Full protection of swine against foot-and-mouth disease by a bivalent B-cell epitope dendrimer peptide vaccine

Esther Blanco  David Andreu

Rodrigo Cañas
Patricia de León
M. Jose Bustos
Elisa Torres
Miguel Rodríguez-Pulido
Marga Sáiz
Conventional FMD vaccines

CONVENTIONAL VACCINES
(chemically inactivated viruses)

+ Good protection against antigenically related viruses.
- Its use is instrumental for disease control
- Risk of viral escapes from vaccine production plants
- A cold-chain is required
- Need for differentiation between infected and vaccinated animals
- Antigenic match between field viruses and vaccine strains

“Vaccination to live”: recently gained acceptance

- World Organization for Animal Health (OIE) Code:
  - recognized a new category “FMD-free country/zone where vaccination is practised”.
  - Reduced the waiting periods to recover the status when vaccination is applied during emergencies.
- Vaccination minimizes the dependence on large-scale culling of animals to control the disease.
Peptide-based vaccines

**Pros**
- No infectious agent involved
- High structural flexibility. Easily adaptable to new, different strains
- Combination of B and T cell epitopes
- Total differentiation between infected and vaccinated animals (DIVA)
- Safe storage and transport (no cold-chain required)
- Fast, affordable large scale production

**Cons**
- Most epitopes are conformational (discontinuous)
- Conformational epitopes difficult to reproduce by chemical means
- Immunogenicity usually needs to be enhanced by: adjuvants, multimerization
VP1 GH loop: a B cell antigenic site in FMDV capsid used for peptide vaccines design

- A continuous B cell site that can be mimicked using synthetic peptides
- Highly variable in sequence: serotype specific
- Limited immunogenicity of GH loop linear peptides

Site D

Site C

GH loop (Site A)

Integrin-Binding Motif (RGD)
An improved synthetic peptide vaccine: pig as a model

Multivalent, dendrimeric scaffolds (dendrimers)

- Optimal replicas of continuous B cell epitopes
- GH loop
- T helper (CD4+) lymphocytes
- Sequence conservation among serotypes
- Widely recognised by porcine MHC class II

3A 21-35

The dendrimeric peptide of type C FMDV

<table>
<thead>
<tr>
<th>Peptide</th>
<th>FMDV protein residues</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>VP1 [136-154]</td>
<td>YTASARGDLAHLLTHARH-amide</td>
</tr>
</tbody>
</table>

* Thioether linkage (C-terminal)
Arrow indicates a putative cathepsin D cleavage site

Solid, Sterilizing Protection

Neutralizing Ab
Specific IgA in serum and mucosae
FMDV-specific T cell responses

Peptides as vaccines against foot-and-mouth disease virus

Extension to other FMDVs epidemiologically relevant: type O (O UK 2001)

Dissecting the B4T components responsible for the immunity observed: Improved, cost-effective dendrimeric constructions

- Mouse (screening model)
- Swine

Patents P20130101063, P20130101063, 2013800289123
Is tetravalency essential?
$B_4T$ vs. $B_2T$ constructs (O UK 2001)

Swiss mice “outbred”
(100 µg peptide/mouse)

B epitope: VP1 140-160
T epitope: 3A 21-35

thioether linkage (C-terminal)

FMDV antibody titters (log 10)

Days postimmunization

Day 19 pb

2. Orientation and attachment mode also matter!
Protection conferred in pigs by type O tetra - and bivalent dendrimers (O UK 2001)

2 mg peptide/ 2 doses
Montanide ISA 50V2 (commercial oil adjuvant)

n = 6 pigs

Total and neutralizing Ab elicited by type O tetra - and bivalent dendrimers
Bivalent dendrimers elicit higher levels of IFNγ-producing activated T cells

Esther Blanco et al., et al. Antiviral Res. (2016)
Bivalent dendrimer $\text{B}_2\text{T (mal)}$ elicits long lasting neutralizing antibodies associated with the induction of IFNγ-producing T cells.

**VNT**

**ELISPOT IFNγ**
Dendrimers can afford solid FMD protection: ongoing work with this modular approach

- Optimizing conjugation chemistry and inclusion of new T cell epitopes taking advantage of the versatility of the dendrimeric modular approach.

- Incorporation of different B cell peptide sequences in single dendrimers/combination of different dendrimeric molecules
Isotypes of the Ab elicited by type O tetra - and bivalent dendrimers

- This results suggests association between a Th-1 biased isotype balance (lower IgG1/IgG2 ratio) and improved protection

- IgG2 and IgA can enhance T cell responses via an FcR-mediated mechanism