Monoclonal Antibodies against Human Paramyxovirus Type 3 and against SV5 Virus: Preparation and Preliminary Characterization

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SUMMARY

Monoclonal antibodies against the major glycoprotein (HN) and the nucleoprotein (NP) of paramyxovirus SV5 and human paramyxovirus (PF) type 3 have been obtained. Two different epitopes have been detected on the SV5 HN polypeptide, one being specific to SV5 and the other also cross-reacting with PF-2 HN polypeptide. Monoclonal antibody against SV5 NP polypeptide also reacted with PF-2 NP polypeptide. Similar epitopes showing both specific and group cross-reactions were detected with PF-3 HN and NP monoclonal antibodies against PF-3 and Sendai viruses. No cross-reactions were detected between these two groups of paramyxoviruses.

INTRODUCTION

The paramyxoviruses belong to the Paramyxoviridae family (Kingsbury et al., 1978) and although a few members such as Sendai virus and Newcastle disease virus (NDV) have been fairly well characterized, those viruses associated with human diseases have been relatively little studied. Five different species of human parainfluenza (PF) viruses have been recognized, namely mumps, PF-1, PF-2, PF-3 and PF-4. Using the techniques of immune precipitation of [35S]methionine-labelled infected cell polypeptides by polyclonal rabbit antisera and analysis by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and autoradiography, we have been able to show an antigenic relationship between PF-1 and PF-3 and also between PF-2 and paramyxovirus SV5 mediated through the nucleoprotein polypeptides (Goswami & Russell, 1982). The SV5 virus (RQ isolate) had been originally isolated from the bone marrow of a multiple sclerosis (MS) patient after fusion with indicator tissue culture cells (Mitchell et al., 1978) although it was not clear whether the origin of the virus could be ascribed to laboratory contamination or to a real association with the bone marrow material. It is also pertinent to point out that although the prototype SV5 virus had been originally isolated from primary monkey kidney cell cultures there is now reasonable evidence that this virus (or a closely related one) is present in both man and domestic animals and is therefore not necessarily a virus endogenous to simian species (Hsiung, 1972; Evermann et al., 1981; Robbins & Rapp, 1982).

In an effort to investigate the antigenic relationships between these viruses in greater depth and to provide more highly specific reagents we have prepared monoclonal antibodies against human paramyxovirus type 3 and against SV5 virus. These reagents have confirmed our original observations, of cross-reactions between the nucleoproteins (NP) of SV5 and PF-2 and between PF-1 and PF-3. Moreover, we have also been able to obtain monoclonal antibodies that detect epitopes on the major glycoprotein (HN) which are common to both SV5 and PF-2 and to PF-1 and PF-3, as well as epitopes which are specific to the homologous glycoproteins.

METHODS

Viruses. The following paramyxoviruses were used: SV5, PF-2, PF-3 (prototype strains obtained from Central Public Health Laboratory, Colindale, London, U.K.); SV5 virus (RQ strain) was isolated from the bone marrow aspirates of an MS patient (Mitchell et al., 1979; Goswami & Russell, 1982). Sendai virus and avian

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paramyxovirus type 3 were from the virus depository at Mill Hill and bovine paramyxovirus type 3 (strain J121) was obtained from Dr P. K. Russell of the Royal Veterinary College, London.

**Cells.** Vero cells (originally obtained from Flow Laboratories), and BHK21 and MDBK cells were grown in H-21 medium supplemented with 10% calf serum. Foetal calf serum (1%) was used in the medium for the propagation of virus. Chick cells were prepared as described previously (Porterfield, 1960). Cells were screened at regular intervals for mycoplasmas by the fluorescent DAPI technique (Russell et al., 1975).

**Purification of viruses.** Virus from supernatants of infected cells was precipitated by polyethylene glycol and purified by sucrose and Urografin density gradient centrifugation (Goswami & Russell, 1982).

**Immunization and fusion.** BALB/c mice, 4 to 6 weeks of age, were immunized intraperitoneally with 1000 haemagglutinating (HA) units of alum-precipitated purified virus. These immunized mice were given boosting doses of 1000 HA units of purified virus intravenously via the lateral tail vein. Spleens were removed 3 days later and a cell suspension was prepared for fusion as previously described (Russell et al., 1981).

**Production of hybridomas.** Spleen cells were treated with 0.83% ammonium chloride solution to lyse erythrocytes. SP2/O myeloma cells (Schulman et al., 1978) were harvested by centrifugation, washed once in phosphate-buffered saline (PBS) and resuspended in the same buffer at 10^7 cells/ml. Two ml of spleen cell suspension in PBS (at a concentration of 10^7 cells/ml) and 5 ml of the SP2/O myeloma cells were mixed in a plastic centrifuge tube. Fusion with polyethylene glycol was carried out as described previously (Russell et al., 1981). The screening procedure for individual wells was carried out by haemagglutination inhibition (HI), fluorescent antibody assay and radioimmunoassay (see below). Cultures of interest were then cloned by limiting dilution and amplified before storage in liquid nitrogen and passage as ascites in mice.

**Fluorescent antibody procedures.** Cells were grown on plastic (PTFE) multispot microscope slides each containing 12 mini wells (Hendley, Essex, U.K.). They were infected with viruses at an added multiplicity of approx. 1 p.f.u./cell and, after incubation at 37°C until a cytopathic effect was just evident (normally about 48 h), the slides were washed in PBS, the cells fixed in cold acetone, air-dried and stored at −20°C. In some experiments the cells were utilized without being fixed. Tissue culture supernatants and ascitic fluid dilutions were assessed for their ability to bind to the cells by adding anti-mouse fluorescent conjugates as previously described (Russell et al., 1981).

**Radioimmunoassay (RIA).** Nitrocellulose paper BA85 (Schleicher & Schüll) was cut to appropriate size, marked with a ball-point pen into 1 cm squares and transferred into a flat plastic dish (size 10 × 10 cm). One ml of antigen diluted with 4 ml of TBS (0.9% NaCl, 10 mM-Tris–HCl pH 7.6) was added to the dish and the paper soaked for 30 min at room temperature on a shaker. The antigens used were either purified virus (titre approx. 1 × 10^9 p.f.u./ml), or infected cell extracts (1 × 10^7 cells/ml). The excess antigen was then removed and the paper washed in TBS with 3% bovine serum albumin (BSA) for 15 min. The paper was blotted dry and 3 µl of antibody in various dilutions was added to each square and incubated for 1 h at 37°C under humidified conditions. The paper was washed gently twice with TBS containing 0.2% Nonidet P40 (NP40) and then immersed in 5 ml of TBS containing either 125I-labelled Fab2 rabbit anti-mouse IgG or 125I-labelled Protein A (approx. 1 × 10^6 cts/min) for 1 h and finally washed twice in TBS for 15 min. The paper was then dried and mounted for autoradiography (Russell & Blair, 1977). Development of the film after overnight exposure was normally sufficient to show positive reactions.

**Labelling of infected cell polypeptides.** Monolayers of Vero cells in 50 mm plastic Petri dishes were infected with virus at a multiplicity of approx. 5 p.f.u./cell, and after 1 h adsorption at 37°C in an atmosphere of 5% CO2 the excess inoculum was removed and the cells were incubated further in 2 ml of H-21 medium with 2% foetal calf serum. Two to 3 days later, when the cell sheet was beginning to show significant c.p.e., this supernatant was replaced with 1 ml of medium lacking methionine and containing [35S]methionine (50 µCi; 1250 mCi/mmol, Amersham International). After incubation at 37°C for 1 h, the medium was removed, the cells scraped off and washed in PBS and resuspended by resuspending the cell pellets (5 × 10^6 cells) in 250 µl of lysis buffer (20 mM-Tris–HCl pH 7-6, 0-15 M-NaCl, 5 mM-EDTA, 0-5% NP40, 0-1 mM-phenylmethylsulphonyl fluoride, 25 Kallikrein units aprotinin and 0-1 mM-tosyl lysine chloromethylketone) and disrupting by a probe sonicator (MSE) for 5 s. An equal volume of high salt buffer (20 mM-Tris–HCl pH 7-6, 1-25 M-NaCl, 5 mM-EDTA, 0-5% NP40) was added and after centrifugation (10000 g, 10 min) in an Eppendorf centrifuge, the supernatant was used for immunoprecipitation.

**Immunoprecipitation.** About 20 µl of labelled cell extract was mixed with 5 µl of ascitic fluid in various dilutions and after incubation for 1 h at 4°C, formalin-fixed staphyloccocal Protein A was added (Kessler, 1975) and incubated at 4°C for a further 30 min before being pelleted (10000 g, 2 min) in an Eppendorf centrifuge. The pellet was resuspended and washed three times in washing buffer (20 mM-Tris–HCl pH 7.6, 0.15 M-NaCl, 5 mM-EDTA, 1% NP40, 10% sucrose). The final pellet was resuspended in SDS denaturing buffer (80 mM-Tris–HCl pH 6-8, 2% SDS, 0.1% dithiothreitol, 10% glycerol, 0.04% bromophenol blue) and placed in a boiling water bath for 2 min. The Protein A was pelleted and the supernatant analysed by SDS–PAGE and autoradiography as described previously (Russell & Blair, 1977). Purified adenovirus type 5 used as a size marker was iodinated in vitro using the Bolton–Hunter reagent (Rekosh et al., 1977).

**Virus neutralization.** Neutralization of ascites and tissue culture supernatants was carried out as described by
Russell et al. (1981). In each set of experiments rabbit antisera against the appropriate virus with a neutralization index of approx. 1/512 was used as control (Goswami & Russell, 1982).

*Haemagglutination inhibition.* Haemagglutination was carried out using guinea-pig (PF-3) and chick erythrocytes (SV5, PF-2 and Sendai) as described previously (Russell et al., 1967) and the dilution of antisera or ascitic fluid inhibiting agglutination of 4 HA units by 50% was determined.

*Hybridoma antibody class.* This was determined by microimmunodiffusion (Crowle, 1958) tests using standard antisera against the various murine immunoglobulins (Litton Bionetics Laboratory Products, Kensington, Md., U.S.A.; Miles Laboratories).

*Competition assay.* An aliquot of ascitic fluid was placed on a column of Protein A–agarose (Bethesda Research Laboratories) and after washing in PBS the bound immunoglobulin was eluted by adding 50 mM-glycine–HCl buffer pH 3.2 and collecting 10-drop fractions in 500 µl of 100 mM-Tris–HCl buffer at pH 8.6. The protein elution profile was monitored by u.v. absorption at 280 nm and an estimation of the protein content was made using BSA as a standard. An aliquot of the eluted immunoglobulin was then dialysed against 50 mM-sodium borate buffer pH 8.6 and iodinated with Bolton–Hunter reagent as previously described (Rekosh et al., 1977; Russell et al., 1981). Extracts of infected cells in detergent–high salt buffer were prepared as described above and 10 µl aliquots were dispensed onto 5 mm diam. nitrocellulose discs (BA85) placed in the wells of a 24-place plastic culture tray. The discs were then washed in TBS with 3% BSA for 15 min, excess buffer removed, and 125I-labelled anti-mouse globulin added in 50 µl TBS (total counts approx. 5 × 10⁵/ct/min), the dilution of immunoglobulin having been previously determined by assay of the dose–response relationship for binding. After incubation for 1 h at 37 °C the discs were then washed in TBS containing 0.2% NP40 and twice in TBS for 15 min before being dried and then the radioactivity counted. For assessing competition, 5 µl of dilutions of unlabelled globulins were mixed with 5 µl of labelled globulin before adding TBS to the antigen-containing discs.

*Antisera.* Standard typing antisera against SV5 and human parainfluenza types 1, 2, and 4 were obtained from the Department of Microbiological Reagents and Quality Control, Central Public Health Laboratory, Colindale, London, U.K. (These sera were obtained by inoculation of rabbits with tissue culture fluids from infected cells.) Antisera against purified RQ and parainfluenza type 3 viruses were prepared as described by Goswami & Russell (1982). Antisera were also obtained from the mice whose spleens were utilized in the production of hybridomas.

**RESULTS**

*Production of hybridomas*

A number of fusion experiments were performed using spleens from both SV5- and PF-3-immunized mice and, in successful fusions after about 2 weeks growth in HAT-containing medium, two or three colonies visible to the naked eye could be observed in about 10% of the wells. The initial screening of the colonies was by RIA and those wells giving a positive reaction in this test were further analysed both by fluorescence and by HI reactions using the homologous virus. A random selection of cultures which gave strong reactions in these procedures (both HI-positive and HI-negative) were cloned by limit dilution on mouse macrophage feeder layers and amplified before passage into mice and harvesting of ascitic fluids. Four such SV5 and five PF-3 hybridoma cultures were further characterized in more detail as described below.

*Characterization by immune precipitation*

SV5 virus contains five major structural polypeptides: two glycoproteins of mol. wt. 70000 (70K) and 52K (HN and F1 respectively), a nucleoprotein (NP) of 60K closely associated with a P polypeptide of 46K and the matrix polypeptide (M) of mol. wt. 38K. A smaller polypeptide of mol. wt. approx. 10K is sometimes found and is assumed to be the F2 polypeptide derived from the precursor to the glycopolypeptide (F0) of mol. wt. 62K. To ascertain which polypeptide each of the monoclonal antibodies was detecting, dilutions of ascitic fluids were reacted with extracts of SV5-infected cells labelled with [35S]methionine and the labelled immunoprecipitates analysed by SDS–PAGE and autoradiography. Fig. 1 shows that two of the SV5 hybridoma clones (designated A12 and C12) clearly reacted with the glycoprotein HN polypeptide of mol. wt. 70K whilst the other two clones (B12 and C10) precipitated the NP polypeptide of mol. wt. 60K. The PF-3 virus major structural polypeptides have been shown by analogy to other related viruses to be of similar electrophoretic mobilities, the major difference being the nucleoprotein polypeptide and the P polypeptide. Thus, the PF-3 P polypeptide has a mol. wt. of 95K, HN of 80K, NP of 72K, F1 of 50K and M of 40K (Goswami & Russell, 1982). Fig. 2 shows that four of
the PF-3 hybridoma clones (C5, C6, C9 and E9) reacted with the glycoprotein of mol. wt. 80K and one (A5) had a specificity for the 72K nucleoprotein polypeptide.

**Cross-reactions between the different parainfluenza viruses**

**Immunoprecipitation**

The ability of the above clones to cross-react with other parainfluenza viruses was assessed by immunoprecipitation reactions using $^{35}$S-methionine-labelled extracts from cells infected with SV5, PF-2, PF-3 or Sendai (a type 1 murine parainfluenza) viruses. Thus, Fig. 3(a) shows a cross-reaction using the SV5 C12 clone with the HN glycoprotein of PF-2 of mol. wt. 80K, whereas the other SV5 clone reacting with the HN glycoprotein (A12) did not show a cross-reaction with PF-2-infected cells (not shown). No cross-reactions were observed with either of these antibodies against PF-3 or Sendai virus-infected cells. These results imply that these two monoclonal antibodies recognize different epitopes on the HN glycoprotein, one having a broad specificity and the other a more restricted specificity. Fig. 3(a) also shows that another SV5 clone, B12, reacted with PF-2-infected cells immunoprecipitating the nucleoprotein polypeptide. The C10 clone behaved in a similar manner (data not shown), indicating that these hybridomas detecting epitopes on the nucleoprotein polypeptide had broad specificities, although in this case also no reaction was observed with either PF-3 or Sendai virus-infected cell extracts (not shown).

Analogous reactions were also observed using the PF-3 hybridoma clones. Thus, Fig. 3(b) shows that the C5, C6 and C9 clones immunoprecipitated the Sendai virus HN glycopolypeptide.
SV5 and PF-3 hybridomas

whereas the E9 clone failed to precipitate this polypeptide (not shown). The A5 PF-3 clone precipitating the NP polypeptide also reacted with the corresponding Sendai virus polypeptide. None of these PF-3 hybridomas showed any reaction with either SV5- or PF-2-infected labelled cell extracts. Both the PF-3 and SV5 hybridomas were tested by immunoprecipitation against extracts of [35S]methionine-labelled chick cells infected with NDV (an avian paramyxovirus) and no reactions were noted (data not shown).

Radioimmune assay

Table 1 shows the results of testing the ability of the SV5 hybridomas to react with partially purified SV5 and PF-2 virus preparations adsorbed on nitrocellulose as detected by 125I-labelled rabbit anti-mouse globulin. In agreement with the immunoprecipitation results, the C12, B12 and C10 clones showed a reaction with both SV5 and PF-2 whereas A12 was restricted to reaction with SV5 only. No binding was detected with PF-3 and Sendai virus preparations (data not shown). Table 2 shows the results of similar tests using the PF-3 hybridomas with PF-3 and Sendai virus antigen preparations. In this case also there was agreement with the immunoprecipitation procedures showing cross-reactions with Sendai virus when clones C5, C6, C9 and A5 were utilized and only a monotypic reaction with E9. No reactions were detected with SV5 and PF-2 virus (data not shown). The C5, C6 and C9 clones showed a faint but significant cross-reaction with avian parainfluenza type 3 in RIA, a much more prominent binding being evident with the PF-3 A5 monoclonal reacting against the nucleoprotein epitope. There was no reaction of the E9 clone with the avian parainfluenza type 3 antigen.
Fig. 3. (a) Autoradiograms of immunoprecipitates from extracts of PF-2-infected Vero cells labelled with [35S]methionine for 1 h at 48 h after infection following reaction with ascitic fluids and serum at dilutions of 1/10. Lane 1, polyvalent rabbit serum against PF-2; lane 2, SV5 B12; lane 3, SV5 C12. (b) Autoradiograms of immunoprecipitates from extracts of Sendai virus-infected chick embryo cells labelled with [35S]methionine for 1 h at 24 h after infection following reaction with ascitic fluids and serum at dilutions of 1/10. Lane 1, PF-3 C5; lane 2, PF-3 C6; lane 3, PF3 C9; lane 4, polyvalent rabbit antiserum against Sendai virus; lane 5, PF-3 A5. Mobilities of marker adenovirus polypeptides (mol. wt. \( \times 10^{-3} \)) are indicated.

Table 1. Characteristics of SV5 ascitic fluids: relationship to PF-2 virus*

<table>
<thead>
<tr>
<th>Clone no.</th>
<th>SV5</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>PF-2</th>
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<th></th>
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<tr>
<td></td>
<td>IP</td>
<td>HI</td>
<td>N</td>
<td>FA</td>
<td>RIA</td>
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<td>HI</td>
<td>N</td>
<td>FA</td>
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<tr>
<td>A12</td>
<td>HN</td>
<td>2048</td>
<td>1024</td>
<td>1000</td>
<td>&gt;100</td>
<td>ND†</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;5</td>
</tr>
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<td>HN</td>
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<td>2048</td>
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<td>&gt;100</td>
</tr>
<tr>
<td>B12</td>
<td>NP</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>1000</td>
<td>&gt;100</td>
<td>NP</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>1000</td>
</tr>
<tr>
<td>C10</td>
<td>NP</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>1000</td>
<td>&gt;100</td>
<td>NP</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>1000</td>
</tr>
</tbody>
</table>

* Numbers refer to dilutions of ascitic fluids giving the 50% endpoint in haemagglutination inhibition (HI), neutralization (N), fluorescent antibody (FA) and RIA. The polypeptide precipitated in immunoprecipitation experiments is indicated (IP).
† ND, Not detected.

Characterization of antibodies

Determination of antibody class

The antibody classes of the ascitic fluids were determined by a microimmunodiffusion technique with results as shown in Table 3.
SV5 and PF-3 hybridomas

Table 2. Characteristics of PF-3 ascitic fluids: relationship to Sendai and avian and bovine PF-3 viruses*

<table>
<thead>
<tr>
<th>Clone no.</th>
<th>PF-3</th>
<th>Sendai</th>
<th>AvPF-3</th>
<th>BovPF-3</th>
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<td></td>
<td>IP</td>
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<td>N</td>
<td>FA RIA</td>
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<tr>
<td>E9</td>
<td>HN</td>
<td>2048</td>
<td>2000</td>
<td>100 ND</td>
</tr>
<tr>
<td>C5</td>
<td>HN</td>
<td>4096</td>
<td>2048</td>
<td>2000 100</td>
</tr>
<tr>
<td>C6</td>
<td>HN</td>
<td>4096</td>
<td>2048</td>
<td>2000 100</td>
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<td>C9</td>
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<td>A5</td>
<td>NP</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>2000 100</td>
</tr>
</tbody>
</table>

* For explanation of terms, see footnote to Table 1. Avian paramyxovirus antigen was crude allantoic fluid with an HA titre of 320. Bovine paramyxovirus type 3-infected MDBK cells were utilized for the FA test.

Table 3. Summary of properties of SV5 and PF-3 monoclonal antibodies

<table>
<thead>
<tr>
<th>Clone no.</th>
<th>Designation</th>
<th>Activity against</th>
<th>Specificity</th>
<th>Class of antibody</th>
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<tr>
<td>A12</td>
<td>SV5 HN-1</td>
<td>HN</td>
<td>SV5</td>
<td>IgG2b</td>
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<tr>
<td>C12</td>
<td>SV5 HN-2</td>
<td>HN</td>
<td>SV5 and PF-2</td>
<td>IgG1</td>
</tr>
<tr>
<td>B12</td>
<td>SV5 NP-1</td>
<td>NP</td>
<td>SV5 and PF-2</td>
<td>IgG2b</td>
</tr>
<tr>
<td>C10</td>
<td>HuPF-3 HN-1</td>
<td>HN</td>
<td>PF-3</td>
<td>IgG2a</td>
</tr>
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<td>PF-3</td>
<td>IgG1</td>
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<tr>
<td>C6</td>
<td>HuPF-3 NP-1</td>
<td>NP</td>
<td>Sendai, AvPF-3</td>
<td>IgG1</td>
</tr>
<tr>
<td>C9</td>
<td>HuPF-3 NP-1</td>
<td>NP</td>
<td>PF-3</td>
<td>Sendai, AvPF-3 BovPF-3</td>
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</table>

Neutralization and haemagglutination inhibition

Tables 1 and 2 show that good neutralization and HI activity were obtained with all the monoclonal antibodies that reacted with the HN glycopolypeptide when using the homotypic virus, whereas no reaction was observed with other viruses even when a cross-reaction was evident by fluorescence and RIA tests. None of the antibodies detecting epitopes on the nucleoprotein polypeptides showed neutralization or HI activity.

Fluorescent antibody tests

Tables 1 and 2 also show the results of a number of fluorescent antibody experiments and it can be seen that the results once again confirmed those seen using the techniques of immunoprecipitation and RIA. There was variable distribution of antigen, the cells showing both granular and diffuse cytoplasmic staining. The monoclonal antibodies reacting against the HN polypeptides reacted with antigens either close to or on the plasma membrane; this was particularly noticeable when using unfixed cells, whereas the monoclonal antibodies detecting NP antigens gave granular inclusions. In some cases, it should be noted that hybridomas reacted in high dilutions (1 in 2000) even against the heterotypically infected cells. Table 2 also suggests that the spectrum of activity of the PF-3 HN monoclonal antibodies does not extend to the bovine (Bov) paramyxoviruses, since no reaction was observed with either the C5, C6 or C9 clones using MDBK cells infected with the J121 strain of bovine paramyxovirus type 3 although a fluorescence was readily demonstrated with the A5 hybridoma reacting against the nucleoprotein.

Competition studies

It is clear that in all the above tests the C5, C6 and C9 clones detecting epitopes on the PF-3 HN polypeptide and the SV5 NP monoclonal antibodies, the B12 and C10 clones detecting epitopes on the SV5 NP polypeptide, could not be distinguished in any tests; this suggests that they were detecting the same or overlapping epitopes. In order to test this assumption, a
competition assay was devised in which the ability of a labelled specific immunoglobulin (derived from a hybridoma) to bind to its particular site on antigen adsorbed on nitrocellulose was blocked by unlabelled immunoglobulin derived from another hybridoma. Fig. 4(a) shows that C9 and C6 immunoglobulin from these clones both successfully competed with labelled clone C5 immunoglobulin for binding sites whereas immunoglobulin from the related E9 HN clone failed to block the binding. Fig. 4(b) shows that unlabelled C10 immunoglobulin successfully competed with $^{125}$I-labelled B12 with similar kinetics to the unlabelled B12, whereas unlabelled A5 (reacting with the PF-3 nucleoprotein) failed to compete. These results indicate that the C5, C6 and C9 hybridomas detect similar epitopes on the HN PF-3 glycoprotein and that the B12 and C10 hybridomas react in a similar fashion with an SV5 nucleoprotein epitope.

**DISCUSSION**

These studies amply demonstrate the utility of the monoclonal antibody technique in dissecting complex antigenic structures. As well as confirming our previous investigations (Goswami & Russell, 1982), which implied that the human paramyxoviruses showed interrelationships via the nucleoprotein antigens, these experiments have clearly shown that similar relationships are also evident in the glycoproteins. Thus, both the nucleoproteins and HN glycoproteins of SV5 and PF-2 and of PF-1 and PF-3 show antigenic relationships which indicate that these two groups of viruses can be logically subgrouped. Moreover, the monoclonal antibodies described here should have some diagnostic value, particularly if fluorescent antibody, RIA (and possibly ELISA) tests are utilized. Furthermore, should monoclonal antibodies having narrower specificities be obtained for PF-2 and PF-1 viruses and with clarification of the reactions with the PF-4 group and mumps, it should be possible to obtain a small 'bank' of monoclonals which would provide a rapid and efficient diagnostic assay for parainfluenza viruses. With more understanding of the number and disposition of the various epitopes it may also be feasible to consider the possibility of different subtypes and possible relationship to disease patterns.

It is interesting that these monoclonals have demonstrated a cross-reaction among the glycoproteins which was not readily apparent using heterotypic rabbit and mouse sera. Whether this represents a selection of a minor epitope or is related to the relative avidities of the antibodies is not at all clear. It is also interesting that in the case of the paramyxoviruses the glycoproteins show, at least in these relatively limited experiments, a similarly parallel specificity to the
nucleoproteins, the only apparent discrepancy being with the bovine parainfluenza viruses. This is in contrast to the influenza viruses where the nucleoprotein shows a much wider reaction than the glycoproteins (e.g., see Russell, 1983). This in turn may reflect the more stable nature of the paramyxoviruses which are apparently not subject to the same degree of antigenic variation as the influenza glycoproteins. It will be interesting to analyse the distribution of the two epitopes on the HN polypeptides in greater detail, particularly in terms of the associated neuraminidase activity and it seems also very likely that further epitopes will be detected by screening more hybridomas. In this respect it is pertinent to note that using monoclonal antibodies four antigens have been detected on the HN polypeptide of 6/94 virus (a type 1 paramyxovirus related to Sendai virus) all showing HI activities but differing significantly in their abilities to inhibit or enhance neuraminidase activity and in their ability to neutralize (Yewdell & Gerhard, 1982).

Table 3 summarizes the properties of the monoclonal antibodies described here and for ease of reference they have been re-designated in terms of the immunizing virus and the antigen detected, thus avoiding the use of trivial names. All the monoclonal antibodies are of the IgG isotype and although they comprise different subclasses it seems very unlikely that this could account for their different properties.

It is evident that these monoclonal antibodies will be valuable in providing much more specific tools to analyse any possible association of paramyxoviruses with disease patterns. In particular they can be used to re-examine the possibility that the SV5 isolates from bone marrow aspirates (Mitchell et al., 1978; Goswami & Russell, 1982) may be directly associated with the tissues rather than the result of adventitious contamination.

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REFERENCES


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