JOURNAL OF VIROLOGY, Oct. 1994, p. 6299–6304
0022-538X/94/$04.00+0

Vol. 68, No. 10

Binding of Measles Virus to Membrane Cofactor Protein (CD46): Importance of Disulfide Bonds and *N*-Glycans for the Receptor Function

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Received 5 April 1994/Accepted 30 June 1994

Two cellular proteins, membrane cofactor protein (MCP) and moesin, were reported recently to be functionally associated with the initiation of a measles virus infection. We have analyzed the interaction of measles virus with cell surface proteins, using an overlay binding assay with cellular proteins immobilized on nitrocellulose. Among surface-biotinylated proteins from a human rectal tumor cell line (HRT), measles virus was able to bind only to a 67-kDa protein that was identified as MCP. The virus recognized different isoforms of MCP expressed from human (HRT and HeLa) and simian (Vero) cell lines. The binding of measles virus to MCP was abolished after cleavage of the disulfide bonds by reducing agents as well as after enzymatic release of N-linked oligosaccharides. By contrast, removal of sialic acid or O-linked oligosaccharides did not affect the recognition of MCP by measles virus. These data indicate that the receptor determinant of MCP is dependent on a conformation of the protein that is maintained by disulfide bonds and *N*-glycans present in the complement binding domains. Our results are consistent with a role of MCP as primary attachment site for measles virus in the initial stage of an infection. The functional relationship between MCP and moesin in a measles virus infection is discussed.

Measles virus (MV) is an enveloped negative-strand RNA virus that contains two surface glycoproteins: the hemagglutinin (H), responsible for attachment to cells as well as for agglutination of erythrocytes, and the fusion protein (F). The entry of MV occurs by binding to specific protein receptors on the host cell and subsequent fusion of the viral with the plasma membrane, resulting in the release of the nucleocapsid into the cytoplasm (for a review, see reference 5).

MV shows a very restricted host range: humans are the only naturally infected species. For the propagation of the virus in cell culture, human or simian cells can be used. Lack of suitable receptors may be responsible for the resistance of many cells from species other than higher primates, although MV can be adapted to cells of many species, with replication continuing on low levels. For many years the cellular counterparts in the binding reaction have remained elusive, although several studies have suggested some receptor candidates. The substance P receptor has been reported to promote the fusion activity of MV (13). Proteins of 20 and 35.5 kDa that were isolated from membranes of Vero cells and African green monkey erythrocytes were able to inhibit virus attachment, infectivity, and hemagglutination activity, but they have not been characterized further (15). Recently, conclusive evidence was presented that membrane cofactor protein (MCP; also designated CD46) is an MV receptor (11, 22, 25). After transfection of nonpermissive cells (mouse or hamster) with MCP cDNA, MV was able to infect these cells. MCP is a C3-binding membrane protein and inhibits complement activation on host cells. It is widely expressed on human cells examined but is absent from erythrocytes. Alternative splicing gives rise to different MCP isoforms that differ in both amino acid sequence and glycosylation (for a review, see reference 21). In a recent report, another membrane protein, moesin, has been shown to be also linked with susceptibility of cells to MV infection (12). Moesin, a membrane-organizing extension spike protein, is largely localized at the inner side of the plasma membrane but also accessible to antibodies on the cell surface (12, 18). Treatment of cells with antibodies to moesin reduces the proliferation rate of smooth muscle cells (17). As described for MCP (27), moesin was found to be downregulated on cells persistently infected with MV (12). Antibodies to both proteins, CD46 as well as moesin, inhibit the infection of cells with MV (12, 25).

With the increasing information about virus receptors, it has become obvious for several viruses that more than one cellular surface component may be involved in the initial interaction between a virus and a cell. Evidence for this concept has been presented for human immunodeficiency virus (6, 8, 10, 14) and poliovirus (24, 31). To understand the molecular events during this early stage of infection, it is important to determine whether a proposed receptor is involved in the attachment of the virus to the cell or in the subsequent fusion reaction between the viral and cellular membrane. If a receptor is required for virus binding, it has to be established whether this molecule directly interacts with the virus or whether it assists another surface component in accomplishing the attachment of the virion to the plasma membrane.

We have started to analyze the interaction of MV with cellular receptors (MCP and moesin) by establishing an in vitro binding assay. Here we show that MV can bind to MCP immobilized on nitrocellulose. We present evidence that disulfide bonds and glycosylation are critical for maintaining a conformation of MCP that is recognized by MV.

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(This work was done in partial fulfillment of the requirements for the degree Dr.rer.physiol. for A.M., Fachbereich Humanmedizin, Philipps-Universität Marburg.)

MATERIALS AND METHODS

Cells and virus. HRT (human rectal tumor), HeLa (human cervical carcinoma), and Vero (African green monkey kidney) cells were propagated in Dulbecco's modified Eagle's minimal essential medium supplemented with 10% fetal calf serum. The Edmonston strain of MV was grown in Vero cells. Virus was harvested from the supernatant when 50 to 80% of the cells showed a cytopathic effect. After clarification of the medium by low-speed centrifugation (3,000 × *g*, 20 min), virus was sedimented by ultracentrifugation at 100,000 × *g* for 90 min. The pellet was suspended in phosphate-buffered saline (PBS) and again pelleted at 150,000 × *g* for 35 min. After resuspension in PBS, the partially purified virus concentrate was stored at −80°C.

Preparation of biotinylated MV glycoproteins. Freshly prepared virus concentrate was labeled with sulfosuccinimidobiotin (S-NHS-biotin; Pierce) by a modification of the protocol described by Clayson et al. (9). Virus samples containing 1 mg of viral protein in 1 ml of PBS were added to 0.66 ml of S-NHS-biotin dissolved in dimethyl sulfoxide (120 μg/ml) and incubated for 4 h at room temperature. After dilution with PBS, biotinylated virus was sedimented by ultracentrifugation (150,000 × *g*, 35 min). Following the biotinylation of virus, the viral glycoproteins were isolated. Biotinylated virus suspended in 800 μl of PBS was incubated in the presence of 5% *n*-octylglucopyranoside (Sigma) for 10 min at room temperature. After centrifugation for 10 min at 12,000 × *g*, the supernatant was further incubated for 20 min at 4°C. Following centrifugation for 30 min at 100,000 × *g*, the supernatant was layered on a sucrose gradient (5 to 30% [wt/wt] on a cushion of 60% sucrose) in PBS containing 1% octylglycoside. After centrifugation for 17 h at 200,000 × *g* in an SW55.1 rotor (Beckman), fractions of 0.5 ml were collected from the bottom of the tube. The samples were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). To determine the fraction containing the MV glycoproteins, the gel was stained with Coomassie brilliant blue. To check the biotinylation, the polyacrylamide gel was blotted to nitrocellulose and the biotinylated proteins were detected by incubation with streptavidin-biotinylated horseradish peroxidase complex (1 h) followed by an incubation with PBS–4-chloro-1-naphthol–H_2O_2 (500:100:1). The fractions used for the glycoprotein overlay protein binding assays (GOBPA) consisted predominantly of MV H protein.

Isolation of biotinylated cell surface proteins. Cell surface proteins were labeled as described by Lisanti et al. (20). After repeated washings with ice-cold PBS containing 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$ (PBS-C/M), cells were incubated twice with S-NHS-biotin in PBS-C/M (0.5 mg/ml) for 30 min at 4°C. After the treatment, the cells were washed once with PBS containing 0.1 M glycine and four times with PBS. The cells were scraped from the culture flask into a solution of PBS containing 1 mM EDTA. After lysis of the cells with radioimmunoprecipitation assay (RIPA) buffer (1% Triton, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 mM iodoacetamide, 0.14 mg of aprotinin per ml, 20 mM Tris-HCl [pH 7.5]), the biotinylated proteins were precipitated by addition of 50 μl of packed streptavidin-agarose (Pierce). Following an incubation for 2 h on ice, the precipitates were washed and eluted by boiling in electrophoresis sample buffer lacking mercaptoethanol. The proteins were separated on an SDS–12% polyacrylamide gel under reducing or nonreducing conditions. After SDS-PAGE, the proteins were blotted to a nitrocellulose sheet. As described for the biotinylated MV glycoproteins, the biotinylated cell proteins were detected by incubation with streptavidin-biotinylated horseradish peroxidase complex and peroxidase substrate.

Immunoprecipitation of MCP. Unlabeled or surface-biotinylated cells were lysed with RIPA buffer, and immunoprecipitation was carried out with a monoclonal antibody (MAb) directed against MCP: J4/48 (Dianova), GB24 (1), or TRA2-10 (2). After an incubation for 2 h on ice, the mouse immunoglobulins were precipitated by addition of 50 μl of protein A-Sepharose (Sigma) preincubated with rabbit anti-mouse immunoglobulins. The precipitates were washed and either used for glycosidase treatments or, after boiling in sample buffer, subjected to SDS-PAGE. The proteins were separated on an SDS–12% polyacrylamide gel under nonreducing conditions and blotted to nitrocellulose.

Glycosidase treatments. For the removal of sialic acid, MCP bound to protein A-Sepharose was incubated for 2 h at 37°C with 250 mU of neuraminidase from *Vibrio cholerae* (VCNA; Behring, Marburg, Germany). For the treatment with other glycosidases, MCP was eluted from the beads by heating at 96°C in a 50 mM phosphate buffer (pH 7.0) containing 0.1% SDS. To release the N-linked oligosaccharides, the eluate was digested overnight with 1 U of *N*-glycosidase F (Boehringer, Mannheim, Germany) at 37°C. For the removal of O-linked oligosaccharides, the eluate containing VCNA-treated MCP was incubated for 18 h at 37°C with 5.6 mU of O-glycosidase (endo-α-*N*-acetylgalactosaminidase; Boehringer). Prior to SDS-PAGE, the digested samples were boiled at 96°C for 5 min in sample buffer (0.1 M Tris-HCl [pH 6.8], 10% glycerol, 2% SDS) and electrophoresed in 12% polyacrylamide gels. After blotting to nitrocellulose, the digested proteins were analyzed by GOPBA.

Binding assays. Binding of MV to proteins blotted to nitrocellulose was analyzed by a virus overlay protein binding assay (VOPBA) (7) or by a modified GOPBA with biotinylated MV glycoproteins. Prior to the binding assay, the nitrocellulose sheet was kept in a solution of 10% nonfat dry milk in phosphate-buffered saline (PBS) overnight at 4°C. Following three washes with PBS containing 0.1% Tween, the nitrocellulose was incubated for 45 min at 4°C with 5 to 10 μg of partially purified MV in 400 μl of PBS-Tween (VOPBA) or with 10 μg of biotinylated glycoproteins in PBS (GOPBA). In the VOPBA, bound MV was detected, after washing with PBS-Tween, with MAb K83 directed to the MV H protein (19) diluted 1:100 in PBS-Tween. After washing, the blot was incubated with horseradish peroxidase (POX)-conjugated anti-mouse immunoglobulins (anti-mouse/POX) from rabbit (dilution 1:400 in PBS-Tween). In the GOPBA, the bound biotinylated glycoproteins were detected with streptavidin-biotinylated POX (streptavidin/POX) complex (dilution 1:500 in PBS-Tween). All incubation and washing steps were performed at 4°C. The proteins recognized by MV or MV glycoproteins were stained by the detection of the bound POX by using the enhanced chemiluminescence system (ECL; Amersham). The resulting light emission was visualized by exposure of the nitrocellulose to an autoradiography film (Hyperfilm ECL; Amersham).

Western immunoblotting. The semidry Western blot method (16) was performed by the modification described by Schultze et al. (30). MAb J4/48 (Dianova) at a dilution of 1:50 in PBS-Tween was used to detect MCP on nitrocellulose

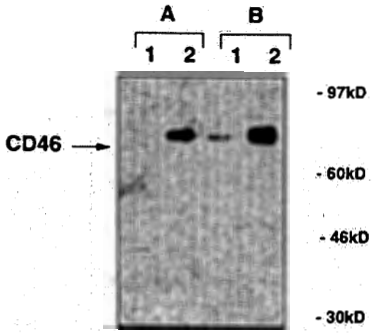

FIG. 1. Binding of MV and J4/48 to proteins from HRT cells. HRT cells were surface biotinylated and lysed with RIPA buffer. Proteins were separated by SDS-PAGE before (lane 1) and after (lane 2) precipitation with streptavidin-agarose. After blotting to nitrocellulose, the immobilized proteins were used for a VOPBA (A) with MV or a Western blot (B) with J4/48. Binding of virus or MAb, respectively, was detected with an enzyme-linked immunoassay. The band of about 40 kDa in lane 1 of panel A is not virus specific because it was also stained in the absence of virus.

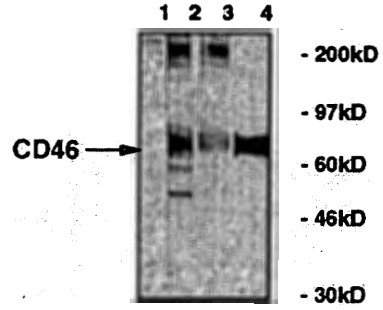

FIG. 2. Binding of MV to isolated MCP. MCP was immunoprecipitated from HRT cell lysates with J4/48, separated by SDS-PAGE, and blotted to nitrocellulose. The immobilized proteins were incubated in the absence (lane 1, control) or in the presence of J4/48 (lane 2), MV (lane 3), or biotinylated MV glycoproteins (lane 4). The binding of antibody and virus (lanes 2 and 3) was detected by immunostaining with anti-mouse/POX. The binding of biotinylated glycoproteins (lane 4) was visualized with streptavidin/POX.

sheets. After incubation with anti-mouse/POX, the protein was visualized by ECL.

RESULTS

Binding of MV to a 67-kDa surface protein from HRT cells. We attempted to detect the direct binding of MV to cell surface proteins by using a VOPBA. After surface biotinylation and lysis of HRT cells, the cellular proteins were separated by SDS-PAGE and blotted to nitrocellulose. The immobilized proteins were used for a binding assay with MV (Fig. 1A) and for a Western blot with J4/48 (Fig. 1B). If the cell lysate was directly analyzed, no specific binding of MV to any cellular protein could be detected (Fig. 1A, lane 1). However, if the biotinylated proteins were isolated with streptavidin-agarose, and thereby concentrated, a protein of 67 kDa was recognized (lane 2). As shown in Fig. 1B, J4/48 reacted with a protein of the same size, suggesting that virus and antibody bind both to the same protein. The antibody even recognized a weak band in the cell lysate (lane 1), indicating that the Western blot has a higher sensitivity than the VOPBA.

As a control, surface proteins from a nonhuman cell line (Madin-Darby canine kidney cells) were isolated and also analyzed, but neither MV nor the MAb recognized any protein (data not shown).

Binding of MV and isolated glycoproteins to immunoprecipitated MCP. To ensure that the 67-kDa protein recognized in the VOPBA was indeed MCP, the protein was immunoprecipitated from HRT cell lysates with J4/48. After SDS-PAGE under nonreducing conditions and blotting to nitrocellulose, MCP was detected both in a Western blot with J4/48 (Fig. 2, lane 2) and in a VOPBA with MV (Fig. 2, lane 3). In contrast to the binding assay with HRT cell surface proteins (Fig. 1), the assay with immunoprecipitated MCP shows binding to both intracellular and surface MCP (Fig. 2). The lower bands that were stained in addition to the 67-kDa protein probably represent intracellular, incompletely processed forms of MCP.

To determine whether the binding to MCP could also be detected with solubilized viral glycoprotein, a preparation of biotin-labeled MV glycoproteins containing predominantly H protein was used for a GOPBA (Fig. 2, lane 4). Like the virions, the isolated glycoprotein bound efficiently to the 67-kDa form of MCP. The lower-molecular-weight bands were recognized less efficiently and were detectable in the overlay binding assay only after longer exposure times (not shown). The bands that can be seen at the top of the gel (lane 1 [control], lane 2 [Western blot] and lane 3 [VOPBA]) are due to the MAbs used for immunoprecipitation of MCP. They were recognized by the POX-labeled anti-mouse antibodies that were necessary for immunostaining of bound virus and bound J4/48. The anti-mouse immunoglobulins were not used in the GOPBA (lane 4) because in this assay biotinylated viral glycoprotein was visualized by streptavidin/POX. The result from Fig. 2 indicates that MV directly interacts with MCP and that the binding is due to MV glycoproteins.

Binding of MV to the nonreduced but not to the reduced form of MCP. To analyze the importance of the protein conformation of MCP for the binding of MV, biotinylated HRT cell surface proteins were isolated with streptavidin-agarose and subjected to SDS-PAGE under nonreducing and reducing (5% mercaptoethanol) conditions. Both MV (Fig. 3A) and J4/48 (Fig. 3B) recognized the nonreduced (lane 1) but not the reduced (lane 2) form of the 67-kDa protein. A faint staining of the reduced form in the Western blot was detected only when the exposure time was extended from 10 to 45 s (Fig. 3B, lane 2'). Detection of biotinylated reduced and nonreduced MCP with streptavidin/POX showed that no degradation had taken place in the reduced sample (data not shown). The result from Fig. 3 indicates that disulfide bonds are critical for maintaining a conformation of MCP that is recognized by MV as well as by the MAb.

Binding of MV glycoprotein to MCP from different cell lines. To compare the binding of MV to MCP from different cells, three MAbs to MCP were used for immunoprecipitation of biotinylated surface MCP from three cell lines (HRT, HeLa, and Vero). As shown in Fig. 4A, J4/48 used for the experiments described above precipitated MCP only from human cells, HRT and HeLa. MAb TRA2-10, known as an antibody specific for the SCR1 domain of MCP (1), showed the same reactivity pattern (Fig. 4B). Vero cells were also included because they are infected by MV more efficiently than HRT or HeLa cells (data not shown). The antibodies mentioned above failed to detect a protein in the monkey cell line. However, immunoprecipitation with a third antibody, GB24, resulted in the precipitation of MCP from all three cell lines (Fig. 4C). This

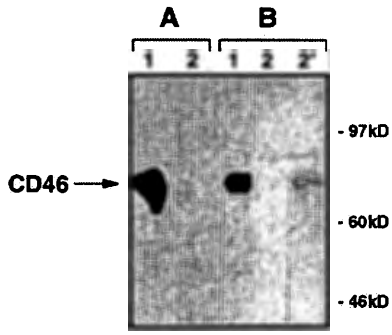

FIG. 3. Binding of MV and J4/48 to surface proteins after electrophoretic separation under nonreducing and reducing conditions. Surface proteins of HRT cells were biotinylated and precipitated with streptavidin-agarose. Precipitates were disrupted in sample buffer in the absence (lanes 1) or presence (lanes 2) of 5% mercaptoethanol, analyzed by SDS-PAGE, and blotted to nitrocellulose. (A) The blot was incubated with MV. (B) The blot was incubated with J4/48. Binding of virus or MAb was detected with an enzyme-linked immunoassay. The exposure times were 10 s for lanes 1 and 2 and 45 s for lane 2'.

antibody is specific for the short consensus repeat (SCR) 3 and 4 region of MCP (1).

The three cell lines appear to express different isoforms of MCP. On the surface of HRT cells, a 67-kDa protein was the major form and a 60-kDa protein was the minor form. On HeLa cells, two isoforms of about 55 and 65 kDa were expressed, the smaller form being more abundant than the larger one. On Vero cells, only a 60-kDa protein was detected. These results are similar to those of others analyzing human and primate cell lines (4, 23, 32).

To determine whether there is a difference between the various MCP isoforms concerning the recognition by MV, a binding assay with the GB24 immunoprecipitates from HRT, HeLa, and Vero cells was performed. Nonbiotinylated cells were used for the immunoprecipitation, and biotinylated MV glycoproteins were used for the binding assay. Bound glycoprotein was detected with streptavidin/POX. As shown in Fig. 5, the MV glycoproteins recognized MCP from all three cell lines (compare with Fig. 4C). The binding to the HeLa cell protein is somewhat weaker than the binding to MCP from HRT, although there were similar amounts of MCP precipi-

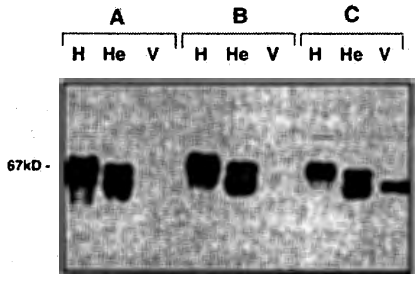

FIG. 4. Immunoprecipitation of MCP from different cell lines with different MAbs. HRT (H), HeLa (He), and Vero (V) cells were surface biotinylated and lysed in RIPA buffer. Immunoprecipitation was performed with J4/48 (A), TRA2-10 (B), and GB24 (C). The samples were separated by SDS-PAGE under nonreducing conditions and blotted to nitrocellulose. Biotinylated proteins were detected with streptavidin/POX.

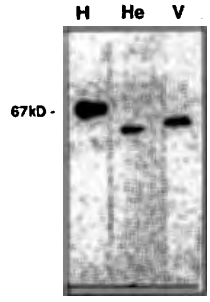

FIG. 5. Binding of biotinylated MV glycoproteins to MCP from different cell lines. HRT (H), HeLa (He), and Vero (V) cell lysates were immunoprecipitated with MAb GB24. The immunoprecipitates were separated by SDS-PAGE under nonreducing conditions and blotted to nitrocellulose. The binding of biotinylated MV glycoproteins was detected with streptavidin/POX.

tated by GB24 from both cells (compare Fig. 4C and 5). The binding to the less abundant 65-kDa isoform of HeLa cells is detectable after longer exposure times (not shown).

Dependence of MV binding on the glycosylation of MCP. To analyze the importance of the carbohydrate moieties of MCP for the binding by MV, the protein was immunoprecipitated with J4/48 and treated with different glycosidases. VCNA was used for the removal of sialic acids. N-linked oligosaccharides were released by incubation with *N*-glycosidase F. O-linked oligosaccharides were cleaved by treatment with VCNA and *O*-glycosidase (neuraminidase was required because *O*-glycosidase acts only on desialylated oligosaccharides). After SDS-PAGE and blotting to nitrocellulose, the digested samples were analyzed in a binding assay with biotinylated MV glycoproteins (Fig. 6). Analysis of the digested samples by Western blotting confirmed that MCP had not been degraded during the enzyme treatment (not shown).

In the case of HeLa cells, the treatment of MCP with glycosidases led to a pronounced decrease in size (Fig. 6). The digestion of the 65- and 55-kDa isoforms with VCNA (lane 2) resulted in two proteins with estimated molecular masses of 55 and 48 kDa, respectively, indicating a high degree of sialylation of the HeLa MCP. Removal of the sialic acids improved the binding of the MV glycoprotein. Further digestion of the

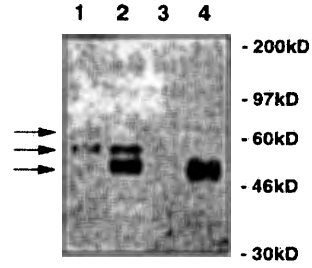

FIG. 6. Binding of biotinylated MV glycoproteins to glycosidase-treated MCP from HeLa cells. Immunoprecipitated MCP was treated with different glycosidases: lane 1, untreated; lane 2, 250 mU of VCNA; lane 3, 1 U of *N*-glycosidase F; lane 4, 250 mU of VCNA and 5.6 mU of *O*-glycosidase. The digested samples were separated by SDS-PAGE under nonreducing conditions and blotted to nitrocellulose. The blot was incubated with biotinylated MV glycoproteins, and binding was detected with streptavidin/POX. The arrows mark different (undigested and digested) forms of MCP.

VCNA-treated MCP (55 and 48 kDa) with *O*-glycosidase (lane 4) resulted in a single broad protein band of about 48 kDa, indicating that only the upper band of MCP contained O-linked oligosaccharides. This result is in agreement with previous data that the difference between the isoforms is mainly due to variation in the amount of O glycosylation (3, 32). The *O*-glycosidase-treated MCP still carries N-linked oligosaccharides and is recognized by MV glycoproteins as efficiently as the desialylated form. Treatment of HeLa MCP (65 and 55 kDa) with *N*-glycosidase F resulted in two main forms of 47 and 40 kDa (not shown). The upper band carries *O*-glycans, whereas the lower band represents the deglycosylated form of MCP. The *N*-glycosidase F-treated HeLa MCP was not recognized by MV glycoproteins (lane 3).

Also, in the case of HRT cells, treatment with VCNA or with VCNA plus *O*-glycosidase did not diminish the binding of MV glycoproteins to MCP, whereas treatment with *N*-glycosidase F abolished the recognition of MCP by MV (data not shown). To summarize, treatment of MCP with *N*-glycosidase F abolished virus binding; neuraminidase and *O*-glycosidase did not diminish but rather improved the recognition of MCP by MV.

DISCUSSION

In this study, using in vitro binding assays, we have demonstrated the direct binding of MV and MV glycoproteins to MCP (CD46). This membrane glycoprotein widely distributed throughout human tissue, normally binds complement factors (C3b and C4b), and protects the cell from complement-mediated damage (21, 28). The MCP gene is transcribed into multiple alternatively spliced mRNAs coding for two groups of MCP isoforms with molecular masses of about 50 to 58 kDa and 59 to 68 kDa (29). The differences in molecular mass are mainly due to different O glycosylation. O-linked oligosaccharides are attached to membrane-proximal domains of MCP that are designated STPs, because they are enriched in serines, threonines, and prolines. Alternative splicing affects the number of STP regions (one, two, or three) and concomitantly the number of O-linked oligosaccharides. The three cell lines used in this study express different MCP isoforms in different quantities. MV was found to bind to all of them. The HeLa MCP seemed to be recognized with lower efficiency than the other MCPs. The weak binding appears to be due to the high extent of sialylation of HeLa MCP, because the removal of sialic acids improved the recognition of this protein by MV. We do not know whether the detrimental effect of sialic acid is due to steric hindrance or due to the negative charge of this acidic sugar. Enzymatic release of the O-linked oligosaccharides did not diminish virus binding. Therefore, the STP domains including the *O*-glycans appear not to be required for the recognition of MCP by MV. This finding is substantiated by the finding that the four primary isoforms of MCP all support MV infection (22).

The receptor determinant is more likely located at the N terminus of the ectodomain which is composed of four repeating units designated SCR 1 to 4. These domains contain three N-linked oligosaccharides (on SCRs 1, 2, and 4) and are responsible for the binding of complement factors C3b and C4b as well as for the cofactor activity of MCP (1). Specifically, SCRs 3 and 4 are required for C3b binding and SCRs 2 to 4 are required for C4b binding, while SCR 2 may play a special role in cofactor activity (21). MV is able to recognize both human and simian MCP; i.e., its binding characteristics are similar to those of MAb GB24, which recognizes an epitope in the SCR 3 and 4 domain. Future work is needed to determine whether this domain also contains the receptor determinant on MCP for attachment of MV.

In contrast to O glycosylation, *N*-glycans appear to play an important role in the binding of MV to MCP. Release of N-linked oligosaccharides from MCP by treatment with *N*-glycosidase F abolished the recognition by MV. The importance of N-linked carbohydrates had been suggested by a previous study. Following inactivation of receptors by protease treatment, regeneration of receptors was prevented in the presence of tuncamycin, an inhibitor of N glycosylation (26). However, these authors have not shown whether the lack of *N*-glycans directly affects the binding of MV or whether MCP lacking Asn-linked oligosaccharides is not transported to the cell surface or proteolytically degraded. Our results suggest that N glycosylation has a direct effect on the binding of MV to MCP. It is unlikely that the oligosaccharides themselves constitute the receptor determinant, because this would require a unique carbohydrate structure present only on MCP. However, the *N*-glycans may be part of the receptor determinant. Another possibility is that MV recognizes a conformational protein domain that is maintained only in the presence of N-linked oligosaccharides. Carbohydrates are known to affect the conformation of glycoproteins. In the case of MCP, lack of the three N-linked oligosaccharides is not expected to have a dramatic effect, because J4/48 interacted very efficiently with *N*-glycosidase F-treated MCP. This antibody recognizes a conformational epitope, as shown by its poor reactivity with the reduced form of MCP. MV also failed to recognize MCP after cleavage of the disulfides bonds, indicating that the receptor determinant on MCP is conformation dependent. Thus, our data indicate that MV binds to a conformational epitope of MCP that requires the presence of N-linked oligosaccharides.

Our results are consistent with MCP constituting the primary attachment site for MV on the cell surface. At present we do not know the relationship between this virus receptor and moesin, another cellular protein involved in the initial stage of an MV infection. Among surface-biotinylated cellular proteins, MV was able to recognize only MCP, not a 75-kDa protein. The failure to detect binding to moesin does not exclude a direct interaction between MV and this protein. It may be explained by the poor biotinylation of moesin or by the low amount present on the cell surface. Binding assays with isolated protein are required to demonstrate the direct binding of MV to moesin. As MAbs to both MCP and moesin inhibit infection by MV (12, 26), it is unlikely that the two proteins function as alternative receptors. Our results favor a role for MCP as the primary attachment site for MV. It is conceivable that moesin plays a role in a postadsorption step. Alternatively, MCP and moesin may interact with each other and form a complex that represents the actual receptor for MV. Future work is needed to determine the relationship between MCP and moesin with respect to an MV infection.

ACKNOWLEDGMENTS

Financial support was provided by grants from Deutsche Forschungsgemeinschaft to G.H. (He 1168/3-1 and 2) and J.S.-S. (Schn 320/2-3).

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