominant B cell epitopes by site-directed mutagenesis, with the aim of directing the immune response towards other, subdominant, B cell epitopes of the virus. One of these is a linear B cell epitope, designated site A [1], comprised of the G–H loop of VP1, while the other is the conformational site D epitope [1], which is comprised of amino acids from VP1, VP2 and VP3. Both epitopes are major targets for the humoral immune response against this particular virus, and both epitopes have been mapped in detail, enabling the specific design of mutant antigens. The mutations have been designed to replace highly antigenic with less antigenic amino acids [11]. Mice were vaccinated using plasmid DNA encoding eight different mutated constructs, and the humoral immune response was analysed towards both homologous and heterologous virus. In addition, the vaccinated mice were challenged with a lethal dose of homologous FMDV to test whether changes could be introduced without negatively affecting the protective capacity of the immune response induced by the modified antigens.

2. Materials and methods

2.1. Construction of plasmid DNA encoding mutated FMDV capsids

A DNA plasmid, pVax1/P1/3C, encoding the P1-region and the 3C-protease from serotype C-S8 (Frimann et al., submitted for publication), was used as template for site directed mutagenesis. Eight different mutated plasmids were produced (Table 1).

The QuickChange XL Site-Directed Mutagenesis Kit (Stratagene, CA, USA) was used as described by the manufacturer to introduce single point mutations, substitutions or deletions. Both the mutagenic primers contained the desired mutation and annealed at the same part of the sequence but on opposite strands of the plasmid (Table 2).

To introduce multiple point mutations simultaneously, as in Mutant 4D and Mutant 1A4D, the same system was used. Primers from the same strand of the template plasmid were used (Table 2), and each of them contained one of the desired mutations. Three mutated plasmids involving site A in VP1 (the G–H loop) were constructed. In Mutant 1A the entire G–H loop from aa 136–150 of VP1 was replaced with 10 glycine residues using the sense primer VP1/136–150/1 and antisense primer VP1/136–150/2.

Mutant 2A was created by replacing part of the G–H loop (aa 139–146) of VP1 with three glycine residues using the following sense and antisense primers VP1/139–146/1 and VP1/139–146/2. For construction of Mutant 3A, the RGD_{143} motif was deleted using primers VP1ΔRGD/1 and VP1ΔRGD/2.

Four different plasmids with modifications in the conformational antigenic site D were constructed and termed 1D, 2D, 3D and 4D, respectively. Mutants 1D, 2D and 3D contained single point mutations, while in mutant 4D each of these three mutations were combined in one construct. For Mutant 1D sense primer VP1/193/1 and antisense primer VP1/193/2 was used to substitute Thr(193) in VP1 (site desig-

Table 1
Modifications were introduced in two different antigenic sites (sites A and D) of FMDV serotype C-S8 by site directed mutagenesis

Mutant	Antigenic site	Mutation
Mutant 1A	A	aa 136–150 in VP1 replaced with 10 Gly
Mutant 2A	A	aa 139–146 in VP1 replaced with 3 Gly
Mutant 3A	A	RGD_{143} deleted
Mutant 1D	D (D1)	VP1: Thr(193) → Ala
Mutant 2D	D (D2)	VP2: Asn(74) → Ala
Mutant 3D	D (D3)	VP3: Glu(58) → Ala
Mutant 4D	D	VP1: Thr(193) → Ala VP2: Asn(74) → Ala VP3: Glu(58) → Ala
Mutant 1A4D	A and D	VP1: aa 136–150 replaced with 10 Gly VP1: Thr(193) → Ala VP2: Asn(74) → Ala VP3: Glu(58) → Ala

Replacements and substitutions are shown.