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Glycosyl-Phosphatidylinositol-Anchored and Transmembrane Forms of CD46 Display Similar Measles Virus Receptor Properties: Virus Binding, Fusion, and Replication; Down-Regulation by Hemagglutinin; and Virus Uptake and Endocytosis for Antigen Presentation by Major Histocompatibility Complex Class II Molecules

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The CD46 molecule is a receptor for measles virus (MV), CD46, which protects autologous cells from complement-mediated damage, exists in several isoforms which are variably expressed in different human tissues. These isoforms differ in their cytoplasmic and transmembrane regions and in a small portion of their proximal extracytoplasmic regions. To examine the role of the cytoplasmic and transmembrane regions of CD46 in MV infection, mouse M12 B cells stably expressing a transmembrane or a chimeric glycosyl-phosphatidylinositol (GPI)-anchored form of CD46 (CD46-GPI) were used. Both the GPI-anchored and transmembrane CD46 forms were able to mediate MV binding. MV binding mediated by the GPI-anchored form but not that mediated by the transmembrane form was abolished after treatment with phosphatidylinositol phospholipase C. MV infection of both M12.CD46 and M12.CD46-GPI cells but not parental M12 cells resulted in MV replication. Expression of hemagglutinin induced cell surface down-regulation of both CD46 and CD46-GPI. Both M12.CD46 and M12.CD46-GPI cells were able to efficiently capture MV for presentation of viral antigens by major histocompatibility complex class II molecules to T cells. This presentation was blocked by chloroquine, indicating some virus endocytosis. These data imply that the extracytoplasmic region encompassing the four N-terminal invariable short consensus repeat regions of CD46 is sufficient to act as a receptor for MV and that the cytoplasmic and transmembrane regions of CD46 may not play a major role in the signal for the hemagglutinin-induced down-regulation of CD46 and/or endocytosis of MV.

Acute measles virus (MV) infection is one of the primary causes of infant mortality in the developing world. On rare instances, MV infection can lead to chronic neurological disorders such as subacute sclerosing panencephalitis and measles inclusion body encephalitis. Recently, there have also been sporadic outbreaks of measles in industrialized countries despite vaccinations. Profound but transient cellular immunosuppression is one of the major features of the physiopathology of measles infection (28), but the underlying mechanisms remain to be elucidated. The recent identification of CD46 as a cellular receptor for MV has opened a new area of investigation.

MV is an enveloped, negative-strand RNA virus belonging to the family *Paramyxoviridae*. Its envelope contains two glycoproteins, the hemagglutinin (H) and fusion (F) proteins, and surrounds the nucleocapsid, which mainly consists of the nucleoprotein (N) and the viral genome. H is responsible for viral binding to the cell surface, and F induces fusion between the viral envelope and the plasma cell membrane. MV infection brings about a characteristic cytopathic effect by inducing cell-cell fusion, i.e., syncytium formation. Recently, it was shown in our laboratory that the CD46 molecule acts as a cellular receptor for MV (25), a finding confirmed by another group (6). Indeed, CD46 expressed after transfection of a murine B-cell line which is nonpermissive to MV allows MV binding to the cell surface, MV-induced cell-cell fusion, and MV replication (25). This effect is likely to be mediated through interaction between the MV H envelope glycoprotein and CD46, as recently evidenced (10). In addition, although after interaction with CD46 MV fuses with the plasma membrane, resulting in the association of the envelope H and F glycoproteins with the plasma cell membrane and the injection of the nucleocapsid into the cytosol for viral replication, CD46 was also found to mediate uptake and endocytosis of some MV particles. Indeed, when an infectious or UV-inactivated virus was used as a source of antigens, expression of CD46 by murine B cells resulted in a highly efficient major histocompatibility complex (MHC) class II-restricted presentation of both the envelope transmembrane H glycoprotein and the internal nucleoprotein N to T cells through a chloroquine-sensitive, antigen-processing pathway (10). Moreover, the expression of MV H envelope glycopro-

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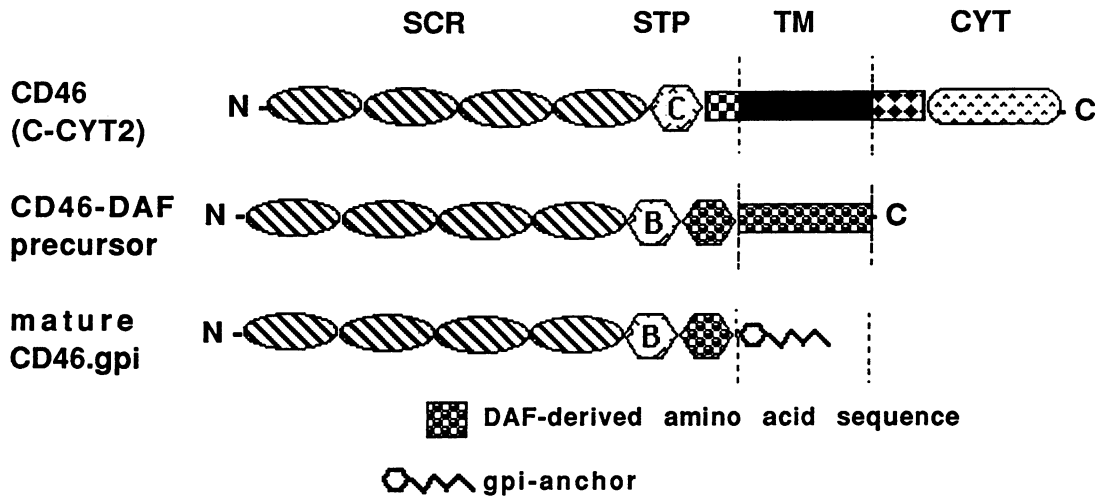

FIG. 1. Diagrammatic representation of transmembrane CD46 and chimeric CD46-GPI molecules. The mature CD46-GPI molecule results from the cleavage of the DAF GPI-anchoring hydrophobic signal (4) from the CD46-DAF chimeric precursor (according to Lublin and Coyne [22]). The two small regions flanking the transmembrane region (TM) of the CD46 molecule are encoded by separate exons and are present in all or most CD46 isoforms. CYT, cytoplasmic tail.

tein in human cells results in a strong down-regulation of cell surface expression of CD46 because of enhanced internalization (27).

The transmembrane protein CD46, or membrane cofactor protein, is a member of the cluster of genes that regulate complement activation and protects cells from autologous complement-mediated damage (19). CD46 can occur in as many as 14 different isoforms because of alternative splicing of its mRNA (29). All isoforms share, at the N terminus, a fourfold repeat of the short consensus repeat (SCR) region, a characteristic feature of the regulators of complement activation located close to the plasma membrane and the first part of the transmembrane domain. The isoforms differ in their use of three exons encoding three STP (Ser, Thr, and Pro)-rich regions (called A, B, and C) and in the exons encoding the second part of the transmembrane region and the cytoplasmic tail (the two major ones are called CYT1 and CYT2; see Fig. 1 for the schematic structure of the C-CYT2 CD46 isoform). The expression of the various CD46 isoforms varies from one tissue to another (29) and may potentially govern tissue sensitivity to MV infection. However, all the CD46 isoforms, including CYT1 and CYT2, tested so far were found to be able to mediate MV infection when expressed in rodent cells (6, 9, 23, 25). To find out whether the transmembrane and cytoplasmic regions of CD46 nonetheless play a crucial role in mediating MV infection, the ability of a chimeric glycosyl-phosphatidylinositol (GPI)-anchored form of CD46 (CD46-GPI) to act as an MV receptor was tested and compared with that of a transmembrane CD46 form after expression in a mouse B-cell line.

MATERIALS AND METHODS

Cell lines. All cell lines were grown in Dulbecco's modified Eagle's medium supplemented with 6% fetal calf serum, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 2 mM glutamine, 5×10^{-5} M 2-mercaptoethanol, and 50 μg of gentamicin per ml. The cell lines used were the M12 B-cell line and M12 cells transfected with either a C-CYT2 isoform of CD46 or a chimeric GPI-anchored form of CD46. The M12.CD46 cell line has been described previously (10, 25, 27), and the M12.CD46-GPI cell line was obtained after electroporation with the pSFFV-Neo-MCP-PI plasmid (kindly provided by D. M. Lublin [22]), selection in G418, three cycles of cell sorting for CD46 expression, and cloning, as detailed elsewhere (25). The MCP-PI construct encoded amino acids 1 to 269 of CD46 isoform BC-CYT2 and amino acids 307 to 347 of decay accelerating factor (DAF) (4, 19, 21, 24), which is a signal for GPI addition (Fig. 1). The mature protein contains the four N-terminal SCR regions, the STP-B region of CD46 attached to a small portion of the STP region, and the glycolipid anchor of DAF (Fig. 1).

Fluorescence-activated cell sorter analyses and virus binding assay. For the detection of CD46, cells were incubated for 30 min at 4°C with MCI20.6 antibody (26), washed, incubated for 20 min with a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin, and then subjected to flow cytometry. The incubations were carried out in phosphate-buffered saline (PBS) containing 1% bovine serum albumin and 0.1% NaN_3. For the virus binding assay, 2×10^5 cells were incubated for 1 h at 37°C with 50 hemagglutinin units of the Halle strain of MV (25). The cells were washed three times and then incubated with clone 55, an anti-MV hemagglutinin antibody (25). The cells were washed prior to incubation with an FITC-conjugated goat anti-mouse immunoglobulin. All the flow cytometric analyses were carried out with a FACStar (Becton Dickinson). The number of hemagglutinin units was determined by hemagglutination of vervet monkey erythrocytes (8).

GPI anchor assay. Cells (10^6) were incubated with 0.01 U of phosphatidylinositol phospholipase C (PIPLC) (Immunotech, Marseille, France) in 0.1 ml of Dulbecco's modified Eagle's medium for 1 h at 37°C. The cells were washed twice, and the viral binding assay was performed as described above.

Viral fusion and replication assay. Cells (10^6) were washed three times with complete culture medium and then incubated in 1 ml with 1 PFU of the Halle strain of MV per cell at 37°C. Six hours later, the cells were washed three times in culture

medium and once in PBS supplemented with 1 mM EDTA and 0.25% trypsin, incubated for 5 min at 37°C in this solution, and then washed twice in culture medium. The last wash medium was kept to determine the residual infectious MV contents. After a 96-h incubation at 37°C, cell-free supernatant was collected and assayed for plaque formation on Vero cells as previously described (25).

Down-regulation of cell surface CD46. Cells were infected overnight with either the wild-type vaccinia virus or the recombinant vaccinia virus coding for MV hemagglutinin (kindly provided by T. F. Wild) at 2 PFU per cell, and then flow cytometry was done by staining with the anti-CD46 MCI20.6 or the clone 55 anti-H antibody and FITC-labelled anti-mouse immunoglobulin G.

Antigen presentation assay. M12.4.1, M12.CD46, or M12.CD46-GPI cells (3×10^4 each) were incubated with various dilutions of MV and 10^5 hybridoma T cells for 24 h at 37°C in a final volume of 0.2 ml in 96-well microplates (10). MV was used either as a supernatant of infected Vero cells or as virus purified by sedimentation through a sucrose gradient as described previously (8). Subclones of previously described H-specific $I\text{-}E^d$-restricted THV124 and N-specific $I\text{-}A^d$-restricted TNP408 (10) selected for high expression of CD4 and greater sensitivity were used. The interleukin-2 secretion in the supernatant was measured by the MTT colorimetric CTL-L2 bioassay (10). All experiments were repeated with similar results, and the standard deviations between replicates were usually well below 5%. In some experiments, 50 μM chloroquine was added throughout the T-cell stimulation as described elsewhere (20) and was well tolerated by CTL-L2 cells.

RESULTS

Cell surface expression of CD46-GPI after transfection of M12.4.1 cells. M12.4.1 cells were transfected with an expression vector encoding a chimeric GPI-anchored form of CD46 (Fig. 1). A clone which stably expressed a level of CD46 similar to that expressed by the M12.CD46 transfectant (Fig. 2) as detected by the MCI20.6 antibody was selected. This antibody was initially selected on the grounds of its ability to block MV binding and infection of human cells (26).

Expression of a GPI-anchored form of CD46 in M12 murine B cells enables them to bind MV. M12.4.1, M12.CD46, and M12.CD46-GPI cells were tested for the ability to bind MV. The cells were incubated with MV for an hour and washed, and MV binding was detected by flow cytofluorometry with an anti-MV H antibody. It was found that expression of CD46-GPI enabled murine B cells to bind MV (49%) (Fig. 3f) as efficiently as murine cells expressing the transmembrane isoform (41%) (Fig. 3e). But no significant MV binding was observed on the parental M12.4.1 cells (4%) (Fig. 3d). The background labelling in the absence of MV was within a 2 to 4% range (Fig. 3a to c). These data indicate that the expression of the extracytoplasmic part of CD46 is necessary and sufficient for MV binding.

Loss of MV binding ability of M12.CD46-GPI cells after PIPLC treatment. To ascertain whether the MV binding ability of M12.CD46-GPI cells was related to CD46 anchored by GPI, the cells were treated with PIPLC, which cleaves GPI-anchored proteins. As expected, M12.CD46-GPI cells lost most of their ability to bind MV after treatment with PIPLC (17%) (Fig. 3h), whereas the efficiency of MV binding of M12.CD46 cells was not affected after PIPLC treatment (41%) (Fig. 3g). This result is also illustrated by expressing the data as median

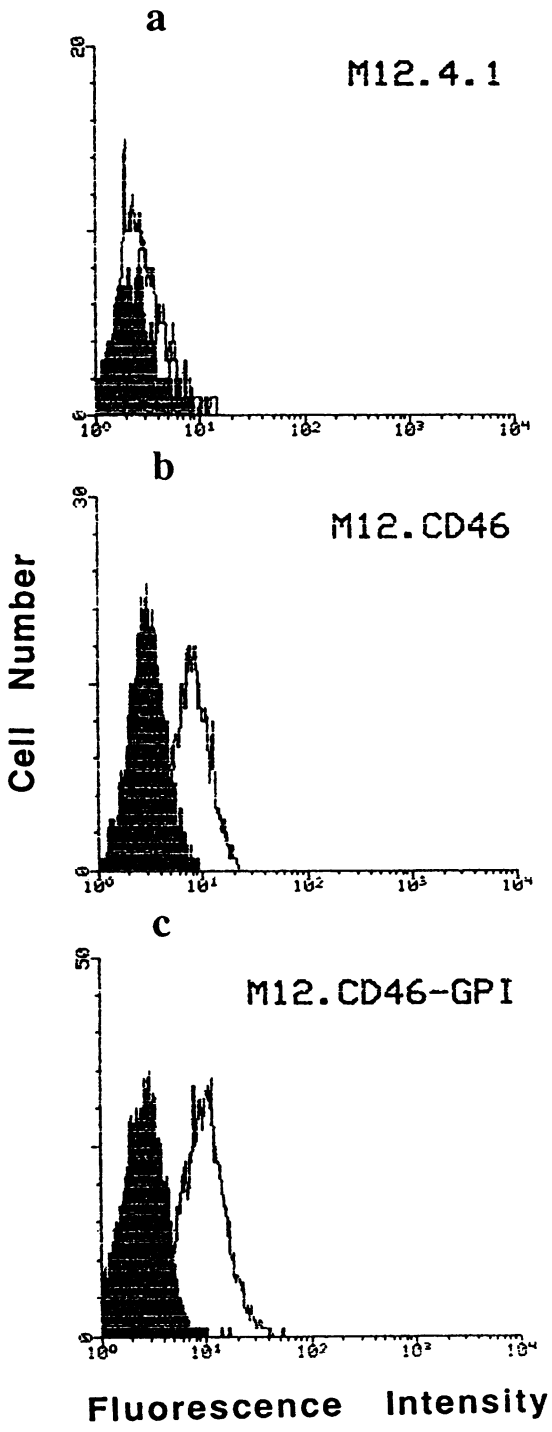

FIG. 2. Expression of CD46 and the GPI-anchored CD46 in M12.CD46 (b) and M12.CD46-GPI (c) cells, respectively. The fluorescence intensity was measured after the molecules were labelled with MCI20.6 antibody and subsequently with FITC-conjugated goat anti-mouse immunoglobulin. The level of CD46 expression is indicated by the shift of the open histogram (MCI20.6 labelling) to the right of the solid control histogram (FITC conjugate alone). Nontransfected M12.4.1 cells show no fluorescence (a).

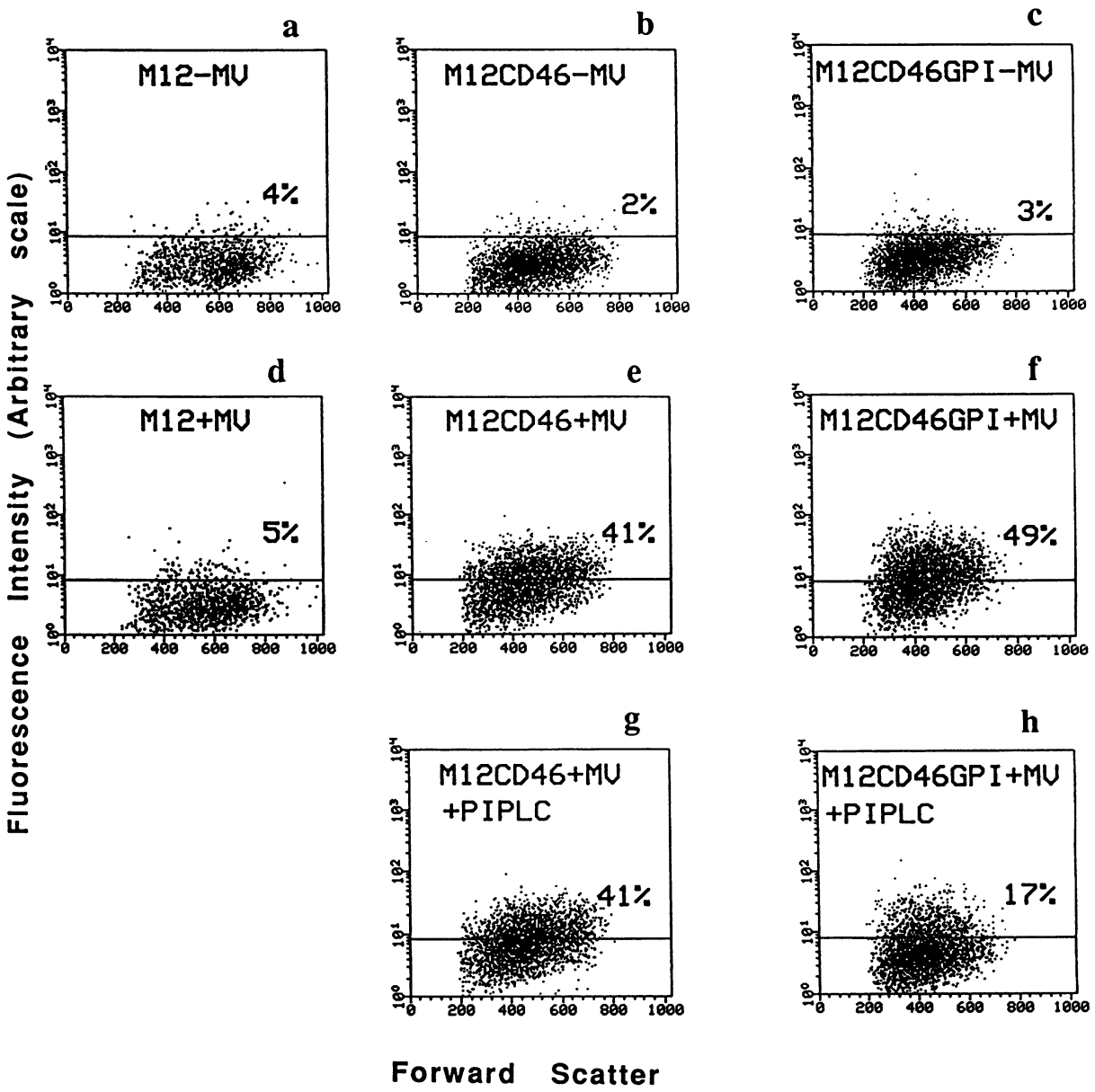

FIG. 3. The GPI-anchored form of CD46 mediates binding of MV as efficiently as the transmembrane form. M12.4.1 (a and d), M12.CD46 (b, e, and g), and M12.CD46-GPI (c, f, and h) cells were incubated alone (a to c) or with 50 hemagglutinin units of MV (d to h) and then labelled with anti-H monoclonal antibody and FITC-anti-mouse immunoglobulin G conjugate and analyzed by flow cytometry. Prior to incubation with MV, the cells were left untreated (a to f) or were pretreated with PIPLC (g and h). The level of MV binding is given as percent cells above the horizontal line.

peak channels. The median peak channels observed for MV binding on M12.CD46-GPI cells were strongly reduced from 8.4 to 4.4 but not abolished after PIPLC treatment, whereas those for binding on M12.CD46 cells remained stable (median peak channels, 7.3 and 7.4, respectively) (Table 1). Accordingly, cell surface CD46 expression by M12.CD46-GPI cells but not that by M12.CD46 cells was strongly reduced after PIPLC treatment, as detected by flow cytofluorometry with the MCI20.6 antibody. The median peak channels were reduced from 9.9 to 4.9 for PIPLC-treated M12.CD46-GPI cells and were largely unmodified (from 9.2 to 9.9) for PIPLC-treated M12.CD46 cells (Table 1). The residual MV binding activity of PIPLC-treated M12.CD46-GPI cells (a median peak channel

TABLE 1. Levels of CD46 expression and MV binding before and after PIPLC treatment of M12.CD46 and M12.CD46-GPI cells

Cell and treatment	Fluorescence median peak channel value		
	Background[a]	CD46 expression	MV binding
M12.CD46, none	2.5	9.2	7.3
M12.CD46, PIPLC	2.5	9.9	7.4
M12.CD46-GPI, none	3.1	9.9	8.4
M12.CD46-GPI, PIPLC	3.1	4.9	4.4

[a] Background fluorescence values are for cells incubated with FITC anti-mouse immunoglobulin alone and were identical to the self-fluorescence values of the cells.

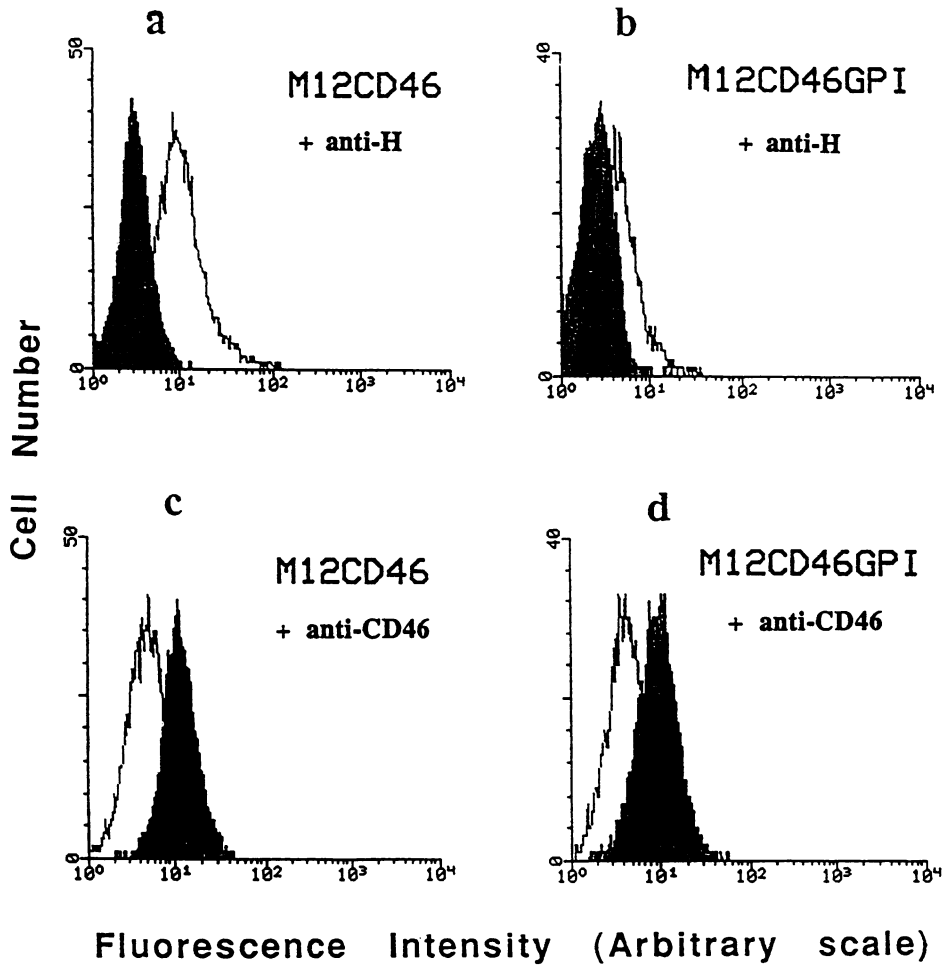

FIG. 4. Transmembrane CD46 and GPI-anchored CD46 show equal sensitivities to H-induced down-regulation. M12.CD46 (a and c) and M12.CD46-GPI (b and d) cells were either not infected (solid histograms) or infected (open histograms) overnight with 2 PFU of vaccinia virus H per cell. The cells were then treated with anti-H clone 55 antibody (a and b) or anti-CD46 MCI20.6 antibody (c and d) followed by FITC-conjugated goat anti-mouse immunoglobulin and analyzed by flow cytometry.

of 4.4, compared with a median peak channel of 3.1 for the fluorescence background of M12.CD46-GPI cells) (Table 1) corresponds to their residual CD46 expression (a median peak channel of 4.9) and was likely due to some CD46-GPI molecules that were not accessible to PIPLC digestion, as has been usually observed when other GPI-anchored proteins have been stripped by PIPLC (4, 15, 16).

Expression of CD46-GPI enables MV replication in mouse B cells. M12.4.1, M12.CD46, and M12.CD46-GPI cells were then tested as hosts for replication of MV. Ninety-six hours after infection with 1 PFU of MV per cell, the supernatants of the M12.CD46 and M12.CD46-GPI cells contained 38,000 and 18,300 PFU/10^6 infected cells, respectively, whereas that of M12.4.1 cells showed only a limited amount of infectious viral particles (480 PFU/10^6 cells), likely corresponding to a carryover of the MV input. Indeed, even after thorough washing, 42, 63, and 149 PFU/10^6 infected cells (M12, M12.CD46, and M12.CD46-GPI, respectively) were recovered in the last wash (6 h postinfection) prior to the 4-day incubation. Thus, the expression of the extracytoplasmic region of CD46 is necessary and sufficient to mediate MV entry and replication.

Cell surface expression of both the C-CYT2 CD46 isoform and CD46-GPI chimeric protein is down-regulated after expression of MV H glycoprotein. Cell surface CD46 expressed on human cells is down-regulated after infection with MV and/or expression of MV H glycoprotein (27). We therefore tested the sensitivities of the transmembrane CD46 isoform and the GPI-anchored CD46 form expressed in mouse B cells to MV H-induced down-regulation. M12.CD46 and M12.CD46-GPI cells were infected overnight either with wild-type vaccinia virus or with the recombinant vaccinia virus coding for MV H, and the expression of MV H and CD46 was tested by flow cytometry. MV H was expressed in both cell lines (Fig. 4a and b), and this resulted in the disappearance of cell surface expression of both CD46 and CD46-GPI molecules (Fig. 4c and d). The control infection of M12.CD46 and M12.CD46-GPI cells with wild-type vaccinia virus did not change the level of cell surface expression of CD46; this is similar to results previously reported (data not illustrated) (27). The levels of down-regulation of CD46 and CD46-GPI were similar, although more H was expressed on M12.CD46 cells. This may indicate either that the level of H expressed on M12.CD46-GPI is already sufficient to bring

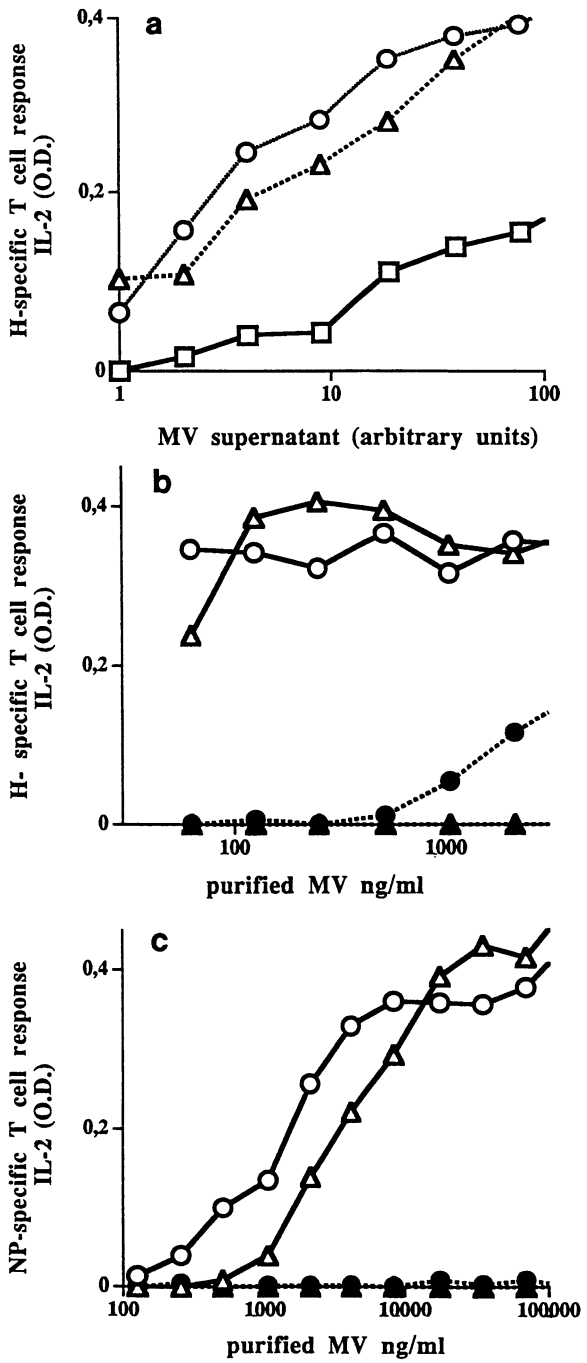

FIG. 5. Expression of GPI-anchored CD46 allows murine B cells to efficiently capture and process MV antigens for MHC class II-restricted presentation to T cells. M12 (squares), M12.CD46 (circles), and M12.CD46-GPI (triangles) cells were incubated with MV supernatants (a) or purified MV (b and c) and used to stimulate H-specific THV124 (a and b) or N-specific TNP408 (c) T hybridoma cells for 24 h in the absence (open symbols) or presence (solid symbols) of 50 μM chloroquine (b and c). The secreted interleukin-2 (IL-2) in the cell-free supernatants was measured by the colorimetric MTT CTL-L2 bioassay. O.D., optical density. NP, nucleoprotein.

down all the CD46 molecules expressed at the cell surface or that the chimeric CD46-GPI form is more sensitive to H-induced down-regulation. Similar results were obtained (i) when another anti-CD46 monoclonal antibody (GB24) which reacts with another epitope of CD46 not involved in MV binding and infection was used and (ii) when cell surface CD46 molecules were prelabelled with anti-CD46 antibodies prior to infection with recombinant vaccinia virus H (data not shown). So, the notion that the lower level of labelling of H-expressing M12.CD46 and M12.CD46-GPI cells with anti-CD46 antibodies was due to competition between the antibodies and H for the same binding site on the CD46 molecule can be excluded. These results thus show that the expression of the extracytoplasmic part of CD46 is sufficient for it to be a target for H-induced down-regulation.

The chimeric CD46-GPI molecule efficiently mediates MV uptake and MHC class II presentation of MV antigens through a chloroquine-sensitive pathway. M12.CD46-GPI cells were incubated with MV and used to stimulate an H-specific MHC class II-restricted T-cell hybridoma. As was observed when M12.CD46 cells were used as antigen-presenting cells, M12.-CD46-GPI cells were found to efficiently capture MV and to present viral H to the THV124 T cells (Fig. 5a), whereas the parental M12 cells were roughly 100 times less efficient. The efficiency with which M12.CD46-GPI cells act as antigen-presenting cells was similar to that observed with M12.CD46. A similarly efficient antigen presentation of the internal viral nucleoprotein N resulting from CD46-GPI-mediated MV capture was observed (data not shown). MHC class II presentation of both MV H and N proteins by M12.CD46-GPI was found to be strongly inhibited in the presence of 50 μM chloroquine (Fig. 5b and c). A similar inhibition of MV H and N presentation by M12.CD46 cells was observed (Fig. 5b and c), as previously reported (10). In the presence of a large amount of MV, the level of chloroquine inhibition of H presentation appeared to be somewhat lower for M12.CD46 than for M12.CD46-GPI (Fig. 5b), but this small difference was not reproducibly observed and was not seen when presentation of the N protein was studied (Fig. 5c).

DISCUSSION

This report shows that, when expressed in mouse B cells, a chimeric GPI-anchored CD46 molecule (Fig. 1) encompassing the four SCR regions and the STP-B region of CD46 that are attached to a small portion of the STP region and the glycolipid anchor of DAF (a glycolipid-anchored protein belonging to the same family [4, 19, 21, 24]) displays the MV receptor properties of the transmembrane CD46 molecule: it allows virus binding, virus entry, and replication; it is sensitive to H-induced down-regulation; and it mediates virus internalization in a chloroquine-sensitive cell compartment involved in the generation of peptide-MHC class II complexes, i.e., virus endocytosis.

Binding of MV via the viral H ligand is the primary function of CD46 as a virus receptor. Since the chimeric CD46-GPI molecule contains only the four SCR regions and the STP-B region of CD46, and since the C-CYT2, BC-CYT2, B-CYT2, BC-CYT1, and C-CYT1 isoforms are able to mediate MV binding and MV infection (6, 9, 23, 25), STP-B and/or STP-C regions are not involved in the virus receptor properties of CD46. The DAF-derived STP region of the chimeric molecule is unlikely to play any role in the interaction of CD46-GPI with MV since the DAF molecule cannot mediate

any MV binding. Indeed, human erythrocytes which express a high level of DAF but no CD46 (21) are not agglutinated in the presence of MV. Therefore, the H binding site on CD46 is likely to be localized within the SCR regions, which are also the regions involved in the complement cofactor activity of CD46 (1, 22).

After binding to its target cell, MV has to fuse with the plasma membrane. Although virus envelope fusion to the cell membrane is difficult to detect, the MV-induced cell-cell fusion which is mediated by MV H and F glycoproteins and CD46 (25, 34) is likely to involve similar molecular mechanisms. Indeed, M12 B cells expressing transmembrane CD46 do form syncytia when infected with a double recombinant H-F vaccinia virus (25) or after MV infection (unpublished data). Formation of syncytia after recombinant H-F vaccinia virus or MV infection of M12 expressing the chimeric CD46-GPI molecule was rarely observed. However, CD46-GPI-mediated fusion of MV with the cell membrane should have occurred since the virus was able to replicate in M12.CD46-GPI cells. In addition, when CD46-GPI was expressed in 3T3 mouse fibroblasts, syncytium formation was readily observed after infection with the H-F recombinant vaccinia virus (unpublished data). It is possible that the poor syncytium formation after MV or H-F recombinant vaccinia virus infection of M12.CD46-GPI cells is related to their growth characteristics as poorly adherent cells.

As a consequence of MV binding and fusion to the plasma cell membrane mediated by the chimeric CD46-GPI molecule, MV replication occurred, indicating that the cytoplasmic and the transmembrane hydrophobic parts of CD46 are dispensable. Intriguingly, the corresponding domains of CD4 (5, 15), ICAM-1 (32), and the poliovirus receptor (17) are also dispensable in mediating human immunodeficiency virus (HIV), major rhinovirus, and poliovirus infections, respectively. For example, the chimeric GPI-anchored CD4 molecule and the ICAM-1 molecule can both mediate HIV and rhinovirus infections, respectively, as do their transmembrane counterparts.

The apparent functional mimicry between transmembrane CD46 and GPI-anchored CD46 extends beyond mediating MV binding and replication. Both were found to be sensitive to the H-mediated down-regulation of CD46 cell surface expression previously reported to be due, at least partly, to the H-induced internalization of CD46 (27). Moreover, they both mediate MV endocytosis, as evidenced by their ability to mediate MV uptake and internalization in a chloroquine-sensitive compartment, allowing H- and N-antigen presentation by MHC class II molecules. The H molecules used for presentation by MHC class II molecules could have come from H glycoproteins embedded in the plasma membrane after virus-cell fusion, thus following the usual endocytic fate of cell surface glycoproteins and efficient MHC class II-restricted presentation (2). But the internal virion N protein, after virus-cell fusion, is released into the cytosol and probably cannot reach the lumens of endosomal compartments where peptide-MHC class II complexes are formed (3, 12, 14). Accordingly, specific inhibition of virus-cell fusion by the z-Phe-Phe-Gly tripeptide has been shown to have no effect on the efficiency of MV N presentation by MHC class II molecules (10). Thus, at least for the N protein, the source of peptides presented by MHC class II molecules is most likely derived from endocytosed MV particles. Compared with the amount of MV which fuses with the plasma membrane, the amount of endocytosed MV is rather limited (2 to 3% of the total virus captured by the target cells) (11). The sensitivity of the GPI-anchored CD46 molecule to H-induced down-regulation and its ability to mediate MV endocytosis indicate that it is mainly if not solely the four SCR regions of CD46 that are involved in these two processes. The STP-B and -C regions would have no or a limited role.

Altogether, these data indicate that the transmembrane regions and cytoplasmic tails of CD46 appear to be dispensable and that GPI anchoring is their functional equivalent. Indeed, GPI-anchored cell surface molecules can transduce intracellular signals by pathways identical to (18) or different from (30) those used by their transmembrane counterparts. Comparison of a CD46-derived transmembrane region and cytoplasmic tail with the DAF-derived GPI anchor by using the chimeric DAF-CD46 protein has shown that only the latter is able to transduce intracellular signals via two protein tyrosine kinases (31). Thus, if any CD46-mediated intracellular signalling is involved in mediating MV entry (by fusion or endocytosis), MHC class II-mediated antigen presentation, and/or MV replication, the different intracellular signal delivered through the DAF-derived GPI anchor should result in similar final effects. For example, the abilities of CD46 and CD46-GPI to mediate MV endocytosis may look functionally similar at the level of peptide-MHC class II complex formation but involve distinct endocytic pathways in the initial step, as recently reported for spontaneous internalization of transmembrane CD4 and GPI-anchored CD4 (16).

As was hypothesized for the interactions between CD14 and endotoxin (18), is it possible that the sole function of CD46 in MV entry is to be the primary ligand, allowing the virus envelope to be brought near additional membrane components present on both human and rodent cells and responsible for viral fusion and entry? Indeed, molecules other than the CD4 receptor are required for infection by HIV, which cannot replicate in human CD4-expressing rodent cells (15). Molecules other than CD46, such as the substance P receptor (13) and moesin (7), have been shown by antibody inhibition to contribute to cell fusion and/or replication by MV, but their function in the absence and presence of CD46 remains to be established. Are those two molecules involved in nonspecific events occurring just after the MV entry mediated by the CD46 receptor?

In conclusion, we showed that the cell surface expression of the four SCR regions of CD46 is sufficient to mediate MV binding to the cell surface, intracellular virus entry after fusion with the plasma membrane, virus replication, virus endocytosis, and sensitivity to virus hemagglutinin-mediated cell surface down-regulation of CD46. Thus, it can be predicted that any of the CD46 isoforms may support MV infection since they all share these structural elements. Accordingly, up to now, every CD46 isoform has been found to mediate MV binding and infection (6, 9, 23, 25). This fits with the broad tissue distribution of CD46 expression, with the notable exception of erythrocytes (19), and to our knowledge, the ability of any human cell except erythrocytes to sustain MV infection or MV binding. Although this report does not show any role for the transmembrane region and cytoplasmic tail of CD46 in its MV receptor properties, it remains to be confirmed that they do not play a more subtle role in MV infection and physiopathogenesis. Indeed, in the case of HIV, a recent report has demonstrated that the cytoplasmic tail of CD4 could control the level of HIV replication (33).

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