Monospecific Antibody to the Haemagglutinin of Measles Virus

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SUMMARY

A hybrid between a murine myeloma cell line and spleen cells from a mouse immunized with measles has been produced. Two stable clones produce antibody with identical immunochemical and biological properties. This antibody reacts with the 76,000 mol. wt. protein present in the lysates and on the surface of cells persistently infected with measles. It exhibits HAI and neutralizing activity.

The production of monospecific antiserum by the fusion of immune cells to mouse myeloma cells, as developed by Köhler & Milstein (1975), provides a powerful tool which can be used to assess the molecular components of many biological systems. We are applying this approach to the study of measles virus, which comprises six polypeptides. This report describes the biological activity residing in monospecific antibody against the 76,000 mol. wt. component of measles previously designated as the HA polypeptide (Tyrrell & Norrby, 1978).

A Balb/c mouse was immunized intraperitoneally (i.p.) with purified Edmonston strain of measles virus (Bellini et al. 1979) emulsified in complete Freund's adjuvant. The inoculum contained 250 µg of Mycobacterium tuberculosis and 15 µg of virus. Approximately 4 weeks later a booster dose of 10 µg virus was given intravenously and 2 days later, the mouse was sacrificed and a spleen suspension was prepared. The red blood cells were lysed by incubation with 0.83% NH₄Cl for 5 min. Immune spleen cells were mixed with P₃ × 63Ag8(×63) cells in a ratio of 5:1 and fused with 50%, polyethylene glycol following the technique described by Koprowski et al. (1977). Cells were suspended at a concentration of 10⁶/ml in hypoxanthine/aminopterin/thymidine (HAT) selective medium. Cultures containing 1 × 10⁵ cells in 100 µl were established in 96-well Linbro plastic plates (Flow Laboratories; McLean, Va., U.S.A.). These were observed for appearance of colonies; the latter appeared after 2 to 3 weeks and were transferred into 24-well Linbro plastic plates (Flow Laboratories) in 0.5 ml of HAT medium.

Supernatant fluids from 105 wells were screened for anti-measles antibody by a modification of a solid-phase radioimmunoassay (RIA) previously described (McFarland & McFarlin, 1979). The antigen consisted of a human prostate cell line (MA 160; Microbiological Associates, Inc., Walkersville, Md., U.S.A.) persistently infected with the Mantooth strain of measles virus. Uninfected cells were used as control antigen. These cells were grown in soft plastic wells (Cooke Engineering, Alexandria, Va., U.S.A.), fixed in 0.025% glutaraldehyde and washed in PBS. Test samples were applied to wells of both infected and uninfected cells; after washing, immunoabsorbent–purified goat anti-mouse immunoglobulin (Cappel Laboratories) labelled with ¹²⁵I was applied. After subsequent washing the plate was cut and individual wells counted. In each assay, control tissue culture medium as well as serum from mice hyperimmunized with measles (MAM) were assayed. A sample showing counts on infected cells which were twice those seen on uninfected cells was considered to have significant antibody activity (McFarland & McFarlin, 1979). The supernatant from one culture (C-0) showed consistent antibody activity even after dilution of 1:100. The anti-measles reactivity in this supernatant fluid was also studied by indirect immunofluorescence,
using both fixed and unfixed infected MA 160 cells. C-o produced bright fluorescence throughout the cytoplasm and along the surface of fixed cells. Prominent membrane fluorescence was observed in unfixed cells. No fluorescence was seen with uninfected MA 160 cells.

The C-o cells were expanded in culture and cloned in soft agar. Culture fluids from two clones, C-1 and C-2, contained significant anti-measles antibody by RIA. Immunofluorescence showed bonding patterns identical to each other and to the parent C-o. The supernatants from these clones also produced surface fluorescence with HEp-2 cells persistently infected with the Edmonston strain of measles (HEp-2 PI) (Hayes et al. 1978).

The C-1 and C-2 cells have been passed 14 times, remained stable and continued to produce anti-measles antibody. In addition they have been propagated as ascites tumours. Balb/c mice were primed with 0.5 ml pristane (Aldrich Chemical company, Milwaukee, Wis., U.S.A.) and 2 weeks later were given 5 x 10^6 tissue culture cells i.p. Significant amounts of ascites fluid accumulated within 2 to 3 weeks and contained anti-measles reactivity which was 100- to 1000-fold greater than that observed with tissue culture supernatants from the same clones.

When ^125I-staphylococcal protein A (SPA) was used in the RIA instead of ^125I-rabbit anti-mouse immunoglobulin, markedly less anti-measles reactivity was demonstrable with ascites fluids from C-1 (C-1 AF) and C-2 (C-2 AF). This indicated that the molecular species of immunoglobulin responsible for the anti-measles reactivity resided in either the IgG1 subclass or one of the other classes of murine immunoglobulins with low reactivity for SPA (Kronvall et al. 1970). In order to identify the immunoglobulin molecules responsible and to see if these were the same in both C-1AF and C-2AF, an indirect RIA was performed. Microtitre wells containing infected and uninfected MA 160 cells were fixed as described above and used to study C-1AF and C-2AF in parallel. Following incubation with each ascites fluid, the wells were washed and replicates were incubated with one of the following: rabbit anti-mouse IgA, goat anti-mouse IgM and rabbit antisera specific for each of the mouse IgG subclasses, as well as for light chain types (Litton Bionetics). After washing, this was followed by ^125I-SPA and subsequent counting. Significant binding in the infected wells containing C-1AF and C-2AF was obtained when anti-IgG1 and anti-kappa were used. Significant binding was not obtained with antisera directed against the other species of immunoglobulins. Because the X63 cell line is a secretor and further because the immunoglobulin produced is IgG1k it was imperative that in each serological and immunofluorescence assay the findings with C-1AF and C-2AF should be compared with X63 ascites fluid (X63AF). On no occasion was evidence of reactivity between X63AF and measles seen.

The HAI and neutralization capacity of C-1AF and C-2AF were tested using methods previously described (Bellini et al. 1979). The commercial measles antigen (Microbiological Associates, Inc.) used for HAI was prepared from the Philadelphia strain of measles virus. Neutralization was studied using the Edmonston strain. Both C-1AF and C-2AF possessed strong neutralization and HAI activities (Table I).

The reactivity C-1AF and C-2AF with individual measles virus polypeptides was studied with an immune precipitation assay. HEp-2 PI cells were labelled with ^35S-methionine (Hayes et al. 1978); cell lysates were prepared with RIPA buffer (Lamb et al. 1978) containing 1 mM-PMSF and 500 K units/ml of aprotinin. These were stored at -20 °C until used. At the time of assay 100 µl of the lysate containing 2 x 10^6 ct/min were added to 400 µl of RIPA buffer containing 0.5% myoglobin and 10 µl of the ascitic fluid to be assayed. After 2 h, 10 µl of immunoabsorbent-purified rabbit anti-mouse IgG (0.5 mg/ml) was added; this step was necessary because of the low affinity of IgG1 for SPA. One h later, this was followed by 100 µl of the 10% suspension of Staph. aureus, Cowan I strain (Enzyme
Table 1. Immunological activity against measles virus glycoprotein in two clones of hybrid cells

<table>
<thead>
<tr>
<th>Ascites fluid</th>
<th>RIA titre*</th>
<th>HAI titre</th>
<th>Neutralization titre†</th>
<th>Immuno-fluorescent pattern</th>
<th>Immunoglobulin subclass and type</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1</td>
<td>$10^6$</td>
<td>$6 \times 10^5$</td>
<td>$1 \times 10^4$</td>
<td>Surface</td>
<td>IgG1 k</td>
</tr>
<tr>
<td>C-2</td>
<td>$10^5$</td>
<td>$2 \times 10^5$</td>
<td>$4 \times 10^5$</td>
<td>Surface</td>
<td>IgG1 k</td>
</tr>
</tbody>
</table>

* Maximal dilution factor giving significant binding to RIA.
† Maximal dilution factor giving 50% reduction in plaque formation.

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![Fluorographic assessment of immunoprecipitates](image)

**Fig. 1.** Fluorographic assessment of immunoprecipitates: cell lysates from ^35^S-methionine-labelled HEp-2 PI cells incubated with: (A) X63AF, (B) C-1AF, (C) C-2AF, (G) Serum 2526; ^125^I-surface labelled HEp-2 PI cells after reaction with: (D) C-1AF, (E) C-2AF, (F) rabbit anti-measles.

Center, Inc., Boston, Mass., U.S.A.). After incubation for 1 h the specimens were centrifuged at 10000 rev/min in a Beckman microfuge B. The pellets were washed with RIPA four times, treated with gel sample buffer and subjected to SDS-polyacrylamide gel electrophoresis, (Laemmli, 1970) using a 9% running gel and 5% stacking gel. After electrophoresis, the gels were prepared for fluorography (Bonner & Laskey, 1974). Anti-measles reactivity in serum from an individual following acute measles infection (no. 2526) was assayed in a similar manner except that the rabbit anti-mouse IgG was omitted. Incubation of X63AF (Fig. 1, lane A) resulted in mild binding to a number of the cellular components. This degree of binding was identical to that seen with *Staph. aureus* alone and was regarded as non-specific. Serum 2526 reacted with all of the measles polypeptides (lane G). In contrast, incubation of C-1AF and C-2AF produced prominent reactivity with only the
Short communications

76 K peptide. Parallel studies in our laboratory have shown that this component is glycosylated. This conclusion is based on the incorporation of \(^{6}\text{H}\)glucosamine as well as reactivity with \textit{Lens culinaris} lectin. (W. J. Bellini, unpublished results).

The nature of the antigen on the cell surface was ascertained in a similar manner. The surface components of HEp-2 PI cells were radio-iodinated with lactoperoxidase (Glorioso & Smith, 1977). Subsequently the cells were lysed by resuspension in PBS containing 0.5\% NP-40 and 10 mm-MgCl\(_{2}\) and centrifuged at 3000 rev/min to remove the nuclei. Specimens of C-1AF and control X63AF were incubated with 200 \(\mu l\) of the radiolabelled cell lysate containing 200000 ct/min; this was followed by rabbit anti-mouse immunoglobulin and pelleting with \textit{Staph. aureus} as described above. Hyperimmune rabbit anti-measles serum (Bellini \textit{et al.} 1979), 10 \(\mu l\), was assayed in parallel. No binding was obtained with control X63AF. The rabbit anti-measles serum reacted with two components, the 76 K peptide, and a smaller peptide, approx. 41 K, presumably the F\(_{1}\) protein (Fig. 1, lane F). C-1AF (lane D) and C-2AF (lane E) reacted with only the 76 K peptide. No reactivity for uninfected surface-labelled HEp-2 cells was observed with the hyperimmune rabbit serum or either of the clones.

The interpretation of our observations is dependent upon the premise that the antibodies produced by C-1 and C-2 were monospecific. The data strongly support this. Both clones were derived from well-isolated individual colonies; the immunological reactivity of each was qualitatively indistinguishable and the immunochemical properties of the two were identical. The low reactivity of each when SPA was used in the indirect RIA is consistent with the observations of others (Kronvall \textit{et al.} 1970) concerning the low affinity of IgG\(_{1}\) subclass of mouse IgG. This demonstration suggests caution to investigators attempting to seek antibody activity in an indirect assay using SPA exclusively.

C-1AF and C-2AF reacted with three strains of measles virus, Edmonston, Mantooth and Philadelphia, and therefore are probably recognizing a shared antigenic determinant. In fact, the manner in which our experiments were conducted, i.e. immunizing with the Edmonston strain and using the Mantooth virus to screen for antibody activity, would be expected to identify cross-reacting determinants. Identification of strain-specific determinants, which may exist, would require using the same strain of measles virus for immunization and screening of antibody.

The present observations provide formal proof of the prevailing views concerning the 76 K polypeptide of measles virus (Tyrrell & Norrby, 1978). This glycoprotein resides on the surface of cells infected with the virus and is responsible for haemagglutination. It is of considerable interest that antibody against this protein possessed neutralizing activity. Quite recently, it has been reported (Armstrong \textit{et al.} 1979) that human anti-measles sera, which were depleted of HAI antibodies by absorption, retained antibody against haemolysin as well as neutralizing activity. Thus, antibody to either of the surface components of measles apparently has neutralizing capabilities. This, however, is not a universal property of antibody against all virus surface antigens. For example, monospecific antibodies against some of the surface components of herpes simplex virus do not contain neutralizing activity (Howes \textit{et al.} 1979).

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