Demonstration of Neutralizing and Non-neutralizing Epitopes on the Trypsin-sensitive Site of Foot-and-Mouth Disease Virus

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SUMMARY

The isolation of monoclonal antibodies directed against the trypsin-sensitive site on the 140S particle of foot-and-mouth disease virus (FMDV) has enabled the demonstration of at least three distinct epitopes within this site. Reaction with two of these resulted in neutralization of virus infectivity. None of the epitopes appeared to be present on the 12S particles, and one of the neutralizing epitopes was sensitive to even milder configurational changes of the particle.

The ability to stimulate neutralizing, i.e. protective, antibody is largely associated with the nucleocapsid (140S particle) of foot-and-mouth disease virus (FMDV). This property is significantly reduced following treatment of the virus with trypsin (Wild & Brown, 1967). The primary immunogenic determinant on the 140S particle appears to be located on the virion polypeptide (VP1) which is the only polypeptide cleaved by trypsin in situ (Wild et al., 1969) and confined to one or two small tracts containing no more than about 20 amino acid residues (Bachrach et al., 1982; Bittle et al., 1982; Strohmaier et al., 1982). However, information on the nature of the antigenic structures formed by these peptides on their own and perhaps in association with other structures is lacking. For example, although VP1 remains intact in the 12S virion subunit, this particle is far less efficient at provoking neutralizing antibody than the 140S particle (Brown & Newman, 1963; Cartwright et al., 1982). We have therefore analysed the antigenic structure of the trypsin-sensitive site of FMDV using monoclonal antibodies as a probe.

Monoclonal and hybridoma cell lines secreting antibody of the IgG immunoglobulin class to virus strain O1 BFS 1860 were isolated from both mouse (using P3-NS1/Ag4-1 myeloma cells and BALB/c mice) and rat (using the Y3 Ag 1.2.3 myeloma line described by Galfre et al., 1979 and Lou F strain rats) fusions. For the preparation of hybridoma cell lines, sucrose-purified 140S antigen either inactivated with acetylethyleneimine or not inactivated was emulsified in Freund's incomplete adjuvant and inoculated intraperitoneally at 28-day intervals. The second or third injection was given without adjuvant, intravenously 3 to 4 days before the spleens were collected. The techniques for the fusion of myeloma cells with spleen cells and the subsequent isolation and cultivation of antibody-secreting cell lines were essentially those described by McMaster & Williams (1979). Both the indirect sandwich enzyme-linked immunosorbent assay (ELISA) (Ouldridge et al., 1982) and neutralization tests (Booth et al., 1978) were used to detect anti-FMDV-secreting cell lines. Briefly, for the indirect sandwich ELISA, rabbit anti-FMDV IgG was absorbed to flexible PVC microtitre plates; this capture antibody was then used to trap purified 140S antigen. Tissue culture supernatants were then reacted with this antigen and the reaction detected with anti-mouse or -rat IgG antibody linked to horseradish peroxidase. For the detection of neutralizing antibody, serial twofold dilutions of test antibody were mixed with 100 tissue culture infective doses (TCID₅₀) of virus, incubated for 1 h at room temperature and the residual infectivity detected in BHK cell monolayers. The titre or SN₅₀ of the sample was defined as that dilution of serum which neutralizes 50% of virus infectivity.
Two hybridoma cell lines secreting neutralizing antibody grew exceptionally slowly, and because difficulty was experienced in cloning these lines, they have been studied as hybridoma cultures. The lines have, however, been secreting high titre antibody of the same specificity in tissue culture for more than 6 months and therefore appear to be stable cell lines.

The antibodies were characterized in the indirect and the indirect sandwich ELISA (Ouldridge et al., 1982) and in neutralization tests (Booth et al., 1978; Skinner, 1953) and by immune electron microscopy. The antibodies in this study were all classified as reactive with the trypsin-sensitive site on the basis of their strong reaction in the indirect sandwich ELISA performed as described above with the purified intact 140S particle and failure to react with purified trypsin-treated 140S particles (Fig. 1, Table 1). One clone (F30.21) showed a slight reaction with the trypsin-treated antigen (Fig. 1 b). The remaining preparations showed no significant reaction (e.g. Fig. 1 a).

The indirect ELISA involved the reaction of antibody with purified antigens absorbed directly to the plate. In this test none of the clones reacted with 12S antigen. Furthermore, one clone (F24.31) had barely detectable reactions with any antigen, even with intact 140S particles, in this test. Clones F10.22.6.4 and F30.21 reacted mainly with the 140S particle but also slightly with the trypsin-treated 140S antigen (Table 1).

Two of the cell lines were capable of neutralizing virus infectivity as measured in the test described above. The other two were screened in a further two tests, the enhanced neutralization test being performed as described above with the addition of anti-IgG before incubation with BHK cells and the mouse index test being performed as described by Skinner (1953). Both of these tests, although probably more sensitive than the first assay, still failed to demonstrate neutralization in either of these two clones.

Immune electron microscopy was performed by mixing purified 140S particles with dilutions of antibody or control antibody preparations on Formvar-coated grids. After incubation at room temperature for 1 h, the grids were washed twice in 0·04M-phosphate buffer and stained with 2%
Table 1. Properties of monoclonal and hybridoma antibodies to the trypsin-sensitive site of O$_1$BFS 1860

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Indirect sandwich ELISA</th>
<th>Indirect ELISA</th>
<th>Neutralization</th>
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<tbody>
<tr>
<td></td>
<td>Trypsin-treated 140S</td>
<td>Trypsin-treated 140S</td>
<td>Heterotypic 140S†</td>
</tr>
<tr>
<td>F10.22.6.4§</td>
<td>4.26 NR</td>
<td>4.38 NR</td>
<td>NR</td>
</tr>
<tr>
<td>F10.46.4.5§</td>
<td>4.29 NR</td>
<td>4.93 NR</td>
<td>NR</td>
</tr>
<tr>
<td>F24.31 II</td>
<td>1.86 Trace</td>
<td>1.20 Trace</td>
<td>NR</td>
</tr>
<tr>
<td>F30.21 II</td>
<td>2.19 Trace</td>
<td>2.10 Trace</td>
<td>NR</td>
</tr>
</tbody>
</table>

* The dose–response curves for antibody titrations fit sigmoidal saturation curves as described by Hingley & Ouldridge (1983). $K$ is defined as the antibody dilution giving an absorbance of 50% of the maximum value (see Fig. 1).
† The heterotypic 140S used was prepared from A$_{24}$ Cruzeiro virus.
‡ $\text{SN}_{50}$ is defined as the dilution of serum which neutralizes 50% of virus infectivity.
§ Ascites preparations of monoclonal antibodies from NS1 cell–BALB/c mouse spleen cell fusions.
‖ Tissue culture supernatants from hybridomas. F24.31 from mouse fusion as above; F30.21 from fusion of rat Y3 Ag 1.2.3 cells with spleen cells from Lou F strain rats.
¶ NR, No detectable reaction; ND, not determined.
phosphotungstic acid, pH 7-6. Grids were examined for evidence of complex formation at a magnification of between $\times 20000$ and $\times 40000$ in a Siemens Elmiskope 1A electron microscope. Supernatants from the two neutralizing hybridoma cultures agglutinated 140S particles (Fig. 2, Table 1).

The results presented here indicate that, within the trypsin-sensitive site of FMDV, epitopes exist that provoke both neutralizing and non-neutralizing antibody. The failure of the two non-neutralizing antibodies even to coat 140S particles would seem to indicate that these two antibodies react with an internal epitope of the 140S particle only revealed either by the mildly disruptive conditions used for binding 140S antigen to the solid phase in the indirect ELISA (Ouldridge et al., 1982) or by reaction with the first phase serum in the indirect sandwich ELISA.

At least two different epitopes appeared to be involved in the neutralization of virus infectivity. One is lost when virus is bound to the solid phase in an indirect ELISA. Since antibody to this epitope neutralizes virus infectivity and binds to 140S in the indirect sandwich ELISA, this failure would seem to reflect the conformational nature of the epitope which is altered when the virus is bound to the solid phase. The conformational nature of this site may explain the relatively poor ability in raising neutralizing antibody of isolated VP1 or VP1 in 12S particles when compared with VP1 in 140S particles (Cartwright et al., 1982; Meloen & Briaire, 1980). It is also similar to the situation found in poliovirus where extracted VP1 failed to raise neutralizing antibody under the same conditions used for FMDV VP1 (Meloen et al., 1979), yet an important neutralizing determinant has been demonstrated on poliovirus VP1 using other techniques (Minor et al., 1983).

The remaining epitope appears to be less dependent on conformation and may be at least partially retained after the 140S particle has been cleaved by trypsin, and antibody to this site may be partly representative of the neutralizing antibody raised by the trypsin-cleaved virus.

Fig. 2. Immune electron microscopy with hybridoma antibody F24.31. (a) No agglutination of O1 BFS 1860 140S in the presence of control tissue culture fluid; (b) agglutination of 140S by F24.31. Magnification between $\times 20000$ and $\times 40000$. 

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By contrast, Meloen et al. (1983) have reported the isolation of FMDV-neutralizing monoclonal antibodies which were directed against the trypsin-resistant antigenic determinant(s) on the 140S particle. Some of these may have properties similar to those of F30.21 in our studies. Further definition of the FMDV-neutralizing epitopes would seem to be warranted and may require competition studies with monoclonal antibodies from different sources similar to those described by Breschkin et al. (1981).

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REFERENCES


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