Extensive antigenic diversification of foot-and-mouth disease virus by amino acid substitutions outside the major antigenic site

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The antigenic sites A and C (the G-H loop and the C terminus, respectively) in VP1 of foot-and-mouth disease virus (FMDV) have been considered the immunodominant regions of the virus involved in the induction of protection. Other antigenic sites have been described but their involvement in protection has not been established. Here we report that two closely related but serologically different FMDVs (the field isolate C₃ Argentina/84 and the vaccine strain C₃ Resende Br/55) have identical A and C sites but differ at other antigenic sites. Such differences have been documented by reactivity with a panel of 28 monoclonal antibodies (MAbs). The two viruses reacted to the same extent with each of 13 MAbs which recognized epitopes within sites A or C, but reacted differently with six out of 15 MAbs that recognized other sites. Accordingly, sequencing of the entire region coding for the capsid proteins, for both viruses, revealed four amino acid substitutions at three antigenic sites other than A and C. The results suggest that identity of sites A and C may not be sufficient to induce cross-protection, and provide the first evidence of significant antigenic diversification of FMDV in the field mediated by amino acid substitutions outside sites A or C.

Control of foot-and-mouth disease of cattle by vaccination is complicated by the extensive antigenic heterogeneity of its causal agent, foot-and-mouth disease virus (FMDV). Seven serotypes with multiple subtypes (Perreira, 1977) and serological variants have been identified. Moreover, and in agreement with the quasispecies structure of FMDV (Domingo et al., 1990, 1992), studies with monoclonal antibodies (MAbs) suggest that each viral isolate may be unique in its fine epitopic make-up (Mateu et al., 1988) and may include multiple genetic and antigenic variants (Domingo et al., 1980; Mateu et al., 1989).

The region of the FMDV capsid that is considered immunodominant includes the central region of loop G-H within protein VP1 (site A, around amino acids 135 to 160; Strohmaier et al., 1982; Bittle et al., 1982; Pfaff et al., 1982). This domain constitutes a protruding, disordered loop on the virion (Acharya et al., 1989), involved in delineating multiple continuous and/or discontinuous epitopes in serotypes O, A and C (Xie et al., 1987; Mateu et al., 1987, 1990; Thomas et al., 1988; Parry et al., 1989a; Bolwell et al., 1989). Site A is the most variable part of the capsid, and the correspondingly extensive antigenic heterogeneity, even within a serotype, has been well documented (Mateu et al., 1988; Martinez et al., 1991).

Additional antigenic sites of FMDV have been identified (Thomas et al., 1988; Kitson et al., 1990). These domains involve exposed loops of VP1, VP2 and VP3 that correspond roughly to those found in antigenic regions of other picornaviruses (Kitson et al., 1990; Minor, 1990). The contribution of such sites to the antigenic diversification of FMDV has not been explored. It has been suggested that variation of FMDV in the field during an epizootic outbreak affects mainly VP1 (Sobrino et al., 1989), but a recent comparison of capsid protein sequences of different FMDV C subtypes (and even of different isolates of the same subtype) indicates considerable variation outside VP1 (Martinez et al., 1992).

The present study concerns two closely related viruses of the same subtype, C₃ Resende Br/55 and C₃ Argentina/84 [isolated in General Roca, Córdoba,
Argentina, in May 1984; Servicio Nacional de Sanidad Animal (SENASA) code number for this isolate 32682], and provides evidence for the relevance of domains other than site A in their antigenic differences. FMDV C₃ Resende Br/55 was used as a vaccine strain in Argentina until 1985; C₃ Argentina/84 was isolated from cattle in the field during an important epizootic wave that occurred in that country in 1984 to 1986, and recognized potency may also have affected the cross-protection results. A drastic reduction in the number of outbreaks resulted from including in the vaccine C₃ Argentina/84 in vaccine trials (Bergmann et al., 1988). These results suggest that the differences between the two viruses must reflect differences in antigenic specificity, although it cannot be completely ruled out that variations in vaccine potency may also have affected the cross-protection results. A drastic reduction in the number of outbreaks resulted from including in the vaccine C₃ Argentina/84 and C₃ Argentina/85 antigens (the latter is a virus from a different lineage, which was cocirculating at the time), instead of C₃ Resende Br/55 (Bergmann et al., 1988). The comparison of VP1 of C₃ Argentina/84 with C₃ Resende Br/55 revealed no amino acid replacements at sites A or C (Piccone et al., 1988). Thus, the antigenic differences between the two viruses remained unexplained.

Here we report the nucleotide sequences of the entire capsid-coding regions of C₃ Resende Br/55 and of C₃ Argentina/84 and identify the amino acid substitutions that underlie their antigenic specificity. The latter was studied with a panel of 28 MAbs in immunoassays using described procedures (Mateu et al., 1988). In order to help in correlating amino acid sequences with antigenic specificity, population heterogeneity was minimized by plaque purification. Plaque-purified C₃ Resende Br/55 and C₃ Argentina/84 were compared in complement fixation tests, yielding in repeated assays a serological relationship R of 64% (r₁ = 0.66; r₂ = 0.63), a value comparable to that obtained with the uncloned virus populations by us and, independently, by Bergmann et al. (1988). Serological relationships established by complement fixation tests are often a reasonable indicator of the extent of cross-protection to be expected for the corresponding vaccines. In some cases, even with higher R values, only partial cross-protection was observed between closely related isolates (Martínez et al., 1988). Thus, all the available evidence suggests that the viruses used in the present study, as are uncloned populations from which they were derived, are significantly different antigenically. The uncloned and cloned populations showed the same amino acid sequence at antigenic site A (Piccone et al., 1988; Piccone, 1989).

C₃ Resende Br/55 and C₃ Argentina/84 were compared in their reactivity with a panel of neutralizing MAbs elicited against FMDV type C (Fig. 1). No differences were noted when the two viruses were tested with a set of 11 MAbs that recognize 11 distinct, non-conserved, continuous epitopes within sites A and C; (b) MAbs that define discontinuous epitopes outside site A. EID assays were done as described in Mateu et al. (1988); 10-fold dilutions of MAbs were tested. For each MAb, a relative reactivity of any virus with respect to either C₃ Santa Pau-Sp/70 or C₃ Indaiá-Br/71 is given as: ■, positive (a similar reactivity is observed); □, weak (a 10 to 100-fold higher amount of MAb is needed to give a similar signal); ○, negative (more than a 100-fold amount of MAb is needed). For MAbs in (a), the results of EID assays (upper left half in each frame) are compared to those obtained in Western blot assays (with uncloned viruses; Martínez et al., 1991) (lower right half). Discrepancies between the two types of assays were found only with MAbs 4C4 and 7EE6. They are under investigation.

![Diagram](https://via.placeholder.com/150)

**Fig. 1.** Reactivity of plaque-purified viruses C₃ Resende Br/55 and C₃ Argentina/84 with MAbs in enzyme immunodot (EID) assays. (a) MAbs that recognize distinct continuous epitopes within sites A and C; (b) MAbs that define discontinuous epitopes outside site A. EID assays were done as described in Mateu et al. (1988); 10-fold dilutions of MAbs were tested. For each MAb, a relative reactivity of any virus with respect to either C₃ Santa Pau-Sp/70 or C₃ Indaiá-Br/71 is given as: ■, positive (a similar reactivity is observed); □, weak (a 10 to 100-fold higher amount of MAb is needed to give a similar signal); ○, negative (more than a 100-fold amount of MAb is needed). For MAbs in (a), the results of EID assays (upper left half in each frame) are compared to those obtained in Western blot assays (with uncloned viruses; Martínez et al., 1991) (lower right half). Discrepancies between the two types of assays were found only with MAbs 4C4 and 7EE6. They are under investigation.
relevant with respect to antigenicity. Three substitutions are not in recognized antigenic sites. The four remaining ones (Fig. 2) are located in surface-exposed loops that have been implicated in antigenic domains different from sites A and C (Thomas et al., 1988; Kitson et al., 1990). One replacement, Glu (58)→Gly, occurred at the B-B ‘knob’ of VP3 [identified as part of antigenic site 4 by Kitson et al. (1990)]; two differences, Ser (45)→Leu and Arg (47)→Gly, were located at the B-C loop of VP1 [antigenic site 3 of Kitson et al. (1990)]; an additional one, Leu (171)→Val, was found in the H-I loop of VP1 [identified as antigenically relevant by Thomas et al. (1988), and located very close to the VP1 B-C loop; the two loops may constitute a single antigenic site]. We propose that one or a combination of these four exposed substitutions are responsible for the immunological differences of the field isolate C³ Argentina/84 when compared to the vaccine strain C³ Resende Br/55.

There is considerable evidence that variation of site A plays an important role in the antigenic diversification of FMDV. At least for serotype C, the antigenic divergence of this site (mediated by accumulation of amino acid substitutions or by single, critical replacements), in general correlates with serological results (Mateu et al., 1988; Martinez et al., 1991). Peptides representing site A mimicked the serotype, subtype and strain specificities of the complete virus (Bittle et al., 1982; Clarke et al., 1983; Rowlands et al., 1983; Mateu et al., 1989, 1992). Also, sequencing of C³ variants that failed to confer cross-protection of swine showed that most replacements in exposed capsid domains had occurred within site A (Sobrino et al., 1989). Heterologous cross-protection of laboratory animals was reported with some peptide vaccines which included sites A and C (Parry et al., 1989b; Doel et al., 1990). However, other evidence suggests that site A may not suffice to evoke a broadly protective response. Unlike a whole-virus vaccine, a recombinant vaccine based on those same antigenic regions of FMDV O, Campos did not protect against O₁ Caseros, which differed in two amino acids within site A (Giavedoni et al., 1991). Although a different presentation of these sites in the recombinant and the peptide vaccines may account for this, an alternative interpretation of this observation is the participation of regions other than sites A and C in the response to whole-virus vaccines. Additional evidence for the immunodominance of such regions in the response of infected animals is provided by FMDV C⁴ Tierra del Fuego Arg/66 which was classified as a new subtype. This virus differs from C³ Resende Br/55 in two antigenically non-critical amino acids within site A, but in 12 residues at other antigenic sites (Martinez et al., 1991, 1992). As observed with the type C viruses studied here, some serological variants of FMDV type O described by Ouldridge et al. (1986) showed identical sequences at site A, suggesting the relevance of modifications elsewhere in the virion on the antigenicity of this virus. Also, Bolwell et al. (1992) isolated in cell culture a variant of an A₂₂ strain which failed to cross-protect cattle against its parental virus despite having a site A identical both in sequence and MAb reactivity.

The antigenic VP1 B-C loop (Fig. 2) has long been recognized as a second hypervariable region of FMDV. It appears that substitutions within this loop directly affect its interaction with antibodies and induce topological alterations of site A (Kitson et al., 1990, 1991; Parry et al., 1990; Minor, 1990). The substitutions found at the VP1 B-C loop, or the nearby H-I loop, in FMDV C³ Argentina/84 as compared with C³ Resende Br/55 did not affect the reactivity of any of 16 antibodies directed to at least 11 distinct epitopes within site A. Thus, so far, no evidence for interaction between the B-C and G-H loops has been found in FMDV of serotype C. This point is under study.

### Fig. 2. Alignment of amino acid sequences of the four capsid proteins of plaque-purified viruses C³ Resende Br/55 and C³ Argentina/84. For the latter, only amino acids that differ from those of C³ Resende Br/55 are indicated. The single-letter amino acid code is used. Asterisk represents undefined amino acid. Above each line secondary structure motifs are indicated assuming that, upon alignment, they correspond to the equivalent residues of FMDV O₁ BFS (Acharya et al., 1989). Symbols: thin lines, N and C termini and loops; arrows, β-sheet, wavy lines, α-helix. Regions where mutations conferring resistance to MAbs have been found in FMDV of serotypes A, O and/or C are boxed.

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In conclusion, sequencing of site A or VP1 alone is insufficient for the characterization of FMDV strains, and MAb's directed to the seven independent antigenic sites are needed for an appropriate description of the antigenic specificity of new isolates. Variation in domains other than antigenic site A are relevant to the overall antigenic diversification of FMDV in the field. Our results suggest that site A may not be the only immunodominant region of the virus, and that additional sites must be considered in the design of synthetic vaccines.

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References


Short communication


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