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Heterogeneity of the Polyribocytidylic Acid Tract in Aphthovirus: Biochemical and Biological Studies of Viruses Carrying Polyribocytidylic Acid Tracts of Different Lengths†

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In this paper we report a study of a sample of foot-and-mouth disease virus carrying two polyribocytidylic acid [poly(C)] tracts of different lengths. By plaque purification in tissue culture, we isolated two populations of particles, one carrying the long poly(C) tract and the other carrying only the short homopolymer. The fingerprints of both viruses were indistinguishable from each other and from that of the virus present in the original sample, suggesting that the main difference between the two types of particles is limited to the poly(C) tracts of their genomic RNAs, to the flanking sequences of the poly(C) tract, or to both. In addition, some biological properties of these viruses are reported, such as stability upon serial passages in different cell lines, plaque size, and pathogenicity for cattle. The results indicate that the size of the poly(C) tract is not directly related to the virulence of these viruses. However, the size of the homopolymer could play a role in determining their efficiency of replication, and it appears that the particles with the short poly(C) tract might have some replicative advantage over those carrying the long one.

The presence of a discrete polyribocytidylic acid [poly(C)] tract within the genomic RNA of a picornavirus was first reported by Porter et al. (12) in encephalomyocarditis virus. This observation was later confirmed and extended by Brown et al. (4) who also found a similar poly(C) tract within the genomes of mengovirus and foot-and-mouth disease virus (FMDV). The absence of this tract in the RNAs of other members of the picornavirus family, such as rhinovirus and enterovirus, was also reported, indicating that the poly(C) fragment is a distinctive feature of the cardiovirus and aphthovirus groups (3). The location of this homopolymer near or at the 5′ end of the genomic RNA was first reported by Chumakov and Agol for encephalomyocarditis virus (5), by Harris and Brown for FMDV (8), and by Perez-Bercoff and Gander for mengovirus (11).

A more-precise location of the tract at about 400 nucleotides downstream from the 5′ end of the genomic RNA of FMDV was achieved by Rowlands et al. (13). The length of the poly(C) tract varies from ca. 80 to >200 nucleotides in different serotypes of encephalomyocarditis virus and FMDV (3). Moreover, variation in the length of this tract among different virus isolates is high and can be used as a valuable tool for a more-precise identification of variants and varieties of this virus (10). The biological function of the poly(C) tract and the relevance of its length variation are at present unknown. Harris and Brown (9) reported that the genomic RNA of a SAT-1 type of FMDV which was attenuated by serial passages in BHK cells had a poly(C) tract considerably shorter than that of its parental strain. However, the direct implication of this tract in the mechanism of attenuation was not established.

In this work, we have studied a sample of the virus C_3 Resende (RES) carrying two poly(C) tracts of different lengths which are present in approximately the same molar ratio. After plaque purification two different virus populations were isolated, one having the long poly(C) tract present in the original virus sample and the other carrying only the short tract. Since the two viruses were indistinguishable by current serological methods and no differences were detected in their T_1 maps, these viruses could be suitable models for studying the function of the poly(C) tract. Therefore, we have studied some biological properties of these viruses, such as infectivity in different cell lines, plaque size, stability upon serial passages in BHK cells, and pathogenicity in cattle.

The results indicate that, at least for these two viruses, there is no direct correlation between the size of the poly(C) tract and the pathogenicity of the virus. The results also suggest that a competitive selection of particles can take place when viruses carrying poly(C) tracts of different sizes replicate simultaneously in tissue culture. In this case, it appears that particles carrying the short poly(C) tract are more efficient than those carrying the long tract.

MATERIALS AND METHODS

Cells and viruses. BHK-21 cells (ca. 2×10^7) in Falcon disposable plastic bottles were infected at high multiplicity (10 to 100 PFU/cell) with FMDV serotype O_1 Caseros, A Argentina (ARG)/79, or A ARG/68 or with one of two different samples of virus C_3 RES, one obtained from the Servicio Nacional de Sanidad Animal of Argentina and the other obtained from the Pan American Foot-and-Mouth Disease Center of Brazil, and designated C_3 RES(SEN) and C_3 RES(PAN), respectively. Both C_3 RES viruses originated from the same seed virus at the Pan American Foot-and-Mouth Disease Center and are currently maintained in both laboratories by limited cattle passages and by not more than three BHK cell passages. Both viruses are indistinguishable by serological methods, T_1 maps (fingerprints), or both methods. Two field isolates of virus C_3 RES, designated C_3 31233 and C_3 31296, were also studied. These viruses were provided by the Servicio Nacional de Sanidad Animal and

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† This paper is dedicated to the memory of Cesar Vasquez, Director of Centro de Virologia Animal, deceased on 19 April 1983.

used for these studies after four passages in BHK cells. Viruses A_{27} Cundinamarca-Col/76 and A_{32} Venezuela/70 were isolated from the field in Colombia and Venezuela, respectively (1). The viruses were maintained by serial passages in bovine tongue epithelium and fingerprinted after no more than four passages in BHK cells.

Selection of clones. To isolate single clones of virus C_3 RES(SEN) from the original seed, we plated different dilutions of the sample to yield one to three plaques per plate. The centers of well-isolated plaques were punched out to isolate the virus. Of 22 different clones isolated, 6 (10, 4A, 5, 9B, 12, and 3B) were selected at random and studied further. Each clone was replicated five or six times in BHK cells to increase the virus titer.

Titration of viruses in different cell systems. Titration in BHK cells, primary bovine kidney cells, and a swine kidney-derived cell line, IB-RS-2, were performed as described by P. Augé de Mello (2).

Cattle. The cattle used in this study were 12-month-old crossbred Holstein-Zebu obtained from an FMDV-free farm outside of Rio de Janeiro, Brazil. The cattle were selected by previous examination of their sera for the absence of neutralizing antibodies for the virus C_3 RES.

Cattle inoculation. Virus suspensions corresponding to clones 3B [short-poly(C) tract] and 12 [long-poly(C) tract] were titrated in IB-RS-2 cells. The virus concentrations of both samples were 7.5 PFU/ml. Each virus suspension (0.1 ml/suspension) was inoculated intradermally at 10 different points on the tongues of two cattle. A third bovine was inoculated in the same way with a 1-ml suspension containing an equal mixture of both viruses.

Preparation of ^{32}P-labeled RNA from the cytoplasm of infected cells. Monolayers of BHK cells in Falcon bottles were infected with each of the viruses indicated above. The viruses were labeled at 2 h postinfection with 300 μCi of [^{32}P]orthophosphoric acid (New England Nuclear Corp.) per ml in the presence of 5 μg of actinomycin D per ml; the single-stranded cytoplasmic RNA was extracted and processed as previously described by La Torre et al. (10).

Enzymatic digestion of RNA with RNase T_1. RNA suspensions were pelleted (10,000 × *g*; 15 min), dissolved in 300 mM sodium acetate buffer (pH 5.0), and precipitated with ethanol at −70°C for 1 h. The precipitates were collected by centrifugation at 17,000 × *g* for 5 min, dried in vacuo, redissolved in T_1 buffer (20 mM Tris-hydrochloride, 2 mM EDTA [pH 7.6]), and digested for 1 h at 37°C with RNase T_1 (Collaborative Research, Inc.) at an enzyme/substrate ratio of 1:5.

Isolation of the poly(C) tract by affinity chromatography. To isolate the poly(C) tract(s), we diluted a fraction of the RNase T_1 digest with 500 mM NaCl–10 mM Tris-hydrochloride (pH 7.5)–0.2% sodium dodecyl sulfate (binding buffer) and chromatographed the fraction twice at room temperature on oligodeoxyguanylic acid [oligo(dG)]-cellulose microcolumns which were previously equilibrated with binding buffer. Fractions containing unbound material, nonpolyribocytidylic acid-containing [poly(C)−] fractions, were pooled and precipitated with 2.5 vol of ethanol. The polyribocytidylic acid-containing [poly(C)+] fractions were eluted with elution buffer (10 mM Tris-hydrochloride [pH 7.5] plus 0.2% sodium dodecyl sulfate) and also precipitated with ethanol at −20°C overnight.

Separation of oligonucleotides by two-dimensional electrophoresis. The method for separation of oligonucleotides was that described by Frisby et al. (7) with minor modifications.

Separation of oligonucleotides by one-dimensional electrophoresis. RNase T_1 digests were precipitated with ethanol, dissolved in formamide sample buffer, and analyzed as previously described (10).

Sizing of the poly(C) tracts. The sizes of the poly(C) tracts of virus C_3 RES(SEN) were determined by using as size markers *Hin*dI restriction fragments of DNA from plasmid pBR322. The plasmid was labeled in vivo with [^{32}P]orthophosphoric acid, extracted, and purified by electroelution. The purified plasmid was digested with the enzyme, boiled for 2 min in 0.3 N NaOH, and electrophoresed on a 10% sequencing gel (10). Samples of RNA labeled and prepared as described above were run in parallel within the same gel.

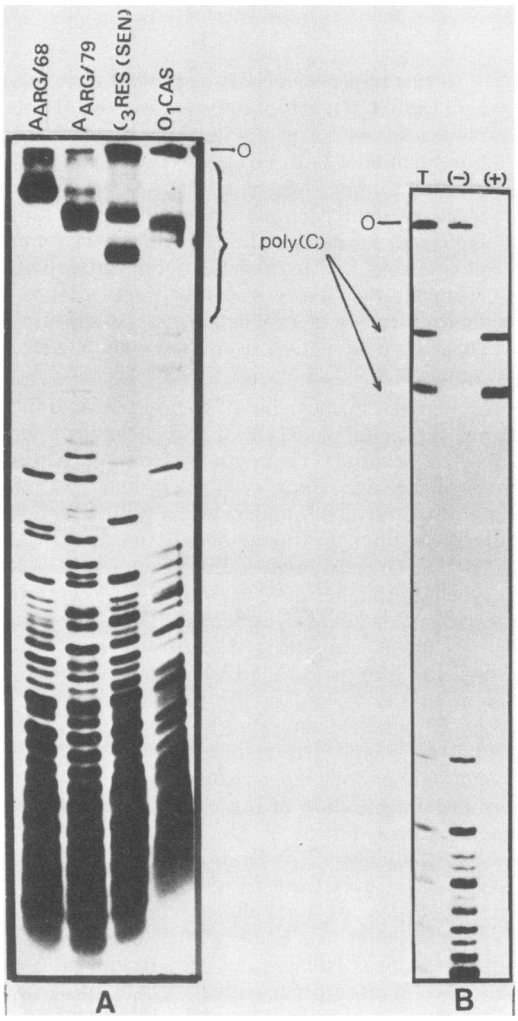

FIG. 1. RNase T_1 one-D maps of induced FMDV RNAs. (A) ^{32}P-labeled induced RNAs from four different FMDV serotypes were extracted, hydrolyzed with RNase T_1, and analyzed on 0.3-mm-thick polyacrylamide gels as previously described (10). Gels were run at 1,500 V and 35 mA until bromophenol blue had run 22.5 cm and xylene cyanol had run 12.3 cm. Gels were then covered with plastic wrap and exposed overnight at −70°C with regular X-ray film and with a Cronex Hi-Plus (E. I. du Pont de Nemours & Co., Inc.) screen. O, Origin of the gel. Bracket indicates the region of the gel at which the poly(C) tract(s) run. (B) A sample of virus C_3 RES(SEN) was fractionated on an oligo(dG)-cellulose microcolumn, as described above, and analyzed as above except that the gel was run until xylene cyanol had reached the bottom of the gel. T, Total unfractionated sample; (−), unbound counts [poly(C)−]; (+), counts bound to the column and eluted at low ionic strength [poly(C)+]; O_1CAS, O_1 Caseros.

RESULTS

Variation of the poly(C) tract among different FMDV types. Analysis of the T_1 hydrolysates of several FMDV types on one-dimensional (one-D) polyacrylamide gels (Fig. 1A) shows that each virus has a distinctive pattern of bands that allows its quick and precise identification (10). The T_1-resistant fragment(s) running near the top of the gel corresponds to the poly(C) tract, a feature which is also distinctive of each virus (4, 10). Some viruses had additional bands, usually present at submolar ratios, in the region of the gel at which the poly(C) ran, suggesting the possibility that, at least with regard to the homopolymer, more than one virus population was present in the sample (see Fig. 1A, viruses A ARG/79 and O_1 Caseros). To identify which oligonucleotide band is the poly(C) tract, we have used two criteria: (i) the ability of the poly(C) tract to bind to oligo(dG)-cellulose columns and (ii) the base composition of the poly(C) tract. In virus A ARG/79, both bands behaved as poly(C)-rich fragments, whereas in virus O_1 Caseros, only the main band was identified as the poly(C) tract of the virus (6). As in virus A ARG/79, virus C_3 RES(SEN) also had two poly(C) tracts which, in this case, were present at approximately the same molar ratio (Fig. 1A). The identity of both oligonucleotides as poly(C) tracts was again established by their ability to bind to oligo(dG) columns (Fig. 1B) and by the fact that both bands are composed of >90% cytosine residues (data not shown). The sizes of both poly(C) tracts were established as described above. The large poly(C) tract was ca. 230 nucleotides long, whereas the short one was ca. 145 nucleotides long.

Two virus populations differing only in the length of their poly(C) tracts. To determine whether both poly(C) tracts of C_3 RES(SEN) are due to the presence of a single fragment interrupted somewhere near its middle by a guanosine residue or whether the tracts originated from two different virus populations, the original seed of C_3 RES(SEN) was plaque purified once in tissue culture (see above). Viruses from each plaque were passaged three to four times in BHK cells to increase their titers. In the last passage, infected cells were labeled with ^{32}P, and the T_1 hydrolysates of the induced RNA were prepared and analyzed on one-D gels (see above).

The patterns of bands elicited by the different plaque-isolated virus clones that were run in parallel with the original uncloned virus seed are shown in Fig. 2. Clones 4A, 5, 9B, and 3B had only the short poly(C) tract, whereas clone 12 had only the long one (Fig. 2). Overexposure of the gel did not show additional bands in the region of the poly(C) tract (data not shown). Clone number 10 had both poly(C) tracts, suggesting that it was not a single isolate. Both the short and the long poly(C) tracts were indistinguishable from those present in the original virus sample [C_3 RES(SEN)] as shown by coelectrophoresis of the T_1 hydrolysates from both types of clones and the original virus sample (Fig. 2B).

Besides the differences in the lengths of the poly(C) tract, analysis of other oligonucleotides on the one-D gels indicates that the T_1 maps from all of the clones analyzed were indistinguishable from each other and from the T_1 map of the original virus seed (Fig. 2A and B). Moreover, the identity of those viruses was further confirmed by analyzing the T_1 hydrolysates on two-dimensional (two-D) gels. A comparison of the fingerprints shown in Fig. 3 clearly indicates that there are no detectable structural differences between the genomes of the viruses carrying either the short or the long poly(C) tracts as well as between these viruses and the original C_3 RES(SEN) strain (Fig. 3A, B, and D, respectively).

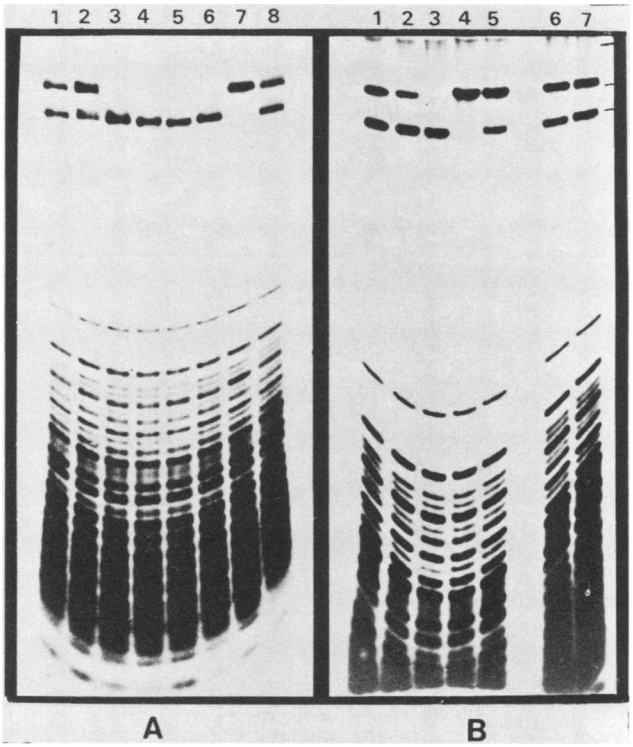

FIG. 2. RNase T_1 one-D maps of the RNAs of different clones of virus C_3 RES(SEN). The RNAs were prepared and analyzed as described in the legend to Fig. 1A. (A) Lanes 1 and 8, original (uncloned) samples of the virus; lane 2, clone 10; lanes 3 to 7, clones 3B, 4A, 5, 9B, and 12, respectively. Bars on the left of (A) indicate the positions of the two poly(C) tracts of the original viruses. (B) Coelectrophoresis of T_1 hydrolysates of clones carrying the short or long poly(C) tracts. Lanes 1 and 7, original (uncloned) samples of the virus; lane 2, coelectrophoresis of the original sample with clone 3B; lane 3, clone 3B; lane 4, clone 12; lane 5, coelectrophoresis of clone 12 and the original sample; lane 6, coelectrophoresis of the original sample and clones 3B and 12. Bars on the right of (B), lane 7, indicate the position of both poly(C) tracts.

These results strongly suggest that the original virus C_3 RES(SEN) is composed of a mixed population of particles bearing similar genomes but different poly(C) tracts. For this reason, this virus sample could be a suitable model to study the biological role of the poly(C) tract.

Pathogenicity of the viruses carrying the short and the long poly(C) tracts in tissue cultures and cattle. To establish whether there are any detectable differences in the infectivity of both viruses in tissue cultures, clones 3B [short poly(C) tract] and 12 [long poly(C) tract] were titrated in BHK, IB-RS-2, and primary bovine kidney cells. No significant differences in the titers of the two viruses were detected in those cells. However, the plaques formed by both viruses in the cell systems tested were heterogeneous, and the virus carrying the long poly(C) tract (clone 12) produced an additional population of plaques which were larger than any of the ones formed by the virus carrying the short poly(C) tract (clone 3B) (data not shown).

Suspensions of virus from clones 3B [short poly(C) tract] and 12 [long poly(C) tract] and a suspension containing an equal mixture of both were injected intradermally into the tongues of three cattle (see above). The cattle were exam-

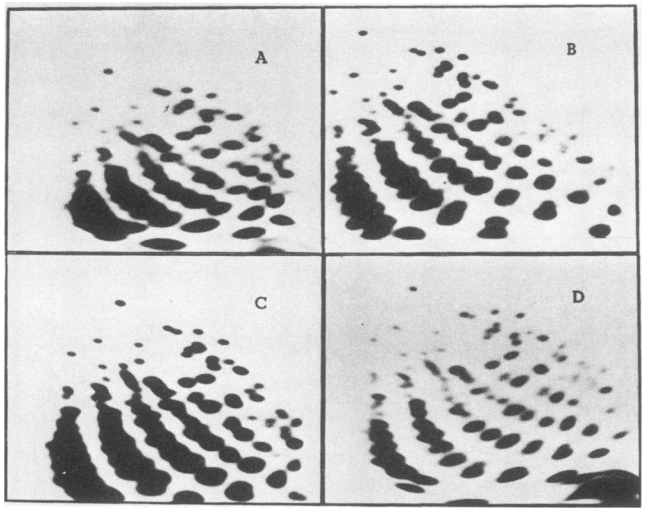

FIG. 3. RNase T_1 two-D gels (fingerprints) of (A) clone 3B [short poly(C) tract], (B) clone 12 [long poly(C) tract], (C) coelectrophoresis of clones 12 and 3B, and (D) virus C_3 RES(SEN), the original uncloned virus.

ined daily after inoculation. The three bovines presented vesicular lesions in the tongue and feet 48 to 120 h after inoculation, indicating that there are no detectable differences in the pathogenicity among the virus samples and that the size of the poly(C) tract is not directly involved in the virulence of these strains. Furthermore, in a survey of aphthovirus in South America, we have studied virus A_{27} Cundinamarca-Col/76 which was originally isolated from the field and is used at present as a prototype strain for vaccine production in Colombia (1). The poly(C) tract of this virus was strikingly shorter than those present in the genomic RNA of viruses A ARG/79 and A_{32} Venezuela/70 (Fig. 4). Moreover, the poly(C) tract of this virus was the shortest detected among a large number of viruses analyzed in this survey. The length of this poly(C) tract was even 11% shorter than that of the short poly(C) tract of virus C_3 RES(SEN) (ca. 130 nucleotides). However, the pathogenicity of this virus for cattle does not appear to have been altered since this strain was isolated from vaccinated animals and is maintained in the laboratory by serial passages in bovine tongue epithelium.

Behavior of the poly(C) tract in different virus isolates. The observation that similar virus isolates carry different poly(C) tracts (10; Bergmann et al., in preparation) could be indicative that this sequence can undergo significant changes in size probably due to modification, selection or both, produced during passages in the field, in tissue culture in the laboratory, in both passages in the field and tissue culture in the laboratory, or during vaccine preparation. For this reason, we have studied the poly(C) tracts from samples of different C_3 RES viruses. Two of those were field isolates, and the others were the C_3 RES prototype strains which are currently in use in Argentina [C_3 RES(SEN)] and at the Pan American Foot-and-Mouth Disease Center [C_3 RES(PAN)] as reference strains or for vaccine production. Comparison of the fingerprints of the four viruses (Fig. 5A) indicates that three of those, the prototypes from Argentina and the Pan American Foot-and-Mouth Disease Center and field isolate C_3 31296, are indistinguishable from each other, whereas field isolate C_3 31233 has a fingerprint which is similar to but clearly distinguishable from those of the others. This latter strain had six additional and five missing spots compared with the prototype virus (Fig. 5B). Comparison of the T_1 hydrolysates of the four virus-induced RNAs on one-D gels (Fig. 6C) indicates that these viruses can be easily distinguished by the difference in the migration rate of their poly(C) tracts. The C_3 RES(SEN) strain had the two poly(C) tracts described above, whereas the field isolate, C_3 31296, which is identical to the corresponding prototype on one- and two-D maps, presented only the short poly(C) tract (Fig. 6A).

The results (Fig. 6B) indicate that serial passages of FMDV through tissue culture can promote selection of virus populations carrying different poly(C) tracts; after 14 passages in BHK cells, the original sample of virus C_3 RES-(SEN) showed an important enrichment of the virus particles carrying the short poly(C) tract (Fig. 6B). These results suggest that field isolate C_3 31296 could have originated from the vaccine strain C_3 RES(SEN) which had lost the virus particles carrying the long poly(C) tract during cell culture passages for vaccine production or while circulating in the field. On the other hand, when the clones carrying either the short or the long poly(C) tracts were also passaged 14 times under the same condition (data not shown), no alterations in the length, behavior, or both length and behavior of their

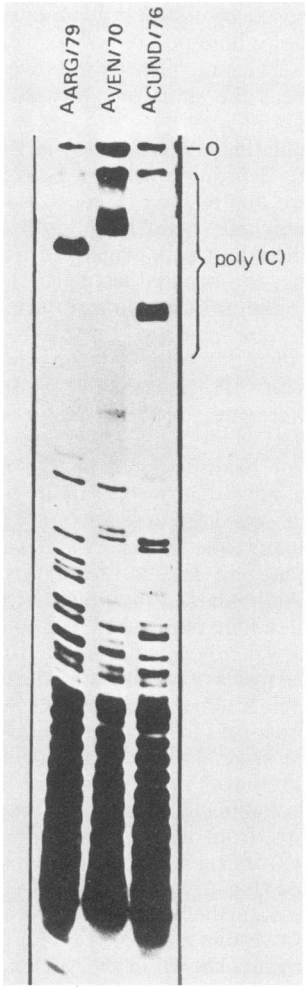

FIG. 4. RNase T_1 one-D gel of viruses A ARG/79, A Venezuela/70 (A VEN/70) and A Cundinamarca-Col/76 (A CUND/76). O, Origin.

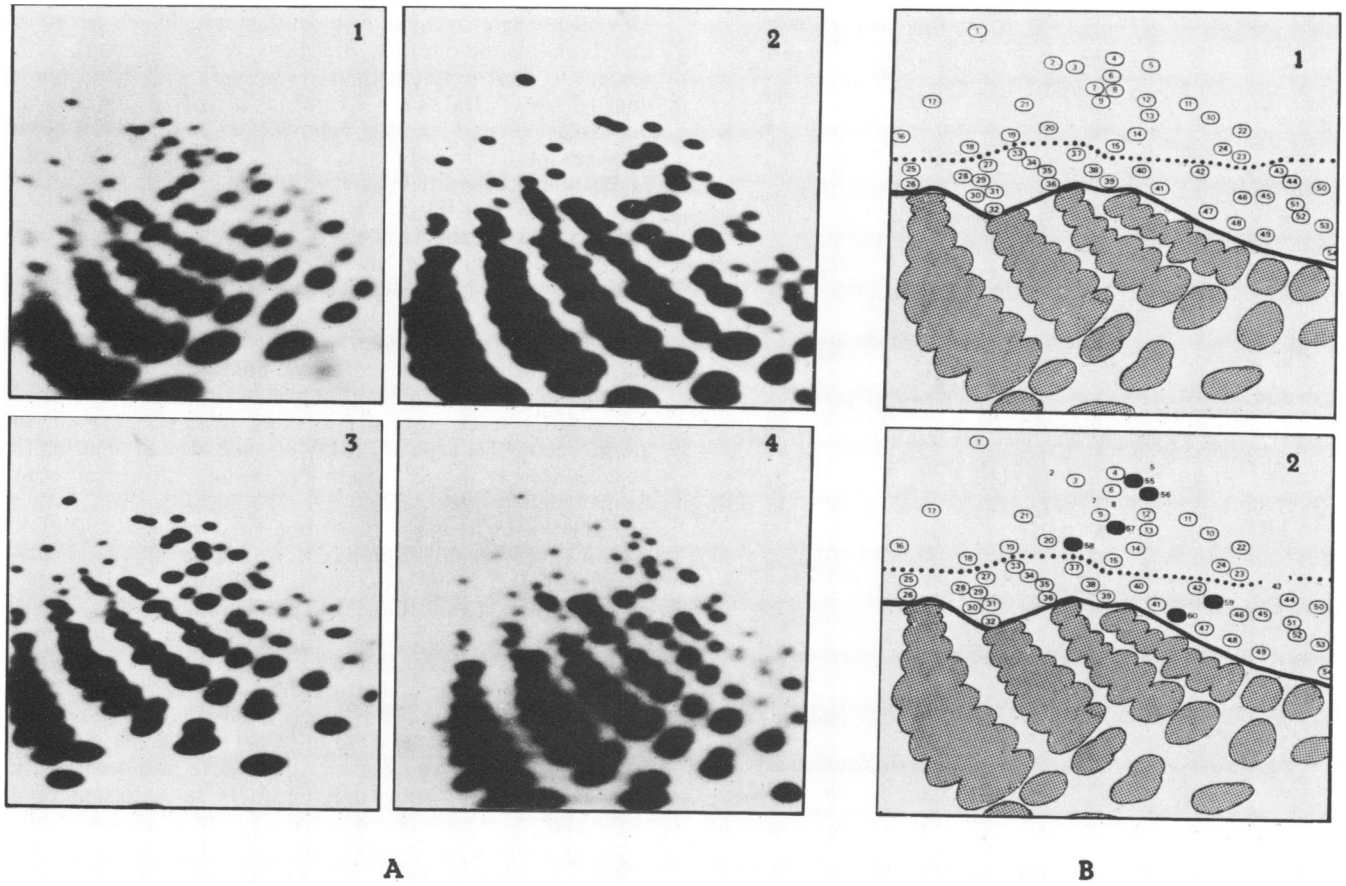

FIG. 5. RNase T_1 two-D maps and comparative drawings of reference strains and field isolates. (A) RNase T_1 two-D maps of the following strains: 1, C_3 RES(SEN); 2, C_3 RES(PAN); 3, field isolate C_3 31296; 4, field isolate C_3 31233. (B) Schematic drawings of the fingerprints of reference virus strain C_3 RES(SEN) (1) and of field isolate C_3 31233 (2). Black spots numbered 55 to 60 represent additional oligonucleotides present in the field isolate that were not present in the reference strain. Uncircled numbers 2, 5, 7, 8, and 43 represent the oligonucleotides of the reference strain that were not present in the field isolate. The black line marks an arbitrary limit between large (upper part) and small (lower part) oligonucleotides. Spots located below this line are not considered suitable. For comparison, the dotted line is an additional division of the larger oligonucleotides which, in this case, was not taken into consideration.

poly(C) tracts were observed, suggesting that the selection could be due to a direct competition between both virus populations present in the original seed virus while replicating in BHK cells.

Besides selection, the poly(C) tract of FMDV could also undergo changes in size. Analysis of the poly(C) fragment of field isolate C_3 31233 (Fig. 6C) on one-D gels clearly illustrates this point. This virus had only one poly(C) tract but of a different size than those constituents of the original C_3 RES(SEN) sample. Analysis of the fingerprint (Fig. 5B) indicates that in this case the modification of the poly(C) tract was also accompanied by detectable modifications in the rest of the genome, suggesting that virus C_3 31233 is a variant of the prototype strain, C_3 RES, probably produced and selected in the field by passages in different susceptible hosts carrying suboptimal levels of neutralizing antibodies. In this sense, another example of changes in the poly(C) tract is shown in Fig. 6D. As can be seen, virus C_3 RES(PAN) had only one poly(C) tract which was longer than the largest one of virus C_3 RES(SEN). However, in this particular case, the fingerprints of both viruses were indistinguishable (compare Fig. 5A and B), indicating that the only detectable change in the genome is restricted to the poly(C) tract, to the flanking sequences of the poly(C) tract, or to both.

DISCUSSION

The results presented in this paper show that the original seed virus of prototype strain C_3 RES(SEN) is composed of two populations of particles which carry poly(C) tracts of different lengths. The fingerprints of both viruses are indistinguishable from each other and from those of the viruses present in the original seed, suggesting that the only detectable changes between both viruses are limited to the poly(C) regions of their genomic RNA, to the flanking sequences of the poly(C) tracts, or to both. For this reason, we have used these viruses as models to study the possible involvement of the poly(C) tract in determining virulence.

Regarding the mechanism(s) by which both types of particles could have originated, it is tempting to think that the viruses carrying the short poly(C) tract were derived from those having the long homopolymer, presumably through a deletion(s) occurring during replication of the virus in different susceptible cells, natural hosts, or both. This mechanism was first proposed by Harris and Brown (9) to explain the shortening of the poly(C) tract of an attenuated SAT-1 type of FMDV. Once originated, the fate of both types of particles would strongly depend on their ability to survive mechanisms of selection during replication. In the case of virus C_3 RES(SEN), at the moment of the study,

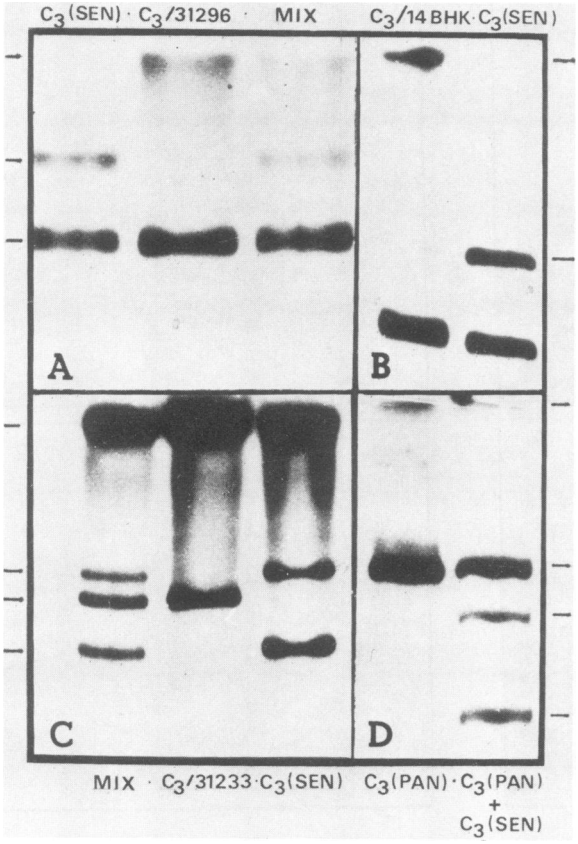

FIG. 6. RNase T_1 one-D maps of FMDV RNA of different C_3 RES viruses showing the region of the gel at which poly(C) tract(s) run. (A) C_3 RES(SEN), Original sample; C_3/31296, field isolate; MIX, coelectrophoresis of both samples. (B) Virus C_3 RES(SEN) passaged 14 times in BHK cells (C_3/14 BHK) and original virus C_3 RES(SEN) [C_3(SEN)]. (C) Virus C_3 RES(SEN) [C_3(SEN)], field isolate C_3 31233, and a coelectrophoresis of both samples (MIX). (D) Virus C_3 RES(PAN) [C_3(PAN)] and a coelectrophoresis of C_3 RES(PAN) and C_3 RES(SEN). Conditions of the run: (C) and (D) were run as described in the legend to Fig. 1A; (B) was run as described in the legend to Fig. 1B. Bars indicate, from top to bottom, origin of the gel and positions of the poly(C) tract(s) of the viruses analyzed.

both poly(C) tracts were present at approximately the same molar ratio. However, after 14 consecutive passages in BHK cells, there was an evident tendency of the particles carrying the long poly(C) tract to disappear. This result suggests that a competitive selection of particles may take place in a mixed population of viruses which replicate simultaneously. This possibility is reinforced by the observation that the cloned virus populations having either the short or the long poly(C) tracts replicated with similar efficiency even after 14 passages in BHK cells.

To investigate whether there is any detectable difference in the biological behaviors of viruses having the short or long poly(C) tract, we have plaque titrated the viruses carrying either the short or the long poly(C) tract in different cell systems. The results indicated that there are not significant differences in the titers of the two viruses in bovine, swine, or hamster cells. However, with respect to plaque size, we have found that only the virus with the long poly(C) tract has the ability to form plaques of large size in addition to small- and medium-size plaques, whereas the virus carrying the short poly(C) tract produces only small- and medium-size plaques. This plaque heterogeneity is in contrast with the observation that both plaque-purified viruses were homogenous with respect to their poly(C) tracts. However, this result is not unusual for FMDV since several plaque-purified strains of this virus elicited similar behavior even after very few passages in tissue culture (P. Sutmöller and P. Augé de Mello, unpublished data). Therefore, it appears difficult to make a direct correlation between plaque size, virulence, and structure of the viral RNA. A similar conclusion was reached by Stohlman et al. with mouse hepatitis virus (14). For that reason, it was decided to test the infectivity of both viruses in cattle. The results demonstrated that viruses carrying either the long or the short poly(C) tract were pathogenic for bovines, as was the mixture of both types.

Regarding the relationship between virulence and size of the poly(C) tract, the size of this homopolymer in an attenuated SAT-1 type of FMDV strain has been reported to be considerably shorter than that present in the original virus from which it was derived (9). We have confirmed that observation in four different attenuated strains of FMDV (Costa Giomi et al., in preparation). However, our results show that, in two of these attenuated strains, the poly(C) tract was only slightly shortened compared with those tracts of the original corresponding virus. Moreover, the extent of the shortening of the poly(C) tract in those four FMDV strains was more dependent on the host in which the virus was attenuated than on the intrinsic degree of attenuation.

Also, in a large-scale survey of FMDV in South America (Bergmann et al., in preparation), we have found strains of the virus with very short poly(C) tracts but with unaltered pathogenicity for cattle (see above). These observations, together with the results of the present work, suggest that for FMDV the changes in the size of the poly(C) tract are not directly related to the virulence of the virus. Nevertheless, the involvement of the poly(C) tract in determining the efficiency of virus replication cannot be ruled out and is at present under investigation in our laboratory.

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