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Rapid Selection of Genetic and Antigenic Variants of Foot-and-Mouth Disease Virus during Persistence in Cattle†

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Rapid evolution of foot-and-mouth disease virus (FMDV) is documented during persistent infections of cattle. The carrier state was established experimentally with plaque-purified FMDV of serotype C_3. Virus was recovered from the esophageal pharyngeal area of the animals up to 539 days postinfection. Analysis of capsid proteins by electrofocusing and by electrophoretic mobility of the genomic poly(C)-rich tract suggested heterogeneity in several isolates and sequential dominance of viral subpopulations. Nucleotide sequences of the VP1-coding region of the parental FMDV C_3 clones and of seven isolates from the carrier cattle showed point mutations that represented rates of fixation of mutations of 0.9×10^{-2} to 7.4×10^{-2} substitutions per nucleotide per year; 59% of the base changes led to amino acid substitutions, some of which were located within residues 135 to 151, a region involved in neutralization of FMDV. In the esophageal pharyngeal fluid samples, FMDV C_3-neutralizing activity was present. Antigenic variation was demonstrated with monoclonal antibodies raised against FMDV C_3. Two isolates from carrier cattle differed from the parental virus by 10^2- or 10^3-fold decreased reactivity with neutralizing monoclonal antibodies. We suggest that persistent, inapparent infections of ruminants, in addition to being a reservoir of virus, may promote the rapid selection of antigenically variant FMDVs.

Foot-and-mouth disease virus (FMDV) is a picornavirus that causes an acute disease of domestic and wild cloven-hooved animals that has a dramatic impact on livestock production and trade (reviewed in references 5 and 36). Several epidemiological investigations have shown that inapparent persistent infections by FMDV are common in areas where the disease is enzootic (4, 8, 22, 48–50). The virus may persist in the esophageal pharyngeal (OP) region of cattle and other ruminants (48–50) for up to several years (11) without causing signs of disease. This type of infection may arise as a sequel from an acute episode or from immunization with live attentuated vaccines, or it may be established by nasal exposure to virus (4, 48, 49). A few early studies showed that FMDVs from carrier animals differed from the corresponding parental viruses associated with acute disease in being temperature sensitive and producing small plaques on cell monolayers (19, 45, 46). Also, generation and selection of viral subpopulations with altered size of the genomic poly(C)-rich tract occurs during persistence in cattle (M. P. Costa Giomi, I. Gomes, B. Triaboschi, P. Augé de Mello, I. E. Bergmann, E. A. Scodeller, and J. L. La Torre, Virology, in press). Recent experiments indicate that emergence of variants of FMDV stems from its extreme genetic heterogeneity (15, 16, 42) through selection or random drift of variants continuously arising during viral replication (16, 38, 42). Although there is substantial experimental evidence that mutational (15, 38, 42, 43) along with recombinational (28, 29) events occur at high frequency during FMDV replication, the mechanisms that mediate fixation of genomic variations in natural viral populations are not well understood. It has been suggested that antigenic variants are selected upon replication of FMDV in immune or partially immune hosts (15, 18, 25, 31). It has also been proposed that since a few particles suffice to infect a susceptible host (17a, 21, 41), viral transmission constitutes a population bottleneck by which one or a few particles, randomly sampled from the heterogeneous parental populations, are amplified and thus replace the previously dominant genomes (15, 16). Clearly, it is important to understand the mechanisms that mediate both generation and fixation of mutations in viruses.

In the present study, persistent infections of cattle were initiated with either one or a mixture of two plaque-purified clonal pools of FMDV C_3 Resende-Brasil/55 (C_3 Res-Br/55). Then, sequential viral samples were isolated for up to 18 months and analyzed by electrofocusing of capsid proteins, size of the genomic poly(C) tract, nucleotide sequencing, and reactivity with monoclonal antibodies (MAbs). The results document a rapid fixation of mutations in the viruses replicating in carrier cattle and selection of antigenically variant FMDVs.

MATERIALS AND METHODS

Cells, viruses, and infections. The persistent infection of cattle was established previously and has been the object of former studies (12; Costa Giomi et al., in press). Cattle were inoculated intranasally with 10^5 50% infectious doses (ID_{50}) of FMDV C_3 clone c3B or c12 or a mixture of about equal amounts of the two clones. FMDV c3B [short poly(C) tract] and c12 [long poly(C) tract] were derived from FMDV C_3 Res-Br/55 (obtained through Servicio Nacional de Sanidad Animal, Argentina) by plaque purification as described previously (12; Costa Giomi et al., in press). The animals were

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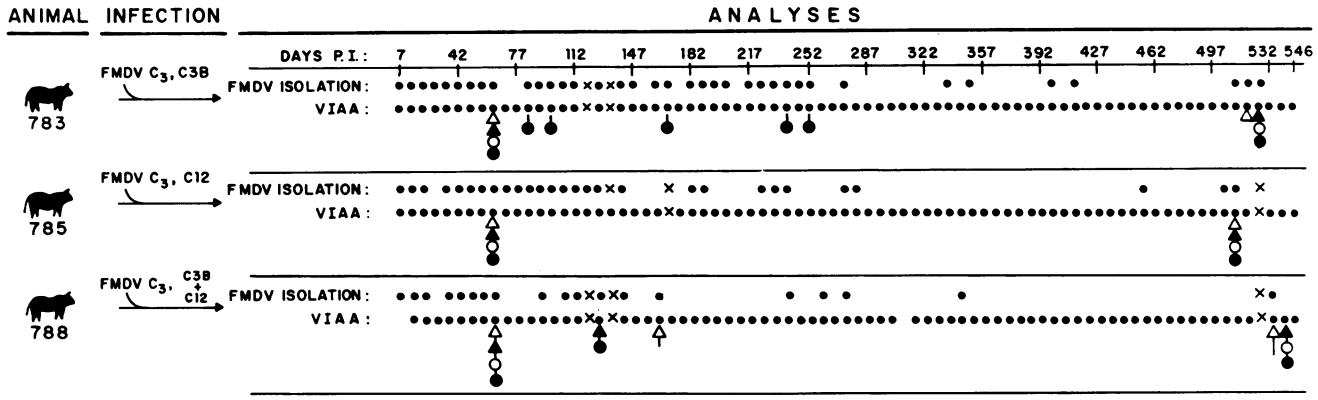

FIG. 1. Scheme for the establishment of persistent infections in cattle with FMDV C_3 c3B, c12, or a mixture of the two viruses. OP and serum samples were obtained at 7-day intervals from each cattle. FMDV isolations were attempted with each clarified OP fluid as detailed in Materials and Methods. The VIAA determination was carried out with each serum sample. Positive viral isolations or VIAA determinations are indicated by a dot. A blank indicates negative FMDV isolation or negative VIAA. Other symbols: ×, sample not tested; ▲, viral samples analyzed by electrofocusing; △, genomic viral RNAs subjected to nucleotide sequence analysis; ●, poly(C)-rich tract size determination; ○, viruses analyzed with MAbs.

unvaccinated zebu steers from a farm in which FMD had not occurred for several years. They were negative for antibodies against FMDV O_1 Campos, A_{24} Cruzeiro, and C_3 Res-Br/55. OP fluid samples from the steers were obtained by probang extraction (48, 50). They were immediately diluted with 1 volume of Earle salt solution containing 1,000 IU of penicillin, 1 mg of streptomycin, and 125 IU of fungizone per ml. Samples were stored at −70°C until used. The FMDV-neutralizing activity of the OP samples was tested by a PFU reduction assay (3). For virus isolation, the OP suspension was mixed vigorously with 7 volumes of trichlorotrifluoroethane and centrifuged at 800 × *g* for 30 min. BHK-21 cell monolayers were inoculated with the clarified supernatant. Isolations were considered positive when cytopathic effect was observed 24 to 48 h after inoculation. Viruses were passaged to the minimal extent needed to provide antigen and RNA for the analyses; this generally involved amplification of about 10^3 to 10^4 PFU to about 10^8 PFU. A similar amplification of natural FMDV isolates in tissue culture did not lead to fixation of mutations at the levels observed among the samples from carrier cattle analyzed here. Thus, the amplification of FMDV in cell culture did not influence the quantitation of variations fixed during replication in vivo. Viral samples are identified by a cattle number followed by the day of isolation: i.e., FMDV RNA 785/518 is viral RNA from animal 785, obtained at day 518 after exposure to virus. FMDV was purified either by sucrose gradient sedimentation (14, 15) or by pelleting through a sucrose cushion (29a, 31). $^{32}P_i$ labeling of FMDV RNA and determination of the electrophoretic mobility of the genomic poly(C)-rich tract were performed by the methods of Costa Giomi et al. (12; in press). Viral infection-associated antigen (VIAA) was determined by described procedures (1).

Electrofocusing. FMDV was labeled with [^{35}S]methionine, and capsid proteins were immunoprecipitated as described (34) except that the labeling period was 1 h, starting at 3 h postinfection (p.i.) and it was followed by a 1-h chase with a 100-fold molar excess of unlabeled methionine. Electrofocusing of FMDV VP1, VP2, and VP3 was done in a 5% mixture of ampholytes with a pH 3 to 10 gradient (27, 34).

RNA sequencing. FMDV RNA was extracted from purified virions and sequenced by oligodeoxynucleotide primer extension and dideoxy chain termination (42, 51) with oligodeoxynucleotides complementary to positions 111 to 126, 264 to 281, 465 to 485, and 471 to 490 of the VP1-coding region and to 34 to 54 of the P14 gene (sequences and numbering as in reference 43) used as primers. The sequencing autoradiograms showed ambiguous readings at several positions. Some of them were resolved by treatment of the reaction mixtures with terminal deoxynucleotidyl transferase (13). Routinely, field gradient gels (2) were used. Some band compressions were eliminated by using standard linear gels under various running conditions. Ambiguous positions not solved by those procedures are indicated in the corresponding nucleotide and amino acid sequences and were considered invariant in the computation of mutations.

Reactivity with MAbs. The procedures used for the production of hybridoma cell lines secreting anti-FMDV C_3 MAbs and the enzyme-linked immunoelectrotransfer blot (EITB) assay have been described (26, 31). The MAbs used in the present study were 7AH1, 7AB5, 7EE6, 7FC12, 7JD1, 7CA8, 7CA11, and 7JA1, raised against FMDV C_3 Indaial-71 (M. G. Mateu et al., manuscript in preparation).

RESULTS

Charge alterations in the capsid proteins of FMDV isolated from persistently infected cattle. Three 12-month-old cross-bred Holstein-Zebu steers were inoculated with 10^5 ID_{50} of FMDV C_3 c3B or c12 or with a mixture of the two cloned viruses (12; Costa Giomi et al., in press). Upon recovery from an initial acute episode, a persistent infection was established as judged by the presence of infectious FMDV in the OP samples and by a positive VIAA (Fig. 1). To test whether variations in the overall charge of the FMDV capsid proteins VP1, VP2, and VP3 had occurred during viral replication, the parental FMDV C_3 clones c3B and c12 and seven viral isolates from the carrier cattle were analyzed by nonequilibrium pH gradient gel electrophoresis (NEPHGE), a method previously used successfully to identify FMDV mutants (27). Charge shifts were detected in VP1, VP2, or VP3 of several of the isolates (Fig. 2), which in some cases evolved to species distinguishable from those of the corresponding parental viruses. Heterogeneity within one isolate was suggested by double bands at the positions of VP1 and VP3 for virus 785/63 and of VP3 for virus 785/511 (Fig. 2B, lanes 3 and 5). The results suggest the occurrence of amino acid substitutions in capsid proteins and the coexistence of viral subpopulations in persistently infected cattle.

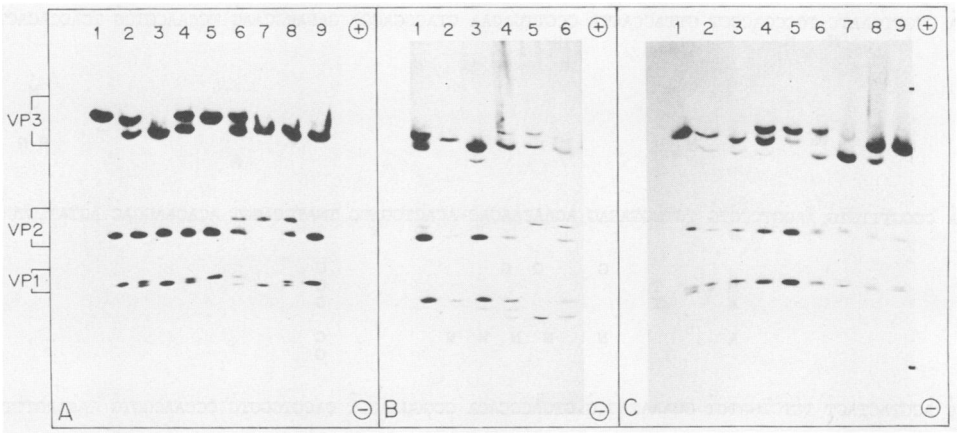

FIG. 2. Analysis of structural proteins VP1, VP2, and VP3 by electrofocusing. [^{35}S]methionine-labeled viral proteins were immunoprecipitated (38) and applied to NEPHGE on slab (29 by 13 by 0.15 cm) gels, and run as described (27, 34). (A) Lanes: 1, C_3 Res-Br/55; 2, mixture of samples c3B and C_3 Res-Br/55; 3, c3B; 4, c3B and 783/63; 5, 783/63; 6, 783/63 and 783/525; 7, 783/525; 8, c3B and 783/525; 9, c3B. (B) Lanes: 1, C_3 Res-Br/55 and c12; 2, c12; 3, 785/63; 4, 785/63 and 785/511; 5, 785/511; 6, c12 and 785/511. (C) Lanes: 1, c3B and c12; 2, 788/63, c12, and c3B; 3, 788/63; 4, 788/63 and 788/126; 5, 788/126; 6, 788/126 and 788/539; 7, 788/539; 8, 788/539, c12, and c3B; 9, c3B and c12.

Serial dominance of genome subpopulations. The genomic poly(C)-rich tract of FMDV (7) is subject to considerable size variations in the course of viral replication during 128 days of persistence in animals (Costa Giomi et al., in press), providing a marker of genomic evolution. Upon replication of c3B in animal 783, a gradual increase in the size of the poly(C) tract was observed up to day 168, then a decrease with two poly(C) populations at day 252 (Fig. 3A), and finally, genomes with a long poly(C) dominant at day 525 (Fig. 3B, lane 3). In animal 785, and consistent with the electrofocusing analysis, two subpopulations of genomes were defined by the presence of a minor band of a short poly(C) at day 63, with dominance of a long poly(C) at day 511 (Fig. 3B, lanes 5 and 6). In the mixed infection of animal 788 by c3B and c12, three species of poly(C) tract coexisted at day 63 and then a species of a size that did not correspond to any of the two parental genomes dominated in the population (Fig. 3C). The poly(C)-rich tract size ranged from about 145 to 280 residues, and this suggests rapid genetic changes of FMDV in carrier cattle.

Mutations in the VP1-coding region. To quantitate the genetic variation of FMDV C_3 replicating in persistently infected cattle, the VP1-coding region from FMDV c3B, c12, and isolates 783/63, 783/518, 785/63, 785/511, 788/63, 788/161, and 788/532 was sequenced by primer extension and dideoxy chain termination. VP1 includes epitopes critical for FMDV neutralization (6, 47). Multiple point mutations but no insertions or deletions were fixed during FMDV replication in carrier animals (Fig. 4). The VP1 gene of each animal isolate differed from that of the corresponding parental virus in 0.4 to 2.5% of positions, with 69% of all recorded mutations being transitions and 31% being transversions. The rate of fixation of nucleotide substitutions was 2.7×10^{-2} substitutions per nucleotide (s/nt) per year for isolate 783/63 and 1.7

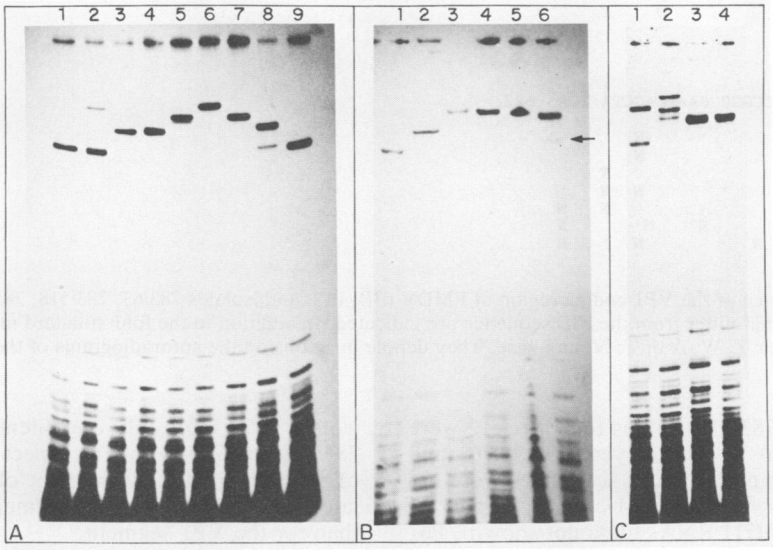

FIG. 3. Poly(C)-rich tract analysis by one-dimensional electrophoresis of ^{32}P-labeled FMDV RNA. Procedures are those described by Costa Giomi et al. (12; in press). (A) Lanes: 1, c3B; 2, C_3 Res-Br/55; 3, 783/63; 4, 783/84; 5, 783/98; 6, 783/168; 7, 783/238; 8, 783/252; 9, c3B. (B) Lanes: 1, c3B; 2, 783/63; 3, 783/525; 4, c12; 5, 785/511; 6, 785/63. (C) Lanes: 1, mixture of c3B and c12; 2, 788/63; 3, 788/126; 4, 788/539. The arrow points to a minor poly(C) species in 785/63.

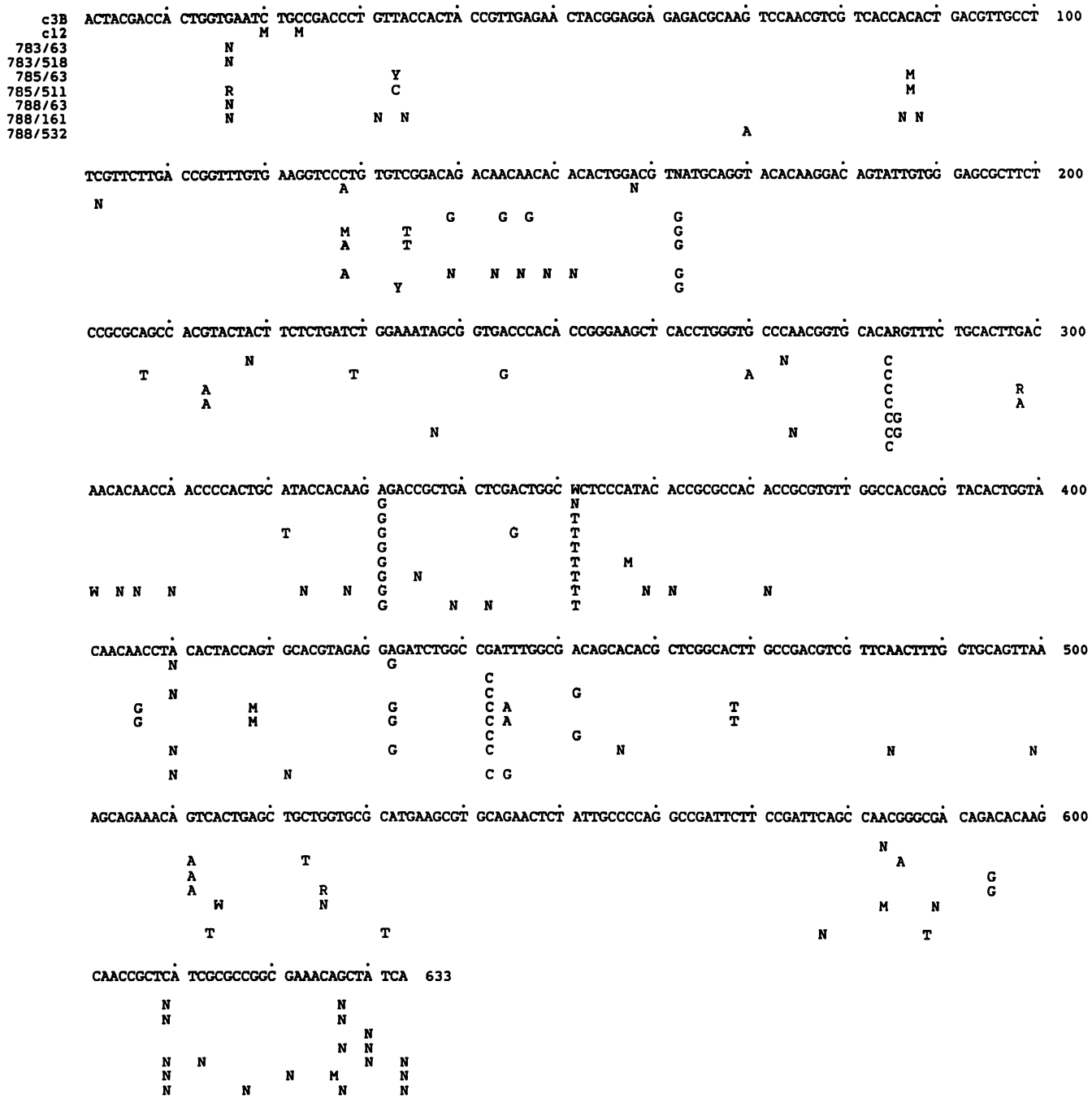

FIG. 4. Nucleotide sequence of the VP1-coding region of FMDV c3B, c12, and isolates 783/63, 783/518, 785/63, 785/511, 788/63, 788/161, and 788/532. Only positions that differ from the c3B sequence are indicated. In addition to the four standard nucleotides, other symbols are: R, A or G; M, A or C; Y, C or T; W, A or T; N, any base. They denote positions on the autoradiograms of the sequencing gels where more than one band was seen.

$\times 10^{-2}$ s/nt per year for 783/518; values for virus c12 were 7.4×10^{-2} and 0.9×10^{-2} s/nt per year for 785/63 and 785/511, respectively. For comparison, sequences were determined for 820 residues from the P61-coding segment of c3B, c12, 783/518, and 785/511 RNAs (data not shown). P61 (or 3D) is the viral subunit of the FMDV RNA replicase, and it is relatively conserved among FMDVs and other picornaviruses (30). While the sequenced segment differed in 8% of residues from the corresponding segment in FMDV C_1 isolate C-S8 (30), only one silent mutation was scored for 783/518 and 785/511 with respect to their parental c3B and c12 viruses. Thus, the number of mutations and the calculated fixation rates for this segment were 5- to 20-fold lower than for the VP1 segment.

Amino acid substitution in VP1. In the VP1-coding segment, 59% of the recorded base changes led to amino acid substitutions. Alignment of the predicted amino acid sequences (Fig. 5) indicates that several substitutions are

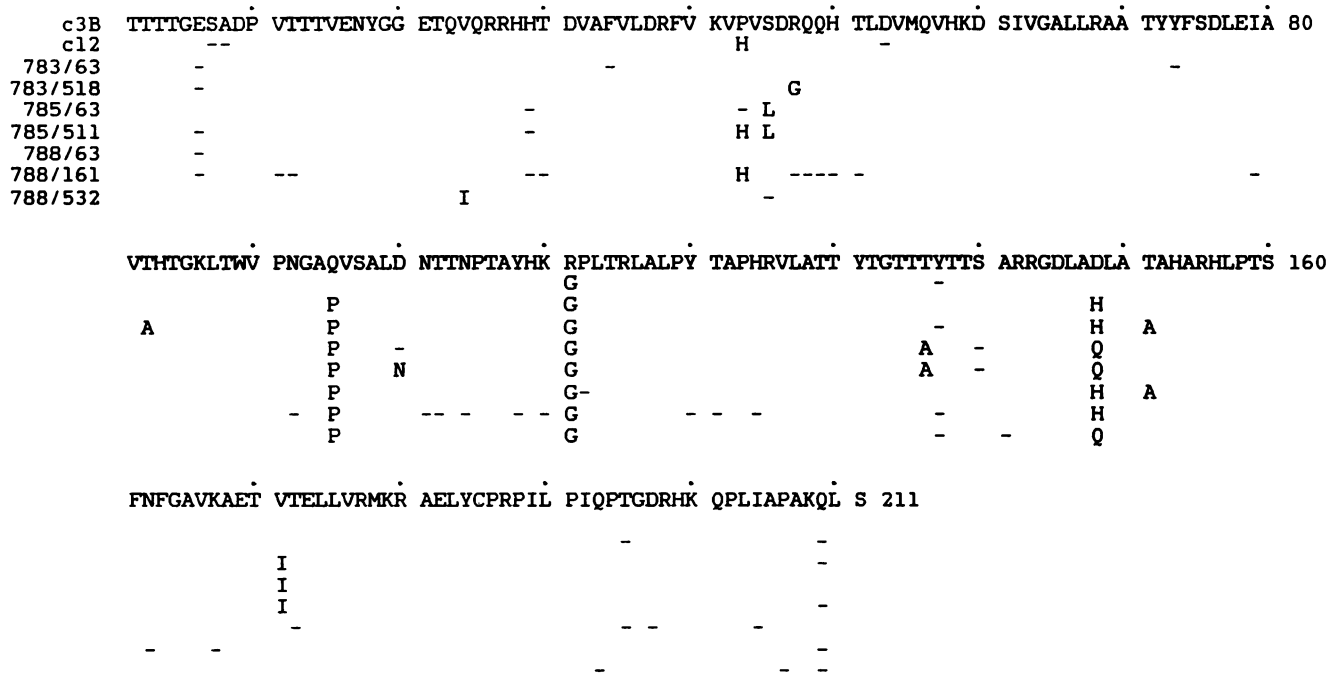

FIG. 5. Amino acid sequences of VP1 deduced from the corresponding nucleotide sequences (Fig. 4). Only amino acids that differ from those of c3B are indicated. The sequence of c3B differs from that of C_3 Res-Br/55 determined by Piccone et al. (37) in positions 95 (Q→P), 111 (R→G), and 148 (D→H). Dash indicates an undefined amino acid.

located within positions 40 to 50 and 135 to 151, the latter segment being involved in the interaction of FMDV with neutralizing antibodies (6, 31, 47). The occurrence of substitutions at those sites suggests that antigenically variant FMDVs were selected during viral replication in cattle. To investigate this possibility, the ability of OP fluids to neutralize FMDV was measured, and the reactivity with MAbs of the viral isolates was quantitated.

Antigenically variant FMDV C_3 are rapidly selected in carrier cattle. FMDV C_3-neutralizing activity was present in OP fluid samples from animals 783, 785, and 788 (Table 1), in agreement with previous work with convalescent and carrier animals (see references 20 and 32 and references therein). However, the polyclonal nature of the anti-FMDV antibodies in OP fluids renders those materials unsuitable to reliably establish differences of antigenicity among related viruses. Therefore, we tested the variation of individual epitopes involved in FMDV neutralization by quantitating the binding of several VP1-specific neutralizing MAbs (nMAbs) to VP1 of FMDV C_3 Res-Br/55, c3B, c12, 783/63, 783/525, 785/63, 785/511, 788/63, and 788/539 in an EITB assay (31) (Table 2). nMAbs 7JA1 and 7CA11 had similar binding titers with the VP1 of each virus under study (Fig. 6E and F and Table 2). In contrast, nMAbs 7AH1, 7AB5, 7EE6, 7FC12, 7JD1, and 7CA8 reacted very weakly with VP1 of viruses 785/63 and 785/511 compared with the VP1 of the other viruses (Fig. 6A and C and Table 2). The binding titer of nMAbs 7AH1 and 7AB5 was 10^3-fold lower and that of MAbs 7FC12 and 7JD1 was about 10^2-fold lower with the persistent virus 785/511 than with its parent, c12 (Fig. 6B and D and Table 2). Since all these nMAbs (exceptnMAb 7JA1) recognized a synthetic peptide spanning residues 138 to 156 of C-S8 VP1 (M. G. Mateu, D. Andreu, C. Navalpotro, E. Giralt, and E. Domingo, unpublished experiments), it is likely that substitutions within residues 139 to 158 (the equivalent to 138 to 156 in FMDV C-S8 after sequence alignment) occurring in VP1 of isolates 785/63 and 785/511 contributed to the decreased binding of these viruses to some MAbs. We conclude that selection of variants at the major VP1 antigenic site of FMDV occurred within the first 63 days of persistence of FMDV C_3 in cattle.

DISCUSSION

Since van Bekkum and his colleagues described persistence of FMDV in cattle (see reference 50 and references therein for early observations), carrier animals have been recognized as an important reservoir of this virus (4, 33, 48,

TABLE 1. Neutralizing activity of OP fluids from carrier cattle

OP fluid[a]	Virus	Neutralizing titer[b]
783/63	783/63	0.85
783/63	c3B	1.08
783/525	783/525	1.25
783/525	c3B	1.77
785/63	785/63	0.93
785/63	c12	0.76
785/511	785/511	0.28
785/511	c12	0.34
788/63	788/63	0.78
788/63	c3B	1.08
788/63	c12	0.60
788/539	788/539	0.53
788/539	c3B	0.76
788/539	c12	0.20

[a] OP fluids were obtained from the indicated cattle by probang extraction and processed and diluted as described in Materials and Methods.

[b] The neutralization titer is given as the log inverse dilution of OP fluid that caused a reduction of 70% in the PFU of the indicated virus (3). Equivalent dilution of OP samples from uninfected cattle caused no detectable PFU reduction of FMDV C_3. The OP samples from cattle 783, 785, or 788 did not neutralize FMDV O_1 Campos or A_{24} Cruzeiro.

TABLE 2. Reactivity of MAbs with VP1 of FMDV C_3 from persistently infected cattle

Antigen[a]	Reactivity with MAbs[b] (relative titer)							
	7AH1	7AB5	7EE6	7FC12	7JD1	7CA8	7CA11	7JA1
C_3 Indaial-71	+	+	+	+	+	+	+	+
C_3 Res Br/55	+	+	+	+	+	+	+	+
c3B	+ (3.0)	+ (3.0)	+	+ (3.0)	+ (3.0)	+	+ (3.0)	+ (3.0)
c12	+ (3.0)	+ (3.0)	+	+ (3.0)	+ (3.0)	+	+ (3.1)	+ (3.0)
783/63	+	+	+	+	+	+	+	+
783/525	+ (2.6)	+ (2.9)	+	+ (2.9)	+ (2.7)	+	+ (3.0)	+ (2.9)
785/63	−	−	−	±	±	±	+	+
785/511	− (0.0)	− (0.0)	−	± (0.7)	± (1.0)	±	+ (2.7)	+ (3.1)
788/63	+	+	+	+	+	+	+	+
788/539	+ (3.0)	+ (3.0)	+	+ (3.0)	+ (2.7)	+	+ (3.1)	+ (3.1)

[a] The amount of each viral protein used as antigen was adjusted to 2 μg, as monitored by densitometry of Coomassie blue-stained electropherograms (31).

[b] Reactivities in an EITB assay (31). Symbols: +, binding was more than about 50% of that observed with the virus used as eliciting antigen; ±, binding was about 1%; −, binding was about 0.1%. Further quantifications were carried out with serial dilutions of MAbs and densitometry of bands; numbers given in parentheses are normalized titers (log inverse dilution) of the MAb with the antigen under analysis relative to a titer of 3 with antigen c3B. The MAbs were prepared by using FMDV C_3 Indaial-71 as the antigen, and they neutralize the infectivity of FMDV C_3 Indaial-71.

49). FMDV was maintained in African buffalo for at least 24 years and through several generations (11), and it was transmitted to susceptible contact cattle, which then developed acute FMD (23). The mechanisms that underlie long-term, limited, asymptomatic replication in the OP area of ruminants are unknown. The duration of the carrier state in cattle is variable (45, 48). In the experimental infections with FMDV C_3 (Fig. 1), the frequency of positive viral isolations from OP fluids decreased with time. Virus was not isolated for several months and then became detectable again, suggesting fluctuations in the level of viral replication. Amino acid substitutions at critical antigenic sites (Fig. 5 and Table 2) could contribute to the escape of viral subpopulations that would otherwise have been cleared as a result of the immune response. Previous work with cattle showed that upon exposure to FMDV, a peak of neutralizing activity caused by a rise of specific immunoglobulin M (IgM) and IgA was followed by a second rise in IgA 20 to 60 days p.i. (20). The sequential dominance of novel antigenic variants was documented in persistent infections of visna virus (10, 40) and of equine infectious anemia (39). It is not possible to unambiguously attribute the decreased reactivity of 785/63 or 785/511 VP1 with some MAbs (Table 2) to a particular amino acid substitution. Even though substitution D-148→Q is a candidate, it cannot be excluded that during the EITB assay some refolding of VP1 occurred and that amino acids outside the 139 to 158 segment (perhaps residue 45 or 136) affected the reactivity of VP1 with MAbs that recognize the main 139 to 158 site. Competition experiments are now in progress to try to detect in cattle OP and serum samples antibodies directed to the antigenic determinants recognized by our panel of MAbs. Thus, persistent infections of ruminants with FMDV are not only an important natural reservoir of the virus (4, 22, 33, 48, 49) but also a potential source of antigenically variant viruses. The number of amino acid substitutions fixed in VP1 in a few days of replication in cattle is similar to the number fixed after several years of evolution in acute episodes (31, 43).

The observed size fluctuations and heterogeneity of the genomic poly(C)-rich tracts (Fig. 3) confirm and extend the conclusions of Costa Giomi et al. (12; in press) with viruses from acutely infected, convalescent, and persistently infected cattle. These poly(C) tract variations, along with the multiple nucleotide (Fig. 4) and amino acid (Fig. 5) substitutions, document the view that each viral isolate is a fleeting representative of a continuously evolving heterogeneous population (15–17, 24). The amino acid in position 43 of VP1 is P in c3B and H in c12, and they are conserved in the respective progeny viruses of each clone (Fig. 5). In positions 45, 136, and 148, the two isolates from animal 783 include the same amino acid, which is distinct from that found at the corresponding positions in animal 785. In contrast to these consistencies, examination of other positions (residues 24, 47, 82, and 100) indicates that each isolate is genetically unique and that occasionally the same substitution has occurred in FMDV of animals 783 and 785 (positions 95, 111, and 171). Occurrence of the same substitution at equivalent VP1 positions among different FMDV C_1 and C_3 isolates from acute disease episodes has been noted in several instances (9, 29a, 37, 43). This suggests that either some mutations occur with higher frequency than others or, more likely, there are important limitations in the number, nature, and compatibility of substitutions in VP1 to maintain FMDV viability. This phenomenon of repeated substitutions along with unique mutations frequently seen in isolates blurs relationships among FMDVs and conclusions on possible genome dominances in mixed infections, such as in animal 788 (Fig. 5).

Extreme genetic heterogeneity (that is, each viral RNA molecule differs in several positions from the other molecules of the same preparation) has been documented for several RNA genetic elements (15a, 16, 24, 35, 44). The viral subpopulations revealed by our analyses could have been produced either during FMDV replication in cattle or during amplification of c3B and c12 in cell culture. In the latter case, the variants were kept at a very low level, as predicted by the population equilibrium model for RNA genomes (15a). According to this model, each viral population (whether clonal or not) is a complex and indeterminate mixture of variants that are rated in competition with all other variants (15a, 16, 17, 24) and thus kept at low concentration unless endowed with a selective advantage in the environment considered. It is in very clear agreement with this concept for RNA viruses that genomic heterogeneities and fluctuations were abundant during FMDV replication in cattle. It is thus not surprising that each natural FMDV isolate sequenced to date—whether from acute (15a, 29a, 43) persistent (14; this report; J. Díez, A. Villaverde, F. Gebauer, and E. Domingo, unpublished experiments) infections—is genetically unique. Several recent studies show, in addition, that

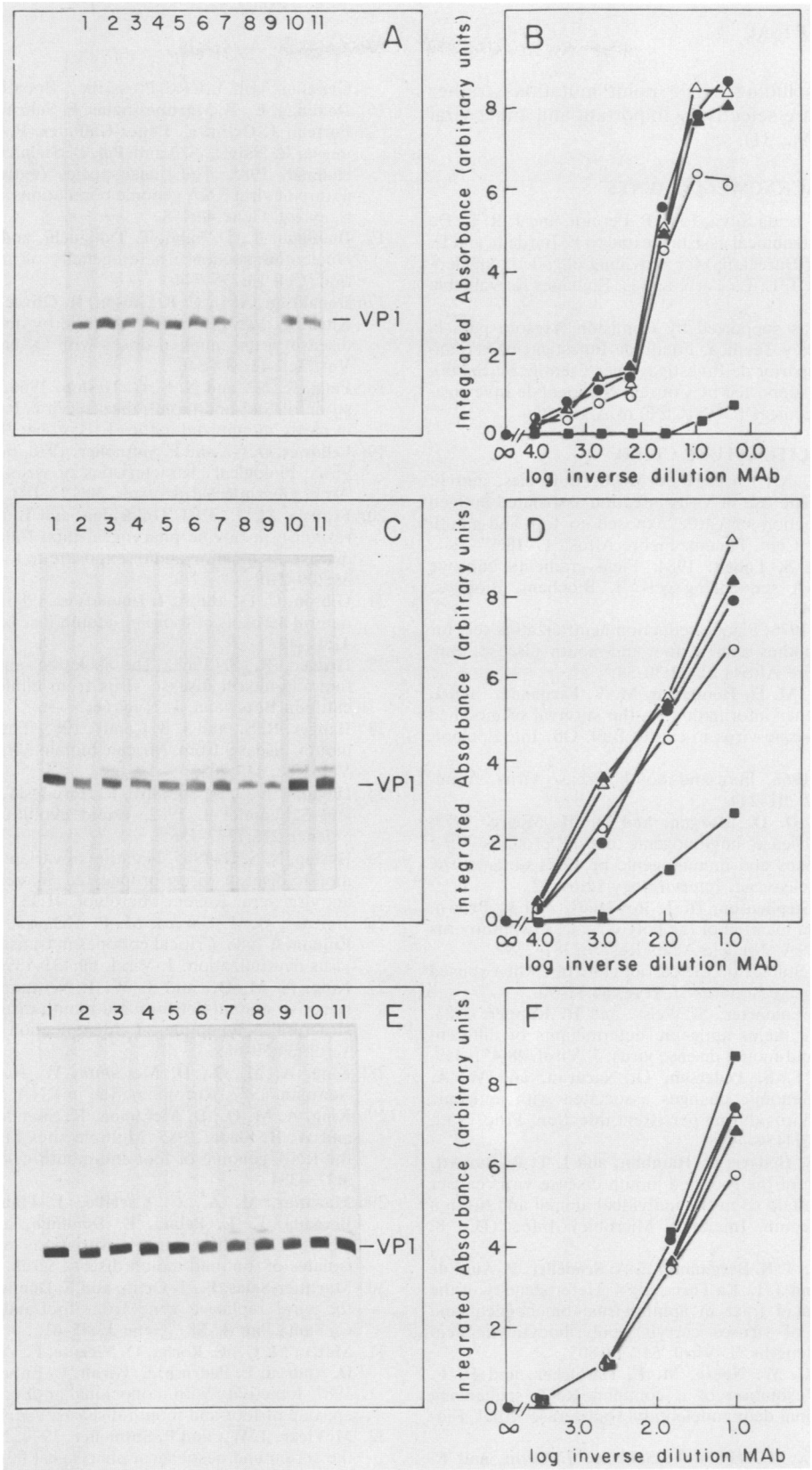

FIG. 6. Reactivity of FMDV C_3 with MAbs in EITB assays. Purified FMDV (2 μg of VP1 in each assay) was electrophoresed and transferred to nitrocellulose as described (31). In each of the blots shown in panels A, C, and E the antigen present was: 1, C-S8; 2, C_3 Indaial-71; 3, C_3 Res-Br/55; 4, c3B; 5, c12; 6, 783/63; 7, 783/525; 8, 785/63; 9, 785/511; 10, 788/63; 11, 788/539. C-S8 is an isolate of FMDV C_1 (31). The reaction given by a 1:10 dilution of MAbs 7AH1, 7JD1, and 7JA1 is shown in panels A, C, and E, respectively. Panels B, D, and F depict the integrated absorbance values obtained with the serial dilutions of Mabs 7AH1, 7JD1, and 7JA1, respectively, indicated on the abcissae; ∞, absence of MAb; △, c3B; ▲, c12; ○, 783/525; ■, 785/511; ●, 788/539. The assay without MAb was also carried out with each of the antigens. Quantitations with MAbs 7AB5, 7FC12, and 7CA11 yielded similar results to those with MAbs 7AH1, 7JD1, and 7JA1, respectively, and are not shown (see Table 2).

many variations, including single point mutations, rather than being neutral, are selectively important and affect viral behavior (15a, 16, 29a, 31).

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