A Hybridoma Cell Line Secreting Antibody to Poliovirus Type 3 D-Antigen: Detection in Virus Harvest of Two D-Antigen Populations

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SUMMARY

A mouse hybridoma cell line (Mo56) secreting IgG antibody to poliovirus type 3 D-antigen was obtained by fusion of a mouse myeloma cell line with spleen cells from mice immunized with Saukett virus. The monoclonal antibody was specific for Saukett virus strains in virus-neutralization and single-radial-diffusion tests. In immunoprecipitation tests the monoclonal antibody reacted with intact infectious virus particles (155S) and with a previously undescribed 70S poliovirus particle with D-antigenic reactivity.

Well-characterized monoclonal antibody preparations produced by mouse hybridoma cells have been successfully employed in the study of the antigens of several viruses (for review, see Koprowski, 1980), including influenza (Koprowski et al., 1977; Gerhard & Webster, 1978) and rabies viruses (Wiktor & Koprowski, 1978). These highly specific reagents have been valuable in the study of antigenic variation in the surface antigens of these two groups of viruses, in the detection of individual antigenic specificities of the different structural components of the viruses, and in investigations of the comparative frequencies of antigenic mutants in virus populations (Lubeck et al., 1980; Webster & Laver, 1980; Yewdell et al., 1979).

There is little precise information on the antigenic determinants of picornaviruses and their relationships to structural components of the virions. Monoclonal antibodies are of great potential value for studies in this field and for the precise antigenic identification of virus isolates for epidemiological purposes. The present report describes the derivation and properties of a mouse hybridoma cell line, Mo56, which secretes antibody specific for the D-antigen of the Saukett strain of poliovirus type 3. The studies revealed a previously undescribed, slowly sedimenting D-antigen particle. Saukett virus is of particular interest because of its wide usage in the preparation of inactivated polio vaccines.

Hybridoma cell lines were prepared according to a modification (Koprowski et al., 1977) of the method of Kohler & Milstein (1975) by fusion of an 8-azaguanine-resistant clone of MoPC-21 mouse myeloma cells (P3/X63-Ag8) with spleen cells from immunized Balb/c mice employing polyethylene glycol for cell fusion. Spleens were obtained from mice immunized with purified D-antigen (155S) particles of Saukett virus prepared from infected Hep-2 cell cultures (Minor et al., 1980). The strain of Saukett virus (NIB strain) used was from the collection of this Institute and originally obtained in 1956, via Connaught Laboratories, Canada, from Dr J. Salk. Three separate injections of antigen were given at 30-day intervals: the first subcutaneous 0.1 ml dose in Freund's complete adjuvant was followed by two intraperitoneal injections without adjuvant. The spleens were collected 3 days after the final dose at a time when the mice were shown to have a neutralizing antibody titre of >1:300 to homologous Saukett virus and was also strongly positive by single-radial-diffusion (SRD) tests (Schild et al., 1980). Following fusion, hybrids were selected in medium containing hypoxanthine, aminopterin and thymidine (HAT) (Littlefield, 1964). The culture fluids from wells showing vigorous growth were screened at confluence by solid-phase radioimmunoassay (RIA) using concentrated purified Saukett virus as antigen (Esposito, 1976).
Table 1. Strain specificity of antibody from Mo56 hybridoma cell line in poliovirus neutralization tests and in single-radial-diffusion (SRD) tests

<table>
<thead>
<tr>
<th>Poliovirus type</th>
<th>Strain</th>
<th>Origin</th>
<th>Year of isolation</th>
<th>Virus-neutralization test*</th>
<th>Direct SRD reaction (stained zones)†</th>
<th>Antigen-blocking activity in SRD test‡</th>
<th>Autoradiographic SRD reaction†</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>30</td>
<td>Canada</td>
<td>1952</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
<td>&lt;2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>715</td>
<td>India</td>
<td>1958</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
<td>&lt;2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>374</td>
<td>Japan</td>
<td>1957</td>
<td>&lt;2 &lt;2</td>
<td>ND§</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>Saukett</td>
<td>U.S.A. (NIB)</td>
<td>1950</td>
<td>67 20</td>
<td>+</td>
<td>640</td>
<td>+ II</td>
</tr>
<tr>
<td>3</td>
<td>Saukett</td>
<td>U.S.A. (CDC)</td>
<td>1950</td>
<td>10 10</td>
<td>+</td>
<td>320</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Leon</td>
<td>U.S.A.</td>
<td>1939</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
<td>&lt;2</td>
<td>-</td>
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<tr>
<td>3</td>
<td>77730</td>
<td>U.S.A.</td>
<td>1952</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>3</td>
<td>77750</td>
<td>U.S.A.</td>
<td>1954</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
<td>&lt;2</td>
<td>-</td>
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<tr>
<td>3</td>
<td>1</td>
<td>U.S.A.</td>
<td>1957</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
<td>&lt;2</td>
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<tr>
<td>3</td>
<td>10</td>
<td>U.S.A.</td>
<td>1957</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
<td>&lt;2</td>
<td>-</td>
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<tr>
<td>3</td>
<td>23</td>
<td>U.S.A.</td>
<td>1959</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
<td>&lt;2</td>
<td>-</td>
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<tr>
<td>1</td>
<td>Mahoney</td>
<td>U.S.A.</td>
<td>1941</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
<td>&lt;2</td>
<td>-</td>
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<tr>
<td>1</td>
<td>Sabin</td>
<td>U.S.A.</td>
<td>1956</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
<td>ND</td>
<td>-</td>
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<td>2</td>
<td>MEF</td>
<td>U.S.A.</td>
<td>1942</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
<td>&lt;2</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>5148</td>
<td>U.S.A.</td>
<td>1965</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
<td>ND</td>
<td>-</td>
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<tr>
<td>2</td>
<td>Sabin</td>
<td>U.S.A.</td>
<td>1956</td>
<td>&lt;2 &lt;2</td>
<td>-</td>
<td>ND</td>
<td>-</td>
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</tbody>
</table>

* Reciprocal dilution of ascitic fluid neutralizing 100 TCID<sub>50</sub> poliovirus.
† Obvious reactions (clearly stained zones or distinct autoradiographic zones) in gels containing 10 µl ascitic fluid/ml gel are indicated as +, no reaction -. Control SRD tests were carried out with normal ascitic fluid. The potency of each test antigen was confirmed by SRD reactions with polyclonal sera (see Schild et al., 1980).
‡ Dilution of ascitic fluid blocking all SRD activity of 10<sup>4</sup> to 10<sup>9</sup> TCID<sub>50</sub> of viral antigen.
§ ND, Not done.
‖ Autoradiographic zones shown to correspond to Saukett antigen of D-antigenic character only.
One of 48 wells seeded with ‘fused’ spleen cells from mice immunized with Saukett virus was positive by RIA and cells from this culture were cloned in soft agar. Individual colonies were picked into 24-well Linbro plates and the fluids retested for RIA activity. Clones positive by RIA were ‘expanded’ by growth in 25 cm² culture flasks. Hybridoma line Mo56 was isolated from a single RIA-positive colony after one cycle of cloning. Additional serial cloning of this cell line yielded cells which produced antibodies of characteristics identical to those of the original cultures, providing preliminary evidence of the monoclonal nature of the hybridoma. Detailed studies were carried out on ascitic fluids generated by injecting 10⁷ cells intraperitoneally into pristane-treated mice and the ascitic fluids collected 7 to 15 days later.

Ascitic fluids were tested for virus-neutralization activity against a collection of poliovirus type 3 strains differing in their geographical origin and chronological isolation (Table 1) including homologous Saukett virus (NIB) and a strain of Saukett virus obtained from Dr Olen Kew, Centre for Disease Control, Atlanta, Ga., U.S.A. (CDC strain). Of the strains tested, neutralization was detected only with the NIB and CDC strains of Saukett virus. However, the titres were lower than those typically encountered with immune animal sera to poliovirus 3 strains (see Schild et al., 1980).

Studies of immunodiffusion reactions in gels were done using direct SRD tests (Schild et al., 1980) employing stained reaction zones. Ascitic fluids of Mo56 were incorporated into agarose gels at concentrations ranging from 1 to 30 μl/ml of gel, and concentrated poliovirus preparations were added to wells in the gels. After 48 h the gels were washed and stained with Coomassie Brilliant Blue. Of the 11 poliovirus type 3 strains tested (Table 1) only Saukett antigens (NIB and CDC strains) gave SRD zones. The limiting concentration of ascitic fluid for the production of distinct stained reaction zones with Saukett was 5 μl/ml. Saukett concentrates did not give SRD reactions after heating (56 °C for 30 min), a procedure known to inactivate poliovirus D-antigen activity.

In antigen-blocking assays, 10 μl vol. of serial twofold dilutions of Mo56 ascitic fluid were mixed with equal volumes of poliovirus 3-strain concentrates containing 10⁶ to 10⁹ tissue culture infectious doses (TCID₅₀) of virus per 10 μl. The latter, when tested alone, gave clear SRD zones (5 mm diam.) when added to 3 mm diam. wells in immunoplates containing immune polyclonal rabbit serum to poliovirus 3 (Sabin strain, Schild et al., 1980). After incubation (30 min at 22 °C) the mixtures were added to wells in immunoplates. The lowest concentration of fluid which completely blocked the SRD activity of the antigen was recorded (Table 1). For the Saukett virus strains, high blocking activity was detected but there was no blocking activity against other poliovirus 3 strains.

Autoradiographic SRD tests (Minor et al., 1980) were carried out in gels containing similar concentrations of Mo56 ascitic fluid. Poliovirus 3 antigens, labelled with ³⁵S-methionine and prepared in sucrose gradients as the rapidly sedimenting (15S) or slowly sedimenting (70S to 80S) radioactive peaks, were added to wells in the immunoplates and the serological reactions detected by autoradiography on X-ray film. Of the strains tested (see Table 1) only Saukett gave autoradiographic SRD reactions. Distinct SRD activity was detected for the Saukett strain with the antigens of both the 15S and the 70S to 80S peak.

Evidence that the virus-specific antibody secreted by Mo56 cells is of the IgG class was provided by the finding that SRD activity with Saukett virus sedimented at approx. 7.5S in sucrose gradients. In addition, the serological reactions were inhibited by incubating the ascitic fluid with specific antiserum to mouse IgG but not with anti-mouse IgM. Additionally, these findings are supported by immunoglobulin precipitation studies (see below) since IgG, but not IgM, precipitated with Staphylococcus aureus protein A.

To investigate the nature of the poliovirus particles which react with Mo56 antibody, ³⁵S-methionine-labelled Saukett virus was prepared and purified on 15 to 45% sucrose gradients fractionated into 0.5 ml vol. as described by Minor et al. (1980) except that
centrifugation was at 20000 rev/min for 16 h (Beckman L5-50, SW27 head). The distribution of radioactivity in a representative gradient is shown in Fig. 1. Ten μl vol. of each fraction were assayed for antigen by autoradiographic SRD tests. The plates either contained immune guinea-pig serum specific for poliovirus type 3 D-antigen or immune rabbit serum for type 3 C-antigen (Minor et al., 1980; Schild et al., 1980). Immunoplates containing monoclonal antibody (Mo56 ascitic fluid) were also similarly assayed for antigen (Fig. 1). The D-antigen-specific plate detected antigenic activity in fractions 2 to 5 (155S) and 25 to 28
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(approx. 70S) while the C-antigen plate detected activity in fractions 16 to 22 (80S). The plates containing monoclonal antibody detected antigen in fractions 2 to 5 and 25 to 28 only, suggesting that the antibody was D-antigen-specific.

Additional evidence for the specificity of the monoclonal antibody was provided by immune precipitation (Kessler, 1975). Gradient fractions (25 μl) containing 35S-labelled Saukett virus were mixed in tubes with NETN buffer (50 mM-tris-HCl pH 7.5, 150 mM-NaCl, 5 mM-EDTA, 0.05% NP40, 50 μl vol.) 10% bovine serum albumin (5 μl) and monoclonal antibody (5 μl ascitic fluid) and left at 4 °C overnight. A 20 μl vol. of 10% (v/v) suspension of formalin-inactivated S. aureus (Cowan strain) washed in NETN was then added to each tube and the mixtures incubated at 22 °C for 40 min. The tubes were then spun in a microfuge and the pellets washed three times in NETN before radioactive counting. As shown in Fig. 1 radioactive counts in the 70S peak and the 155S (infectious virus) peak, but not the 80S C-antigen peak, precipitated under these conditions. Counts in control tubes without serum were not precipitated. These findings indicated that the antibody from Mo56 cells is exclusively D-antigen-specific.

Studies with specific polyclonal sera (Minor et al., 1980) have identified a 130S C-antigen particle for certain poliovirus 3 strains in addition to 155S D-antigen (infectious virus) and 80S C-antigen (empty capsids). Slowly sedimenting poliovirus particles of D antigenicity have not been described previously. The biological and antigenic significance of the 70S D-antigen and its existence for virus strains other than Saukett require further investigation. The relatively low degree of activity of Mo56 antibody in virus-neutralization tests and its potent activity in antigen-blocking tests with high doses of virus (D-antigen) suggested that the antibody, although reacting efficiently with virus capsids, was not directed against the antigenic sites important for virus neutralization.

The unique specificity of Mo56 for Saukett virus suggests that panels of monoclones for polioviruses, when available, will be of value in the precise intratypic identification of polioviruses for epidemiological purposes. The potential of these preparations for detailed antigenic analysis of virus preparations is also illustrated by the detection in our studies of a new slowly sedimenting poliovirus particle with D-antigenic reactivity. Since Saukett virus is commonly used in the preparation of inactivated poliovaccines, monocline Mo56 may have practical use as a reagent for immunoassays of the antigen content of vaccines (Schild et al., 1980) or for affinity chromatography in purification of D-antigen in commercial vaccine production.

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