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VP1 of Serotype C Foot-and-Mouth Disease Viruses: Long-Term Conservation of Sequences

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The nucleotide sequences of the VP1-coding regions of several isolates of serotype C_3 foot-and-mouth disease virus (FMDV) were determined. The deduced amino acid sequences were compared with those of serotype C_1 FMDV. The results provide evidence for two different lineages of FMDV C_3 and document the potential for both long-term conservation and rapid evolution of FMDV.

Foot-and-mouth disease (FMD), economically the most important virus disease of cattle, is enzootic in most South American countries, where hundreds of outbreaks are recorded each year (1, 24). The causative agent is FMD virus (FMDV), a picornavirus that displays remarkable genetic and antigenic variability (reviewed in references 3 and 24 and in E. Domingo and J. J. Holland, *in* E. Domingo, J. J. Holland, and P. Ahlquist, ed., *RNA Genetics*, in press). Capsid protein VP1 includes antigenic determinants able to induce neutralizing antibodies and to protect animals against FMDV infection (4, 5, 19, 31).

Protection has also resulted from administration of VP1 expressed in bacteria (16) or of synthetic peptides that are copies of the relevant sequences (9, 11, 25). Two problems have hampered the application of synthetic vaccines: (i) the low immunogenicity of most synthetic vaccine formulations tested to date and (ii) the occurrence of variant viruses in FMDV populations (12, 29) with amino acid substitutions which alter the interaction of FMDV with antibodies (22, 27). A premise for vaccine design is a knowledge of the amino acid sequences at critical epitopes and, if possible, of the variations they are likely to undergo during FMDV circulation in the field.

Here we report the nucleotide sequences of the VP1-coding regions of viruses C_3 Resende-Br/55, C_3 Argentina-84, and C_3 Argentina-85 (isolated in Resende, Brazil, in 1955, and in Argentina in 1984 and 1985, respectively, and abbreviated as C_3 Res-55, C_3 Arg-84, and C_3 Arg-85, respectively) and compare the deduced amino acid sequences with those of FMDV C_3 Indaial-71 (C_3 Ind-71) (10), FMDV C_3 Indaial-78 (C_3 Ind-78) (20), and C_1 viruses (7, 29). The results provide evidence for two different lineages of FMDV C_3 in Argentina and document the potential for both long-term conservation and rapid evolution of the FMDV genome.

Serotype C_3 FMDVs were provided by Servicio Nacional de Sanidad Animal, Buenos Aires, Argentina. Their serological relationship and their use as vaccine strains in Argentina have been described previously (1, 2). Viruses were from bovine lingual epithelium and, after isolation, were passaged in BHK-21 cell monolayers not more than five times before virus purification and RNA extraction (26). Primary cultures of fetal bovine kidney cells and of fetal bovine testicular cells were grown as described previously (G. Kaplan, Ph.D. thesis, University of Buenos Aires, Buenos Aires, Argentina, 1986). The cDNAs of the structural protein-coding segment of the FMDV C_3 genomes were synthesized by using as a primer an oligodeoxynucleotide complementary to positions 34 to 55 of the nonstructural protein P14-coding region and were cloned in plasmids by standard procedures (21). Cloned cDNA was sequenced by the method of Maxam and Gilbert (23). FMDV RNA coding for VP1 was also sequenced by primer extension and dideoxy chain termination (32). The primers used were synthetic oligodeoxynucleotides complementary to positions 109 to 126, 264 to 282, 379 to 395, 478 to 495, and 523 to 540 of the VP1-coding region and 34 to 55 of the P14-coding region (Fig. 1).

Two lineages of FMDV C_3. Although FMDV C_3 Res-55, C_3 Arg-84, and C_3 Arg-85 are serologically distinct (1), a closer relationship of C_3 Res-55 to C_3 Arg-84 than to C_3 Arg-85 was suggested by complement fixation tests, cross-protection of guinea pigs or cattle, comparison of T1 oligonucleotide maps of genomic RNA (1), and cross-reactivity with polyclonal sera (G. Zuloaga, E. Domingo, and E. L. Palma, unpublished results). In sodium dodecyl sulfate-urea-polyacrylamide gel electrophoresis, each virus showed a distinct migration pattern for VP1, VP2, and VP3 (Fig. 2). The nucleotide sequences of the VP1-coding regions of C_3 Res-55, C_3 Arg-84, and C_3 Arg-85 have been aligned with those of FMDV C_3 Ind-71 and C_3 Ind-78 (Fig. 3). The computation of the number and nature of base substitutions between pairs of viruses reveals a striking lack of correspondence between time and genetic distances among these RNAs. The VP1-coding region of C_3 Res-55 differs from that of C_3 Arg-84 in 12 nucleotides, while those of C_3 Arg-84 and C_3 Arg-85 differ in 76 (Fig. 3 and Table 1). We suggest that two lineages of C_3 viruses have circulated in Argentina: C_3 Arg-84 is related to C_3 Res-55, and C_3 Arg-85 is related, albeit more distantly, to C_3 Ind-71 and C_3 Ind-78. In agreement with this proposal, the triplet CAA at positions 145 to 147 was found in RNAs of C_3 Res-55 and C_3 Arg-84 but not in others. The deduced amino acid sequences of VP1 have been aligned with those of viruses of serotype C_1 (Fig. 4). VP1s of any two C_3 viruses differ in 4 to 21 residues; between any one C_1 virus and any one C_3 virus the difference is of 13 to 19 residues. Two regions of high heterogeneity are located within segments 43 to 61 and 136 to 155 (Fig. 4), the latter segment being the main epitope involved in the neutralization of FMDV (9, 11, 25, 31). Since VP1 of C_3 Res-55 and VP1 of C_3 Arg-84 are

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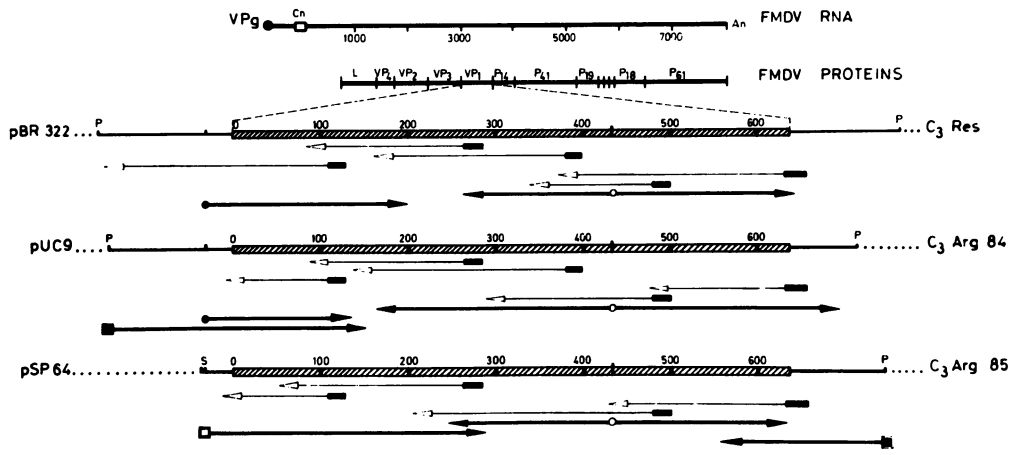

FIG. 1. FMDV genome, encoded proteins (14), and strategy for the nucleotide sequence determination. RNA was sequenced by oligodeoxynucleotide primer extension (32); the location of primers is indicated by black rectangles and thin arrows. cDNAs of C_3 Res-55, C_3 Arg-84, and C_3 Arg-85 were cloned in plasmids pBR322, pUC9, and pSP64, respectively (21). DNA restriction fragments were labeled at their 5′ ends with [γ-^{32}P]dATP and polynucleotide kinase. Symbols: ○, *Bgl*II; ■, *Hin*dIII; ●, *Sac*I; □, *Eco*RI. The labeled ends are indicated by dots, the extent of sequencing is indicated by thick arrows, and the S and P at the ends of the lines indicate restriction sites of cloning for *Sac*I and *Pst*II, respectively.

identical around positions 136 to 155, the serological differences seen between these two viruses (1) must be due to variations elsewhere in the virions. Although the segment from positions 40 to 60 does not appear to be immunogenic, a distal effect of substitutions at amino acid 43 or 48 of VP1 on the recognition by a monoclonal antibody of an epitope composed of amino acids from segments 146 to 150 and 200 to 213 has recently been reported in FMDV O_1 BFS 1960/67 (N. R. Parry, P. V. Barnett, A. D. Syred, D. J. Rowlands, and F. Brown, Abstr. 5th Meet. European Group Mol. Biol. Picornaviruses, Mallorca, Spain, abstr. no. D2.1 P2.27, 1987). Thus, it is possible that the radical substitution S (position 45) to L or R (position 47) to G could contribute to serological differences between C_3 Res-55 and C_3 Arg-84. This point is under investigation.

Potential for long-term conservation and rapid evolution of FMDV genome segments. A rate of fixation of mutations of 5×10^{-3} substitutions per nucleotide per year was calculated for the two C_3 Indaial isolates; this value was in the range found for some time periods in the evolution of FMDV C_1 (29). However, a 10-fold-lower rate was computed for C_3 Res-55 and C_3 Arg-84. We considered four possibilities to explain the relative conservation of the VP_1-coding segment of these two viruses, isolated over a 29-year interval: (i) convergent evolution effected by passage of the viruses in cell culture following their isolation; (ii) accidental reintroduction of vaccine viruses in the field; (iii) persistent infections of cattle as a reservoir of viruses undergoing little variation; and (iv) potential for long-term conservation of genomes as a property of highly heterogeneous RNA viruses. We favor possibility iv for the following reasons. (i) Plaque-purified C_3 Res-55 was serially passaged 15 times in BHK-21 cells or primary fetal bovine kidney or testicular cell cultures (Kaplan, Ph.D. thesis). Genomic RNA from each resulting viral population was sequenced at positions 400 to 520; no mutations were found. This result and previous estimates of fixation of mutations upon passage of FMDV in cell cultures (28) suggest that the sequences reported here were not altered in a quantitatively significant manner by the passage history of the viruses. (ii) Since C_3

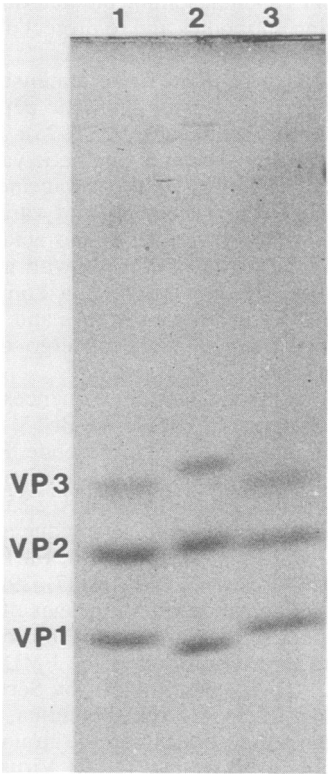

FIG. 2. Electropherogram of purified FMDV C_3 Arg-84, C_3 Arg-85, and C_3 Res-55 (lanes 1, 2, and 3, respectively) obtained by the method of Laemmli (18) with 8 M urea in the gel. Each viral preparation (about 5 μg of protein) was disrupted by being heated at 100°C for 1.5 min in a buffer containing 80 mM Tris hydrochloride (pH 6.3), 10% sodium dodecyl sulfate, 8 M urea, 1.2 M 2-mercaptoethanol, 18% glycerol, and 0.02% bromophenol blue.

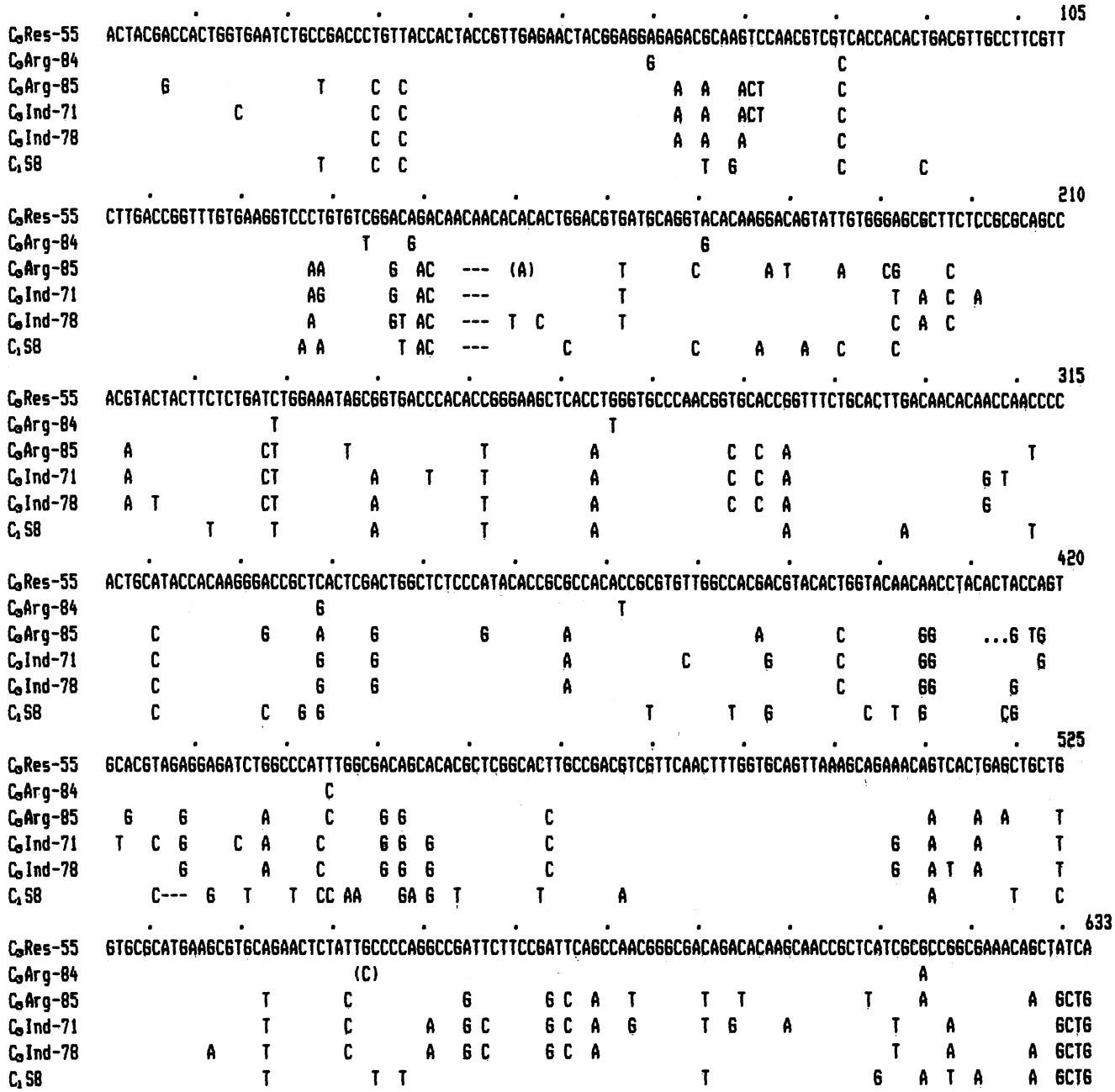

FIG. 3. Nucleotide sequence of the VP1-coding region of FMDV C_3 and FMDV C_1 S8, Santa Pau (Girona) (29). Only residues that differ from those of C_3 Res-55 are indicated. Dashes indicate the absence of bases. Dots indicate ambiguities in the sequencing gels. Parentheses indicate bases deduced by the RNA sequencing method. Positions 151 and 554 were determined to be guanine by the chemical degradation method with cloned cDNA. The sequences of C_3 Ind-71 and C_3 Ind-78 are those reported by Cheung et al. (10) and Makoff et al. (20), respectively.

Res-55 and C_3 Ind-71 have been used as vaccine strains in Argentina and Brazil, respectively (2), they might have been reintroduced accidentally in the field. It was recently suggested that some FMD outbreaks in Europe had such an accidental onset (8). Given the enzootic nature of FMD in South America, with a continuously replicating pool of viruses (1, 24), the isolation of a vaccine-derived FMDV would seem an unlikely event. However, there is not enough information to rule out this possibility. (iii) Sequencing of RNA from C_3 FMDV which has persisted in cattle has shown a rapid sequential fixation of amino acid substitutions in the VP1 of viruses from individual cattle (F. Gebauer et al., unpublished results). It is thus unlikely that persistence could contribute to the long-term conservation of VP1. (iv) The high mutation rates operating during replication of RNA genomes (6, 15, 30; Domingo and Holland, in press) and the extremely heterogeneous nature of RNA genomes do not necessarily lead to their rapid evolution. Depending on the selective constraints, the equilibrium pools of genomes may be maintained around the same average sequence (13, 15;

TABLE 1. Nucleotide and amino acid substitutions among the VP1-coding regions of FMDVs of serotype C_3

Virus	Differences among viruses[a]											
	C_3 Res-55 Aa	C_3 Arg-84			C_3 Arg-85			C_3 Ind-71			C_3 Ind-78	
		Ts	Tv	Aa	Ts	Tv	Aa	Ts	Tv	Aa	Ts	Tv
C_3 Res-55		9	3		57	14		60	13		52	12
C_3 Arg-84	4				60	16		65	14		57	13
C_3 Arg-85	18			21				40	6		36	7
C_3 Ind-71	15			18			10				23	3
C_3 Ind-78	12			15			10			7		

[a] For each pair of VP1-coding regions, the number of point mutations (Ts, transitions; Tv, transversions) and the deduced number of amino acid substitutions (Aa) are given. Deletions are counted as amino acid substitutions. The computations are based on the sequences given in Fig. 3 and 4.

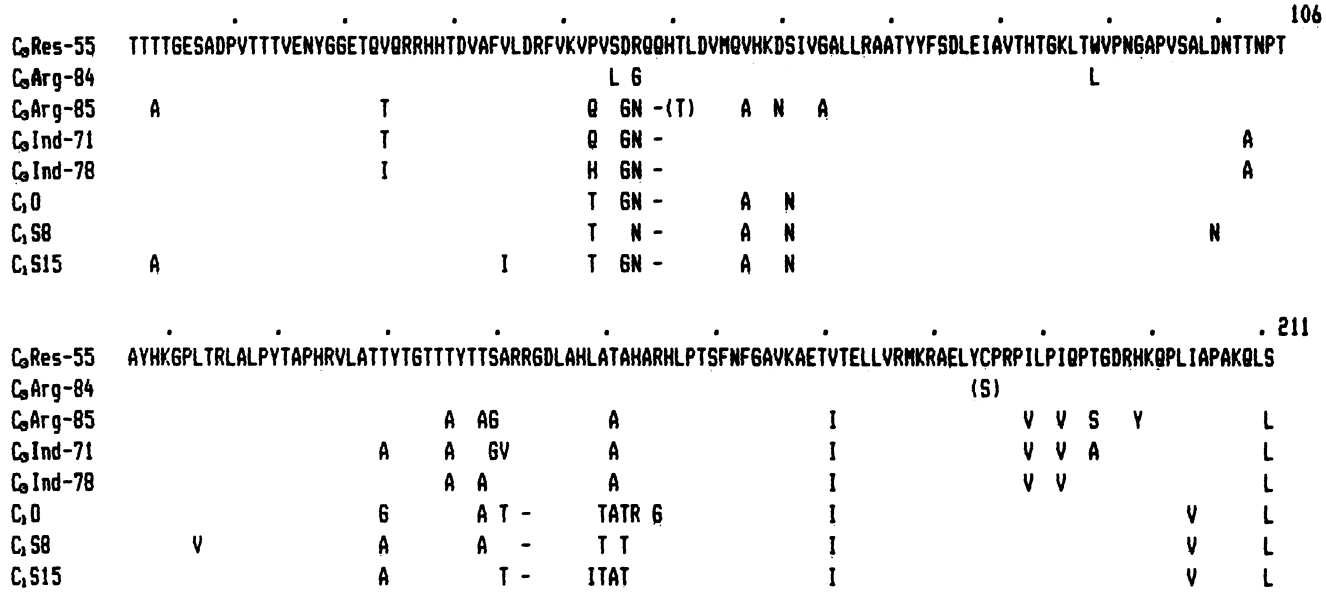

FIG. 4. Amino acid sequence of VP1 of FMDV C_3 viruses deduced from the nucleotide sequence and aligned with the corresponding sequences of FMDV C_1 S8 and C_1 S15 (29) and C_1 O (7). Parentheses indicate amino acids deduced by the RNA sequencing method; dashes indicate absence of amino acid. Positions 51 and 185 are A and C, respectively, as predicted by cDNA sequencing.

Domingo and Holland, in press). This dual potential for variation or conservation has been experimentally demonstrated for vesicular stomatitis virus, which can be genetically stable in serial dilute passage or made to evolve rapidly in the presence of defective interfering particles (15; Domingo and Holland, in press). Also, despite the documented high variability of human immunodeficiency viruses, remarkable conservation has been found among simian T-cell lymphotropic viruses and human T-cell lymphotropic virus type 4 (17).

For FMDV, we suggest that the nature and number of amino acid substitutions compatible with a viable virion must be very limited. Note that several amino acid substitutions are shared by a subset of C_3 and C_1 viruses (Fig. 4, positions 3, 24, 46, 47, 57, 130, 136, 139, 140, 141, 151, 171, 189, 192, 195, and 211). New viable genomes may arise by minor adjustments of sequences or by multiple mutations on the same genome which require adjustment (via compensating substitutions, selection of groups of mutations, etc.) to one of the limited set of viable sequences. Multiple mutations on the same genome are expected at a frequency of $10^{-m \times n}$ (n being the number of mutations and 10^{-m} being the mutation rate per nucleotide and RNA doubling, with $m = 4$ being a likely value) (6, 15, 30; Domingo and Holland, in press). Fixation of such simultaneous mutations will thus appear as "sequence jumps," which may explain the different lineages seen in both C_3 and C_1 viruses (22, 29). In this respect, it is noteworthy that FMDV C_3 Res-55 and C_3 Arg-84 have an S residue instead of an L residue at the predicted carboxy end of VP1, implying an unusual cleavage site at the VP1-P14 junction (manuscript in preparation) not found in any other FMDV sequenced to date (8, 29).

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