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Human Membrane Cofactor Protein (CD46) Acts as a Cellular Receptor for Measles Virus

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A monoclonal antibody (MCI20.6) which inhibited measles virus (MV) binding to host cells was previously used to characterize a 57- to 67-kDa cell surface glycoprotein as a potential MV receptor. In the present work, this glycoprotein (gp57/67) was immunopurified, and N-terminal amino acid sequencing identified it as human membrane cofactor protein (CD46), a member of the regulators of complement activation gene cluster. Transfection of nonpermissive murine cells with a recombinant expression vector containing CD46 cDNA conferred three major properties expected of cells permissive to MV infection. First, expression of CD46 enabled MV to bind to murine cells. Second, the CD46-expressing murine cells were able to undergo cell-cell fusion when both MV hemagglutinin and MV fusion glycoproteins were expressed after infection with a vaccinia virus recombinant encoding both MV glycoproteins. Third, M12.CD46 murine B cells were able to support MV replication, as shown by production of infectious virus and by cell biosynthesis of viral hemagglutinin after metabolic labeling of infected cells with [^{35}S]methionine. These results show that the human CD46 molecule serves as an MV receptor allowing virus-cell binding, fusion, and viral replication and open new perspectives in the study of MV pathogenesis.

Measles virus (MV) is responsible for an acute respiratory tract infection and immunodepression and is among the primary causes of infant mortality in developing countries (3). On rare occasions, MV infection can lead to severe acute and chronic neurological disorders known as subacute sclerosing panencephalitis and measles inclusion body encephalitis. The mechanisms of MV pathogenesis are not well elucidated, and a better understanding of MV entry into host cells is crucial.

The first step in viral infection is attachment of the virus to a cell surface receptor. The importance of this step has been shown with the identification of cell receptors for a number of human pathogenic viruses, including poliovirus, rhinovirus, Epstein-Barr virus, and human immunodeficiency virus (HIV) (7, 9, 11, 14, 19, 22, 26). Entry of MV, like that of other enveloped viruses, occurs by binding to cell surface receptors followed by fusion of viral and cell membranes either at the plasma membrane, for viruses such as MV and HIV, or after internalization into acidic endosomes, for other enveloped viruses such as influenza virus. MV, a member of the *Morbillivirus* genus in the *Paramyxoviridae* family, is a negative-strand enveloped single-stranded RNA virus containing two membrane glycoproteins, the hemagglutinin (H) glycoprotein and the fusion (F) glycoprotein. During MV infection of human cells, the two MV envelope glycoproteins are expressed at the surface of the infected cells and induce cell-cell fusion leading to syncytium formation, characteristic of measles cytopathic effect. It has been shown for various paramyxoviruses that both the H and F proteins are necessary to produce syncytia. In the case of MV, after infection of human cells with a vaccinia virus recombinant encoding the MV H and F glycoproteins (VV-H/F), characteristic MV-like syncytia are observed. Further, it was shown that a cellular component correlating with the permissiveness of a cell line to MV infection is also necessary to obtain MV-like cytopathic effect (28).

MV exhibits a very limited host range; humans are the only known reservoir, although the virus can infect some monkey species. We thus speculated that the receptor could be important in determining the limited tropism of MV and could also be important in MV H/F-induced cell-cell fusion. We previously isolated a monoclonal antibody (MAb), called MCI20.6, for its ability to block VV-H/F-induced syncytium formation. MCI20.6 blocked MV binding and infection and recognized a 57- to 67-kDa membrane glycoprotein present in human and simian cells but not in cell lines of species nonpermissive to MV (20).

In this work, N-terminal amino acid sequencing identified gp57/67 as human membrane cofactor protein (CD46). CD46 is a member of the regulators of complement activation gene cluster and is involved in protecting cells from autologous complement-mediated damage (16, 17). Transfection of murine cell lines, which are nonpermissive to MV, with a CD46 cDNA has shown that CD46 acts as an MV receptor allowing virus binding, fusion, and replication.

MATERIALS AND METHODS

Cells and virus. All cell lines were grown in Dulbecco's modified Eagle's medium supplemented with 6% fetal calf serum, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 2 mM glutamine, 5×10^{-5} M 2-mercaptoethanol, and 50 μg of gentamicin per ml. G418 (1 mg/ml) was added to medium for transfected cells selected for neomycin resistance, and hypoxanthine-aminopterin-thymidine was added for cells selected for aminopterin resistance. Human epithelial (HeLa and HeLaT4) and T-cell (Jurkat) lines and murine fibroblast (L) and B-cell (M12) lines were used in the experiments. The Hallé strain of MV was grown on Vero cells and purified on a discontinuous sucrose gradient as described previously (20).

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Affinity chromatography and microsequencing. Purified MAb MCI20.6 was coupled to CNBr-activated Sepharose 4B (Pharmacia) according to Pharmacia's instructions, and the column was equilibrated with 0.2% Nonidet P-40 in phosphate-buffered saline (PBS). Cells were lysed in 1% Nonidet P-40 in 10 mM Tris hydrochloride (pH 7.8)–0.15 M NaCl–0.5 M KCl–5 mM EDTA. The lysate was batch incubated with the resin before transfer to a column. Unadsorbed protein was washed from the column with 0.2% Nonidet P-40 in PBS. Elution of bound protein was carried out with 0.2 M glycine (pH 2.8)–0.15 M NaCl–0.2% Nonidet P-40. A fraction of the cells were surface labeled with ^{125}I as a tracer of gp57/67-containing fractions.

The fractions obtained were concentrated, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred onto a polyvinylidene difluoride filter. Coomassie blue R-50 staining and autoradiography localized the protein, which was cut from the filter and microsequenced with the help of M. Marie Boutillon and M. Van der Rest (Institut de Biologie et de Chimie des Proteines, Lyon, France).

Transfections. Fibroblasts (Ltk^- cells) and B-cell lymphoblastoma (M12) cells were transfected with a recombinant expression vector containing the cDNA isoform of CD46 containing exons 1 to 6, 9 to 12, and 14 (6) by the $CaPO_4$ technique (5). An *Eco*RI 1.5-kb fragment was subcloned downstream of the β-actin promoter into the *Eco*RI site of the pIRV expression vector coding for neomycin resistance (1). The cells were then either transfected with pIRV alone (M12 cells) or cotransfected with plasmid pAG0 containing the herpesvirus thymidine kinase gene (Ltk^- cells). Drug-resistant cells underwent four cycles of sorting on a FACStar cell sorter (Becton Dickinson) on the basis of their expression of CD46 recognized by MAb MCI20.6. A fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin was used as the second antibody. The cells were then cloned by limiting dilution.

Fluorescence analyses and virus binding assay. For CD46 detection, cells were incubated for 30 min at 4°C with MCI20.6, washed, and then incubated for 30 min with an FITC-conjugated goat anti-mouse immunoglobulin. The incubations were carried out in PBS containing 1% bovine serum albumin and 0.1% NaN_3. Flow cytometry was then performed. For the virus binding assay, 2×10^5 cells were incubated for 2 h at 4 or 37°C with 50 hemagglutinin units of the Hallé strain of MV. Cells were washed three times and then incubated with clone 55, an anti-MV H antibody (10). The cells were washed prior to incubation with an FITC-conjugated goat anti-mouse immunoglobulin. All of the flow cytometric analyses were carried out on a FACStar (Becton Dickinson). The hemagglutinin units were determined by hemagglutination of vervet monkey erythrocytes as described previously (20).

Fusion assay and virus infectivity determination. For the fusion assay, the vaccinia virus recombinant VV-H/F was used (27). Human cells were infected with VV-H/F at 0.1 PFU per cell, and murine cells were infected at 1 PFU per cell. The cytopathic effect was observed 18 h postinfection (p.i.) and photographed on an Olympus inverted microscope.

For virus infectivity determination, cells were infected with MV at 1 PFU per cell for 6 h at 37°C, washed three times, and put into culture. Two to three days p.i., cells were centrifuged and the supernatant was assayed for plaque formation on Vero cells. For this assay, 5×10^5 Vero cells were plated in six-well dishes and incubated with 10-fold dilutions of infected-cell supernatants. Three to four days later, cells were fixed in 10% formalin and stained in a methylene blue solution, and plaques were counted. Background was determined from supernatants taken 1 h after cell washes.

a.

NH2- X -glu-glu-pro- X - X -phe-glu-ala-met-glu-leu-ile-gly-lys

b.

NH2-cys-glu-glu-pro-pro-thr-phe-glu-ala-met-glu-leu-ile-gly-lys

FIG. 1. The N-terminal amino acid sequence of gp57/67 immunopurified with MAb MCI20.6 corresponds to the N-terminal sequence (minus the signal sequence) of human membrane cofactor protein (CD46 [17]). The 15 N-terminal amino acids of gp57/67 (a; the X indicates nondetermined amino acids) are aligned with the N-terminal amino acids of the CD46 molecule (b; underlined amino acids correspond to the unknown residues in the gp57/67 sequence).

Cell labeling and immunoprecipitation. For surface iodination of cells, the glucose-lactoperoxidase technique was used as described previously (12). For metabolic labeling, cells were incubated in labeling medium (methionine- and cysteine-free medium containing dialyzed fetal calf serum) for 1 h prior to a 2-h incubation in labeling medium containing 50

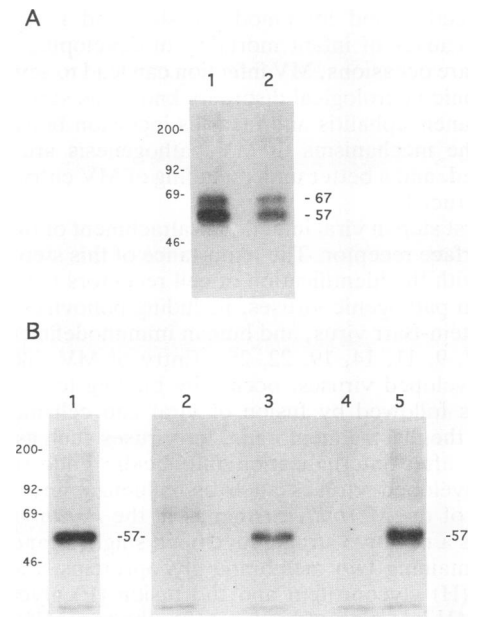

FIG. 2. Expression of the CD46 molecule on human cells and CD46 transfectants. (a) MCI20.6 and GB24 immunoprecipitates have identical electrophoretic profiles. ^{125}I-surface-labeled polypeptides from HeLa cell extracts were immunoprecipitated with MCI20.6, an anti-MV receptor antibody (lane 1), or GB24, a CD46 antibody (lane 2). (b) L.CD46 and M12.CD46 transfectants express CD46. M12.CD46 (lane 1), M12 (lane 2), L.CD46 (lane 3), Ltk^- (lane 4), and Jurkat (lane 5) cells were surface labeled with ^{125}I and immunoprecipitated with MCI20.6. SDS-PAGE analysis of CD46 from Jurkat cells yields one diffuse 57-kDa band, while that of HeLa cell lysates yields two diffuse bands. This difference is due to alternate splicing of the mRNA and to differences in glycosylation (25). The transfected cells express only one isoform. Sizes are indicated in kilodaltons.

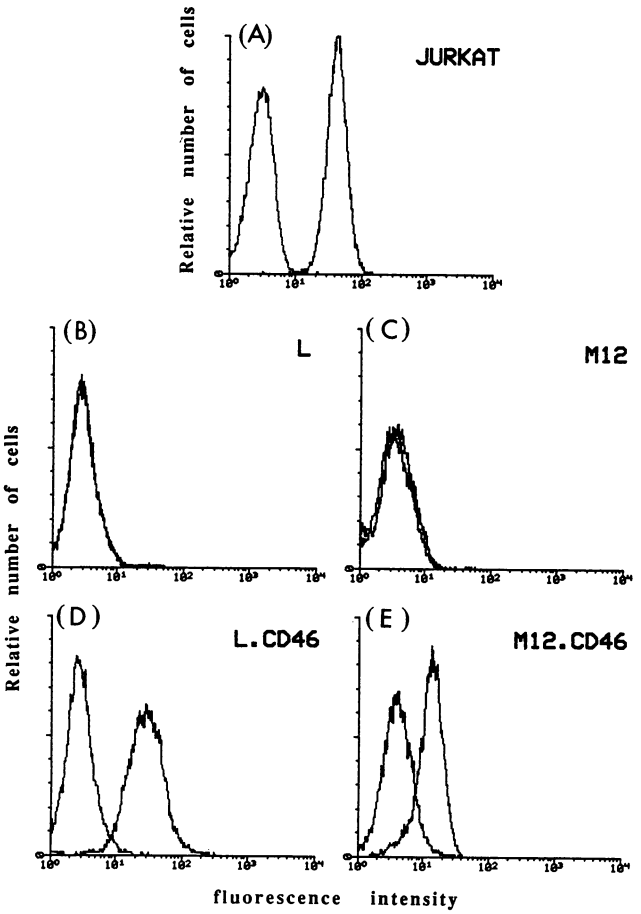

FIG. 3. L.CD46 and M12.CD46 express CD46 at the cell surface. Flow cytometry shows MCI20.6 staining of Jurkat (A), L.CD46 (C), and M12.CD46 (E) cells but not of Ltk$^-$ (B) or M12 (D) cells. Control antibody is represented by the left histogram, and specific CD46 staining is represented by a displacement to the right.

μCi of Expres^{35}S (ICN Biomedicals Inc.) per 10^6 cells. Cells were then washed in PBS and lysed in 1% Nonidet P-40-containing lysis buffer. Immunoprecipitation from the cell lysates was carried out with MCI20.6 or GB24 (8, 24) (both CD46 antibodies) or clone 55 anti-MV H antibody (10) followed by incubation with protein A-Sepharose (Pharmacia) previously coupled to goat anti-mouse immunoglobulin G (BioSys). The immunoprecipitates were then washed, and proteins were separated on an 8 or 10% polyacrylamide gel in reducing conditions, dried, and autoradiographed.

RESULTS

N-terminal amino acid sequencing identifies gp57/67 as the CD46 molecule. MAb MCI20.6 inhibits MV binding to target cells and recognizes a glycoprotein with a molecular mass of between 57 and 67 kDa. This glycoprotein was purified from Jurkat cells by immunoaffinity chromatography using MAb MCI20.6, and the first 15 amino acids were determined by N-terminal amino acid sequencing. Figure 1a shows that the amino acid sequence obtained corresponds to the N-terminal sequence of human membrane cofactor protein (CD46 [17]) (Fig. 1b). Immunoprecipitation of ^{125}I-labeled HeLa cells with MCI20.6 and GB24 (a CD46 antibody) produced iden-

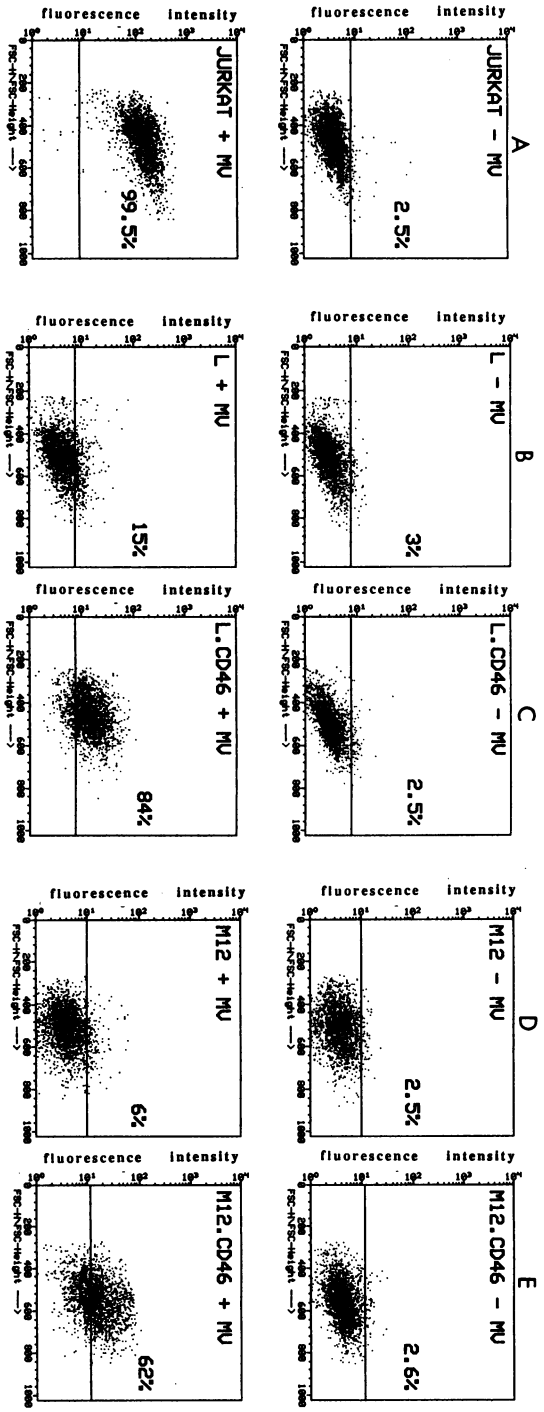

FIG. 4. Expression of human CD46 in murine cells confers MV-binding capacity. MV binds to Jurkat (A), L.CD46 (C), and M12.CD46 (E) cells but not to Ltk$^-$ (B) or M12 (D) cells. Cells were incubated with MV and washed, and flow cytometry was carried out with an anti-MV hemagglutinin antibody. The percentage of cells above the cursor is indicated. The cursor is set on the dot plots of the control cells (without virus) treated with an anti-MV H antibody (represented in the upper graph). The background observed in the absence of MV is approximately 3%.

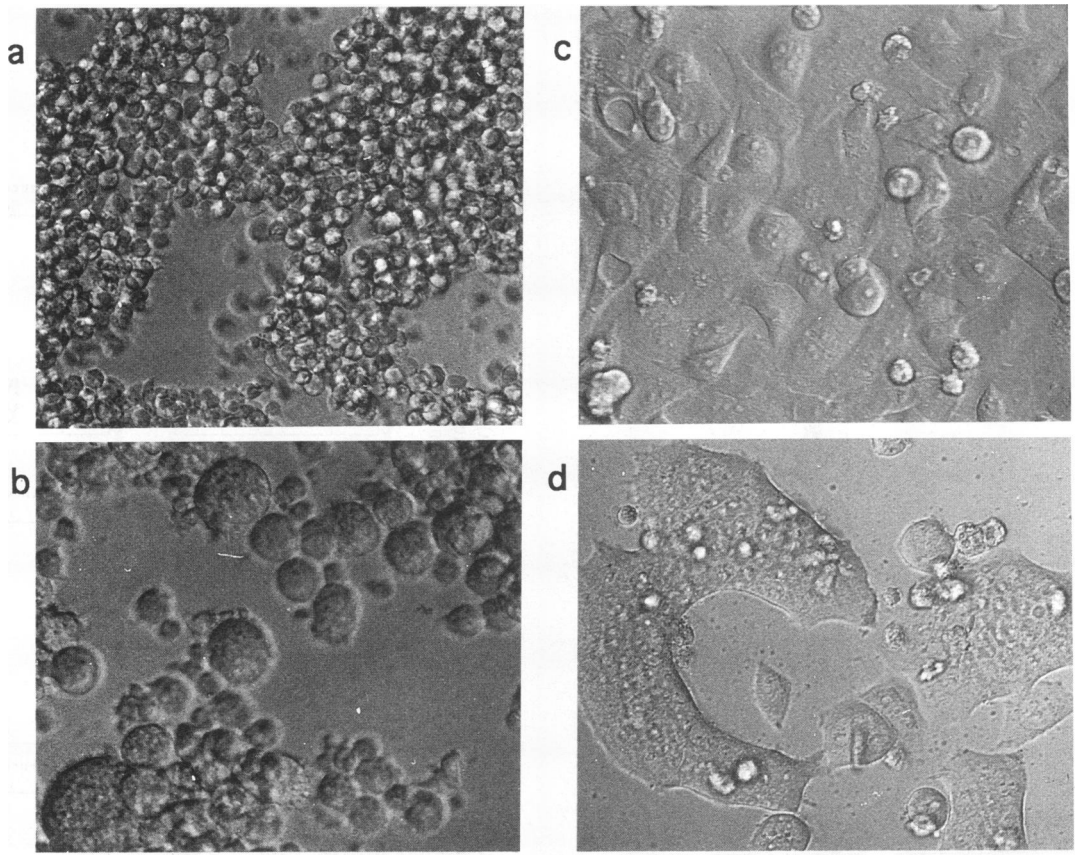

FIG. 5. Expression of human CD46 in murine cells confers cell-cell fusion capacity after infection with VV-H/F. Cells were infected with VV-H/F and observed under a light microscope 18 h p.i. Jurkat (b), HeLaT4 (d), L.CD46 (h), and M12.CD46 (l) cells underwent syncytium formation, and Ltk$^-$ (f) and M12 (j) cells did not. Uninfected counterparts are shown in panels a, c, e, g, i, and k.

tical electrophoretic profiles, providing further evidence that MCI20.6 recognized the CD46 antigen (Fig. 2A). Expression of CD46 is complex, and because of alternate splicing events and variation in N and O glycosylation, the CD46 gene gives rise to proteins of heterogeneous M_r (25).

Stable expression of CD46 in murine cells enables them to bind MV. We previously showed that murine cells were not able to bind MV. Thus, to determine whether the CD46 molecule could confer MV-binding capacity to cells nonpermissive for MV infection, murine fibroblast (Ltk$^-$) and B-cell (M12) lines were transfected with CD46 cDNA under the control of the β-actin promoter; stable cell lines expressing CD46 were obtained, as shown by immunoprecipitation of the CD46 molecule with MAb MCI20.6 from ^{125}I-surface-labeled transfected cells and by flow cytometry (Fig. 2B and 3). These transfected cell lines, L.CD46 and M12.CD46, were incubated with various amounts of MV and assayed for MV binding. MV bound to human Jurkat (99%), L.CD46 (84%), and M12.CD46 (63%) cells (Fig. 4, lower graphs) but not to the untransfected L or M12 cells. The higher background level observed for MV binding to L cells likely represents nonspecific attachment due to the adherent properties of these cells. Binding of MV to L.CD46 cells was thus reduced to 62%. MV binding to human, L.CD46, and M12.CD46 cells occurred in a dose-dependent manner (data not shown).

CD46-expressing murine cells can undergo H/F-induced cell-cell fusion. Expression of MV H and F by infection of human cells with the vaccinia virus recombinant VV-H/F induces MV-like syncytium formation. Murine cells, however, do not exhibit cell-cell fusion after infection with VV-H/F (28). To determine whether CD46, besides allowing MV binding to murine cells, could also confer cell-cell fusion and thus possibly virus-cell fusion, L.CD46 and M12.CD46 cells were infected with VV-H/F and examined for syncytia. VV-H/F-infected Jurkat (Fig. 5b) and HeLaT4 (Fig. 5d) cells and the CD46-transfected murine cells (Fig. 5h and j) produced syncytia, whereas the untransfected L and M12 cells did not (Fig. 5f and j). Infection of cells with wild-type vaccinia virus did not result in syncytia in any of the cells tested (data not shown). The extent and appearance of cell-cell fusion cannot be quantitated with the VV-H/F system, since infection efficiencies for different cell types as well as the relative fragility of the cells vary. For example, adherent cells produce more extensive syncytia. To determine whether MV infection could also lead to cell-cell fusion, cells were infected with MV at 1 PFU per cell; 72 h p.i., syncytium formation was observed in M12.CD46 cells but not in L.CD46 cells. This result suggested that infected M12.CD46 cells were synthesizing viral glycoproteins whereas infected L.CD46 cells were not.

A CD46-expressing murine B-cell line can support MV replication. Since both types of CD46-transfected murine cells could bind MV and undergo fusion after expression of H and F by VV-H/F but only M12.CD46 cells underwent fusion after MV infection, we tested the MV-infected

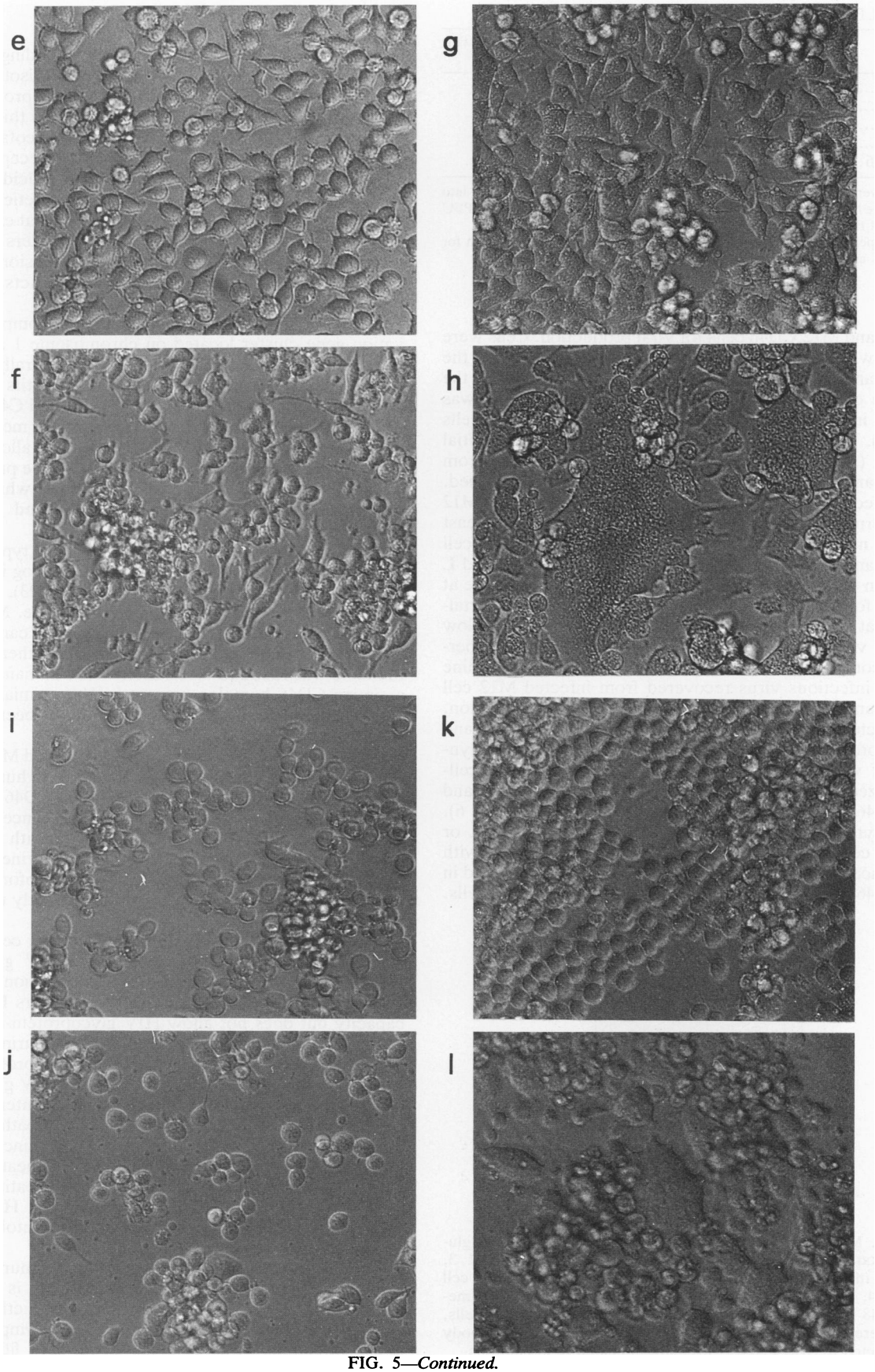

FIG. 5—*Continued.*

TABLE 1. Replication of MV in human and mouse cells[a]

Cell type	MV produced in supernatant (PFU/ml) at 72 h p.i.[b]
Jurkat	5×10^5
L	20
L.CD46	0
M12	600
M12.CD46	5×10^4

[a] Cells were infected with 1 PFU per cell for 6 h, washed, and put into culture. One hour later, supernatants were tested for MV. The number of PFU observed at this time (1 h p.i.) was 40 in all cases.

[b] The supernatant was taken prior to extensive cell mortality, at 48 h for Jurkat cells and 72 h for murine cells.

L.CD46 and M12.CD46 cells for viral production. Cells were infected with MV at 1 PFU per cell, and 48 or 72 h p.i., the supernatants of these MV-infected cells were assayed for the presence of infectious viral particles. MV replication was detected in the supernatants of Jurkat and M12.CD46 cells (Table 1). The PFU due to virus carried over from the initial infection (approximately 40 PFU/ml) was determined from supernatant taken 1 h after infected cells had been washed. Little infectious virus could be recovered from infected M12 cell supernatants, and in four separate experiments, at least 100-fold more virus was recovered from M12.CD46 cell supernatants. No virus could be detected in MV-infected L cells or in L.CD46 supernatants. These assays were done at 48 h p.i. for Jurkat cells (i.e., prior to extensive cell mortality) and at 72 h p.i. for murine cells. At 48 h p.i., only low levels of virus were detected in infected M12.CD46 supernatants compared with levels found at 72 h p.i. To determine whether infectious virus recovered from infected M12 cell supernatants was carried over from the initial infection, MV-infected cells were metabolically labeled and lysed, and immunoprecipitation was carried out to detect cell biosynthesis of viral H glycoprotein. Figure 6 shows that cell-synthesized labeled MV H is present in Jurkat and M12.CD46 cells but not in M12 cells (lanes 2, 4, and 6). Newly synthesized MV H could not be detected in L or L.CD46 cells (data not shown). Thus, in agreement with MV-induced fusion results, viral replication was observed in M12.CD46 cells and not in L.CD46 or parental murine cells.

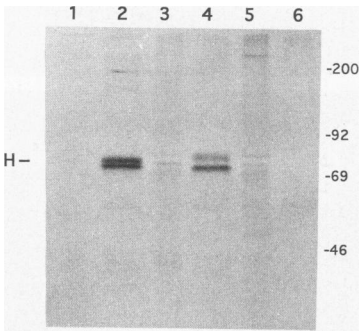

FIG. 6. MV-infected M12.CD46 cells synthesize viral hemagglutinin. Jurkat, M12.CD46, and M12 cells were uninfected (lanes 1, 3, and 5) or infected with 1 PFU of the Hallé strain of MV per cell (lanes 2, 4, and 6). After metabolic labeling of cells with [^{35}S]methionine 48 h p.i. for Jurkat cells and 72 h p.i. for murine cells, lysates were immunoprecipitated with clone 55 anti-MV H antibody and subjected to SDS-PAGE. Sizes are indicated in kilodaltons.

DISCUSSION

A MAb (MCI20.6) which inhibits MV binding, syncytium formation, and infection was previously isolated in our laboratory (20). MCI20.6 recognizes a glycoprotein of 57 to 67 kDa (gp57/67), depending on cell type. In this report, we have identified gp57/67 as human membrane cofactor protein (CD46) and shown that it acts as a cellular receptor for MV. In addition to identical N-terminal amino acid sequences, gp57/67 and CD46 have identical electrophoretic profiles and M_r heterogeneity. We have further shown that expression of CD46 in nonpermissive murine B cells renders them sensitive to MV binding and MV H/F induced fusion and allows viral replication, thus confirming that CD46 acts as a cellular receptor for MV.

CD46 is a member of the regulators of complement activation gene cluster located on chromosome 1 at q3.2 (17). The members of this gene cluster are structurally related, as they all possess 60-amino-acid repeating motifs called short consensus repeats, and they all bind C3b and C4b (reviewed in reference 15). CD46 has cofactor activity, meaning that it binds to membrane-bound C3b and C4b and allows C3b and 4b to be degraded by factor I, a plasma serine protease (15). CD46 thus provides a control mechanism which protects cells from autologous complement-mediated self-damage (16).

CD46 is expressed on most human cell types with the exception of erythrocytes. The CD46 homolog is, however, expressed on certain primate erythrocytes (23). This finding is in accordance with the MV binding profile. MV binds to most human tissues except erythrocytes and can agglutinate erythrocytes of certain primate species. Furthermore, MAb MCI20.6 was found to cross-react with a simian gp67, likely a simian CD46 homolog present in Vero simian cells (20). The murine homolog of CD46 has not yet been characterized.

Expression of CD46 in murine cells allowed MV to bind to the cells but in a less efficient manner than to human cells. It has been shown that alternate splicing of CD46 mRNA can give rise to 14 isoforms of CD46 (25). Since the CD46-expressing murine cells were transfected with one cDNA, the weaker binding to CD46-expressing murine cells could reflect the importance of certain CD46 isoforms in MV-binding efficiency. This hypothesis is currently under investigation.

The expression of human CD46 in mouse cells was also sufficient to render them sensitive to MV glycoprotein-induced fusion. In the case of HIV, expression of the HIV receptor, human CD4, in mouse cells confers HIV-binding capacity but does not allow HIV glycoprotein-induced cell fusion (18). This finding suggests that the murine cells used may lack a factor necessary for HIV glycoprotein-induced cell fusion but possess one necessary for MV glycoprotein-induced cell fusion. HIV has been shown to enter $CD4^-$ cells by a complement component C3-dependent pathway (4, 13). This can be excluded in the case of MV since all of our experiments were done in the presence of heat-inactivated heterologous serum (fetal calf serum). Heating destroys C3-binding activity, and it was shown for HIV that the C3-mediated pathway of entry requires autologous non-heated serum (13).

Our results show that depending on the murine cell line used, expression of CD46 in murine cells is not always sufficient to allow MV replication and production of infectious virus. Indeed, M12.CD46 (a B-cell lymphoma) produced infectious virus whereas L.CD46 (a fibroblast cell

line) did not. Furthermore, cell-cell fusion after MV infection was observed only for M12.CD46 cells, since these infected cells were synthesizing viral glycoproteins. To induce fusion between L.CD46 cells, H and F had to be expressed by a vaccinia virus recombinant. The absence of MV glycoprotein synthesis and of viral replication in L.CD46 cells may be due to a lack of a cellular factor necessary for MV replication in these cells, since after microinjection of L cells with MV nucleocapsids, no virus replication was detected (2). Whether the difference in replication ability between M12.CD46 and L.CD46 cells is related to cell type, rate of cell proliferation, or simply the nature of the cell lines is currently being studied.

Work on viral receptors has revealed a number of viruses that use receptors of the immune system: CD4 for HIV (7, 14), CR2 for Epstein-Barr virus (9, 22), and ICAM-1 for major group rhinoviruses (11, 26). MV also uses a receptor involved in the immune system and more specifically in the complement pathway. Certain cytopathic effects of MV infection could thus be related to the influence of the virus on complement regulation via CD46. We have previously shown that surface expression of CD46 (published as gp57/67) is downregulated by MV infection and more precisely by an interaction with MV H which triggers internalization of surface CD46 (21). The significance of MV H-induced downregulation of surface CD46 in MV pathogenesis is unknown. We are currently investigating the consequences of MV infection on the sensitivity of cells to complement-mediated lysis. This could prove critical in determining whether alterations in complement regulation play a role in the pathogenesis of acute and chronic MV infection leading to MV-induced immunodepression and neurological complications. Finally, with very few animal models available to study MV pathogenesis, the identification of CD46 as a cellular receptor for MV opens new perspectives in developing transgenic animal models with which to study MV pathogenesis and the immune response relevant to vaccination.

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