Immunization against Foot-and-Mouth Disease with Synthetic Peptides Representing the C-terminal Region of VP1

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SUMMARY

Foot-and-mouth disease virus challenge experiments in guinea-pigs and immunoassays with a range of peptides equivalent to either or both of the sequences 141 to 158 and 200 to 213 of VP1 showed the most effective structure, in terms of protection, to be one in which both 'sites' were present with a minimum of additional amino acids. An 80 residue peptide comprising amino acids 134 to 213 was considerably less effective than 40 or 45 residue peptides. The major site for the induction of protection was deduced to be in the region 141 to 158. Thus, protection with the 40 or 45 residue peptide did not appear to be due to the presence of antibody directed solely to the 200 to 213 sequence. Finally, induction of antibody to the latter site appeared to be dependent on both the size of the peptide and the disposition of 'sites' within it.

In a previous communication, we demonstrated protection of cattle against challenge with foot-and-mouth-disease virus (FMDV) by immunization with a synthetic peptide (DiMarchi et al., 1986). The sequence used was based on the published data of Strohmaier et al. (1982) for the coat protein, VP1 of the O, Kaufbeuren (O, K) strain of the virus and was Cys-Cys-200-213-Pro-Pro-Ser-141-158-Pro-Cys-Gly (throughout this communication sequences unique to VP1 are shown numerically, whereas 'additional' amino acids used in the construction of the peptide are shown by their three-letter codes). The peptide differed from those described by Bittle et al. (1982) and Pfaff et al. (1982) in a number of important respects. First, it incorporated the 200 to 213 sequence of VP1 which had been recognized by Strohmaier et al. (1982) and Bittle et al. (1982) as capable of inducing low levels of neutralizing antibody. Second, the 200 to 213 and 141 to 158 sequences were linked via Pro-Pro-Ser and terminated with Cys-Cys (N terminus) and Pro-Cys-Gly (C terminus). The broad aim of this construction was the formation of a cyclic/polymerized peptide that would not require a carrier protein. Protection tests in guinea-pigs showed this carrier-free peptide (P40) to be considerably more effective than either 141–158-Pro-Cys-Gly (P21) or P21 linked to keyhole limpet haemocyanin. These results prompted questions on the role of the 200 to 213 sequences in the induction of a protective immune response.

We have directed our attention to delineating the structural basis for the action of P40 and have prepared by solid-phase synthetic methods (Merrifield et al., 1982) a number of peptides which represent regions of VP1 of the O, K strain of FMDV. Each peptide was purified to near homogeneity by preparative reverse-phase chromatography. The following peptides were synthesized and evaluated: P14, 200–213; P27, 134–160; P40, Cys-Cys-200–213-Pro-Pro-Ser-141–158-Pro-Cys-Gly; P45, (134–160) (196–213); P53, 161–213; P80, 134–213.

For a given peptide, a range of concentrations was prepared in 0.04 M-phosphate buffer pH 7.6 and emulsified with an equal volume of Freund's complete adjuvant. A guinea-pig dose of 0.2 ml contained either 125, 25, 5 or 1 nmol of the stated peptide. Each vaccine was injected by the subcutaneous route into four Dunkin Hartley guinea-pigs. Blood samples were taken at 27
**Table 1. Protection of guinea-pigs vaccinated with different synthetic peptides**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Dose of peptide vaccine (nmol)</th>
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<tr>
<td></td>
<td>125</td>
<td>25</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>P14</td>
<td>0/4*</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
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<tr>
<td>P27</td>
<td>3/4</td>
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<tr>
<td>P40</td>
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<td>P45</td>
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<td>P53</td>
<td>0/4</td>
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<tr>
<td>P80</td>
<td>1/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Control</td>
<td>0/5</td>
<td></td>
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</tbody>
</table>

* Number of guinea-pigs protected/number challenged.

days post-vaccination (p.v.) and pools of the individual sera within a group of four guinea-pigs were made. All animals were challenged 28 days p.v. with 3000 guinea-pig infectious doses of virulent O₁K virus by the intraplantar route. This challenge procedure is used routinely in our laboratory to evaluate conventional FMDV vaccines, although more recent studies with P40 in guinea-pigs suggest that antibody rises to maximum titres at 21 rather than 28 days p.v.

The titres of serum antibody to peptide and FMDV were determined by ELISA. Briefly, microtitre plates (Nunc Immunoplates II) were coated with either 100 µl per well of 1 µg/ml pure O₁ BFS 1860 virus or 1 µg/ml of P14, 2 µg/ml of P27, 3 µg/ml of P40, 3 µg/ml of P45, 4 µg/ml of P53 or 6 µg/ml of P80. These concentrations were chosen to give approximately equimolar coating solutions. Sera were titrated on the plates and specific antibodies were detected using horseradish peroxidase conjugated to rabbit anti-bovine or anti-guinea-pig immunoglobulins. Antibody titres were determined as the log₁₀ of the antiserum dilution giving 1 A₄₉₂ unit. Titres of 1.7 (equivalent to 50-fold dilution of antiserum) or less were considered negative.

Table 1 gives the results of the guinea-pig challenge experiment. The most effective peptides were P40 and P45. Each of these peptides induced comparable levels of protection, the criterion of protection being the absence of lesions other than at the site of challenge. Only one other peptide, P27, protected a significant number of animals. P27 appeared to have similar activity to the sequence 141–158-Pro-Cys-Gly which we showed previously to protect guinea-pigs at the highest dose used (DiMarchi et al., 1986). P14 and P53, which lack the 141 to 158 sequence, were completely ineffective and the largest peptide, P80, protected only one guinea-pig.

Sera from both convalescent and virus-vaccinated cattle were tested for anti-peptide activity and a typical set of data is shown in Fig. 1. In this particular experiment, a cow was vaccinated with inactivated virus and serum taken at 21 days p.v. The predominant activity was against P40 and P45. Antibody titres against the other peptides were lower, and in the case of P14 were negative.

The anti-virus titres of the guinea-pig sera are shown in Fig. 2. Consistent with the guinea-pig protection data, the highest antibody titres were seen with P40, P45 and, to a lesser extent, P27. Neither P14, P53 nor P80 induced significant levels of antibody to virus at any dose of peptide used. The same sera were also assayed in a microneutralization test. Essentially identical results were observed, indicating that the ELISA titres were directed primarily against intact virus (results not shown).

In the final experiment, the sera from those guinea-pigs vaccinated with the highest dose of each peptide were assayed both for antibody against the immunizing peptide (homologous) and the other peptides (heterologous). Fig. 3 shows the results of this experiment. In common with its general lack of activity in the previous experiments, P14 failed to stimulate homologous or heterologous antibody. P27 stimulated antibody to itself, and this antibody reacted similarly with P40 and to a lesser extent with P45, hardly at all with P80 and not at all with P53 and P14. P40 stimulated a high level of homologous antibody which, in descending order, reacted to a lesser extent with P45, P27, P80 and P53. Considering that the only sequence common to P53 and P40 is 200 to 213, it is interesting that anti-P40 antibody reacted with P53 and not P14. The antibody induced by P45 was clearly different from that induced by P40, notably having very
Fig. 1. ELISA of bovine antiserum to the O, BFS strain of FMDV with different synthetic peptides. V indicates the reaction in wells coated with 1 μg/ml of virus. The arrow indicates log₁₀ 1.7, below which sera were regarded as negative.

Fig. 2. ELISA of pooled guinea-pig antisera from the experiment shown in Table 1, titrated against O, BFS virus on the ELISA plate. For each peptide vaccine, the four bars represent, from left to right, groups of guinea-pigs receiving a dose of 125, 25, 5 or 1 nmol of peptide.

Fig. 3. ELISA of pooled guinea-pig antisera equivalent to the 125 nmol peptide vaccine groups of Table 1, titrated against each of the peptides. For each peptide vaccine, indicated below the figure (P14 etc.), the six bars represent, from left to right, P14, P27, P40, P45, P53 and P80 peptide-coated wells. For clarity, the 'homologous' reaction is shown as a solid bar.

much higher anti-P53 and P80 titres although not as high as the homologous and anti-P40 titres. Perhaps most significant is the fact that P45 did induce anti-P14 antibody. P53 induced high antibody titres against all of the peptides with the exception of P27, the highest titre being against a heterologous peptide, P45. P80 induced an almost identical antibody profile to that of P53 with the exception of a slightly reduced homologous titre.
Our results demonstrate clearly the dominance of the 141 to 158 sequence of VP1 in relation to protection. Neither P14 nor P53, which lack this sequence but contain the 200 to 213 sequence, protected guinea-pigs. Indeed, P14 did not induce anti-virus or anti-peptide antibodies, nor did convalescent or virus vaccine sera appear to contain antibody to P14. However, anti-virus serum did contain antibody to P53. One explanation for this is that antibody to the virus recognizes sequences within P53 other than 200 to 213. Alternatively, P14 may present residues 200 to 213 in a conformation quite different from that which exists in the larger peptides or virus. This idea is supported by the observation that antibody to P40 failed to recognize P14 but did recognize P53, the only common sequence being 200 to 213. It was possible to induce antibody to P14 by immunizing with P45, P53 or P80.

Perhaps the most intriguing result was the difference between P40 and P45. Whereas P40, in which the 200 to 213 sequence was N-terminal to the 141 to 158 sequence, failed to induce P14 antibody, P45, in which the 200 to 213 sequence was C-terminal to the 141 to 158 sequence, induced high levels of antibody to P14. There are other minor differences between the two peptides, namely the fact that P45 has only one Cys, which is located at the amino terminus, and possesses five additional amino acids. However, we have shown previously that a version of P40 lacking the two Cys residues at the amino terminus (P38) was just as effective as P40 in guinea-pig protection tests (DiMarchi et al., 1986). It should also be noted that the junctions between the two 'sites' in both P40 and P45 are similar and are, respectively, Pro-Pro-Ser and Pro-Pro-Thr. Preliminary studies with a version of P40 in which Pro-Pro was replaced by Val-Val appear to indicate that the two proline residues are required for optimum activity (results not shown).

Despite the fact that P80 contains both the 200 to 213 sequence and the 141 to 158 sequence and all of the intervening amino acids, it failed to induce significant titres of anti-virus antibody and protected only one guinea-pig. It was recognized by bovine anti-virus serum. Furthermore, P80 failed to induce antibody to the 134 to 160 sequence (P27) suggesting that the reaction between P80 antibody and P40 or P45 was directed primarily against the 200 to 213 sequence.

On the basis of the current evidence, a peptide of approximately 40 amino acids comprising both 141 to 158 and 200 to 213 appears optimal. The sequence 141 to 158 appears to be the major site for generation of neutralizing and protective antibody. However, the additional presence of the sequence 200 to 213 once again significantly increased the potency of the peptide (DiMarchi et al., 1986). The absence of any significant concentration of P40 antibody directed against P14 strongly suggests that the presence of antibody directed solely against 200 to 213 is not the reason for the increased potency of P40 relative to P27. The failure of P14, P53 and P80 to protect guinea-pigs further supports this conclusion. However, a definitive answer can only be reached when selective removal of antibody against 200 to 213 is achieved with a peptide that is conformationally identical to that which exists in virus. Finally, the largest peptide, P80, despite containing both 141 to 158 and 200 to 213, appeared to be quite different from P40 and P45 in view of its failure to induce antibody to 134 to 150. We assume that this is due to masking of the 141 to 158 sequence during folding of P80 by virtue of the fact that antibody to P27 does not bind to P80.

We wish to thank Len Pullen for conducting the guinea-pig challenge work.

REFERENCES


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