Monoclonal Antibodies Against Measles Virus

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SUMMARY

Monoclonal antibodies were produced in vitro by fusing mouse myeloma cells (SP2) with spleen cells derived from Balb/c mice immunized with purified measles virus. Fifteen independent hybrid cell lines, isolated from two separate fusions, were maintained in culture for up to 5 months without loss of their antibody-secreting activity. Radioimmunoprecipitation and polyacrylamide gel electrophoresis showed that in five of the hybrid lines the antibodies were directed against haemagglutinin, in two against the nucleoprotein, and in one against L protein. The remaining seven hybridomas did not precipitate viral antigens under the experimental conditions employed even though they gave positive immunofluorescence against measles virus-infected cells. Monoclonal haemagglutinin antibodies displayed anti-haemagglutinating activity and neutralized measles virus infectivity but not canine distemper virus (CDV).

INTRODUCTION

Measles virus, normally associated with acute childhood infections, has been implicated in a slow persistent infection in children and young adults, namely subacute sclerosing panencephalitis (SSPE) (Baublis & Payne, 1968; Bouteille et al., 1965; Connoly et al., 1967; Horta-Barbosa et al., 1969), and has also been linked with multiple sclerosis (Cathala & Brown, 1972; Levy et al., 1976) and Paget's bone disease (Baslé et al., 1979). Measles virus is antigenically stable, a single infection giving life-long immunity. Measles virus isolates from SSPE patients may show less avidity for measles virus antibodies (Payne & Baublis, 1973; Wild & Huppert, 1980), but, in general, polyclonal antibody fails to distinguish strains. It has been suggested by Holland et al. (1979) that during persistent infections, viruses can accumulate mutations which cannot be retained in the acute system. To investigate this at the immunological level, it is necessary to use antibody against restricted antigenic sites. The work of Köhler & Milstein (1975) has shown the possibility of making such antibodies. In this study we describe the methodology for isolation and characterization of hybrid cell lines which secrete measles virus monoclonal antibody.

METHODS

Cells and virus. The myeloma cells SP2/OAg14 (supplied by Dr Buttin, Paris, France) were grown according to the method of Cotton et al. (1973). BGM cells (an African green monkey kidney line) and a measles virus persistently infected line, BGM/Hallé, were cultivated as previously described (Wild & Dugrê, 1978). The Hallé strain of measles virus (Horta-Barbosa et al., 1971) was grown in monolayer cultures of BGM cells at 33 °C. Virus was concentrated from the supernatant by adding an equal volume of saturated ammonium sulphate pH 7.6 resuspended in phosphate-buffered saline (PBS) and clarified at 10000 g for 15 min. The supernatant was centrifuged on a discontinuous sucrose gradient of 20 to 60% (w/w) in PBS at 25000 rev/min for 3 h in the SW27 Beckman rotor. The virus band was
removed, diluted in PBS and pelleted at 35,000 rev/min for 60 min in the SW41 rotor. The pellet was resuspended in PBS and stored at -70 °C. Cells were checked for the presence of mycoplasmas by the method of Schneider et al. (1974), and were found to be uniformly negative.

**Immunization of mice.** Balb/c mice were immunized intraperitoneally with an emulsion of measles virus (2 × 10⁹ p.f.u.) in Freund's complete adjuvant (1:1). Two weeks later, the same mixture was inoculated both intraperitoneally and into the hind foot pads. The animals were sacrificed after a further 3 days.

**Fusion and isolation of hybrid cell lines.** Spleen cells from the immunized mice were fused with SP2 cells by the method of Buttin et al. (1978). Hybrid cell colonies selected in HAT medium were visible from day 11. These were tested for measles virus antibody synthesis, and the positive colonies cloned at terminal dilution in microplates.

**Detection of measles virus antibody**

**Immunofluorescence.** BGM and BGM/Hallé cells (Wild & Dugré, 1978) were grown in Labteck chamber slides. The cells were used either directly or after fixation with acetone (10 min at 4 °C). Tissue culture fluid from the hybrid cultures was incubated with the BGM and BGM/Hallé cells, followed by a rabbit anti-mouse γ-globulin and then a fluorescein-conjugated anti-rabbit IgG (Dako, Copenhagen, Denmark). In some experiments, a specific anti-mouse IgG or IgM (Miles Laboratories, U.S.A.) was used to determine the type of antibodies secreted.

**Radioimmune precipitation.** BGM cells were infected with measles (Hallé) virus at 2 p.f.u./cell. After 24 h the cells were labelled with 50 μCi/ml ³⁵S-methionine (800 mCi/mmole; The Radiochemical Centre, Amersham) for 2 h and then solubilized in RIPA buffer (0-15 m-NaCl, 10⁻³ m-EDTA, 0-002 m-tris pH 7-2, 1% Triton, 1% DOC, 0-1% SDS) containing 0-28 trypsin-inhibiting (T.I.) units/ml aprotinin. The extract was centrifuged at 10000 g for 20 min and then stored at -20 °C. For immunoprecipitation, the ³⁵S extract was added to 2 vol. RIPA containing 5 mg/ml bovine serum albumin (BSA) and then centrifuged at 80000 g for 1 h. The supernatant was absorbed with an equal volume of a 10% suspension of *Staphylococcus aureus* (gift from Institut Mérieux, Lyon, France) to absorb proteins that bind non-specifically. Then, 100 to 200 μl ³⁵S antigen were incubated with either 50 μl of the hybrid culture supernatant or 5 μl ascites fluid for 1 h at room temperature. A 100 μl amount of 10% S. aureus was added and incubated for a further 15 min. The complex was washed twice in RIPA containing 5 mg/ml BSA, 0-28 T.I. units/ml aprotinin, and twice in RIPA, boiled for 2 min in electrophoresis loading buffer (Laemmli, 1970) and analysed by SDS-polyacrylamide slab-gel electrophoresis.

**SDS-polyacrylamide gel electrophoresis (PAGE).** This was performed according to Laemmli (1970). The ¹⁴C-marker proteins: myosin (200K), phosphorylase b (92-5K), BSA (69K), ovalbumin (46K) and carbonic anhydrase (30K), were obtained from The Radiochemical Centre, Amersham. The K values indicate mol. wt. of proteins × 10⁻³. Fluorography was performed according to the method of Bonner & Laskey (1974).

**Biological assays.** Haemagglutination inhibition (HI), haemolysin inhibition (HLI) and neutralization assays were performed as previously described (Wild & Huppert, 1980).

**RESULTS**

**Isolation of hybrid cell lines**

Spleen cells from mice immunized with purified measles virus were fused with SP2 cells. From two fusions, 198 hybrid cell cultures were obtained. By immunofluorescence, 28 of these cultures were shown to secrete measles virus antibody. After cloning (cloning efficiency
Measles monoclonal antibody

Table 1. Properties of measles virus antibodies from cloned hybrid cell lines

<table>
<thead>
<tr>
<th>Immuno-fluorescence group</th>
<th>Hybrid line</th>
<th>Class of immunglobulin</th>
<th>Immuno-precipitation</th>
<th>Designation</th>
<th>HI*</th>
<th>Measles</th>
<th>CDV†</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15</td>
<td>IgG</td>
<td>+</td>
<td>HA</td>
<td>640</td>
<td>&gt;5120</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>IgG + IgM</td>
<td>+</td>
<td>HA</td>
<td>160</td>
<td>&gt;5120</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>IgM</td>
<td>+</td>
<td>HA</td>
<td>640</td>
<td>2560</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>IgG</td>
<td>+</td>
<td>HA</td>
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<td>ND</td>
<td>ND</td>
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<tr>
<td></td>
<td>55</td>
<td>IgG</td>
<td>+</td>
<td>HA</td>
<td>640</td>
<td>&gt;5120</td>
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<tr>
<td>II</td>
<td>3</td>
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<td>-</td>
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<td>0</td>
<td>&lt;10</td>
<td></td>
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<tr>
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<td>5</td>
<td>IgG</td>
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<td>0</td>
<td>&lt;10</td>
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<tr>
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<td>6</td>
<td>IgG</td>
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<tr>
<td></td>
<td>30</td>
<td>IgG</td>
<td>-</td>
<td>?</td>
<td>0</td>
<td>&lt;10</td>
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<tr>
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<td>36</td>
<td>IgG</td>
<td>-</td>
<td>?</td>
<td>0</td>
<td>&lt;10</td>
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</tr>
<tr>
<td>III</td>
<td>22</td>
<td>IgG + IgM</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>IgG</td>
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<td>NP</td>
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<td>&lt;10</td>
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</tr>
<tr>
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<td>34</td>
<td>IgG</td>
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<td>NP</td>
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<td>&lt;10</td>
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<tr>
<td></td>
<td>44</td>
<td>IgG</td>
<td>+</td>
<td>L</td>
<td>0</td>
<td>&lt;10</td>
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* Haemagglutination inhibition (HI) and neutralization tests were performed on ascites fluids. In the case of hybrid line 48 anti-mouse IgM was also added.
† CDV, Canine distemper virus.
‡ ND, Not determined.

0 to 50%), 15 of these cultures gave stable lines which were maintained in cultures for up to 5 months.

To obtain large quantities of antibodies, the hybrid cell lines were passaged in Balb/c mice. The mice were pretreated with Pristane and γ-irradiated before intraperitoneal injection with 1 × 10⁶ to 5 × 10⁶ hybrid cells. Under these conditions all mice produced ascites within 7 to 10 days. Some of the hybrid ascite cells (between 20 and 30%) lost their antibody-secreting properties on passage in mice. This phenomenon did not appear to be related to a particular line or clone as the same clone inoculated into several mice could give antibody-producing or -non-producing cells.

Characterization of antibody synthesized by hybrid cell lines

For our subsequent studies using monoclonal antibodies as immunological probes, it was necessary to determine the class of γ-globulin secreted by the cells. Tissue culture fluid from the hybrid lines was incubated with measles virus-infected cells and subsequently with a fluorescein-conjugated anti-mouse (rabbit serum) IgG or IgM. By this method, 12 hybrid lines were classified as IgG, 1 as IgM and 2 contained a mixture of IgG and IgM (Table 1).

The nature of the secreted antibody was further analysed by radiolabelling the hybrid cells with 35S-methionine for 6 h, precipitating the 35S antibody from the culture medium with an anti-mouse total γ-globulin and analysing the precipitate by PAGE. Fig. 1 shows an example of some of the results obtained. All the clones which were classified as IgG by immunofluorescence contained heavy chains with mol. wt. of 56 to 68K and light chains of 21 to 23K (Table 2). The clones classified as IgM or IgG+M gave bands at 84 to 88K and 23 to 26K. Minor bands at 60K were observed in lines 18 and 22.

Specificity of antibodies

After the initial screening by immunofluorescence of the antibody secreted by the hybrid cell lines, those reacting with non-infected cells were discarded and the remaining cells were
Fig. 1. PAGE of $^{35}$S antibody secreted by hybrid cell lines. The hybrid cell lines were labelled with $^{35}$S-methionine for 6 h and the antibody precipitated from the medium with an anti-mouse $\gamma$-globulin. The precipitates were analysed by PAGE. Hybrid cell lines were: (a) 6, (b) 18, (c) 3, (d) 26 (not cloned), (e) 29, (f) 44, (g) 16, (h) 30, (i) 55, (j) 25, (k) 1 (l) SP2 cells and (m) 53. The mol. wt. $\times 10^{-3}$ (K) of the marker proteins are shown (right side), and the heavy (H) and light (L) chains are indicated (left side).

Table 2. Mol. wt. of immunoglobulins secreted by hybrid cell lines*

<table>
<thead>
<tr>
<th>Hybrid line</th>
<th>Mol. wt. (K) of immunoglobulin</th>
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<tbody>
<tr>
<td></td>
<td>IgM</td>
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<tr>
<td>3</td>
<td>–</td>
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<td>5</td>
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<td>6</td>
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<tr>
<td>48</td>
<td>84</td>
</tr>
<tr>
<td>55</td>
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</table>

* $10^7$ hybrid cells in 10 ml methionine free minimum essential medium were labelled with $100 \mu$Ci $^{35}$S-methionine for 6 h. The medium was clarified at 10'000 g for 30 min and the $^{35}$S antibody precipitated with an anti-mouse total immunoglobulin serum. The precipitates were analysed by PAGE.
Measles monoclonal antibody

Fig. 2. Classification of measles virus monoclonal antibody based on the type of immunofluorescence on BGM/Hallé cells. (a, b) class I; (c, d) class II; (e, f) class III; (g, h) polyclonal antibody. (a, c, e, g) cells fixed in acetone; (b, d, f, h) cells unfixed.

further characterized. These could be divided into three major groups (Fig. 2). Groups I and II were characterized by both intracellular and membrane fluorescence, whereas group III did not give immunofluorescence on unfixed tissue. Group I gave a diffuse pattern in contrast to the spickled pattern in group II.
Fig. 3. Immunoprecipitation (direct) of measles virus proteins by monoclonal antibody. Antibody was incubated with extracts of $^{35}$S measles virus-infected cells and the complexes precipitated with S. aureus. The precipitates were analysed by PAGE. (a) hybrid cell line 15; (b) hybrid line 55; (c) a non-producing line; (d) hybrid line 53; (e to g) three different clones of hybrid line 25; (h, i) hybrid line 44; PS, rabbit polyclonal serum.

The specificity of the antibodies was further tested in a radioimmunoprecipitation test employing $^{35}$S measles virus-induced proteins. The precipitation was performed either directly with S. aureus (Fig. 3), or after the addition of an anti-mouse IgG or IgM (Fig. 4). Antibody from lines 15, 53 and 55 precipitated the haemagglutinin (HA) polypeptide, line 25 the nucleoprotein (NP) and line 44 the L protein in the direct immunoprecipitation, whereas lines 18 and 48 precipitated the HA and line 34 the NP after the addition of a second antibody. In the indirect assay some nucleoprotein was non-specifically precipitated when a second antibody was added (Fig. 4 negative control).

**Biological activity**

The tissue culture and ascites fluids (where available) of the hybrid cell lines were tested for HI and neutralizing activity (Table 1). Clones from group I both neutralized measles virus infectivity and had HI activity. In the case of clone 48 (IgM), it was necessary to add an anti-mouse IgM serum to observe this activity. These antibodies did not cross-neutralize the closely related canine distemper virus (CDV). The three IgG-secreting lines 15, 53 and 55 did not have any HLI activity although lines 18 and 48 did display a certain level of HLI (data not shown).

**DISCUSSION**

In our present study, we have established several hybrid cell lines which secrete measles virus-specific antibodies. Despite the instability of such hybrid lines in viability and antibody secreting properties, 15 hybrid lines were maintained in culture for more than 5 months.
Measles monoclonal antibody

Fig. 4. Immunoprecipitation (indirect) of measles virus proteins by monoclonal antibody. In (a) the antibody from hybrid cell lines 18, 34 and 48 were incubated with extracts of 35S measles virus-infected cells. The antibody complexes were either precipitated directly (1) with *S. aureus*, or after further incubation with an anti-mouse IgG (2) or IgM (3). A rabbit polyclonal serum (PS) was included as a positive control. In (b) indirect assay (lanes 1 to 3) was used as a negative control; PS, rabbit polyclonal serum.

The identification of the specificity of the hybrid cell lines by radioimmunoprecipitation was in accordance with that obtained by immunofluorescence. Group I contained monoclonal antibody against the HA and group III against the nucleoprotein, L or NP. We have, however, failed to identify the members of group II. In a kinetic study, we were able to show that the viral polypeptide identified by this group had a different location in the cell than those recognized by the other groups. It is possible that these antibodies cannot recognize the dissociated structure. This is particularly relevant as the biological activity of the haemolysin (HL) antigen is sensitive to the detergent treatment used in the radioimmunoprecipitation assay. In our present work we have failed to identify an anti-HL-producing hybrid.

McFarlin *et al.* (1980) have previously shown that monoclonal antibody of two clones from the same hybrid line inhibited HA activity and neutralized measles virus infectivity. We have confirmed these observations with our anti-HA monoclonal antibody and have also shown that they do not cross-neutralize CDV. Previous studies by Norrby & Gollmar (1975) suggest that the anti-HL antibodies are responsible for neutralization. However, Merz *et al.* (1980) have recently shown that the two antigens of Sendai virus, HA and HL, both promote neutralizing activity. We await the isolation of an anti-HL hybrid cell line to clarify the situation.

A more detailed characterization of epitope specificity of the available monoclonal antibodies is in progress.
We would like to thank Dr J. Huppert for pointing us in the direction of monoclonal antibodies and Dr Buttin (Paris) for help in overcoming technical difficulties. This study was supported by grant I.N.S.E.R.M. No. C.R.L. 80 1041.

REFERENCES


