Monoclonal Antibodies to Three Non-glycosylated Antigens of Herpes Simplex Virus Type 2

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SUMMARY

The production and properties of three monoclonal antibodies, designated LP1, LP4 and AP2, directed against non-glycosylated polypeptides of herpes simplex virus type 2 are described. LP1 is specific for polypeptide VP16 and cross-reacts with HSV-1; LP4 reacts with the major DNA-binding protein and is type-specific. AP2 is directed against the major capsid antigen of HSV-1 and HSV-2.

INTRODUCTION

The genomes of herpes simplex viruses type 1 and type 2 code for at least fifty polypeptides (Honess & Roizman, 1973; Powell & Courtney, 1975; Marsden et al., 1976): this is likely to prove a considerable underestimate (Haarr & Marsden, 1981). Although some of these polypeptides have been purified and their functions investigated (Powell & Purifoy, 1977; Strobel-Fidler & Franke, 1980; Chen & Prussof, 1978; Powell et al., 1981), the majority of the virus-specified polypeptides are defined only by their molecular weight, their kinetic class, their presence or absence in the virion and whether or not they are glycosylated or phosphorylated. Furthermore, the complexity of the polypeptide pattern following acrylamide gel electrophoresis of virus-specific polypeptides has caused considerable problems in polypeptide nomenclature.

Antiserum against individual virus polypeptides (monoprecipitin sera) define proteins by serological criteria and have proved of considerable value both in analysing the synthesis and function of some virus antigens and in establishing serological relationships between members of the herpesvirus group (e.g. Watson & Wildy, 1969; Baucke & Spear, 1979; Eberle & Courtney, 1980; Powell et al., 1981). An extension of this approach is to prepare monoclonal antibodies against herpes simplex virus antigens. Since such antibodies are directed against single antigenic sites they can be used to dissect complex antigenic mixtures. Many monoclonal antibodies against herpes simplex virus types 1 and 2 have already been described (Zweig et al., 1979; Pereira et al., 1980; Balachandran et al., 1981; Showalter et al., 1981), and, although this work is in its infancy, this approach has led to the identification of a previously unrecognized glycoprotein (Balachandran et al., 1981). Furthermore, a recent paper demonstrates the value of monoclonal antibodies in distinguishing HSV-1 isolates from HSV-2 isolates and in further subdividing the herpes simplex types (Pereira et al., 1982).

In this paper we describe the properties and target antigens of three monoclonal antibodies directed against non-glycosylated polypeptides of HSV-2.

METHODS

Cells and media. BHK-21 and Hep-2 cells were grown in Glasgow-modified Eagle’s medium (GMM) supplemented with 10% tryptose-phosphate broth and 10% newborn calf serum. Balb-3T3 cells were grown in Dulbecco’s modified Eagle’s medium (DMM) supplemented with non-essential amino acids and 10% foetal calf serum (FCS). The mouse myeloma cell line P3. NS1/1Ag-3.4.1 (NS1, a gift from Dr C. Milstein) was maintained in GMM supplemented with 5% FCS.

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Hybrid cell lines derived by fusing NS1 cells with mouse spleen cells were initially maintained in GMM containing hypoxanthine, aminopterin and thymidine (HAT medium; Littlefield, 1964) and 20% FCS. Once established, hybridomas were grown in GMM containing 5% FCS.

**Viruses.** Strains of herpes simplex virus used were as follows. Type 1 strains were Cl (101) of Dubs & Kit (1964), strain HFFEM of Watson et al. (1966) and strain F of Ejercito et al. (1968). Type 2 strains were Fry of Thouless & Skinner (1971), strain 25766 (isolated by K. R. Dumbell, Wright Fleming Institute, London, U.K.) and strain 333 (isolated by W. Rawls, McMaster University, Ontario, Canada).

Virus preparations required for infecting or immunizing mice were grown in Balb-3T3 cells. Purified virus was prepared from culture supernatants from infected Hep-2 cells as described by Powell & Watson (1975). For all other purposes, virus was grown in BHK-21 cells.

**Immunization.** Six- to nine-week-old Balb/c mice (obtained from Bantin and Kingman, Ltd.) were inoculated with 2 × 10^4 p.f.u. HSV-2 (strain 25766) in the ear pinna to establish latent infection (Hill et al., 1975). Two intraperitoneal inoculations of 5 × 10^4 and 10^5 p.f.u. were given after a further 4 and 6 weeks. A final inoculation of 10^6 p.f.u. of formaldehyde-inactivated virus was given intravenously 3 days before cell fusion.

**Cell fusion.** The method used for cell fusion was essentially as described by Galfre et al. (1977). Three days after the final immunization, mice were killed and the spleens were removed and carefully macerated. 10^8 spleen cells were washed in GMM + 2% FCS, mixed with 10^7 NS1 cells and the cell mixture was pelleted. The pellet was resuspended in 1 ml 50% polyethylene glycol 1500 in serum-free medium for 1 min at 37°C. Increasing amounts of serum-free medium were added over the next 8 min, to a final total volume of 20 ml. The cells were pelleted, resuspended in 100 ml GMM + 20% FCS and distributed among the wells of two 24-well Linbro trays. After 24 h, 1 ml of medium was removed from each well and replaced with 1 ml HAT medium. This was repeated every 48 h. Colonies of hybrid cells were observed after 4 or 5 days and the supernatant fluids were assayed for the presence of anti-HSV-2 antibodies after a further 4 to 6 days. Cells from positive wells were passaged in 50 ml tissue culture flasks and those cultures which remained positive after four passages were cloned in soft agar. Positive clones were frozen in liquid nitrogen in 30% GMM + 60% FCS + 10% glycerol.

**Radioimmune assay.** BHK-21 cells were grown to confluency in 48-well Linbro trays and infected at a multiplicity of infection (m.o.i.) of 10. After 8 h the monolayers were fixed in 0.25% glutaraldehyde in phosphate-buffered saline (PBS) for 5 min at room temperature, washed exhaustively in PBS, then dried and stored at −20°C. For assay, 50 μl of antibody was added to each well. After 1 h at room temperature, the cell monolayers were extensively washed in PBS and the bound antibody was detected using 125I-labelled *Staphylococcus aureus* protein A (sp. act. approx. 5 μCi/μg). Bound 125I was measured with a gamma-counter. To reduce non-specific binding, target wells were always pretreated with a drop of heat-inactivated FCS.

Where purified virus was required as a radioimmune assay target, a suspension of 10^10 particles per ml in PBS was made 1% with respect to Nonidet P40 and 50 μl of the mixture was added to each well of a 96-well Linbro tray. Adsorption of protein to the plastic was allowed to proceed overnight at room temperature; the virus suspension was then removed. Adsorbed antigen was fixed with 0.25% glutaraldehyde and, with washing with PBS, the targets were ready for use. The assay procedure was as described for infected-cell targets.

**Preparation of isotope-labelled antigen.** Monolayers of BHK-21 cells were infected with HSV-1 or HSV-2 at an m.o.i. of 10 and labelled from 3 to 16 h after infection in methionine-free medium containing 50 μCi/ml [35S]methionine (sp. act. >600 Ci/mmol; Amersham International). 32P-labelled antigens were prepared by incubating cells from 3 to 16 h post-infection in phosphate-free medium containing 200 μCi/ml carrier-free [32P]orthophosphate (Amersham International). Cells labelled with either isotope were harvested in PBS at 10^7 cells/ml. Cell suspensions were lysed by addition of 0·1 vol. of lysis buffer (0·9 m-NaCl, 0·01 m-Tris–HCl pH 7·4, 7·5 mm-L-methionine, 10% Triton X-100, 10% deoxycholate), and incubated for 15 min at 0°C. Insoluble material was removed by centrifugation at 10000 g for 10 min and the supernatants were made 0·2 mM with respect to phenylmethylsulphonyl fluoride.

**Immunoprecipitation.** 20 μl of isotope-labelled antigen was mixed with 5 μl antibody (ascites fluid or serum) and incubated for 30 min at room temperature. 25 μl of a 50% suspension of Protein A–Sepharose C14B beads (Pharmacia) was added together with 150 μl 0·15 m-NaCl, 0·01 m-Tris–HCl pH 7·4, 75 mm-L-methionine, 1% Triton X-100, 1% sodium deoxycholate. This mixture was kept at room temperature for 2 h, with continuous agitation to keep the beads in suspension. The beads were collected by centrifugation, washed three times in 0·5 m-LiCl, 0·1 m-Tris–HCl pH 7·4, 1% 2-mercaptoethanol, and the immune complexes were dissolved by addition of 50 μl 0·025 m-Tris–HCl pH 7·0, 2% SDS, 20% glycerol, 1·5% dithiothreitol, 0·02% bromophenol blue. Samples were heated to 100°C for 2 min and electrophoresed for about 4 h at 12 V/cm in 10% or 12.5% acrylamide gels cross-linked with 0·15% bisacrylamide. The buffer systems used for electrophoresis were as described by Laemmli (1970). After electrophoresis the gels were stained in 0·1% Coomassie Brilliant Blue in methanol/acetic acid/water (50:10:40), destained in the same solvent, dried on to filter papers and exposed to X-ray film for 2 to 10 days.

**Identification of target antigens by treatment of acrylamide gels with antibody.** As an alternative to immunoprecipitation followed by electrophoresis, in some experiments unlabelled extracts of virus-infected cells or unlabelled purified virus were subjected to SDS–polyacrylamide gel electrophoresis and the target antigen...
Monoclonal antibodies to HSV

Monoclonal antibodies to HSV were identified by treatment of the gel with antibody. 9% polyacrylamide gels were cross-linked with N,N'-dialyltartardiamide (Heine et al., 1974) and antigens were disrupted prior to electrophoresis by incubating for 10 min at 45 °C in 2% SDS, 5% 2-mercaptoethanol, 3% sucrose in 0.05 M-Tris-HCl pH 7.0. After electrophoresis, gels were fixed in methanol/acetic acid/water (46:8:46) at -20 °C for at least 12 h and were then washed for 24 h in three changes of PBS. The gels were then shaken for 5 h in a sealed plastic bag with enough antibody solution to keep the gel surface moist (about 5 ml for a 5 × 11 cm gel slice). Ascites fluids containing monoclonal antibodies were diluted 100-fold in PBS containing 200 µg/ml ovalbumin. After reaction with antibody the gels were washed for 36 h in PBS and incubated in PBS containing 200 µg/ml ovalbumin and 2 µCi 125I-labelled protein A (sp. act. approx. 10 µCi/µg). Gels were again washed for 36 h to remove unreacted protein A and were then stained with Coomassie Brilliant Blue, destained and dried on to filter paper before autoradiography.

Immunofluorescence. Subconfluent BHK monolayers were grown on glass microscope slides and infected with HSV-2 at an approximate m.o.i. of 3. Eight h after infection the cells were fixed in methanol at -20 °C overnight. Fixed cells were reacted with antibody for 1 h at room temperature, washed in PBS and then reacted with FITC-labelled, affinity-purified sheep anti-mouse Fab (a gift from Dr A. Munro) for 1 h at room temperature.

RESULTS

Production of hybridomas

The radioimmune assay procedure used for screening hybridoma supernatants has some disadvantages in that it will select for those classes of antibody which bind to protein A (Kronvall et al., 1970) and will identify only those antibodies whose target epitopes are resistant to glutaraldehyde fixation. The use of glutaraldehyde as a fixative is unlikely to restrict the number of available targets severely, since this fixative has been shown to have little modifying effect, at least on cell surface antigens (Heusser et al., 1981).

The antibodies described in this paper are derived from three independent fusion experiments. In each experiment, over half of the culture supernatants from the microtitre plates gave positive results when screened by radioimmune assay for the presence of anti-HSV-2 antibodies. Cells from those wells giving the highest binding ratios were passaged for 3 weeks and the supernatants were re-tested. About half the cultures became negative on passage and were discarded. Positive cultures were cloned in soft agar. The cloned hybridomas were established as ascites tumours by intraperitoneal injection of 10⁷ cells in 6-week-old Balb/c mice, and unless otherwise stated all results were obtained using ascites fluids or sera. The monoclonal nature of each hybridoma was established by showing (i) that antibody secreted by different subclones precipitated the same target antigen from extracts of HSV-2-infected cells and (ii) that the light chains secreted by different subclones were of identical molecular weight as judged by SDS–polyacrylamide electrophoresis.

Preliminary characterization of antibodies

The three antibodies described in this paper were designated LP1, LP4 and AP2. Antibodies LP4 and AP2 were shown to be of immunoglobulin class IgG2a and LP1 of class IgG1 by precipitation in double-diffusion agar plates using class-specific rabbit antiserum against mouse immunoglobulins and using culture supernatants as a source of the monoclonal antibody. The identification of LP1 as an IgG1 was surprising since this mouse antibody class is thought not to bind to protein A at neutral pH (Kronvall et al., 1970). However, Ey et al. (1978) have shown that mouse IgG1 shows considerable heterogeneity in its protein A-binding properties and it is likely that generalizations drawn from experiments with polyclonal immunoglobulin mixtures will not always hold true for monoclonal antibodies. None of the antibodies neutralized HSV-2 in the presence or absence of guinea-pig complement. By radioimmune assay, antibodies LP1 and AP2 reacted with both HSV-1-infected cells (strain HFEM) and HSV-2-infected cells (strain 25766), while antibody LP4 failed to react with HSV-1 infected cells. To investigate further the specificity of LP4, radioimmune assays were repeated using target cells infected by different HSV strains. The results, in Table 1, showed that while LP1 and hyperimmune serum to HSV-2 reacted with all infected targets, LP4 showed specificity for type 2. The same conclusion was drawn from the results of immunofluorescence experiments with HSV-1- and HSV-2-infected cells; LP4 reacted only with HSV-2-infected cells. However, using a more sensitive
Fig. 1. Electrophoresis of LP1 immunoprecipitates. Extracts of HSV-2-infected cells labelled either with $^{32}$P orthophosphate or $^{35}$S methionine from 3 to 16 h post-infection were mixed with LP1 antibody and the complexes selected using Protein A-Sepharose. Samples were electrophoresed in 12.5% acrylamide gels. Lane 1, total $^{32}$P-labelled extract; lane 2, immune precipitate; lane 4, total $^{35}$S methionine-labelled extract; lane 3, immune precipitate. Mol. wt. (given $\times 10^{-3}$) are of unlabelled marker proteins. The polypeptides corresponding to major capsid protein (MCP, mol. wt. approx. 157000) and major DNA-binding protein (MDBP, mol. wt. approx. 30000) are marked.

Table 1. Type specificity of antibodies LP1 and LP4*

<table>
<thead>
<tr>
<th>Herpes virus</th>
<th>Strain</th>
<th>LP1</th>
<th>LP4</th>
<th>Immune†</th>
<th>Pre-immune</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25766</td>
<td>8100</td>
<td>2300</td>
<td>4800</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>Bry</td>
<td>2100</td>
<td>2000</td>
<td>3500</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>333</td>
<td>2600</td>
<td>700</td>
<td>1300</td>
<td>60</td>
</tr>
<tr>
<td>Type 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>3100</td>
<td>80</td>
<td>1300</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>CI (101)</td>
<td>400</td>
<td>60</td>
<td>290</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>HFEM</td>
<td>550</td>
<td>80</td>
<td>640</td>
<td>90</td>
</tr>
<tr>
<td>Uninfected cells</td>
<td></td>
<td>110</td>
<td>60</td>
<td>110</td>
<td>40</td>
</tr>
</tbody>
</table>

* Infected target cells were reacted with antibody, immune serum or pre-immune sera at $10^{-3}$ dilution and bound antibody detected by addition of $^{125}$I-labelled protein A. Results are expressed as ct/min bound.
† Hyperimmune rabbit serum raised against HSV-2-infected RK-13 cells.

immunoperoxidase staining technique, K. L. Powell (personal communication) has detected weak reactivity of LP4 with HSV-1-infected cells. For most practical purposes, LP4 can be regarded as HSV-2-specific but may show weak cross-reaction with HSV-1.

Identification of target antigens

Antibody LP1

Extracts of HSV-2-infected cells labelled with $^{35}$S methionine or $^{32}$P orthophosphate were mixed with antibody LP1 and the immune complexes selected on Protein A-Sepharose. After denaturation, the samples were subjected to SDS–polyacrylamide electrophoresis, and the results (Fig. 1) showed that LP1 reacts with a phosphorylated polypeptide of approximately 65000 mol. wt. Identical results were obtained with HSV-1-infected cell extracts. Several groups
have reported the presence of phosphorylated polypeptides of approx. 65,000 mol. wt. in HSV-infected cells. A prominent structural polypeptide of this mol. wt. has been designated VP16 in HSV-1 and HSV-2 (Spear & Roizman, 1972; Honess & Roizman, 1973; Cassai et al., 1975) and is phosphorylated (Gibson & Roizman, 1974). This probably corresponds to the polypeptide designated M65 and P65' in HSV-1-infected cells by Marsden et al. (1978) and to ICSP 31 in HSV-2-infected cells (Powell & Courtney, 1975). Knopf & Kaerner (1980) found a phosphoprotein of 65,000 molecular weight associated with the chromatin of infected cells but could not detect the same polypeptide in purified virions. To establish whether the target antigen of LP1 is a structural polypeptide, purified HSV-1 particles were disrupted and subjected to electrophoresis in parallel to extracts of uninfected cells, HSV-1-infected cells and HSV-2-infected cells. The gel was then reacted with LP1 antibody followed by 125I-labelled protein A as described in Methods. The resulting autoradiograph (Fig. 2a) shows that the target antigen was present in purified particles and of similar molecular weight in type 1- and type 2-infected cells. The interpretation of this result is, of course, dependent upon the quality of the purified virus. The purified preparation contained 10 to 20 μg protein per 10^10 particles and its polypeptides are shown in the stained gel in Fig. 2(c). Note that track 4 in Fig. 2(a) contains 2 to 3 μg purified virus protein but the infected-cell tracks contain 30 μg protein. Purification of the virus therefore enriches for the target antigen of LP1. Similar experiments were attempted using antibodies LP4 and AP2 but no reaction was obtained with infected cell extracts after gel electrophoresis; we presume that the target epitopes of these antibodies are sensitive to SDS denaturation.

The properties of the LP1 target antigen suggest that it corresponds to polypeptide VP16 in
HSV-1 and ICSP31 in HSV-2. However, this conclusion should be treated with caution in view of the complexities of the published HSV polypeptide patterns in this region of SDS–acrylamide gels and the current confusion in HSV polypeptide nomenclature. In any event, antibody LP1 should prove useful in helping to achieve a consistent nomenclature among different groups of workers using different virus isolates and different gel electrophoresis systems.

Antibody LP4

Electrophoresis of immunoprecipitates prepared from [35S]methionine-labelled extracts of HSV-2-infected cells (Fig. 3a) showed that the target antigen of LP4 was a polypeptide of approx. 130000 mol. wt. The electrophoretic behaviour of this polypeptide suggested that it might correspond to the HSV-2 major DNA-binding protein, designated ICSP 11/12 by Purifoy & Powell (1976). This was confirmed by showing that LP4 would react with the purified HSV-2 major DNA-binding protein (Powell et al., 1981). Purified ICSP 11/12 (a gift from Dr K. Powell) was precipitated by LP4 but not by a different monoclonal antibody of the same sub-class (Fig. 3b). LP4 is therefore directed against the major DNA-binding protein of HSV-2.

Antibody AP2

Electrophoresis of immunoprecipitates prepared with antibody AP2 (Fig. 4) showed that the antibody reacted with a polypeptide with the mobility of the major capsid antigen designated...
Fig. 5. Immunofluorescent staining with LP1 and LP4. Uninfected BHK cells (a, c) or HSV-2-infected BHK cells (b, d) were fixed in methanol 8 h after infection and treated with antibody LP1 (a, b) or LP4 (c, d). Bound antibody was stained and detected using a fluorescein-labelled sheep anti-mouse Fab, as described in Methods.

Table 2. Antibody binding to purified virus antigens

<table>
<thead>
<tr>
<th>Antibody*</th>
<th>HSV-1 Ct/min bound x 10^-2</th>
<th>HSV-2 Ct/min bound x 10^-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMS†</td>
<td>4.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Anti-HSV-1‡</td>
<td>140</td>
<td>115</td>
</tr>
<tr>
<td>LP1</td>
<td>34</td>
<td>46</td>
</tr>
<tr>
<td>AP2</td>
<td>62</td>
<td>41</td>
</tr>
<tr>
<td>LP4</td>
<td>3.5</td>
<td>5.7</td>
</tr>
<tr>
<td>AP5</td>
<td>3.0</td>
<td>61</td>
</tr>
</tbody>
</table>

* All results are for antibodies used at 1/200 dilution.
† Normal mouse serum.
‡ Hyperimmune rabbit serum raised against HSV-1-infected RK13 cells.

VP5, ICP5 for HSV-1 (Spear & Roizman, 1972; Honess & Roizman, 1973) and ICSP 9 for type 2 (Powell & Courtney, 1975). To distinguish VP5 from VP6 unambiguously, immune precipitates were prepared from infected cells labelled with [32P]orthophosphate. No polypeptides could be detected (Fig. 4). The target antigen for AP2 is not phosphorylated and therefore cannot correspond to VP6 (Gibson & Roizman, 1974). Confirmation that AP2 reacts with a virion polypeptide was obtained by using purified virus as a radioimmune assay target. The data in Table 2 show that, as expected, LP1 and AP2 bind to purified HSV-1 and HSV-2. LP4 failed to bind to HSV-1 and gave a marginal result with purified HSV-2. However, the binding of LP4 to HSV-2 was so low that these data could not be taken as evidence that the major DNA-binding protein is a structural polypeptide. Antibody AP5, a type 2-specific antibody whose probable target is gC, was included as a control and bound only to purified HSV-2.

Immunofluorescence

Indirect immunofluorescence with all three antibodies resulted in a predominantly nuclear fluorescence in HSV-2-infected cells, consistent with the target antigens of these antibodies. Antibody LP1 gave a homogeneous nuclear fluorescence while the distribution of antibody LP4
was heterogeneous (Fig. 5). Results obtained with AP2 were indistinguishable from those obtained with LP1 (not shown).

DISCUSSION

We describe in this paper the production of three monoclonal antibodies against non-glycosylated polypeptides of herpes simplex virus type 2. Antibody LP1 is directed against the virion polypeptide designated VP16 in HSV-1 and HSV-2 (Cassai et al., 1975). This is the first report of an antiserum against this polypeptide and LP1 should prove useful in the characterization of the structure and function of VP16. Antibody LP4 reacts type-specifically with the major DNA-binding protein, ICSP 11/12, of HSV-2. This antigen is highly conserved among members of the herpesvirus group (Killington et al., 1977; Ye et al., 1981; Littler et al., 1981) and has recently been purified (Powell et al., 1981). Antibodies to the major DNA-binding protein are apparently elevated in the sera of patients with cervical carcinoma (Anzai et al., 1975; Melnick et al., 1976) and the protein has been detected by serological techniques in HSV-2-transformed cells (Flannery et al., 1977) and in cervical carcinoma biopsies (Dreesman et al., 1980). This evidence has been interpreted so as to implicate HSV-2 in cervical carcinoma, but, as Powell et al. (1981) point out, there is a considerable cross-reactivity between the major DNA-binding proteins of different herpesviruses, and so the use of polyclonal sera to detect this antigen does not define its origin. Since LP4 is type 2-specific this antibody should prove a more precise probe for detecting ICSP 11/12 in cervical carcinoma biopsies. Showalter et al. (1981) have recently described a type-specific monoclonal antibody whose tentative target is the HSV-1 major DNA binding protein (ICP 8). It is notable that despite the structural conservation of this antigen among different herpesviruses (Littler et al., 1981) and the broad cross-reactivity of polyclonal sera raised against it (Powell et al., 1981), both monoclonal antibodies to this antigen are type-specific.

Antibody AP2 is directed against a type-common site on the major capsid antigen of herpes simplex virus. A type-common antibody of different subclass directed against VP5 has been described by Showalter et al. (1981).

We wish to thank Drs K. Powell for a gift of purified ICSP 11/12, A. Munro for FITC-labelled sheep anti-mouse Fab and M. Parkhouse for class-specific rabbit anti-mouse IgG. Dr David Secher provided valuable advice in overcoming problems of monoclonal antibody production. C. McLean thanks the Cancer Research Campaign for support and A. Buckmaster is a recipient of an MRC Training Grant. This work was supported by the Medical Research Council.

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Monoclonal antibodies to HSV


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