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Human Cell Receptor CD46 Is Down Regulated through Recognition of a Membrane-Proximal Region of the Cytoplasmic Domain in Persistent Measles Virus Infection

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Monkey cells persistently infected by measles virus (MV) Biken strain (Biken-CV-1 cells) showed no cytopathic effects and lacked surface expression of a homolog of human cell receptor, membrane cofactor protein CD46. Transfection of a human CD46 gene into these cells induced extensive cell fusion, indicating that down regulation of the endogenous CD46 homolog was essential for the maintenance of a noncytopathic mode of infection. Surface expression of the exogenously introduced human CD46 was also drastically down regulated in the persistently infected cells compared with uninfected cells. The down regulation was specific for CD46 and did not affect surface expression of exogenously introduced CD4. Exogenous human CD46 was synthesized efficiently in the persistently infected cells, but it did not accumulate on the cell surface. Fusion of Biken-CV-1 cells required the extracellular hemagglutinin (H-protein)-binding domain but not the cytoplasmic domain. Replacing the transmembrane and cytoplasmic domains of CD46 with a glycosylphosphatidylinositol anchor did not prevent cell fusion but completely alleviated down regulation of the glycosylphosphatidylinositol-anchored CD46 in Biken-CV-1 cells. Deletion analyses revealed that the membrane-distal sequences of the CD46 cytoplasmic domain were not only unnecessary but also inhibitory for CD46 down regulation. By contrast, the six amino acid residues proximal to the membrane contained a sequence required for CD46 down regulation in the persistently infected cells. These results indicate that CD46 is down regulated in the persistently infected cells by a mechanism that recognizes a membrane-proximal sequence in the CD46 cytoplasmic domain.

Measles virus (MV), a morbillivirus in the paramyxovirus family, attaches to human cells through interaction between the virus-encoded hemagglutinin (H protein) and a cellular receptor (3, 34). Upon binding to the host cells, the H protein cooperates with the viral fusion (F) protein to fuse the viral envelope with the plasma membrane, allowing virus entry into the host cells (31, 52). Interaction between these viral and cellular proteins also induces fusion between infected and uninfected cells to form multinucleated giant cells (syncytia) (34). Thus, the interaction between the MV glycoproteins and the cellular receptors plays a crucial role in the entry and cell-to-cell transmission of the virus.

The major human cell receptor for MV has been identified as the membrane cofactor protein (MCP or CD46) (11, 32). CD46 is a complement-regulating protein whose normal function is to protect the host from autologous complement attack, by binding complement components C3b and C4b and facilitating their cleavage by factor I (24, 43). CD46 is a type 1 transmembrane protein with an amino-terminal extracellular domain and a carboxy-terminal cytoplasmic domain. Most human cells synthesize differentially spliced CD46 mRNAs encoding multiple 45- to 65-kDa CD46 isoforms. The largest isoform contains four short consensus repeats (SCR1 to -4) and three serine-, threonine-, and proline-rich regions (STP-A to -C), followed by a short sequence which links these extracellular regions to a transmembrane anchor and a cytoplasmic domain. Other CD46 isoforms have the same SCR domains, but they differ in having different STP domains and one of two alternative cytoplasmic termini called Cyt 1 and Cyt 2 (24). Each of the SCR1, -2, and -4 domains contains an N-linked glycosylation site, and each STP domain possesses multiple O-linked glycosylation sites (26, 36, 37). All of the common CD46 isoforms can serve as receptors for MV (14, 29). The SCR1 and SCR2 domains constitute the binding determinant for the MV H protein, whereas the SCR3, SCR4, and to some extent SCR2 domains are important for the complement-regulating function (1, 19, 30) (Fig. 1A).

Infection by MV causes down regulation of surface expression of CD46 (33), which renders cells more susceptible to complement-mediated lysis (42). This effect has been postulated to contribute to the pathogenicity of MV (39, 40). Interestingly, many newly isolated lymphotropic MV strains do not down regulate CD46, even though the infectivities of some of those strains are inhibited by anti-CD46 antibodies (39, 40). Recently, the abilities to interact with and down regulate CD46 have been linked to specific amino acid changes in the MV H protein, possibly as a result of adaptation to replicate in cultured fibroblasts (23). There is also circumstantial evidence suggesting that the H protein-CD46 interaction may be involved in immunosuppression. Peripheral blood mononuclear cells infected by MV suppress the mitogenic response of uninfected peripheral blood mononuclear cells in a mixed culture (38), and antibodies against MV abolish the immunosuppressive effect (38, 54). The finding that the MV H protein can trigger CD46 down regulation through cell contact lends support to a role for these viral and cellular proteins in intercellular communication (21, 41).

Studying the interaction between MV and CD46 in a per-

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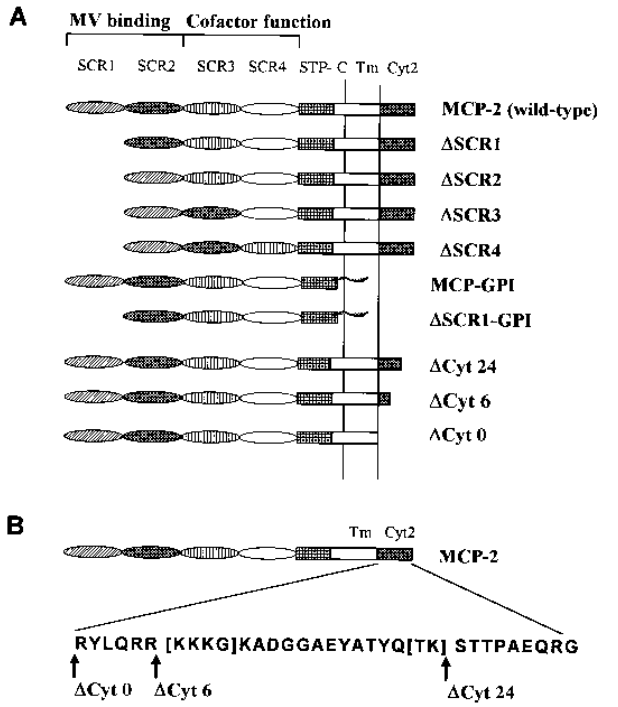

FIG. 1. Diagrammatic representation of the structures of CD46 and mutants. (A) Comparison of wild-type CD46 and mutants used in this study. (B) Amino acid sequence (in single-letter amino acid code) of the Cyt 2 CD46 cytoplasmic domain. Residues in brackets are predicted to be in β-turns. Arrows show the termini of the carboxy-terminal deletion mutants. Tm, transmembrane domain.

sistent infection is a useful approach to gain knowledge of the CD46 down regulation mechanisms. MV occasionally causes a persistent infection in the human brain, which leads to a rare fatal central nervous system disease subacute sclerosing panencephalitis (SSPE) (47). A number of SSPE-associated MV strains have been isolated and maintained in persistently infected cell cultures. Biken-CV-1 cells are African green monkey kidney (CV-1) cells persistently infected by the Biken strain, a well-characterized SSPE-associated MV evolved from a known progenitor acute MV Nagahata strain (2, 17, 18, 49, 51, 53). Biken-CV-1 cells are 100% infected and constitutively express the Biken viral H and F proteins, yet these persistently infected cells show no cytopathic effects (16). In this study, we examined the expression of human CD46 and mutants by transfection into Biken-CV-1 cells and found that the persistently infected cells specifically down regulate CD46 by a mechanism that recognizes a membrane-proximal region of the CD46 cytoplasmic domain.

MATERIALS AND METHODS

Cell cultures. Uninfected African green monkey kidney CV-1 and Biken-CV-1 cells persistently infected by Biken strain SSPE-associated MV (16, 49) were maintained in Eagle's minimum essential medium supplemented with 10% newborn calf serum.

Plasmids encoding CD46 and mutants. Figure 1A shows the CD46 gene constructs used for this study. MCP-2 encodes a human CD46 isoform with STP-C and Cyt 2 domains (26). The deletion mutants ΔSCR1, ΔSCR2, ΔSCR3, and ΔSCR4 lacking different SCR domains were generated by site-specific mutagenization (19). The MCP-GPI construct, encoding a glycosylphosphatidylinositol (GPI)-anchored form of CD46, was generated by PCR to join the sequences encoding amino acids 1 through 293 of MCP-2 to the sequences encoding amino acids 308 through 347 of decay-accelerating factor (25), which provided a signal for GPI addition. The presence of a GPI anchor was confirmed by treatment with phosphatidylinositol phospholipase C (18a). The ΔSCR1-GPI construct was generated by replacing the carboxy-terminal sequences of ΔSCR1 with the corresponding sequences from MCP-GPI by using a common *Avr*II site (nucleotides 963 and 964). Cytoplasmic domain deletion mutants (ΔCyt 24, ΔCyt 6, and ΔCyt 0) were generated from the MCP-2 clone by PCR site-specific mutagenization to introduce translational stop codons at the positions depicted in Fig. 1B. The exact sequences of all of the mutagenized constructs were confirmed by DNA sequencing. The human CD4 cDNA (28) was kindly provided by Michael Emmerman (Fred Hutchinson Cancer Research Center, Seattle, Wash.). For expression studies, all of the cloned genes were placed in the pME18S vector under control of a modified simian virus 40 early promoter, SRα (46).

Antibodies. Monoclonal antibody (MAb) M177 against CD46 has been described previously (44). The epitope of M177 was mapped to SCR2 (19). MAb MH61 was against a sperm-specific antigen identified as the sperm CD46 (35) and was kindly provided by Masaru Okabe (Research Institute for Microbial Diseases, Osaka, Japan). The epitope of MH61 was mapped to SCR3 (19). The MAb against human CD4 (SIM.2) was obtained from the NIH AIDS Research and Reference Reagent Program.

Transfection and analysis of surface and intracellular CD46. CV-1 or Biken-CV-1 cells were transfected with 10 to 15 μg of cDNA per 7×10^5 cells by the calcium phosphate method (15). The transfected cultures were shocked with 10% dimethyl sulfoxide for 10 min at 4 to 5 h posttransfection. The surface and intracellular CD46 was recovered by sequential immunoprecipitation (27) as follows. At approximately 45 h posttransfection, the transfected cells were washed twice with ice-cold phosphate-buffered saline (PBS) containing 1% bovine serum albumin and then incubated in 200 μl of the same solution containing 1 μl of MAb at 4°C for 1 h with frequent agitation. Unbound antibody was removed by washing the cultures three times with ice-cold PBS, and cells were lysed in 200 μl of PBS containing 1% Triton X-100 or radioimmunoprecipitation assay buffer (10 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% sodium dodecyl sulfate [SDS]). Nuclei were removed by centrifugation, and the surface antibody-bound proteins were precipitated with protein G-Sepharose (Pharmacia). The supernatant was subjected to another round of immunoprecipitation with 1 μl of MAb to recover the intracellular proteins. The surface and intracellular proteins were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) under nonreducing conditions and detected by enhanced chemiluminescence Western blotting (immunoblotting) (Amersham Life Science), using the same MAb as for immunoprecipitation.

Metabolic labeling and pulse-chase analysis. At approximately 45 h posttransfection, cells were labeled for 1 h with 60 μCi of [^{35}S]methionine (Du Pont NEN Research Products) per culture. For pulse-chase analysis, cells were labeled with 100 μCi of [^{35}S]methionine per culture for 30 min, washed with medium containing 10 mM L-methionine, and incubated in the same medium for 2 h. The surface and intracellular labeled proteins were sequentially immunoprecipitated as described above and analyzed by SDS-PAGE and fluorography.

RESULTS

Biken-CV-1 cells lack an endogenous CD46 homolog for supporting syncytium formation. Biken-CV-1 cells produce all the viral proteins, including the H and F proteins (16, 18, 51), yet these cells do not form syncytia and are morphologically similar to uninfected CV-1 cells (Fig. 2F and A, respectively). Transfection of a human CD46 cDNA (MCP-2) into Biken-CV-1 cells induced extensive syncytia (Fig. 2G). Transfection of the same CD46 cDNA into uninfected CV-1 cells caused no morphological changes (Fig. 2B). The ΔSCR1 and ΔSCR2 mutants lacking the H-protein-binding domain (Fig. 1A) (19, 30) did not induce syncytia in either CV-1 or Biken-CV-1 cells (Fig. 2C and H, respectively; similar data for ΔSCR2 not shown). The ΔSCR3 mutant (Fig. 1A) lacking the cofactor function (19, 30) caused extensive syncytia in Biken-CV-1 but not CV-1 cells (Fig. 2I and D, respectively).

These results show that the absence of syncytia in Biken-CV-1 cells is due to lack of a functional CD46 homolog that can support MV-induced cell fusion. The H-protein-binding domain of CD46 is required, but the complement-regulatory cofactor function of CD46 is nonessential for syncytium formation in Biken-CV-1 cells.

Down regulation of exogenous human CD46 in Biken-CV-1 cells. To gain insight into the basis of CD46 down regulation in Biken-CV-1 cells, we studied expression of the human CD46 and mutants in the transfected CV-1 and Biken-CV-1 cells. We first ruled out possible differences in transfection efficiency by comparing the intracellular CD46-specific RNAs by Northern (RNA) blot analysis. In both CV-1 and Biken-CV-1 cells, the

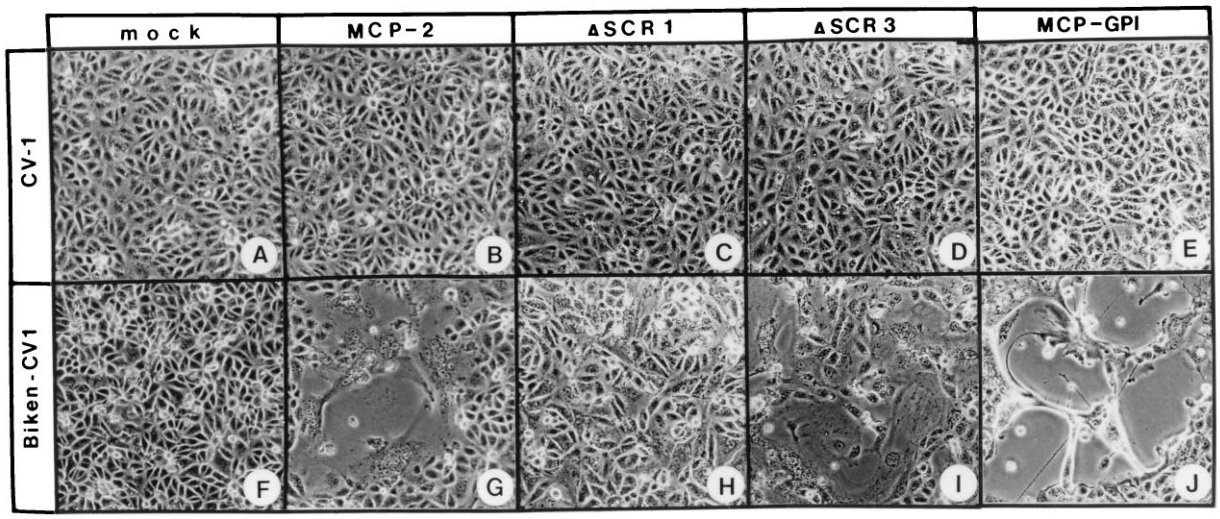

FIG. 2. Syncytium formation induced by human CD46 in Biken-CV-1 cells. CV-1 or Biken-CV-1 cells were transfected with cDNAs encoding wild-type CD46 (MCP-2) (B and G), ΔSCR1 (C and H), ΔSCR3 (D and I), or MCP-GPI mutant (E and J). Syncytium formation was studied by light microscopy at about 45 h posttransfection.

exogenously introduced human CD46 constructs produced equivalent levels of CD46-specific RNA (data not shown). We then compared the surface-associated and intracellular CD46 protein by immunoprecipitation with MAb M177 against human CD46 (44) as described in Materials and Methods.

MAb M177 detected low levels of the simian endogenous CD46 homolog on the surface and in the interior of the mock-transfected CV-1 cells (Fig. 3A, CV-1, lanes 1 and 5, respectively). The mock-transfected Biken-CV-1 cells expressed very low levels of intracellular CD46 homolog, which was not detectable on the cell surface (Fig. 3A, Biken-CV-1, lanes 5 and 1, respectively). The low expression of endogenous CD46 on the Biken-CV-1 cell surface was confirmed by fluorescence-activated cell sorter analysis (20a). Human epidermoid (KB) cells persistently infected by the Biken strain MV also expressed very low levels of CD46 on the cell surface (20a). Therefore, the low levels of CD46 on the Biken-CV-1 cell surface were not simply due to inefficient detection of the simian CD46 homolog by the MAb against the human CD46.

After transfection with MCP-2, CV-1 cells expressed high levels of 50- to 65-kDa CD46 proteins on the cell surface and intracellularly (Fig. 3A, CV-1, lanes 2 and 6, respectively). By contrast, Biken-CV-1 cells transfected with the same CD46 cDNA produced the 50- to 65-kDa CD46 proteins plus an additional 40-kDa CD46-related protein (Fig. 3A, Biken-CV-1, lane 6). These CD46-related proteins were detected mainly intracellularly, but very low levels of these proteins were seen on the transfected Biken-CV-1 cell surface (Fig. 3, Biken-CV-1, lane 2). Both the ΔSCR1 and ΔSCR3 mutants were expressed efficiently on the surface and in the interior of the transfected CV-1 cells (Fig. 3A, CV-1, lanes 3, 4, 7, and 8). The ΔSCR1 mutant showed higher electrophoretic mobility than the ΔSCR3 mutant (Fig. 3A, CV-1, lanes 7 and 8) as a result of the loss of an N-linked glycosylation site in the SCR1 domain but not in the SCR3 domain (19). Deleting the SCR3 domain did not alleviate the down regulation of CD46 on the Biken-CV-1 cell surface (Fig. 3, Biken-CV-1, lane 4). The effect of SCR1 deletion was more difficult to judge, since the ΔSCR1 mutant was expressed poorly both on the surface and intracellularly in Biken-CV-1 cells (Fig. 3A, Biken-CV-1, lanes 3 and 7). We also tested the ΔSCR2 and ΔSCR4 mutants and found that deletion of the SCR2 or SCR4 domain also affected expression in Biken-CV-1 cells (data not shown).

MAb M177 is against an epitope in the SCR2 domain involved in H-protein binding (19, 30). To test the possibility that this epitope was masked by the Biken virus H protein in the Biken-CV-1 cells, we also studied CD46 expression with MAb

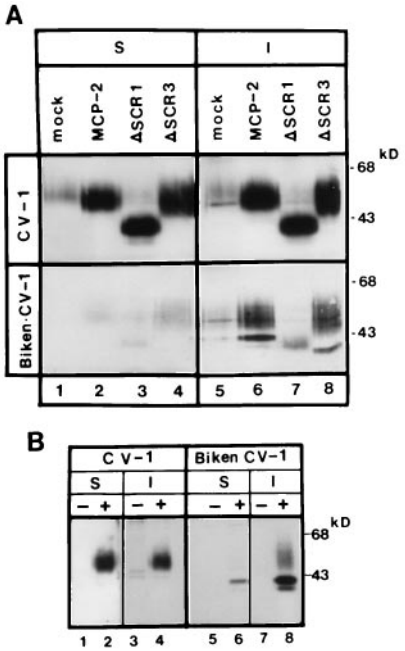

FIG. 3. Comparison of surface and intracellular expression of CD46 and mutants. (A) CV-1 or Biken-CV-1 cells were transfected with no DNA (lanes 1 and 5), MCP-2 (lanes 2 and 6), ΔSCR1 (lanes 3 and 7), or ΔSCR3 (lanes 4 and 8). Surface (S) and intracellular (I) proteins were immunoprecipitated with MAb M177, resolved by SDS-PAGE, and detected by immunoblotting with the same MAb. (B) CV-1 or Biken-CV-1 cells were transfected with no DNA (−) or MCP-2 (+). Surface (S) and intracellular (I) proteins were immunoprecipitated with MAb MH61, resolved by SDS-PAGE, and detected by immunoblotting with the same MAb.

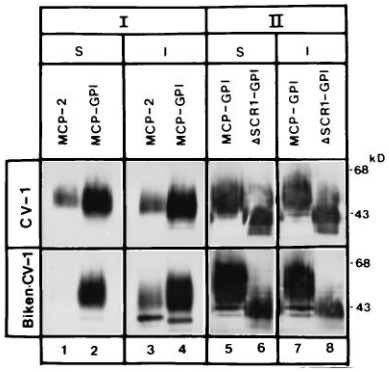

FIG. 4. Comparison of surface and intracellular expression of the GPI-anchored form of CD46. CV-1 or Biken-CV-1 cells were transfected with MCP-2 (lanes 1 and 3), MCP-GPI (lanes 2, 4, 5, and 7), or ΔSCR1-GPI (lanes 6 and 8). Surface (S) and intracellular (I) proteins were immunoprecipitated with MAb M177, resolved by SDS-PAGE, and detected by immunoblotting with the same MAb.

MH61 (35) against an epitope in SCR3, which is not involved in H-protein binding (19, 30). MAb MH61 did not detect the endogenous simian CD46 homolog in the untransfected CV-1 or Biken-CV-1 cells efficiently (Fig. 3B, lanes 1, 3, 5, and 7), but it detected the human CD46 expressed from MCP-2 on the surface and inside the transfected CV-1 cells (Fig. 3B, lanes 2 and 4). MAb MH61 detected 50- to 65-kDa and 40-kDa proteins in the MCP-2-transfected Biken-CV-1 cells (Fig. 3B, lane 8). The 40-kDa and the additional lower band are incompletely glycosylated CD46 species generated by interaction with the Biken virus H protein during exocytic transport of CD46 (16a) (see Discussion). MAb MH61 reacted with the incompletely glycosylated CD46 species more efficiently than MAb M177 (compare Fig. 3A and B). Most important, MAb MH61 also detected very low levels of CD46, including the incompletely glycosylated species on the Biken-CV-1 cell surface (Fig. 3B, lane 6). Taken together, the studies with both MAb M177 and MAb MH61 show that surface expression of CD46 is down regulated in the Biken-CV-1 cells, and this effect is not simply due to epitope masking by the Biken H protein.

Cytoplasmic and transmembrane domains of CD46 are essential for down regulation in Biken-CV-1 cells. To test whether the transmembrane and cytoplasmic domains of CD46 play a role in the down regulation of CD46, we transfected CV-1 or Biken-CV-1 cells with the MCP-GPI construct encoding a human CD46 variant lacking the transmembrane and cytoplasmic domains. MCP-GPI was identical to MCP-2 except that the transmembrane and cytoplasmic domains were replaced by a GPI anchor (Fig. 1A). Surface and intracellular levels of the GPI-anchored CD46 variant were compared with those of the transmembrane form of CD46 expressed from MCP-2. Confirming the results presented above, the transmembrane form of CD46 was expressed efficiently both on the surface and in the interior of the transfected CV-1 cells (Fig. 4, CV-1, lanes 1 and 3) but was drastically down regulated on the surface of Biken-CV-1 cells (Fig. 4, Biken-CV-1, lanes 1 and 3). In sharp contrast, the GPI-anchored CD46 variant was highly expressed on the cell surface and inside both CV-1 and Biken-CV-1 cells in repeated experiments (Fig. 4, lanes 2, 4, 5, and 7). A portion of the GPI-anchored CD46 variant expressed in Biken-CV-1 cells also appeared as a partially glycosylated lower band. Deleting the SCR1 domain from the GPI-anchored CD46 variant (ΔSCR1-GPI) (Fig. 1A) reduced the size of the protein but did not affect the surface expression in both CV-1 and Biken-CV-1 cells (Fig. 4, lanes 6 and 8). The MCP-GPI mutant caused more extensive syncytia than the transmembrane-anchored CD46 in Biken-CV-1 cells (Fig. 2J). Thus, the transmembrane and cytoplasmic domains of CD46 appear to be required for CD46 down regulation in a persistent MV infection, and the lack of these domains may allow the GPI-anchored CD46 mutant to escape down regulation and cause extensive syncytium formation in Biken-CV-1 cells.

To determine whether other transmembrane proteins are also down regulated in Biken-CV-1 cells, we transfected the CD4 gene under control of the same promoter into CV-1 and Biken-CV-1 cells and analyzed CD4 expression by the same procedures. Unlike CD46, the 55-kDa CD4 protein was expressed on the surface and inside both the transfected CV-1 and Biken-CV-1 cells (Fig. 5, lanes 2 and 4). These results suggest that CD46 is down regulated by a mechanism that specifically recognizes the transmembrane or cytoplasmic domain of CD46.

CD46 is synthesized efficiently in Biken-CV-1 cells but does not accumulate on the cell surface. To determine whether the low level of CD46 in Biken-CV-1 cells was due to inefficient protein synthesis, we studied the surface-associated and intracellular CD46 by metabolic labeling with [^{35}S]methionine. CD46 was labeled very inefficiently, and its detection required the use of high concentrations of radioisotope and long exposure of the autoradiogram, which caused a high background. Nonetheless, newly synthesized intracellular CD46 was detected after 1 h labeling in CV-1 cells transfected with the MCP-2 cDNA and various mutants (Fig. 6A, CV-1, lanes 7 to 11, asterisks). In the same labeling period, newly synthesized intracellular CD46 was also detected in Biken-CV-1 cells transfected with the MCP-2 cDNA and mutants (Fig. 6A, Biken-CV-1, lanes 7 to 11, asterisks). These cell types showed markedly different levels of labeled CD46 on the cell surface. The newly synthesized mutant CD46 proteins appeared on the transfected CV-1 cell surface within 1 h (Fig. 6A, CV-1, lanes 2 to 5, asterisks). MCP-2 CD46 appeared to be transported to the CV-1 cell surface more slowly than the various CD46 mutants but became detectable along with the mutant proteins after a 2-h chase (Fig. 6A, CV-1, lanes 1 to 5; Fig. 6B, CV-1, lane 2, bracket). By contrast, only the GPI-anchored forms of CD46 were detectable on the Biken-CV-1 cell surface after the 1-h labeling period (Fig. 6A, Biken-CV-1, lanes 4 and 5, asterisks), but MCP-2 and CD46 mutants with a transmembrane anchor were not detected on the Biken-CV-1 cell surface (Fig. 6A, Biken-CV-1, lanes 1 to 3). After a 2-h chase, the intracellular CD46 proteins were reduced in Biken-CV-1 cells (Fig. 6A, Biken-CV-1, lanes 7 to 10). However, only the MCP-GPI

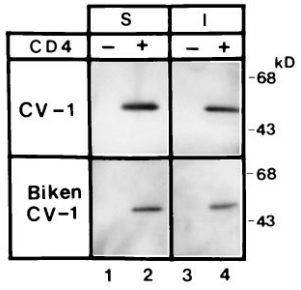

FIG. 5. Comparison of surface and intracellular expression of CD4. CV-1 or Biken-CV-1 cells were transfected with a CD4 cDNA, and surface (S) or intracellular (I) proteins were immunoprecipitated with MAb SIM.2 against human CD4. Immunoprecipitated surface and intracellular proteins were analyzed by SDS-PAGE and immunoblotting with the same MAb.

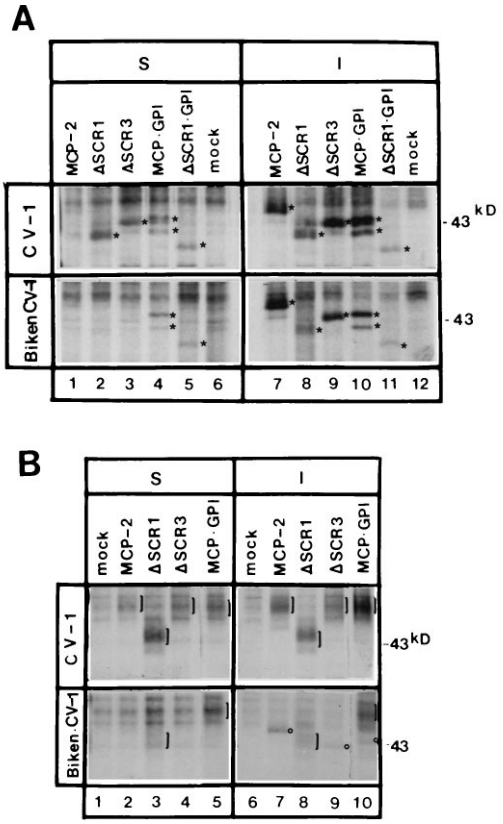

FIG. 6. Synthesis and accumulation of CD46 and mutants studied by metabolic labeling. (A) CV-1 or Biken-CV-1 cells were transfected with MCP-2 (lanes 1 and 7), ΔSCR1 (lanes 2 and 8), ΔSCR3 (lanes 3 and 9), MCP-GPI (lanes 4 and 10), ΔSCR1-GPI (lanes 5 and 11), or no DNA (lanes 6 and 12). At about 45 h posttransfection, cells were labeled with [^{35}S]methionine for 1 h. The labeled proteins were immunoprecipitated with MAb M177 and analyzed by SDS-PAGE. (B) CV-1 and Biken-CV-1 cells were transfected with the CD46 and mutant genes as for panel A but were labeled with [^{35}S]methionine for 30 min and chased with unlabeled medium for 2 h before analysis. S and I, surface and intracellular proteins, respectively.

construct and possibly the ΔSCR1 mutant accumulated to levels above background on the Biken-CV-1 cell surface after the chase (Fig. 6B, Biken-CV-1, lanes 5 and 3, respectively, brackets). The labeled CD46 proteins on the surface and inside CV-1 cells appeared to be fully glycosylated and migrated as broad bands (Fig. 6B, CV-1, lanes 2 to 5 and 7 to 10, brackets). By contrast, CD46 and variants with an intact H-protein-binding domain (e.g., MCP-2 and ΔSCR3) remained incompletely glycosylated in Biken-CV-1 cells after the chase and migrated as sharp bands (Fig. 6B, Biken-CV-1, lanes 7 and 9, circles). These incompletely glycosylated proteins were sensitive to endoglycosidase H treatment, suggesting that they contain unmodified high-mannose N-linked oligosaccharides (16a). Deleting the SCR1 domain allowed more efficient glycosylation of the mutant in Biken-CV-1 cells (Fig. 6B, Biken-CV-1, lane 8, bracket), and the ΔSCR1 mutant was transported more efficiently to the cell surface (Fig. 6B, Biken-CV-1, lane 3). Despite its more efficient transport, the ΔSCR1 mutant did not accumulate on the surface or inside Biken-CV-1 cells, as shown by Western blotting (Fig. 3A, Biken-CV-1, lanes 3 and 7), suggesting that this mutant was unstable. Most of the GPI-anchored CD46 (MCP-GPI) in Biken-CV-1 cells became fully glycosylated after the chase, but a fraction remained incompletely glycosylated (Fig. 6B, Biken-CV-1, lane 10, bracket and circle, respectively).

These results show that the exogenous human CD46 is synthesized efficiently in both CV-1 and Biken-CV-1 cells. However, CD46 with a transmembrane anchor accumulates on the surface of CV-1 but not Biken-CV-1 cells. CD46 forms with an intact H-protein-binding domain are inefficiently glycosylated and transported to the Biken-CV-1 cell surface. Removing the SCR1 domain facilitates glycosylation and transport but also appears to render the protein unstable in Biken-CV-1 cells. Most important, replacing the transmembrane and cytoplasmic domains of CD46 with a GPI anchor alleviates the restriction on surface expression of CD46 in Biken-CV-1 cells.

The membrane-proximal region of the CD46 cytoplasmic domain is critical for CD46 down regulation. To determine the role of the cytoplasmic domain in CD46 down regulation, we generated CD46 deletion mutants ΔCyt 24, ΔCyt 6, and ΔCyt 0, which possess 24, 6, and no residues, respectively, in the cytoplasmic domain (Fig. 1B; see Discussion for rationale). We expressed these mutants in CV-1 and Biken-CV-1 cells and compared the surface and intracellular levels of the mutant proteins. Deletions in the CD46 cytoplasmic domain increased the electrophoretic mobilities of the mutants in both CV-1 and Biken-CV-1 cells (Fig. 7). Removal of the nine membrane-distal carboxy-terminal residues (ΔCyt 24) did not prevent CD46 down regulation. The ΔCyt 24 mutant protein was expressed efficiently on the surface and interior of CV-1 cells (Fig. 7, CV-1, lanes 3 and 9, respectively) but was expressed at a lower level on the Biken-CV-1 cell surface than intracellularly (Fig. 7, Biken-CV-1, lanes 3 and 9, respectively). Interestingly, further truncation of the CD46 cytoplasmic domain (ΔCyt 6) augmented the down regulation effect. The ΔCyt 6 mutant with only six remaining cytoplasmic amino acid residues was greatly down regulated on the Biken-CV-1 cell surface compared with intracellular expression (Fig. 7, Biken-CV-1, lanes 4 and 10, respectively). By comparison, wild-type CD46 with the entire Cyt 2 cytoplasmic domain was less down regulated on Biken-CV-1 cells than the ΔCyt 6 mutant (Fig. 7, Biken-CV-1; compare lanes 1 and 7 with lanes 4 and 10). The level of surface expression of the ΔCyt 6 mutant on CV-1 cells was also slightly lower than the intracellular level (Fig. 7, CV-1,

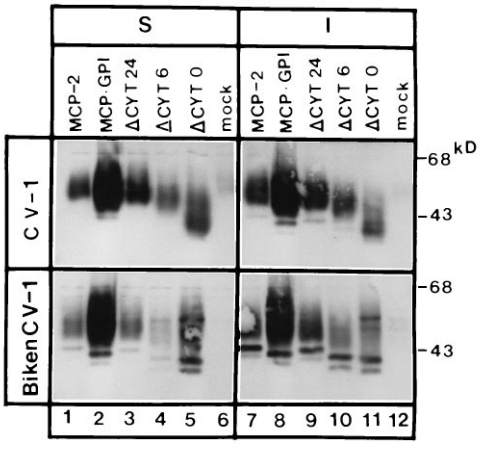

FIG. 7. Comparison of surface and intracellular expression of CD46 cytoplasmic deletion mutants. CV-1 and Biken-CV-1 cells were transfected with MCP-2 (lanes 1 and 7), MCP-GPI (lanes 2 and 8), ΔCyt 24 (lanes 3 and 9), ΔCyt 6 (lanes 4 and 10), or ΔCyt 0 (lanes 5 and 11). Surface (S) or intracellular (I) proteins were immunoprecipitated with MAb M177 and analyzed by SDS-PAGE and immunoblotting with the same MAb.

lanes 4 and 10). Most important, removing the remaining six membrane-proximal residues in the CD46 cytoplasmic domain (ΔCyt 0) alleviated the down regulation, and the ΔCyt 0 mutant was expressed at higher levels on the surface than intracellularly in both CV-1 and Biken-CV-1 cells (Fig. 7, CV-1 and Biken-CV-1, lanes 5 and 11, respectively). Thus, complete removal of the CD46 cytoplasmic domain had an effect on surface expression of CD46 similar to that of replacing the transmembrane and cytoplasmic domains with a GPI anchor, which allowed efficient expression of the truncated CD46 on the Biken-CV-1 cell surface (Fig. 7, lanes 2 and 8). The ΔCyt 0 mutant also caused more extensive syncytia than wild-type CD46 or the ΔCyt 6 and ΔCyt 24 mutants (data not shown), indicating that high levels of CD46 on the infected cells increased the syncytium-forming cytopathic effects of MV.

These data show that the cytoplasmic domain of CD46 is not required for syncytium formation but plays an important role in the down regulation of surface expression of CD46 in a persistent MV infection. In particular, the six membrane-proximal amino acid residues in the CD46 cytoplasmic domain contain a sequence critical for CD46 down regulation.

DISCUSSION

MV-induced down regulation of CD46 was first observed in Jurkat T-lymphoma cells infected by Halle strain, a replication-competent MV isolated from an SSPE patient (33). Both wild-type CD46 and a GPI-anchored mutant lacking the transmembrane and cytoplasmic domains were found to be down regulated by the Halle H protein overexpressed from a recombinant vaccinia virus vector (33, 50). The Halle H protein expressed on mouse cells down regulated CD46 on neighboring Jurkat T-lymphoma cells through cell-to-cell contact, and this response was blocked by antibodies to either the H protein or CD46 (21). Likewise, the H protein expressed on MV-infected U937 human monocytic cells down regulated CD46 on uninfected U937 cells through cell contact (41). Those studies suggest that the MV H protein alone can trigger CD46 down regulation, and the transmembrane and cytoplasmic domains of CD46 are unnecessary for CD46 down regulation.

This study shows that down regulation of CD46 is required for the maintenance of a noncytopathic persistent MV infection. Removing the cytoplasmic tail of CD46 or replacing the transmembrane and cytoplasmic domains with a GPI anchor prevents CD46 down regulation in the persistently infected cells (Fig. 4, 6, and 7), even though the tailless CD46 mutants can interact with the H protein to support cell fusion (Fig. 2 and data not shown). In light of the present findings, it is likely that CD46 is down regulated by multiple factors, and the MV H protein may represent only one aspect of the CD46 down regulation mechanisms. For instance, the MV H protein may interfere with processing or transport of CD46 in the exocytic pathway. In support of this hypothesis, CD46 with an intact H-protein-binding domain often produces an aberrant 40-kDa protein in Biken-CV-1 cells. The 40-kDa protein possesses mainly high-mannose N-linked oligosaccharides, indicating that the protein has not been modified by Golgi-associated enzymes (16a). The 40-kDa protein also reacts better with MAb MH61 against SCR3 than with MAb M177 against SCR2 (Fig. 3B). Since SCR2 overlaps with the H-protein-binding region (19, 30), the 40-kDa protein may exist as a complex with the H protein which masks the M177 epitope. Such an interaction may be analogous to that between gp160 of human immunodeficiency virus type 1 and its cognate receptor CD4, which interferes with transport of CD4 from the endoplasmic reticulum (10, 20). However, the MV H protein does not completely prevent CD46 transport, since some 40-kDa protein is detectable by immunoprecipitation on the Biken-CV-1 cell surface (Fig. 3B and 7).

The MV H protein may also play a role in CD46 internalization from the cell surface. Overexpression of the MV H protein accelerates the disappearance of CD46 from the cell surface (33). Numerous studies have shown that sequences in the cytoplasmic domain are important for internalization of many receptors (48). Tyrosine-containing sequences located near a tight turn have been identified as signals for internalization of constitutively internalized proteins such as the transferrin and low-density lipoprotein receptors (4, 7, 22, 45, 48). Some receptors (e.g., epidermal growth factor receptor) that are internalized upon ligand-binding contain tyrosine residues in a turn-helix-turn motif (6, 8). Computer analysis using algorithms proposed by Chou and Fasman (9) and Garnier (13) predicts that the Cyt 2 cytoplasmic domain possesses two β-turns located at amino acid residues 7 to 10 and 23 to 24 (Fig. 1B, brackets). We therefore tested the expression of deletion mutants lacking one (ΔCyt 24) or both (ΔCyt 6) of the predicted β-turns or without a cytoplasmic domain at all (ΔCyt 0). The ΔCyt 24 mutant lacking the predicted membrane-distal β-turn is still down regulated in Biken-CV-1 cells, but the tailless ΔCyt 0 mutant is no longer down regulated (Fig. 7). Interestingly, the ΔCyt 6 mutant retaining six membrane-proximal cytoplasmic residues but lacking both predicted β-turns is consistently more down regulated than wild-type CD46 (Fig. 7). These results suggest that a determinant for CD46 down regulation resides within the six membrane-proximal residues in the CD46 cytoplasmic domain. It is possible that the membrane-distal portion of the CD46 cytoplasmic tail prevents access to the membrane-proximal sequences by cellular factors involved in receptor internalization. Interaction between the Biken viral H protein and CD46 may facilitate access to the membrane-proximal sequences. Alternatively, Biken-CV-1 cells may possess factors that interact with the CD46 cytoplasmic domain, and the H protein may play no role in this interaction. We have found that CD46 isoforms with either a Cyt 1 or Cyt 2 cytoplasmic domain are both down regulated in Biken-CV-1 cells (16a). Notably, the membrane-proximal amino acid residues important for CD46 down regulation are conserved between the Cyt 1 and Cyt 2 cytoplasmic tails (24).

A third possible mechanism of CD46 down regulation is by altering the intracellular trafficking of CD46. Pulse-chase analysis shows that CD46 is synthesized at comparable rates in CV-1 and Biken-CV-1 cells (Fig. 6A). The newly synthesized intracellular CD46 decreases with time in Biken-CV-1 cells, but the protein does not accumulate at the cell surface (Fig. 6B). One interpretation is that a significant portion of the newly synthesized CD46 is degraded in the endoplasmic reticulum of Biken-CV-1 cells. Alternatively, it is possible that the newly synthesized CD46 in Biken-CV-1 cells is delivered directly from the *trans*-Golgi network to the lysosomal compartments without reaching the plasma membrane. Lysosome-associated membrane proteins (e.g., LAMP-1) are transported directly to lysosomal compartments in this manner (5). The transferrin receptors can also be found in endosomal compartments before appearing on the cell surface (12). Indeed, intracellular degradation rather than internalization of CD46 could more easily explain why Biken-CV-1 cells normally show no signs of syncytium formation. However, it is not known whether the endogenous CD46 homolog in Biken-CV-1 cells is down regulated by the same mechanisms that down regulate the exogenous human CD46. Regardless of the mechanism, identifying a cytoplasmic region required for CD46 down regulation opens the possibility that CD46 is regulated by cytoplasmic compo-

nents. Further dissection of the interactions between CD46 and these components may provide useful insight into mechanisms that regulate cellular susceptibility to complement and how MV influences these regulatory mechanisms.

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