Distinct Kinetics for Binding of the CD46 and SLAM Receptors to Overlapping Sites in the Measles Virus Hemagglutinin Protein*

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Measles virus (MV) is a human pathogen using two distinct cell surface receptors for entry into host cells. We present here a comparative analysis for binding of the MV receptors CD46 and SLAM to the measles virus hemagglutinin protein (MVH, Edmonston strain). Soluble monomeric and dimeric MVH variants were prepared in mammalian cells and their conformation assessed using a panel of monoclonal antibodies. The two receptor molecules specifically bound to the MVH protein with distinct binding modes. The association rate ($k_a$) for SLAM binding to MVH was very low (3000 s$^{-1}$), about 20 times lower than the $k_a$ determined for CD46 binding. However, SLAM bound tighter to the virus protein than CD46, as revealed by a 5-fold lower dissociation rate ($k_d$, 1.5 x 10$^{-3}$ s$^{-1}$). These data suggest that the SLAM receptor binds to a less accessible and more hydrophobic surface on MVH than the CD46 receptor, as illustrated in a binding model. Despite the differences in kinetics, receptor competition binding experiments revealed that they recognize overlapping sites in MVH. Indeed, a panel of anti-MVH monoclonal antibodies equally inhibited binding of both receptor molecules. The similar immune reactivity of the two receptor binding sites suggests that the shift in receptor usage by MV may not be driven by immune responses.

A large variety of cell surface receptors are used by viruses for entry into cells. Even though there is specificity in virus-receptor recognition, flexibility in receptor usage has been reported for several enveloped and non-enveloped viruses (1). Virus strains differing in the cellular receptor used for attachment and entry appear during the course of the infection. A well documented example is provided by measles virus (MV) (2–9), which can use cell surface receptor molecules from two distinct families, CD46 (membrane cofactor protein) and SLAM (signaling lymphocyte activation molecule, CDw150).

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The abbreviations used are: MV, measles virus; MVH, measles virus hemagglutinin; SLAM, signaling lymphocyte activation molecule; HA, hemagglutinin; CHO, Chinese hamster ovary; ICAM-1, intercellular adhesion molecule 1; NDV, Newcastle disease virus; HIV, human immunodeficiency virus.

MV, a highly contagious agent infecting millions of individuals worldwide, is one of the most serious diseases in children in developing countries, causing more than 1 million deaths annually (10). The virus belongs to the *Morbillivirus* genus of the Paramyxovirus family (11). The virus particle contains a negative strand RNA genome packed in a ribonucleoprotein structure surrounded by an envelope or lipid bilayer with two transmembrane glycoproteins, the hemagglutinin (MVH) and the fusion protein (MVF). MVH is a type II membrane protein that forms disulfide-linked homodimers on the surface of the virus. The extracellular region of the protein has been predicted to fold into a C-terminal $\beta$-propeller domain connected by a protein stalk to the membrane-spanning domain, as described for related proteins of members of the Paramyxovirus family (12, 13). MVF is a type I membrane protein formed by two disulfide-linked polypeptides (F1 and F2) in its mature conformation (11). It is expected to form tetramers at the virus membrane and associate with the MVH dimers. The precise nature of these interactions and the processes linking receptor binding to membrane fusion are currently unknown.

Characterized strains or isolates of MV differ in their cellular tropism (4–7). These differences appear to be related to differences in cellular receptor recognition for cell entry. The ubiquitously expressed CD46 was first characterized as the receptor for the vaccine or Edmonston strain of MV (2, 3). The CD46 ectodomain contains four short consensus repeats domains (SCR1–SCR4), and the two N-terminal repeats bind to MV. The structure of the MV binding fragment of CD46 revealed a large binding surface with a central protruding loop bearing a critical virus binding residue (14). MV strains differ in their affinity for CD46 (8). Although virus variants grown in fibroblast-like cells bind well to CD46 on the cell surface, primary isolates or viruses grown in lymphoid cells have low affinity or undetectable binding to this receptor. These differences correlate with a single Asn-to-Tyr mutation in the MVH glycoprotein (7, 8). Recently, the lymphocyte-specific receptor molecule SLAM was identified as a receptor for MV (9). Primary isolates, lymphotropic, and laboratory-adapted virus variants all bind to SLAM on the cell surface with comparable affinity (9, 15). These data suggest that SLAM could be the primary receptor during measles virus infections and that new virus species with increased affinity for the ubiquitous CD46 receptor could arise during the course of the infection, facilitating spreading of the virus throughout the body. Moreover, primary infection of lymphocytes by the virus may explain the immune suppression associated with some MV infections (9).

The determinants of the flexibility in receptor usage by MV or any other virus are currently unknown. Moreover, no comparative analysis on binding of several monomeric receptors to a single virus has yet been reported, including the binding of
CD46 and SLAM to MVH. There are several reports on interactions of viruses with individual receptors, which have provided information about the nature of virus-receptor recognition (16–20). Monomeric receptor proteins bind to viruses with moderate affinities, from about 0.1 to 1 μM. The binding kinetics for virus-receptor interactions are highly variable, reflecting differences among the conformation of the receptor binding sites in viruses as well as in the residue composition of the virus-receptor binding interfaces.

We have developed an experimental approach for the analysis of monomeric CD46 and SLAM receptors binding to soluble MVH (Edmonston) using surface plasmon resonance. The Edmonston variant of MV was chosen because of its ability to bind to both receptor molecules. Our data show that CD46 and SLAM bind to overlapping sites on the MVH protein with significantly higher kinetic rates for CD46 than SLAM. A model for binding of the virus protein to the receptors is presented. Neutralizing monoclonal antibodies inhibited the binding of both receptors to MVH very efficiently.

MATERIALS AND METHODS

Antibodies

The monoclonal antibody recognizing the HA epitope (anti-HA) present in the recombinants MVH proteins (see below) is from Roche Molecular Biochemicals, the SLAM antibody, IPO3, from PharMingen, and the IgG1-horseradish peroxidase rabbit anti-human antibody from Dako. Mouse monoclonal antibodies binding to MVH (I-12, I-29, I-41, I-44, 16-CD11, and 16-DE6) were prepared from hybridomas (21), and anti-MVH-specific MV4, MV12, and MT14 were human recombinant neutralizing Fabs derived by the phage display method (22).

Cloning, Expression, and Purification of Recombinant Proteins

Soluble MVH—Recombinant MVH (Edmonston strain) cDNAs were cloned in-frame with the murine Ig κ-chain leader sequence and a hemagglutinin A epitope (HA epitope) present in the vector pDisplay (Invitrogen). The cDNAs starting at N-terminal residues 61, 135, 149, 156, 179, 193, and 288, respectively, for variants 1–7 (Fig. 1A) and with a translation stop codon at position 617 were generated by PCR from pTM-H/MV (23) using Pfu I polymerase (Stratagene). They were cloned into the unique SacI and SalI sites of pDisplay (Invitrogen). 293T cells were transiently transfected with the constructs in pDisplay for immunoprecipitations (see below). MVH protein variants 3 and 4 were also

![Fig. 1. Design and expression of soluble MVH variants. A, schematic presentation of the regions of the MVH proteins, including the cytoplasmic region (CYT), transmembrane domain (TM), and the predicted stalk and globular fragments in the extracellular or soluble region. The approximate locations of the glycosylation sites are indicated by lollipops. Recombinant soluble variants were engineered by placing a signal peptide (SP) and an influenza hemagglutinin epitope (HA) at the 5′-end of the MVH (Edmonston) coding region (bold lines) as described under “Materials and Methods.” The N-terminal residue of the MVH protein is indicated for each one of the variants 1–7. Cysteine (C) residues used as references for preparation of the constructs are included. B, SDS-PAGE of immunoprecipitates of soluble MVH variants under reducing (left) and nonreducing (right) conditions. Lanes 1–7 include immunoprecipitates of the corresponding variants presented in A. Lane C includes immunoprecipitates from radiolabeled supernatants of vector, mock transfected 293T cells. An antibody against the HA epitope was used (see “Materials and Methods”). Migration and size (kDa) of molecular mass markers are indicated.](image-url)
Receptor Binding to Measles Virus Hemagglutinin

At the I-12 clonal antibodies belonging to antigenic sites I (Stratagene). The sequence of the clone was identical to the reported primers and subcloned into the unique site of the expression vector pPICZA determined from the amino acid composition.

Yeast supernatants by chelate affinity chromatography (Amersham Biosciences). Protein was purified from protein to the supernatant were selected with Zeocine as recombination vector pPICZA.

Six His residues at its C-terminal end. Yeast clones secreting the protein to the supernatant were selected with Zeocine as recombinant vector pPICZA.

The fusion protein was purified by protein A affinity chromatography, and the SLAM fragment was released from the Fe by thrombin treatment at 30 °C. Final purification of the soluble SLAM included a size exclusion chromatogram.

Immunoprecipitation of Soluble MVH Variants

293T cells (2.5 × 10^6 cells) were plated in 10-mm-diameter tissue culture plates and transiently transfected with 15 μg of DNA/plate using the calcium phosphate method. Cells were labeled 1 day post-transfection with ^35S-Met (75 μCi/ml) and ^35S-Cys (25 μCi/ml) in minimum essential medium and incubated at 37 °C for 3 days. Supernatants were collected, clarified by centrifugation, and divided into identical aliquots for immunoprecipitation with purified monoclonal antibodies anti-HA and anti-MVH at 2 μg/ml concentration and protein A-Sepharose (Amersham Biosciences). Immunoprecipitates were analyzed by SDS-PAGE.

Receptor Competition for Binding to MVH

Experiments were carried out with plastic-coated MVH variant 4 (MVH4). A 96-well plate (Nunc) was coated with 1 μM MVH4 protein in phosphate-buffered saline for 1 h at 37 °C, and blocked with 200 μl of 2% bovine serum albumin in phosphate-buffered saline for 1 h at 37 °C. Purified CD46/SCR1–4, CD46/SCR1–2, and CD46/SCR3–4 were added to cell supernatants having SLAM-Fc to reach final concentrations of 0.025, 0.05, 0.1, 0.25, 0.5, 1, and 2 μM. Mixtures were added to triplicate wells with plastic-coated MVH and incubated for 1 h at room temperature. Binding of the fusion protein was monitored using a horse radish peroxidase-labeled anti-human IgG1-Fc polyclonal antibody (Dako). Inhibition (%) of SLAM-Fc binding to MVH was determined by the ratio between the A_{490} from wells having and lacking CD46 proteins.

Binding of Soluble CD46, SLAM, and Antibodies to MVH in BIAcore

Surface plasmon resonance was applied to monitor protein-protein interactions using a BIAcore 2000 instrument and CM5 sensor chips. HEPES buffer (10 mM HEPES, 150 mM NaCl, pH 7.5) was used as running buffer. First, the anti-HA antibody was covalently immobilized in the dextran surface of the chip via primary amino groups using the amine coupling kit (BIAcore). Anti-HA (30 ng/ml) was injected in 10 mM sodium acetate, pH 5.5, and about 13,000 resonance units were usually immobilized. For receptor binding experiments, MVH variant 3 or 4 was first injected through the surface with anti-HA antibody at 3 μl/min followed by the injection of the soluble receptors molecules at 10–20 μl/min. Proteins were released from the antibody for surface regeneration by a pulse of 50 mM citric acid, pH 3.0. Successive cycles of binding and regeneration were usually performed on the same monolonal antibody surface with no apparent change in its activity. Binding kinetics were determined from analysis of the sensograms with the BIA Evaluation 3.0 program in two different ways. First, a simple Langmuir fitting model was applied to sensograms corrected by drifting of the base line from the release of antibody-captured MVH during receptor injection. The correction was done by subtraction of a control sensorgram recorded with buffer instead of receptor. Kinetics were also analyzed by applying the drifting baseline model included in the BIA Evaluation 3.0 software. In this case a baseline drifting rate calculated from the dissociation of MVH from the monoclonal antibody was applied to the fitting model. Both methods gave similar kinetic rates. Affinity dissociation constants were determined either from the kinetic rates (k_d) or from the amount of receptor bound at steady-state conditions by Scatchard plots. The amount of bound receptor was determined 20 s after the end of the injection and corrected by the base-line drifting and the unspecified binding of the receptor to surfaces lacking MVH.

Kinetic rates determined at four different temperatures were used to calculate the activation energy of the association and dissociation reactions as described (16). Enthalpy (ΔH°) was determined from the differences between activation energy for association and dissociation reactions.

**Fig. 2. Recognition of soluble MVH variants by monoclonal antibodies.** A, reducing SDS-PAGE of immunoprecipitates from radiolabeled supernatants of 293T cells transfected with cDNAs for the indicated MVH variant (1–7) and mock transfected cells (–). The monoclonal antibody I-44 belonging to the antigenic site IV of MVH was used (21). Migration and size of molecular mass markers are indicated. B, immunoprecipitation of MVH variants 3 and 4 with anti-HA and monoclonal antibodies belonging to antigenic sites I (1-12 and 1-29), II (16-CD11), and III (I-41). The MVH variant number presented in Fig. IA is at the top of the corresponding track.

Expressed in CHO Lec 3.2.8.1 (CHO-Lec) cells using the glutamine expression vector pEF-BOS (25). A thrombin cleavage sequence was introduced at the 3′-end of the SLAM cDNA. The SLAM-Fc fusion protein was purified from transiently transfected 293T cells. Protein secretion to the cell supernatant was monitored by a sandwich ELISA (enzyme-linked immunosorbent assay) with antibodies against SLAM (IPO-3) and human IgG1-Fc (Dako). The fusion protein was purified by protein A affinity chromatography, and the SLAM fragment was released from the Fe by thrombin treatment at 30 °C. Final purification of the soluble SLAM included a size exclusion chromatogram.

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FIG. 3. Specific binding of CD46 to MVH in BIAcore. A, sensorgrams monitoring response in resonance units (RU) for two cycles of soluble CD46 binding to sensor chip surfaces having or lacking the MVH protein. Either MVH variant 4 (continuous line sensorgram) or HBS buffer (discontinuous line) were first injected (Injection) at a flow rate of 3 μl/min at 25 °C. The end of the injection is marked Buffer 1. CD46 was subsequently injected and signal recorded during and after injection (Buffer 2) in each of the cycles. Surfaces were finally regenerated as described under "Materials and Methods." The inset shows SDS-PAGE of purified CD46/SCR1–4 (left) and CD46/SCR1–2 (right) with migration and size of molecular mass markers indicated. B, overlay plot of normalized sensorgrams monitored during injection of CD46 variants through a surface with captured MVH4. 40 μl of the CD46 proteins (1 μl) were injected at a flow rate of 20 μl/min at 25 °C. Sensorgrams were corrected by unspecific receptor binding to a surface lacking MVH and by base-line drifting during the injection. The range of the association and dissociation phases is indicated.
RESULTS

Expression of Soluble MVH Proteins—The MVH glycoprotein has the transmembrane domain at its N terminus, so that the expression of soluble MVH variants required replacement of the cytoplasmic and transmembrane domains by the murine Ig H9260 leader sequence (Fig. 1A). Several constructs having different fragments of the extracellular region of the protein were prepared using the Cys residues as reference. Protein expression was analyzed by transient expression in 293T cells, radiolabeling, and immunoprecipitation (Fig. 1B). All variants except one that lacked Cys 287 were secreted to the cell supernatant and presented an electrophoretic mobility under reducing conditions consistent with their size. The size of variants 1–3 was about two times higher under nonreducing than under reducing conditions, showing that they formed disulfide-linked dimers. MVH4 appeared monomeric, showing that Cys residue 154 was involved in the intermolecular association of the longest variants. MVH6 appeared partially as a disulfide-linked dimer, most likely formed by an intermolecular disulfide bond involving the Cys residue paired with the deleted Cys188.

The conformation of the MVH soluble variants was assessed using a panel of monoclonal antibodies recognizing four different antigenic sites previously defined in the MVH protein (21). Monoclonal antibodies representing all four antigenic sites immunoprecipitated the six expressed soluble variants of the Edmonston strain of MV (Fig. 2). Differences in the amount of protein among the different variants could be related to differences in protein expression.

Receptor Binding to Soluble MVH—Soluble protein fragments of CD46 having the two N-terminal short consensus repeats (CD46/SCR1–2) or the four SCR of the protein (CD46/SCR1–4) were prepared in CHO-Lec cells and presented a low glycosylation-related heterogeneity (Fig. 3A, inset). A BIAcore 2000 instrument was used to monitor CD46 binding to soluble MVH. The MVH protein carrying a HA epitope at the N terminus (Fig. 1) were captured on surfaces having covalently immobilized anti-HA antibody (Fig. 3A). CD46 was subsequently injected through surfaces having or lacking captured MVH protein, and the increase in the base line after CD46 injection (buffer 2, Fig. 3A) was recorded specifically in the surfaces having MVH protein. Binding was specific for soluble CD46 receptors with SCR1–2 and SCR1–4 (Fig. 3B). About two times higher binding (shown in resonance units (RU)) for CD46/SCR1–4 than CD46/SCR1–2 was monitored at the end of the receptor injection. This was consistent with the difference in size between the two proteins, which suggested similar binding affinities. The CD46/SCR3–4 protein lacking the MV binding site did not bind to the sensor chip surface with captured MVH (Fig. 3B).

The complete extracellular region of the SLAM receptor was also expressed in mammalian cells and purified for analysis of binding to MVH in BIAcore (Fig. 4). The size of the soluble receptor was as expected (Fig. 4, inset), and it bound to the monoclonal antibody IPO-3 after final purification. SLAM bound specifically to BIAcore surfaces having captured MVH protein (Fig. 4). However the binding sensorgrams were strikingly different from those recorded during the injection of CD46 (Fig. 5).

Affinity and Kinetics for Binding of Monomeric Soluble Receptor to MVH—The determination of the kinetics for receptor binding to MVH required recording several cycles of receptor association and dissociation using different receptor concentrations (Fig. 5). Binding of the receptor to the surface with immobilized MVH increased with the receptor concentration. About a 10-fold higher concentration of SLAM receptor than CD46 was required to have similar binding. However, the
CD46 protein dissociated faster from MVH than SLAM. Sensorgrams recorded for CD46 plateau, indicating higher binding kinetic rates for CD46 than for SLAM.

Kinetic and affinity constants were first determined from the recorded binding sensorgrams at 25 °C (Table I). The affinities for CD46 binding to dimeric (MVH3) or monomeric (MVH4) proteins determined from either the kinetic constants ($K_a$) or the MVH-bound receptor at steady-state conditions ($K_D$) were not significantly different. CD46 binding affinity was similar to that reported (120 nM) for monomeric receptor binding to membrane bound MVH (26). The kinetic association constants ($k_\text{a}$) for binding of both CD46 and SLAM to the MVH3 variant were slightly higher, although the $k_d$ rates were almost identical. Kinetic rates did not change with the flow (10–80 μl/min) or amount of captured MVH (not shown), indicating an absence of mass transfer or rebinding during receptor association and dissociation phases.

The differences between the kinetics for binding of CD46 and SLAM to both MVH proteins were highly significant. Association and dissociation kinetic rates were about 20 and 5 times higher, respectively, for CD46 binding as compared with SLAM, resulting in a higher affinity (lower $K_D$) for the CD46 receptor. Dissociation affinity constants increased with the temperatures for binding of both receptors to MVH, showing a release of heat upon receptor binding to the MVH protein (Table I).

Overlapping CD46 and SLAM Binding Sites in MVH—A receptor binding competition experiment was carried out with plastic-coated MVH protein, monomeric soluble CD46 molecules, and a dimeric SLAM-Fc protein (Fig. 6A). The IgG1-Fc region fused to the SLAM receptor facilitated detection of the protein bound to MVH (see “Materials and Methods”). Increasing concentrations of soluble CD46 variants were added together with a SLAM-Fc protein to wells immobilized MVH protein, and progressively higher inhibition of SLAM binding to MVH was observed with CD46/SCR1–4 and CD46/SCR1–2 (Fig. 6A). The smaller CD46 variant with only 2 repeats gave an inhibitory effect similar to that of the longer variant. These results indicated overlapping binding sites for the two receptors on the viral protein. No binding inhibition was observed with CD46/SCR3–4, a protein that does not bind to MVH (see Fig. 3B).

Further analysis of the receptor binding epitopes on MVH was carried out in BLAcore using anti-MVH neutralizing monoclonal antibodies (Fig. 6B). The antibodies used are clustered in

### Table I

| Interaction   | $k_a \times 10^{-3}$ | $k_d \times 10^3$ | $K_D$ | $K_D'$ |
|---------------|---------------------|------------------|-------|--------|
| CD46-MVH3    | 72 (30)             | 7.3 (2.5)        | 100 (20) | 126 (10) |
| CD46-MVH4    | 58 (13)             | 7.5 (0.8)        | 130 (32) | 227 (60) |
| SLAM-MVH3    | 5 (0.8)             | 1.4 (0.2)        | 265 (50) | ND      |
| SLAM-MVH4    | 2 (0.4)             | 1.7 (0.5)        | 770 (150) | ND      |

### Table II

| Interaction   | $K_D$ 15° | $K_D$ 20° | $K_D$ 25° | $K_D$ 30° | ΔH$^\ddagger$ |
|---------------|-----------|-----------|-----------|-----------|--------------|
| CD46-MVH4    | 95 (10)   | 140 (20)  | 180 (20)  | 250 (40)  | −11          |
| SLAM-MVH4    | 180 (140) | 460 (140) | 690 (110) | 800 (60)  | −17          |
four antigenic sites: I (I-12 and I-29), II (16-CD11), III (I-41, 16-DE6, MV12, and MT14), and IV (I-44) (21, 22). MV4 has not been clustered yet. The receptor-binding inhibitory properties of those antibodies were determined in BIAcore using surfaces with varying amounts of captured MVH (Fig. 6B). Purified antibody and Fab molecules were injected at saturating concentrations through the MVH surfaces prior to the injection of the receptor proteins. Antibodies from antigenic sites I (I-29), II, and III abolished binding of both receptors to MVH. I-12 and I-44 were partially inhibitory. All antibodies gave similar inhibition of binding of both receptor molecules to MVH. These data confirm that the binding sites of the CD46 and SLAM receptors are overlapping and show that neutralizing antibodies prevent receptor binding.

**DISCUSSION**

We have analyzed virus-receptor interactions with soluble MVH proteins and the two receptor molecules used by MV for entry into host cells. The soluble variants used here were recognized by monoclonal antibodies directed to four MVH antigenic sites (21), and therefore it is presumed that they must have the same conformation as the protein present in the virus envelope. Our data revealed that the CD46 and SLAM receptors bind to overlapping but distinct interacting surfaces in MVH, as shown by the large differences in their binding kinetics. Binding kinetics provide information on the nature of protein-protein interactions. The rate of association is dependent on the rate of diffusion and can be modulated by electrostatic forces (27). On the other hand, the rate of dissociation can be directly correlated with the strength of the binding interaction. High kinetic rates arise from electrostatic protein-protein interactions, whereas relatively hydrophobic binding surfaces result in lower rates (28).

The kinetic rates determined for the binding of the SLAM receptor to the MVH protein (Table I) are low and surprisingly similar to the binding of the ICAM-1 receptor to human rhinovirus (16). Thus, the low rate of association presented by SLAM
suggests a hydrophobic virus-receptor binding interface and, as reported for major group rhinoviruses (29), binding of the receptor molecule to a relatively inaccessible surface. By contrast, association of CD4 to MVH was about 20 times faster, indicating that the binding surface for this receptor molecule is more accessible and therefore more hydrophilic. Indeed, the higher dissociation rate for CD4 also suggests a weaker and more electrostatic interaction than that for SLAM.

The receptor binding region of the MVH protein is expected to have a β-propeller fold (12), as shown for the related hemagglutinin-neuraminidase of Newcastle disease viruses (NDV) (13). The low kinetic association rate of the SLAM molecule suggests binding of the receptor molecule to the relatively inaccessible inner cavity of the β-propeller, where sialic acid binds to the NDV neuraminidase. The N-terminal domain of SLAM is the MV-binding domain (30). Therefore, as shown for binding of ICAM-1 to rhinoviruses (29), we predicted that the loops in the tip of the MV-binding domain of SLAM would penetrate into the cavity of the propeller, as illustrated in Fig. 7. It is however unlikely that the large measles virus surface of the CD46 receptor could penetrate extensively into the propeller cavity (14). Moreover, the Tyr481 residue critical for CD4 binding is modeled outside of the cavity, on the side of the MVH propeller domain (Fig. 7). We expected the Tyr481 would contact with the MV binding residue Pro199 on the protruding D-loop located in the first repeat of CD46 (14). The second repeat of CD46 could overlap with the SLAM binding surface on the top of the propeller. The differences between the MVH binding surfaces of the CD46 and SLAM receptors proposed here are consistent with the differences in the binding kinetic rates determined.

The oligomerization state of the different MVH length variants shows that residue Cys154 is involved in formation of soluble disulfide-linked homodimers as reported for the membrane-bound hemagglutinin (31, 32). Receptors bound to monomeric and dimeric MVH variants with similar affinities and almost identical $k_{d}$, indicating that the virus-receptor binding surface is located in a single virus protein and that the interaction is equimolar. The higher $k_{d}$ determined with the dimeric MVH molecule is not very significant, but it could reflect a more accessible binding site in the dimer.

Binding of both receptor molecules to MVH was exothemeric, as described for the binding of CD4 to the HIV gp120 protein (19). We would expect that the energy released by binding of the receptor molecule to one of the spike proteins could trigger the molecular events that follow receptor binding and lead to fusion. Indeed, HIV and MV entry do not require acidification and must therefore be mediated actively by receptor binding. Further studies on receptor binding to the complete spike protein complex are needed to understand the link between receptor binding and fusion events in MV.

The overlapping nature of the CD46 and SLAM receptor binding sites was demonstrated by a receptor binding competition experiment and by antibody blocking of receptor binding to MVH (Fig. 6). Antibodies belonging to antigenic sites I (1-29), II, and III completely inhibited receptor binding to MVH, suggesting that these sites are in the vicinity of the receptor binding site and on the top of the β-propeller domain (Fig. 7). Antigenic site IV must be more distant because the antibody I-44 had lower inhibitory activity. These data show that the receptor binding surface in MVH is a major target for antibody responses neutralizing virus infectivity (21, 22). The similarities in blocking receptor binding to MVH among the different monoclonal antibody tested suggest that the shift in receptor usage by MV may not necessarily be driven by specific immune responses. A major force behind it may be the marked differences in tissue distribution between the CD46 and CD4 receptors. Initial measles infection may develop in lymphoid cells expressing the SLAM receptor. Populations of virus with increased affinity for the CD46 receptor may appear during the course of the infection and move from lymphoid cells into other cell types in immunosuppressed individuals. The use of the CD46 receptor leads to MV pathogenesis in animal models (33) and may be needed for the spread of the infection to different tissues, including the central nervous system.

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