Objectives

Previous research on C-S strains of FMDV has shown that the GH loop can be generally reproduced by small 15-mer peptides. The study of the biospecific interactions between such peptides and anti-FMDV neutralising antibodies is therefore central to an understanding of the molecular mechanisms involved in viral infection and may help in the design of synthetic vaccines. The present research work had, in this context, three major goals:

1 Development of an SPR kinetic assay for the screening of small antigenic peptides from foot-and-mouth disease virus

The features of SPR biosensors such as BIAcore 1000 make them more atractive than ELISA or other immuno-enzymatic techniques for the fast and detailed screening of synthetic antigens.

Given the large numbers of variant FMDV peptides usually synthesised and evaluated in our group, the only practical approach to perform such screenings consists on antibody immobilisation and peptide injection. Unfortunately, direct detection of analytes smaller than 5 kDa is unadvisable with standard BIAcore 1000 instruments, and our peptides are only 30% of this molecular weight cut-off. The first objective of this work was, therefore, the search and optimisation of an adequate experimental methodology for the systematic antigenic evaluation of pentadecapeptides from the GH loop (site A) of foot-and-mouth disease virus as soluble analytes.

2 Analysis of the antigenic determinants within site A of FMDV C_1-Barcelona

An FMDV field variant, isolate C_1-Barcelona (also named C-S30), contains four changes within the main antigenic site A, at least one of which involving a highly conserved position, critical for antibody recognition in the reference strain C-S8c1. However, FMDV C-S30 behaves similarly to C-S8c1 toward site A-directed monoclonal antibodies such as 4C4. This remarkable behaviour directly led to the second objective, namely the SPR characterisation of synthetic peptides displaying all possible combinations of the four mutations. The study was further complemented by ELISA and NMR analyses.

3 Study of multiply-substituted peptides combining antigenicity-enhancing amino acid replacements

Previous ELISA studies on the effect of single-residue changes on the antigenicity of site A peptides pointed out to five replacements which turned out to be equally or even more antigenic than the native C-S8c1 sequence towards a panel of seven anti-site A monoclonal antibodies. The synthesis and systematic SPR screening of peptides reproducing all possible combinations of such substitutions, in an attempt to find antigenicity-enhancing effects, was the third target of the present research. Further ELISA and structural analysis complemented the study of the peptides.