Selection of Antigenic Variants of Foot-and-Mouth Disease Virus in the Absence of Antibodies, as Revealed by an \textit{in situ} Assay

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\textbf{SUMMARY}

Antigenic variants of foot-and-mouth disease virus (FMDV) of serotype C (isolate C-S8cl) were selected upon serial passage of the virus in cell culture in the absence of anti-FMDV antibodies. The variants rose from frequencies of $<10^{-2}$ in the initial plaque-purified FMDV C-S8cl preparation, to 0.1 to 1 in three passaged populations. The proportion of antigenic variants was quantified using a new \textit{in situ} plaque immunotest. A nitrocellulose filter is applied to the agar overlay of a FMDV plaque assay, and allows recovery of infectious virus from individual plaques. A second filter is placed directly on the cell monolayer and binds enough virus to permit colorimetric visualization of plaques by an enzyme-linked assay using monoclonal antibodies (MAbs). Either all or a fraction of plaques from passaged FMDV failed to react with MAb 4G3, an antibody that recognizes an epitope located within residues 144 to 150 of capsid protein VP1. Some variants rapidly dominated the viral population, and others were maintained at low levels. RNA from unreactive viruses included mutations that resulted in amino acid substitutions at the epitope recognized by MAb 4G3. We discuss models for the selection of antigenic variants of FMDV in the absence of antibodies, and implications for the antigenic diversification of RNA viruses.

\textbf{INTRODUCTION}

RNA viruses are genetically heterogeneous in that each population consists of a distribution of related, non-identical genomes, rather than single, defined species (for recent reviews see Domingo & Holland, 1988; Domingo, 1989). Such distributions have been called quasispecies, a term introduced by M. Eigen and colleagues to describe informational molecules in early life (Eigen & Schuster, 1979; Eigen & Biebricher, 1988). The designation quasispecies reflects both the heterogeneity of viral RNA populations and the competitive replication among continuously arising variant genomes (Domingo \textit{et al.}, 1985). For foot-and-mouth disease virus (FMDV), an aphthovirus of the \textit{Picornaviridae} family that causes the economically most important disease of cattle (reviews in Bachrach, 1968; Brown, 1979; Pereira, 1981; Domingo \textit{et al.}, 1989), a quasispecies structure was initially proposed as a result of the analysis of genomic RNA from field isolates (Domingo \textit{et al.}, 1980). Each RNA from closely related viruses had a unique nucleotide sequence, and genetic heterogeneity was detected within a single isolate (Domingo \textit{et al.}, 1980). A subsequent study showed that FMDV variants arise frequently during replication in cell culture, and that in clonal preparations of FMDV types O or C each infectious genome differed from the consensus sequence of the population in a mean of two to eight mutations (Sobrino \textit{et al.}, 1983). At least some of the substitutions fixed in cell culture were biologically relevant, since the passaged virus showed a larger yield of infectious particles per cell than the parental virus (Sobrino \textit{et al.}, 1983). Genetic and antigenic heterogeneity have been documented in field isolates of FMDV (King \textit{et al.}, 1981; Rowlands \textit{et al.}, 1983; Piccone \textit{et al.}, 1988; Mateu \textit{et al.}, 1988). The antigenic diversity of the virus limits the geographical area in which a given vaccine can be successfully applied. Seven serotypes (A, O, C, SAT1, SAT2, SAT3 and Asia 1), more than 65 subtypes (Pereira, 1977) and many antigenic variants that often
coclute in a given geographical location (Arrowsmith, 1975, 1977; Sobrino et al., 1986; Martínez et al., 1988; Mateu et al., 1987, 1988) have been described. Such variants may presumably be selected upon infection of partially immune hosts (Hyslop, 1965), but other environmental changes may also lead to selection of viruses with markedly different antigenic properties. This is the case of a variant of FMDV A22 Iraq 24/64 selected upon adaptation of the virus to suspension spinner cultures, described by Bolwell et al. (1989).

We are interested in the mechanisms of antigenic diversification of FMDV. Here we report that serial passage of the three times plaque-purified FMDV C-S8c1 in BHK-21 cell monolayers in the absence of any detectable anti-FMDV antibodies resulted in the selection of antigenic variants. Their proportion has been quantified using a new in situ assay that allows visualization of the reactivity of individual viral plaques with monoclonal antibodies (MAbs). The procedure has been designed to recover infectious virus from preselected plaques. This has permitted identification of the mutations responsible for the antigenic change. We discuss the implications of these observations for the antigenic variation of RNA viruses.

METHODS

Cells, viruses and infections. BHK-21 (Cl-13) cells were cloned by endpoint dilution, and used at passages seven to 20 after cloning. They were grown as monolayers in DMEM (Dulbecco's modification of Eagle's medium) with 5% foetal calf serum (FCS) (Flow Laboratories). FMDV C-S8c1 is a three times plaque-purified derivative of isolate FMDV C1, Santa Pau (Spain, 1970). All these reagents as well as the procedures for infection of cell monolayers in liquid medium and for plaque assays have been described previously (Domingo et al., 1980; Sobrino et al., 1983; de la Torre et al., 1985).

Transfer of FMDV from individual plaques to nitrocellulose filters and recovery of infectivity. Plaque assays were carried out in 55 cm² dishes (Nunc) containing 12 to 15 ml of agar medium overlay per dish, and with a maximum of about 100 plaques per dish. At 30 to 40 h after plating, a dry nitrocellulose filter (Scharlau) was applied onto the agar for 5 to 10 min. FMDV was bound to the nitrocellulose, as expected from the diffusion of virus from the plaque at the monolayer towards the agar surface; the virus remained viable and localized at the agar surface for several hours after plaque formation (Mosley & Enders, 1961). The filters were stored at −70 °C and, for at least 1 month, infectivity was consistently recovered from nitrocellulose portions that had been in contact with the agar at the sites juxtaposed to plaques. The filter was aligned with the corresponding dish, in which the position of the plaques (visible without staining) had been marked. Then, pieces of nitrocellulose cut following the outline of well separated plaques were placed on BHK-21 monolayers; c.p.e. was seen after 12 to 24 h of incubation. Fragments of nitrocellulose that had been in contact with agar in areas where no plaques were present did not produce c.p.e.

In situ reactivity of virus from plaques with MAbs. To transfer virus from individual plaques to nitrocellulose for in situ immunological tests, the agar overlay was removed and a dry nitrocellulose filter was placed for 1 to 2 min directly on the cell monolayer. Then, a few drops of phosphate-buffered saline (PBS) were scattered on the filter to moisten it slightly. After 1 to 2 min, the nitrocellulose was removed and placed on filter paper, the side that had been in contact with the monolayer facing up. The nitrocellulose was then incubated with a 3% bovine serum albumin (BSA) solution in PBS for either 2 h at room temperature or overnight at 4°C. The reaction with one or a mixture of MAbs was as described by Mateu et al. (1987), except that goat anti-mouse antibody coupled to peroxidase was used instead of rabbit anti-mouse serum and Protein A–peroxidase. MAbs 4G3 (IgG1), 7CA11 (IgG1) and 5D6 (IgG1) have been described (Mateu et al., 1987, 1988). The MAb concentration needed to visualize viral plaques with a clear signal-to-background ratio was about 20 μg/ml. Supernatant of hybridoma culture was used; ascitic fluid (without purification of the immunoglobulin fraction) yields a high colour background and is unsuitable for this test. Fig. 1 shows plaques of FMDV C-S8c1 and FMDV R100 [a small-plaque variant recovered from cell cultures persistently infected with FMDV C-S8c1 (de la Torre et al., 1985, 1988)] visualized colorimetrically by the in situ assay. Note the accumulation of viral antigen at the rim of the plaques; the ring appearance probably reflects a high local concentration of reactive antigen in the cells that support active viral replication at the time of transfer to the nitrocellulose.

Nucleotide sequencing. FMDV RNA was prepared as described (Mateu et al., 1987). Genomic RNA was sequenced by primer extension and dideoxynucleotide incorporation by reverse transcriptase using procedures previously described by Sobrino et al. (1986).

RESULTS

Selection of antigenic variants of FMDV in cell culture

A three times plaque-purified FMDV C-S8c1 (Sobrino et al., 1983) was divided into three portions that were serially passaged by infection of BHK-21 cell monolayers, as depicted in Fig. 2.
Fig. 1. *In situ* reaction of viral plaques with MAb 7CA11. After plaque development (35 h post-inoculation), monolayers were transferred to nitrocellulose filters and incubated with a 1:5 dilution of supernatant of hybridoma culture; details are given in Methods. (a) FMDV C-S8cl; (b) FMDV R100, a small-plaque derivative of FMDV C-S8cl (de la Torre et al., 1985).

Fig. 2. Scheme of serial passages and clonal analysis of FMDV C-S8cl. ■, Plaque isolation; ○, uncloned preparation. The horizontal arrows in series 1, 2 and 3 represent the serial infections of about $10^6$ cells with $10^4$ to $10^5$ p.f.u. from the previous infection. Vertical arrows indicate the populations subjected to the *in situ* immunoassay of individual plaques (see Methods).

Table 1. Reactivity of individual viral plaques from the parental FMDV C-S8cl and from passages 2, 10, 20 and 30 with MAbs*

<table>
<thead>
<tr>
<th></th>
<th>Reactive plaques/total plaques analysed</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>C-S8cl p.2† p.10† p.20† p.30†</td>
</tr>
<tr>
<td>MAb 4G3</td>
<td>479/479 22/22 34/34 22/22 2/54 41/41 31/31 0/61 36/41 22/28 0/69 31/45 58/63</td>
</tr>
<tr>
<td>MAb 7CA11</td>
<td>129/129 31/31 38/38 42/42 68/68 32/32 34/34 50/50 44/44 27/27 51/51 58/58 56/56</td>
</tr>
</tbody>
</table>

* The passage history of FMDV C-S8 is shown in Fig. 2. Reactivity of viral plaques with MAbs was assayed with the *in situ* procedure, as described in Methods.
† For each passage, 1, 2 and 3 correspond to the three series, as described in Fig. 2.

Samples from the parental population FMDV C-S8cl as well as from passages 2, 10, 20 and 30 were plated and, when plaques formed, they were analysed by the *in situ* procedure described in Methods. The results of reactivity with MAbs 4G3 and 7CA11 (Table 1) show that virus from all plaques analysed reacted positively with MAb 7CA11. Upon serial passage of the virus, variants unreactive with MAb 4G3 were detected. They gradually displaced the parental particles and became dominant in passage series 1, and were kept at a low level in series 2 and 3 (Table 1). The most immediate interpretation of this result is that the culture medium contained anti-FMDV.

Antigenic variants of FMDV
Fig. 3. In situ colorimetric immunoassays with MAbs 7CA11 (filters a, b) or 4G3 (filters c, d) of virus recovered from plaques 1 (a, c) or 4 (b, d) from population passage 30, series 2 (compare Fig. 2 and Table 3).

Table 2. Thermal inactivation of FMDV C-S8c1 in the presence or absence of FCS*

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Medium</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>11</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>DMEM</td>
<td>7.7 x 10^6</td>
<td>3.3 x 10^4</td>
<td>3.8 x 10^3</td>
<td>4.0 x 10^3</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>DMEM, 0.5% FCS</td>
<td>9.7 x 10^5</td>
<td>2.9 x 10^4</td>
<td>3.6 x 10^3</td>
<td>3.0 x 10^3</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>DMEM, 10% FCS</td>
<td>1.1 x 10^6</td>
<td>3.6 x 10^4</td>
<td>4.0 x 10^3</td>
<td>1.2 x 10^3</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>DMEM, 0.5% BSA</td>
<td>1.2 x 10^6</td>
<td>4.5 x 10^4</td>
<td>3.5 x 10^3</td>
<td>5.5 x 10^2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>22</td>
<td>DMEM</td>
<td>1.3 x 10^6</td>
<td>4.7 x 10^3</td>
<td>5.0 x 10^4</td>
<td>2.7 x 10^4</td>
<td>1.1 x 10^2</td>
</tr>
<tr>
<td></td>
<td>DMEM, 0.5% FCS</td>
<td>1.1 x 10^6</td>
<td>4.1 x 10^3</td>
<td>3.4 x 10^4</td>
<td>2.9 x 10^4</td>
<td>2.1 x 10^2</td>
</tr>
<tr>
<td></td>
<td>DMEM, 10% FCS</td>
<td>1.3 x 10^6</td>
<td>4.6 x 10^3</td>
<td>2.5 x 10^4</td>
<td>3.1 x 10^4</td>
<td>2.1 x 10^2</td>
</tr>
<tr>
<td></td>
<td>DMEM, 0.5% BSA</td>
<td>1.1 x 10^6</td>
<td>4.0 x 10^3</td>
<td>8.0 x 10^4</td>
<td>2.7 x 10^4</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

* Duplicate samples of FMDV C-S8c1 were incubated in the medium and at the temperature shown. At the indicated times, samples were withdrawn and titrated. Values are the mean of the two samples.

antibodies that selectively inactivated FMDV C-S8c1. This was, however, extremely unlikely since the only possible source of antibodies was the FCS. As expected, no neutralizing activity was detected when the virus was incubated with a 20-fold larger amount of FCS than that present in the culture medium during the serial infections (Table 2).

To ascertain that positive or negative in situ plaque reactivity was an inheritable characteristic
Antigenic variants of FMDV

Table 3. Reactivity of virus recovered from individual plaques from population passage 30, series 2*

<table>
<thead>
<tr>
<th>Original plaque</th>
<th>Reactivity with MAb 4G3</th>
<th>Filter</th>
<th>MAb</th>
<th>Reactive plaques/total plaques</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>a</td>
<td>7CA11</td>
<td>24/24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b</td>
<td>4G3</td>
<td>16/16</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>a</td>
<td>7CA11</td>
<td>20/20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b</td>
<td>4G3</td>
<td>15/15</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td>a</td>
<td>7CA11</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b</td>
<td>4G3</td>
<td>0/6</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>a</td>
<td>7CA11</td>
<td>56/56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b</td>
<td>4G3</td>
<td>0/54</td>
</tr>
</tbody>
</table>

* Procedures for recovering virus from plaques 1 to 4 transferred to nitrocellulose and for the in situ immunoassay are detailed in Methods. Filters a, b from plaques 1 and 4 are shown in Fig. 3.

Fig. 4. Nucleotide sequences of VP1 RNA (residues 415 to 450), and deduced amino acid sequences (residues 139 to 150), from the indicated FMDV populations (compare Fig. 2 and 3). Two nucleotides in a given position indicate band heterogeneity in the autoradiograms of the sequencing gels. RNA sequencing was by oligodeoxynucleotide primer extension, as described by Sobrino et al. (1986).

of a viral subpopulation, virus from passage 30, series 2 was further analysed. Virus was rescued from pieces of nitrocellulose cut around the outline of two plaques that were positive with MAb 4G3 and of two that gave no signal with MAb 4G3. Upon replating, each virus produced the expected in situ reaction, identical to that of the respective parental plaques (Table 3 and Fig. 3). We conclude that antigenic variants of FMDV C-S8cl were selected upon serial passage of the virus in monolayer cultures of BHK-21 cells in the absence of detectable anti-FMDV antibodies.

Fixation of amino acid substitutions at the main antigenic site of FMDV C-S8cl

MAbs 4G3 and 7CA11 recognize VP1 amino acids 144 to 156 of FMDV C-S8cl, as shown by reactivity with synthetic peptides (Mateu et al., 1989, and unpublished results). FMDV C-S8cl mutants resistant to neutralization by MAb 4G3 included substitutions at positions 146, 148, 149 or 150 of VP1 (M. G. Mateu and others, unpublished results). To examine whether virus which was unreactive with MAb 4G3 in the in situ immunoassay (Tables 1 and 3) contained substitutions at the above VP1 positions, virus from the uncloned populations at passage 30, series 1, 2, 3 as well as from plaques 1 and 3 from passage 30, series 2 (Table 3) was purified and the genomic RNA was analysed by nucleotide sequencing (Fig. 4). In passage 30, series 1, the substitution Thr(148)→Lys was fixed in the population. Although this change is not represented in our collection of MAb 4G3-resistant mutants, another substitution at this position [Thr(148)→Pro] is represented. No other replacements were detected in the virus from passage 30, series 1 at the epitope recognized by MAb 4G3, suggesting that Thr(148)→Lys is responsible for the negative reactivity of this virus in the in situ assay. No mutations were detected in the uncloned population of passage 30, series 2. However, VP1 of viral plaque 3 from that
population included the substitution Thr(150)→Lys, found also among the MAb 4G3-resistant variants of FMDV C-S8c1. In this clone, a second substitution, Ser(139)→Arg, was found; some MAb SD6-resistant mutants include Ser(139)→Arg as the only substitution in VP1 and they show a positive reaction with MAb 4G3 (M. G. Mateu and others, unpublished results). Thus, this substitution cannot contribute to the decrease of reactivity with MAb 4G3 of virus from plaque 3 of passage 30 series 2 (Table 3 and Fig. 3). As expected, this virus did not react with MAb SD6 in the in situ assay (results not shown). Plaque 1 from the same population reacted with MAb 4G3 and showed no substitutions in the 140–160 VP1 region, except for some heterogeneity at nucleotide A (415) (Fig. 4); the origin of this heterogeneity was not investigated. In uncloned virus from passage 30, series 3, no substitutions other than a small proportion of A at nucleotide residue 449 was detected in this VP1 region (Fig. 4), consistent with the positive reactivity of most of the viruses in that population with MAb 4G3. These results show that clonal populations of FMDV C-S8c1 passaged in cell culture are antigenically heterogeneous and that at least part of the heterogeneity is due to fixation of substitutions at the main antigenic site of VP1.

Equilibrium among antigenic variants

The data in Table 1 were plotted to depict the proportion of unreactive plaques as a function of passage number (Fig. 5). The graph shows that the variant selected in series 1 rapidly dominated the population, whereas the variants in series 2 and 3, after an initial rise, were maintained at a low level in several passages. This is in agreement with the population equilibrium model for RNA genomes (Domingo & Holland, 1988; see Discussion). The number of variants is not restricted to those with alterations in the epitope of MAb 4G3. In several of the populations derived from FMDV C-S8c1 that have been analysed, heterogeneity with regard to the in situ reactivity with MAb SD6 was also noted (results not shown). In particular, the virus from plaque 1 of passage 30, series 1 (compare Table 1) segregated into particles which were reactive or unreactive with MAb SD6; this observation is consistent with the heterogeneity detected at position 139 (Ser/Arg; Fig. 4), a residue that belongs to the epitope recognized by MAb SD6 (Mateu et al., 1989).

In conclusion, multiple antigenic variants of FMDV may coexist in a dynamic equilibrium during propagation of the virus in cell culture. Some variants were maintained at low levels, and could be detected using an in situ method to sample the reactivity of individual viral plaques with antibodies.
DISCUSSION

Serial infections of cell cultures with plaque-purified FMDV lead to genetically heterogeneous viral populations (Sobrino et al., 1983). The results reported here indicate that some of the variant viruses generated include amino acid substitutions at the main antigenic determinant site of FMDV (Strohmaier et al., 1982; Bittle et al., 1982; Pfaff et al., 1982). Some mutants were revealed using a new in situ colorimetric immunoassay that allows the testing of the reactivity of individual plaques with MAbs, as well as recovering infectious virus from preselected plaques. A number of $10^2$ to $10^3$ p.f.u. is readily accessible to sampling by transferring to nitrocellulose filters the plaques formed on two to 20 55 cm$^2$ Petri plates, as detailed in Methods. Antigenic variants can be isolated using non-neutralizing MAbs as screening reagents, since the identification of variants is solely based on the degree of binding to antibody. The procedure should be extendible to other viruses, including the identification of variant foci induced by retroviruses, provided that a sufficiently high concentration of viral antigen is localized on a monolayer to ensure a specific reaction with antibodies. We are presently using this in situ test to evaluate the antigenic heterogeneity of field isolates of FMDV.

MAb-resistant mutants of picornaviruses occur at frequencies of about $10^{-4}$, although values ranging from $10^{-2}$ to $10^{-6}$ have been reported (Prabhakar et al., 1985; Diamond et al., 1985; Sherry et al., 1986; Xie et al., 1987; C. Carrillo, M. A. Martinez & E. Rocha, unpublished results). Thus, the generation of antigenic variants of FMDV was not surprising except for the fact that their proportion increased with passage number (Fig. 5). Which mechanisms may be in operation to endow antigenic variants with a selective advantage in cell culture? One possibility is that the increased fitness was due to mutations elsewhere in the genome, the substitutions fixed at the antigenic determinant being passenger mutations, selectively neutral. The three-dimensional structure of FMDV VP1 residues 137 to 156 to be an exposed, disordered loop (Acharya et al., 1989). The corresponding segment of FMDV C-88c1, where the substitutions have been found (Fig. 4), would be expected to tolerate replacements due to limited structural constraints. In this view, since immunologically active domains of viruses often correspond to exposed loops, they may be prone to accept substitutions due to random mutation events. Genomic fluctuations would be determined by other, unrelated mutations. In such a situation, the occurrence of replacements at antigenic domains would precede exposure to the selective activity of the immune system, that would normally mediate in their fixation. This sequence of events would be to the advantage of viruses such as FMDV that induce potent humoral immune responses in their host organisms. An alternative possibility is that the replacements at the main antigenic site of FMDV C-88c1 were not strictly neutral. They may have subtle effects on other residues of the capsid or they may modify slightly the accessibility of the conserved stretch Arg-Gly-Asp (amino acids 141 to 143 in Fig. 4), thought to interact with the cellular receptor (Fox et al., 1989). The selective advantage of the antigenic variants could result from the prior history of FMDV C-88 in the field. This virus was isolated in an area where FMD is enzootic (Domingo et al., 1980). During its circulation in the field, particles with their antigenic sites modified to cope with host immune responses may have been selected (Martinez et al., 1988). Upon passage in cell culture, evolution towards more optimal sequences would entail modification of those amino acid stretches which varied most in the field. Fixation of such replacements would require many generations if the selective coefficients involved were small. At present we cannot distinguish among those two possibilities, neither of which involves a direct participation of an immune response as the selective agent.

The in situ plaque immunoassay has shown that FMDV variants can be maintained at unequal levels in an evolving population (Fig. 5), as predicted by the population equilibrium model of RNA genomes (Domingo & Holland, 1988). A dynamic equilibrium, with little variation in the relative proportion of variant genomes was previously described for phage Qb, in which a genomic sequence (represented by one T1 oligonucleotide) amounted to about 80% and its variant counterpart to about 20% in a multiply passaged phage population (Domingo et al., 1978). The different fitness exhibited by the antigenic variants (Fig. 5) points to the difficulty of relating mutation rates to the proportion of MAb-resistant mutants in clonal viral


The present results, as well as the previous observations by Bolwell et al. (1989), suggest the interesting possibility that shifts in the population equilibrium of RNA genomes, brought about by any of a number of environmental changes, may lead to antigenic variation with high probability. The FMDV C-S8c1 variants that we have analysed included amino acid replacements at position 139, 148 or 150 of VP1 (Fig. 4), and the absence of binding to the MAbs was paralleled by resistance to neutralization (data not shown). We have no evidence that substitutions at other sites of the viral capsid influenced the epitopes recognized by MAbs 4G3 was paralleled by resistance to neutralization (data not shown). We have no evidence that substitutions at other sites of the viral capsid influenced the epitopes recognized by MAbs 4G3 and SD6 to date included a substitution within the 138–150 VP1 segment (Mateu et al., 1989, and unpublished results; C. Carrillo and others, unpublished results). Furthermore, the effect of naturally occurring substitutions on the interaction of FMDV C-S8c1 virions or isolated VP1 with MAb SD6 was quantitatively mimicked with synthetic peptides that included the relevant replacements (Mateu et al., 1989).

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