Comparison of the Antibodies Elicited by the Individual Structural Polypeptides of Foot-and-Mouth Disease and Polio Viruses

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SUMMARY

Antibody produced against preparations of VP₁, one of the four structural polypeptides of foot-and-mouth disease virus, neutralized the virus and reacted with both full and empty particles in radioimmunoassays (RIA). Antiserum against VP₂ reacted with artificial empty particles of the virus but not with full particles. In contrast, none of the individual polypeptides of poliovirus produced antisera which neutralized the virus nor reacted with it in RIA. However, antisera produced with VP₁ and VP₂ reacted with artificial empty particles in RIA.

Picornavirus capsids are composed of four major polypeptides and with all the viruses studied only one of them has been shown to be susceptible to chemical modification in situ (Carthew & Martin, 1974; Rowlands et al. 1975; Lonberg-Holm & Butterworth, 1976; Beneke et al. 1977; Lund et al. 1977). It has been suggested that this evidence implies a surface location of the polypeptide. This polypeptide is usually termed VP₁ or α from a nomenclature based on the apparent mol.wt. of the capsid proteins in polyacrylamide gel electrophoresis, VP₁ or α migrating most slowly. More significant evidence of the identity of the capsid polypeptides of different picornaviruses has been derived from their relative positions on the genome (Rekosh, 1972; Sangar et al. 1977). In the presence of high concentrations of urea, the order of migration of polypeptides VP₁ and VP₃ of FMDV is reversed so that VP₃, as defined above, then migrates more quickly than VP₂ and VP₃. To avoid confusion we shall refer to the three larger polypeptides of FMDV as VP₁, VP₂ and VP₃ to make them directly comparable to the poliovirus proteins, even though VP₁ and VP₃ migrate in the reverse order on the high urea gels used to purify them. By using this nomenclature, FMDV VP₁ is equivalent to VP₁ of poliovirus and α of cardiovirus but to VP₃ in the numbering system used by Bachrach et al. (1975) and Kaaden et al. (1977) for FMDV.

Purified preparations of VP₁ from FMDV types O and A and α from Mengovirus have been shown to induce the production of antibodies that can neutralize and precipitate complete virus particles (Laporte et al. 1973; Bachrach et al. 1975; Lund et al. 1977). The other capsid proteins either did not stimulate the production of antibody or produced antibody which precipitated virus but did not neutralize its infectivity.

In view of reports that significant differences may exist between the antigenic structure of the enteroviruses and other picornaviruses (Breindl, 1971; Crowell & Philipson, 1971; Rowlands et al. 1975) we compared the properties of sera produced in guinea pigs against the isolated polypeptides of poliovirus type 1 (Mahoney) and FMDV, type A. The structural proteins of FMDV, purified on sucrose gradients (Rowlands et al. 1975) were separated on 10% polyacrylamide gels using the discontinuous system of Laemmli (1970) in the presence of 0.1% SDS and 8 M-urea (Bachrach et al. 1975). The proteins of purified polio virus were separated on continuous phosphate buffered 10% gels in the presence of 0.1% SDS and 0.5 M-urea (Burroughs et al. 1971). The gels were stained with Coomassie blue and the clearly resolved stained bands were cut from the gels, fragmented and dried at 40 °C under
Short communications

Table 1. Serological activity of the sera produced by the isolated proteins of poliovirus type I (Mahoney) and FMDV type A (subtype 10, strain 61)

<table>
<thead>
<tr>
<th>Antiserum produced against</th>
<th>Neutralization test*</th>
<th>Virus</th>
<th>Artificial empty particles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum dilution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poliovirus VP₁</td>
<td>≥3</td>
<td>1500</td>
<td>420  $</td>
</tr>
<tr>
<td>VP₂</td>
<td>≥3</td>
<td>950</td>
<td>400</td>
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<tr>
<td>VP₃</td>
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</tr>
<tr>
<td>VP₄</td>
<td>≥3</td>
<td>950</td>
<td>400</td>
</tr>
<tr>
<td>Virus</td>
<td>≥3</td>
<td>1500</td>
<td>420</td>
</tr>
<tr>
<td>FMDV VP₁</td>
<td>≥3</td>
<td>1500</td>
<td>420</td>
</tr>
<tr>
<td>VP₂</td>
<td>≥3</td>
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<td>VP₃</td>
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</tr>
<tr>
<td>VP₄</td>
<td>≥3</td>
<td>1500</td>
<td>420</td>
</tr>
<tr>
<td>Virus</td>
<td>≥3</td>
<td>1500</td>
<td>420</td>
</tr>
<tr>
<td>12S subunit</td>
<td>≥3</td>
<td>1500</td>
<td>420</td>
</tr>
</tbody>
</table>

* Log titre.
† Serum dilution which precipitates 30% of the antigen.
‡ —, No activity in neutralization test and less than 20 in RIA test.
§ Estimate obtained by extrapolation.
|| nd, Not done.

Serological activity of the sera produced by the isolated proteins of poliovirus type I (Mahoney) and FMDV type A (subtype 10, strain 61)

The dried gel fragments were rehydrated in PBS containing 0.1% SDS and kept overnight at 56 °C. An equal volume of complete or incomplete Freund's adjuvant was added and each mixture emulsified before being injected intramuscularly into guinea pigs. Four guinea pigs were used for each mixture and each animal received material from 0.6 mg virus. Positive control sera were prepared by inoculating 0.001 mg/per animal of acetylatedene-imine-inactivated virus, emulsified in the same adjuvant. In the case of FMDV a preparation of the 12S capsid subunit purified on sucrose gradients after acid disruption of the virus was also inoculated into a group of four guinea pigs. Each animal received 0.001 mg of the 12S particle emulsified in Freund's adjuvant. The animals in all the groups were inoculated a second time after 4 weeks. Two weeks later the animals were exsanguinated and the sera separated.

In all the sera the neutralizing activity was determined as described by Rowlands et al. (1975) while the precipitating activity was measured by radioimmune assay (Crowther, 1977) with 35S-labelled virus and artificial empty particles. The empty particles of poliovirus were prepared by heating the virus at 56 °C for 1 h; those of FMDV were obtained by dialysing against 0.01% EDTA in 0.02 M-tris buffer, pH 7.6, for 18 h at 20 °C. The empty particles were purified by sucrose gradient centrifugation.

None of the structural proteins of poliovirus-induced neutralizing antibodies and only one VP₃ antiserum gave a reaction in RIA with complete virus (Table 1). This reaction was rather weak and is of dubious significance. In contrast, complete virus induced both neutralizing and precipitating activity. When the virus was disrupted into empty capsids, both anti-VP₁ and anti-VP₂ sera reacted, indicating a conformational change in the capsid particles which is consistent with the results obtained by other methods (Lonberg-Holm & Butterworth, 1976; Beneke et al. 1977).

The results with FMDV (Table 1) are consistent with those obtained by Laporte et al. (1973), Bachrach et al. (1975) and Kaaden et al. (1977); only one structural protein, VP₁ according to our nomenclature, induced neutralizing antibodies and antibodies precipitating with complete virus. In addition, we have found that artificial empty particles of FMDV not
only react with anti-VP₃ but also with anti VP₂-sera, again indicating a conformational change as reported by Rowlands et al. (1975).

Anti-VP₁ sera of polio virus reacted with the artificial empty particles in RIA showing that antibodies had been induced by this polypeptide. However these sera do not react with complete virus. This is rather puzzling since VP₁ of polio virus appears to be located on the surface of the virion (Lonberg-Holm & Butterworth, 1976; Beneke et al. 1977) as with the other picornaviruses studied so far (Carthew & Martin, 1974; Rowlands et al. 1975; Lund et al. 1977). In contrast, polypeptide VP₁ of FMDV and Mengovirus (Laporte et al. 1973; Bachrach et al. 1975; Lund et al. 1977) induces neutralizing antibodies and antibodies precipitating with the complete virion. Thus polio virus appears to be different from the other picornaviruses in this respect. It remains to be shown, however, whether these reactions are typical of the enterovirus group. Our results do not necessarily support the results of Breindl (1971) who reported that VP₄ is the protein carrying the D reactivity, as we did not observe any antibody in VP₄ sera. It is possible that VP₁ in the polio virion exposes antigens which are lost after denaturation.

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REFERENCES


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