Protection of cattle and swine against foot-and-mouth disease, using biosynthetic peptide vaccines

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SUMMARY

A single dose of foot-and-mouth disease (FMD) virus protein 1 (VP1) peptide, expressed in *Escherichia coli* as a fusion protein with 190 amino acids (AA) of the LE' protein of the tryptophan operon of *E coli*, elicited an immune response in steers sufficient to withstand the challenge of exposure to animals with acute FMD. The 58-μg dose of viral peptide, composed of a segment of the VP1 from the A12 strain (A12) of FMD virus (FMDV; A12-32dimer) in a tandem repeat configuration of AA137 through 168 and emulsified with oil adjuvant, elicited a serologic response in cattle equivalent to that obtained using conventional whole virus vaccines. Two groups of swine were vaccinated, 1 with the A12-32dimer as used in cattle and 1 with AA131 through 157 from VP1 of the A24 strain (A24) of FMDV (A24-peptide), expressed in the same system as A12-32dimer, but as a single copy per molecule. In swine, the 58-μg dose of the A12-32dimer repeated at 28 days was an effective immunogen; all swine were protected against A12 and, in addition, the vaccine protected 50% of the swine against A24. The 29-μg dose of A24-peptide, administered according to the same schedule, elicited protection against A24 in 50% of the vaccinates and, in addition, protected 25% of those vaccinates against A12. The serologic response elicited by A12-32dimer against A24 virus was considerably greater than the response elicited by A24-peptide against A12 virus. The evidence of multiple immunogenic epitopes between AA131 and AA168 was evaluated.

Foot-and-mouth disease (FMD) is a highly contagious viral vesicular disease of cloven-hoofed animals. The 7 distinct serotypes of FMD virus (FMDV) constitute the *Aphthovirus* genus of the family Picornaviridae.[1] Vaccines against FMD are used extensively in food animals of all continents except North America and Australia, which are free of the disease at this time.[2]

Enzootic FMD results in major losses of productivity in food, fiber, and draft animals. The risks of loss ascribed to FMD, owing mainly to its extreme contagiousness, can be directly correlated with the intensity of the animal production scheme used. Thus, the developed countries, where animal production is intense and the potential for loss through FMD is great, have established severe restrictions on importation of possibly contaminated products from countries where FMD is enzootic. These circumstances led to a policy of stamping out epizootics of FMD (slaughter of infected and exposed animals) in countries where the disease was not enzootic. The reality of the numbers of animals that would be slaughtered in a widespread FMD epizootic has led to the serious consideration of control through vaccination.

Conventional FMD vaccines (inactivated virus) are prepared from either FMDV-infected cultures of cattle tongue epithelium or cultured cell lines.[3] Production of these vaccines is complex, expensive, and fraught with the risk of infective virus surviving chemical inactivation.[4] Furthermore, the immunogenic diversity of the 7 types of FMDV is such that serologic matching to subtype (more than 60) is often required for the formulation of efficacious vaccines.[5]

The FMD virion is a positive strand of RNA packed into a capsid composed of 4 viral proteins (VP1 through 4).[5] Monoclonal antibody analysis of the FMD virion has indicated that either conformation or sequential epitopes can elicit infectivity-neutralizing antibodies.[6-9] Evaluations of the immunogenicity of the virus proteins indicated that neutralizing antibodies could only be elicited by use of VP1 (serotypes A and O).[5] Immunogenicity or protection in large animals has not been reported for virus-derived VP1 of serotype O, and results with synthetic peptides based on this serotype have not been as clearcut as those obtained with peptides based on serotype A. Biosynthetic FMDV immunogens, copies of the amino acid (AA) sequence of the VP1 of type A virus, have been used successfully to protect cattle from FMD.[10]

The objective of the study reported here was to evaluate the immunogenicity of 2 genetically engineered fusion proteins: the first, given to cattle and swine, contained short AA sequences identical with those of FMDV type A strain 12 (A12) VP1 (A12-32dimer), and the second, given to swine only, contained FMDV type A strain 24 (A24) VP1 (A24-peptide). The homologous and heterologous protection afforded swine through the use of these peptides was evaluated.

Materials and Methods

Vaccines—The FMDV A12 large plaque ab variant[11,12] with VP1 AA sequence 137 through 168 was expressed as a tandem repeat configuration (A12-32dimer), the monomers being separated by an aspartic acid molecule and fused with 190 AA related to the *Escherichia coli* tryptophan operon.[13] The FMDV A24 Cruzeiro isolate sequence (A24-peptide) was expressed in the same system and with the same fusion partner, but as a mon-

omer.[14] The FMD VP1 AA sequences (Table 1) were prepared as described,[13,14] and were used as immunogens. Each cattle or swine vaccine dose consisted of 250 μg of fusion protein. This represented 58.6 μg of A12-32dimer or, in the case of A24-peptide, 29 μg of VP1 sequence, the remainder being *E coli*-related protein. A dose-response curve was developed in guinea pigs, using this dose and 2 tenfold dilutions of it. The antigen preparations, in $0.05M$ histidine (pH 6.0) with 0.1% mercaptoethanol, were emulsified with oil adjuvant and used at a dose volume of 2 ml in all 3 animal species.[15]

Vaccination—Animals were kept in a high-containment isolation facility throughout the study period. Hereford steers, approximately 18 months old (each weighing 320 kg), Yorkshire pigs (gilts and barrows, each weighing 35 kg), and female Dunkin-Hartley guinea pigs (each weighing 450 g) were the vaccination subjects. Vaccine was administered SC: in cattle, a single vaccination in the center of the lateral area of the neck; in swine, 2 vaccinations immediately caudal to the ear (original on the left and booster on the right); and in guinea pigs, a single vaccination under the loose skin just off the dorsal midline over the rib cage. Vaccination site reactions were evaluated at weekly intervals throughout the course of the study.

Serologic evaluation—Serum samples were obtained from blood samples collected from cattle (jugular vein), swine (cranial vena cava), and guinea pigs (cardiac puncture) and kept at −20 C until tested. Fifty percent mouse protective dose (MPD_{50})[10] values were developed as described. Plaque-reduction test values were established in a bovine kidney cell line[16] and were based on 70% reduction in plaque number. Determination of the MPD_{50} and the plaque-reduction test were performed, using aliquots of the virus isolates that had been used to establish the AA sequences (Table 1). Ouchterlony diffusion reaction was used to test for antibodies to FMD virus infection-associated antigen (FMD-VIAA).[17]

Challenge of immunity—The immunity of the vaccinated cattle and swine against FMD was challenged by exposure[18,19] to animals infected with the same virus stocks as those used to establish the AA sequences (Table 1). Challenge of immunity in cattle was started at postvaccination day (PVD) 28 and in swine was started at PVD 59, 31 days after booster vaccination.

Groups of 5 cattle were housed together (3 vaccinated, 1 untreated control, and 1 infected by intradermolingual inoculation of 10,000 mouse infective doses of FMDV A12 large plaque ab variant). Swine were housed as 2 test groups, 11 swine in each, composed as follows: 4 vaccinated with A12-32dimer, 4 vaccinated with A24-peptide, 1 untreated contact control, and 2 in-

oculated controls infected by foot-pad inoculation of 10,000 mouse infective doses of the indicated FMDV. One group of swine had immunity challenged with FMDV subtype A12 and the other, with FMDV subtype A24 Cruzeiro. Rectal temperature was recorded daily throughout the challenge period. The muzzle (or snout), nares, lining of the buccal cavity, tongue, soft palate, and feet of the intentionally infected controls were examined for lesions of FMD 24 hours after virus inoculation. All test animals were examined after postchallenge days (PCD) 5 and 14. Postmortem examination (cattle at PCD 22 and swine at PCD 14) was performed on these areas as well as on internal organs. Detection of a lesion at any of these locations at any point in the study constituted break in immunity.

Results

Vaccination of cattle with FMDV A12-32dimer—Analysis of the PVD-7 serum samples indicated negligible serologic response to vaccination; however, at PVD 14 and later, response was excellent (Table 2). There was a variation of nearly 2 orders of magnitude in the MPD_{50} response among the vaccinates at PVD-28, with minimal response being adequate and maximal response being equivalent to that of animals convalescing from FMD. Samples from the vaccinates at PCD 14 and 22 had only minimal changes in serologic results after challenge of immunity (Table 2).

All controls had generalized FMD and developed fever at the time of or before the appearance of lesions. The vaccinates developed neither vesicular lesions nor fever, with the exception of steer VAC8, which developed fever on PCD 6 and 7. Antibodies to FMD-VIAA were detected in the PCD-14 and -22 serum samples from controls, but were not detected in serum of the vaccinates.

Vaccination of guinea pigs with FMDV A12-32dimer—Single injection of A12-32dimer elicited a serologic response of greater magnitude (Table 3) than that obtained in cattle and swine. The tenfold-dose intervals elicited a clear-cut dose-response effect. The lower doses did not elicit a response at PVD 28, but were effective at PVD 56.

Vaccination of swine with FMDV A12-32dimer—Adequate response against the homologous virus was detected at PVD 28; however, the cross-neutralization response to A24 was minimal, with 2 exceptions (Table 4). After

TABLE 1—Amino acid (AA) sequences of virus protein 1 (VP1) from foot-and-mouth disease viruses (FMDV) type A strain 12 isolate large plaque ab (A12) and type A strain 24 isolate Cruziero (A24)

Virus	AA sequence														
AA No.*	129				133					138					143
A12	val	tyr	asn	gly	thr	asn	lys	tyr	SER	ALA	SER	GLY	SER	GLY	VAL
A24	val	tyr	ASN	GLY	THR	SER	LYS	TYR	ALA	VAL	GLY	GLY	SER	GLY	ARG
AA No.	144				148					153					158
A12	ARG	GLY	ASP	PHE	GLY	SER	LEU	ALA	PRO	ARG	VAL	ALA	ARG	GLN	LEU
A24	ARG	GLY	ASP	MET	GLY	THR	LEU	ALA	ALA	ARG	VAL	VAL	LYS	GLN	leu
AA No.	159				163					168		170			
A12	PRO	ALA	SER	PHE	ASN	TYR	GLY	ALA	ILE	LYS	ala	glu			
A24	pro	ala	ser	phe	asn	tyr	gly	ala	ile	lys	ala	asp			

* AA No. = the AA sequence number of the VP1 chain of the indicated virus (A12 or A24); the AA are given in the standard 3-letter code. The series of AA listed in upper case were part of the immunogen, whereas those listed in lower case are for purposes of comparison, but were not a part of the immunogen. Where sequences from the 2 viruses are identical, the AA are underlined.

TABLE 2—Response elicited by vaccination of cattle with FMDV A12-32dimer and challenge exposure

Steer No.	After vaccination 14* MPD	After vaccination 14* PRT	After vaccination 21 MPD	After vaccination 21 PRT	After vaccination 28 MPD	After vaccination 28 PRT	A12 CHALLENGE	After challenge exposure 14 MPD	After challenge exposure 14 PRT	After challenge exposure 22 MPD	After challenge exposure 22 PRT	Final results FMD	Final results Anti-VIAA
VAC3	1.6	2.1	2.3	2.1	2.1	1.9	C	2.0	1.8	2.3	2.7	NVL	Neg
VAC4	>1.7	2.3	3.3	3.7	2.8	3.3	H	3.3	3.2	2.7	2.1	NVL	Neg
VAC5	>1.7	2.9	2.7	2.3	2.3	2.7	A	2.6	2.2	2.1	2.9	NVL	Neg
VAC6	>1.7	3.0	>3.1	7.1	3.8	5.6	L	3.8	3.9	3.4	4.5	NVL	Neg
VAC7	1.4	1.7	2.0	2.9	3.4	3.7	L	2.7	3.0	2.7	3.6	NVL	Neg
VAC8	>1.7	1.8	2.8	2.3	2.7	2.8	E	2.6	3.2	2.9	3.7	NVL	Neg
							N						
CC6	CC				<0.3	ND	G	3.5	ND	3.4	3.2	Gen	Pos
IC7	IC				<0.3	ND	E	3.9	ND	3.0	3.6	Gen	Pos
IC8	IC				<0.3	ND		4.0	ND	3.5	3.3	Gen	Pos
CC9	CC				<0.3	ND		3.7	ND	3.9	3.4	Gen	Pos

* At postvaccination days (PVD) 0 and 7, there was no measurable serologic response to FMDV.
MPD = reciprocal of the serum dilution (10^x) that provides protection to 50% of the suckling mice exposed to 100 infective doses of FMDV A12 isolate large plaque ab variant (A12 Lp ab); PRT = reciprocal of the serum dilution (10^x) that results in a 70% reduction of virus plaques; CHALLENGE = at PVD 28, 2 inoculated cattle (IC) housed with 6 vaccinates (VAC) and 2 untreated contact controls (CC), were given intradermolingual inoculations of FMDV A12 LP ab; they developed acute FMD 5 days later, thus exposing all to FMD; NVL = no visible lesions; Gen = generalized FMD. Anti-VIAA = results of Ouchterlony analysis for antibodies to FMD virus infection-associated antigen (ie, FMDV RNA polymerase). Pos = positive result; Neg = negative result; ND = not determined.

TABLE 3—Serologic response elicited by vaccination of guinea pigs with FMDV A12-32dimer

Dose* (µg)	PVD 28	PVD 56
250.0	5.2	4.6
25.0	< 0.3	3.4
2.5	< 0.3	1.0

* Each dose group consisted of 5 guinea pigs, from which blood samples were obtained at PVD 28 and 56. Serum obtained from blood samples from guinea pigs of each group was pooled and tested. Twenty-three percent of the weight of the fusion peptide is virus related (dosages of 58.6, 5.9 and 0.6 µg of viral sequence).

Data are expressed as MPD; *see* Table 2 for key.

FMDV A12 challenge exposure, and developed neither fever nor antibodies to FMD-VIAA. After challenge exposure with FMDV A12, there was little change in the serotest results in the A12-32dimer vaccinates to either FMDV A12 or A24. Challenge exposure to the A12-32dimer vaccinates with FMDV A24 resulted in an overall increase in titer to A12 (with exception of No. SVAC85) and a plateau in the relationship between anti-A12 and anti-A24 serotiters. During the challenge-exposure period, only those animals that develoepd FMD lesions developed fever or antibodies to FMD-VIAA. Controls infected with A12 had anti-A12 titer similar to that in A12-32dimer vaccinates, but had higher anti-FMDV A24 titer. The animals challenge exposed with FMDV A12 overall developed higher antiviral titer to both viruses than did the controls (Table 4). The swine (No. SVAC87) protected against A24 had no visible lesions and developed much increased antiviral titer, but did not have signs of FMD.

Vaccination of swine with FMDV A24-peptide—A minimal 28-day serologic response against A12 and A24 was detected; however, the response to booster vaccination indicated anamnestic response to both viruses. Only 50%

booster vaccination, the response to A12-32dimer was of nearly the same magnitude as that in animals convalescing from FMD (controls), and the cross-neutralization response to A24, although not as great as that in the convalescents (FMDV A12), was never the less significant. All A12-32dimer-vaccinated animals were resistant to

TABLE 4—Response elicited by vaccination of swine with FMDV A12-32dimer and subsequent challenge exposure with either FMDV A12 or A24

Swine No.	PCV 28 A12	PCV 28 A24		PCV 59 A12	PCV 59 A24	Virus*	PCD 14 A12	PCD 14 A24	Challenge-exposure results FMD	Challenge-exposure results Anti-VIAA
SVAC65	2.6	0.7		3.8	2.7	A12	3.3	2.0	NVL	Neg
SVAC67	1.6	0.5	B	3.6	1.5	A12	3.5	1.2	NVL	Neg
SVAC68	2.0	0.4	O	3.4	1.6	C A12	3.6	0.7	NVL	Neg
SVAC73	2.7	0.2	O	3.5	1.8	H A12	4.2	1.9	NVL	Neg
SVAC75	2.5	0.9	S	3.6	<1	A A24	4.1	4.7	Gen	Pos
SVAC82	1.8	2.0	T	3.8	2.5	L A24	4.5	4.2	Gen	Pos
SVAC85	2.6	1.2	E	3.6	1.7	L A24	3.4	3.0	Gen	Pos
SVAC87	2.1	0.9	R	3.9	1.8	E A24	4.5	4.2	NVL	Neg
						N				
SIC70	IC			0.4	0.3	G A12	4.0	2.8	Gen	Pos
SCC79	CC			0.7	0.3	E A12	4.2	3.2	Gen	Pos
SIC81	IC			0.7	0.3	A12	3.6	3.1	Gen	Pos

* During challenge exposure, swine of the A24 and A12 groups were housed in separate containment facilities; control swine for the A24 challenge exposure are listed in Table 5.
BOOSTER = second vaccination; IC = inoculated control; CC = contact control.
Numerical data are expressed as MPD, which was established with FMDV A12 and A24; *see* Table 2 for key.

TABLE 5—Response elicited by vaccination of swine with FMDV A24-peptide and subsequent challenge exposure with either FMDV A12 or A24

Swine No.	PCV 28 A12	A24		PCV 59 A12	A24	Virus	PCD 14 A12	A24	Challenge-exposure results FMD	Anti-VIAA
SVAC66	0.8	1.3		0.6	2.8	A12	4.2	3.0	Gen	Pos
SVAC69	1.4	0.2	B	2.5	2.2	A12	4.5	3.1	NVL	Neg
SVAC71	0.3	0.3	O	1.1	2.2	C A12	NS		NVL	NS
SVAC72	0.3	0.9	O S	0.7	1.8	H A12	4.0	2.8	Inf	Pos
SVAC74	1.4	0.3	T	1.9	3.3	L A24	2.5	2.8	NVL	Neg
SVAC76	0.3	0.6	E	0.4	1.9	L A24	4.2	4.2	Gen	Pos
SVAC78	0.3	0.3	R	0.7	1.3	E A24	2.8	3.0	NVL	Neg
SVAC84	0.3	0.9		1.0	2.1	N G A24	4.3	3.7	Gen	Pos
SCC80	IC			0.2	<0.3	E A24	3.7	4.0	Gen	Pos
SIC83	IC			0.3	<0.3	A24	4.1	4.1	Gen	Pos
SCC86	CC			0.6	<0.3	A24	3.2	4.0	Gen	Pos

* During challenge exposure, swine of the A24 and A12 groups were housed in separate containment facilities; control swine for the A12 challenge exposure are listed in Table 4.
NS = no sample; Inf = localized lesion on 1 foot.
See Table 4 for key.

of the swine had more than minimal serologic response to A12 (Table 5). The boost in titer that followed challenge exposure with A12 was largest against A12. The 2 swine with the highest anti-FMDV A12 titers were protected against A12; however, 1 of the protected swine (No. SVAC69) developed fever during the challenge-exposure period. One (No. SVAC72) of the 2 nonimmune swine developed only a local lesion and had no fever, the other had generalized FMD, and both developed antibodies to FMD-VIAA. With the exception of swine SVAC84 and SCC86, all other swine (the disease resistant as well as those that developed FMD in response to A24) had similar titer to both viruses after challenge exposure. On the other hand, swine inoculated with A12 (No. SVAC-66, SVAC-69, and SVAC-72, Table 4; and No. SIC-70, SIC-81 and SCC79, Table 5) developed much higher titer against A12 than against A24. All A24 vaccinates developed fever during challenge exposure. The 2 vaccinates that developed FMD lesions also developed detectable amounts of antibody to FMD-VIAA; whereas the one lesionless swine, for which a sample was available, did not develop such antibodies.

Vaccine site reactions—Swellings as large as 25 to 30 mm were visible in affected cattle and swine (about 90% of the vaccinates) for as long as 4 weeks after vaccination. In other animals, they could only be detected by palpation. At the postmortem examination (6 weeks after the last vaccination), the site reactions were not visible externally and could be detected only by palpation or dissection. The site reactions were more or less the same for both peptides, and 2 of 6 of the controls given only oil adjuvant and buffer developed small, but readily detectable, vaccine site reactions.

Discussion

The neutralizing antibody response elicited to A12-32dimer vaccination in cattle at PVD 28 was equivalent to what would have been anticipated from the administration of a conventional FMD vaccine.[15,19] All the vaccinates withstood challenge exposure at PVD 28, and the serologic data would predict that the animals were protected by PVD 14. At PVD 28, the response elicited by 58 µg of virus-related A12-32dimer was comparable with that elicited by as much as 655 µg of inactivated whole virus at PVD 35 in cattle.[18] In a previous dose-response study,[18] it was not possible to distinguish a 0.16-µg dose of whole virus from a 655-µg dose by the amount of antibody activity elicited at PVD 35; however, at later periods, the larger dose elicited superior response. There was considerable variation between animals in response to the dimer; however, the smallest serologic response in cattle (No. VAC3) was adequate to resist infective challenge exposure. The response elicited by use of this dimer peptide was much larger than that elicited by use of the whole VP1 molecule (doses of 100 µg), whether derived from the virion or biosynthesized in the *E coli* system.[10,20]

It has been reported that fusion proteins of FMDV type 01 strain BFS (VP1 AA137 through 162), which contain multiple copies of immunogen on one molecule, are more immunogenic than those containing only a single copy.[21] In that experiment, 4 swine were reported to be protected by a dose of 40 µg of virus-related AA sequence in a tetrameric presentation. Our data supported that conclusion.

The variation in response to the A12-32dimer between animals was larger than is usual in response to a whole virus vaccine, owing principally to the exceptionally great response of cattle VAC6 and VAC7 (Table 2). This could be a reflection of the individual's genetic capacity to respond to a particular epitope. With the virion and its variety of epitopes, the elicitation contribution of an individual epitope may be lost in the overall neutralization titer; whereas, in the case of a small peptide, the response (or lack thereof) elicited by a single epitope becomes a much more detectable portion of the whole.

During challenge exposure, the vaccinated cattle did not develop clinical FMD lesions. In addition, large serologic changes were not detected in the vaccinates during challenge exposure. This was a sign of solid immunity, as contrasted to the changes in swine that, despite low initial titer, withstood challenge exposure, but had a large increase in titer during challenge exposure (No. SVAC78; Table 5). Considering the latter swine and the very high anti-FMD titer in the cattle, less immunogen would have perhaps been sufficient to elicit immunity.

A vaccine dose antigenicity-response curve was estab-

lished in guinea pigs; the response elicited by A12-32dimer can be compared with other anti-FMD vaccine preparations (Table 3). The guinea pig response to 58 μg of antigen at PVD 28 and 56 was equivalent to the previously reported response elicited by 655 μg of whole virus.[22] The guinea pig response to 5.9 μg of virus-related peptide was equivalent to that elicited by 2.6 μg of whole virus.[22] However, for the 2 lower doses, the response was delayed until after PVD 28. A similar phenomenon was the lack of a PVD-7 response by cattle and swine to the peptides used in these experiments (data not shown).

The vaccination of swine with 58.6 μg of A12-32dimer elicited adequate serologic response against A12 virus at PVD 28 (Table 4). In the test system used here, MPD_{50} titer of 2.0 is usually predictive of protection against challenge. After revaccination, the serologic response at PVD 59 was indistinguishable from that subsequently detected in the convalescent controls. Under most circumstances, convalescent MPD_{50} values are grater than those elicited by conventional FMD vaccines. The serologic cross protection (MPD_{50}) elicited by A12-32dimer against A24 at PVD 28 was low, but after revaccination at PVD 59, it was equivalent to what might be expected in response to a conventional vaccine.

The A12-32dimer vaccinates were solidly immune to challenge exposure with infective FMDV A12. Signs of FMD were not observed during challenge exposure, whereas controls became ill and developed all the classic lesions of FMD. Immune status was further validated by the fact that there was no change in either the anti-A12 or -A24 MPD_{50} titer during challenge exposure. Had the A12 virus replicated, one would have anticipated that the anti-A24 MPD_{50} titer would have been higher, based on the ratio of A12-to-A24 MPD_{50} values for the FMDV A12-infected controls. The lack of postchallenge-exposure change in the A12-to-A24 MPD_{50} ratio further supported the conclusion that the virus did not replicate in the A12-32dimer vaccinates during challenge exposure. In addition, these vaccinates developed neither fever nor antibodies to FMD-VIAA during the challenge-exposure period.

Challenge exposure of the A12-32dimer vaccinates with FMDV A24 indicated that only 1 of 4 swine was resistant to the virus. The anti-A24 MPD_{50} values would have predicted that possibly 3 of 4 swine would have been protected. The MPD_{50} titer in all A24-infected swine (vaccinates and controls; Tables 4 and 5) were nearly the same against FMDV A12 and A24, whereas swine infected with A12 all had greater MPD_{50} titer to A12 than to A24. On the basis of serotest results, FMDV A12 elicited more antibody activity against FMDV A24 than A24 elicited against A12. The large increases in MPD_{50} values during challenge exposure indicated that FMDV-A24 replicated in all 4 A12-32dimer vaccinated animals, even in swine SV87, which did not develop FMD (Table 4).

Most of the MPD_{50} values against A12 in the swine challenge exposed with FMDV A24 were slightly increased, whereas all the anti-FMDV-A24 MPD_{50} values were greatly increased. The infected vaccinates (A12-32dimer vaccinates challenge exposed with A24) developed higher MPD_{50} values than did infected controls, indicating an anamnestic response against epitopes common to the peptide and the virus (Table 4). Despite the fact that the immune system was primed for response, it was overwhelmed by the rapidly replicating virus, which produced lesions be-

fore it was neutralized. Following this line of reasoning, swine SVAC87 (Table 4) was one in which the immune system mobilization outpaced virus replication, and the swine did not succumb to challenge exposure.

Vaccination of swine with 29.8 μg of A24-peptide elicited a poor serologic response at PVD 28 (Table 5). Revaccination resulted in response of a magnitude comparable with that obtained in response to initial inoculation with A12-32dimer and was similar in effect to use of conventional FMD vaccines. Based on the serotest results, this vaccine was less immunogenic than the A12-32dimer vaccine. The serologic cross-neutralization (MPD_{50} titer) elicited by A24-peptide against A12 was almost nonexistent at PVD 28 and was minimal and sporadic (notable response in 4 of 8 vaccinates) after hyperimmunization at PVD 59 (Table 5).

Comparison of the AA of the peptides (Table 1) indicated that in the area covered by the A24-peptide (AA131 through 157) AA triplets are the largest areas of identity between the 2 peptides. This area of FMDV A12 contains a neutralization-related epitope centered on AA 152, and the monoclonal antibody that identifies this epitope does not neutralize A24.[23] Polyclonal antiserum to each peptide neutralizes both viruses, thereby documenting the existence of at least 2 neutralization-related epitopes on each peptide. However, from AA 157 through 168, an area covered by the A12-32dimer, but not by the A24-peptide, there is an identical 12 AA sequence in the VP1 of both viruses. This AA sequence, present in A12-32dimer and absent in A24-peptide, but present in VP1 of both viruses, offers an explanation for the unequal cross-reactions of the antisera elicited by the 2 peptides. The A12-32dimer antisera is a more effective neutralizer of A24 virus than is A24-peptide antisera against the A12 virus. There are, of course, many other possible explanations, one of which is that the peptide has a secondary structure, which results in a nonsequential epitope. On the other hand, the fact that the A12 virus elicits a higher serologic response against A12 than against A24, whereas A24 virus elicits a more or less equal response against both viruses (controls; Tables 4 and 5), could be interpreted as documentation of a shared epitope involved in the neutralization of A12, but not A24. One can clearly conclude from this information that these peptides contain multiple neutralization-related epitopes.

The A24-peptide was a much less efficacious vaccine than the A12-32dimer, because only 2 of 4 animals in the homologous system (A24-peptide vaccinates vs A24 virus) were protected from clinical FMD. All A24-peptide vaccinates challenge exposed with A24 developed fever during challenge exposure. However, the MPD_{50} titer of the animals without visible lesions, although high, was not boosted to the point reached by the nonresistant vaccinates or the controls, thereby indicating a lesser effect of the infection on these animals. That these are valid degrees of immunity was further supported by the fact that only those animals with visible FMD lesions developed immunodiffusion-detectable amounts of antibodies to FMD-VIAA. The A24-peptide elicited protection against A12 in 2 of 4 of the vaccinates and amelioration of the disease in swine SVAC72 (Table 4), which had a single foot lesion but never fever. The prechallenge-exposure titer against A12 was a reasonable indicator of the challenge-exposure results (animals with MPD_{50} value > 1.0 were protected).

The lack of visible lesions seemed a valid criterion for protection during challenge exposure. On the other hand, it seems clear that swine SVAC69 (Table 4) became infected and, by this criteria, was protected (no visible lesions). Thus, an effective vaccine protects the vaccinates from lesions of the disease, but not necessarily from FMDV infection. This situation is not unique to the FMD peptide vaccines, but is regularly seen during evaluations of conventional (inactivated whole virus) FMD vaccines. Whether the infected, but lesionless, animals (eg, swine SVAC69 and SVAC78) excrete enough virus to influence the perpetuation of the disease is an unanswered question of considerable economic importance. If these 2 swine were innocuous to the remainder of the swine and cattle population, more potent vaccination would have been wasteful.

Selected FMD VP1 AA sequences are effective immunogens, which can be produced biosynthetically without fear of contagion. These experiments indicate that FMD VP1-based peptide vaccines can be effective immunogens and that, potentially, the spectrum of protection afforded can be broadened by blending or combining several peptides into a single molecule.

References

1. Brooksby JB. Portraits of viruses: foot-and-mouth disease virus. *Intervirology* 1982;18:1–23.
2. Della-Porta AJ. Current status of foot-and-mouth disease vaccines including the use of genetic engineering. *Aust Vet J* 1983;60:129–135.
3. Bahnemann HG. Binary ethylenimine as an inactivant for foot-and-mouth disease virus and its application for vaccine production. *Arch Virol* 1975;47:47–56.
4. Beck E, Strohmaier K. Subtyping of European foot-and-mouth disease virus strains by nucleotide sequence determination. *J Virol* 1987;61:1621–1629.
5. Bachrach HL. Foot-and-mouth disease virus: properties, molecular biology, and immunogenicity. In: Romberger JA, ed. *Beltsville Symposium in Agricultural Research I. Virology in Agriculture*. Montclair, NJ: Allanheld, Osmun & Co, 1977;3–32.
6. Morgan DO, Moore DM, McKercher PD. Vaccination against foot-and-mouth disease. In: Mizrahi A, Hertma I, Klingly MA, et al, eds. *New developments with human and veterinary vaccines*. New York: Alan R. Liss Inc, 1980;169–178.
7. Morgan DO, Robertson BH, Moore DM, et al. Aphthoviruses: control of foot-and-mouth disease with genetic engineering vaccines. In: Kurstak E, ed. *Control of virus diseases*. New York: Marcel Dekker Inc, 1984;135–145.
8. Stave JW, Card JL, Morgan DO. Analysis of foot-and-mouth disease virus type 01 Brugge neutralization epitopes using monoclonal antibodies. *J Gen Virol* 1986;67:2083–2092.
9. Stave JW, Card JL, Morgan DO, et al. Neutralization sites of type 01 foot-and-mouth disease virus defined by monoclonal antibodies and neutralization-escape virus variants. *Virology* 1988;162:21–29.
10. McKercher PD, Moore DM, Morgan DO, et al. Dose response evaluation of a genetically engineered foot-and-mouth disease virus polypeptide immunogen in cattle. *Am J Vet Res* 1985;46:587–590.
11. Cowan KM. Immunological studies of foot-and-mouth disease. V. Antigenic variants of virus demonstrated by immunodiffusion analysis with 19S but not 7S antibodies. *J Exp Med* 1969;129:333–350.
12. Martinsen JS. The effect of diethylaminoethyl dextran and agar overlay pH on plaque formation by two plaque-size variants of foot-and-mouth disease virus. *Can J Comp Med* 1970;34:13–19.
13. Kleid DG, Dowbenko DJ, Bock LA, et al. Production of recombinant vaccines from microorganisms: vaccine for foot-and-mouth disease virus. In: Leive L, ed. *Microbiology—1985*. Washington, DC: American Society for Microbiology, 1985;405–408.
14. Yansura DG, Dowbenko D, Weddell GN, et al. Biosynthetic vaccine for foot-and-mouth disease. In: Downey K, Voellmy RW, eds. *Advances in gene technology: molecular genetics of plants and animals*. New York: Academic Press Inc, 1983;479–493.
15. Gametchu B, Morgan DO, McKercher PD, et al. Immunogenicity of foot-and-mouth disease virus type 01 replicated in either monolayer or suspended BHK cell systems. *Comp Immunol Microbiol Infect Dis* 1983;6:19–29.
16. Baxt B, Morgan DO, Robertson BH, et al. Epitopes on foot-and-mouth disease virus outer capsid protein VP1 involved in neutralization and cell attachment. *J Virol* 1984;51:298–305.
17. Morgan DO, Moore DM, McKercher PD. Purification of foot-and-mouth disease virus infection-associated antigen, in *Proceedings* US Anim Health Assoc Annu Meet 1978:82:277–283.
18. Morgan DO, McKercher PD, Bachrach HR. Immunogenicity of nanogram to milligram quantities of inactivated foot-and-mouth disease virus. II. Comparative response of guinea pigs and steers, in *Proceedings*. US Livestock Assoc Annu 1968;72:407–415.
19. Morgan DO, McKercher PD, Bachrach HL. Quantitation of the antigenicity and immunogenicity of purified foot-and-mouth disease virus vaccine for swine and steers. *Appl Microbiol* 1970;20:770–774.
20. Bachrach HL, Morgan DO, McKercher PD, et al. Foot-and-mouth disease virus: immunogenicity and structure of fragments derived from capsid protein VP3 and of virus containing cleaved VP3. *Vet Microbiol* 1982;7:85–96.
21. Broekhuijsen M, Van Rijn JMM, Blom AJM, et al. Fusion proteins with multiple copies of the major antigenic determinant of foot-and-mouth disease virus protect both the natural host and laboratory animals. *J Gen Virol* 1987;68:3137–3143.
22. Morgan DO, Bachrach HL, McKercher PD. Immunogenicity of nanogram to milligram quantities of inactivated foot-and-mouth disease virus. I. Relative virus-neutralizing potency of guinea pig sera. *Appl Microbiol* 1969;17:441–445.
23. Moore DM, Vakharia VN, Morgan DO. Identification of virus neutralizing epitopes on variants of type A12 foot-and-mouth disease virus. *Virus Res* 1989;in press.