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CD46 Short Consensus Repeats III and IV Enhance Measles Virus Binding but Impair Soluble Hemagglutinin Binding

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The binding of a recombinant soluble form of the measles virus (MV) hemagglutinin (sH) to cells expressing hybrid CD46/CD4 proteins was compared to that of purified virus. For binding of both ligands, both CD46 external short consensus repeats I and II (SCR I and II) in the natural order were essential. The addition of SCR III and IV enhanced virus binding but inhibited sH binding. Accordingly, this lowered the ability of sH to compete with MV binding. Antihemagglutinin monoclonal antibodies selectively inhibited the binding of either sH or MV. Thus, sH and MV share a common binding site in SCR I and II but differ in their apparent avidity to CD46 under the influence of SCR III and IV.

The attachment of a virus to its cellular receptor is the first step in infection and controls the efficiency of virus entry. The next step, fusion with the cell membrane, can critically depend upon an appropriate scaffolding of the cellular receptor and the virus envelope proteins (4). A detailed understanding of the molecular interactions between the viral attachment proteins and the cellular receptor is a prerequisite for understanding the structural changes involved in fusion.

Attachment and fusion of the measles virus (MV) envelope with the plasma cell membrane is mediated by the hemagglutinin (H) and fusion (F) proteins, respectively (see reference 9 for review). These proteins are organized in a regular array of tightly packed spikes made of H tetramers (dimers of disulfide bridge-linked homodimers) and F trimers (17, 21, 24, 25).

CD46, or membrane cofactor protein, acts as the primary MV receptor (7, 23). CD46 is a member of the regulator of complement activation family. From its amino terminus, the ectodomain of the type I glycoprotein CD46 consists of short consensus repeats I to IV (SCR I to IV, 60-amino-acid modules characteristic for regulator of complement activation proteins), up to three serine-, threonine-, and proline-rich regions (STP A, B, and C) containing O-linked carbohydrate chains, and a short sequence next to the transmembrane region.

The two external CD46 SCR are necessary and sufficient to allow MV binding, fusion, and replication (4, 12, 18), whereas the complement regulatory function maps to SCR II, III, and IV (see reference 27 for review). The N-linked oligosaccharide of SCR II, but not that of SCR I, plays a crucial role in determining the conformation of the MV binding site (14, 22). Moreover, SCR III and/or IV contribute to MV binding (4), and like several anti-SCR I and anti-SCR II monoclonal antibodies (MAbs), one anti-SCR III MAb inhibits MV entry (11). STP domains have subtle effects on MV binding and fusion (3, 11).

MV-receptor interactions are primarily mediated by the binding of H to CD46 (6, 8, 15, 16), as shown by the reciprocal binding of purified recombinant ectodomains of CD46 and H (6). Moreover, binding of recombinant soluble sCD46 to membrane-anchored H is enhanced in the presence of F protein (6).

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To better understand the molecular events involved in MV binding to CD46, a recombinant soluble homodimeric form of H (sH) (6) was compared with purified MV for its ability to bind to CD46 and CD46/CD4 chimeric molecules varying in their structure and size.

SCR I and II are required for sH binding. The respective abilities of sH, produced as previously described (6), and MV to interact with CD46/CD4 hybrid molecules consisting of various CD46 SCR (I to IV) linked to one or more immunoglobulin (Ig)-like domains (1 to 4) of CD4 was compared after transient expression in Ltk cells with T7 promoter-based plasmids and infection with a recombinant vaccinia virus encoding the T7 polymerase (4). The sH and MV binding was determined by cytofluorometry after labelling with an anti-H antibody and the results were normalized to binding to the full-length CD46 (4, 6). Both sH and MV bound to cells expressing hybrid molecules with CD46 SCR I and II (Fig. 1c and d). No sH binding was observed with cells expressing the hybrid molecule with only SCR I or II (Fig. 1a, I/3-4 and II/3-4), similar to that observed with control cells expressing CD4 or with untransfected cells (data not shown). A duplication of SCR I or II (Fig. 1a and b, I-I/4 and II-II/4) or an inversion of these two SCR (Fig. 1a and b, II-I/4) did not allow any significant sH or MV binding. These data show that like MV binding, sH binding to CD46 requires both SCR I and II in the natural order.

Receptor length influences sH binding. Since the length of the receptor has an influence on MV binding (4), we tested whether a similar effect can be detected with sH. Receptor proteins of various lengths were obtained by directly linking SCR I and II to the CD4 transmembrane region or to one, two, or four CD4 Ig-like domains. sH bound, albeit with low efficiency, to the shortest molecule (Fig. 1c, I-II), whereas no specific MV binding could be detected (Fig. 1d, I-II). Steric hindrance by large cell surface glycoproteins may not be relevant for this effect, because the large-enveloped Epstein-Barr virus can bind the first two SCR of CD21, which serve as its receptor, even if they are moved close to the cell membrane (5). Lack of detectable virus particle binding to the shortest receptor may result from loss of secondary binding to SCR III and IV (see below) or from defective CD46 oligomerization, relying on SCR III and IV or on CD4 domains 3 and 4 (4, 26).

sH binding increased with the length of the hybrid molecules

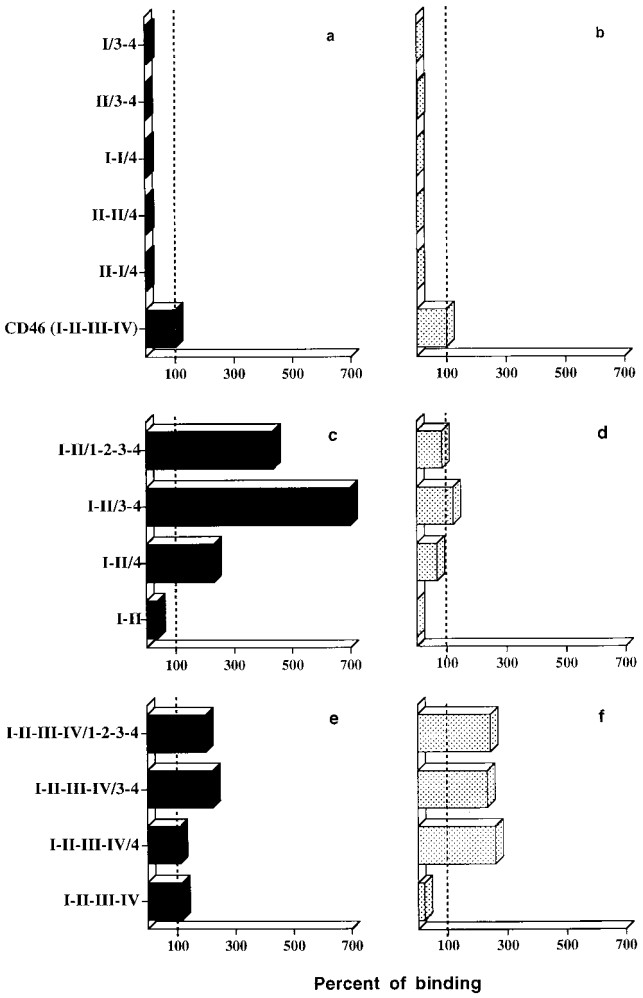

FIG. 1. Requirement of SCR I and II for sH binding and influence of SCR III and IV. Ltk cells transiently expressing CD46 or CD46/CD4 hybrid molecules were incubated with saturating amounts of sH (black columns, left histograms) or MV (grey columns, right histograms), and binding levels were determined by flow immunocytofluorometry with H-specific 48cl6 MAb and cl55 MAb as probes for bound sH and MV, respectively. Results are expressed in percentages with the sH and MV binding level on CD46.BC1 [CD46 (I-II-III-IV)] being respectively taken as 100% (vertical dotted line). From top to bottom: binding of cells expressing I/3-4, II/3-4, I-I/4, II-II/4, II-I/4, and CD46.BC1 (a and b); I-II/1-2-3-4, I-II/3-4, I-II/4, and I-II (c and d); and I-II-III-IV/1-2-3-4, I-II-III-IV/3-4, I-II-III-IV/4, and I-II-III-IV (e and f) molecules.

(Fig. 1c, I-II, I-II/4, I-II/3-4, I-II/1-2-3-4) reaching a level seven times that of the standard CD46-BC1 and then slightly decreasing. This length effect parallels the one previously described for MV binding (4) (Fig. 1d).

SCR III and IV have an opposite effect on sH and MV binding. Since SCR III and IV enhance MV binding (4), their effect on sH binding was also tested. Surprisingly, the three longer I-II/CD4 hybrids bound more sH than the corresponding I-II-III-IV/CD4 hybrids (compare Fig. 1c and e), whereas the I-II-III-IV/CD4 hybrids are better binders of MV (Fig. 1d and f). This effect was independent of molecule length because I-II/3-4 and I-II/1-2-3-4 proteins bound sH more strongly than I-II-III-IV and I-II-III-IV/3-4 proteins expected to be of similar size, respectively (4). The negative effect of SCR III and IV was not likely due to the masking of the binding site of the sH-detecting antibody, since similar results were obtained with two different anti-H MAbs, 19H40 and 48cl6 (10), and the monkey polyclonal anti-MV-BIL serum BMS94 (29).

The positive effect of SCR III and IV on virus binding may be indirect: these modules may affect the conformation or the positioning of SCR I and II. Alternatively, SCR III and IV may be secondarily interacting with the H protein tetramer or with the F protein trimer (4). In favor of the latter hypothesis, a soluble form of the CD46 ectodomain more efficiently binds cells expressing the MV H protein when F protein is coexpressed (6). Such a secondary interaction, by cross-linking the H, F, and CD46 proteins, would structure the virus-receptor complex so as to implement virus entry. The involvement of SCR III and IV in MV entry may explain how one MAb directed against SCR III can inhibit MV infection (11).

Mode of sH binding to CD46. Stable CHO cell lines expressing CD46.B2, I-II/3-4, and I-II-III-IV/3-4 hybrid molecules were used for equilibrium sH binding assays. The incubation of CHO.CD46.B2 cells with increasing amounts of sH resulted in increasing amounts of bound sH until saturation was reached (Fig. 2a). By using the representation of Lineweaver and Burk (Fig. 2a, insets), the avidity (50% binding) of sH for CD46.B2 was estimated in four independent experiments to be 5.2 ± 1.9 nM. A binding curve of similar shape was observed for CHO.I-II-III-IV/3-4 (Fig. 2b), with a similar avidity of 5.3 ± 2.2 nM. This indicates that linking the four SCR of CD46 to the Ig-like domains 3 and 4 of CD4 has no major effects on sH/SCR I and II interactions. In contrast, the binding curve observed for CHO.I-II/3-4 (Fig. 2b) was different. The first five points of the curve were very similar to those of the curve for sH binding to the I-II-III-IV/3-4 hybrid molecule and CD46.B2, but at higher protein concentrations, binding increased. This may indicate additional cooperative interactions. It is conceivable that in the absence of SCR III and IV, dimeric sH molecules oligomerize on receptor molecules, whereas when receptor molecules are composed of all four SCR, oligomerization is inhibited. Natural transmembrane-anchored homodimeric H is indeed able to oligomerize (17).

SCR III and IV reduces competition of MV binding by sH. The effects of SCR III and IV on sH equilibrium binding and MV binding capacity were further explored by testing the ability of various amounts of sH to compete with MV for binding (Fig. 3). The best MV binding inhibition by sH was observed on CHO.I-II/3-4 cells. At the highest concentration, inhibition was almost complete. The MV binding inhibition on CHO-CD46.B2 cells paralleled that on CHO.I-II/3-4 cells but was lower and reached a plateau at around 40%. At low sH concentrations, the MV binding inhibition on CHO.I-II-III-IV/3-4 cells was low, but at higher concentrations an intermediate level plateau was reached. The high inhibition of MV binding to I-II/3-4 reflects the high sH avidity and the low MV binding ability of this receptor protein.

sH and MV binding to CD46 differ in sensitivity to inhibition by certain H-specific MAbs. CHO-CD46.B2 cells were incubated with nonsaturating amounts of sH or MV in the presence of various amounts of the purified H-specific MAbs cl55, 19H40, or 48cl6, recognizing different conformational epitopes (10) on both MV and sH (6). Cl55 more efficiently inhibited the binding of sH than that of MV (Fig. 4). In contrast, 48cl6 MAb strongly reduced MV binding but had no effect on sH binding, and 19H40 partially inhibited only sH binding. A control MAb directed against human C3 (WM1) did not inhibit sH or MV binding. Similar observations were made when sH binding to immobilized purified recombinant soluble CD46 was studied by enzyme-linked immunosorbent assay (data not shown). Since the experiments were performed

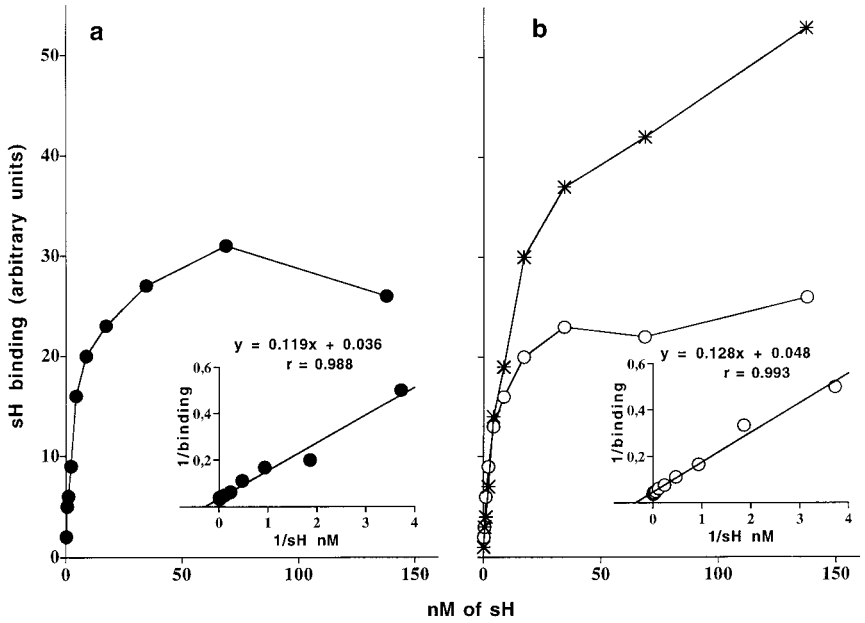

FIG. 2. Influence of SCR III and IV on sH equilibrium binding. CHO.CD46.B2 (solid circles) (a), CHO.I-II/3-4 (asterisks) (b), and CHO.I-II-III-IV/3-4 (open circles) (b) cells were incubated with various amounts of sH, and binding levels were determined by flow immunocytofluorometry. Insets: Lineweaver-Burk plot of sH binding data of CHO.CD46.B2 (a) and CHO.I-II-III-IV/3-4 (b) cells.

as preincubations, antibodies may have indirectly induced alterations in the CD46 binding site of the native transmembrane H but not in the recombinant sH. Alternatively, the interactions of sH and of the virus-bound hemagglutinin with CD46 involved overlapping but distinct sites.

Interaction of receptors with viruses and purified attachment proteins. Altogether, our data suggest that the mode of interaction of the MV attachment protein with its receptor differs profoundly when this protein is geometrically organized in the envelope of a viral particle or when it is in a soluble form. The fact that three MAbs competed differently with the binding of sH or virus particles is coherent with this interpretation. During virus-to-cell attachment and fusion, H is likely to undergo some conformational changes. It is possible that, when in soluble homodimeric form, H adopts one of these intermediate conformations.

Similarly, the binding efficiencies of several human immunodeficiency virus isolates to the CD4 receptor show greater variations than that of soluble gp120 molecules purified from these viruses (20).

It is also important to note that the avidity constant of several virus receptor interactions as measured with purified proteins (6, 19, 20, 28) or virus particles (1, 2, 13) is in the nanomolar range. It is conceivable that secondary and tertiary interactions, which occur only when attachment proteins are geometrically organized in a virus particle, do not significantly improve the binding constant but organize the scaffold necessary for viral entry.

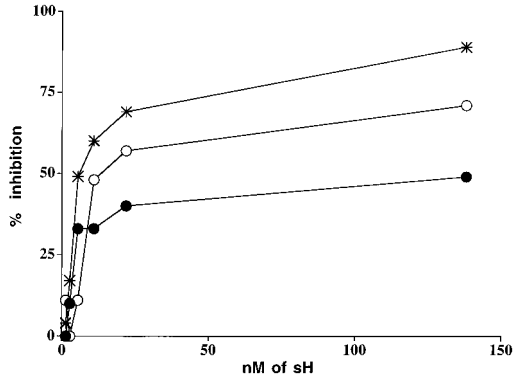

FIG. 3. SCR III and IV reduce the inhibition of MV binding by sH. CHO.I-II/3-4 (asterisks), CHO.I-II-III-IV/3-4 (open circles), and CHO.CD46.B2 (solid circles) cells were incubated with MV in the presence of various amounts of sH, and MV binding levels were determined by flow immunocytofluorometry by using an F-specific MAb as a probe. The results are expressed in percent inhibition calculated as described previously (6).

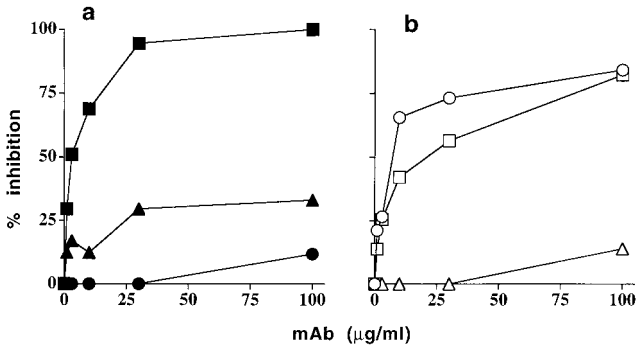

FIG. 4. sH and MV binding to CD46 differ in sensitivity to inhibition by H-specific MAbs. CHO.CD46.B2 cells were incubated with a nonsaturating amount of sH (a) or MV (b) in the presence of various amounts of cl55 (squares), 19H40 (triangles), or 48cl6 (circles) MAbs, and the binding levels were determined by flow immunocytofluorometry with monkey MV-specific BMS94 antibody and biotinylated F-specific MAb as probes for bound sH and MV, respectively.

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