A monoclonal antibody recognizes a human cell surface glycoprotein involved in measles virus binding

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Measles virus (MV) has a very limited host range, humans being the only natural reservoir of the virus. This restriction may be due to the absence of an MV receptor on the surface of non-primate cells. We have studied the MV-binding ability of several cell lines and attempted to characterize the receptor by studying the binding of 35S-labelled MV and by a rosette formation technique. We confirmed that all the human cell lines examined (HeLa, Raji and Jurkat) bound MV and that the murine cell lines (BW and L) did not. The glycoprotein nature of the receptor activity was demonstrated by the fact that it could be removed from the cell membrane using proteolytic enzymes and by its failure to be re-expressed in the presence of a protein synthesis inhibitor or an N-glycosylation inhibitor. A monoclonal antibody isolated after immunization of mice with Raji cells specifically inhibited MV binding and infection of human cells, and recognized human and simian but not murine cells. Depending on the cell line (HeLa, Raji, Jurkat or Vero), this antibody immunoprecipitated one or two glycoproteins with apparent Mr's of 57K and/or 67K from human and simian cells, but not from murine cells.

Introduction

Measles virus (MV) is the causative agent of an acute childhood disease; in rare cases, persistent infection can induce subacute sclerosing panencephalitis and measles inclusion body encephalitis, lethal diseases of the central nervous system. MV, a member of the Morbillivirus group, is a negative-strand enveloped ssRNA virus containing two membrane glycoproteins, the haemagglutinin (H) glycoprotein, responsible for binding to the host cell, and the fusion (F) glycoprotein, which mediates fusion of viral and cell membranes and penetration into the cell. Infected cells fuse with neighbouring cells to give syncytia, which are characteristic of the MV c.p.e. By using recombinant vaccinia viruses (VV), Wild et al. (1991) have demonstrated that the H and F glycoproteins, as well as a cellular component correlating with the permissiveness of a cell line to MV infection, are necessary to obtain MV-like c.p.e.

Humans are the only known hosts of MV, although the virus can infect and induce disease in some monkey species. The first step in virus infection is attachment to a cell surface receptor, and this can govern the host range and tissue tropism of the virus (for review, see Marsh & Helenius, 1989). Indeed, the host range of rhinovirus, poliovirus and murine ecotropic retrovirus, as well as the tissue specificity of Epstein–Barr virus and human immunodeficiency virus, correlates with the expression of their respective receptors (Dalgleish et al., 1984; Fingeroth et al., 1984; Greve et al., 1989; Klatzmann et al., 1984; Mendelsohn et al., 1989; Nemerow et al., 1985; Staunton et al., 1989; Tomassini et al., 1989; Wang & Kavanagh, 1991; Woo Kim et al., 1991). Therefore we hypothesize that the species specificity of MV infection is due to virus binding to a cell surface receptor expressed only on cells of human or simian origin. We have studied this by measuring MV binding to cells. MV-specific attachment occurred only with human and not murine cells. We then confirmed the glycoprotein nature of the binding activity (Krah, 1989; Valdimarsson et al., 1975). We developed a monoclonal antibody (MAb) which blocked MV binding and infection of a human cell line and used it to characterize the potential receptor glycoprotein for MV further.

Methods

Cells and virus. All cell lines were grown in Dulbecco's modification of Eagle's medium (DME) supplemented with 6% foetal calf serum (FCS), 10 mM-HEPES, 2 mM-glutamine, 5 x 10^-5 M-2-mercaptoethanol and 50 µg/ml gentamicin. Human epithelial (HeLa), B cell (Raji) and T cell (Jurkat) lines, simian fibroblast (Vero) cell line, and murine T cell (BW) and fibroblast (L) cell lines were used in the experiments.

The Halle strain of MV was grown in Vero cells. Supernatant from infected cells was used as stock virus. Vero cells were infected at
0.01 p.f.u./cell, and the virus was harvested from the supernatant 4 to 5 days later when c.p.e. was 30 to 50%. Virus was purified by first clarifying (3000 g, 30 min) the supernatant and then concentrating by ultrafiltration on 100K membranes using the Miniten device (Millipore). The concentrate was layered onto a 30% to 60% (w/w) sucrose gradient and centrifuged for 2 h at 110000 g. The virus band at the interface was collected and pelleted by centrifugation in PBS containing 1 mM-EDTA. The pellet was resuspended in PBS and stored at -70 °C.

Purified [35S]methionine-labelled MV was prepared in the same manner after metabolically labelling MV-infected Vero cells at 30 to 50% c.p.e. with [35S]methionine (Amersham) at 25 µCi/ml for 24 h.

The Ferrand strain of mumps virus and parainfluenza virus types 1 and 2 were kindly provided by M. Aymard and N. Kessler (Lyon, France).

**Virus binding assays.** Binding of [35S]methionine-labelled MV to target cells was done using 5 x 10⁵ cells in 100 µl DMEM containing 6% FCS. MV (10⁵ c.p.m.) was added and incubated with the cells at 4 °C for 2 h. Cells were then washed by layering them in PBS containing 0.1% BSA and 0.1% NaN₃, onto a 1 ml cushion of FCS, and centrifuging to recover the cell pellet. After three washes, cells were lysed in 0.1% SDS prior to scintillation counting in a Packard Tri-Carb scintillation analyser.

The rosette formation assay was performed on cells in suspension; adherent cell lines were removed from tissue culture flasks with PBS/1 mM-EDTA. 10⁵ cells were resuspended in 100 µl culture medium and incubated with 50 haemagglutination units (HAU) MV at 4 °C for 2 h. After three washes in DMEM containing 0.1% BSA and 0.1% NaN₃, and resuspension in 200 µl PBS, cells were incubated with 0.2% final concentration of vervet monkey erythrocytes (VMEs) for 1 h at 37 °C. The cells forming rosettes were gently resuspended and counted in a haemocytometer. Cells with three VMEs or more were counted as rosettes.

Rosette formation assays for mumps virus and parainfluenza viruses were done in the same manner except that all incubations were at 4 °C to prevent dissociation from cells due to viral neuraminidase action, and guinea-pig erythrocytes were used.

For virus haemagglutination assays, VMEs were washed three times in PBS and suspended to 0.5% in PBS. VMEs (100 µl) were then added to 100 µl PBS containing 4 HAU MV and incubated in round-bottom well plates at 37 °C for 1 h before determination of haemagglutination.

**Protease, cycloheximide and tunicamycin treatment of cells.** Cells were washed, suspended to 10⁶ cells/ml in DMEM without FCS and incubated at 37 °C for 30 min with 0.6 to 1 mg/ml pronase [7 units (U)/mg], 1 to 2 mg/ml trypsin (40 to 110 U/mg), or for 1 h with 100 µg/ml papain (30 U/mg) (all enzymes were from Boehringer Mannheim). The cells were then washed three times and suspended in DMEM. The assay of virus binding was then performed as described above.

To study the regeneration of virus-binding activity, pronase- or papain-treated cells were washed and suspended in DMEM containing 6% FCS alone, or containing 10 µg/ml cycloheximide or 10 µg/ml tunicamycin, and incubated at 37 °C for 4 h prior to the virus-binding assay.

**Isolation of a MAb inhibiting MV-like c.p.e.** Adult BALB/c mice were injected intraperitoneally with 5 x 10⁴ Raji cells in 0.5 ml PBS and complete Freund's adjuvant. Thirty days later the mice received a second intraperitoneal injection of Raji cells in PBS and incomplete Freund's adjuvant. One to 3 months later, the mice were boosted intravenously with 10⁴ Raji cells in 0.4 ml PBS. Three to 5 days after the last immunization, the spleen cells were fused with the SP2/0 myeloma line, following a procedure described by Harlow & Lane (1988). Hybridoma cell supernatants were assayed for their ability to inhibit MV-like syncytium formation between HeLa cells infected with a VV recombinant encoding the MV H and F proteins (VV-H/F). VV-H/F was constructed by inserting both genes in opposite orientations behind the 7-5K promoter of the thymidine kinase gene of VV (R. Drillien et al., unpublished results). HeLa cells were plated in 96-well plates at 3 x 10⁴ cells/well, allowed to adhere overnight and then incubated with 100 µl of hybridoma cell supernatant for 30 min before adding 0.075 p.f.u./cell VV-H/F. The c.p.e. was observed 18 h after infection. Hybridomas scoring positive were subcloned and used to make ascites fluid. Mouse ascites fluid from hybridoma MCI20.6 was purified on a Protein G-Sepharose column (Pharmacia).

**Assay for protection of cells against MV infection.** Purified MAb MCI20.6 was assayed for its ability to protect cells from death induced by MV infection. The level of MV infection was determined by measuring the cell survival using a colorimetric test based on the ability of live cells to metabolize a tetrazolium dye which forms dark cytoplasmic crystals that can be quantified (Gerlier & Thomasset, 1986; Mosmann, 1983).

Jurkat cells were seeded in 96-well plates at 10⁴ cells/well in DMEM containing 8% FCS, and incubated for 1 h at 37 °C with different concentrations of purified MAb. Virus was added at a multiplicity of 0.1 p.f.u./cell. After 6 days incubation, 20 µl 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in PBS at 7.5 mg/ml was added to the wells and incubated at 37 °C for 4 h. Medium was removed and 100 µl DMSO containing 0.04 M-HCl was added to dissolve the crystals. The optical density (OD) was read on an ELISA microplate reader at 490 nm with correction for cell turbidity at 650 nm.

**Flow cytometric analyses.** Cells (5 x 10⁶) were incubated for 45 min at 4 °C with purified MAb MCI20.6 or an unrelated antibody in 100 µl PBS containing 1% BSA and 0.1% NaN₃. Cells were then washed three times in this solution and incubated with a 1:20 dilution of fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Jackson) in 100 µl PBS for 30 min at 4 °C. After three washes, flow cytometric analyses were performed on a FACScan (Becton Dickinson).

**Immunoprecipitation.** HeLa cells were metabolically labelled in DMEM containing 5% of the normal amount of methionine and cysteine, and [35S]methionine (50 µCi/ml) and [35S]cysteine (25 µCi/ml). After 15 h at 37 °C, cells were washed three times and lysed (10⁶ cells/ml) in lysis buffer (1% NP40, 2% BSA, 10 mM-Tris-HCl, 0.15 M-NaCl, 0.5 M-KCl, 5 mM-EDTA, pH 7.8) containing anti-proteases (20 µM-E-64, 100 µM-DCI, 100 µM-penanthrolone; Sigma). After a 30 min incubation at 4 °C, lysates were centrifuged for 30 min at 100000 g.

For surface iodination, the glucose oxidase/lactoperoxidase technique was used (Hubbard & Colin, 1972). Cells (5 x 10⁶) were incubated at 23 °C for 20 min with 400 µCi Na¹²¹I (Amersham) in 500 µl PBS containing 25 mM-glucose, 12 U/ml lactoperoxidase and 0.1 U/ml glucose oxidase (Boehringer Mannheim). Cells were washed three times in PBS and lysed as described above.

Prior to immunoprecipitation, a clearing step was done by incubating lysates from 5 x 10⁶ cells with 4 µl normal mouse serum for 1 h. Packed Protein A-Sepharose CL-4B beads (25 µl; Pharmacia) were then added and left for 1 h, and supernatant was recovered and subjected to immunoprecipitation. After incubation for 2 h with 8 µl mouse ascites fluid containing 1 mg/ml MAb MCI20.6, 25 µl packed Protein A-Sepharose, previously coupled to goat anti-mouse IgG (BioSys), was added and incubated for 1 h. The immunoprecipitates were washed once in lysis buffer containing 1% deoxycholate, three times in lysis buffer and once in PBS, lyophilized and kept at 4 °C. To deglycosylate glycoproteins, immunoprecipitates were boiled for 5 min in denaturation buffer containing 50 mM-Tris-HCl, 1% SDS and 2% 2-mercaptoethanol. The sample was diluted with buffer containing 1%
NP40 instead of SDS, and incubation was carried out with 4-3 U/ml N-glycosidase F (Boehringer Mannheim; specific activity 25000 U/mg) at 37 °C for 18 h before SDS-PAGE.

For SDS-PAGE, immunoprecipitates were boiled at 100 °C for 5 min in sample buffer (62-5 mm-Tris-HCl pH 6-8, 10% glycerol, 2% SDS, 5% 2-mercaptoethanol, 0-05% bromophenol blue) and electrophoresed on 8% polyacrylamide gels. After fixing and drying, the gels were autoradiographed at −70 °C with Hyperfilm Ilmax for 35S and MP for 125I (Amersham).

Results

**MV binds to human but not to murine cells**

By using two different binding assays we attempted to ascertain whether human and murine cells bound MV. Cells were incubated with purified [35S]methionine-labelled MV and cell-associated radioactivity was determined. Human cells consistently bound labelled virus (1000 to 2000 c.p.m.) and mouse cells did not (1000 c.p.m.). 35S-labelled MV binding to human cells was shown to be specific and thus could be inhibited by incubation with unlabelled MV (results not shown). MV is very heterogeneous in size and tends to aggregate, and therefore this assay proved difficult to use routinely. Therefore we developed a rosette formation assay. MV agglutinates VMEs and therefore forms rosettes when bound to a cell.

Cells were incubated with virus and then with VMEs, and cells were counted in a haemocytometer as described in Methods. After incubation with virus, 85 to 90% of human epithelial B and T cell lines (HeLa, Raji and Jurkat) formed rosettes with VMEs, whereas mouse T (BW) and fibroblastic L cell lines did not form rosettes. Vero cells also bound MV (results not shown). As a control, cells incubated with VMEs in the absence of virus did not form rosettes (see Table 1).

**Protease sensitivity of MV binding**

To investigate the biochemical nature of the MV receptor, cells were treated with different proteases and MV binding was assessed by determining rosette formation. Cells treated with pronase (800 µg/ml) lost their ability to bind MV and form rosettes: 85 to 90% of untreated cells formed rosettes, only 10% did so after pronase treatment. MV binding was also sensitive to treatment with other proteases (100 µg/ml papain, 2 mg/ml trypsin) (data not shown).

**Recovery of MV receptor activity after protease treatment**

To determine whether the receptor activity destroyed by the proteases could be re-expressed and whether this recovery required protein synthesis and N-glycosylation, cells were treated with pronase and then incubated in the presence of cycloheximide or tunicamycin. A virus binding assay was performed 4 h later. Without this treatment, nearly 100% of cells formed rosettes; after pronase treatment, 14% of cells formed rosettes. When cells treated with pronase were incubated for 4 h in medium alone, up to 80% of the control level of rosette formation was recovered. However, if cycloheximide or tunicamycin was present, the proportion of rosette-forming cells remained between 11 and 18%. This shows that both protein synthesis and N-glycosylation are required for expression of MV binding activity at the cell surface.

### Table 1. Specific inhibition of MV binding to human cells by MAb MCI20.6 as measured by a rosette formation assay

<table>
<thead>
<tr>
<th>Virus</th>
<th>No virus</th>
<th>Virus + PBS</th>
<th>Virus + MCI20.6*</th>
<th>Virus + CD14**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles‡</td>
<td>0</td>
<td>81</td>
<td>29</td>
<td>75</td>
</tr>
<tr>
<td>Mumps§</td>
<td>0</td>
<td>65</td>
<td>67</td>
<td>ND†</td>
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<tr>
<td>Parainfluenza 2§</td>
<td>0</td>
<td>85</td>
<td>93</td>
<td>ND†</td>
</tr>
</tbody>
</table>

* Assay done with 75 µg antibody.
† CD14, MAb specific for the CD14 molecule.
‡ Rosette formation assayed with VMEs as described in Methods.
§ Rosette formation assayed at 4 °C with guinea-pig erythrocytes.
† ND, Not determined.

**Isolation of an MAb inhibiting MV-like c.p.e.**

To characterize the MV receptor activity further, we proceeded to develop an anti-receptor antibody. For practical reasons, we used a screening procedure involving a VV–MV recombinant virus encoding both H and F glycoproteins. Previous studies have shown that the fusion produced by this recombinant virus can be inhibited by anti-H protein MAbs (Wild et al., 1991). Therefore we assumed that antibodies to the MV receptor would also inhibit the fusion activity. Hybridoma cell supernatants were tested for their ability to inhibit the c.p.e. of VV–HA/F on HeLa cell monolayers. Over 3000 hybridomas were screened, and those giving 100% inhibition were subcloned twice by limiting dilution and used to produce mouse ascites fluid. MAb MCI20.6 was selected and used in the following studies.

Fig. 1 shows the c.p.e. observed in HeLa cells infected with VV–H/F (b) and the inhibition when cells were incubated with MCI20.6 supernatant or purified antibody (c). The inhibition of c.p.e. observed with MAb MCI20.6 was similar to that observed using an anti-F or anti-H protein MAb (Fig. 1d; Wild et al., 1991). Normal mouse serum had no effect on c.p.e.
Inhibition of MV binding and infection using MAb MCI20.6

MAb MCI20.6 was tested for its ability to block virus binding. Incubation of cells with various concentrations of MAb MCI20.6 before incubation with MV inhibited virus binding in a dose-dependent manner, as measured by rosette formation (Fig. 2). As a control, the same experiment was carried out with a MAb recognizing the CD14 molecule expressed at the surface of the cell lines tested. Incubation of cells with this control antibody did not inhibit MV binding significantly, as measured by rosette formation (Table 1). Incubation of VMEs with MAb MCI20.6 prior to haemagglutination with MV had no effect on virus binding (data not shown).

The inhibition of MV binding by MAb MCI20.6 was shown to be specific because even a large amount of antibody (75 μg) did not inhibit the binding of mumps, parainfluenza type 1 or parainfluenza type 2 virus, as measured by assaying rosette formation with guinea-pig erythrocytes (Table 1).

To investigate whether MAb MCI20.6 could block MV infection, cells were incubated with the antibody prior to infection with MV and cell survival was assayed by the colorimetric MTT test. Since HeLa and Raji cells multiply quickly and c.p.e. is not very pronounced at a low m.o.i., we used the Jurkat cell line which, when infected at 0.1 p.f.u./cell, shows cell mortality 6 days after infection.

Incubation of Jurkat cells with MAb MCI20.6 prior to MV infection prevented cell death at day 6. This protection correlated with the amount of antibody added. Maximal MV cell protection occurred with 10 to 100 μg/ml of MAb MCI20.6, at which cell viability was 50 to 60% that of uninfected cells (Fig. 3).
**MAb MCI20.6 specifically stains human and Vero cells**

To determine whether MAb MCI20.6 is specific for human cells, we analysed its binding to different cell lines by flow cytometry. Purified MAb MCI20.6 strongly stained three different human cell types (epithelial, B and T cells), bound poorly to Simian Vero cells and did not bind at all to murine cell lines (Fig. 4). Binding of MAb MCI20.6 to HeLa cells was unchanged when 5, 15 or 30 μg antibody was used (Fig. 4a; data not shown). However, MAb MCI20.6 binding to Vero cells increased with antibody concentration and 15 to 30 μg antibody was necessary to obtain a significant fluorescence intensity (Fig. 4e, f). An unrelated antibody was used as a negative control (Fig. 4d). Thus, MV and MAb MCI20.6 have the same specific binding properties with cell lines of different origins.

**Immunoprecipitation of 57K and 67K proteins with MAb MCI20.6**

To identify the protein recognized by MAb MCI20.6, immunoprecipitation studies on human, simian and murine fibroblast L cells were undertaken. SDS–PAGE of immunoprecipitates from[^35]S]methionine/cysteine-labeled HeLa cell extracts revealed proteins with approximate Mr of 57K and 67K (Fig. 5a, lane 1). These proteins were not immunoprecipitated by normal mouse serum, nor from mouse L cell extracts (Fig. 5a, lanes 2, 3 and 4). To confirm that these proteins were in their mature form and present on the cell surface,[^125]I surface-labelled cells were solubilized and subjected to immunoprecipitation and SDS–PAGE.[^125]I-labelled HeLa cell extracts contained proteins with approximate Mr of 57K and 67K (Fig. 5b, lane 1). Immunoprecipitates of Raji cell extracts contained a more diffuse band between 57K and 67K, and Jurkat cell extracts gave a band migrating similarly to the lower Mr protein immunoprecipitated from HeLa cells, at 57K (Fig. 5b, lanes 3 and 5). However, MAb MCI20.6 did not recognize any labelled protein on the surface of mouse L cells (Fig. 5b, lane 7). The same profiles were observed when SDS–PAGE was done under non-reducing conditions without 2-mercaptoethanol (data not shown). Immunoprecipitation of labelled Vero cell extracts with MAb MCI20.6 revealed a 67K band which could be detected only when a greater amount of antibody was used, and after overexposure of the autoradiograph. This band was specifically immunoprecipitated; it was not present in the background material precipitated by normal mouse serum (Fig. 5b, lanes 8 and 9).
Fig. 5. SDS-PAGE of immunoprecipitation of extracts of different cell lines with MAb MCI20.6. Cells were lysed in 1% NP40 lysis buffer, and immunoprecipitation with MAb MCI20.6 (lanes 1, 3, 5, 7 and 8) or normal mouse serum (lanes 2, 4, 6 and 9) was performed on (a) HeLa (lanes 1 and 2) and L cells (lanes 3 and 4) metabolically labelled with [3sS]methionine/cysteine, or (b) on HeLa (lanes 1 and 2), Raji (lanes 3 and 4), Jurkat (lanes 5 and 6), L (lane 7) and Vero cells (lanes 8 and 9) surface-labelled with 125I. Samples were electrophoresed on 8% polyacrylamide gels.

Fig. 6. SDS-PAGE of immunoprecipitates of HeLa cell extracts treated with N-glycosidase F. HeLa cell immunoprecipitates were denatured and treated with 1 U N-glycosidase F for 18 h at 37 °C. Treated (lane 1) and untreated immunoprecipitates (lane 2) were analysed on 8% polyacrylamide gels.

57K and 67K glycoproteins may share a 43K protein core

To determine whether the heterogeneity in Mr, observed in HeLa cell immunoprecipitates is due to differently glycosylated forms of one protein, the immunoprecipitates were treated with N-glycosidase F, which removes N-linked sugars by hydrolysing the bond between asparagine and the innermost N-acetylglucosamine. SDS-PAGE showed that after the treatment one major band at 43K and two minor bands of slightly higher Mr were visible (Fig. 6, lane 1). Immunoprecipitates subjected to the same treatment without glycosidase contained the 57K and 67K proteins only. The two minor bands observed after N-glycosidase treatment may correspond to only partially digested glycoproteins.

Discussion

In this study, using two independent virus binding techniques, we have demonstrated that, in agreement with Valdimarsson et al. (1975), MV specifically binds to human and simian cells, but not to murine cells. This MV binding activity is destroyed by proteases, and its re-expression is inhibited by cycloheximide and tunicamycin. This strongly supports the hypothesis that MV binds to a surface glycoprotein component as has been suggested previously (Krah, 1989; Valdimarsson et al., 1975). We have isolated a MAb (MCI20.6) which exhibits all the properties expected of an anti-MV receptor antibody: (i) inhibition of MV binding to human cells, (ii) inhibition of an MV-like c.p.e. on HeLa cells induced by simultaneous expression of H and F glycoproteins after infection with a double VV recombinant and (iii) inhibition of c.p.e. after MV infection of human Jurkat cells. This antibody only inhibits cell-cell fusion induced by the VV recombinant and does not inhibit the syncitium formation induced by a VV recombinant expressing the human T cell lymphotrophic virus type 1 glycoprotein (L. Gazzolo, personal communication). MAb MCI20.6 recognized a cell surface protein.
present on human and Vero cells but not on murine cells and, depending on the cell type used, immunoprecipitated one or two glycoproteins in the Mr range of 57K to 67K. Treatment of immunoprecipitates of HeLa cells with \( N \)-glycosidase F suggested that a single 43K core protein may be differentially glycosylated to give the 57K and 67K proteins.

MAb MCI20.6 showed a binding profile similar to that of MV, i.e. binding to various human and simian cells and not to murine cells. MV infects a number of tissues in vivo, infection beginning in the respiratory tract and disseminating into circulating lymphoid cells (Yamanouchi et al., 1973). We can thus propose that the MV receptor has a wide if not ubiquitous distribution on human tissue types. MAb MCI20.6 recognized one or two proteins of 57K and/or 67K depending on the cell type. HeLa cells express both glycoproteins, whereas Jurkat and Raji cells express only the 57K and 67K glycoproteins respectively. As these three cell lines bind MV equally well, the presence of at least one of the proteins favours the idea that they are different forms of the same protein. This is supported by preliminary data showing that the two glycoproteins may share a common 43K protein core. The possible existence of different \( N \)-glycosylated forms suggests that carbohydrate moieties are not involved in MV binding to its receptor. However addition of complex carbohydrates does seem important for the folding and/or transport of the protein to the cell surface, as shown by the inhibition of MV receptor regeneration with tunicamycin.

So far we have no evidence supporting the existence of the putative MV receptor in oligomeric form. Electrophoretic profiles are identical when immunoprecipitates are analysed by SDS–PAGE in the absence of 2-mercaptoethanol or without boiling (data not shown), i.e. no higher \( M_r \) species is observed. This suggests that if a oligomer exists, it is not held together by a disulphide bond and it is sensitive to SDS.

MAb MCI20.6 was raised against human cells and binds poorly to Simian Vero cells, as shown by flow cytometric analyses. Accordingly, MAb MCI20.6 weakly immunoprecipitates a 67K glycoprotein from these cells, which is revealed only after long exposure of the autoradiograph. The increased amount of MAb necessary to bind to Vero cells, together with its poor ability to immunoprecipitate a 67K band, could be due to either low expression of the protein by Vero cells or a lower binding affinity of MAb MCI20.6 for the Vero cell protein. Since Vero cells bind MV and are infected as well as the human cell lines used, the latter hypothesis is more plausible. It is possible that MAb MCI20.6 binds to an epitope encompassing a part of the virus-binding site and an adjacent region. The whole epitope may be present on human cells, and may be different in Vero cells. The virus-binding site is likely to be conserved, but the adjacent region included in the epitope may be slightly different in Vero cells, and thus would be recognized poorly by the MAb. MCI20.6 does not inhibit MV haemagglutination of VMEs either because of the large number of VMEs used or because it recognizes the VME surface receptor only weakly or not at all. It should be stressed that in the case of MV haemagglutination, the receptor on the erythrocytes may not necessarily be the same as that on the host cell because certain anti-H protein MAbs neutralize virus infectivity, but do not inhibit haemagglutination (Giraudon & Wild, 1985). Therefore the erythrocyte structure involved in haemagglutination may be different to the cellular receptor, and not recognized by MAb MCI20.6.

A crucial property of an anti-receptor antibody is that it blocks attachment and subsequent infection by the virus. MAb MCI20.6 inhibits MV binding to human cells in a dose-dependent fashion, with half the maximal inhibition at 10 \( \mu \)g of antibody for 10^5 cells; 100 \( \mu \)g did not block the binding of three other paramyxoviruses. This shows that MAb MCI20.6 is able to recognize a cell surface component involved in the initial attachment of MV virions. The epitope recognized may be the entire virus binding site or a part of it. As expected, the binding of MAb MCI20.6 to the cell surface does inhibit MV infection of Jurkat cells.

Very few attempts at characterizing the MV receptor have been published (Harrowe et al., 1990; Krah & Choppin, 1988; Valdimarsson et al., 1975). Interestingly, Harrowe et al. (1990) have recently established a correlation between MV–cell fusion and substance P neuropeptide (SP) binding to human cells, by inhibiting MV–target cell fusion with SP. They also have results suggesting that the SP receptor is a 58K protein. The relationship between our 57K to 67K glycoprotein isomorphs and their 58K protein, which may correspond to the SP receptor, are currently under investigation.

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