Chimeric Polioviruses That Include Sequences Derived from Two Independent Antigenic Sites of Foot-and-Mouth Disease Virus (FMDV) Induce Neutralizing Antibodies against FMDV in Guinea Pigs

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Five poliovirus recombinants containing sequences corresponding to foot-and-mouth disease virus (FMDV) antigenic sites were constructed. Viable virus was recovered from four of these plasmids, in which the VP1 βB-βC loop (antigenic site 1) of poliovirus type 1 Sabin had been replaced with sequences derived from the VP1 βG-βH loop (antigenic site 1) of FMDV 0, Kaufbeuren (O,K), chimera O1.1 (residues 141 to 154), chimera O1.2 (residues 147 to 156), and chimera O1.3 (residues 140 to 160) or from the βB-βC loop of VP1 (antigenic site 3) in chimera O3.1 (residues 40 to 49). One chimera (O1.3) was neutralized by FMDV-specific polyclonal serum and monoclonal antibodies directed against antigenic site 1 of FMDV. Chimeras O1.3 and O3.1 induced site-specific FMDV-neutralizing antibodies in guinea pigs. Chimera O1.3 was capable of inducing a protective response against FMDV challenge in some guinea pigs.

The capsid of picornviruses consists of 60 copies of four proteins, VP4 (1A), VP2 (1B), VP3 (1C), and VP1 (1D). The three-dimensional structures of representatives of this group of viruses have been determined by X-ray crystallography, namely, rhinovirus 14 (33), poliovirus (10), mengo virus (13), and foot-and-mouth disease virus (FMDV) (1). It has been established in each case that VP4 is entirely internal and that the other three proteins are all partially exposed on the surface. The major structural differences among the capsid proteins of the different viruses are in the loops which connect the conserved β-barrel cores. The antigenic sites of poliovirus (reviewed in reference 20), rhinovirus 14 (36), and FMDV (12, 39) have been mapped by sequence analysis of monoclonal antibody (MAb)-resistant mutants and are located on these surface-exposed loops.

This information has permitted the design of antigenic chimeras which contain one or more antigenic sites from one virus substituted by a sequence corresponding to an antigenic site from another virus. Initial studies replaced antigenic site 1 of one poliovirus serotype by the corresponding sequence from a different serotype (5, 14, 25, 26). These intertypic recombinants display composite antigenicity and immunogenicity. A further extension of this approach is replacement of poliovirus sequences by antigenic regions of other proteins. Site 1 of poliovirus type 1 Sabin has been replaced by residues 735 to 752 of gp41 from human immunodeficiency virus 1 (8) and a 16-residue sequence from human papillomavirus type 16 L1 protein (11). The chimeric polioviruses were recognized by anti-gp41 MAbs and human papillomavirus type 16 L1-specific MAbs, respectively, and induced production of antibodies specific for the inserted antigenic site. The ability of such a sequence to induce an immune response capable of conferring protection against challenge from its parental pathogen has not been demonstrated.

Four functionally distinct antigenic sites have been defined in the O1, Kaufbeuren (O, K) strain of FMDV (12, 15). Site 1 corresponds to the residue 140 to 160 region (the large βG-βH loop) and the carboxy terminus of VP1 (up to residue 213); key residues have been shown to be 144, 148, and 154 and 208, respectively. Most MAbs which recognize this site also recognize isolated VP1 and the 140- to 160-residue peptide derived from it. In the crystallographic structure of type O FMDV, this region has not been resolved (1); this probably reflects variability in the conformation of this loop. The 140- to 160-residue peptide has been shown to induce a protective response in guinea pigs (4), and thus a chimera containing this sequence would provide a useful tool for determination of the potential of chimeric picornviruses as alternative antigen presentation systems. The other sites are much more dependent on the structural integrity of the virus particle. Site 2 has been mapped by escape mutations to the βB-βC loop of VP2, and site 4 has been mapped to the knob in VP3 (residues 56 and 58). Site 3 is found on the βB-βC loop of VP1 (residues 43 to 45) and behaves totally independently from site 1 (12, 15). In other studies, substitutions have been identified at residues 43 and 48 of VP1 in MAb-resistant variants of the closely related virus O1, BFS 1860 (2). However, this MAb was also reported to react with peptides from the residue 140 to 160 region of VP1 in an antigen inhibition enzyme-linked immunosorbent assay (ELISA) and it was concluded that these mutations were "acting at a distance" by inducing a conformational change in the βG-βH loop (27, 28).

Poliovirus/FMDV chimeras provide an opportunity to examine the properties of the individual antigenic sites in isolation. In this study, we constructed such chimeras and examined the antigenicity and immunogenicity of these
viruses. We show that residues corresponding to FMDV sites 1 and 3 can be appropriately presented on the surface of poliovirus and can independently induce site-specific anti-FMDV neutralizing antibodies, thus supporting the view that these regions of FMDV are separate antigenic sites.

MATERIALS AND METHODS

Construction of poliovirus/FMDV antigen chimeras. Poliovirus/FMDV chimeras were constructed by using the pCAS-1 Sabin 1-based expression vector (6), which contains unique Sall and DraI restriction sites. Synthetic oligonucleotides were synthesized on an Applied Biosystems 381A machine. Complementary oligonucleotides were annealed and ligated with Sall-DraI-digested pCAS-1 and used to transform competent Escherichia coli MC1061 cells as previously described (6). For chimera 01.3, four oligonucleotides were used to specify the FMDV sequence and were annealed and ligated into the vector in the same manner. In each case, introduction of the oligonucleotides was confirmed by DNA sequence analysis.

Recovery of viable poliovirus/FMDV chimeras. The recombinant pCAS-1/FMDV plasmids were linearized with Nael, which cuts within vector sequences of the construct, and used as the template in a T7 transcription reaction (40). The RNA transcription reaction product was transfected into subconfluent HeLa-2C monolayers by using DEAE-dextran. A cytopathic effect was observed 2 to 5 days following transfection. Purified RNA from recovered chimeric virus was sequenced through antigenic site A1C.1 (prechallenge) and were sequenced through antigenic site VP1 residues 140 to 160 was a gift from T. Doel (Institute for Animal Health, Pirbright, England).

Virus neutralization assays. Plaque reduction neutralization assays were performed as described previously (42). Neutralization titers were expressed as reciprocal log10 antibody dilutions that neutralized 105 PFU of virus by 50%.

ELISA. FMDV sandwich ELISAs were performed essentially as previously described (17). Serum from guinea pigs immunized with poliovirus chimeras was pretreated with whole bovine serum-agarose (Sigma Chemicals Ltd., Poole, England) to remove nonspecific antibodies. ELISA antibody titers were expressed as reciprocal log10 antibody dilutions which gave an optical density values of 0.4 above the background.

Guinea pig immunization and FMDV challenge. Guinea pigs (Dunkin Hartley) were inoculated subcutaneously with 2 to 5 μg of sucrose gradient-purified virus in 0.1 ml of Freund complete adjuvant, and 28 days later the guinea pigs were inoculated with a second dose of 2 to 5 μg of purified virus in Freund incomplete adjuvant. The guinea pigs were challenged with 1,000 guinea pig-infectious doses of guinea pig-adapted O3 K FMDV 42 days post initial inoculation. Guinea pigs were examined daily for 7 days for the appearance of lesions following FMDV challenge. Serial bleeds were taken on days 0 (preinoculation), 14, 28 (preboost), 42 (prechallenge), and 49 (postchallenge). The second immunization experiment was performed by the same protocol, except that the guinea pigs were inoculated with two doses of 50 μg of partially sucrose-cushion-purified virus (32).

Selection of neutralization-resistant variants. Neutralization-resistant variants of chimera 01.3 were selected by using the procedure for the selection of poliovirus mutants described previously (23). Presumptive mutants were plaque purified once and screened by using the selecting antibody.

RESULTS

Construction of poliovirus/FMDV antigen chimeras. The oligonucleotide sequences inserted into the pCAS vector are shown in Fig. 1. Three recombinants containing sequences from the βG-βH loop of VP1 of type O3 K FMDV were constructed. In chimera 01.1 (containing residues 141 to 154), the size of the poliovirus βB-βC loop is increased by two amino acids and retains the VDN sequence, which is conserved in all three serotypes of poliovirus; thus, the residue equivalent to proline 142 of FMDV VP1 is replaced by an aspartic residue in the chimera sequence. The length of the VP1 βB-βC loop in chimera 01.2 (O3 K residues 147 to 156) is the same as that of Sabin type 1 poliovirus. Chimera 01.3 contains the entire residue 140 to 160 sequence of FMDV VP1; thus, the length of the βB-βC loop is increased by 12 amino acids in this construct. Recombinant A1C.1 contains a sequence corresponding to the carboxy terminus of VP1 (residues 200 to 213) from type A10 FMDV. This region of VP1 has been shown to be antigenic and forms part of antigenic site 1 of FMDV (3, 39, 42). The sequence of type A10 FMDV was chosen, as this had been shown to react with MAb 18 in a pepscan assay (19). The length of the βB-βC loop is increased by five amino acids in this construct. Chimera 03.1 (FMDV VP1 residues 40 to 49) contains a sequence corresponding to FMDV O3 K antigenic site.
3 (12). Antigenic site 3 of FMDV is analogous to antigenic site 1 of poliovirus; in the three-dimensional structure of these viruses, these antigenic sites (on the ββ-βC loop of VP1) are located around the fivefold axis of the virion. The size of the ββ-βC loop is increased by one amino acid in this chimera.

Recovery of viable virus. A cytopathic effect was observed 2 to 5 days following transfection of HEp-2c monolayers with RNA transcripts of constructs 01.1, 01.2, 01.3, and 03.1. The nucleotide sequence of the recovered virus around the region encoding poliovirus antigenic site 1 was determined by sequencing of purified viral RNA and found to be correct for all four recovered chimeric viruses (data not shown). Recombinant AIC.1, containing residues 200 to 213 of FMDV VP1, failed to produce virus following repeated transfections (data not shown), although the DNA sequence of the engineered site was correct.

Growth characteristics of the recovered viruses. The growth efficiencies of recovered viruses 01.3 and 03.1 were compared with that of unmodified Sabin type 1 poliovirus in a one-step growth curve experiment (Fig. 2). Replication of both chimeras was slightly impaired compared with type 1 Sabin virus; chimeras 01.3 and 03.1 produced maximum titers of 10^{8.1} and 10^{8.0} PFU/ml, respectively, compared with a titer of 10^{9.0} PFU/ml for type 1 Sabin virus. Chimeras 01.1 and 01.2 displayed growth characteristics similar to those of chimera 03.1 (data not shown). The plaque morphologies of the different chimeras were indistinguishable from those of Sabin type 1 virus.

Antigenic properties. The antigenic characteristics of the chimeras were assessed in neutralization and immune diffusion assays. Assays using poliovirus type 1-specific MAbs (21, 22) demonstrated loss of reactivity with antigenic site 1-specific MAbs 952 and 955 but retained reactivity with MAbs directed against poliovirus antigenic sites 2 and 3 (data not shown). Chimeras containing sequences corresponding to FMDV antigenic site 1 were tested against anti-FMDV MAbs B2, D7, D9, 13DB, and 13DD (which are specific for FMDV site 1) (12), guinea pig polyclonal hyperimmune anti-O, K FMDV serum, and guinea pig polyclonal antisemur to the synthetic peptide (O, K VP1 residues 140 to 160). Chimera 01.3 (containing the entire residue 140 to 160 region) was neutralized efficiently by MAbs B2, D7, and 13DB and polyclonal anti-FMDV serum, while polyclonal anti-residue 140 to 160 peptide serum and MAbs D9 and 13DD displayed only weak neutralizing activities against this chimera (Table 1). Similar reactions were observed when an immune diffusion assay was used (data not shown). The neutralization titers of MAbs 13DB and D7 against chimera 01.3 were similar to the neutralization titers against FMDV O, K (Table 1); however, the titers of MAb B2 and polyclonal serum were substantially reduced. In contrast, the antipeptide serum and the MAbs failed to react with chimera 01.1 or 01.2 in either neutralization (Table 1) or immune diffusion assays (data not shown). Weak reactivity of polyclonal anti-FMDV serum with chimera 01.1 in both neutralization and immune diffusion assays was observed.

Chimera 03.1, containing sequences corresponding to FMDV antigenic site 3 (VP1 residues 40 to 49) was tested against polyclonal anti-FMDV serum and FMDV site 3-specific MAbs C8, LMK1, O4, and O10. No reactivity was observed in neutralization assays (Table 1) or immune diffusion assays.

None of the chimeras reacted with MAbs specific for FMDV antigenic site 2 or 4 in immune diffusion assays (data not shown).

Selection of neutralization-resistant variants of chimera 01.3. As shown above, chimera 01.3 was neutralized by both anti-FMDV MAbs and polyclonal serum. To determine whether the FMDV sequence in this chimera was recognized in the same manner in the chimera as in FMDV itself, neutralization-resistant variants of chimera 01.3 were selected by using FMDV-specific MAbs B2, D7, and 13DB and polyclonal serum. Of six presumptive mutants analyzed, two were resistant to the selecting antibody. Mutant 01.3/M6 was selected with MAb 13DB, and mutant 01.3/M9 was selected with guinea pig polyclonal anti-FMDV serum. The phenotypes of these two independent variants were identical when examined by a plaque reduction neutralization assay using a panel of anti-FMDV antibodies (Fig. 3, top). Sequence analysis of purified virion RNA through this region of VP1 identified a single amino acid substitution of proline to leucine at residue 102 (equivalent to residue 148 in the FMDV sequence) in both chimera variants (Fig. 3, bottom). This is known to be a key residue in the region of FMDV recognized by these antibodies (42).

Immunogenicity of poliovirus/FMDV chimeras. The poli-
VDAVPNLQDLQVLAQKVARTLPK

P

CTT → CCT

FIG. 3. Isolation and characterization of neutralization-resistant mutants of poliovirus/FMDV chimera O1.3. Presumptive neutralization-resistant mutants of O1.3 (M2 to M10) were rescreened against the selecting antibody and others, as indicated, in a neutralization assay (top). The RNA sequence of mutants M6 and M9 was determined, and the inferred protein sequence (identical in these independent mutants) is indicated (bottom). The residue numbers within chimeric VP1 are indicated along with the original FMDV residue positions. ND, not determined.

virus/FMDV chimeras were tested for the ability to stimulate production of FMDV-specific antibodies and confer protection against FMDV challenge in two immunization experiments. The first experiment involved immunization of five groups of four guinea pigs with 2 to 5 µg of purified immunogen (see Materials and Methods). Each set of animals was immunized with two doses of chimera or Sabin virus (control groups) on days 0 and 28. The animals were challenged with O, K FMDV 42 days post initial inoculation and observed for the appearance of FMDV-specific lesions. Sera obtained from serial bleeds were tested for the presence of FMDV-specific antibodies in ELISA and virus neutralization assays. Sera from guinea pigs immunized with chimera O1.3 possessed prechallenge FMDV neutralization titers of 1.1 to 2.8 (log_{10} reciprocal antibody dilution) (Table 2). One (4R) of four guinea pigs immunized with this chimera was protected against FMDV challenge, and antisera from this animal possessed the highest FMDV-neutralizing and ELISA antibody titer before challenge (Table 2). The development of the neutralizing anti-FMDV antibody response in this guinea pig is shown in Fig. 4A. The anti-FMDV response was increased postchallenge, indicating that the animal had received FMDV although no disease was detected.

The response to chimera O3.1 was more variable: two guinea pigs showed almost no FMDV-specific response in both neutralization assays and ELISA, while animal 3R possessed low levels of FMDV-neutralizing antibody. However, guinea pig 3Y was partially protected against FMDV challenge; only 1 lesion appeared 5 days postchallenge (compared with 6 to 13 lesions in nonprotected animals). This animal also possessed the second highest prechallenge anti-FMDV neutralizing antibody titer (log_{10} reciprocal antibody dilution, 1.6) (Table 2). Guinea pigs immunized with chimera O1.1 or O1.2 were not protected against FMDV challenge; prechallenge sera from these animals reacted weakly, or not at all, with FMDV in ELISA or virus neutralization assays (Table 2). Control guinea pigs which received two doses of Sabin virus were also not protected against FMDV challenge, and sera from these animals did not contain FMDV-specific antibodies. Prechallenge sera from the guinea pigs were also tested for the presence of poliovirus-neutralizing antibodies, and all of the animals possessed significant levels of poliovirus-neutralizing antibodies; however, the neutralizing titers of sera from guinea pigs immunized with either chimera O1.1 or O1.2 were lower than those of sera from the other animals (Table 2).

The second immunization experiment involved immunization of two groups of four guinea pigs with two doses of 50 µg of partially purified chimera O1.3 or O3.1 by using a similar inoculation regimen. The guinea pigs were challenged 42 days postimmunization with FMDV. Challenge sera from guinea pigs immunized with chimera O1.3 possessed FMDV-neutralizing antibody titers of 2.0 to 2.7 (log_{10} reciprocal antibody dilution) (Table 2). One guinea pig (11N), which possessed the highest FMDV-neutralizing antibody titer, was protected against FMDV challenge. Sera from guinea pigs immunized with chimera O3.1 possessed prechallenge FMDV-neutralizing antibody titers of 0.8 to 1.5 (log_{10} reciprocal antibody dilution) (Table 2). One guinea pig (12R) was partially protected against FMDV challenge; this guinea pig possessed the highest FMDV-neutralizing antibody titer of the guinea pigs which received chimera O3.1. The anti-FMDV antibody titers in ELISA were higher than the neutralizing titers for sera from both guinea pigs immunized with either chimera O1.3 or O3.1 (Table 2). However, no correlation between ELISA antibody titer and protection against challenge was observed. Sera from both groups of guinea pigs possessed high levels of poliovirus-neutralizing antibodies.

Specificity of anti-chimera sera. The specificity of the neutralizing antibodies generated in guinea pigs against chimeras O1.3 and O3.1 was assessed by determination of the neutralization titer against FMDV antigenic site 1 or 3 MAb neutralization-resistant mutants 480 (site 1 mutant, amino acid substitution at residue 148 of VP1) and 625 (site 3 mutant, amino acid substitution at residue 44 of VP1) (12, 42). Antiserum 4R, raised against chimera O1.3, showed a greatly reduced neutralization titer against MAb-resistant variant 480 compared with the titer against parental virus (Fig. 4B). Postchallenge serum from this guinea pig possessed similar neutralizing titers against both viruses. An analogous result was observed with serum 3Y raised against chimera O3.1; a reduction in neutralization titer of prechallenge serum was observed against site 3 mutant virus 625 compared with the titer against parental virus (Fig. 4C). Following FMDV challenge, little difference in neutralization titer against the two viruses was observed. Guinea pig 3Y was partially protected against FMDV challenge, despite a considerably lower level of anti-FMDV neutralizing antibody than in other animals immunized with chimera O1.3, which were not protected. Therefore, the serum from this guinea pig was tested for the presence of nonneutralizing anti-FMDV antibodies which may help to limit FMDV infectivity in vivo. Neutralization assays were performed, including the use of Pansorbin to sediment infectious nonneutralized virus-antibody complexes. A 10-fold increase in
antibody titer against FMDV was observed with this assay (Fig. 4D).

**DISCUSSION**

Five poliovirus/FMDV recombinant plasmids were constructed, and viable chimeric virus was recovered from four of these. The reasons for the nonviability of construct A1C.1 are unclear; however, it has been suggested that the presence of basic residues at the amino end of the βB-βC loop compromises virus viability (4a). Although chimera 01.3 shows reduced virus yield compared with wild-type Sabin type 1 poliovirus, the replacement of 9 amino acids in the wild-type sequence with 21 amino acid residues in the chimera demonstrates the remarkable flexibility of the βB-βC of poliovirus VP1 to accommodate extensive foreign amino acid sequences.

The three chimeras containing sequences from the βG-βH loop of VP1 varied considerably in the extents of their FMDV antigenicity. Results from the sequencing of FMDV antigenic site 1 MAb neutralization-resistant mutants have shown that residues 144, 148, and 154 of VP1 contribute to the epitopes of MAb B2, D7, 13DB, 13DD, and D9 (12, 15, 42). Chimera 01.2 does not contain all of these residues; thus, the lack of MAb reactivity with this chimera is not surprising. The sequence inserted into chimera 01.1 does encompass these residues, but no reactivity with any of the FMDV site 1-specific Mabs was observed and only weak neutralizing reactivity was observed with polyclonal anti-FMDV serum. This suggests that other residues around this region contribute to the antibody epitopes or that the sequence adopts a conformation in the chimera which is not recognized by the antibodies. The results obtained with these chimeras are consistent with the findings from studies with synthetic peptides which suggested that the length of the peptide was important in determining the antiviral response (30).

In contrast to the lack of reactivity of chimeras containing only part of antigenic site 1, chimera 01.3, which includes the entire VP1 residue 140 to 160 region of FMDV, was neutralized by a number of different FMDV-specific antibodies. This chimera was efficiently neutralized by polyclonal anti-FMDV serum and MAb B2, 13DB, and D7, suggesting that the loop can adopt a conformation similar to that found in FMDV. In contrast, 01.3 reacted weakly with MAb D9 and 13DD and polyclonal anti-residue 140 to 160 peptide serum. Between these two extremes, MAb B2 and polyclonal anti-FMDV serum efficiently neutralized this chimera but with a much lower titer than against FMDV. Some of these differences in reactivity can be explained by the fact that not all of the FMDV residues involved in antibody recognition are present in the βG-βH loop. This would obviously apply to the polyclonal anti-FMDV serum, but also MAb D9 is known to interact with residue 208 close to the C terminus of VP1 (42). It was a surprise that anti-residue 140 to 160 peptide serum displayed only a weak neutralizing titer against chimera 01.3 but neutralized FMDV effectively. This serum can probably recognize a wide range of conformations of the peptide; it may be that this loop is more constrained in the chimera (and hence is recognized by fewer antibodies) than in FMDV, where the residue 138 to 157 region of VP1 has an undefined structure by crystallography (1).

The selection of neutralization-resistant variants of chimera 01.3 by using MAb 13DB and polyclonal anti-FMDV serum, each containing a substitution of the residue corresponding to residue 148 of FMDV VP1, confirms the importance of this residue in the antigenicity of antigenic site 1. This residue has been previously shown to be important in studies using MAb neutralization-resistant variants of

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**TABLE 2. Anti-FMDV responses generated in guinea pigs inoculated with poliovirus/FMDV chimeras**

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<th>Chimera used as immunogen (FMDV residues inserted), dose (µg)</th>
<th>Serum</th>
<th>Guinea pig protection (no. protected/total)</th>
<th>Mean (range of prechallenge day 42 serum neutralization titer)</th>
<th>Mean (range of FMDV ELISA titer on day 42)</th>
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* Each dose was administered twice as described in Materials and Methods.

* +, no spread of FMDV lesions from the challenge site; −, generalization of FMDV lesions; ±, delayed and limited spread of lesions.
FIG. 4. Characterization of anti-FMDV antibodies produced in guinea pigs inoculated with poliovirus/FMDV chimeras. (A) Serum collected at days 14 (○), 28 (■), 42 (△), and 49 (□) post FMDV challenge from guinea pig 4R was assayed for its ability to neutralize wild-type FMDV O1 K. (B) 4R serum collected at days 42 (△ and △) and 49 (□ and □) postchallenge was assayed for its ability to neutralize wild-type FMDV O1 K (open symbols) or site 1 mutant 480 (VP1 residue 148 substituted) (closed symbols). (C) Serum from guinea pig 3Y (inoculated with chimera O3.1) taken at day 42 (△ and △) or 49 (□ and □) postchallenge was assayed for its ability to neutralize wild-type FMDV O1 K (open symbols) or site 3 mutant 625 (VP1 residue 44 substituted) (closed symbols). (D) 3Y serum taken at day 42 (△ and △) or 49 (□ and □) was assayed for its ability to neutralize wild-type FMDV O1 K alone (open symbols) or with Pansorbin (closed symbols) as described in Materials and Methods. Use of preimmune serum with Pansorbin is also indicated (*). Neutralization assays were performed with HEP-2C cells as described in Materials and Methods. Data are presented as the percentages of the input virus (PFU) not neutralized.

FMDV (37, 42) and in studies using synthetic peptides (29, 34). This result further suggests that this sequence must be presented within the chimera in a manner highly analogous to that found in FMDV, despite the poor reactivity with the anti-residue 140 to 160 peptide serum discussed above.

O1.3 induced significant FMDV-specific responses in all of the guinea pigs immunized with this chimera. The response was shown to be directed against FMDV antigenic site 1, since a much lower neutralization titer against site 1 mutants of FMDV was observed. In both immunization experiments, one of four guinea pigs was protected against FMDV challenge. Protection correlated with the highest levels of prechallenge anti-FMDV neutralizing antibody. This is the first demonstration of protection using a poliovirus antigen chimera containing an inserted sequence from another virus.

Chimera O3.1 and O1.3 contain the RGD amino acid sequence, which is reported to be part of the cell attachment site of FMDV (9). None of the chimeras had acquired the ability to grow on BHK, IBRS2 (pig kidney), or primary bovine thyroid cell monolayers. Furthermore, chimera O1.3 did not bind specifically to BHK cells, nor did preabsorption of this virus onto BHK cells block the ability of FMDV to infect the cells (data not shown).

Chimera O3.1 was recognized by neither anti-FMDV polyclonal serum nor antigenic site 3-specific MABs. Antigenic site 3 of O1 K FMDV is a conformation-dependent site; MABs which recognize this site react poorly, if at all, with subviral components or isolated proteins (15, 16, 18). Amino acid substitutions which confer resistance to neutralization by site 3 MABs have been identified only in the βB-βC loop of VP1 (12). However, it is possible that residues from loops adjacent to the βB-βC loop also contribute to the epitopes of site 3 MABs, a situation that has been observed with the analogous antigenic site 1 of poliovirus (41). Furthermore, residues from the adjacent VP1 βH-βI loop of type A10 and A12 FMDV have been shown to contribute to an analogous antigenic site (3, 39). Therefore, the lack of reactivity of chimera O3.1 with FMDV-specific antibodies probably reflects the absence of residues which contribute to the epitopes of site 3 MABs in the chimera. Despite this, chimera O3.1 was capable of inducing production of site 3-specific FMDV-neutralizing antibodies, implying that its structure
does mimic at least part of this conformational site. However, this response was variable among different animals immunized with this chimera. In both immunization experiments, one of four animals appeared to be partially protected against FMVD challenge, as judged by the reduced and late appearance of lesions. These two animals possessed the highest FMVD-neutralizing antibody titers of the animals immunized with chimera O3.1. Thus, a correlation between the neutralizing antibody titer and reduced severity of disease symptoms was observed.

The results obtained with this chimera further support our conclusion from the study of MAb neutralization-resistant mutants that VP1 residues 43 to 45 contribute to antigenic site 3 and that this site is distinct from antigenic site 1 (12). It has been suggested, on the basis of studies with the closely related O1 BFS 1860 strain of FMVD, that a MAB 24.31-selected antigenic variant was resistant to neutralization by the selecting MAB because of the distant effect of a mutation at residue 43 of VP1 on the conformation of the residue 140 to 160 loop of VP1 (28). Binding of MAB 24.31 to virus in an antigen inhibition ELISA was inhibited by high concentrations of peptides covering the residue 140 to 160 region of VP1 (27). It seems likely that this is a nonspecific effect, since binding of this MAB in this assay was inhibited to the same degree by either trypsin-treated or untreated virus (2). Trypsin treatment cleaves type O1 FMVD at residues 138, 154, and 200 of VP1 (38), resulting in loss of residues 139 to 154 and 201 to 213 from the virus particle, consistent with the lack of reactivity of antipeptide serum in ELISA or radiolabeled precipitation assays (27). It should also be noted that previous studies from this laboratory (12, 42) and others (31, 37) have demonstrated that MABs which recognize the residue 140 to 160 region of VP1 permit selection of escape mutants with substitutions in this region. Furthermore, the studies presented here demonstrate that antibodies generated against the VP1 ββ-BC loop in chimera O3.1 can neutralize FMVD and that mutations in this loop confer resistance. Thus, it seems most likely that the modified conformation of the βG-βH loop in mutants selected with MAB 24.31 (28) reflects a second phenomenon unrelated to the antigenic change in the ββ-BC loop. All previous studies with picornaviruses and influenza virus support the view that the escape mutations occur at antibody contact residues.

The results obtained with chimera O3.1 suggest that it is possible to induce antibodies against conformation-dependent antigenic sites, as well as to neutralize poliovirus chimeras. The neutralizing response generated by these chimeras might be improved by introducing FMVD sequences at more than one site into the poliovirus structure. It is probable that such viruses can be generated, since it has been demonstrated that poliovirus antigenic site 2 can also be modified (24). Bovine enterovirus is common in cattle and has now been cloned and sequenced (7). The introduction of similar FMVD sequences into single or multiple sites in bovine enterovirus may generate a replicating but nonpathogenic chimeric picornavirus capable of conferring protection against FMVD on cattle. Studies designed to test this are in progress in this laboratory.

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