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Multiple isoforms of CD46 (membrane cofactor protein) serve as receptors for measles virus

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ABSTRACT **Measles virus (MV) causes a productive infection in humans and certain simian hosts. Rodent cells such as Chinese hamster ovary (CHO) and murine cell lines normally resist MV infection. Human CD46, or membrane cofactor protein, a complement regulatory protein, recently has been reported as the cellular receptor for MV. Multiple isoforms of the CD46 protein exist; four of these isoforms are commonly expressed on human cells. Expression of each of the four isoforms in CHO cells followed by exposure to MV led to the appearance of viral proteins within the cells and on the cell surface as detected by immunofluorescence. Syncytium formation also was observed in the cultures. CHO cells expressing any of the four isoforms and exposed to MV formed infectious centers when plated on Vero cell monolayers, indicating that the cells can transmit virus to uninfected cells. The murine cell line MC57 expressing the BC1 isoform of CD46 also stained positively for MV antigens and was positive in the infectious center assay after exposure to MV. Treatment of CD46-expressing cells with antibody to human CD46 inhibited MV binding in a dose-dependent manner. These observations indicate that any of the four primary isoforms of CD46 are able to serve as a receptor for MV.**

Measles virus (MV) is among the most contagious infections of humans. Despite the development and use of an effective vaccine, >1 million infants and young children die each year from measles (1). MV infects epithelial cells and cells of the immune system resulting in pneumonia, diarrhea, and profound immunosuppression leading to secondary infections. In addition, MV can cause a post-infectious hyperallergic condition, an inclusion-body encephalitis, and a persistent infection of neurons called subacute sclerosing panencephalitis (SSPE) (2). The mechanism by which MV causes immunosuppression and the pathogenesis of the acute or persistent MV infections are not well understood.

MV is a member of the genus Morbillivirus in the family paramyxoviridae. It is an enveloped virus with a single-stranded RNA genome. MV contains two membrane glycoproteins, hemagglutinin (MV-H) and fusion (MV-F). Both glycoproteins are required for infection and for MV-associated syncytium formation; it is thought that MV-H is involved in virus binding to the host cell and MV-F induces fusion of viral and host cell membranes (2, 3).

Expression of MV-H activates the alternative complement pathway, resulting in deposition of complement C3b protein on the surface of infected cells (4). CD46, or membrane cofactor protein, is a member of the regulators of complement activation gene cluster and functions to inhibit deposition of complement proteins C3b and C4b on host cells (5). Recently, CD46 has been reported to be the cellular receptor for MV (6, 7). Multiple isoforms of CD46 exist, of which four (C1, C2, BC1, and BC2) are commonly expressed on human cells. Certain tissues, however, may preferentially express different isoforms [e.g., kidney, salivary gland, and brain (8)]. CD46 isoforms primarily consist of an extracellular domain of four short consensus repeat sequences, combinations of serine/threonine-rich regions, and a variable-length cytoplasmic tail. Recently, it was shown that expression of the C2 isoform of CD46 allowed MV replication in a murine B-cell line but not in a murine fibroblast line (6). Further, expression of the C2 or BC2 isoforms on CHO cells enabled MV infection (7).

Here we document that the four common isoforms (C1, C2, BC1, and BC2) of CD46 confer MV tropism on CHO cells. These CD46-expressing cells form syncytia after exposure to MV and can transmit virus to uninfected cells. These observations indicate that multiple isoforms of CD46 function as receptors for MV.

MATERIALS AND METHODS

Cells, Media, and Virus. MV (Edmonston strain) was obtained from the American Type Culture Collection and was passaged in Vero cells. Virus stocks were generated in Vero cells infected at a multiplicity of infection (moi) of 0.3. After 48 h, cells and medium were harvested, frozen–thawed, and sonicated for 90 sec. Cell debris was removed by centrifugation and the supernatant was stored in aliquots at −70°C. CHO cells were maintained in either Ham's F-12 medium or RPMI 1640 medium. Vero cells were maintained in minimal essential medium (MEM). MC57 cells, a mouse fibroblast cell line (9), were maintained in RPMI 1640 medium. All media were supplemented with 2 mM L-glutamine and 7% (vol/vol) fetal calf serum.

CD46 Receptor Expression. The cDNA for each CD46 isoform (BC1, BC2, C1, and C2) was expressed under control of the human β-actin promoter in the vector pHβApr-1-neo (10, 11). CHO cell lines expressing CD46 isoforms and a control CHO cell line containing the CD46 cDNA in the reverse orientation have been described (11, 12). MC57 cells expressing the CD46-BC1 isoform were generated by calcium phosphate transfection of plasmid K5-23 (11, 13), followed by selection with G418 (Geneticin, GIBCO/BRL) at 0.4 mg/ml. Expression of CD46 in the MC57 cells was confirmed by immunofluorescence assay using a monoclonal antibody (GB24) against CD46 (14).

Immunofluorescence Assays. CD46-expressing or nonexpressing cells were plated on glass coverslips and infected with MV at a moi of 3. After 48 h, cells were fixed with 100% ether/95% ethanol, 1:1 (vol/vol), for 10 min followed by 95% ethanol for 20 min. After washing, cells were stained either

Abbreviations: MV, measles virus; SSPE, subacute sclerosing panencephalitis; MV-H, MV hemagglutinin; MV-F, MV fusion; moi, multiplicity of infection; STP, serines, threonines, and prolines.

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with a polyclonal human serum from an SSPE patient followed by a goat anti-human antibody conjugated to fluorescein isothiocyanate (FITC) or with mouse monoclonal antibodies to MV-H, MV-F, or nucleoprotein followed by a rat antibody to murine IgG conjugated to FITC. The origin of the monoclonal antibodies, the immunofluorescence staining procedure, and the use of the fluorescent microscope are detailed elsewhere (15, 16).

Infectious Center Assays. Cells were infected with MV at a moi of 3 and after 24 or 48 h were harvested by trypsinization, washed four times in ice-cold phosphate-buffered saline (PBS), and resuspended at serial dilutions from 2×10^6 cells per ml to 2×10^2 cells per ml. Cells were overlaid on Vero cell monolayers in 6-well tissue culture plates at 0.5 ml per well. As a control, duplicate samples were frozen–thawed and then plated, to measure the number of plaques formed by virus in the supernatant or adhering to the cells. Plates were incubated at 37°C for 1 h and then overlaid with 1× MEM/0.5% agarose. After 6 days at 37°C, monolayers were fixed in 4% (vol/vol) formaldehyde in PBS and stained with crystal violet, and plaques were counted. Uninfected cells (1×10^6 cells per well) were also plated as a control. Values obtained are expressed as number of infectious centers per number of cells plated minus the values obtained from the frozen–thawed samples.

Blocking of MV Infection with Antibody to CD46. Serial 1:5 dilutions of a polyclonal antibody to human CD46 (rabbit antiserum to recombinant soluble CD46; a gift of Cytomed, Cambridge, MA) were incubated with 5×10^4 HeLa, Vero, CHO, or CHO-CD46 BC1 cells in 100 μl. After 45 min at 37°C, MV (1.5×10^5 plaque-forming units) was added and the cells were plated on glass coverslips in 24-well plates. Cells were allowed to adhere, washed three times with PBS, and overlaid with fresh medium. After 24 h at 37°C, cells were fixed and stained for immunofluorescence as described above, by using a polyclonal human antiserum from a SSPE patient to detect MV. Fluorescence-positive cells were counted using an Olympus fluorescence microscope.

RESULTS

Expression of Each of Four Primary Isoforms of CD46 Confers Permissivity for MV on CHO Cells. MV is normally tropic for human and monkey cells, but not for cells of other species (2). To determine whether multiple isoforms of human CD46 could function as a MV receptor, CHO cells expressing the C1, C2, BC1, or BC2 isoform were exposed to MV and after 48 h were stained with a polyclonal antiserum to MV (obtained from a patient with SSPE). Fig. 1 shows examples of immunofluorescence staining of control CHO cells (not expressing CD46) and of CHO cells expressing CD46 isoforms C1, C2, BC1, and BC2. Control CHO cells show only a few scattered fluorescence-positive cells (<0.5% of the population; Fig. 1*A*), and CHO cells expressing the four isoforms (shown in Fig. 1 *B–E*) showed strong staining in multiple experiments. In a representative experiment, the isoforms showed 66%, 70%, 71%, and 73% MV-positive cells for C1, C2, BC1, and BC2 isoforms, respectively. Similar values were obtained when cells were stained and analyzed by fluorescence-activated cell sorting (data not shown). In addition to polyclonal SSPE sera, monoclonal antibodies to MV-H, MV-F, and nucleoprotein routinely stained MV-infected CD46-expressing cells, and CHO cells expressing each of four isoforms were positive for MV-H, MV-F, and nucleoprotein antigens by immunofluorescence to approximately the same extent (data not shown). The CHO cell lines described above were not clonal populations; however, one CHO cell line derived from a single G418-resistant clone

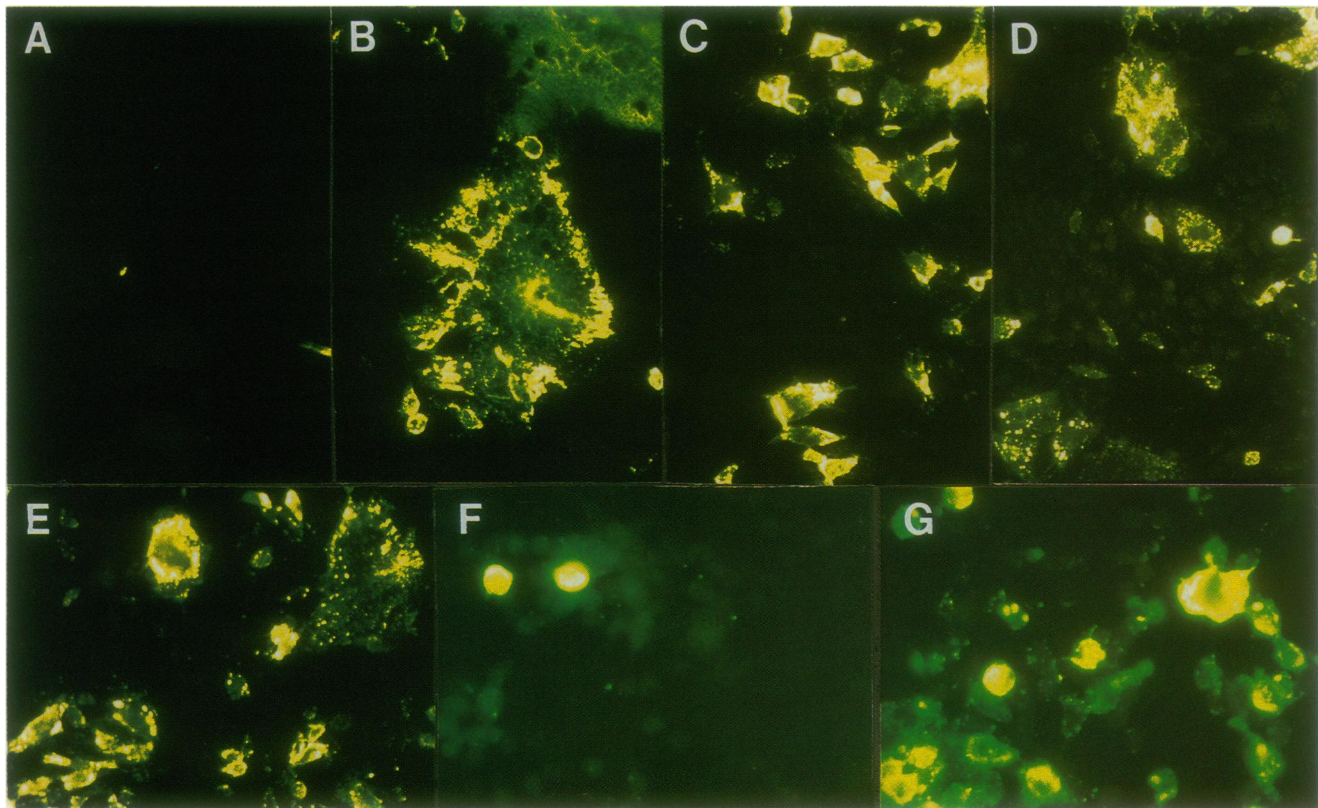

FIG. 1. Immunofluorescence staining of MV-infected CD46 receptor-expressing cells. All cells were plated on glass coverslips and infected with MV at a moi of 3. After 48 h, cells were fixed and stained with a human antiserum from a patient with SSPE. (*A*) CHO control cells infected with MV. (*B–E*) CHO cells expressing CD46-C1 isoform (*B*), CD46-C2 isoform (*C*), CD46-BC1 isoform (*D*), or CD46-BC2 isoform (*E*) and infected with MV. (*F*) MC57 cells infected with MV. (*G*) MC57-CD46 cells (BC1 isoform) infected with MV. (×200.)

(BC1 isoform) was tested and was positive for MV antigens in ≈98% of the cells. As expected, MV proteins were detected in the cytoplasm. MV-H and MV-F were also found on the surface of infected living CHO cells expressing CD46 when such cells were evaluated by fluorescence-activated cell sorting (see ref. 15). Additionally, CHO cells expressing any one of the four isoforms formed syncytia when exposed to MV, whereas CHO control cells did not form syncytia (Fig. 1). These results indicate isoforms C1, C2, BC1, and BC2 can function as a receptor for MV.

MV-Infected CHO-CD46 Cells Are Able to Transmit Virus. To determine whether CHO-CD46 cells were infected with MV could pass the infection to adjacent cells, MV-infected CHO-CD46 cells were tested for infectious center formation on Vero cell monolayers. CHO cells expressing CD46 isoforms C1, C2, BC1, or BC2 or control CHO cells were infected with MV, harvested after 48 h, and overlaid on Vero cell monolayers. Table 1 shows the number of infectious centers formed after plating serial 1:10 dilutions from 10^6 to 10^2 infected cells on Vero cell monolayers. CD46-expressing MV-infected cells showed between 30-fold (BC2 isoform) and >100-fold (C1 and C2 isoforms) increases in infectious center formation compared to control CHO cells. MV-infected CHO control cells did form a small number of infectious centers (≈0.1% of the cells). This result is concurrent with the numbers of positive cells detected by immunofluorescence (<0.5%) and suggests the presence of a CD46-like native protein or that a secondary receptor other than CD46 may be utilized on CHO cells. As a control, uninfected cells from each of the lines were also plated and did not form any infectious centers (data not shown). Each of the isoforms tested was able to form large numbers of infectious centers compared to control CHO cells, again indicating that each isoform is able to function as an MV receptor and enable a productive infection of CHO cells.

MV Infection of CD46-Expressing Murine MC57 Cells. Next we determined the ability of murine MC57 cells expressing the CD46-BC1 isoform to be infected with MV. MC57 cells, a murine fibroblast line (9) expressing the BC1 isoform of CD46, were infected with MV at a moi of 3. After 48 h, 8% of control MC57 cells stained positively for MV (Fig. 1*F*). In contrast, 88% of MC57-CD46 BC1 cells stained positively for MV antigens in the cytoplasm (Fig. 1*G*). MC57-CD46 cells formed syncytia in the cultures (Fig. 1*G*). Additionally, MC57-CD46 cells were tested in the infectious center assay and were positive for infectious center formation, whereas control MC57 cells were not (Table 1).

Table 1. Infectious center formation on Vero cell monolayers

Cell line/isoform	No. infectious centers formed/ no. cells plated				
	10^6	10^5	10^4	10^3	10^2
Experiment 1					
Vero	TM	TM	TM	TM	4
CHO-CD46 C1	TM	TM	TM	TM	15
CHO-CD46 C2	TM	TM	TM	57	14
CHO-CD46 BC1	TM	TM	TM	65	9
CHO-CD46 BC2	TM	TM	TM	39	0
CHO	TM	TM	12	0	0
Experiment 2					
Vero	TM	TM	112	14	0
MC57-CD46 BC1	TM	TM	45	6	0
MC57	0	8	0	0	0

Infectious centers formed by MV-infected CHO cell lines expressing CD46 isoforms (experiment 1) or MC57 cells expressing CD46 BC1 (experiment 2). From 10^2 to 10^6 cells were plated. Cells were infected at a moi of 3. After 48 h, cells were harvested by trypsinization and overlaid on Vero cell monolayers in 6-well plates. Plaques were counted after 6 days and scored as number of infectious centers per number of cells plated. TM, too many to count.

Antibody to CD46 Blocks MV Infection. Fig. 2 shows that a rabbit antibody made against CD46 blocks MV infection of HeLa cells, which express human CD46 (5), and CHO-CD46 cells in a dose-dependent manner. Background levels of MV staining on control CHO cells were not affected by the level of anti-CD46 antibody added. As a control, HeLa or CHO-CD46 BC1 cells incubated with a 1:4 dilution of rabbit serum not containing antibody to CD46 showed no blocking of MV infection, with 92% and 85% of cells staining positive for MV, respectively.

DISCUSSION

This report shows that the common isoforms of the CD46 protein (C1, C2, BC1, and BC2) can serve as receptors for MV, enabling a productive infection and syncytium formation in otherwise nonpermissive CHO cells. Further, CD46-expressing murine MC57 cells were productively infected with MV. These data, in conjunction with the recent report that expression of the C2 isoform in murine B cells allowed MV replication (6), strongly implicate the human CD46 protein as the receptor for MV.

CD46 is a type I membrane glycoprotein consisting of at least four regularly expressed isoforms that serve to inhibit complement activation on host cells (5). CD46 is a member of the regulators of complement activation family and functions as a cofactor, in concert with plasma serine protease factor I, to proteolytically degrade C3b and C4b deposited on host tissue. The isoforms of CD46 result from the alternative splicing of its gene (17, 18). The extracellular N terminus of CD46 consists of four of the C3/C4 binding modules known as short consensus repeats, the building blocks of the regulators of complement activation (Fig. 3; for review see ref. 19). Adjacent to this is a region enriched in serines, threonines, and prolines (STP), a site of extensive O-linked glycosylation. The STP region consists of either 14 or 29 amino acids, depending on whether STP exon B (15 amino

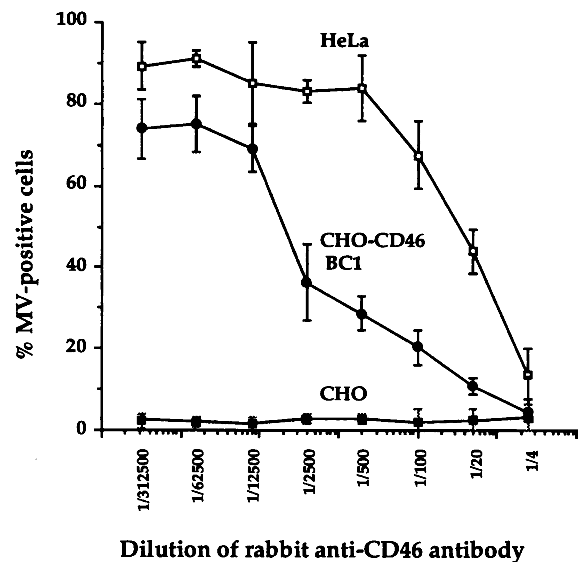

FIG. 2. Blocking of binding of MV to HeLa (open squares), CHO-CD46 BC1 (solid circles) or CHO (solid squares) cells by using a polyclonal antiserum against CD46. Cells were incubated with serial 1:5 dilutions of CD46 antibody. MV was added and the cells were plated on coverslips. After 24 h at 37°C, cells were stained for the presence of MV antigen. Cells were counted and at least three fields were used to determine the percentage of MV-positive cells per sample. Points indicate the mean value for each sample, with error bars depicting ±2 SDs.

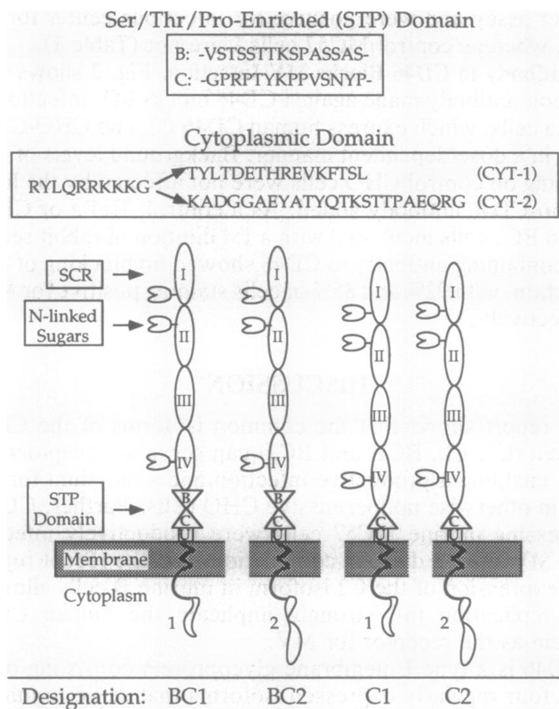

FIG. 3. Schematic of CD46 (MCP) protein illustrating the four most commonly expressed isoforms that arise by alternative splicing. Amino acid sequence of the alternatively spliced STP domains and cytoplasmic tails are boxed. The lower portion of the diagram depicts the structural organization of the common isoforms of CD46: designated BC1, BC2, C1, and C2. Regions of N-linked glycosylation are indicated. SCR, short consensus repeat.

acids) is spliced out. After a small tract (12 amino acids) of unknown functional significance are transmembrane and cytoplasmic domains. Alternative splicing produces one of two cytoplasmic tails of 16 (CYT-1) or 23 (CYT-2) amino acids. CD46 isoforms are named on the basis of the alternative splicing in the STP (BC or C) and cytoplasmic (1 or 2) regions resulting in four primary isoforms: C1, C2, BC1, and BC2 (Fig. 3). These isoforms are expressed in differing proportions on human epithelial, endothelial, fibroblast, and peripheral blood mononuclear cells (except erythrocytes), thus paralleling the tropism of MV (2, 5, 20, 21).

Expression of MV-H activates the alternative complement pathway, resulting in the deposition of complement C3b on infected cells in the absence of MV-specific antibody (4). This phenomenon may serve as a primary host defense mechanism against MV infection. Alternatively, induction of C3b deposition on MV-infected cells may constitute a strategy by the virus to promote virus spread by enhancing the interaction of MV with CD46 on uninfected cells, perhaps by using C3b to enhance the affinity for CD46 for MV or by using the C3b–CD46 interaction as a bridge between MV-infected and uninfected CD46-expressing cells.

We have tested the four most commonly expressed human CD46 isoforms for their ability to serve as MV receptors: C1, C2, BC1, and BC2 (5). Each of the four isoforms confers permissivity on receptor-expressing CHO cells that otherwise are not permissive for MV. Further, we have shown that CHO cells expressing any one of the four CD46 isoforms tested are able to form syncytia after exposure to MV, display viral antigens as detected by immunofluorescence, and transmit the virus to uninfected cells. Naniche *et al.* (6) demonstrated that expression of the C2 isoform of CD46 on murine B cells allows virus replication while C2 expressed on murine fibroblast L cells did not and that neither of these cell types exhibited syncytium formation when exposed to MV. These authors suggest that the different isoforms of CD46 may have differential ability to function as MV receptors and that isoforms other than C2 might allow replication in mouse fibroblast cells. We have demonstrated that the four isoforms tested here, including C2, are able to allow replication and syncytium formation in CHO cells to a similar extent. Further, we have demonstrated that expression of the BC1 isoform in MC57 fibroblast cells allows MV replication and syncytium formation. Our results suggest that, rather than a difference in the particular isoform used, some other block to MV replication is present in particular murine fibroblast cell lines. We have evidence that murine NIH 3T3 cells expressing CD46 replicate MV but fail to produce infectious progeny (M.M., M.K.L., J.P.A., and M.B.A.O., unpublished data).

We have detected MV antigens and infectious centers in control cells not expressing CD46. This low-level infection of control cells (0.5–8% in various lines) is unaffected by addition of CD46 antibody. Such results suggest that there may be a native CD46-like protein or a secondary receptor for MV that is utilized on rodent cells (29).

Work in other viral systems (22–28) implicates cellular receptors in tissue tropism and pathogenesis. Virus receptors have important roles in the early steps of infection such as binding, penetration, and uncoating. It will be important to examine the physical interaction between CD46 and MV. Furthermore, there currently is no small animal model in which the host genetics and immune system can be manipulated for studying MV pathogenesis and immunity. Expression of the human CD46 receptor in transgenic mice may lead to development of such a model.

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