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# Epitopes on Foot-and-Mouth Disease Virus Outer Capsid Protein $VP_1$ Involved in Neutralization and Cell Attachment

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**Foot-and-mouth disease virus structural protein $VP_1$ elicits neutralizing and protective antibody and is probably the viral attachment protein which interacts with cellular receptor sites on cultured cells. To study the relationships between epitopes on the molecule related to neutralization and cell attachment, we tested monoclonal antibodies prepared against type $A_{12}$ virus, isolated $A_{12}$ $VP_1$, and a CNBr-generated $A_{12}$ $VP_1$ fragment for neutralization and effect on viral absorption. The antibodies selected for analysis neutralized viral infectivity with varying efficiencies. One group of antibodies caused a high degree of viral aggregation and inhibited the adsorption of virus to cells by 50 to 70%. A second group of antibodies caused little or no viral aggregation but inhibited the adsorption of virus to cells by 80 to 90%. One antibody, which is specific for the intact virion, caused little viral aggregation and had no effect on the binding of virus to specific cellular receptor sites. Thus, at least three antigenic areas on the surface of foot-and-mouth disease virus which were involved in neutralization were demonstrated. One of the antigenic sites appears to have been responsible for interaction with the cellular receptor sites on the surface of susceptible cells.**

The neutralization of virus by antibody is a complex phenomenon which can occur via a number of different mechanisms (38, 39). In the case of picornaviruses, the introduction of monoclonal antibody technology has helped elucidate the mechanisms of viral neutralization (12, 19–21, 32), characterize the epitopes involved in neutralization (22, 41–43), locate those epitopes on various morphogenic structures (11, 13, 19, 23, 31, 40, 50), and type picornavirus strains (16, 18, 30, 43).

Foot-and-mouth disease virus (FMDV) is an aphthovirus in the Picornaviridae family. The viral capsid consists of 60 copies each of four proteins designated $VP_1$, $VP_2$, $VP_3$, and $VP_4$ (1). The surface location and exposure of $VP_1$ on intact FMDV has been determined by protease sensitivity and iodination of intact virus (10, 45, 48).

Although the exact three-dimensional arrangement of $VP_1$ on the virion surface is not known, purified $VP_1$ from FMDV type A is able to elicit neutralizing antibodies and protect animals from virus challenge (2, 3, 34). In addition, the fragments derived from CNBr treatment of isolated $VP_1$, which generates a 13-kilodalton (kd) fragment (residues 55 to 179), or limited trypsin digestion of intact virus, which generates a 16-kd fragment of $VP_1$ (residues 1 to 144) (45), are also immunogenic and induce protective immunity (3, 4, 33). Trypsin treatment of purified type A or O virus, however, results in a loss of both infectivity and the ability of the virus to interact with its cellular receptor site (CRS) on intact cells (1, 7, 10, 14). These data indicate that $VP_1$ acts both as the carrier of major antigenic determinants of FMDV and as the viral attachment protein which reacts with the CRS (14).

That trypsin-treated type A virus could still induce neutralizing antibodies and protective immunity yet could no longer interact with its CRS led us to speculate that there were multiple functional regions on $VP_1$. We have explored this question with a series of seven monoclonal antibodies, all of which neutralize type $A_{12}$ FMDV. These antibodies define at least three neutralizing regions on the viral surface, of which one appears to be involved in cell attachment. A detailed report of the physical epitope mapping of five of these seven antibodies will be presented in a subsequent communication (B. H. Robertson, D. O. Morgan, and D. M. Moore, submitted for publication). Two of the antibodies have been described (44; C. A. Timpone, M.S. thesis, Cornell University, Ithaca, N.Y., 1982).

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## MATERIALS AND METHODS

**Cells and virus.** Foot-and-mouth disease virus type $A_{12}$ strain 119, large-plaque ab variant ($A_{12}$) (17), was produced in 2-liter roller bottle cultures of BHK-21 cells. Virus, labeled with [$^3$H]uridine, was prepared at 5 to 7 h after infection and purified as described previously (10). Unlabeled virus was concentrated by polyethylene glycol precipitation (51) and purified by CsCl density gradient centrifugation (5). The number of virus particles per milliliter was determined as previously described (10). Bovine kidney (BK) cells were derived from frozen stocks prepared from trypsinized calf kidneys and were used at either passage level 4 or 5. A continuous BK cell line, LF-BK (L. M. Swaney, F. K. Bishop, and C. A. House, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, T38, p. 256), was used for plaque assays.

**Hybridomas.** The production and characterization of hybridomas from mice immunized with purified inactivated virus, isolated $VP_1$, or the 13-kd, CNBr-derived $VP_1$ fragment and hybridomas generated by standard techniques will

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TABLE 1. Monoclonal antibodies used in this study

| Antibody | Antigen[a] | Isotype[b] | Reactivity[c] |
|---|---|---|---|
| 2PD11.12.8.1 | Intact virus | IgG2b | V |
| 2FF11.11.4 | Intact virus | IgG3 | V, 12S |
| 7SF3.1.H3 | 13-kd | IgG3 | V, 12S, $VP_1$, 13-kd |
| 6HE4.1.1 | $VP_1$ | IgG3 | V, 12S, $VP_1$, 13-kd |
| 6FF5 | $VP_1$ | ND | V, 12S, $VP_1$, 13-kd |
| 6HC4.1.3 | $VP_1$ | IgG2b | V, 12S, $VP_1$, 13-kd |
| 6EE2.1.2 | $VP_1$ | IgG2a | V, 12S, $VP_1$, 13-kd |

[a] Antigens used to elicit the antibodies are listed. Intact virus is ethyleneamine-inactivated, purified FMDV $A_{12}$ strain 119 ab. $VP_1$ (6), isolated, intact structural protein; 13-kd, isolated CNBr fragment of $VP_1$ spanning the region from amino acid 55 (glutamine) to amino acid 179 (methionine).

[b] Isotype was determined by agar gel diffusion with commercial isotyping sera (Litton Bionetics). IgG, Immunoglobulin G; ND, not determined (see the text).

[c] The reactivity of each antibody with intact virus (V) was determined in solution with *S. aureus* protein A (see Table 4). Reactivity with other antigens was determined by adsorption of antibodies to plastic dishes (Robertson et al., submitted for publication). 12S refers to subunit pentamers of the viral capsid containing $VP_1$, $VP_2$, and $VP_3$.

be described in a separate report (D. O. Morgan, B. H. Robertson, and J. Card, manuscript in preparation).

**Plaque reduction neutralization assay.** Serial twofold dilutions of antibody were made in phosphate-buffered saline (PBS) containing 1% calf serum (CS). To 0.5 ml of each dilution, an equal volume of purified $A_{12}$ virus was added. The virus was diluted in PBS with 1% CS to a titer of ca. $10^3$ PFU/ml before it was mixed with antibody. The virus-antibody mixture was incubated for 2 h at room temperature. Of each mixture, 0.2 ml (ca. 100 PFU) was injected onto 60-mm tissue culture dishes containing ca. $3 \times 10^6$ LF-BK cells per dish and was adsorbed for 1 h at 37°C, after which the inoculum was removed and the cells were washed once with PBS. Triplicate plates were used for each dilution. The cells were overlaid with methylcellulose (25) and incubated for 3 days. Cell sheets were stained with crystal violet-Formalin and the plaques were counted. Neutralization titers and slopes were calculated by the logit-log transformation method (49).

**Viral adsorption studies.** Monoclonal antibodies were diluted in PBS with 1% CS and mixed with an equal volume of [$^3$H]uridine-labeled $A_{12}$ virus diluted 1:135 in the same buffer. Final antibody dilutions are given in the figure legends. The virus-antibody mixture was incubated for 2 h at room temperature. Binding assays were performed on BK cells as previously described (9) with some modifications. To 100 μl of cells at $5 \times 10^7$ cells per ml, in 1.5-ml conical tubes, 5 μl of virus-antibody mixture was added (multiplicity of infection, 800 particles per cell) and allowed to incubate at 4°C. At various times, tubes were removed, and the cells were washed with PBS followed by pelleting in a microfuge (Eppendorf) for 3 s. Cell-associated radioactivity was determined by trichloroacetic acid (TCA) precipitation as previously described (9).

**Isotope.** [5,6-$^3$H]uridine (specific activity, 40 Ci/mmol) was purchased from ICN Corp., Irvine, Calif.

## RESULTS

**Monoclonal antibodies.** The hybridomas secreting monoclonal antibodies were generated during three separate fusions with mice immunized with either purified, inactivated virus (44; Timpone, M.S. Thesis, 1982), isolated $VP_1$ (45), or the 13-kd CNBr cleavage fragment of $VP_1$ (4). A list of these antibodies, including the entire antibody designation for each, and some of their characteristics are provided in Table 1. The first two antibodies, 2PD11.12.8.1 (2PD11) and 2FF11.11.4 (2FF11), were from mice immunized with intact virus. Antibody 2PD11 reacted only with intact virus. It did not react significantly with 12S protein subunits, nor did it react with denatured viral proteins by Western blotting (M. J. Grubman, personal communication). It could, however, immunoprecipitate virus-like structures from cell-free protein-synthesizing systems (24). Antibody 2FF11 reacted with both intact virus and 12S protein subunits (44; Timpone, M.S. thesis, 1982). Antibody 7SF3.1.H3 (7SF3) was from mice immunized with the 13-kd CNBr fragment of $VP_1$, and the remaining four antibodies, 6HE4.1.1 (6HE4), 6FF5, 6HC4.1.3 (6HC4), and 6EE2.1.2 (6EE2), were from mice immunized with $VP_1$ (Morgan et al., manuscript in preparation). These antibodies reacted with intact virus and 12S protein subunits, as well as with $VP_1$ or $VP_1$-derived fragments (Robertson et al., submitted for publication). Each antibody-producing hybridoma was cloned at least twice by limiting dilution. The only exception was the 6FF5-producing hybridoma. Upon cloning these cells, we could not detect antibody-producing clones. In addition, culture supernatant from the original cell culture did not react with any of the mouse isotyping antisera, even though there was indication of antibody-like reaction with virus and its components. All of the antibodies were used as mouse ascites fluids, with the exception of 6FF5 and 6EE2 which were used as culture supernatants from growing hybridoma cells.

**Neutralization of $A_{12}$ FMDV by monoclonal antibodies.** The results of plaque reduction neutralization assays of the monoclonal antibodies with purified $A_{12}$ virus are shown in Table 2. Five of the antibodies neutralized virus with 50% endpoint titers ranging from 4.5 to >6.5 logs of activity for mouse ascites fluids, and 6EE2, which was a hybridoma culture supernatant, neutralized virus with an endpoint titer of 2.3 logs. Antibody 6FF5, also a hybridoma supernatant, had a very low titer in this assay.

The endpoint titer is probably a reflection of the actual amount of antibody present in either the ascites fluid or the hybridoma supernatant. However, analysis of the slopes of the dose-response curves indicates that the antibodies fall into two classes. Antibodies 2FF11, 7SF3, and 6HE4 had slope values ranging from 0.24 to 0.30. In contrast, the slopes for antibodies 2PD11, 6HC4, and 6EE2 were at least twofold higher, indicating a steeper dose response. Although the slope of 6FF5 was even higher, the extremely low titer of this antibody (50% endpoint dilution was ca. 1:15 versus ca. 1:209 for the antibody 6EE2) did not allow for an accurate slope determination.

TABLE 2. Plaque reduction neutralization of monoclonal antibodies[a]

| Antibody | Log 50% endpoint ± SE[b] | Slope[c] |
|---|---|---|
| 2FF11 | 4.64 ± 0.32 | 0.24 |
| 7SF3 | 4.83 ± 0.43 | 0.24 |
| 6HE4 | 4.48 ± 0.24 | 0.30 |
| 2PD11 | 6.68 ± 0.25 | 0.65 |
| 6HC4 | 5.08 ± 0.08 | 0.75 |
| 6EE2 | 2.32 ± 0.10 | 0.58 |
| 6FF5 | 1.17 ± 0.02 | 1.06[d] |

[a] Plaque reduction neutralization assays were performed as described in the text.

[b] Calculated by computer and use of the logit-log transformation (49).

[c] Calculated by the same computer program used to calculate endpoint, and is obtained by plotting plaque number versus antibody dilution.

[d] This may not be an accurate value (see text).

TABLE 3. Neutralization of $^3$H-FMDV by monoclonal antibodies[a]

| Antibody (dilution) | Expt 1 | | Expt 2 | |
|---|---|---|---|---|
| | PFU/ml $\times 10^{-7}$ | % Control | PFU/ml $\times 10^{-7}$ | % Control |
| No-Mo-AF (1:15)[b] | 20.8 | 100 | 15.2 | 100 |
| 7SF3 (1:25) | 5.53 | 27 | ND[c] | |
| 6EE2 (1:2) | 13.2 | 63 | ND | |
| 6HE4 (1:25) | ND | | 3.43 | 23 |
| 6HC4 (1:25) | ND | | 4.55 | 30 |
| 6FF5 | ND | | ND | |
| 2PD11 (1:50) | ND | | 0.735 | 4.8 |
| 2FF11 (1:50) | ND | | 0.291 | 1.9 |

[a] Each antibody was diluted in PBS with 1% CS and mixed with an equal volume of virus diluted 1:135 in the same buffer. The mixture was incubated for 2 h at room temperature. Plaque titer was determined on LF-BK cells with a methylcellulose-HLH overlay (25).

[b] No-Mo-AF, Normal mouse ascites fluid obtained by injecting 180/TG sarcoma cells (46) intraperitoneally into an unimmunized mouse.

[c] ND, Not determined.

In addition, the antibodies could reduce the plaque titer of the radioactive seed used in all of the subsequent assays (Table 3). Antibodies 2PD11 and 2FF11 both reduced the titer by >95%. Antibodies 7SF3, 6HE4, and 6HC4 reduced the titer from 70 to 75%, whereas 6EE2 reduced the titer by only 40%. The latter antibody also had the lowest plaque reduction neutralization titer. With all the antibodies, the reduction in plaque titer was not as dramatic as that of the titers in the plaque reduction assay. This could probably be attributed to the very high particle/PFU ratios in FMDV seeds (47). In the labeled seed used in this study, this ratio was $4.28 \times 10^3$. This seed was diluted to the same level as was used in all subsequent experiments. Therefore, the initial particle titer which was reacted with antibody was ca. $7.7 \times 10^{11}$ particles per ml. In addition, the virus that contained radioactive label probably did not represent a large proportion of the total population. Thus, at the dilutions used, there was a large amount of virus that was not labeled and that also may not have been infectious but could still react with antibody.

**Effect of monoclonal antibodies on viral particle integrity.** Recent studies have indicated that some antipoliovirus monoclonal antibodies cause aggregation of viral particles (12, 32), and, in one case, aggregation has been implicated as the direct cause of viral neutralization (12). FMDV-antibody complexes were analyzed by velocity sucrose gradient sedimentation, and the results (Fig. 1) indicate that the antibodies can again be divided into two general classes. Antibodies 7SF3 (Fig. 1a and b), 6HE4 (Fig. 1c and d), and 2FF11 (Fig.

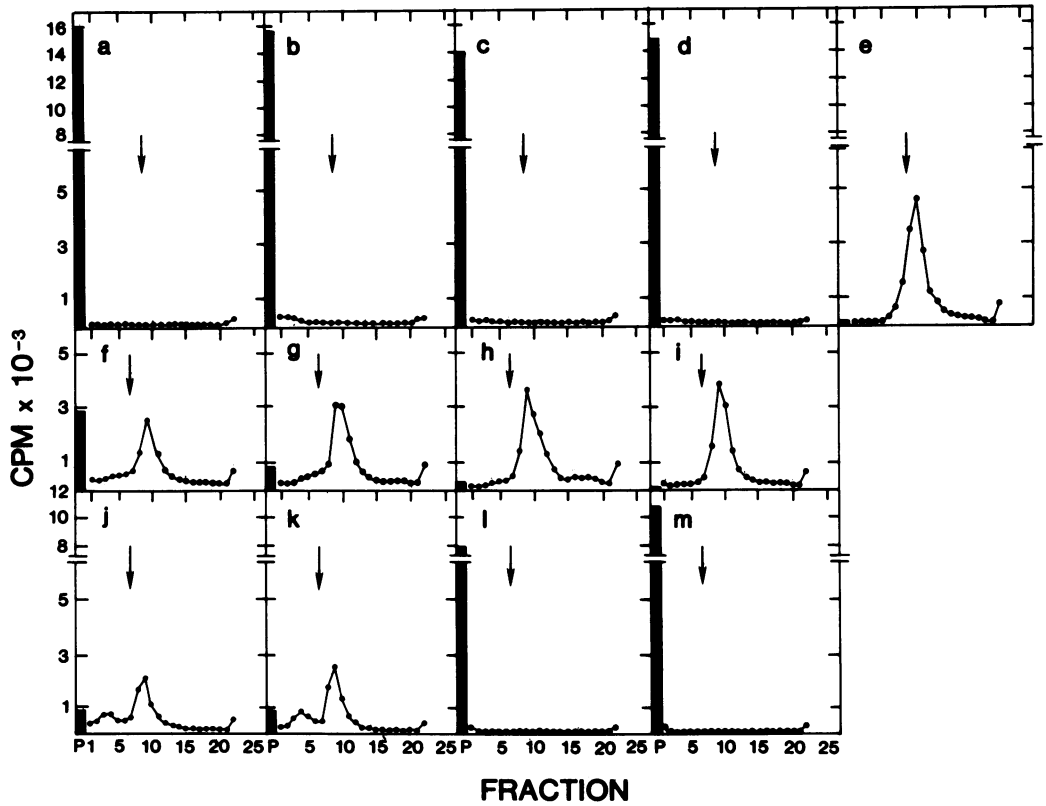


FIG. 1. Sucrose gradient analysis of monoclonal antibody-virus complexes. Monoclonal antibodies were diluted in PBS with 1% CS and mixed with an equal volume of $^3$H-$A_{12}$ strain 119 ab diluted 1:135 in the same buffer. The virus antibody mixtures were incubated for 2 h at room temperature and then layered on a 16 to 30% (wt/wt) sucrose gradient in PBS with 0.1% bovine serum albumin. The gradients were spun at 40,000 rpm for 40 min at 20°C in an SW55 rotor and fractionated into 200-μl fractions, each of which was TCA precipitated. The arrows represent the position of virus incubated with ascitic fluid from an unimmunized mouse (1:25) and centrifuged into a parallel gradient. The bars (P) at the left side of each panel represent the TCA-precipitable counts present in the pellet, which were determined by rinsing the bottom of the tube with 200 μl of 0.15 N NaOH. Centrifugation was from right to left. The antibodies used were 7SF3 at 1:50 (a) and 1:100 (b), 6HE4 at 1:25 (c) and 1:50 (d), 6FF5 at 1:2 (e), 6HC4 at 1:25 (f) and 1:50 (g), 6EE2 at 1:2 (h) and 1:5 (i), 2PD11 at 1:50 (j) and 1:100 (k), and 2FF11 at 1:50 (l) and 1:100 (m).

TABLE 4. Radioimmunoassay of monoclonal antibodies with $^3$H-FMDV[a]

| Antibody | Source | Dilution | Counts per minute bound[b] | % Bound |
|---|---|---|---|---|
| No-Mo-AF | MAF | 1:25 | 756 | 8 |
| PBS | | | 94 | 1 |
| 7SF3 | MAF | 1:25 | 8,831 | 95 |
| | | 1:50 | 8,903 | 96 |
| 6HE4 | MAF | 1:25 | 9,830 | 100 |
| | | 1:50 | 9,635 | 100 |
| 6FF5 | H/SN | 1:2 | 6,150 | 66 |
| 6HC4 | MAF | 1:25 | 10,567 | 100 |
| | | 1:50 | 10,165 | 100 |
| 6EE2 | H/SN | 1:2 | 9,520 | 100 |
| | | 1:5 | 9,150 | 99 |
| 2PD11 | MAF | 1:50 | 10,986 | 100 |
| | | 1:100 | 10,118 | 100 |
| 2FF11 | MAF | 1:50 | 10,017 | 100 |
| | | 1:100 | 8,707 | 94 |

[a] A sample (25 μl) of each monoclonal antibody, diluted in PBS with 1% CS, was mixed with an equal volume of [$^3$H]uridine-labeled $A_{12}$ strain 119 ab diluted 1:135 in the same buffer. The mixture was incubated for 2 h at room temperature at which time 100 μl of a 10% suspension of protein, after A-containing *S. aureus* was added to each tube. The reaction proceeded for an additional 15 min, and the bacteria were washed twice with NET–Nonidet P-40–bovine serum albumin (50 mM Tris-hydrochloride [pH 8.2], 150 mM NaCl, 5 mM EDTA, 0.05% Nonidet P-40, 1 mg of bovine serum albumin per ml). The bound antigen-antibody complex was eluted in 100 mM Tris-hydrochloride (pH 7.2)–150 mM NaCl–2% sodium dodecyl sulfate at 37°C for 15 min. The *S. aureus* suspension was pelleted, and the supernatant was precipitated with TCA. No-Mo-AF, Normal mouse ascites fluid; MAF, mouse ascitic fluid; H/SN, culture supernatant from growing hydridoma cells.

[b] Total counts added to each tube, 9,287 cpm.

1l and m) caused complete viral aggregation since all of the viral radioactivity was recovered in the pellet of the gradients. In contrast, antibodies 6HC4 (Fig. 1f and g), 6EE2 (Fig. 1h and i), 2PD11 (Fig. 1j and k), and 6FF5 (Fig. 1e), although causing some aggregation, essentially left the virus as single virion particles which sedimented slightly more slowly than did virus reacted with ascites fluid from an unimmunized mouse. Antibody 2PD11 also caused some intermediate aggregation as evidenced by the appearance of a small peak at fractions 4 and 5 (Fig. 1j and k). Thus, at neutralizing concentrations, antibodies 7SF3, 6HE4, 6HC4, and 2FF11 caused viral aggregation, whereas antibodies 2PD11, 6EE2, 6HC4, and 6FF5 caused little or no aggregation (Fig. 1). The classification of antibodies by their effects on virion sedimentation rate is identical to their classification by dose-response curve slopes as seen in the previous section.

The antibodies were used at two different dilutions in these experiments, but there was little dose effect observed, indicating that the antibody was probably in excess. The final dilution of the virus was at 1:270. This dilution was chosen so that, in the cell adsorption experiments, the multiplicity of added virus would be ca. 800 particles per cell (see below). The same seed of labeled virus was used throughout this study. At these dilutions, however, with the exception of 6FF5, all of the labeled virus reacted with antibody since all of the counts could be bound to protein A-containing *Staphylococcus aureus* (Table 4). Thus, the lack of aggregation by 6HC4, 6EE2, 2PD11, and 6FF5 was not due to virus which had not reacted with antibody. Furthermore, when the virus peaks from these gradients were incubated with protein A-containing *S. aureus*, the majority of the counts were bound to the bacteria, indicating that antibody was not removed during sedimentation (data not shown).

**Effect of monoclonal antibodies on the adsorption of $A_{12}$ virus to cellular receptor sites.** To determine whether any of the monoclonal antibodies interfered with the interaction of FMDV and the BK cell CRS, we performed binding studies with virus-antibody complexes. The results obtained with the antibodies which caused viral aggregation are shown in Fig. 2. In each case, the results were similar. The antibodies inhibited adsorption of virus to BK cells by 50 to 70%. These results can be contrasted to those presented in Fig. 3, in which identical experiments were done with the antibodies which did not cause viral aggregation. Two of the antibodies, 6HC4 (Fig. 3a) and 6EE2 (Fig. 3b), caused almost total inhibition of viral adsorption. Antibody 2PD11, however, was unable to inhibit viral binding to BK cells (Fig. 3c). Antibody 6FF5 caused a small amount of inhibition. Thus, the two categories of neutralizing antibodies which have been defined can be increased to three as follows: antibodies that aggregate virus, those that do not aggregate but do inhibit viral adsorption, and one antibody that neither aggregates nor inhibits viral adsorption.

**Specificity of adsorption of virus-antibody complexes.** Since some of the antibodies were allowing either partial or total viral adsorption to BK cells, it was important to determine whether those viral-antibody complexes were adsorbing to

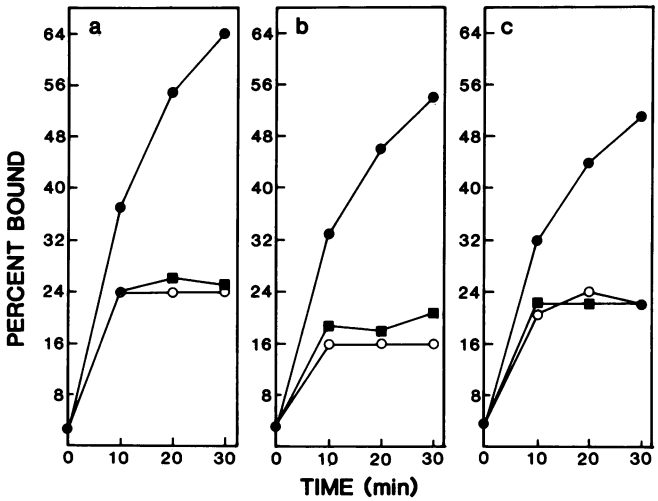


FIG. 2. Adsorption of virus-antibody complexes to BK cells. Monoclonal antibodies were diluted in PBS with 1% CS and mixed with an equal volume of $^3$H-$A_{12}$ strain 119 ab diluted 1:135 in the same buffer. The virus-antibody mixture was incubated for 2 h at room temperature. Binding assays were performed on BK cells. To 100 μl of cells at $5 \times 10^7$ cells per ml, 5 μl of the virus-antibody mixture was added (multiplicity of infection, 800 particles per cell) and allowed to incubate at 4°C. At the time points indicated, tubes were removed, the cells were washed twice with PBS, and TCA precipitates were prepared to determine cell-associated radioactivity. Cell-associated counts are represented as the percentage of counts added to each tube. (a) Control (●) and 7SF3 at 1:25 (○) and 1:50 (■); (b) control (●) and 6HE4 at 1:25 (○) and 1:50 (■); (c) control (●) and 2FF11 at 1:50 (○) and 1:100 (■).

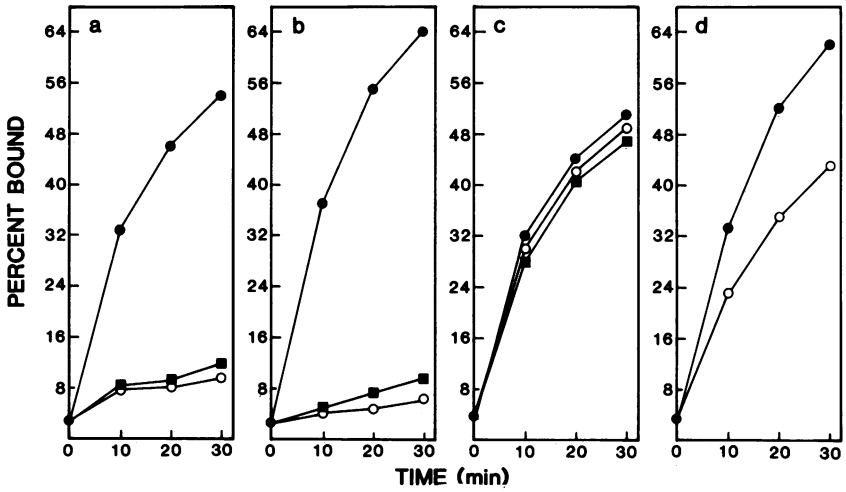


FIG. 3. Adsorption of virus-antibody complexes to BK cells. Conditions for these experiments are identical to those described in the legend to Fig. 2. (A) Control (●) and 6HC4 at 1:25 (○) and 1:50 (■); (b) control (●) and 6EE2 at 1:2 (○) and 1:5 (■); (c) control (●) and 2PD11 at 1:50 (○) and 1:100 (■); (d) control (●) and 6FF5 at 1:2 (○).

specific receptor sites. We have previously shown that there are a finite number of FMDV receptor sites on the surface of susceptible cells and that these sites can be saturated with excess virus (9, 47). The results of competition-adsorption experiments are presented in Fig. 4 for two of the antibodies. Whereas excess unlabeled $A_{12}$ virus almost totally inhibited the adsorption of labeled $A_{12}$ virus, the same amount of unlabeled virus did not inhibit the binding of labeled virus reacted with 7SF3. In fact, adsorption was enhanced. The reason for this phenomenon is not known, but it has also been observed in a poliovirus system (20). Similar results were obtained with 2FF11 and 6HE4 although less enhancement was seen in these two cases (data not shown).

Virus reacted with antibody 2PD11 was able to bind to specific CRS on BK cells (Fig. 4b). The binding of the virus-antibody complex was inhibited >50% by unlabeled $A_{12}$ virus. The small amount of virus which is aggregated by this antibody (Fig. 1j and k) probably is the reason that the inhibition of binding by unlabeled virus is not as large as that in the control. A similar result was obtained for antibody 6FF5 (data not shown).

Thus, antibody-induced viral aggregation results in the failure of virus either to bind to cells or to bind to nonreceptor sites on the cell surface, whereas neutralization by 2PD11 and 6FF5 seems to occur at some event subsequent to adsorption.

## DISCUSSION

The results presented in this report indicate that there are at least three antigenic domains on the surface of $A_{12}$ FMDV that are involved in viral neutralization. Two of these domains are present on isolated $VP_1$ and 12S protein subunits, and one is a conformation-specific site only found on intact virus. The mechanisms of viral neutralization which occur when the monoclonal antibodies react with these domains appear to be different. At neutralizing concentrations, one group of monoclonal antibodies caused extensive viral aggregation, and another group inhibited viral adsorption to BK cells, but one antibody (2PD11) neither aggregated virus nor prevented viral adsorption.

Recent reports suggest that there may be as many as seven poliovirus-neutralizing epitopes (20). At least one of these epitopes might be responsible for reaction with the polio CRS on HeLa cells. In addition, neutralizing poliovirus epitopes have been found on isolated $VP_1$ (11, 50).

Antibody 2PD11, which defines the conformation-specific epitope found only on the intact virus, appears to neutralize

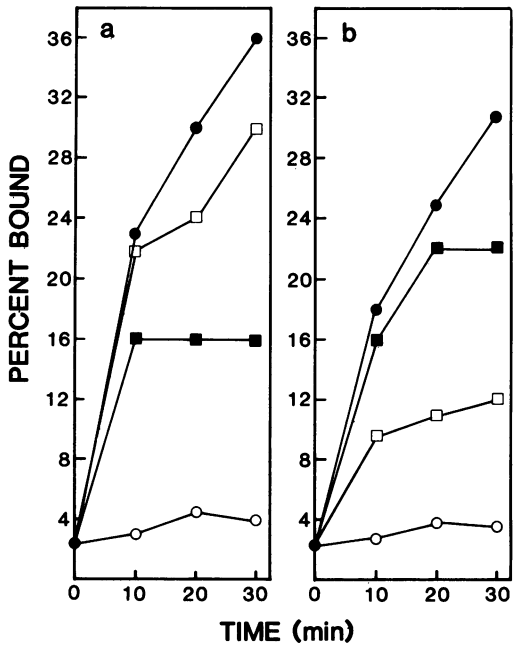


FIG. 4. Specificity of adsorption of virus-antibody complexes to BK cells. Monoclonal antibodies were diluted in PBS with 1% CS, mixed with equal volumes of $^3H$-$A_{12}$ strain 119 ab diluted 1:135 in the same buffer, and incubated for 2 h at room temperature. Unlabeled $A_{12}$ strain 119 ab (multiplicity of infection, $5 \times 10^4$ particles per cell) was adsorbed to 100 μl of BK cells ($5 \times 10^7$ cells per ml) for 1 h at 4°C before the addition of the labeled virus-antibody complex. Cell-associated radioactivity was determined as described in the legend to Fig. 2. (a) Control (●), control in the presence of unlabeled virus (○), 7SF3 at 1:25 (■), and 7SF3 in the presence of unlabeled virus (□); (b) control (●), control in the presence of unlabeled virus (○), 2PD11 at 1:50 (■), and 2PD11 in the presence of unlabeled virus (□).

at some point subsequent to viral adsorption. At an antibody concentration which reduced infectivity by 95%, only minor viral aggregation was noted, and virus-antibody complexes bound to specific receptor sites on BK cells. Mandel (35, 36) had shown previously that polyclonal poliovirus neutralizing antibody inhibits uncoating rather than penetration or attachment and suggested that neutralizing antibody stabilizes the virus in one of two conformational states defined by isoelectric point (37). Recently, Emini et al. (20) reported that six neutralizing monoclonal antibodies, although failing to inhibit viral attachment, alter the isoelectric point of the virion. At least three of these antibodies require bivalent attachment of antibody to virus (21). In contrast, Icenogle et al. (32) reported that a single monoclonal antibody neutralizes virus by cross-linking viral pentamers resulting in an inhibition of uncoating, without changing the electrophoretic profile of the virion. It is possible that 2PD11 may neutralize by one of these two mechanisms.

The epitope which reacts with 2PD11 is not present on 12S protein subunits (Timpone, M.S. thesis, 1982) nor on $VP_1$ (M. J. Grubman, personal communication) and appears to be similar to a number of neutralizing monoclonal antibodies recently described for $O_1$ FMDV (41). In contrast with the $O_1$ monoclonals, however, 2PD11 did not react with trypsinized virus (unpublished observations). This difference, however, might be related to serotype. Although these authors did not study the binding of virus-antibody complexes to cells, they suggested that this epitope is not identical with the cell attachment site on $VP_1$.

Two of the antibodies which neutralize viral infectivity (6HC4 and 6EE2) seem to react with the $A_{12}$ cell attachment site on $VP_1$. Epitope mapping of this site on $VP_1$ places it between amino acid residues 169 and 179 (Robertson et al., submitted for publication). The assignment of the cell attachment site to this region of $VP_1$ seems to satisfy some previous observations on FMDV-cell interactions.

Trypsin cleavage of $VP_1$ on different serotypes or subtypes of intact FMDV results in peptides which have different molecular weights and is probably related to the presence or absence of arginine or lysine residues within the major antigenic variable region (residues 134 to 158). Trypsin treatment of $O_1$ results in a large fragment (residues 1 to 138) and a smaller fragment (residues 155 to 213) which remains virion associated (8, 28, 48). Such treatment of $O_1$ virions did not affect the ability of the virus to induce neutralizing antibody in rabbits, although it did so at a lower level than with intact virus (29). There are conflicting conclusions regarding the effect of trypsinization on the cell attachment region of $O_1$ virions. Barteling et al. (8) have suggested that the cell attachment site for $O_1$ virions resides in the smaller fragment remaining after trypsinization, whereas Strohmaier et al. (48) implicated residues (amino acids 138 to 154) in the region which is not found virion associated after trypsinization. Trypsin treatment of intact $A_{12}$ virus cleaves $VP_1$ at arginine 144, resulting in a 16-kd fragment (3, 45) and a smaller, uncharacterized peptide that appears to remain virion associated (unpublished observations). Such treatment results in the loss of virus cell-binding activity (10), although virions were still able to induce protective immunity in cattle (3). Antibodies 6EE2 and 6HC4, which inhibit the binding of $A_{12}$ virions to the cellular receptor, are unable to react with trypsinized virus (Robertson et al., submitted for publication), indicating that the epitope has been removed, conformationally changed, or made inaccessible to antibody. If the small peptide remaining after trypsinization of $A_{12}$ virions is analogous to the small $O_1$ peptide, then residues 169 to 179 should still be virion associated. If this is the case, we favor the conclusion that, upon trypsinization, the cell receptor region of $A_{12}$ virions has been conformationally changed or made inaccessible to antibody.

Finally, a published comparison of nucleotide and amino acid sequence data from ten different FMDV strains representing three major serotypes detected variable amino acid residues at positions 167 to 173, which are partially located within this epitope (15). This might explain our earlier observations that different serotypes of FMDV adsorb to host cells with different efficiencies (9, 47).

The last functional region is defined by antibodies 7SF3, 6HE4, and 2FF11. These antibodies seem to neutralize by causing massive viral aggregation. This suggests that each antibody is binding two virions. The aggregation results in a partial inhibition of adsorption with the residual adsorption being nonspecific. These antibodies might be analogous to a monoclonal antibody which neutralized poliovirus by a similar mechanism (12).

All of the antibodies which cause FMDV aggregation are of the same isotype (see Table 1). Thus, viral aggregation might be related to the isotype of the antibody. Epitope mapping studies of 7SF3 and 6HE4 on $VP_1$ have placed the epitope between residues 145 and 168 (Robertson et al., submitted for publication). Although the 2FF11-reactive epitope has not been identified, this antibody still reacts with trypsinized virus (unpublished observations) in contrast to 7SF3 and 6HE4 (Robertson et al., submitted for publication). Since the latter two antibodies map very close to the trypsin cleavage point, their epitope might be adjacent to or partially overlapped by the 2FF11 epitope. Whether antibody-induced viral aggregation is isotype or epitope related (or both), however, it still appears to be one mechanism of viral neutralization.

Antibody 6FF5 maps in a region which may overlap the 7SF3 and 6EE2 epitopes (Robertson et al., submitted for publication) and also causes a partial inhibition of virus binding without causing viral aggregation. The residual binding with this antibody, however, is specific.

Of the seven antibodies described, all but one react with both 140S intact virus and 12S protein subunits. One of these cross-reactive antibodies (2FF11) was obtained from mice immunized with intact virus. Both virus-specific and 12S-cross-reactive monoclonal antibodies against $O_1$ FMDV have been described (40) as well as monoclonal antibodies reactive with various poliovirus morphogenic structures (19, 31).

Polyclonal antiserum from animals vaccinated with inactivated FMDV can be fractionated into 140S-specific and 140S- or 12S-cross-reactive components (26). Further work indicated that the cross-reactive antibodies might be sensitizing antibodies that needed antiglobulin for neutralization and that virus reacted with this antibody could bind to a proper receptor site (27). Our results with the cross-reacting monoclonal antibody generated with intact virus (2FF11) indicate that this antibody can neutralize directly without antiglobulin and that the virus-antibody complexes cannot bind to proper receptor sites. It is possible, however, that antibody 2FF11 might not have the properties of the polyclonal antibodies used in the previous study (27).

This study constitutes a comparison of a number of different neutralizing anti-FMDV monoclonal antibodies, and we have attempted to show that the mechanisms of viral neutralization which occur when antibodies of different epitope specificities react with virus are not the same. In this report, we did not measure the antibody-to-virion ratios at

which these events occur. However, we have compared antibodies with approximately the same neutralizing titers and have been able to show differences in the amount of viral aggregation caused by antibody and the effect of antibody on early virus-cell interactions. Antibody 2PD11 had ca. 100-fold-greater plaque reduction-neutralization titer than did most of the other antibodies, yet it caused little viral aggregation and did not interfere with viral adsorption. It has been shown that, for a single antipoliovirus monoclonal antibody, the virion/antibody ratio can affect the extent of viral aggregation (32). It should also be pointed out that different antibodies which react with the same epitopes (i.e., 6EE2 and 6HC4 or 7SF3 and 6HE4) or with epitopes which are closely located (i.e., 2FF11 and 7SF3) all exhibit the same effects on viral aggregation.

In addition, antibody 6EE2 had a 300-fold-lower plaque reduction-neutralization titer than did antibody 6HC4 (Table 2), yet at a 1:5 dilution, 6EE2 inhibited the adsorption of labeled virus to BK cells as well as did 6HC4 at a 1:50 dilution (Fig. 3a and b).

All of the antibodies used in this study were capable of neutralizing in vivo in a mouse protection test (Morgan et al., manuscript in preparation). In addition, one of the antibodies (7SF3), when presented in high concentration, passively protected a pig from FMDV challenge (D. O. Morgan, Conf. Res. Work. Anim. Dis. 1983, p. 211). Thus, the mechanisms of viral neutralization in vitro probably are also operative in vivo and knowledge of the epitopes involved will help determine which types of epitopes would be most advantageous to have in viral vaccines.

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