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Vaccines Prepared from Chimeras of Foot-and-Mouth Disease Virus (FMDV) Induce Neutralizing Antibodies and Protective Immunity to Multiple Serotypes of FMDV

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The G-H loop of VP1 (residues 132 to 159) of foot-and-mouth disease virus (FMDV) is a prominent feature on the virion surface and has an important role in vaccine efficacy, generation of antigenic variants, and cell binding. Using an infectious cDNA of FMDV, we have constructed serotype A viruses in which the G-H loop has been substituted with the homologous sequences from serotype O or C. These chimeric viruses replicated to high titer and displayed plaque morphologies similar to those of wild-type viruses, demonstrating that the functions provided by the loop can be readily exchanged between serotypes. Monoclonal antibody analyses showed that epitopes contained within the loop were transferred to the chimeras and that epitopes encoded by the type A backbone were maintained. Chemically inactivated vaccines prepared from chimeric viruses induced antibodies in guinea pigs that neutralized both type A and either type O or type C viruses. Swine inoculated with the A/C chimera vaccine also produced cross-reactive antibodies, were protected from challenge with the type A virus, and partially protected against challenge with type C. These studies emphasize the importance of epitopes outside of the G-H loop in protective immunity in swine, which is a natural host of FMDV.

Foot-and-mouth disease (FMD) virus (FMDV), a member of the family *Picornaviridae*, causes an economically important viral disease of livestock (2, 34). Chemically inactivated vaccines have been widely used to control FMD but do not confer long-lasting immunity. Furthermore, protection is often restricted to specific serotypes and subtypes which can give rise to antigenic variants.

The high antigenic diversity of FMDV stems from its extreme genetic heterogeneity (28), and antigenic variants can be selected upon replication in immune or partially immune hosts (16). Selection of new variants is facilitated by the existence of an immunodominant site, located between β strands G and H (G–H loop) of VP1, which allows new strains of virus, altered at this site, to be selected in animals whose immune response is focused on the loop. Thus, new variants sharing many surface features with their parental viruses can escape immune surveillance if they contain mutations within one small region of the genome. This evolutionary strategy, coupled with the ability of the virus to establish a persistent infection in certain species (40), helps to explain why FMD has been difficult to control throughout much of the world.

The FMD virion consists of a single-stranded RNA genome packaged in an icosahedrally symmetric protein shell composed of 60 copies each of four structural proteins, VP1 to VP4. VP4 is located inside the mature virus particle, while VP1, VP2, and VP3 form the capsid surface. These latter three proteins have a similar tertiary structures represented by a highly conserved β-barrel core (1, 21).

While the immunodominant site of FMDV has been identified as including the G-H loop of VP1 (site 1), other immunogenic sites have been identified in VP2 and VP3 (7, 19, 41, 42). Extensive characterization of these sites has been performed on type O. Specifically, monoclonal antibody (MAb) escape mutant studies have defined at least five different sites on the type O virion (11, 19). Site 1 includes residues located within the 140–160 region (G-H loop) and carboxy terminus of VP1 (35, 43), site 2 includes residues 70 to 78 and 131 to 134 of VP2 (4, 43), site 3 includes residues 40 to 60 of VP1 (βB-βC loop) and has been shown to modulate epitope presentation at site 1 (33), site 4 includes residues 56 to 58 on VP3 (43), and site 5 maps to a single residue (residue 149) on the G-H loop of VP1 distinct from site 1 (11).

Although epitopes from site 1 presented in many different forms, including synthetic peptides (15), can induce neutralizing antibodies, vaccines based on these immunogens may lack practical application, as they would be more likely to fail because of the presentation of a restricted number of epitopes, inviting antigenic variation. Specifically, Krebs et al. (20) have shown that swine vaccinated with peptides based on the type O G-H loop developed FMD following challenge, and an antigenic variant isolated from one animal was not neutralized by antipeptide sera. Vaccination-challenge studies using synthetic peptides have also failed to demonstrate complete protection in cattle, offering further evidence of failure of vaccines presenting only a single antigenic site (12, 13).

Aside from its immunodominance, the G-H loop of VP1 has other important biological properties. It contains a conserved arginine-glycine-aspartic acid (RGD) sequence, located at its apex (22), which is the cell binding site (5, 14, 25, 35).

Previous studies on molecularly defined intertypic polioviruses have shown that viable hybrids can be obtained and that these hybrids are useful for studying antigenic and biological characteristics of specific serotypes (24, 30, 31, 32). Using a similar strategy, we directly examined the importance of the G-H loop of VP1 in the protective immune response generated by inactivated vaccines. Specifically, we have designed and produced chimeric viruses in which the G-H loop of FMDV type A_{12} has been replaced by the corresponding loops from serotype C_3 or O_1. In this report, we show that sequential epitopes contained within the G-H loops of FMDV serotypes

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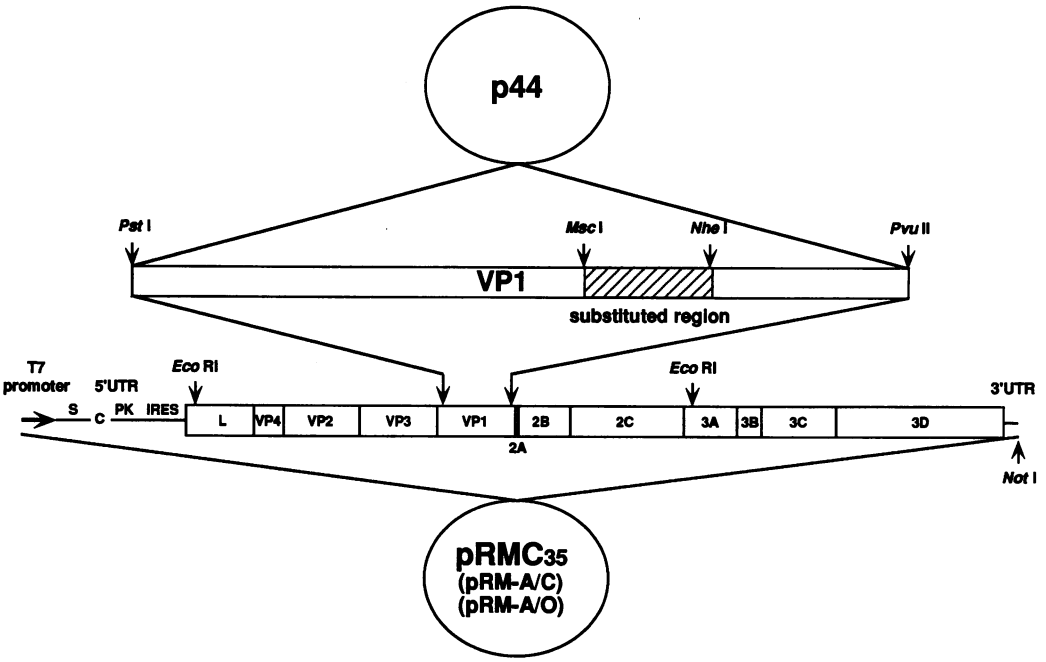

FIG. 1. Diagram of plasmids used to produce the FMDV chimeras. Abbreviations: IRES, internal ribosome entry site; PK, pseudoknots; C, poly(C) tract; S, 5′ portion of the genome or small fragment; UTR, untranslated region.

O and C can be transferred in a functional form onto the A_{12} backbone, producing viruses that are useful in probing the immunogenicity of FMDV.

MATERIALS AND METHODS

Cell lines, viruses, and plasmids. Baby hamster kidney (BHK) cells (strain 21, clone 13) were propagated as described previously (37). FMDV types O_1 British field strain (O_1BFS) and C_3 Resende (C_3RES) and the swine-virulent strain of A_{12} ($A_{12}S_{15}$) were supplied by F. Brown (Plum Island Animal Disease Center [PIADC]), E. Palma (Instituto Nacional de Tecnología Agropecuaria [INTA], Castelar, Argentina), and M. Grubman (PIADC), respectively. The O_1BFS and C_3RES viruses were plaque cloned under agar twice, and cDNAs amplified from the RNA of the twice-cloned viruses were sequenced through the G-H loop, using Sequenase (U.S. Biochemical, Cleveland, Ohio). Virus stocks were prepared and titrated in BHK cells. FMDV type A_{12}, used for vaccination and all in vitro analysis, was derived from the infectious clone $pRMC_{35}$ (37), and all plasmids used to produce the chimeric viruses were derivatives of $pRMC_{35}$. Viruses were purified on sucrose density gradients as previously described (6).

Construction of chimeric cDNAs and recovery of chimeric viruses. A plasmid containing most of the 213-amino-acid VP1 coding region (amino acids 8 to 211; bordered by *Pst*I and *Pvu*II restriction sites) was created, and two new restriction sites (*Msc*I and *Nhe*I) bordering the G-H loop of VP1 were added by using standard PCR techniques (17) (Fig. 1). This plasmid, designated p44, was sequenced to confirm that only the desired changes were introduced during the PCR mutagenesis. Double-stranded oligonucleotides containing *Msc*I and *Nhe*I restriction sites and the G-H loops of O_1BFS or C_3RES were ligated to p44 DNA cleaved with these two restriction enzymes, and the ligation mixtures were transformed into *Escherichia coli*. The chimeric A/O and A/C VP1 cDNA molecules were then excised from the p44 derivatives with *Pst*I and *Pvu*II and inserted into the full-length infectious cDNA, $pRMC_{35}$, via a shuttle vector containing a 4,260-bp *Eco*RI restriction endonuclease fragment extending from within the leader protease gene into 3A (38) (Fig. 1). The full-length cDNA-containing plasmids were then resequenced through the G-H loop as described above.

Transfections of BHK cells with in vitro RNAs produced from *Not*I-linearized, full-length cDNA plasmids were performed by using Lipofectin (GIBCO-BRL, Gaithersburg, Md.) as described by Rieder et al. (37).

MAbs. Neutralizing MAbs against FMDV types A_{12} (2PD11, 6EE2, and 7SF3) and O_1 Brugge (12FE9 and 10GA4) have been characterized previously (6, 7, 41). The FMDV type C_3 Indaial-specific MAbs (7AB5, 7EE6, 7CF12, and 7CA11) were kindly provided by Fernando Osorio and Vincente Astudillo (Centro Panamericano de Fiebre Aftosa [CPFA], Rio de Janeiro, Brazil), and their partial characterization has been published by Mateu et al. (26, 27, 29).

Virus neutralization and 3D antibody assays. Plaque reduction neutralization (PRN) assays using MAb-containing hybridoma culture fluid or polyclonal sera were performed on BHK cells as described previously (6) except that cells were overlaid with 0.6% gum tragacanth containing 1% calf serum and incubated for 40 to 48 h before staining. Neutralization titers are reported as the log of serum dilution yielding a 70% reduction in PFU ($\log_{10}$ PRN_{70}). Antibodies to the 3D subunit of the FMDV RNA polymerase were detected by using radioimmunoprecipitation assays performed with 3 μl of serum and culture fluid harvested from [^{35}S]methionine-labeled cells infected with FMDV type C_3 or A_{12}.

Guinea pig immunizations. Groups of five 500-g guinea pigs were given one intraperitoneal inoculation with 2 μg of gradient-purified binary ethylenimine (BEI)-inactivated virus preparations (3) emulsified in Freund's incomplete adjuvant.

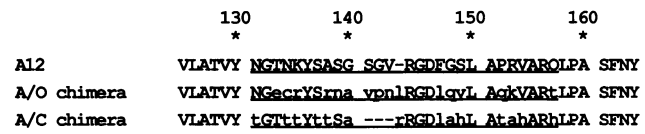

FIG. 2. Alignment of amino acid sequences of the G-H loop of VP1 found in the A_{12} virus and the chimeras. Underlined sequences represent the foreign coding sequences inserted into the A_{12} VP1 cDNA. Lowercase is used to highlight differences; dashes indicate gaps.

Guinea pigs were bled 3 weeks after immunization, and sera were tested for neutralizing antibodies as described above.

Swine immunization and challenge with FMDV. Mixed-breed gilts, 20 to 25 kg, were given a single intramuscular injection with 2 μg of BEI-inactivated virus preparations (see above) emulsified with an equal volume of mineral oil (9:1 Marcol 52/Montanide 888, provided by J. Mesquita, CPFA) or an emulsion of minimal essential medium containing no virus in the same oil. Sera obtained 4 weeks postvaccination were tested for the presence of neutralizing antibodies as described above. One week later, each group was challenged by direct inoculation of one of the four minimal essential medium-vaccinated control pigs present in each room. This source animal was inoculated with 10^7 PFU of either C_3RES or a swine-virulent strain of A_{12} ($A_{12}S_{15}$) by a combination of intravenous, intranasal, and snout dermal scarification routes, and all animals were observed for signs of FMD infection. Classical FMD lesions were observed on all animals that showed signs of infection within 7 days after cohabitation with the source animal. We defined lesions as follows: localized lesions, vesicles observed on snout, lips, or one foot during the postexposure period; generalized lesions, vesicles observed on snout, lips, and one or more feet during the postexposure period. Three-weeks postexposure, all animals were rebled, and sera were tested for the presence of antibodies to 3D as described above.

RESULTS

Production of chimeric viruses. Chimeric full-length type A_{12} cDNA molecules containing the G-H loop of FMDV O_1BFS or C_3RES were generated as shown in Fig. 1 (see Materials and Methods for details). The exact coding sequences inserted into these full-length cDNA plasmids, designated pRM-A/O and pRM-A/C, are shown in Fig. 2. Junctions at codons 130 and 158 were selected on the basis of alignments of the sequences of the A, C, and O viruses (23, 36, 38) and the identities of the last residues visible in the preliminary crystal structure of FMDV type O_1BFS (1). These selections are in agreement with the ends of the loop recently defined by Logan et al. (22) for the reduced form of O_1BFS.

T7 RNA transcripts derived from $pRMC_{35}$, pRM-A/O, and pRM-A/C displayed similar specific infectivities in BHK cells, indicating that the three transcripts were equally viable (Table 1). Furthermore, viruses recovered from these transcripts showed indistinguishable plaque morphologies in BHK cells (results not shown) and grew to similar titers in liquid culture (Table 1). RNA extracted from samples of each virus recovered from infected BHK cells was reverse transcribed, amplified by PCR, and sequenced through the G-H loop (25), confirming the expected amino acid coding sequences (Fig. 2). Chimeric viruses maintained these sequences through four additional passages in BHK cells at low multiplicities of infection (a total of six passages), further demonstrating their genetic stability.

TABLE 1. Comparison of specific infectivities of full-length RNAs and virus yields from BHK cells infected with viruses recovered from transfected cells

RNA source or virus	Specific infectivity[a]	Yield in tissue culture[b]
$pRMC_{35}$	$4.7\ 10^3$	$6.5\ 10^7$
pRM-A/O	$7.8\ 10^3$	$3.0\ 10^7$
pRM-A/C	$4.7\ 10^3$	$4.0\ 10^7$
O_1BFS	ND[c]	$3.3\ 10^7$
C_3RES	ND	$4.0\ 10^7$

[a] PFU per microgram of synthetic RNA obtained with the indicated plasmids.
[b] PFU per milliliter recovered from overnight cultures of BHK cells infected with products of transfection or the indicated virus.
[c] ND, not determined.

Antigenic characteristics of chimeric viruses. Antigenic characteristics of chimeric and parental viruses were examined by using a panel of MAbs (Table 2). Reaction with type A-specific MAb 7SF3, mapped to an epitope in the G-H loop (7), was restricted to the A_{12} parent virus. Neutralization titers obtained with MAb 6EE2, which recognizes a sequential epitope near the C terminus of VP1 (7), indicated that this epitope was displayed equally well by the A_{12} parent and both chimeras (Table 2). Reactions with the discontinuous epitope recognized by 2PD11, which includes residues near the C terminus of VP3 (7), revealed a similar reactivity profile (Table 2). These data indicate that substitution of foreign G-H loops did not significantly influence the structure of other components on the virion surface. Reactions of type O MAbs showed that G-H loop-specific MAb 12FE9 (41) reacted with both O_1BFS and the A/O chimera, whereas 10GA4, which recognizes a conformational epitope, reacted only with O_1BFS (Table 2). All type C-specific MAbs tested, which recognize at least three different epitopes in antigenic site 1 (26, 27, 29), reacted with the A/C chimera (Table 2), indicating that several different epitopes were transferred with the type C loop sequences.

Immunogenicity of chimeric viruses in guinea pigs. Viruses were purified, inactivated, and inoculated into guinea pigs as described in Materials and Methods. Sera collected 3 weeks

TABLE 2. Reactivities of parent and chimeric viruses with serotype-specific MAbs

MAb			Neutralization titer (log PRN_{70}) versus:				
Serotype	Name	Reactivity with G-H loop[a]	A_{12}	A/O chimera	A/C chimera	O_1BFS	C_3RES
A[b]	7SF3	+	2.0	<0.4	<0.4	<0.4	<0.4
	6EE2	−	1.6	1.6	1.6	<0.7	<0.7
	2PD11	−	4.1	3.8	3.5	<0.4	<0.4
O[b]	12FE9	+	<0.7	3.8	<0.7	2.9	<0.7
	10GA4	−	<0.4	<0.4	<0.4	2.9	<0.4
C[c]	7AB5	+	<0.4	<0.4	3.5	<0.4	3.5
	7EE6	+	<0.4	<0.4	2.9	<0.4	2.9
	7FC12	+	<0.4	<0.4	3.8	<0.4	3.8
	7CA8	+	<0.4	<0.4	4.4	<0.4	4.4
	7CA11	+	<0.4	<0.4	4.4	<0.4	4.1

[a] +, recognizes an epitope in the G-H loop; −, recognizes an epitope outside the G-H loop.
[b] Produced at PIADC.
[c] Provided by CPFA.

TABLE 3. Neutralization of parental and chimeric viruses with guinea pig antisera

Immunogen[a]	Neutralization titer[b] versus:				
	A_{12}	O_1BFS	C_3RES	A/O chimera	A/C chimera
A_{12}	3.4	<1.0	<1.0	2.2	2.2
O_1BFS	<1.0	2.2	<1.0	<1.0	<1.0
C_3RES	<1.0	<1.0	2.5	1.0	2.5
A/O chimera	2.2	2.2[c]	<1.0	3.4	1.9
A/C chimera	2.2	<1.0	2.2[d]	2.2	3.1

[a] BEI-inactivated virus.
[b] Log PRN_{70} values from sera pooled from all five animals in each group (PRN titers of <1.0 were detected in preimmune sera from all groups).
[c] Log PRN_{70} titers of individual sera: 1.6, 1.6, 1.6, 2.2, 2.5.
[d] Log PRN_{70} titers of individual sera: 2.5, 1.6, 1.6, 2.2, 2.2.

later were tested by PRN assays (Table 3). As expected, the highest PRN_{70} titers obtained for each immunogen were against the homologous virus. These titers varied from 2.2 logs for O_1BFS to over 3 logs for A_{12} or the A_{12}-based chimeras. Antibodies produced against type A_{12} reacted with both chimeras, and antibodies elicited by C_3RES recognized C_3RES and the A/C chimera (Table 3). In the case of O_1BFS, the homologous reaction was the poorest detected, and PRN_{70} reactivity could not be detected against the A/O chimera at a 1:10 serum dilution (Table 3). Chimeric viruses induced high levels of homologous antibodies that cross-reacted with both of their parents. In the case of the A/O chimera, the homotypic reaction was over 3 logs, and reactions with type O_1, type A_{12}, and the A/C chimera were about 2 logs each (Table 3). Interestingly, the A/O immunogen induced levels of neutralizing antibodies to O_1BFS that were equal to those induced by immunization with O_1BFS itself (Table 3). The A/C chimera elicited a homologous reaction of 3 logs and cross-reactions with type C_3, type A_{12}, and the A/O chimera of over 2 logs (Table 3). Antibodies produced against C_3RES also showed a weak reaction to the A/O chimera, consistent with low levels of cross-serotype responses reported in other studies (for a review, see reference 9). Although we did not measure the PRN_{70} of every individual serum with each virus, all five sera raised to the A/O and A/C chimeras reacted well with O_1BFS and C_3RES, respectively (Table 3, footnotes *c* and *d*).

Swine protection experiments. To test the protective potential of the chimeric viruses in an animal naturally susceptible to the disease, we designed a swine challenge study. Because of the poor performance of the O_1BFS vaccine in guinea pigs, only type A, type C, and the A/C chimera were used in swine. Pigs were divided into two separately housed groups, one for A_{12} challenge and another for C_3 challenge. The groups were four swine inoculated with minimal essential medium (mock vaccination), five swine vaccinated with A_{12} vaccine, five swine vaccinated with the C_3RES vaccine, and five swine vaccinated with the A/C chimera vaccine. Four weeks postvaccination, all of the animals were bled, and sera were tested for neutralization of C_3RES (Table 4) or A_{12} (Table 5). One week later, all animals were challenged by contact exposure to either C_3RES or $A_{12}S_{15}$ (see Materials and Methods) and examined for signs of disease (Tables 4 and 5). For both groups, 100% of mock-vaccinated animals (minimal essential medium controls) showed clinical signs of infection indicating that efficient transmission had occurred. Three weeks postexposure, animals were rebled and sera were tested for the presence of antibodies to the nonstructural protein 3D, an indicator of viral replication in infected animals (10, 39) (Tables 4 and 5).

TABLE 4. Responses of pigs to vaccination with A_{12}, C_3, or A/C chimera vaccines and C_3 challenge

Pig no.	Vaccine[a]	PRN titer vs C_3[b]	Response to challenge with C_3[c]	
			Lesions[d]	3D[e]
51	None	<1.0	++	+
52	None	<1.0	++	+
53	None	<1.0	++	+
54	None	<1.0	++	+
55	A_{12}	<1.0	−	+
56	A_{12}	<1.0	++	+
57	A_{12}	<1.0	++	+
58	A_{12}	<1.0	−	+
59	A_{12}	2.2	++	+
60	C_3	2.8	−	−
62	C_3	3.4	−	−
63	C_3	3.4	−	−
64	C_3	2.8	−	−
65	C_3	>3.7	−	−
66	A/C chimera	3.4	−	−
67	A/C chimera	2.2	+	+
68	A/C chimera	2.5	++	+
69	A/C chimera	2.8	−	−
70	A/C chimera	3.1	−	−

[a] BEI-inactivated virus.
[b] Log PRN_{70} versus C_3RES of sera collected 28 days postvaccination.
[c] Animals were challenged with FMDV type C_3 by inoculating one of the mock-vaccinated animals (animal 51) with virus at 34 days postvaccination and allowing the infection to spread among all of the jointly housed animals.
[d] Scoring of clinical signs: −, no visible lesions, +, localized gross lesions; ++, generalized lesions.
[e] Presence of antibodies to the nonstructural protein 3D in sera collected 22 days postchallenge, determined by radioimmunoprecipitation. −, no specific immunoprecipitation; +, specific immunoprecipitation.

Vaccination with the A/C chimera produced high titers of antibodies cross-reactive with both A_{12} and C_3; however, titers against A_{12} were generally higher (Tables 4 and 5). These data contrast with the results for guinea pigs, which showed similar levels of neutralization against C_3 and A_{12} among the A/C chimera-vaccinated animals (Table 3). Swine protection correlated well with neutralization data. In the C_3 challenge group, all animals that received mock vaccination and three of five A_{12}-vaccinated animals showed generalized FMD lesions (Table 4). Furthermore, all swine in these two groups developed antibodies to 3D (Table 4), demonstrating that they had become infected with the challenge virus and confirming the effectiveness of the contact challenge. Although one of the A_{12}-vaccinated animals (animal 59) developed a low level of antibodies that neutralized C_3, it was not protected from C_3 challenge. All animals vaccinated with the C_3 vaccine were protected from disease and viral infection after C_3 challenge (Table 4). Of the five A/C chimera-vaccinated animals, one animal developed generalized lesions, one showed localized lesions, and the remaining three animals had no overt clinical signs of infection after C_3 challenge. Both swine with lesions also had responses to 3D, whereas the absence of 3D antibodies in the other three animals (which had higher prechallenge PRN titers) indicated that they had been protected against infection by the C_3 virus (Table 4). In the A_{12} challenge group, all of the mock-vaccinated and C_3-vaccinated animals had FMD lesions and developed antibodies to 3D (Table 5). All animals vaccinated with the A_{12} vaccine were protected from disease and infection after A_{12} challenge (Table 5). None of the A/C chimera-vaccinated animals showed overt clinical signs of infection or antibodies to 3D after A_{12} challenge,

TABLE 5. Responses of pigs to vaccination with A_{12}, C_3, and A/C chimera vaccines and A_{12} challenge

Pig no.	Vaccine[a]	PRN titer vs A_{12}[b]	Response to challenge with $A_{12}S_{15}$[c]	
			Lesions[d]	3D[e]
71	None	<1.0	++	+
72	None	<1.0	++	+
73	None	<1.0	++	+
74	None	<1.0	++	+
75	A_{12}	>3.7	−	−
76	A_{12}	3.7	−	−
77	A_{12}	3.4	−	−
78	A_{12}	>3.7	−	−
79	A_{12}	>3.7	−	−
80	C_3	1.2	+	+
81	C_3	1.2	++	+
82	C_3	1.0	+	+
83	C_3	1.2	+	+
84	C_3	<1.0	++	+
85	A/C chimera	3.1	−	−
86	A/C chimera	>3.7	−	−
87	A/C chimera	>3.7	−	−
88	A/C chimera	>3.7	−	−
89	A/C chimera	>3.7	−	−

[a] BEI-inactivated virus.
[b] Log PRN_{70} versus A_{12} of sera collected 28 days postvaccination.
[c] Animals were challenged with $A_{12}S_{15}$ by inoculating one of the mock-vaccinated animals (animal 71) with virus at 35 days postvaccination and allowing the infection to spread among all of the jointly housed animals.
[d] Scoring of clinical signs: −, no visible lesions; +, localized gross lesions; ++, generalized lesions.
[e] Presence of antibodies to the nonstructural protein 3D in sera collected 21 days postchallenge, determined by radioimmunoprecipitation. −, no specific immunoprecipitation; +, specific immunoprecipitation.

indicating that they had been completely protected by the chimeric vaccine (Table 5).

DISCUSSION

Intertypic chimeras of FMDV type A_{12} containing the sequences of the immunodominant G-H loops of type C_3 or O_1 have been created and characterized. These viruses grow well in tissue culture and display plaque phenotypes indistinguishable from that of the type A_{12} parent. Chimeric viruses stably maintained the O_1 or C_3 sequence on passage, indicating that G-H loop sequences are interchangeable between viruses of these three serotypes. Viability of these viruses indicates that the G-H loop of the chimeras still functions in cell binding, since the loops contain the only RGD sequence found on chimeric virions and the RGD sequence is absolutely required for growth of FMDV in BHK cells (25).

Efficient neutralization of chimeric viruses by a panel of MAbs known to react with G-H loop residues of types O_1 and C_3 showed that several epitopes, probably including portions of antigenic sites 1 and 5 defined for serotype O_1 (see the introduction) and antigenic site A defined for type C (27), were transferred to these chimeras with the G-H loop sequences. Furthermore, sequential or discontinuous antigenic determinants outside the loop of A_{12} were maintained in chimeras, whereas an epitope mapped to the A_{12} G-H loop was lost upon substitution with the O_1 or C_3 sequence.

Our data support the importance of the G-H loop in antigenicity of FMDV and indicate that the immunodominant site is readily separated from other virion components. These observations are particularly interesting since studies using serotype O virus indicate that the G-H loop interacts with other components on the surface of the virion (3, 22). Specifically, Parry and coworkers (33) demonstrated that amino acid changes outside of the G-H loop could affect the antigenic properties and the three-dimensional structure of the G-H loop. One peculiar feature of the G-H loop of type O_1BFS is a disulfide bond which links Cys-134 of VP1 to Cys-130 of VP2 (1). However, this bond does not appear to be required for biological activity, and the crystal structure of a reduced form of this virus has been described recently (22). Our data suggest that the oxidized form of the G-H loop may not be significant in determining structures of the loop involved in cell binding or antigenic activity, since our A/O chimera cannot form the disulfide bridge and retains the functional and antigenic properties of the loop. However, our data do not rule out the importance of other interactions of VP2 with the loop, since these could be produced in natural and chimeric viruses.

To determine if the transferred antigenic sites retained their immunogenicity, vaccines prepared from the chimeras were tested in guinea pigs and swine. Guinea pigs vaccinated with the chimeras produced similar levels of neutralizing antibodies to A_{12} and either C_3 or O_1, and in the case of the A/O chimera, the neutralizing antibody to O_1 was equal to that obtained with an O_1 vaccine. Similar success in the induction of composite antigenicity and immunogenicity has been shown with a type 1/3 antigenic hybrid of poliovirus (30, 32).

The immune response to the transferred G-H loops is consistent with the well-established immunogenicity of peptides corresponding to the G-H loop in guinea pigs (8, 15) and the immunogenicity of chimeric polioviruses containing the G-H loop of FMDV type O_1 Kaufbeuren (18). Our A_{12}-based chimeras appear to be better immunogens than the poliovirus-O_1 Kaufbeuren chimeras, since a single immunization with 2 μg of immunogen induced significant FMDV-specific responses in all of the animals immunized with our chimeras. The better performance of the FMDV A_{12}-vectored O_1 loop is consistent with the idea that expression of an O_1 loop in an A_{12} context would more closely mimic presentation of this site in the native type O_1 particle.

Vaccination and challenge studies with swine revealed that epitopes outside of the G-H loop are important in protection in this species, which is a natural host of FMDV. Specifically, swine vaccinated with the A/C chimeras developed neutralizing antibodies to type A_{12} similar to the antibodies elicited by vaccination with A_{12}, and all of the animals vaccinated with the chimera were protected from A_{12} infection. The A/C chimera produced significant, albeit lower, levels of neutralizing antibodies to C_3RES, and only 60% of the animals vaccinated with the A/C chimera were protected from C_3 infection. These data, which demonstrate that epitopes outside the G-H loop are important for protecting swine from FMDV, are supported by studies showing that synthetic peptides based solely on the G-H loop poorly protect livestock from FMDV challenge, probably because of the limited number of epitopes presented to the animal (see the introduction).

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