**Immunochemical Studies of Polioviruses: Identification of Immunoreactive Virus Capsid Polypeptides**

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**SUMMARY**

Investigation of the immunological reactions with individual poliovirus capsid polypeptides of antisera and monoclonal antibodies raised against poliovirus type 3 antigens are described. Virus polypeptides were separated by electrophoresis, transferred electrophoretically to nitrocellulose sheets and treated with antibody preparations. Antibody binding specifically to the virus polypeptides was then detected by application of $^{125}$I-labelled anti-immunoglobulin followed by autoradiography. The technique readily enabled the identification of the polypeptides recognized by the antibody. Antibodies present in polyclonal, type-specific neutralizing sera to poliovirus type 3 bound to the two largest capsid polypeptides (VP1 and VP2) of the homotypic poliovirus, and also to the VP1 of poliovirus type 1 and type 2. There was no obvious difference between the antibody binding patterns obtained with neutralizing and non-neutralizing antisera or between C-specific and D-specific antisera. VP1 appeared to be the immunodominant virus polypeptide. Among monoclonal antibodies specific for the C antigen of poliovirus type 3, a proportion reacted homotypically with the VP1 of poliovirus type 3. Other monoclonal antibodies of C antigen or D antigen specificity, or which reacted both with D and C antigens, some of which had potent virus-neutralizing activity, failed to give demonstrable binding reactions. The non-correlation of neutralization and immunoblot reactivity suggests that sequence determinants alone do not mediate virus neutralization which may depend on antigenic determinants specified by complex conformational arrangements of the virus capsid proteins.

The immunochemical characterization of virus proteins may provide useful information about the structural and genetic similarities between strains and types of viruses, and the identity of the antigenic determinants involved in virus neutralization and protection from infection.

There is little precise information concerning the antigenic determinants of polioviruses and their involvement in virus structure and function. Polioviruses have been classified into three serotypes (1, 2 and 3) according to the ability of type-specific antisera to neutralize infectivity. The virus exists in two different antigenic forms: the D antigen, which is associated with the infectious particles, and the C antigen, which is associated with non-infectious particles (Mayer et al., 1957; Le Bouvier et al., 1957; Roizman et al., 1959; Minor et al., 1980). As infectivity is associated with D antigen particles, it has been assumed that this immunogen is largely responsible for the production of neutralizing antibodies. Most immunochemical work with polioviruses, as well as with other picornaviruses, has been carried out with intact virus particles in which the capsid proteins are arranged in complex structural configurations, so that the immunological and antigenic properties of individual virus proteins could not be shown.

For foot-and-mouth disease virus it has been possible to establish that immunization with the trypsin-sensitive protein (VP1) induces neutralizing antibodies (Meloen et al., 1979). However, these authors show that, following a similar protocol with purified poliovirus, VP1 failed to produce neutralizing antibodies.

To investigate the relationship between poliovirus proteins and virus neutralization by antibodies, we have used immune sera to poliovirus type 3 antigens (Minor et al., 1980; Schild et al., 1980) and monoclonal antibodies, some of which showed neutralizing activity, and looked for their reactions with virus coat proteins separated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and then transferred to nitrocellulose sheets. An antibody–antigen reaction was
then carried out on the ‘blot’ and antigen-bound antibody visualized by incubation with $^{125}$I-labelled anti-immunoglobulin, followed by autoradiography.

Poliovirus was grown in Hep2 cells and the 155S infectious virus (D antigen) purified on sucrose gradients as previously described (Minor et al., 1980). Virus was either labelled with $[^{35}S]$methionine or unlabelled, in which case the 155S peak fraction was taken using a parallel gradient of $^{35}$S-labelled virus as a guide. Where necessary, the virus was pelleted for 3 h at 120,000 g. The poliovirus strains used were Mahoney (type 1), MEF (type 2) and 3/10/57 (type 3).

SDS–PAGE was carried out using 15% (w/v) polyacrylamide slab gels and the buffer system of Laemmli (1970). Virus was heated at 100 °C in 2% (w/v) SDS, 2% (w/v) 2-mercaptoethanol and 2 M-urea for 1 min before electrophoresis. Electrophoretic transfer of proteins from SDS–polyacrylamide gels to nitrocellulose sheets (0.45 μm pore size) was carried out using an Electroblot E.C.215 system (E.C. Apparatus Corp., St Petersburg, Fla., U.S.A.) essentially as described by Towbin et al. (1979). A transfer time of 2 h was used at a potential difference of 36 V.

Antibody reaction with C or D antigen was assayed by an antigen-blocking test (G. C. Schild, unpublished results), a modification of the autoradiographic method described by Schild et al. (1980). Purified radioactive D or C antigen prepared as described were incubated with 10-fold serial dilutions of serum for 1 h at room temperature and then added to wells of single-radial-diffusion plates containing low concentrations of homotypic immune poliovirus serum. Zones were detected autoradiographically and the antigen-blocking titre of the serum defined as that dilution of serum reducing the zone diameter to approx. 50% of that produced by the antigen incubated with PBS alone.

Antisera were produced to type 3 poliovirus antigens as described previously (Schild et al., 1980). Rat and mouse monoclonal antibodies were prepared against type 3 poliovirus essentially as described by Köhler & Milstein (1975) (M. Spitz et al., unpublished results; Ferguson et al., 1982).

The reactions of the antisera and monoclonal antibodies in the ‘blotting’ technique, virus neutralization and antigen-blocking tests are shown in Table 1. Rabbit antiserum against unfractionated virus (115B) with potent, type 3-specific neutralizing activity contained immunoglobulins which bound strongly to VP1 and VP2 of type 3 virus (Fig. 1 a). Weak binding to VP3 was seen on autoradiographs exposed for long periods (data not shown). The antiserum also recognized VP1 of type 1 and type 2 viruses. In antigen-blocking tests (Table 1) serum 115B reacted to high titres with both D and C antigens of poliovirus 3, confirming that it contains antibody to both infectious virus and empty capsids. Antiserum R50 and R51, from rabbits immunized with C particles of type 3 virus which did not neutralize virus infectivity and were C antigen-specific in antigen-blocking assays, bound strongly to VP1 of type 3 virus, but only very weakly to VP2. Again cross-reactivity was seen with VP1 of poliovirus type 1 and type 2, although binding to VP1 of type 1 virus was very weak (Fig. 1 b). GP35, a D antigen-specific type 3 neutralizing antiserum, bound strongly to VP1 and less strongly to VP2 of type 3 virus, and cross-reacted appreciably with VP1 of type 1 and type 2 viruses (Fig. 1 c).

NIBY25/2-11 and NIBY25/4-4, which were non-neutralizing monoclonal antibodies raised against type 3 virus and were C antigen-specific in antigen-blocking assays, bound only to VP1 of type 3 virus. No binding with the other polypeptides of type 3 virus or to any polypeptide of
Table 1. Characteristics of the antibodies to poliovirus 3 antigens used in the 'blotting' test

<table>
<thead>
<tr>
<th>Antibody preparations</th>
<th>Virus* neutralization titre</th>
<th>Blocking antigen titre with polio-virus 3 antigens</th>
<th>Poliovirus proteins reacting with antibody on the blot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D particles</td>
<td>C particles</td>
</tr>
<tr>
<td>115B</td>
<td>Polyclonal antiviral</td>
<td>100000</td>
<td>30000</td>
</tr>
<tr>
<td>GP35</td>
<td>Polyclonal anti-D</td>
<td>30000</td>
<td>5000</td>
</tr>
<tr>
<td>R51, R50</td>
<td>Polyclonal anti-C</td>
<td>&lt;2</td>
<td>3</td>
</tr>
<tr>
<td>NIBy25/2-11</td>
<td>Monoclonal anti-C</td>
<td>&lt;2</td>
<td>1</td>
</tr>
<tr>
<td>NIBy25/4-4</td>
<td>Monoclonal anti-C</td>
<td>&lt;2</td>
<td>1</td>
</tr>
<tr>
<td>NIBy25/3-10</td>
<td>Monoclonal anti-C</td>
<td>&lt;2</td>
<td>1</td>
</tr>
<tr>
<td>NIBy25/5-16</td>
<td>Monoclonal anti-D</td>
<td>&lt;2</td>
<td>300</td>
</tr>
<tr>
<td>NIBy25/1-14</td>
<td>Monoclonal anti-D + C</td>
<td>10000</td>
<td>3000</td>
</tr>
<tr>
<td>NIBy25/4-12</td>
<td>Monoclonal anti-D + C</td>
<td>5000</td>
<td>100000</td>
</tr>
<tr>
<td>NIBy27/4-4</td>
<td>Monoclonal anti-D + C</td>
<td>14000</td>
<td>500</td>
</tr>
<tr>
<td>NIBy25/5-5</td>
<td>Monoclonal anti-D + C</td>
<td>150</td>
<td>1000</td>
</tr>
</tbody>
</table>

* Against homologous virus.
† Autoradiographic bands were scored in order of increasing intensity from + (weak) to ++++ (very intense).
Fig. 1. Autoradiographs of immunoblots of poliovirus proteins (see text for technical details). (a) Immunoblot processed with rabbit antiserum 115B. Lanes 1, 3 and 5, antibody-labelled proteins; lanes 2, 4 and 6, biosynthetically labelled proteins. Lanes 1 and 2, poliovirus type 3; lanes 3 and 4, poliovirus type 2; lanes 5 and 6, poliovirus type 1. (b) Immunoblot processed with rabbit antiserum R51. Lanes 1, 3 and 5, antibody-labelled proteins; lanes 2, 4 and 6, biosynthetically labelled proteins. Lanes 1 and 2, poliovirus type 3; lanes 3 and 4, poliovirus type 2; lanes 5 and 6, poliovirus type 1. Antiserum R50 produced an identical blot. (c) Immunoblot processed with guinea-pig antiserum GP35. Lanes 1, 3 and 5, biosynthetically labelled proteins; lanes 2, 4 and 6, antibody-labelled proteins. Lanes 1 and 2, poliovirus type 3; lanes 3 and 4, poliovirus type 2; lanes 5 and 6, poliovirus type 1. (d) Immunoblot of poliovirus type 3 processed with rat monoclonal antibody 25/2-11. Lane 1, antibody-labelled protein; lane 2, biosynthetically labelled proteins. No proteins of poliovirus type 1 or type 2 were recognized.
type 1 or type 2 virus was seen, even after prolonged exposure of the blot to X-ray film (Fig. 1d). A second C antigen-specific monoclonal antibody (25/3-10) failed to react in the immunoblotting technique.

Other rat monoclonal antibodies, which were of D antigenic specificity and failed to neutralize virus infectivity (25/5-16), or which were of D + C antigen specificity and possessed virus neutralization activity (25/1-14, 25/4-12, 27/4-4 and 25/5-5), all failed to produce any bands in the blotting test even after 2 weeks exposure of the X-ray films. Similar results were obtained with mouse monoclonal antibodies (Ferguson et al., 1982) prepared against Sabin type 3 poliovirus.

All the polyclonal antisera to poliovirus type 3 examined, whether raised using D or C particles and with or without virus-neutralizing activity, reacted with VP1 of all three poliovirus serotypes. Thus, it can be concluded that VP1 possesses antigenic determinants common to all three poliovirus types as well as type-specific determinants which are detected by other techniques. Romanova et al. (1981) has also shown intertypic cross-reactivity between poliovirus types by immunoprecipitation with antisera which showed only type-specific virus neutralization.

Monoclonal antibodies NIBy25/2-11 and NIBy25/4-4, which in antigen-blocking tests were poliovirus type 3 C antigen-specific, bound detectably only to VP1 of poliovirus type 3. As neither of these monoclonal antibodies was neutralizing, the type-specific antigenic determinants recognized by these antibodies are unlikely to be responsible singly for virus neutralization. An additional C antigen-specific monoclonal antibody for poliovirus type 3 and all other monoclonal antibodies with D antigen or D + C antigen specificity, including several with virus-neutralizing activity, failed to react in the blotting tests. The failure of these monoclonal antibodies to react in the blotting test with any of the virus polypeptides may be because they are directed against antigenic determinants which are specified by more than a single virus polypeptide or which are determined by tertiary protein structure.

VP1 seems to be immunodominant regardless of the form of the immunizing antigen. A recent report (Blondel et al., 1982) suggests that immunization with VP1 causes the production of neutralizing antibodies and in this respect the immune response to poliovirus VP1 may be similar to that to VP1 of foot-and-mouth disease virus and to VP2 of coxsackievirus (Meloen et al., 1979; Beatrice et al., 1980). Although homotypic antibodies binding to VP2 were detected with hyperimmune sera, no heterotypic cross-reactivity was observed for this protein.

Only one of the hyperimmune sera (115B) bound to VP3, and no evidence for anti-VP4 antibodies was found in any of the antisera tested. The failure to demonstrate any clear difference in virus type or polypeptide specificity between the C- and D-specific polyclonal antisera suggests that the immunochemical reactions observed in this report are not directly correlated with virus neutralization or with the distinction between C and D antigens reported using other techniques (Mayer et al., 1957; Minor et al., 1980).

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REFERENCES


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Short communications


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