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Heterotypic Protection Induced by Synthetic Peptides Corresponding to Three Serotypes of Foot-and-Mouth Disease Virus

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Synthetic peptide vaccines of the general sequence Cys-Cys(200–213)-Pro-Pro-Ser-(141–158)-Pro-Cys-Gly, where the numbered residues refer to VP1 sequences of three different strains of foot-and-mouth disease virus, have been evaluated in cattle and guinea pigs. High levels of serotype-specific (homotypic) antiviral and antipeptide antibody were produced with each peptide. The A- and O-serotype peptides provided complete protection of guinea pigs against their respective virus challenges. The C-serotype peptide appeared to be less effective than the other peptides. In cross-protection studies (heterotypic) in guinea pigs, it was possible to protect A-serotype peptide-vaccinated animals against O-virus challenge and vice versa. Some heterotypic protection was also achieved with the C-serotype peptide. The heterotypic protection observed related more to the presence of cross-reactive antipeptide antibody than to neutralizing antibody.

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hooved animals and, as such, is of great concern to the agricultural economies of the world. The measures used to control the disease include extensive and frequent prophylactic campaigns based on killed virus vaccines. However, there are a number of problems associated with the use of such vaccines. First, FMD virus (FMDV) is relatively unstable with respect to both temperature and mild acid conditions (5, 13). Second, the use and large-scale production of vaccine have been implicated in European outbreaks, where considerable attention is focused because of the low incidence of "natural" disease (1). For these and other reasons, a number of laboratories have attempted to develop alternative vaccines based on either the viral coat protein, VP1 (9), or synthetic peptides equivalent to specific regions of the same protein (2, 4, 12, 15). The 141–160 region of VP1 has been clearly identified as a major site for induction of virus-neutralizing antibody (VNA), and we have used it in the peptide Cys-Cys-(200–213)-Pro-Pro-Ser-(141–158)-Pro-Cys-Gly to protect cattle against intradermolingual challenge with the virulent O_1BFS 1860 strain of FMDV (4). This peptide also contained a second region of VP1, residues 200 to 213, which was initially recognized by Strohmaier et al. (17) as a minor neutralization site. Additional amino acids, denoted by their three-letter codes, were used to link the two FMDV sequences and to provide termini which would facilitate cyclization or polymerization of the peptide. By using such a construction, the need for a carrier protein, such as keyhole limpet hemocyanin, was eliminated.

A common expectation with synthetic peptide vaccines is the generation of highly specific antibodies such that vaccines will not be protected against variant viruses possessing minor mutations in their protein sequences (10; S. J. Barteling and R. Woortmeyer, paper presented at the 4th meeting of the European Group on Molecular Biology of Picornaviruses, 1985). Indeed, the heterogeneity of FMDV is such that it is necessary to produce conventional killed virus vaccines against each of the seven serotypes of the virus. Commercial producers invariably go one stage further and adapt field strains to growth in tissue culture so as to optimize vaccine potency. Furthermore, there is no evidence of protective enhancement between virus strains included in conventional trivalent vaccines (3).

In the present work, we have evaluated the protective capacity of three peptides, based on the VP1 sequences of three FMD serotypes, with particular reference to the question of heterotypic protection.

MATERIALS AND METHODS

Preparation of viruses. The following strains of FMDV were prepared from infected BHK-21 cell monolayers: O_1BFS 1860, O_1BFS 1848, O_1 Campos, O_1 Lausanne, O_1 Kaufbeuren, O SAU 4/83, O IND 53/79, O_2 Brescia, O AUR 1/81, C_3 Indaial, and A_{24} Cruzeiro. Each virus was clarified by centrifugation at 2,000 rpm for 20 min and concentrated by centrifugation at 35,000 rpm for 1 h. Virus pellets were suspended in 0.04 M phosphate buffer, pH 7.6, and centrifuged in 5 to 30% (wt/vol) sucrose gradients in phosphate buffer for 65 min at 40,000 rpm in a Beckman SW40 rotor. Purified virus peaks were identified and isolated by pumping the contents of each centrifuge tube through the flow cell of a spectrophotometer set to 260 nm. Quantification of the virus yields was done as described previously (6).

Preparation of synthetic peptides. The following peptides were prepared by solid-phase synthetic methods (11) and were based, respectively, on the published sequences of the VP1 proteins of the A_{24} Cruzeiro, C_3 Indaial, and O_1 Kaufbeuren strains of FMDV. Single-letter codes are given, with the "additional" amino acids underlined.

Peptide A40 .... C-C-R-H-K-Q-K-I-I-A-P-A-K-Q-L-L-P-P-S-G-S-G-R-R-G-D-M-G-S-L-A-A-R-V-V-K-Q-P-C-G

Peptide C40 .... C-C-R-H-K-Q-P-L-I-A-P-A-K-Q-L-L-P-P-S-A-S-A-R-R-G-D-L-A-H-L-A-A-A-H-A-R-H-P-C-G

Peptide O40 .... C-C-R-H-K-Q-K-I-V-A-P-V-K-Q-T-L-P-P-S-V-P-N-L-R-G-D-L-Q-V-L-A-Q-K-V-A-R-T-P-C-G

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TABLE 1. Protection of guinea pigs with A40, C40, and O40 peptide vaccines following homotypic challenge with FMDV 28 days postvaccination

Peptide	No. of guinea pigs protected/no. challenged with given peptide dose		
	1 mg	0.2 mg	0.04 mg
A40	5/5	5/5	5/5
C40	2/4	5/5	3/5
O40	4/4	5/5	5/5

In addition to these peptides, "constituent" peptides were also synthesized, that is, 141–158 PCG (A21, C21, and O21) and 200–213 (A14, C14, and O14). It should be noted that the numbering system of 141–158 and 200–213 relates to the VP1 sequence of the O virus and that the corresponding A and C sequences were chosen on the basis of alignment of the three sequences. Each peptide was purified to near homogeneity by preparative reverse-phase chromatography.

Vaccination of guinea pigs and cattle. Vaccines were prepared by emulsifying an appropriate peptide in incomplete oil adjuvant. Volumes of 2 (guinea pigs) or 5 (cattle) ml were injected by the subcutaneous route. Sera were obtained at various times prior to challenge of the animals, which was usually 28 days postvaccination. Challenge was with 3,000 guinea pig 50% infective doses by the intraplantar route or 100,000 bovine tongue 50% infective doses by the intradermolingual route. The criterion for protection was that none of the animals developed vesicular lesions other than at the site of challenge.

ELISAs. The titers of serum antibody to peptide and virus were determined by enzyme-linked immunosorbent assays (ELISAs). Microdilution plates (Nunc Immunoplates II) were coated with 100-μl/well amounts of 1 μg of A, C, or O14, 2 μg of A, C, or O21, or 3 μg of A, C, or O40 per ml. For the viruses, plates were either coated directly with 100-μl/well portions of 1 μg of purified virus (different O viruses) per ml or with a 1/4,000 dilution of rabbit antiserum to the A, C, or O viruses. In the case of the trapping-antibody ELISA, the plates were washed prior to exposure to clarified tissue culture preparations of the viruses. Sera were titrated on the plates, and specific antibodies were detected with horseradish peroxidase conjugated to rabbit anti-cow or anti-guinea pig immunoglobulins. Antibody titers were determined as the $\log_{10}$ of the antiserum dilution giving 1 A_{492} unit. Titers of 1.7 (equivalent to a 50-fold dilution of antiserum) or less were usually considered negative.

VNA. Neutralizing antibodies were determined in a microdilution test, using a porcine kidney cell line, IB-RS2, as described previously (8).

RESULTS

Homotypic protection of guinea pigs conferred by peptides A40, C40, and O40. Peptides A40, C40, and O40 were used to immunize guinea pigs. Table 1 shows the results of this experiment and indicates complete protection at all dose levels against homotypic challenge in the case of A and O viruses, whereas the C peptide was completely protective only at the 0.2-mg dose level.

Serological evaluation of bovine antivirus and antipeptide sera. Sera from cattle previously vaccinated with O40 peptide or infected with O_1BFS 1860 virus were titrated on plates coated directly with a number of virus strains of the O serotype. Both sera recognized the different O viruses to a similar extent (Fig. 1).

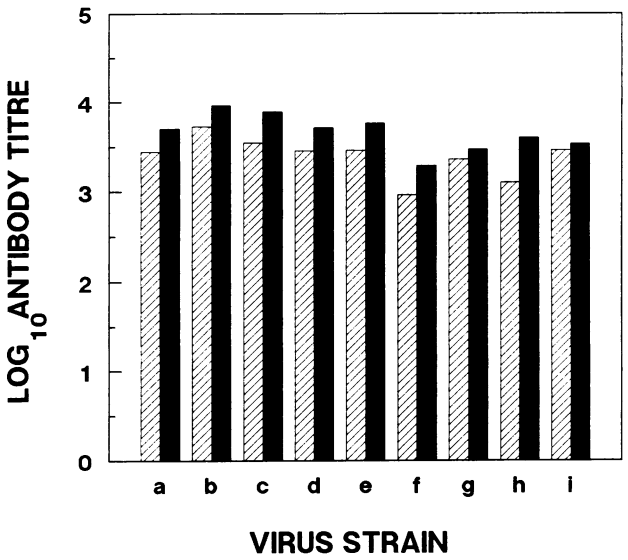

FIG. 1. ELISA of bovine antipeptide and antiviral sera against a range of O-serotype viruses. The peptide serum (hatched bars) was from an animal immunized with O40 peptide, and the virus serum (solid bars) was from an animal previously infected with the O_1BFS strain of FMDV. The viruses used to coat the plates were, from a to i, OBFS 1860, OBFS 1848, O Campos, O Lausanne, O Kaufbeuren, O Saudi, O India, O Brescia, and O Austria.

In a separate experiment, the A40, C40, and O40 peptides were used to immunize cattle. Table 2 summarizes the results. In general, each group of vaccines seroconverted strongly against the virus equivalent to the immunizing peptide. The C40 and O40 peptides appeared to generate a more serotype-specific response than that observed with the A40 peptide. A few cattle showed high heterotypic responses, a particular example being the third animal, which was immunized with A40 and gave titers of 90 and 355 for the A and C viruses, respectively. It was not possible to challenge any of these cattle.

TABLE 2. VNA titers of cattle sera following immunization with A40, C40, or O40[a]

Peptide	VNA titer against:		
	A virus	C virus	O virus
A40	128	22	32
	708	178	22
	90	355	45
	45	64	16
	22	11	16
C40	8	355	≤6
	16	512	90
	8	708	32
	32	708	11
	178	355	45
O40	≤6	32	128
	11	11	708
	16	8	≥1,400
	≤6	22	32
	8	16	708

[a] Cattle were given 1 mg of peptide in incomplete oil adjuvant, and serum samples were taken 28 days postvaccination.

TABLE 3. Protection of cattle to challenge with A_{24} Cruzeiro virus following immunization with A40 and O40 peptides

Animal no.	Treatment	VNA titer[a] against:		Protection
		A virus	O virus	
RA82	A40, 2 doses	708	45	Yes
RA83	A40, 2 doses	512	11	Yes
RA84	A40, 2 doses	128	9	No
RA85	A40, 1 dose	32	≤6	No
RA86	A40, 1 dose	178	≤6	Yes
RA87	A40, 1 dose	1,400	22	No
PX45	A peptides, 2 doses[b]	1,020	22	Yes
PX46	A peptides, 2 doses[b]	2,050	45	Yes
PX47	O40, 2 doses	355	1,400	Yes
PX48	O40, 2 doses	256	708	No

[a] Determined with sera taken the day of challenge. A titer of ≤6 was the lowest detectable.

[b] PX45 and PX46 both received an initial dose of 1 mg of a version of the A40 peptide lacking the terminal Cys-Cys and Pro-Cys-Gly sequences, followed 6 months later by 0.2 mg of A40.

Protection of cattle induced with A and O peptides. Peptide A40 was also evaluated in cattle with challenge. Animals were either vaccinated with a single dose (5 mg) of A40 and challenged 28 days postvaccination with A_{24} Cruzeiro virus or boosted at this time with 0.2 mg of the same vaccine and challenged 14 days later. Two of the three animals given two doses of vaccine were protected (Table 3), whereas only one of those given a single immunization was protected.

Four other animals, which were recovered from another experiment, were included in the challenge. Each of these had received a single dose (1 mg) of A or O peptide followed 6 months later by a booster dose (0.2 mg). Challenge was 14 days after the booster vaccination. The animals receiving two doses of the A peptides were protected (Table 3). More interesting in the context of heterotypic protection was the fact that, of the two animals which received only O40 peptide, one was protected to challenge with the A virus. The control (nonvaccinated) animals were completely susceptible to virus challenge.

VNAs in these cattle were also assayed. The results in Table 3 show high homotypic antibody titer in all animals, with the exception of RA85. None of the A-peptide-immunized animals (RA82-87, PX45, and PX46) seroconverted significantly to the O virus. This is consistent with the results observed with the initial cattle immunization trial shown in Table 2. In contrast, the O-peptide-immunized animals (PX47 and PX48) did show a strong cross-reactivity with A virus. The difference observed relative to the results expressed in Table 2 may have arisen from the booster dose administered at an advanced age.

Heterotypic protection of guinea pigs conferred by peptides A40, C40, and O40. An experiment similar to that detailed in Table 1 was conducted in guinea pigs to establish the validity of the single observation of heterotypic protection in cattle (Table 3). Briefly, two dose levels of each peptide corresponding to the three virus serotypes were used. The results of the challenge are shown in Table 4. It is clear that the results of the homotypic challenge experiment reported in Table 1 are confirmed in this experiment. Complete homotypic protection was achieved with the A and O peptides, whereas the C peptide yielded complete protection at the 5-mg dose only.

A significant degree of cross-protection was also achieved with each of the peptides. This was particularly apparent with both doses of the A peptide and O-virus challenge and the highest dose of O peptide and A-virus challenge. The highest level of heterotypic protection conferred by the C peptide was against O-virus challenge. In general, O-virus challenge appeared to be the easiest to protect against, whereas C-virus challenge was the most difficult. All control (nonvaccinated) animals were completely susceptible to virus challenge.

TABLE 4. Protection of guinea pigs against homotypic and heterotypic FMDV challenge (28 days postvaccination) following immunization with peptide A40, C40, or O40

Peptide	Dose (mg)	No. of guinea pigs protected/no. challenged with given virus		
		A	C	O
A40	0.2	5/5	0/5	4/5
C40	0.2	1/5	3/5	3/5
O40	0.2	1/5	0/5	5/5
A40	5.0	5/5	1/5	3/5
C40	5.0	1/5	5/5	3/5
O40	5.0	4/5	1/5	5/5

Serum pools were prepared from blood samples taken 27 days postvaccination. The VNA titers of these pools are shown in Table 5. It can be seen that all of the guinea pigs seroconverted homotypically, but there was little evidence of heterotypic antibody. Because of this, various sera were also assayed for antibody against the 40-residue peptides and the constituent 21- and 14-residue peptides.

Clearly, the strongest reactions were with the 40-residue peptides, with which high homotypic and heterotypic titers were observed (Table 6). There was some correlation between titer against 40-residue peptide and protection. A particular exception to this was the result with the sera from guinea pigs immunized with C40. Thus, the heterotypic anti-A40 titer was considerably higher than the anti-O40 titer, whereas the inverse was seen in the case of protection. Against the constituent peptides, only the homotypic values were significantly above background and were observed with the 21-residue peptide. Similar conclusions were drawn from ELISA of the cattle sera from the experiment detailed in Table 3, in that most of the antipeptide antibody was directed at the 40-residue peptide rather than the 21- or 14-residue peptide (results not shown).

Table 7 shows the sequence conservation among the peptides. The highest values (64 to 86%) are seen with residues 200 to 213.

DISCUSSION

We have extended our previous studies (4, 7) by demonstrating that peptides representing two specific regions of the

TABLE 5. VNA titers of guinea pig sera following immunization with A40, C40, or O40

Peptide	Dose (mg)	VNA titer against:		
		A virus	C virus	O virus
A40	0.2	512	11	≤6
C40	0.2	≤6	128	90
O40	0.2	11	≤6	≥1,400
A40	5.0	708	11	≤6
C40	5.0	≤6	1,024	≤6
O40	5.0	≤6	8	≥1,400

TABLE 6. Antipeptide titers of guinea pig sera (27 days postvaccination) following immunization with a 5-mg dose of A40, C40, or O40

Peptide serum	ELISA peptide serotype	Antipeptide titer for given ELISA peptide length		
		40 residues	21 residues	14 residues
A40	A	3,262	985	8
	C	339	12	10
	O	1,123	8	10
C40	A	708	31	15
	C	3,877	708	8
	O	108	10	10
O40	A	1,639	12	12
	C	92	46	10
	O	2,908	369	10

VP1 protein of the A_{24} Cruzeiro, C_3 Indaial, and O_1 Kaufbeuren serotypes can confer protection against viral challenge. In two separate experiments, the A and O peptides were found capable of providing complete protection of guinea pigs at all dose levels against their respective virus challenge (Tables 1 and 4). The C peptide provided a high level of protection, but it was less than complete. This particular C virus appeared to be a more stringent test of the peptide-vaccine efficacy in guinea pigs, and it is not immediately clear why this should be so. In cattle challenge experiments, Black et al. (3) have reported the A_{24} virus to be approximately 30 times more immunogenic than the O_1 virus and the C_3 virus to be of intermediate immunogenicity. Consequently, our results may be specific to the guinea pig-adapted viruses or, more likely, an inherent feature of the peptide immunogens used.

Because FMDV exists as seven distinct serotypes and numerous subtypes, it is necessary to produce conventional vaccines which correspond closely to the strains in the field. Furthermore, there is no evidence of synergy between serotypes in conventional multiserotype vaccines (3). This extensive variation has led many workers, for example, Mateu et al. (10), to predict that peptide vaccines would be too specific because of the limited repertoire of epitopes which they possess. Despite these apparent constraints, we have demonstrated here that peptide vaccines are capable of inducing a broad level of reactivity. Cross-reactive antiviral antibody, as measured by ELISA, was observed with anti-O40 peptide sera and a range of O viruses (Fig. 1). Similar results have been reported by Ouldridge et al. (E. J. Ouldridge, B. E. Clarke, P. V. Barnett, A. Brown, N. R. Parry, C. Bolwell, D. J. Rowlands, and F. Brown, paper presented at the 17th OIE Comm. FMD, 1986, p. 197–203). Perhaps more surprising was the demonstration of heterotypic protection. The best experimental evidence for this was with guinea pigs, although one heifer given two doses of O40 peptide was also protected against challenge with A_{24} virus. In general, high levels of heterotypic VNA (Table 5) were not observed, which contrasts with the level of heterotypic antipeptide antibody (Table 6). A similar observation was made by Barteling et al. (S. J. Barteling, D. Voskamp, R. H. Meloen, and H. M. Geysen, Abstr. 6th Int. Congr. Virol. 1984, p. 265) with sera from rabbits immunized with smaller FMD peptides linked to a carrier protein.

TABLE 7. Conservation of sequences among different peptides used in this work

Peptides compared	% Conservation		
	40 residues[a]	21 residues[a]	14 residues
O and A	53	33	79
O and C	53	44	64
C and A	63	44	86

[a] Additional amino acids at the amino or carboxy termini (CC and PCG) or both and the junction sequences (PPS) in the 40-residue peptides were disregarded in these calculations.

Given the sequence diversity among the three peptides (Table 7), it is intriguing that heterotypic protection can be achieved, and this suggests that highly conserved elements exist within the peptides. The highest sequence conservation is seen in the amino-terminal region of the peptides, although the failure in both the present work and a previous report from our laboratory (7) to demonstrate antibody against the 200–213 sequence suggests that this site may not be involved in heterotypic protection. It is not possible, however, to preclude a conformational role for the 200–213 sequence in the 40-residue peptides.

If heterotypic protection relies largely on amino acids within the 141–158 portion of the peptides, then the most likely sequence is R-G-D (145–147) either in isolation or as part of a larger structure involving other conserved amino acids (L-A, 151–152). It is interesting to note that R-G-D is conserved throughout almost all of the many isolates of FMDV that have been sequenced and has been identified as part of the cell attachment site on the virus (7a). Although other workers have identified amino acids at 148 and 153 of an A-serotype virus as being critical in relation to antigenic variability (16), their conclusions were based on cross-neutralization data which are not at variance with the results of the present study. Rather, we have demonstrated cross-reactivity at the levels of antipeptide antibody and protection, indicating a distinction between immunoassay studies which mimic the antigenic properties of the virus and protection studies which appear to depend on different elements within the peptide.

The lack of correlation of protection with VNA was reported in a previous paper from this laboratory (4) and is in direct contrast to conventional FMD vaccines with which manufacturers are able to obtain a relatively accurate estimate of potency by the level of VNA induced (14). This lack of correlation suggests a significant qualitative difference between the antibodies induced by peptide and viral vaccines. It appears that our peptide vaccines do not exactly mimic the immune responses of cattle and guinea pigs to virus, but nonetheless achieve the objective of protection. This may be through the induction of antibody to the sequences involved in binding of the virus to the host cell. Clearly, peptides can be used to induce a unique immune response, in our case, heterotypic protection, which cannot be achieved with the whole virus. This argues well for FMD and other potential peptide vaccines.

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