Neutralization of Foot-and-Mouth Disease Virus. I. Sensitization of the 140S Virion by Antibody Also Reactive with the 12S Protein Subunit

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(Accepted 13 March 1981)

SUMMARY

The in vitro interaction of foot-and-mouth disease virus (FMDV) with an immune serum resulted in a fraction of virus which failed to be neutralized. This inability of antibody to neutralize the entire population of a virus preparation was studied with emphasis on the antigenic specificity of the antibody–virus reaction. Antibody to FMDV recognized multiple antigenic determinants. Immunoadsorbent fractionation of the serum into 12S subunit cross-reactive and 140S virion-specific antibody revealed that these multiple antigenic determinants are factors in determining the neutralizing ability of the antibody. Antibody specific to the infective 140S virion neutralized virus effectively, whereas antibody reactive with both the 140S virion and 12S non-infective component did so ineffectively. Neutralization was independent of viral aggregation, strain, or type heterogeneity, dissociation of the immune complex, heterogeneity of antibody class, or incubation time. The non-neutralized fraction of virus was not due to insufficient antibody in the system, was demonstrated to be complexed with antibody ("sensitized") and could be neutralized with anti-globulin serum. The findings demonstrate the heterogeneity of antibody specificity to FMDV in serum preparations and relate the importance of antibody specificity to the neutralization of virus in vitro.

INTRODUCTION

The interaction of virus, antiviral antibody and host cell is one of the most extensively studied, fundamental and yet controversial phenomenon of viral immunology. The in vitro neutralization reaction ideally results in complete inhibition of viral infectivity. However, non-neutralized fractions resulting from the incomplete neutralization of animal virus populations by homologous antibody have frequently been reported for many viruses, including foot-and-mouth disease virus (FMDV) (Dulbecco et al., 1956; Bradish et al., 1962; Philipson, 1966; Fiala, 1969; Lewenton-Kriss & Mandel, 1972; Mandel, 1976; Rweyemamu et al., 1977; Booth et al., 1978). Non-neutralized fractions have been identified for such a large variety of virus–antiserum–cell systems (Daniels, 1975; Della-Porta & Westaway, 1978) that their existence should perhaps be considered a general characteristic.

The exact mechanism of neutralization, as well as the nature of the non-neutralized fraction, has yet to be elucidated. Properties of each of the three components of the neutralization system (virus, antibody, cell) have been implicated by various studies as the responsible factor in incomplete neutralization. It is generally accepted, however, that the

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non-neutralized fraction of most systems consists of infectious immune complexes (‘sensitized’ virus) (Majer, 1972; Della-Porta & Westaway, 1978). The existence of sensitized virus as the cause of the non-neutralized fraction reflects a heterogeneity of virus–antibody complexes. This heterogeneity has been related to the differences in affinity, the net charge of the antibodies, or both (Svehag, 1968). The present study proposes the hypothesis that antibody specificity is also an important factor. The investigation of a non-neutralized fraction of virus in FMDV–antibody reaction mixtures demonstrated that the fraction was associated only with antibody cross-reactive with virus and the 12S subunit and was absent from antibody–virus mixtures specific for the 140S virion.

METHODS

Viruses. The large-plaque antigenic variant ‘ab’ (Cowan, 1969) of FMDV type A$_{12}$ strain 119 (A$_{12}$) was the principal virus used in this study. The virus was isolated by two successive plaque isolations (Martinsen, 1970). A stock suspension of virus was prepared for neutralization tests by two passages in secondary bovine kidney (2° BK) cells with Hanks’ balanced salt solution containing 10% lactalbumin hydrolysate (HLH) (I.C.N. Pharmaceutical, Cleveland, Ohio, U.S.A.) plus 10% (v/v) foetal bovine serum. Aliquots of virus stock were diluted in 10% (v/v) normal bovine serum and filtered through a 200 nm membrane filter before use. Plaque-isolated virus was propagated for purification by two passages in baby hamster kidney (BHK$_2$) cell cultures in HLH (Polatnick & Bachrach, 1964). In some tests, progeny of virus that escaped neutralization was harvested and tested for neutralization resistance. The virus was obtained by aspirating several isolated plaque zones through the overlay medium, suspended in HLH, and used in a neutralization test.

Type A$_{24}$ of FMDV (A$_{24}$), used in confirming assays, was produced from infected bovine tongue tissue by four passages in BHK cell cultures with HLH as described for A$_{12}$.

Inactivation and purification of virus. The A$_{12}$ BHK$_2$ virus was inactivated with 3 mM-ethylenimine (BEI; Bahnemann, 1975) at 25 °C for 72 h, after which the reaction was stopped by addition of a molar excess of sodium thiosulphate (Brown & Cartwright, 1963). The BEI-inactivated virus was concentrated 100 times by precipitation with polyethylene glycol 6000 (PEG) and resuspended in barbital–glycine buffer containing 0.15 M-NaCl (Cowan et al., 1974). The PEG-concentrated virus was purified by ultracentrifugation into CsCl gradients (Wagner et al., 1970) and dialysed against NaCl solution buffered with tris (TBS; 0.05 M-tris–HCl pH 7.5, 0.3 M-NaCl). Virus concentration was determined spectrophotometrically (Bachrach et al., 1964).

Preparation of antiserum. Antiserum to A$_{12}$ and A$_{24}$ was produced by subcutaneous inoculation of five guinea-pigs (Cowan, 1968) with 10 μg inactivated purified 140S virus antigen emulsified in Freund’s-type incomplete oil adjuvant (Morgan & McKercher, 1977). Sera (GP-a-A$_{12}$, GP-a-A$_{24}$) were collected and pooled at 31 days post-immunization. All sera were heat-inactivated at 56 °C for 30 min.

Production of 12S protein antigen and preparation of 12S immunoadsorbent column. The 12S virus subunit–protein antigen was prepared by acid degradation of purified 140S FMDV antigen (Cowan, 1968) and dialysed against TBS pH 7.5. The capsid polypeptides from the purified 12S antigen and the 140S whole-virus antigen from which it was made were resolved by 12.5% polyacrylamide–SDS gel electrophoresis, stained by Coomassie Brilliant Blue and examined to ensure that the polypeptides were not degraded and, therefore, antigenically changed (Moore & Cowan, 1978).

The 12S protein antigen used in the immunoadsorbent column was dialysed against borate–NaCl buffer pH 8.5 (0.12 M-borate buffer, 0.2 M-NaCl). Fifteen mg of 12S antigen were mixed with 1 g of washed and reswollen cyanogen bromide-activated Sepharose 4B
FMDV sensitized by anti-12S/140S antibody

(Pharmacia) equilibrated with borate-NaCl buffer pH 8.5 (described above) and gently tumbled at 4 °C overnight to allow coupling. The gel was washed twice with the borate-NaCl buffer to remove unreacted 12S subunit antigen and the remaining amino-reactive gel groups were blocked with 0.4 M-tris-HCl buffer pH 8. The column was rinsed with 1 M-sodium thiocyanate (NaSCN) to elute non-covalently bound 12S antigen and reequilibrated with borate-NaCl.

Fractionation of antiserum by immunoadsorbent chromatography. Immune serum (10 ml) in 0.2 M-NaCl was applied to the 12S antigen-Sepharose 4B column (1 g gel, reswollen), which was then rinsed with borate-NaCl buffer. The column was eluted of bound immunoglobulin, first with 1 M-NaSCN in 0.15 M-NaCl, 0.02 M-potassium phosphate (PO₄-NaCl) buffer pH 6.5, and second with 2 M-NaSCN in PO₄-NaCl buffer pH 6.5. Normal guinea-pig serum (NGP; 5%, v/v) was added to the NaSCN fractions as carrier protein. Fractions were compared by immunodiffusion analysis for 140S and 12S antigen cross-reactivity, pooled into 12S antigen-adsorbed and 1 M-NaSCN and 2 M-NaSCN 12S antigen cross-reactive serum eluates and dialysed under negative pressure against TBS pH 7.5. The recovered, dialysed, concentrated serum volumes for the 12S antigen-adsorbed, 1 M-NaSCN and 2 M-NaSCN eluates were 5 ml, 2.5 ml and 2.2 ml respectively.

Solution fractionation of 140S-12S component-reactive serum. The equivalence point of the 12S-anti-12S subunit system was determined by mixing a twofold 12S antigen dilution series (1600 to 50 μg range) with equal volumes of serum and comparing the dilutions by immunodiffusion analysis (Sutmoller & Cowan, 1974). Antibody reactive with the 12S subunit was subsequently batch precipitated with equal volumes (1 ml) of purified 12S antigen at equivalence (400 μg/ml) in TBS and serum. The reaction was carried out at 37 °C for 1 h and at 4 °C for 18 h. The immune precipitate was centrifuged at 12000 g for 20 min at 4 °C and the supernatant fluid aspirated. The 12S subunit-reactive antibody was recovered by dissolving the 12S-anti-12S subunit immune complex with 4 M-NaSCN and then dialysing the preparation with TBS pH 7.5. Concentrations of NaSCN above 3 M were found to degrade the 12S subunit antigen, enabling recovery of free antibody (data not shown).

Immunodiffusion and immunoelectrophoretic analysis. Ouchterlony-type immunodiffusion tests were performed in 1% agar, barbital-glycine buffer with 0.15 M-NaCl (Cowan et al., 1974). Immunoelectrophoretic (IEP) analyses were performed in 1% agar, sodium-barbital buffer (ionic strength 0.03, pH 8.6) according to the method of Hirschfeld (1960). Serum samples (25 μl) were subjected to electrophoresis and purified 140S virus antigen or rabbit-anti-normal guinea-pig (Ra-a-NGP) serum (200 μl) was applied for precipitation.

Cell culture and neutralization tests. The Mengeling and Vaughn foetal porcine kidney cell line (MVPK-1 clone 7) (Dinka et al., 1977) was grown and maintained in Eagle's minimum essential medium (MEM; F-15, Grand Island Biological, Grand Island, N.Y., U.S.A.) containing 1 mM-sodium pyruvate, 5% (v/v) foetal bovine serum, penicillin (100 units/ml) and streptomycin (50 μg/ml). Cell cultures were prepared in four-well (35-mm) plastic tissue culture dishes (Falcon) and incubated at 37 °C in a humidified atmosphere of 2% CO₂ in air.

Neutralizing activities of the antisera were measured in a plaque reduction–neutralization test (PRNT) adapted from McVicar et al. (1974). Serial fivefold dilutions of antisera were prepared in HLH, mixed with equal volumes of stock virus and incubated at 37 °C for 1 h. The virus control was mixed with an equal volume of HLH and treated in the same manner. Cell cultures were inoculated with 0-1 ml of reaction mixture, adsorbed for 1 h at 37 °C, overlaid with 1% methyl cellulose (4000 centipoise) in F-15 and incubated for 48 h at 37 °C in 2% CO₂ in air. The number of plaque-forming units (p.f.u.) was plotted versus antibody dilutions based on original serum volume before fractionation. Neutralizing titres and slopes were calculated by computer use of the logit-log transformation method (Trautman & Harris, 1977).
Secondary neutralization with anti-immunoglobulin G serum. Secondary neutralization was accomplished by the addition of anti-globulin (Majer, 1972). Rabbit-anti-guinea-pig immunoglobulin G (Ra-a-GPIgG) was calibrated for equivalence with NGP serum by radioimmunoprecipitation of 125I-labelled guinea-pig immunoglobulin G (M. M. Hardy & D. M. Moore, unpublished results). Mixtures of virus and antiserum were prepared and incubated in the usual manner except that dilutions of antiserum of 1:250 and higher were supplemented with 1:50 NGP serum to maintain equivalence. After incubation, each mixture was divided, mixed with an equal volume of HLH or 1:5 Ra-a-GPIgG, incubated at 37 °C and assayed for survivors. The virus controls, in 1:50 NGP serum, were also subdivided and mixed with HLH or Ra-a-GPIgG as above.

RESULTS

In vitro neutralization reaction

An example of the type of neutralization response curve obtained by assaying anti-A12 serum with A12 virus in a standard PRNT in this study is shown in Fig. 1, line (a). After an initial reduction in plaque counts with increasing serum concentration, infectivity again increased sharply. At very high serum concentration (<1:50 dilution; \(-\log_{10} = 1.7\)), the infectivity again decreased, approaching zero at 1:2 (\(-\log_{10} = 0.3\)) antiserum dilutions. The apex of this non-neutralized peak fluctuated in different tests between serum dilutions 1:50 and 1:250 (\(-\log_{10} = 2.4\)). Assays were done in duplicate with each point on the response curves representing the mean value. The responses were consistent and reproducible in more than 30 tests performed on different passages of the same cell line. Increasing the virus concentration in the assay failed to alter the shape of the curve. As can be seen from the high standard error and low slope values (insert, Fig. 1) for the response curve, the non-neutralized fraction greatly interfered with an accurate measure of the neutralizing ability of the antiserum. Fig. 1, line (b), represents the computer-predicted response for the 60%, 70% and 80% neutralization endpoints of Fig. 1, line (a), calculated by the logit transformation. Ideally, this line should closely parallel the actual response. Fig. 1, line (b), demonstrates that the empirical application of the endpoint calculation method would be invalid.

Effect of serum fractionation based on specificity on the neutralization of virus

Immunoadsorbent column fractionation

The starting serum and the serum fractions collected from the 12S subunit immunoadsorbent column were compared by immunodiffusion with 12S and 140S purified antigens (Fig. 2). The starting serum formed precipitin lines with both the 12S and 140S antigens, but a specific spur line formed with the 140S virion over the 12S subunit line. The 12S subunit-adsorbed serum was specific for the 140S virion and failed to precipitate 12S antigen (Fig. 2). The antibody eluted with 1 M-NaSCN precipitated both 140S and 12S antigens, but with an apparent 140S virion-specific spur line. The antibody eluted with 2 M-NaSCN also precipitated both 140S and 12S antigens, but with a 12S subunit-specific spur line.

The neutralization characteristics of the starting serum and column-fractionated serum fractions were compared on MVPK cell monolayers by the PRNT method. Neutralization results are shown in Fig. 3. The 12S subunit cross-reactive 1 M-NaSCN eluate displayed a biphasic, non-linear dose-response curve in the area of high antibody concentration which was almost identical to, but somewhat displaced to the left of, the response curve of the starting serum. The 12S subunit cross-reactive 2 M-NaSCN eluate demonstrated a very shallow dose-response curve. The response curve obtained for the 140S virion-specific serum, however, was sigmoidal. Furthermore, computer-calculated 70% and 50% plaque reduction
**FMDV sensitized by anti-12S/140S antibody**

![Graph showing virus neutralization](image)

**Fig. 1.** Neutralization of A12 strain 119 large-plaque 'ab' variant FMDV by guinea-pig antiserum, incubated for 1 h at 37 °C: (a) typical response curve demonstrated by this antiserum; (b) computer-predicted response calculated by logit-log transformation of response (a). The mean virus control value [v.c.(x)] is indicated for response curve (a); 60%, 70% and 80% = computer-calculated plaque reduction endpoints.

![Immunoassay image](image)

**Fig. 2.** Effect of 12S subunit immunoadsorbent column fractionation on specificity of antiserum to A12 strain 119 large-plaque 'ab' variant FMDV. Immunodiffusion analysis of the starting serum (ser), 12S subunit column-adsorbed (12S ADS) and 1 M- and 2 M-NaSCN eluate (EL) fraction pools for 140S and 12S antigen reactivity, are shown.

Endpoints exactly superimposed on the experimental line (Fig. 3). The 140S virion-specific serum pool demonstrated calculated slopes ranging from 0.75 to 1.1; the slope values calculated for the 12S cross-reactive fractions and starting serum were low, ranging from 0.09 to 0.34.
The starting serum and all three fractionated pools completely neutralized the virus at high antiserum concentrations (≤1:2 dilution). However, because of the concentration of the fractionated samples during dialysis, the relative volume of serum added to virus was greater than that possible for unconcentrated serum. The equivalent of undiluted 1 M- and 2 M-NaSCN eluate serum pools demonstrated a non-neutralized infectivity of 0.5 to 1% when allowed to react with virus. This non-neutralized fraction was not seen with the virion-specific pool. The non-neutralized peak of the starting serum was associated only with the 12S subunit-reactive antibody after fractionation.

The starting serum and the 1 M-NaSCN eluate 12S subunit-reactive pool occasionally markedly enhanced infectivity in the non-neutralized peak region of the neutralization response. Also noticeable was a decrease in plaque size in very high serum concentrations (≤1:50 dilution) of the starting serum and 1 M- and 2 M-NaSCN eluate pools. The 140S virion-specific serum pool produced no such plaque-size reduction.

**Solution fractionation**

Immunodiffusion tests on the serum fractions attained by solution fractionation substantiated that the 12S subunit cross-reactive and 140S virion-specific serum pools had the same specificity as the corresponding column-fractionated pools.

When the solution-fractionated pools were tested for neutralizing ability, the aberrant neutralization response was again absent with the 140S virion-specific pool and present with the 12S cross-reactive fraction (data not shown). The column-fractionated serum, therefore, was used in all subsequent tests in this investigation.

**Viral aggregation and viral type or antibody class heterogeneity as factors influencing the neutralization response**

Previous investigations (Wallis & Melnick, 1967) have proposed that the persistence of infectivity in reactions with low dilutions of serum could be due to the presence of large
aggregates of virus that, when used in a neutralization test, become complexed with antibody only on the periphery and dissociate when inoculated on to the cell culture surfaces. This hypothesis was tested by filtering the virus stock before reaction with antibody to remove large aggregates. The filtration had no effect on the shape of the neutralization response curve, indicating that aggregation was not responsible for the non-neutralized fraction. Because of the possible interference from aggregation, however, all data presented in this investigation were obtained with prefiltered virus preparations.

Although the virus stock was obtained by plaque isolation, the appearance of a neutralization-resistant population of virus suggested the possible existence of virus heterogeneity. However, a PRNT with non-neutralized peak-derived virus progeny and the fractionated serum pools demonstrated the same general trend as with the parent population (i.e. the non-neutralized peak was associated solely with 12S subunit-reactive antibody) (data not shown).

Characterization by IEP analysis of the antibody classes present in the four serum fractions indicated that only the immunoglobulin G (IgG) fraction reacted with the 140S virus antigen (Fig. 4). The presence of serum proteins other than IgG in the 1 M- and 2 M-NaSCN 12S-reactive eluates is due to the addition of NGP serum as carrier which
Fig. 5. Primary (---) and secondary (----) neutralization of A₁₂ strain 119 large-plaque 'ab' variant FMDV by: (a) starting serum; (b) 140S virion-specific fraction; (c) 1 M-NaSCN 12S subunit cross-reactive eluate; and (d) 2 M-NaSCN 12S subunit cross-reactive eluate. Secondary neutralization of the primary reaction was accomplished by the addition of rabbit-anti-guinea-pig IgG (Ra-a-GPIgG). v.c. (x) = mean virus control value.

permitted the relative comparison of serum immunoglobulin classes present in the purified immune serum fractions. The heavy Ra-a-NGP precipitin lines appearing in Fig. 4(a) and 4(b) are due to an overload of anti-A₁₂ serum in the centre well which was necessary to produce a virus precipitin line. The overload of 2 M-NaSCN eluate antibody produced only a light precipitin line upon reaction with 140S antigen which is barely visible in Fig. 4(d).

**Dependence of neutralization on reaction time**

Comparison of the neutralization responses of the starting and fractionated pools after incubation for 1 h (37 °C) and 18 h (4 °C) intervals showed that although the height of the non-neutralized peak was slightly diminished by the increased reaction time, the shape of the response was not greatly altered (data not shown). The neutralization response of the 140S virion-specific antibody fraction was completely unaffected.

**Demonstration of infectious immune complexes by secondary neutralization**

Most non-neutralized fractions found in virus populations have been attributed to the presence of virus that has reacted with specific antibody (is sensitized) but has not been neutralized by that antibody (Daniels, 1975; Della-Porta & Westaway, 1978). Whether such infectious immune complexes were present and whether their presence affected the shape of the dose-response curve was studied by exposing the primary neutralization mixture to Ra-a-GPIgG for secondary neutralization. The similarity of response of the two virus control means indicated that the virus infectivity was not affected by the anti-globulin (Fig. 5). However, secondary neutralization markedly altered the shape of the three aberrant dose-response curves and enhanced the antibody neutralizing titre for all four serum samples. The responses of the primary reaction mixtures remained unchanged from previous results.
Table 1. Primary and secondary* 70% plaque reduction log neutralization titres† for anti-\textit{A}_{12} strain 119 large-plaque 'ab' variant FMDV starting serum and 12S subunit column-fractionated serum pools

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<th>Primary neutralization</th>
<th>Secondary neutralization</th>
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<td></td>
<td>Titre</td>
<td>Standard error</td>
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<tr>
<td>Starting serum</td>
<td>1.45</td>
<td>1.40</td>
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<tr>
<td>140S virion-specific fraction</td>
<td>2.33</td>
<td>0.14</td>
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<tr>
<td>1 M-NaSCN eluate 12S-reactive fraction</td>
<td>0.13</td>
<td>9.55</td>
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<tr>
<td>2 M-NaSCN eluate 12S-reactive fraction</td>
<td>0.68</td>
<td>0.74</td>
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* Secondary neutralization of the primary reaction was accomplished by the addition of rabbit-anti-guinea-pig IgG.
† Titres represent those calculated from the responses presented in Fig. 5.

This observed alteration in curve shapes upon second neutralization served to normalize the responses into sigmoid-type curves, allowing the logit calculation of titres with low error and steep dose-response curves (Fig. 5 & Table 1). Enhancement of neutralizing titres by secondary neutralization demonstrated that virus was sensitized with all four serum fractions. The removal of this sensitized virus through secondary neutralization effectively eliminated the non-neutralized peak.

\textit{Non-neutralized fractions with FMDV \textit{A}_{24}}

Several sera to FMDV type \textit{A}_{24} were tested to confirm the occurrence of non-neutralizing aberrant responses with FMDV. A non-neutralized fraction was found and the presence of sensitized \textit{A}_{24} virus was detected, through the use of second neutralization, in a PRNT with a guinea-pig (GP) anti-\textit{A}_{24} hyperimmune (infected) serum, a GP anti-\textit{A}_{24} 140S purified antigen immune serum, and a GP anti-\textit{A}_{24} 12S purified antigen immune serum. Filtration of the \textit{A}_{24} strain virus stock also did not alter the responses.

\textbf{DISCUSSION}

An in-depth study of the neutralization of animal viruses by antibody was first described by Dulbecco \textit{et al.} (1956). They found that neutralization was a first-order reaction, the slope of which was proportional to the concentration of the antibody. The theoretical neutralization curve, therefore, as proposed in the study, was sigmoid or occasionally linear. This type of response curve has come to be known as the expected or usual neutralization response. Svehag (1968) contended, however, that the neutralization plot cannot be expected to be curvilinear if the antibody population is heterogeneous in affinity. The results of this study demonstrate that, at least for this system, efficiency of neutralization is directly linked to the specificity of the antibody, and a non-sigmoid neutralization plot to the heterogeneity of antibody specificities. When the 12S subunit cross-reactive (sensitizing) antibody was removed from the serum, leaving only the 140S virion-specific (neutralizing) antibody, the neutralization reaction was sigmoidal, with the amount of neutralization proportional to antibody concentration. FMDV has been demonstrated to have at least three different antigenic sites that stimulate the production of antibodies (Cowan, 1968; Wild \textit{et al.}, 1969; Brown & Smale, 1970). Brown & Smale (1970), in particular, showed these three immunogenic sites to be a 140S virion/12S subunit shared site, a 140S virion-specific trypsin-sensitive site and a 140S virion-specific site that was not trypsin-sensitive. Rowlands
et al. (1971) demonstrated the latter two sites to produce neutralizing antibody, although in different concentrations. The number of different antigenic determinants on the virus particle and whether all of them are critical are, therefore, important factors.

Two fractions of 12S subunit/140S virion cross-reactive antibody separated by column elution were shown to be of differing specificities by immunodiffusion and neutralization, suggesting at least four different antigenic sites on the 140S intact virion. With the 1 m-NaSCN eluate, the 140S antigen-specific spur line that appeared over the 12S antigen line may indicate the presence of antibody that has a greater affinity or complementarity for the 140S antigen than the 12S component, even though it reacted with the 12S subunit-conjugated column. The absorption of 12S subunit reactivity from the serum in solution, its elution, and subsequent neutralizing results, which paralleled those found for the column fractionation, indicated that the solid-phase 12S subunit column had not been distorted or aggregated to mimic intact virus. The presence of neutralizing activity in the 2 m-NaSCN fraction suggests the presence of at least one type of 140S–12S cross-reacting antibody that has higher affinity for 12S subunits and is different from the 140S–12S cross-reactivity of the 1 m-NaSCN antibody.

Since the neutralization response curves for all serum fractions varied in reactivity, ranging from good to very poor neutralization, the possibility that the effect was due to aggregation or heterogeneity of virus assay stock, as proposed by Wallis & Melnick (1967), is doubtful. In addition to this report, prior filtration was reported as incapable of preventing the formation of a non-neutralized fraction by Ashe et al. (1969); Majer & Link (1970), Lewenton-Kriss & Mandel (1972), Booth et al. (1978). The non-neutralized fraction did not represent a resistant subpopulation of virus for the progeny displayed neutralization patterns identical to those of the parental stock virus (Dulbecco et al., 1956; Ashe & Notkins, 1967; Majer & Link, 1970).

The non-neutralized fraction was not a result of insufficient antibody, since the shape of the neutralization curves was unaltered by increasing virus concentration. Excess antibody was shown by the decrease in plaque size at high concentrations of serum (Kjellen & Schlesinger, 1959; Wecker, 1960). Washing of the inoculated cell cultures before application of the overlay allowed normal plaque growth and size. The numbers of plaques remained unchanged, showing that the excess antibody did not affect the response and that the non-neutralized fraction was not caused by dissociated neutralized virus in the overlay. Neutralization results with different host cells showed an absence of the non-neutralized peak (M. M. Hardy & D. M. Moore, unpublished data), further demonstrating that dissociation did not occur. Dissociation of virus–antibody complexes is highly improbable, as shown by the work of Mandel (1961) and other investigators (Dulbecco et al., 1956; Ashe & Notkins, 1967; Svehag, 1968; Radwan & Burger, 1973). This result also demonstrated that the non-neutralized fraction was not caused by dissociation of viral aggregates which may have passed the filter. Further investigations have indicated that the MVPK cells are more sensitive in discriminating between FMDV infectivity and neutralization (M. M. Hardy & D. M. Moore, unpublished data).

It is widely accepted that the non-neutralized fractions of most viruses are not particles that have failed to react with antibodies, but consist of virus–antibody complexes that can be eliminated by addition of anti-globulin (Mandel, 1958; Hahon, 1970; Wallis & Melnick, 1970; Lewenton-Kriss & Mandel, 1972; Majer, 1972; Booth et al., 1978). Rwiyemamu et al. (1977) demonstrated that treatment of FMDV neutralization mixtures with anti-rabbit-IgG enhanced neutralization without a significant alteration in the relationship between virus and attached antibody. In this study, the degree of sensitization of the virus varied for the four serum fractions tested. The 140S virion-specific serum-reacted virus, although it did not display a non-neutralized peak, was sensitized. This finding supports Majer's (1972) conclusions that sensitized virus is not restricted to the non-neutralized fraction and that
sensitization with neutralizing antisera always precedes neutralization. This sensitization is not perceived by the authors as the first step in a two-step stabilization like that favoured by Svehag (1968). In that theory, the initial monovalent neutralizing antibody–virus reaction is reversible (and sensitizing), while a secondary two combining-site antibody reaction is stabilizing (neutralizing). This study does not support this stabilization theory, because increased incubation time had no major effect on the responses. It is more likely that, as in a frequency distribution, there were not enough antibody-reacted critical sites to neutralize these virus particles. Indeed, Trautman & Harris (1977) proposed such a model for the FMDV neutralization response. This sensitization is different from that caused by sensitizing antibody in that it is due to a population or concentration effect, rather than to antigenic specificity.

The 12S subunit cross-reactive serum fractions contained mostly, if not only, sensitizing antibodies. The 2 M-NaSCN 12S subunit cross-reactive fraction demonstrated the least neutralizing ability but the same degree of sensitization (5 times less than the start) as the 1 M-NaSCN 12S subunit cross-reactive antibody in the secondary reaction. This finding would indicate that they contained the same relative concentrations of antiviral antibody. The fact that the 1 M-NaSCN 12S subunit cross-reactive fraction produced a neutralization curve identical in shape to that produced by the starting serum may reflect a mixture of similar antibody specificities. The presence of the 140S virion-specific antibody and cross-reactive antibody with higher affinity for the 140S virion evidently contribute to the shape of the curve because the 2 M-eluate, which contains neither of these, had the shallowest response curve and lowest titre.

A non-neutralized fraction is more evident with immunoglobulin M (IgM), because of its low affinity for and high dissociability with virus (Gard, 1957; Lafferty, 1963). Lewenton-Kriss & Mandel (1972) were unable to isolate a solely sensitizing antibody and found that IgG and IgM both sensitized and neutralized. The results of this study also demonstrate that IgG both neutralized and sensitized. Brown & Smale (1970) showed that 12S subunit does not react with IgM indicating that IgM would not have been present in the 12S-reactive fractions and would not have been an influencing factor in this study.

Sensitization of virus has been found for several other FMDV systems. R. C. Knudsen (Plum Island Animal Disease Center, unpublished data) found a very poor in vitro neutralization response for a GP-adapted anti-A12 convalescent serum with MVPK, BHK and GP tongue explant cultures. Secondary neutralization detected the presence of sensitizing antibody and resulted in neutralization. Non-neutralized aberrant response curves similar to those of the A12 system have been observed for FMDV type O, strain Casaros (B. Gametchu, unpublished data). These aberrant responses were observed with both monolayer and suspension cell-derived virus, with infected and hyperimmunized GP sera, and with infected hyperimmunized bovine sera. When these sera were adsorbed on a Sepharose–12S subunit column, the 12S subunit-adsorbed sera displayed 'normal' responses, while the responses of the 12S subunit-reactive eluate remained atypical (B. Gametchu, unpublished data). Additionally, antisera to an isolated FMDV capsid protein, VP3 (the external virus protein, also called VP1 or VP-threonin), demonstrated sensitization in in vitro and neutralization in in vivo assay. Guinea-pig antiserum to VP3 displayed protracted non-neutralized atypical neutralization response curves. When this serum was 12S–subunit column adsorbed, it was found to contain only 12S subunit cross-reactive and no 140S virion-specific antibody (D. M. Moore, unpublished observations). In general, non-neutralized fractions and sensitized virus have been reported for such a wide variety of viruses that reports of the absence of sensitization are perhaps more unusual.

This report proposes that the 140S virion-specific antibody is directed against, or specific to, the critical site(s) of the virion. This antibody is directly neutralizing in high enough
concentrations to react with or complete the required number of critical sites. The 12S subunit cross-reactive antibody is primarily sensitizing antibody that is unable to neutralize directly, in vitro, because of its specificity for a non-critical site(s). Any neutralization occurring with antibody of this specificity is due to a blocking of critical sites through steric hindrance.

The authors thank Drs Ellen M. Duffy and Gerald Green for their careful review and constructive criticism of this manuscript. The authors also are grateful for the excellent photographic assistance of Mr Tracy Durham (deceased).

REFERENCES


FMDV sensitized by anti-12S/140S antibody


(Received 26 November 1980)