

# CD46 on Glial Cells Can Function as a Receptor for Viral Glycoprotein-Mediated Cell–Cell Fusion

RICCARDO CASSIANI-INGONI,<sup>1,3</sup> HEATHER L. GREENSTONE,<sup>2</sup> DONATELLA DONATI,<sup>1</sup>  
ANNA FOGDELL-HAHN,<sup>1</sup> ELENA MARTINELLI,<sup>1</sup> DANIEL REFAI,<sup>1</sup> ROLAND MARTIN,<sup>1</sup>  
EDWARD A. BERGER,<sup>2</sup> AND STEVEN JACOBSON<sup>†1\*</sup>

<sup>1</sup>Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

<sup>2</sup>Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland

<sup>3</sup>Department of Human Physiology and Pharmacology, Center of Excellence in Biology and Molecular Medicine, University of Rome La Sapienza, Rome, Italy

## KEY WORDS

MCP; oligodendrocyte; HHV-6; MV; cell fusion; multiple sclerosis

## ABSTRACT

Membrane cofactor protein (CD46) is a regulator of complement activation that also serves as the entry receptor for human herpes virus 6 (HHV-6) and measles virus (MV) into human cells. While it is clear that oligodendrocytes and astrocytes are cell types commonly infected by these viruses, it is unclear whether oligodendrocytes express CD46, or which are the cellular mechanisms underlying the infection. We show that adult oligodendrocytes, as well as astrocytes and microglial cells, express CD46 on the cellular surface. Moreover, we employed a quantitative fusion assay to demonstrate that HHV-6A infection of T lymphocytes enables cell–cell fusion of these cells to astrocytes or to oligodendroglial cells. This fusion is mediated by the interaction between viral glycoproteins expressed on the membrane of the infected cells and CD46 on the glial targets, and is also observed using cells expressing recombinant MV glycoproteins. These data suggest a mechanism that involves cell–cell fusion by which certain viruses could spread the infection from the periphery to the cells in the nervous system. Published 2005 Wiley-Liss, Inc.<sup>†</sup>

## INTRODUCTION

Membrane cofactor protein (CD46) is a type I membrane protein that belongs to a family of glycoproteins that regulates complement activation. One of its most important functions is to confer protection against activated complement-mediated lysis by inactivating C3b/C4b deposited on the membrane of autologous cells (Seya et al., 1999; van Dixhoorn et al., 2000). However, besides its protective function, CD46 is also known to be a receptor for measles virus (MV) and human herpes virus 6 (HHV-6), which is necessary for viral entry into human cells (Cattaneo, 2004).

In the CNS, CD46 is highly expressed on the vascular endothelium and at comparatively lower levels throughout the tissue parenchyma (McQuaid and Cosby, 2002; Shusta et al., 2002). Interestingly, CD46 is not detected

by Western blot on compact myelin membranes from either the peripheral or central nervous system, although it is expressed on Schwann cells (Koski et al., 1996). It is controversial whether oligodendrocytes express this receptor (Scolding et al., 1998; McQuaid and Cosby, 2002). Tissue and fluid levels of CD46 in the CNS are modulated by inflammatory signals and are dysregulated in a number of different neurological conditions, among which subacute sclerosing panencephalitis (SSPE) and multiple sclerosis (MS) are two examples (Ogata et al., 1997; Soldan et al., 2001). In these two conditions, respectively, MV or HHV-6 antigens and genomes are often detected in glial cells (Allen et al., 1996; Cermelli et al., 2003; Goodman et al., 2003). Little is known, however, of the mechanisms used by these viruses to infect cells of the brain.

In vitro, the formation of syncytia has been observed upon infection of glial cells with strains of HHV-6 and MV (Albright et al., 1998; Mori et al., 2002). In many systems tested, CD46 has been demonstrated to mediate this process (Seya et al., 1997; Mori et al., 2003). Albright and colleagues have shown that human adult oligodendrocytes, as well as microglial cells, can be infected when cultured together with HHV-6-infected lymphocytes and that this process results in the formation of multinucleated cells. However, it remains unclear whether free virus mediates fusion of two glial cells or if infected immune cells, expressing virally encoded fusogenic proteins on the surface, can fuse directly to glial cells. Moreover, the evidence for viral-induced syncytia formation on oligodendrocytes is interesting, considering that the only previous study on CD46 expression on adult oligodendrocytes in culture reports the absence of this receptor on the surface (Scolding et al., 1998). If CD46 were not expressed on oligodendrocytes, this

Grant sponsor: Integrative Neural Immune Program (INIP)/National Institute of Mental Health.

\*Correspondence to: Steve Jacobson, Bldg. 10, Rm. 5B16, National Institutes of Health, Bethesda, MD 20892.  
E-mail: jacobsons@ninds.nih.gov

Received 11 January 2005; Accepted 22 March 2005

DOI 10.1002/glia.20219

Published online 26 May 2005 in Wiley InterScience (www.interscience.wiley.com).

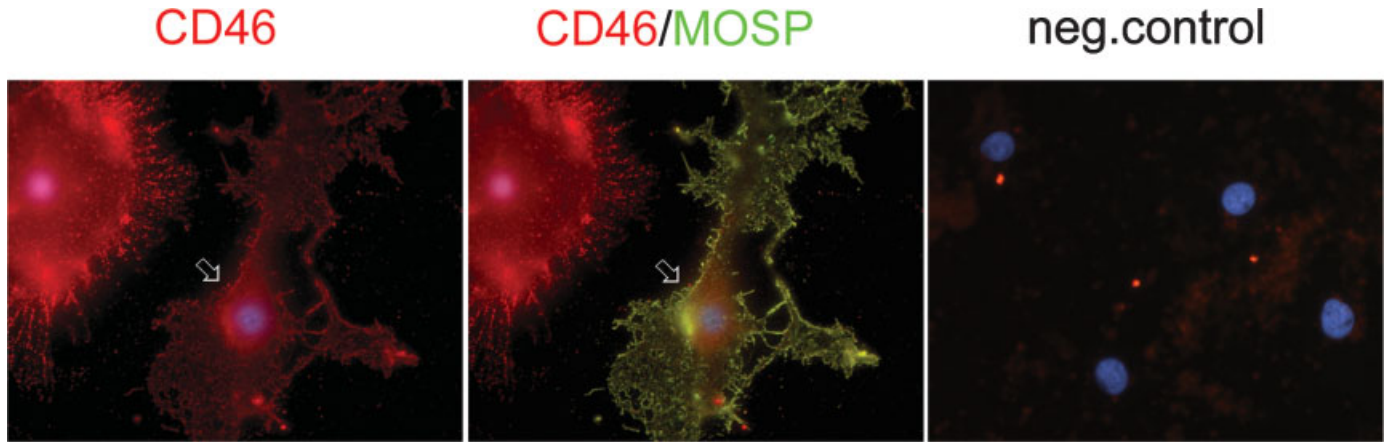


Fig. 1. Detection of CD46 on primary oligodendrocytes. In the left image, glial cells are positively stained with the monoclonal antibody M177 against CD46 (red). In the central image, double-staining for the myelin/oligodendrocyte specific protein MOSP (green) identifies the cell on the right as an oligodendrocyte (arrow); the other cell is a activated microglial cell robustly expressing CD46. In the right image, no nonspecific staining is detected on oligodendrocytes in the negative control (see Methods). (Original magnification: 63 $\times$ )

would clearly suggest the existence of an alternative, CD46-independent mechanism for cell fusion. The mechanism of fusion, itself, may have relevance in understanding viral infection in the CNS.

Therefore, we first investigated the expression of CD46 on glial cells isolated from adult human brain. Mature (GalC-positive) and immature (O4-positive) oligodendrocytes express detectable levels of CD46, although low compared with astrocytes and microglial cells. In addition, we adapted a quantitative cell fusion assay to demonstrate that viral infection of T lymphocytes by HHV-6A enables cell-cell fusion of the immune cells to glial cells. Our results further indicate that this process requires CD46 expression on the surface of the target cell.

## MATERIALS AND METHODS

### Isolation of Primary Glial Cells and Immunofluorescence

Samples of the temporal lobe of five patients undergoing surgical resection for intractable epilepsy were processed as previously described (Donati et al., 2003). After enzymatic digestion, a fraction of the cell suspension was applied on top of a 4-step OptiPrep density gradient (Axa-Shields, Oslo, Norway) in a 15-ml tube and centrifuged for 15 min at 800g. The fraction enriched for oligodendrocytes (>90%) was recovered, washed, and plated on glass coverslips coated with poly-D-lysine inside 6-well plastic culture plates. The cells were cultured for 2 weeks in Neurobasal A Medium supplemented with 2% B27 (both from Gibco-Invitrogen, Carlsbad, CA).

For immunocytochemistry, the coverslips were washed and blocked (Protein Block, Dako, Carpinteria, CA) for 15 min to reduce unspecific binding. Cells were cooled on ice and incubated with different combinations of antibodies recognizing the following surface epitopes: to detect CD46, we used a monoclonal antibody designated M177

(Seya et al., 1995) (1:100, generously donated by Tsukasa Seya, Osaka Medical Center, Osaka, Japan) directed against the extra-cellular subdomain SCR2 of CD46, or a commercial monoclonal antibody J4-48 (Chakrabarti et al., 1997) against the SCR1 domain (1:50, Coulter-Immunotech, Marseilles, France); O4 (1:50, Chemicon), galactocerebroside (GalC) (1:200, Chemicon), or myelin/oligodendrocytes specific protein (MOSP) (1:500, Chemicon, Temecula, CA) were used to detect oligodendrocytes, whereas staining for CD45 (1:500, BD Pharmingen, San Jose, CA) was used to identify microglial cells. After 45 min, the primary antibodies were removed, cells washed and subsequently incubated with the isotype-specific secondary antibodies (1:1,000, Molecular Probes, Eugene, OR) for 30 min. Coverslips were then washed several times, fixed in 4% paraformaldehyde and mounted on glass slides with Anti-fade mounting medium (Molecular Probes) containing DAPI to stain the nuclei. Some preparations were further processed for intracellular staining against glial fibrillary acidic protein (GFAP). These coverslips were fixed for 10 min in a solution of 4% paraformaldehyde and 0.3% Triton 100, blocked for 10 min and incubated with the antibody against GFAP (1:200, Dako) for 30 min. Following incubation with the specific secondary antibody, the slides were mounted as above. Oligodendrocytes from each sample were analyzed for CD46 expression at least three times independently.

To exclude any nonspecific staining of oligodendrocytes, live cells were incubated with a solution containing the primary antibody for MOSP, together with an antibody against the astrocyte-specific and intracellular antigen GFAP (1:50, Sigma, St. Louis, MO) of the same class as the antibody used to detect CD46 in parallel preparations. Following staining with the isotype-specific secondary antibodies, in green to detect MOSP and in red to detect GFAP, the cells were fixed and nuclei were stained with DAPI. In Figure 1 (right), four oligodendrocytes, as identified under the microscope by the green

staining for MOSP, were imaged through the red and blue filters only. Lack of red signal demonstrates (1) no nonspecific staining of oligodendrocytes by an irrelevant primary antibody or by the secondary antibodies used; and (2) at the microscope settings used for acquisition of the red signal, no bleed-through detected in the red channel from the green fluorescence. As a further negative control, no staining for CD46 was detected on the rat neuroblastoma cell line B104 (not shown). All images were acquired on an inverted microscope (Axiovert 200, Zeiss, Thornwood, NY) using the provided hardware and software (Axiovision, Zeiss).

### Fusion Assay

The human T-cell line HSB-2 was obtained from American Type Cell Collection (ATCC, Manassas, VA) and maintained in suspension in RPMI. The oligodendroglial cell line KG-1c (JCRB Cell Bank, ShiniuKu, Japan) (provided by David Mock, University of Rochester, NY), fetal astrocytes and NIH 3T3 cells were maintained in DMEM. All media were supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and gentamicin (10 µg/ml).

Cell-cell fusion mediated by viral glycoproteins was tested according to a previously described vaccinia-based reporter gene assay (Nussbaum et al., 1994) adapted to measure the fusion between effector cells expressing glycoproteins of HHV-6A (Santoro et al., 1999) or of MV (Nussbaum et al., 1995) to oligodendroglial KG-1c cells or to primary astrocytes differentiated from human neurospheres (target cells). Further, T-cell-glial cell interactions were documented under the microscope by labeling the effector cells with a fluorescent dye.

Effector cells were generated using HHV-6A (GS strain) or by using vaccinia recombinants encoding MV-glycoproteins (Edmonston strain). In all cases, the effectors also expressed bacteriophage T7 RNA polymerase encoded by vaccinia recombinant vTF7-3 (Fuerst et al., 1986).

For HHV-6 effectors, following activation with phytohemagglutinin (Sigma) at 5–10 µg/ml for 2–3 days HSB-2 cells were infected with cell-free HHV6-A (strain GS) for 7 days. Every 3–4 days infected cells were mixed with uninfected HSB-2 cells; infected cultures could be maintained in this way for several weeks. For the cell fusion assay, infection of the T cells by HHV-6A was quantified by DNA Taq-Man as previously described (Donati et al., 2003), and was further confirmed by the downregulation of CD46 on these cells (see Fig. 3).

For MV effectors, HSB-2 or NIH 3T3 cells were co-infected with vaccinia recombinants vT7-H<sub>MV</sub> and vT7-F<sub>MV</sub> encoding the glycoproteins hemagglutinin (HA) and fusion (F), respectively (Nussbaum et al., 1995). KG-1c cells or primary astrocytes used as target cells were infected with vaccinia recombinant vCB21R containing the *Escherichia coli* LacZ gene linked to the T7 promoter (Alkhatib et al., 1996).

HHV6 or MV effector cells were washed and mixed with target cells inside a 96-well flat-bottom plate (1:1 ratio;  $1 \times 10^5$  total cells in 100 µl) and incubated 2.5 h at 37°C. The cells were then lysed by addition of 1% NP-40. β-Galactosidase activity in the lysates was quantified colorimetrically, using chlorophenol red-β-D-galactopyranoside as substrate and measuring A<sub>570</sub> (Nussbaum et al., 1994). For direct visualization of effector-target interactions we labeled T-effectors with DiI (Molecular Probes) for 20 min at room temperature to maximize staining of the cytoplasm. 2.5 h after mixing labeled effectors with unlabeled targets to allow cell fusion, these preparations were washed thoroughly multiple times to detach loosely adhering cells, fixed and assayed for β-Galactosidase activity in situ by incubation with X-Gal for 3–5 h. Effector cells not infected with HHV-6A, or not expressing MV glycoproteins, were used as controls in all conditions tested.

Fusion inhibition assays were performed using the anti-CD46 antibody M177. Target cells were pre-incubated for 10 min at 37°C with the designated concentrations of the antibody. Effector cells were then added to the wells and the fusion assay was performed as indicated above.

### Flow Cytometry Analysis

The surface expression levels of CD46 on KG-1c and HSB-2 cells were compared by flow cytometry. Cells were incubated for 10 min with the monoclonal antibody against SCR1, followed by detection with anti-mouse phycoerythrin (Beckman Coulter, CA). Fluorocytometric analysis was performed using a FacScan analyzer (Becton Dickinson, Mountain View, CA). The primary antibody was omitted in the negative controls.

## RESULTS AND DISCUSSION

In this study, we investigated the expression of the complement regulator and viral receptor CD46 on primary human oligodendrocytes and other glial cells. Oligodendrocytes were isolated and cultured from the adult temporal lobe and the expression of CD46 was analyzed by immunofluorescence. After 2 weeks of culture, most oligodendrocytes developed well-branched processes and were positive for myelin/oligodendrocyte specific protein MOSP and for GalC. A small proportion of the cells displayed a more immature phenotype, with short processes and brightly positive with the O4-antibody. A small number of astrocytes and microglial cells were also present in these preparations.

All the cell types analyzed expressed detectable levels of CD46. On oligodendrocytes, the staining was predominant on the cell body (arrow in Fig. 1) as compared with cell processes. GalC-positive and O4-positive oligodendrocytes displayed comparable levels of CD46 expression, although in some cases immature cells were more brightly stained (Fig. 2). Compared with mature oligo-

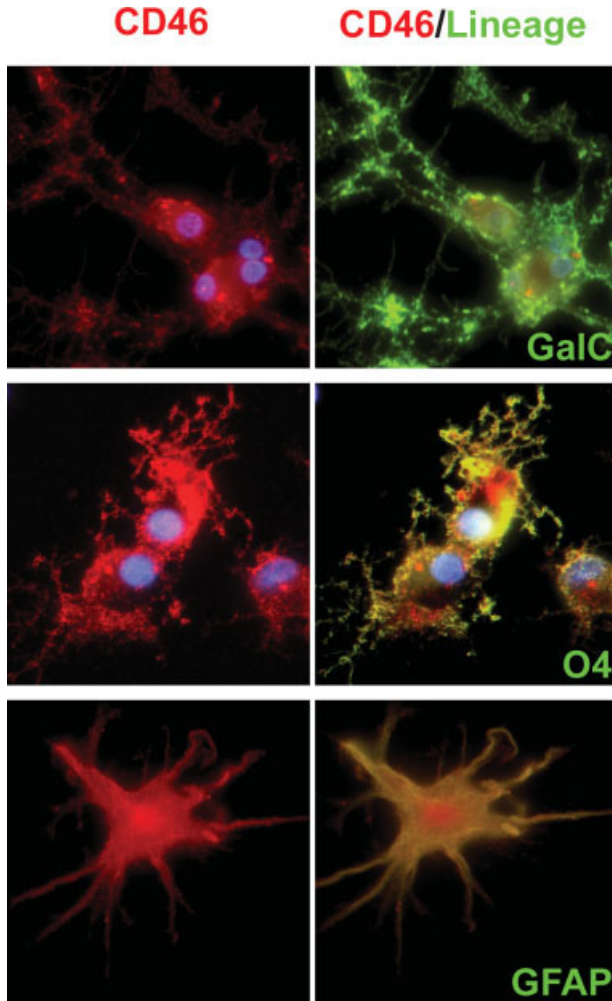


Fig. 2. CD46 expression on primary glial cells. Cells were stained for CD46 (left panels) and double-stained with lineage-specific markers of differentiation (right panels). CD46 was detected on both GalC- and on O4-expressing oligodendrocytes, as well as on GFAP-positive astrocytes. (Original magnification: 20× top and bottom panels, 63× center panels)

dendrocytes, stronger expression was detected on astrocytes (Fig. 2) and on activated microglial cells (Fig. 1). The absence of any nonspecific staining or “bleed-through” in the negative control demonstrates the specificity of the staining procedure used (see Methods). Variable levels of CD46 were also detected on neurons and glial cells differentiated in vitro from fetal neurospheres (not shown), in agreement with previous findings (Singharao et al., 2000) and possibly a reflection of different degrees of cellular activation. Our data are in contrast with the results of a previous study that failed to detect expression of CD46 by adult oligodendrocytes (Scolding et al., 1998). Contrasting results are perhaps explained by differences in the antibodies used, since both the cell source and the staining procedures were similar in the two studies. We used two subdomain specific monoclonal antibodies, M177 (Seya et al., 1995) (that recognizes the SCR2 subunit), as well as another commercially available antibody J4-48 (Chakrabarti

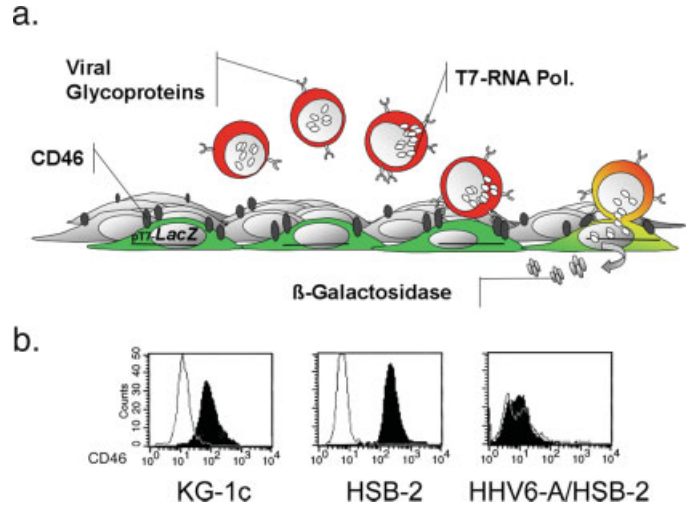


Fig. 3. (a) Schematic depiction of the fusion assay. Effector cells infected with HHV-6A or expressing MV glycoproteins (in red) are added to KG-1c target cells or to astrocytes (in green) endogenously expressing CD46. If the effector cell fuses to a glial target cell, the cytoplasmic T7 RNA-polymerase expressed by the effector cell will activate the LacZ gene in the target cell. The enzymatic activity of  $\beta$ -Galactosidase is used as a measure of the degree of cell-cell fusion. (b) Surface expression of CD46 on oligodendroglial cells, uninfected and infected T-cells. KG-1c cells (left panel) and HSB-2 cells (central panel) express detectable levels of CD46 by flowcytometry. Infection of the HSB-2 lymphocytes used in the assay by HHV-6A is confirmed by the down-regulation of CD46 expression on these cells (right panel). (This figure can be seen in color, online, at [www.interscience.wiley.com](http://www.interscience.wiley.com)).

et al., 1997) (against SCR1), to detect surface expression of this receptor on oligodendrocytes. Notably, expression of CD46 on the oligodendrocyte and absence of this receptor on compacted myelin (Koski et al., 1996), mirrors the situation seen in the PNS, for Schwann cells and peripheral myelin membranes, respectively.

Interestingly, several different infectious agents that rely on CD46 for the infection of the human host have emerged (Cattaneo, 2004). HHV-6 and MV, two viruses that are known to enter the brain and infect glial cells, share the use of overlapping domains of this molecule for infection (Greenstone et al., 2002; Manchester et al., 1997). In vitro experiments have demonstrated that oligodendrocytes can be infected by HHV-6 when co-cultured with other infected cells, and the formation of multinucleated cells has been described (Albright et al., 1998). However, the nature of these syncytia and the possible role of CD46 in these phenomena were not further investigated. In parallel work, we observed that blocking CD46 specifically abrogates productive infection of human astrocytes by HHV-6A in vitro (D. Donati et al., 2005); this effect was accompanied by a reduction in the number of multinucleated syncytia that form following the infection. To test whether infected immune cells can fuse to oligodendrocytes and astrocytes, we employed a previously described vaccinia-based reporter gene assay (Nussbaum et al., 1994) which serves as a measure of fusion activity mediated by viral glycoproteins. We adapted this assay to measure the fusion between cells expressing glycoproteins of HHV-6A (Santoro et al., 1999) or of MV (Nussbaum et al., 1995) to

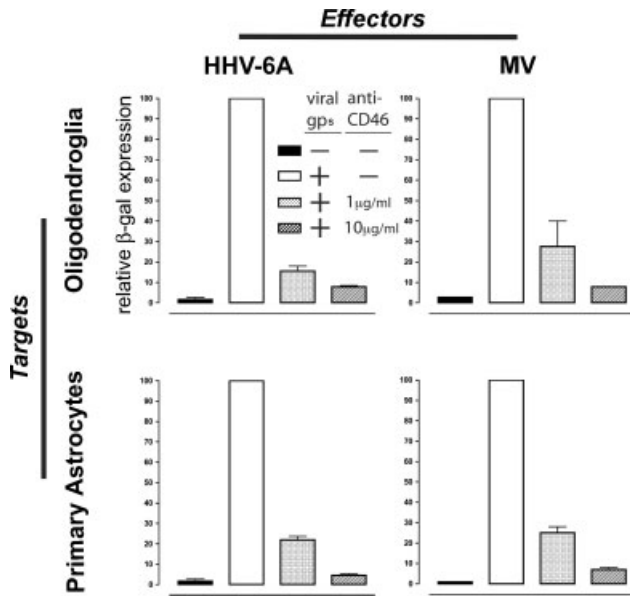


Fig. 4. T-cells infected with HHV-6A or expressing recombinant MV glycoproteins, can fuse to glial-target cells in a mechanism that involves CD46. The bars represent  $\beta$ -Galactosidase activity in the different conditions tested (viral glycoproteins expressed and no antibody defined as 100). No activation was detected when effector cells not expressing viral glycoproteins (control) were mixed with glial target cells, oligodendroglial KG-1c cells (top panels) or primary astrocytes (bottom panels). In contrast, enzymatic activity was detected when effectors infected with HHV-6A or expressing MV glycoproteins were mixed with glial targets. In both cases pre-incubation of the glial cells with the indicated concentrations of the antibody specific for CD46 inhibited fusion almost completely. Error bars indicate the standard deviation for duplicate samples in a representative experiment.

glial target cells. As schematically depicted in Figure 3a, fusion between a virally infected effector cell (that also expresses the T7 RNA polymerase) and a glial target cell (which contains the *LacZ* gene under the T7 promoter) activates the expression of  $\beta$ -Galactosidase, which can be quantified using a sensitive colorimetric assay.

To model infection of oligodendrocytes by HHV-6A, we used the oligodendroglial cell line KG-1c together with T lymphocytes (HSB-2) infected with HHV6-A. KG-1c cells have been shown to share many features with immature oligodendrocytes (Kurihara et al., 2000; Buntinx et al., 2003) and express detectable levels of CD46 by flow cytometry (Fig. 3b). HHV-6A infection of HSB-2 cells used in this assay was quantified by Taq-Man PCR for HHV-6A DNA ( $2.1 \times 10^9$  viral copies/million cells) and was further demonstrated by downregulation of CD46 on these cells (Fig. 3b), as previously reported in peripheral blood mononuclear cells (Santoro et al., 1999). HHV-6-infected cells express viral glycoproteins on the surface as soon as four days after infection (Santoro et al., 1999); these glycoproteins can potentially bind to CD46 on the target cell and mediate cell fusion.

When we mixed HHV-6A infected T cells with oligodendroglial target cells we observed cell-cell fusion between these cell types as determined by the activation of the *LacZ* gene (Fig. 4). In contrast, no enzymatic activity was detected when uninfected (control) cells were used as effectors. The glycoprotein-mediated cell

fusion was dependent on the interaction between the viral glycoproteins expressed on the effector cells and CD46 on the glial target cells, since it was significantly blocked by pre-incubation of the targets with the antibody M177; this antibody blocks the SCR2 subdomain, which is engaged by both HHV-6 and MV glycoproteins. Fusion was not significantly inhibited when the subdomain SCR4 was blocked using the GB24 antibody (Cho et al., 1991) (not shown), consistent with the noninvolvement of this domain for HHV-6 infection (Greenstone et al., 2002). We observed similar results using primary astrocytes as the target cells (Fig. 4, bottom). In addition, we observed fusion using effector cells expressing recombinant MV glycoproteins HA and F on the surface (Fig. 4, right), which are known to bind to CD46 and to mediate MV infection (Nussbaum et al., 1995; Manchester et al., 1997). These data show that CD46 on glial cells can function as a receptor for cell fusion mediated by viral glycoproteins.

To visualize T-cell-glial cell interactions in situ, we performed fusion assays using fluorescently labeled HHV-6A or MV effector cells mixed to unlabeled glial targets. Within 1 h after addition of labeled effector cells, some of these cells were in stable contact with the glial targets. Many fused targets showed faint uptake of DiI in restricted areas of the cytoplasm; 1.5 h later,  $\beta$ -galactosidase activity in DiI-labeled glial targets was confirmed using X-Gal staining (Fig. 5). Fusion was not observed when uninfected labeled control cells were used as effectors.

Our results clearly demonstrate that HHV-6A infection of a lymphocyte can determine fusion of this cell to a glial cell, thus suggesting that direct transmission of the virus to the cytoplasm can take place. Fusion is also observed using lymphocytes expressing recombinant MV glycoproteins on the surface, demonstrating that this event can be completely independent from the release of free virus by infected cells. These observations also suggest that the engagement of the same receptor by both viruses, although through different sets of viral glycoproteins, might reflect a common mechanism of infection. It should be noted that signaling lymphocyte-activation molecule (SLAM) is considered the main receptor for some wild-type strains of MV, while CD46 is used preferentially by vaccine strains and certain other wild-type strains (Schneider-Schaulies et al., 1995). However, because SLAM is not expressed by brain cells (McQuaid and Cosby, 2002; Plumb et al., 2002) other mechanisms are responsible for MV spread in the CNS. It has been shown that a single amino acid substitution in the sequence of HA (from asparagine or lysine to tyrosine in position 481) allows wild-type strains of MV to interact with CD46, while preserving the ability to bind SLAM (Buckland and Wild, 1997). This kind of mutations allows MV to infect cells that don't express the primary receptor, as could be the case of glial cells. Moreover, the evidence that CD46 expression is downregulated in the infected brain regions (Ogata et al., 1997; McQuaid and Cosby, 2002) strongly supports a relevance for MV-CD46 interactions in vivo.

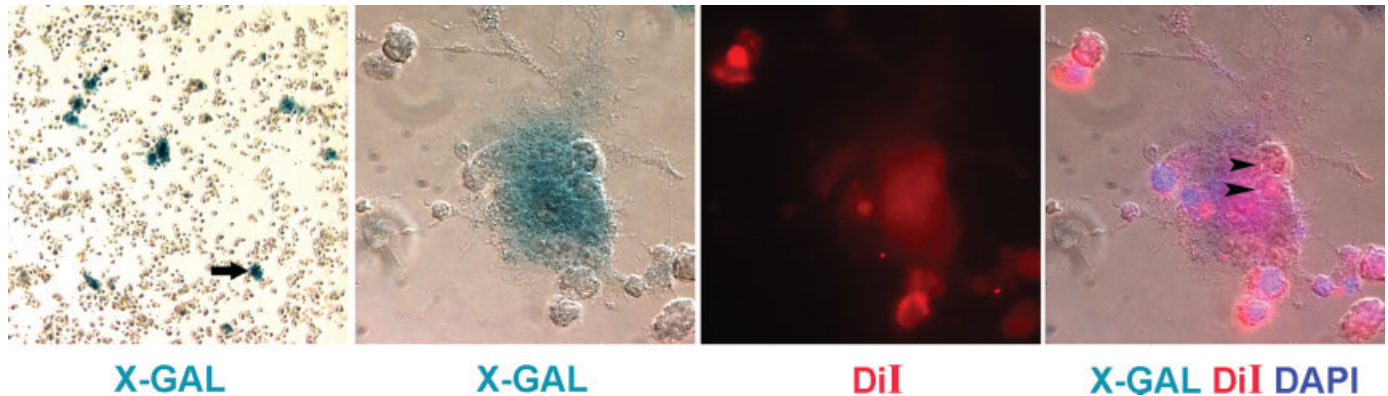


Fig. 5. Fusion of infected T-cells to glial targets. The fusion of DiI-labeled T-effectors to the glial targets activates the expression of  $\beta$ -Galactosidase in these cells, which is detected in situ as a blue X-Gal precipitate (left image). The arrow shows the fusion event imaged at higher magnification on the right: the glial target expresses  $\beta$ -Galactosidase and shows faint DiI-staining in the cytoplasm after fusing with labeled T-cells; arrowheads indicate the sites of fusion between two effector cells and a single glial cell. (Original magnification: 20 $\times$  in the left image, 63 $\times$  in the others)

While it remains unclear which mechanisms viruses adopt to enter the brain and how they manage to spread from cell to cell, the constant trafficking of immune cells into the CNS becomes increasingly appreciated as a common phenomenon, even in nonpathological conditions (Ransohoff et al., 2003). Surprisingly, fusion of blood cells with mature Purkinje neurons has been recently demonstrated both in rodents and in humans, although its physiological significance is still unclear (Alvarez-Dolado et al., 2003; Goodman et al., 2003; Weimann et al., 2003). We propose that viruses could adopt a similar mechanism to spread the infection from circulating cells to the CNS.

Further, the observation that HHV-6A-infected lymphocytes have the ability to fuse to glial cells supports those studies that detect viral genome in multiple areas of the CNS or re-activation of the virus in the presence of inflammation and the relation to underlying neurological conditions (Goodman et al., 2003; Alvarez-Lafuente et al., 2004). Many of the immune cells among those invading the inflamed brain in multiple sclerosis could harbor HHV-6A (Kim et al., 2000) and express viral fusogenic glycoproteins on their surface at that time. Transmission of the virus through cell-cell fusion of these cells with glial cells, and thus detection of the virus in oligodendrocytes and astrocytes, might represent a pathological consequence of CNS inflammation. Nevertheless, the evidence that HHV-6A infected cells can fuse with glial cells further strengthens the association of this virus with brain disease (Soldan et al., 2001; Berti et al., 2002; Alvarez-Lafuente et al., 2004).

#### ACKNOWLEDGMENTS

The authors thank Monique Dubois-Dalcq for reviewing the manuscript and Paolo Muraro for helpful discussions. We gratefully acknowledge the technical assistance of Paul E. Kennedy. R.C.I. is supported by the

Integrative Neural Immune Program (INIP) of the National Institute of Mental Health and is a doctoral candidate in the Research in Neurophysiology program at the University of Rome La Sapienza.

#### REFERENCES

- Albright AV, Lavi E, Black JB, Goldberg S, O'Connor MJ, Gonzalez-Scarano F. 1998. The effect of human herpesvirus-6 (HHV-6) on cultured human neural cells: oligodendrocytes and microglia. *J Neurovirol* 4:486-494.
- Alkhatib G, Broder CC, Berger EA. 1996. Cell type-specific fusion cofactors determine human immunodeficiency virus type 1 tropism for T-cell lines versus primary macrophages. *J Virol* 70:5487-5494.
- Allen IV, McQuaid S, McMahon J, Kirk J, McConnell R. 1996. The significance of measles virus antigen and genome distribution in the CNS in SSPE for mechanisms of viral spread and demyelination. *J Neuropathol Exp Neurol* 55:471-480.
- Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, Alvarez-Buylla A. 2003. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. *Nature* 425:968-973.
- Alvarez-Lafuente R, De las Heras V, Bartolome M, Picazo JJ, Arroyo R. 2004. Relapsing-remitting multiple sclerosis and human herpesvirus 6 active infection. *Arch Neurol* 61:1523-1527.
- Berti R, Brennan MB, Soldan SS, Ohayon JM, Casareto L, McFarland HF, Jacobson S. 2002. Increased detection of serum HHV-6 DNA sequences during multiple sclerosis (MS) exacerbations and correlation with parameters of MS disease progression. *J Neurovirol* 8:250-256.
- Buckland R, Wild TF. 1997. Is CD46 the cellular receptor for measles virus? *Virus Res* 48:1-9.
- Buntinx M, Vanderlocht J, Hellings N, Vandenabeele F, Lambrichts I, Raus J, Ameloot M, Stinissen P, Steels P. 2003. Characterization of three human oligodendroglial cell lines as a model to study oligodendrocyte injury: morphology and oligodendrocyte-specific gene expression. *J Neurocytol* 32:25-38.
- Cattaneo R. 2004. Four viruses, two bacteria, and one receptor: membrane cofactor protein (CD46) as pathogens' magnet. *J Virol* 78:4385-4388.
- Cermelli C, Berti R, Soldan SS, Mayne M, D'Ambrosia JM, Ludwin SK, Jacobson S. 2003. High frequency of human herpesvirus 6 DNA in multiple sclerosis plaques isolated by laser microdissection. *J Infect Dis* 187:1377-1387.
- Chakrabarti S, Sisler JR, Moss B. 1997. Compact, synthetic, vaccinia virus early/late promoter for protein expression. *Biotechniques* 23:1094-1097.
- Cho SW, Oglesby TJ, Hsi BL, Adams EM, Atkinson JP. 1991. Characterization of three monoclonal antibodies to membrane co-factor protein (MCP) of the complement system and quantification of MCP by radioassay. *Clin Exp Immunol* 83:257-261.
- Donati D, Akhyani N, Fogdell-Hahn A, Cermelli C, Cassiani-Ingoni R, Vortmeyer A, Heiss JD, Cogen P, Gaillard WD, Sato S, Theodore WH,

- Jacobson S. 2003. Detection of human herpesvirus-6 in mesial temporal lobe epilepsy surgical brain resections. *Neurology* 61:1405–1411.
- Donati D, Martinelli E, Cassiani-Ingoni R, Ahlqvist J, Hou J, Major EO, Jacobson S. 2005. Variant specific tropism of HHV-6 in human astrocytes. *J Virol* in press.
- Fuerst TR, Niles EG, Studier FW, Moss B. 1986. Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase. *Proc Natl Acad Sci USA* 83:8122–8126.
- Goodman AD, Mock DJ, Powers JM, Baker JV, Blumberg BM. 2003. Human herpesvirus 6 genome and antigen in acute multiple sclerosis lesions. *J Infect Dis* 187:1365–1376.
- Greenstone HL, Santoro F, Lusso P, Berger EA. 2002. Human herpesvirus 6 and measles virus employ distinct CD46 domains for receptor function. *J Biol Chem* 277:39112–39118.
- Kim JS, Lee KS, Park JH, Kim MY, Shin WS. 2000. Detection of human herpesvirus 6 variant A in peripheral blood mononuclear cells from multiple sclerosis patients. *Eur Neurol* 43:170–173.
- Koski CL, Estep AE, Sawant-Mane S, Shin ML, Highbarger L, Hansch GM. 1996. Complement regulatory molecules on human myelin and glial cells: differential expression affects the deposition of activated complement proteins. *J Neurochem* 66:303–312.
- Kurihara H, Zama A, Tamura M, Takeda J, Sasaki T, Takeuchi T. 2000. Glioma/glioblastoma-specific adenoviral gene expression using the nestin gene regulator. *Gene Ther* 7:686–693.
- Manchester M, Gairin JE, Patterson JB, Alvarez J, Liszewski MK, Eto DS, Atkinson JP, Oldstone MB. 1997. Measles virus recognizes its receptor, CD46, via two distinct binding domains within SCR1-2. *Virology* 233:174–184.
- McQuaid S, Cosby SL. 2002. An immunohistochemical study of the distribution of the measles virus receptors, CD46 and SLAMF1, in normal human tissues and subacute sclerosing panencephalitis. *Lab Invest* 82:403–409.
- Mori Y, Seya T, Huang HL, Akkapaiboon P, Dhepakson P, Yamanishi K. 2002. Human herpesvirus 6 variant A but not variant B induces fusion from without in a variety of human cells through a human herpesvirus 6 entry receptor, CD46. *J Virol* 76:6750–6761.
- Mori Y, Yang X, Akkapaiboon P, Okuno T, Yamanishi K. 2003. Human herpesvirus 6 variant A glycoprotein H-glycoprotein L-glycoprotein Q complex associates with human CD46. *J Virol* 77:4992–4999.
- Nussbaum O, Broder CC, Berger EA. 1994. Fusogenic mechanisms of enveloped-virus glycoproteins analyzed by a novel recombinant vaccinia virus-based assay quantitating cell fusion-dependent reporter gene activation. *J Virol* 68:5411–5422.
- Nussbaum O, Broder CC, Moss B, Stern LB, Rozenblatt S, Berger EA. 1995. Functional and structural interactions between measles virus hemagglutinin and CD46. *J Virol* 69:3341–3349.
- Ogata A, Czub S, Ogata S, Cosby SL, McQuaid S, Budka H, ter Meulen V, Schneider-Schaulies J. 1997. Absence of measles virus receptor (CD46) in lesions of subacute sclerosing panencephalitis brains. *Acta Neuropathol (Berl)* 94:444–449.
- Plumb J, Duprex WP, Cameron CH, Richter-Landsberg C, Talbot P, McQuaid S. 2002. Infection of human oligodendrogloma cells by a recombinant measles virus expressing enhanced green fluorescent protein. *J Neurovirol* 8:24–34.
- Ransohoff RM, Kivisakk P, Kidd G. 2003. Three or more routes for leukocyte migration into the central nervous system. *Nat Rev Immunol* 3:569–581.
- Santoro F, Kennedy PE, Locatelli G, Malnati MS, Berger EA, Lusso P. 1999. CD46 is a cellular receptor for human herpesvirus 6. *Cell* 99:817–827.
- Schneider-Schaulies J, Schnorr JJ, Brinckmann U, Dunster LM, Bacsko K, Liebert UG, Schneider-Schaulies S, ter Meulen V. 1995. Receptor usage and differential downregulation of CD46 by measles virus wild-type and vaccine strains. *Proc Natl Acad Sci USA* 92:3943–3947.
- Scolding NJ, Morgan BP, Compston DA. 1998. The expression of complement regulatory proteins by adult human oligodendrocytes. *J Neuroimmunol* 84:69–75.
- Seya T, Hirano A, Matsumoto M, Nomura M, Ueda S. 1999. Human membrane cofactor protein (MCP, CD46): multiple isoforms and functions. *Int J Biochem Cell Biol* 31:1255–1260.
- Seya T, Kurita M, Hara T, Iwata K, Semba T, Hatanaka M, Matsumoto M, Yanagi Y, Ueda S, Nagasawa S. 1995. Blocking measles virus infection with a recombinant soluble form of, or monoclonal antibodies against, membrane cofactor protein of complement (CD46). *Immunology* 84:619–625.
- Seya T, Kurita M, Iwata K, Yanagi Y, Tanaka K, Shida K, Hatanaka M, Matsumoto M, Jun S, Hirano A, Ueda S, Nagasawa S. 1997. The CD46 transmembrane domain is required for efficient formation of measles-virus-mediated syncytium. *Biochem J* 322 (Pt 1):135–144.
- Shusta EV, Zhu C, Boado RJ, Pardridge WM. 2002. Subtractive expression cloning reveals high expression of CD46 at the blood-brain barrier. *J Neuropathol Exp Neurol* 61:597–604.
- Singhrao SK, Neal JW, Rushmere NK, Morgan BP, Gasque P. 2000. Spontaneous classical pathway activation and deficiency of membrane regulators render human neurons susceptible to complement lysis. *Am J Pathol* 157:905–918.
- Soldan SS, Fogdell-Hahn A, Brennan MB, Mittleman BB, Ballerini C, Massacesi L, Seya T, McFarland HF, Jacobson S. 2001. Elevated serum and cerebrospinal fluid levels of soluble human herpesvirus type 6 cellular receptor, membrane cofactor protein, in patients with multiple sclerosis. *Ann Neurol* 50:486–493.
- van Dixhoorn MG, Dekker S, Janssen RW, Verbakel CA, Grosveld F. 2000. Human CD46 rather than CD55 is a key element in protection against complement activation in vitro. *Transplant Proc* 32:916–918.
- Weimann JM, Charlton CA, Brazelton TR, Hackman RC, Blau HM. 2003. Contribution of transplanted bone marrow cells to Purkinje neurons in human adult brains. *Proc Natl Acad Sci USA* 100:2088–2093.