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Effective synthetic peptide vaccine for foot-and-mouth disease in swine☆

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Abstract

We have designed a peptide-based vaccine for foot-and-mouth disease (FMD) effective in swine. The peptide immunogen has a G–H loop domain from the VP1 capsid protein of foot-and-mouth disease virus (FMDV) and a novel promiscuous T helper (Th) site for broad immunogenicity in multiple species. The G–H loop VP1 site was optimised for cross-reactivity to FMDV by the inclusion into the peptide of cyclic constraint and adjoining sequences. The incorporation of consensus residues into the hypervariable positions of the VP1 site provided for broad immunogenicity. The vaccine protected 20 out of 21 immunised pigs from infectious challenge by FMDV O1 Taiwan using peptide doses as low as 12.5 μg, and a mild adjuvant that caused no lesions. A safe chemically-defined product would have considerable advantages for vaccination against FMD.

Keywords: FMD; Vaccine; Peptide

1. Introduction

Highly contagious foot-and-mouth disease virus (FMDV) is responsible for devastating outbreaks of the disease among cloven-hoofed animals. Foot-and-mouth disease (FMD) can be controlled by slaughter of infected and exposed herds, or by vaccination. Slaughter has been the preferred action in countries that are normally free of the disease because under the current regulations of the Office International des Epizooties (OIE) the presence of vaccinated animals leads to loss of FMD-free status and access to export markets [1]. The current vaccines consist of partly purified inactivated virus preparations. These vaccines are successful in preventing the disease, but present the risks of virus escape from production plants [2]. Moreover, vaccination compromises the ability to detect infection by the internationally accepted immunoassays. These tests assay serum for the presence of either neutralising antibodies or antibody to whole virus by ELISA and they cannot distinguish carrier animals from uninfected vaccinates. This serious shortcoming prevents detection of asymptomatic carrier animals that can be found in vaccinated herds. Consequently, OIE regulations specify that vaccinated animals must be destroyed before a country can regain disease-free status [1]. A synthetic subunit vaccine for FMDV has long been sought as an antigenic marker vaccine and for the safety advantages of a product that does not use virus in its manufacturing process.

The prominent G–H loop of the VP1 capsid protein of FMDV, spanning residues 134–158, has been identified as the major immunogenic site for neutralising antibodies [2–5]. This has formed the basis for the peptide approach to vaccination against FMD [3,6,7]. The successful protection of susceptible hoofed species by administration of G–H loop synthetic peptides has not been achieved due to the limited immunogenicity of the peptides. The VP1 region itself lacks T helper (Th) cell epitopes for promiscuous recognition by porcine and bovine MHC alleles [8,9]. Thus, a fully protective VP1 loop peptide vaccine needs

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the addition of promiscuous Th sites from a source outside VP1 [8–10]. An effective peptide vaccine also needs potent B cell sites for the induction of high affinity neutralising antibodies [11,12]. The hypervariability of the immunodominant G–H loop domain has been another major drawback for VP1-based immunogens. Amino acid substitutions in the G–H loop domain alter the interaction of FMDV with neutralising antibodies [8,13,14]. We have designed a novel synthetic peptide vaccine with T and B cell sites optimised for both immunogenicity and antigenic cross-reactivities. This peptide immunogen spans the entire G–H loop domain and extensive flanking sequences (129–169), has a unique consensus sequence to confront the hypervariability of serotype O viruses, and includes a promiscuous artificial Th site [15].

2. Materials and methods

2.1. Peptide synthesis

The peptide antigens for immunoassays and the peptide immunogens for vaccines were produced by synthesis on a solid-phase support using an Applied Biosystems Peptide Synthesiser Model 430A, and Fmoc protection for the α-NH_2 terminus and side chain protecting groups of trifunctional amino acids. Peptides having combinatorial library Th epitopes were prepared by providing a mixture of the desired amino acids at the specified positions. Completed peptides were cleaved from the solid support and side chain protecting groups removed by 90% trifluoroacetic acid. Synthetic peptide preparations, except for the library immunogens, were characterised for correct composition by matrix-assisted laser desorption time-of-flight mass spectrometry using a PerSeptive Biosystems/Vestec LaserTec Benchtop 11 mass spectrometer, and by reverse phase HPLC. The library immunogen for the vaccine was characterised by size exclusion chromatography to a specification that requires 90% of the integrated area to exceed a mass threshold limit value, and by Edman degradation for N-terminal amino acid analysis. Liquid phase cyclisation of the peptide immunogens was accomplished by dissolving the peptides in water at 0.8 mg/ml, pH 3, adding DMSO to 1% (v/v) and adjusting to pH 7.5 with NH_4OH. The solution was incubated at ambient temperature in air and checked daily for 3 days by colorimetric assay using Ellman's reagent until disulphide bond formation was at least 90% complete.

2.2. Vaccine formulations

Vaccine compositions were as described in Table 1. Peptide-based vaccines were water-in-oil single emulsions mixed 1:1 with Montanide ISA 51 (Seppic, Paris). A commercial inactivated virus FMDV O1 Taiwan vaccine was given to one group at a dosage of 1 ml having at least 6 PD_{50}. It was supplied by the manufacturer as an oil/water/oil double emulsion.

2.3. Consensus sequence peptides

VP1 sequences for positions 125–171 from 75 historic and prevalent serotype O isolates were taken from www.iah.bbrc.ac.uk/virus/picornaviridae/aphthovirus/fmd.htm. Sequences were aligned and a consensus amino acid was selected for each variable position.

2.4. Animals

Groups of three Duncan-Hartley guinea pigs were immunised intramuscularly with 100 μg of peptide at week 0 with Freund's complete adjuvant, and with incomplete Freund's adjuvant on week 3. Guinea pig sera were prepared and pooled for each group on week 5 and 1:100 dilutions were analysed for neutralising indices [16].

Specific pathogen-free swine, castrated male or female, 8 weeks old, were obtained and maintained for an immunogenicity trial by Covance Research Products, Inc. (Denver PA.,USA) and the USDA Plum Island Animal Disease Center. Peptide-immunised animals were given 400 μg of UBITh®-O consensus peptide plus a VP1 peptide having the target VP1 sequence of FMDV O1 Taiwan without UBITh® and linker (1:1), formulated with Freund's Incomplete Adjuvant, at weeks 0, 3, and 6. A control group received a commercial FMDV O1 Taiwan vaccine at weeks 0 and 6 per the manufacturer's instructions.

Specific pathogen-free swine, castrated male or female, 8 weeks, seronegative for FMDV reactivity by UBI® FMDV NS EIA (swine) and VN test for neutralising antibodies, were obtained in Taiwan by the Pig Research Institute Taiwan (PRIT). They were immunised by intramuscular injection on weeks 0 and 4. On week 7 they were transferred to the containment facility of the National Institute of Animal Health, Taiwan and on week 8 (day 56) the pigs were challenged by injection into the heelbulb of $10^{4.5}$ $TCID_{50}$ of FMDV O1 Taiwan, grown in BHK cells. Swine were monitored for clinical signs of FMD. These included body temperatures elevated to $\geq 40^{\circ}$ for three successive days, lameness and vesicular lesions on the snout and coronary bands of the legs.

2.5. Serological assays

Virus-neutralising activities of the guinea pig sera and of swine sera from the immunogenicity study were assayed by determining the neutralisation index [16], expressed as logs of input virus ($TCID_{50}$) neutralised to the 50% endpoint, by serum diluted 1:100. Neutralising antibody titres for the pig sera from the challenge trial, expressed as reciprocal dilutions, were determined by a quantitative VN microtest [17]. Peptide-based ELISAs used to analyse swine

sera were the UBI® FMDV VP1 EIA (swine) having the consensus VP1 serotype O peptide without the UBITh1® site and linker for antibody capture, and the UBI® FMDV NS EIA (swine) having the 3B non-structural peptide [18]. ELISA procedures were as reported [18] except that results were reported as signal/cutoff ratios with the cutoff value being $0.23 \times A_{450\,nm}$ of reactive control; the chromagen was 3,3′,5,5′-tetramethyl benzidine (TMB). All sera were tested by ELISA at 1:21 dilutions and the reactive controls were sera from uninfected pigs hyperimmunised with either the VP1 vaccine immunogen or the 3B NS peptide.

3. Results

3.1. Peptide design studies

A series of VP1 peptides corresponding to the G–H loop and flanking regions of a virus of serotype A subtype 12 were synthesised (Table 1) and used to immunise guinea pigs for selection of functionally antigenic designs. The immunogenicity of the designs was evaluated by determining the neutralising indices [16] of the guinea pig anti-peptide immune sera. Longer target site peptides covering the G–H loop domain from amino acids 134–169 were more effective than the 134–159 constructs. The introduction of cyclic constraint by cysteine residues positioned 8 Å apart at residues 134 and 158 was necessary for high neutralisation activity. Constructs containing additional immunostimulatory elements such as the VP1 21–40 Th site [19] and an invasin domain peptide [15], were more effective in the guinea pigs than those that relied on the Th cell epitopes intrinsic to the G–H loop region [20,21].

Later, the VP1 target site of interest was changed to serotype O sequences for activity against the virus that caused the major epidemic in Taiwan in 1997 and the PanAsia O isolates responsible for the current worldwide pandemic [22]. An FMDV O peptide homologue of the third peptide of Table 1 was made having amino acids 134–169 of FMDV O1 Taiwan, with cysteine residues at positions 134 and 158 and cyclisation. The serotype O homologue also elicited neutralising antibody responses in guinea pigs. An extension of the homologue to include amino acids 129–133 (see Table 2 for the O1 Taiwan VP1 sequence) provided further neutralising activity in guinea pigs over the O1 Taiwan 134–169 peptide (neutralising indices against the O1 Taiwan, O1 PI, A12, and Asia1 viruses were 4.0 for the extended peptide anti-serum versus 2.0 for the 134–169 peptide anti-serum against O1 Taiwan, and 4.5 versus 2.0 against O1 PI at week 5; 4.5 versus 2.5 against O1 Taiwan; 2.5 versus 0.5 against A12, and 2.0 versus 1.5 against Asia1 at week 12).

Table 1
Optimisation of design for VP1 peptide immunogen

Description of peptide immunogen	Log_{10} FMDV A_{12} ($TCID_{50}$) neutralised by serum[a]
A_{12}(134–159)	2.5
A_{12}(134–169)	4.5
A_{12}{134(N → C)[b] –158(Q → C)[c] –169}	7.0
Inv[d] -GG-$VP1_{(21–40)}$Th[e] -GG-A_{12}(134–159)	5.5
Inv[d] -GG-$VP1_{(21–40)}$Th[e] -GG-A_{12}(134–169)	6.0
Inv[d] -GG-$VP1_{(21–40)}$Th[e] -GG-A_{12}{134(N → C)[b] –158(Q → C)[c] –159}	7.0
Inv[d] -GG-$VP1_{(21–40)}$Th[e] -GG-A_{12}{134(N → C)[b] –158(Q → C)[c] –169}	8.0

[a] Serum for neutralisation assays diluted 1:100.
[b] N_{134} of the native sequence is replaced by C.
[c] Q_{158} of the native VP1 sequence is replaced by C.
[d] Inv is an invasin immunostimulatory sequence taken from *Yersinia*, TAKSKKFPSYTATYQF [15].
[e] $VP1_{(21–40)}$Th is a VP1 helper T cell site in cattle [19].
Groups of 3 guinea pigs were immunised intramuscularly with the indicated VP1 peptide immunogen on weeks 0 and 3. Sera were prepared on week 5 and the capacity of the anti-peptide antibodies to recognise and neutralise FMDV A_{12} was evaluated by determining the neutralising indices of pooled sera [16].

Table 2
Alignment of corresponding 129–169 VP1 immunogenic sites from prevalent FMDV O isolates and comparison to O consensus site

Consensus O VP1 site	VYNGNCKYGENAVTNVRGDLQVLAQKAAR**C**LPTSFNYGAIK
Corresponding VP1 sequences from FMDV isolates	
O TWN/1/97 (O1 Taiwan)	VYNGSSKYGDTSTNNVRGDLQVLAQKAERTLPTSFNFGAIK
O TWN/2/99	VYNGNCKYGESPVTNVRGDLQVLAQKAARTLPTSFNYGAIK
O UK/1/01	VYNGNCKYGESPVTNVRGDLQVLAQKAARTLPTSFNYGAIK
O CHN/GD/86	VYNGSCKYSDARVSNVRGDLRVLAQKAERALPTSSNYGAIK
O Campos/BR	VYNGECRYSRNAVPNVRGDLQVLAQKVARCLPTSFNYGAIK
O Manisa/TK/69	VYNGNCKYGDGTVANVRGDLQVLAQKAARALPTSFNYGAIK

Cysteine at consensus position 158, in boldface, selected for cyclic constraint.

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ISISEIKGVIVHKIETILF-εK-VYNGNCKYGENAVTNVRGDLQVLAQKAARCLPTSFNYGAIK
   T   RT    TR
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Fig. 1. UBITh1® -O consensus immunogen for FMDV O. T cell help is provided by the combinatorial library UBITh1® site shown [15], linked through an ε-Lysine linker to the consensus O VP1 site from Table 2.

3.2. Consensus VP1 site and promiscuous Th for final immunogen design

Sequences for the VP1 region of interest (129–169) from historic and prevalent O isolates were aligned with the available FMDV O sequences (Section 2.3) and consensus amino acids were selected for each variable position. The derived serotype O consensus sequence is shown in Table 2 and compared to sequences for prevalent viruses. That consensus target sequence was provided with extrinsic T cell help for immunogenicity in porcines by attachment to a linker and a promiscuous artificial Th site derived from measles virus, UBITh1® (Fig. 1). The UBITh1® site belongs to a family of Th sites already proven to be promiscuously immunogenic in several species, including rats, guinea pigs, and baboons as well as swine [15], goats, and dogs (to be submitted), when combined with various peptide B cell epitopes taken from Luteinizing Hormone Releasing Hormone (LHRH or GnRH), somatostatin, and IgE.

Guinea pig antibodies evoked by the chimeric O consensus peptide displayed cross-serotype and cross-subtype neutralising indices against the A12, O-1 PI, O-1 Taiwan, and Asia1 viruses of 2.5, 4.0, 4.5, and 1.5, respectively. In a preliminary immunogenicity study in swine, animals immunised with an inactivated virus O1 Taiwan vaccine had stronger neutralising indices against O Taiwan than against O Manisa and O Campos. Swine immunised with the O consensus peptide and the O Taiwan-specific VP1 sequence also displayed strong neutralising activities against O Taiwan; however, the set of neutralising indices for each peptide-immunised pig was equivalent across all three FMDV O isolates (Table 3).

Table 3
Immunogenicity trial in swine

Vaccine	Animal no.	O Taiwan	O Manisa	O Campos
Killed virus vaccine	210	3.0–3.5	3.0	2.5
	211	3.5	2.0	2.0
	212	2.5	1.5	2.0
	213	3.0–3.5	3.0	2.0
	214	3.0	2.0	2.0
Peptide vaccine	184	3.0	3.0	2.5
	209	2.5	2.5	2.5
	193	2.5	2.5–3.0	2.5
	194	3.0	2.5	2.5
	223	3.5	4.0	3.0
	224	3.5	3.5–4.0	3.5

Swine were immunised either on weeks 0, 3, and 6 with the consensus peptide immunogen plus a sequence specific to O1 Taiwan, or on weeks 0 and 6 with a commercial killed virus vaccine for FMDV O1 Taiwan. Neutralisation indices [16] were determined for sera collected at week 9, diluted 1:100, and tested on serial dilutions of the indicated FMDV O subtype.

3.3. Vaccine trial

The synthetic immunogen shown in Fig. 1 was formulated into vaccines with ISA51 at the doses shown in Table 4. The synthetic vaccines were given by intramuscular injection to groups of three pigs on days 0 and 28. The peptide immunogen and mild adjuvant caused no local adverse reactions. A positive control group receiving a commercial FMDV O1 Taiwan vaccine and a non-immunised control group given only adjuvant were inoculated on the same schedule. The pigs were challenged on day 56.

No neutralising antibodies above background [17] were found in the sera of any of the animals at day 0, indicating a lack of previous exposure. By day 40, almost all the animals immunised with the synthetic peptide had attained significant levels of neutralising antibodies (Table 4). They were monitored throughout by peptide-based enzyme immunoassays for reactivity to the VP1 O consensus target peptide (UBI® FMDV VP1 EIA) and for reactivity to the 3B non-structural viral protein (UBI® FMDV NS EIA) [18]. Serological reactivity to non-structural virus proteins can be used as a differential marker that distinguishes infection from vaccination because production of antibodies against non-structural proteins [18,23–25], of which 3B is the most immunodominant [18], is an indicator of viral replication. Immunoassay results are shown in Figs. 2 and 3 for representative animals from each group. All animals given experimental vaccines or a commercial FMDV O1 Taiwan vaccine seroconverted to VP1 reactivity following immunisation. The pigs of non-immunised control group 8 seroconverted to VP1 reactivity within one week after challenge (Fig. 2) due to their infected status. The control pigs of group 8 with the most apparent signs of FMD seroconverted to NS reactivity by week 2 following challenge, while the other animals remained non-reactive to the 3B NS peptide (Fig. 3).

Pigs were observed daily for clinical signs of FMD after the challenge and were scored as protected or not protected by clinical signs (Table 4). Of the 21 pigs receiving synthetic vaccine formulations (groups 1–7), all but one remained free of clinical signs at all times following exposure. In the non-immunised control group (group 8), pig no. 371 had vesicles on the coronary bands of four legs by the second day post-challenge, no. 387 of that control group had vesicles on the legs by the fourth day and on the snout by day five, no. 372 developed lameness in one leg by the

Table 4
Results of immunogenicity/challenge trial in pigs

Group	Vaccine	Tag no.	NA Titre (day 0)	NA Titre (day 40)	Protection
1	UBITh1®-VP1 100 μg/0.5 ml/dose	368	≤3	64	+
		369	≤3	4	+
		370	≤3	23	+
2	UBITh1®-VP1 50 μg/0.5 ml/dose	373	≤3	32	–
		374	≤3	91	+
		375	≤3	64	+
3	UBITh1®-VP1 50 μg/1.0 ml/dose	377	≤3	45	+
		378	≤3	362	+
		379	≤3	362	+
4	UBITh1®-VP1[a] 50 μg/1.0 ml/dose	394	≤3	181	+
		395	≤3	256	+
		397	≤3	32	+
5	UBITh1®-VP1 50 μg/1.5 ml/dose	381	≤3	256	+
		382	≤3	362	+
		383	≤3	64	+
6	UBITh1®-VP1 25 μg/0.5 ml/dose	386	≤3	32	+
		388	≤3	181	+
		389	≤3	91	+
7	UBITh1®-VP1 12.5 μg/0.5 ml/dose	390	≤3	45	+
		391	≤3	64	+
		392	≤3	45	+
8	Adjuvant 0.5 ml/dose	371	≤3	≤3	–
		372	≤3	≤3	–
		387	≤3	≤3	–
9	Commercial FMDV O1Taiwan vaccine	376	≤3	512	+
		380	≤3	≥724	+
		393	≤3	≥724	–

[a] Consensus FMDV O peptide immunogen and O1 Taiwan peptide immunogen in 1:1 ratio.

Pigs were immunised at weeks 0 and 4 with UBITh1®-O consensus peptide immunogen (Fig. 1) in indicated formulations with ISA51, except for group 4 which also received the VP1 immunogen having the target VP1 sequence of FMDV 01 Taiwan without UBITh® and linker. Neutralising antibody (NA) titres, expressed as reciprocal dilutions, were determined by quantitative VN microtest [17]. Protection was scored by absence of clinical signs of FMD.

fourth day, and all three control animals had elevated temperatures. Of the 24 vaccinated animals, animal no. 393 of the three pigs given commercial killed virus vaccine (group 9) had vesicles on a coronary band by day 11 post-challenge, and animal no. 373, of the group given 50 μg of peptide in 0.5 ml of vaccine (group 2), displayed lameness in one leg on day two followed by the appearance of small coronary vesicles on the affected leg on day 4. Both of these vaccinates also had elevated temperature concurrent with the signs of limited infection. No clinical signs were seen in the two other vaccinates given the killed vaccine or in the other 20 given peptide vaccines throughout a two week period of post-challenge observation.

4. Discussion

Inoculation of 21 pigs with the UBITh1® FMDV O consensus peptide (Fig. 1), in a dose range of 12.5–100 μg, in volumes of 0.5–1.5 ml, induced anti-peptide and neutralising antibodies in all the animals (Fig. 2 and Table 4). All but one of these pigs were protected from challenge with FMDV O1 Taiwan. Synthetic vaccines having an immunogen load of 50 μg of peptide elicited the highest titres of neutralising antibodies, but paradoxically the one peptide-immunised pig that was not protected (no. 373) had been in one of those groups (Table 4). The lack of seroconversion to antibody reactivity against non-structural protein 3B by peptide-immunised animals suggests a lack of generalised infection.

The major design objectives for the FMDV peptide vaccine were maximum immunogenicity combined with broad cross-reactivity against antigenic variants. This was accomplished by (1) disulphide bonds formed between the cysteine residues at positions 134 and 158; (2) by extending the contiguous flanking sequences on both the amino and carboxy terminii; (3) by addition of foreign T cell help; and (4) by use of a consensus sequence for broad cross-reactivity against diverse isolates. As predicted [26], cyclisation by disulphide bonds to stabilise the presentation of a flexible

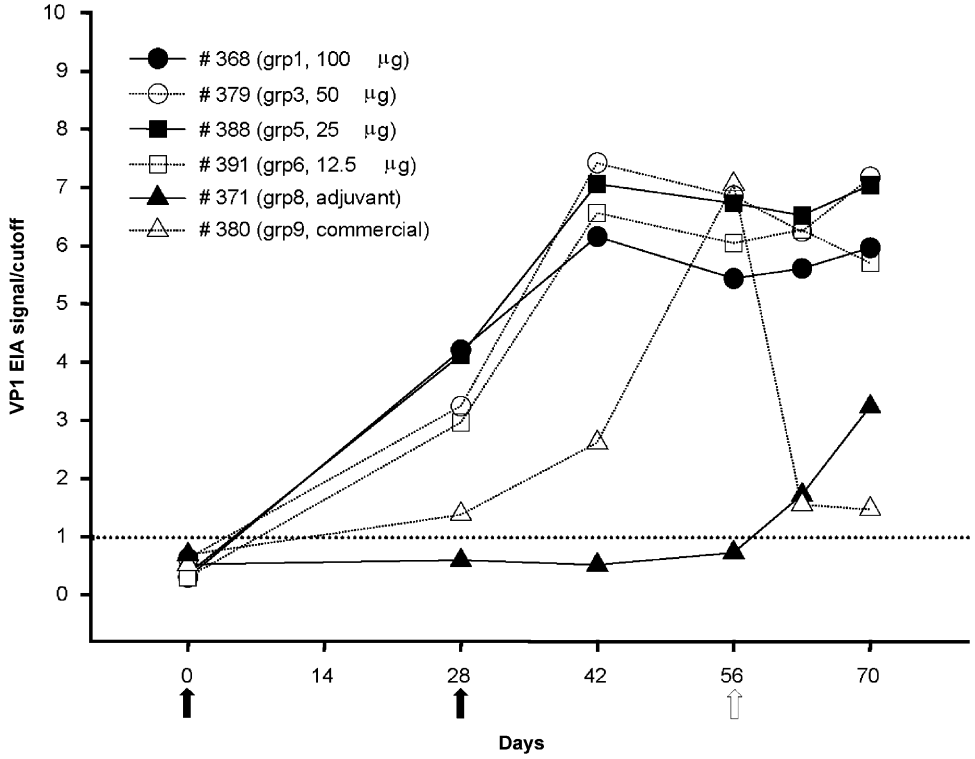

Fig. 2. Seroconversion to VP1 reactivity. Sera were collected from animals at the indicated intervals and tested at a 1:21 dilution for antibodies against the serotype O consensus VP1 site (Table 2) by the UBI® VP1 EIA (SWINE). The solid arrows show days for immunisation and the open arrow is day of challenge with FMDV. ELISA procedures were as reported [18] except that the VP1 EIA used serotype O consensus peptide without the UBITh1® site and linker as antigen for antibody capture; results were reported as signal/cutoff ratios with the cutoff value being $0.23 \times A_{450\,nm}$ of reactive control (cutoff $A_{450\,nm}$ 0.306); and the chromagen was 3,3′,5,5′-TMB. A signal/cutoff ratio of ≥1, shown by dotted line, indicates seroconversion.

G–H loop-like structure provided a better immunogen than the linear equivalent. Extending the flanking sequences beyond the critical 140–160 loop domain may provide additional T cell help and add additional discrete neutralising B cell sites, including epitopes that do not overlap the crucial Arg–Gly–Asp motif of the loop [10,16,21].

A promiscuous foreign T cell helper site, UBITh1®, was used for extrinsic T cell help rather than an autologous FMDV VP1 Th site because broad responsiveness to VP1 is limited in swine by genetic restriction at the Th level [20]. Even among convalescent pigs where about 57% of the neutralising activity maps to the immunodominant G–H loop, some animals remain unresponsive to the VP1 neutralising domain [27]. No such genetic restriction has been observed by us in swine for UBITh1® chimeric immunogens for LHRH, somatostatin, and IgE [15], and here for an FMDV immunogen. Neutralising antibody responses to the VP1 loop domain, that were protective in 20 out of 21 animals, were elicited in all 21 swine by the incorporation of the promiscuous UBITh1® Th cell combinatorial sequence. Foreign T cell help will not prime for FMDV-specific T cell help in the event of exposure, but site-specific anti-VP1 neutralising antibodies have been maintained in swine by the foreign T help through 6 months (data not shown). This duration of protection provided by the priming doses will suffice for production animals who go to market between 24 and 30 weeks of age. The duration of protection beyond 6 months, and hence the interval between boosts, needs further investigation for breeder swine and for application to cattle. The measles-derived UBITh1® site, in combination with the FMDV O VP1 target sequence has also shown immunogenicity in goats and cattle (data not shown), suggestive of widespread T cell responsiveness to the immunogen.

The considerable antigenic variation in FMDV and in other viruses such as those causing influenza and AIDS presents problems in their control by vaccination. In the case of FMDV, the highly variable G–H loop and corresponding peptides often mimic the strain specificities of the viruses from which they are derived [8,13,14]. The much publicised quasi-species described by Taboga and his colleagues [8] may account for the low level of protection seen in those unprotected cattle whose isolated breakthrough virus differed in the sequence of the G–H loop from that of the peptide used for immunisation. Our approach has been to attempt to overcome the quasi-species problem by designing a peptide that incorporated consensus residues in those positions that are hypervariable. The consensus sequence immunogen

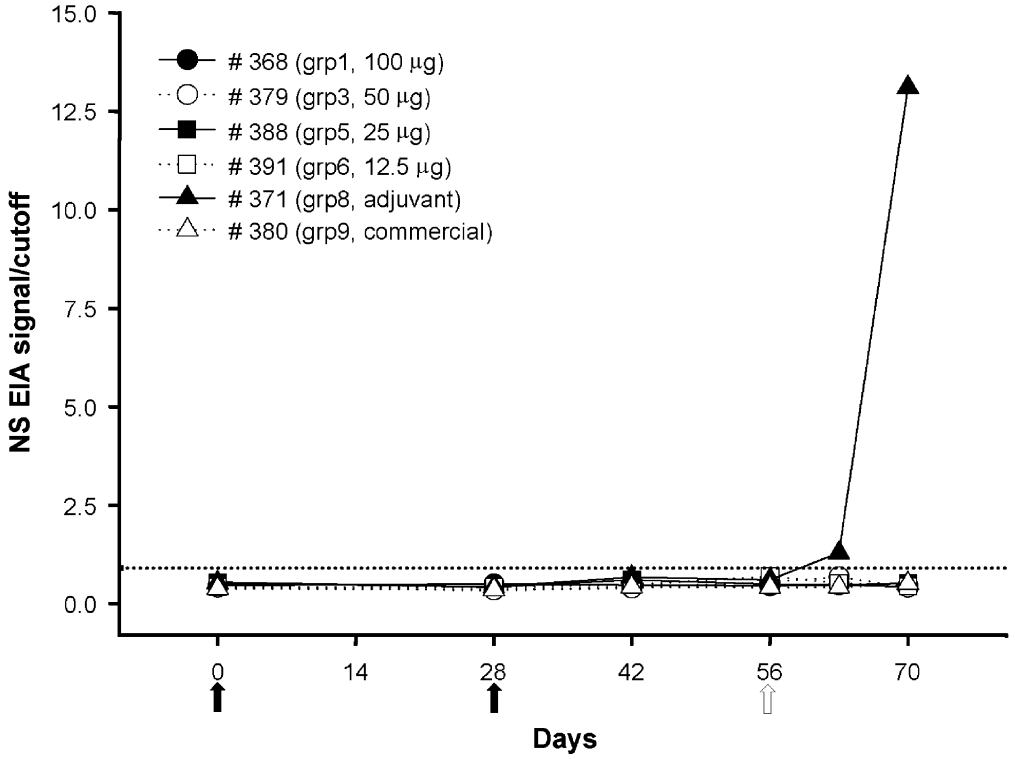

Fig. 3. Seroconversion to NS reactivity. Sera were prepared and tested by ELISA as described in Fig. 2 except that the test used was the UBI® NS EIA (SWINE) having a 3B non-structural peptide as antigen for antibody capture [18]. The cutoff value was $A_{450\,nm}$ 0.189.

selected here is more similar to a UK PanAsia isolate [22] than to the O1 Taiwan virus used here for infectious challenge (Table 2), but it nevertheless provided protection from the Taiwan virus. Moreover, the G–H loop sequences in Table 2 show no drastically antigenic replacements in the 141–160 region centred on and flanking the conserved Arg–Gly–Asp triplet. This is a critical site involved in attachment of the virus to the host cell [28–30]. UBITh® serotype O consensus peptide immunogens were administered to swine in an earlier immunogenicity/challenge trial and the immune sera were tested for neutralising indices against O Manisa and O Campos, in addition to O1 Taiwan. The peptide immunogens elicited more broadly neutralising antibodies against the Manisa, and Campos isolates than a conventional 01 Taiwan killed virus vaccine (Table 3). Consequently, the O consensus peptide immunogen is expected to be broadly protective in pigs against serotype O strains. The consensus approach is being used to design peptide homologues for swine vaccines against the six other serotypes of FMDV. A similar approach to serotype O peptide immunogens is being investigated for cattle.

As has been previously seen with synthetic peptide immunogens [8,12,31,32], there was not a strong correspondence between serum neutralising activity and protection (Table 4). Protection was found in the presence neutralising antibodies of low or moderate titre in many of the animals given the synthetic vaccine, particularly in pig no. 369. In contrast, pig no. 393 given the killed virus vaccine had a high titre by the in vitro neutralising antibody assay but was not protected. Neutralisation of the capacity of the virions to infect susceptible cells in vitro may not be the only functional parameter of the antibody response to an FMDV immunogen that is responsible for protection. Differences in affinity may underlie observed variations in the biological effectiveness of peptide-induced neutralising antibodies [12], and neutralising antibodies may have a significant role in vivo for viral clearance through antibody-dependent enhancement of phagocytosis and the capacity of bound antibodies to fix complement [31,32]. Thus, for evaluation of the efficacy of synthetic peptide-based vaccines for FMD, consideration will be needed for antibody affinity and other functional properties of the antibody response, in addition to in vitro neutralising activity.

The synthetic peptide vaccine is a site-specific antigenic marker vaccine. The chemically-defined immunogen elicits site-specific antibody responses that are readily distinguishable from the complex responses to infection. In combination with a UBI® complementary synthetic-peptide-based system for differential immunodiagnosis [18], an integrated system is provided for the immunosurveillance of infection in the presence of vaccination and for the effectiveness of national vaccine campaigns. A safe chemically-defined vaccine, as part of an integrated system, can expedite eradication through vaccination while minimising the need for control by culling.

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