A CD46CD[55–46] chimeric receptor, eight short consensus repeats long, acts as an inhibitor of both CD46 (MCP)- and CD150 (SLAM)-mediated cell–cell fusion induced by CD46-using measles virus

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According to their cellular receptor use, measles virus (MV) strains can be separated into two phenotypes, CD46-using and CD46-non-using. A long chimeric receptor, CD46CD[55–46], was generated from the CD46 backbone, encompassing the four short consensus repeat (SCR) domains of CD46 linked via a flexible glycine hinge to SCR1 and SCR2 of CD55, SCR3 and SCR4 of CD46 and the STP, transmembrane and cytoplasmic tail of CD46. This chimeric receptor was proficient for MV binding but deficient in mediating MV-induced cell-to-cell fusion and virus replication, possibly due to the extended distance between the MV haemagglutinin (H) binding site (CD46 SCR1–SCR2) and the cell membrane. When coexpressed with either wild-type CD46 or CD150, this fusion-incompetent receptor exerted a dominant negative effect and inhibited both cell-to-cell fusion and entry of MV with CD46-using, but not CD46-non-using, phenotype. A soluble octameric CD46–C4bpα exhibited similar CD46- and CD150-mediated fusion inhibition properties only against CD46-using MV. This suggests that the long CD46CD[55–46] receptor acts by sequestering incoming MV prior to its binding to the shorter functional CD46 or CD150 receptor.

Introduction

pH-independent fusion at the plasma membrane is characteristic of several viruses including retroviruses and paramyxoviruses. In measles virus (MV), both of the envelope proteins, haemagglutinin (H) and fusion (F) proteins, are required for fusion. They are organized as tightly associated complexes of H tetramers and F trimers (Lamb et al., 1999; Malvoisin & Wild, 1993; Plemper et al., 2000). The H protein binds to the receptor on the target cell (Devaux et al., 1990; Tanaka et al., 1998; Tatsu et al., 2000a) and enables the F protein to mediate the fusion process (Cattaneo & Rose, 1993; Tatsu et al., 2000a; Wild et al., 1991), probably through conformational changes required for fusion (Chen et al., 2001).

To date, two cell surface proteins have been identified that act as cellular receptors mediating MV entry, CD46 (or Membrane Cofactor Protein, MCP) (Döreig et al., 1993; Naniche et al., 1993) and CD150 (or Signalling Lymphocytic Activation Molecule, SLAM) (Erlenhoefer et al., 2001; Hsu et al., 2001; Tatsu et al., 2000b).

CD46 allows the entry of MV laboratory strains maintained in epithelial and fibroblastic cell lines, including all attenuated virus strains used as efficient human vaccines (Escoffier & Gerlier, 1999; Hsu et al., 1998; Kobune et al., 1990; Lecouturier et al., 1996; Parks et al., 2001; Schneider-Schaules et al., 1995b; Tanaka et al., 1998). However, CD46 is not used by recent MV isolates grown in simian B95 B cells (Hsu et al., 1998; Kobune et al., 1990; Lecouturier et al., 1996; Murikami et al., 1999), or from throat swabs from infected individuals.

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Wild-type CD46 and CD55 sequences in this paper refer to GenBank accession numbers A18585 and M30142, respectively.
(Ono et al., 2001b). Whether some natural wild-type strains can use CD46 has so far been obscured by their in vitro isolation in cell lines expressing CD46 but not CD150, or in B95 cells, which express CD150 but very little full-length functional CD46 (Hsu et al., 1998; Manchester et al., 2000; Murikami et al., 1999). Thus, depending on the cell type used for virus growth, MV strains use either CD150 or both CD46 and CD150; these virus strains will be referred as CD46-non-using MV and CD46-using MV, respectively. The ability of MV H to bind to CD46 has been mapped to critical residues at positions 211, 451 and 481 of the H protein (Bartz et al., 1997), as confirmed by their 3-D structure study including STP BC and cytoplasmic tail 1. The chimeric CD46CD[55–46] protein and cell transfectants. The wild-type cell-surface CD46 isoform used in this study includes STP BC and cytoplasmic tail 1. The chimeric CD46CD[55–46] protein included the insertion between CD46 SCR4 and STP B of a flexible glycine hinge together with four SCR domains: SCR1 and SCR2 from CD55, and SCR3 and SCR4 from CD46 sequence (Fig. 1). It was generated using splice overlap extension–polymerase chain reaction (SOE–PCR) (Horton et al., 1989), confirmed by sequencing and expressed after ligation of the cDNA into the end-filled XbaI site of the APEX3p vector, which contains the EBV Ori and the APEX3p–CD46CD[55–46] plasmid using the lipofectamine reagent (Invitrogen). The expression of the EBNA protein, in both cell lines, ensured the episomal replication of the APEX3p vector and high levels of EBNA expression. The wild-type cell-surface CD46 isoform used in this study includes STP BC and cytoplasmic tail 1.

**Methods**

### Virus, cell lines, antibodies and reagents.

The Hallé MV strain, which uses CD46 as a cellular receptor (Naniche et al., 1993), was propagated in Vero cells, and the Ma93F and Lys-1 MV strains, which cannot use CD46 (Fayolle et al., 1999; Lecouturier et al., 1996; this paper), were propagated in B95 cells. The following cell lines were used and maintained in Dulbecco’s modified minimum essential medium supplemented with 6% foetal calf serum, gentamycin, t-glutamine and 10 mM HEPES: CHO (Chinese hamster ovary) and CHO.CD46 (Christiansen et al., 2000b) cells, Epstein–Barr virus (EBV)-transformed simian B95a B lymphoblastoid cells, and human 293-EBNA fibroblastic cells expressing the EBV nuclear antigen (Christiansen et al., 2000a). The following antibodies were used: anti-CD150, A12 (Pflaumgen Becton–Dickinson) anti-CD46, MCI20.6 recognizing the MV H binding site on SCR1 (Buchholz et al., 1997; Naniche et al., 1993), GB24 recognizing SCR4 (Adams et al., 1991; Christiansen et al., 2000c) and 11C7 recognizing SCR1–SCR2 (Christiansen et al., 2000c), anti-CD55 recognizing SCR1–SCR2 (Christiansen et al., 2000c), anti-H cl55 recognizing MV H glycoprotein (Giraudon & Wild, 1985) and anti-F Y503 recognizing MV F glycoprotein (Christiansen et al., 2000a). The octameric soluble sCD46–C4bp protein has been described elsewhere (Christiansen et al., 2000a).


Wild-type cell-surface CD46 isoform used in this study includes STP BC and cytoplasmic tail 1. The chimeric CD46CD[55–46] protein included the insertion between CD46 SCR4 and STP B of a flexible glycine hinge together with four SCR domains: SCR1 and SCR2 from CD55, and SCR3 and SCR4 from CD46 sequence (Fig. 1). It was generated using splice overlap extension–polymerase chain reaction (SOE–PCR) (Horton et al., 1989), confirmed by sequencing and expressed after ligation of the cDNA into the end-filled XbaI site of the APEX3p vector, which contains the EBV Ori (Christiansen et al., 2000b). The flanking amino acid sequence of the CD46[SCR4] to CD55[SCR1] junction (CD46 sequence in bold, glycine hinge underlined, CD55 sequence in italics) was CLKV-GGGKG-DCLG, and the CD55[SCR2] to CD46[SCR3] junction was FCKK-VLCT. Stable CHO–K1 fibroblasts expressing the chimeric protein were derived by transfection, selection with puromycin and cloning as previously described (Christiansen et al., 2000c). Transient CD46CD[55–46]-expressing cells were derived from B95 and 293-EBNA cells transfected with APEX3p–CD46CD[55–46] plasmid using the lipofectamine reagent (Invitrogen). The expression of the EBNA protein, in both cell lines, ensured the episomal replication of the APEX3p vector and high levels of EBNA expression.
expression of the CD46CD[55–46] protein in most if not all cells. One day after transfection, over 90% of 293-EBNA cells expressed CD46CD[55–46] molecules and they were used for the infection test with MV, or as the ‘receptor’ cell partner in fusion assays. The B95–CD46CD[55–46] cells were first selected by growth in the presence of puromycin for 10–14 days before use and more than 95% of cells expressed the chimeric receptor.

**Protein expression assays.** The expression of CD46, CD46CD[55–46] and MV H glycoprotein were measured at the cell surface after incubation with appropriate antibodies, labelling with phycoerythrin anti-mouse Ig and flow cytometry as detailed previously (Naniche et al., 1993). The expression was also tested by Western blot after SDS–PAGE separation under non-reducing conditions and use of 12A12 and 11C7 antibodies as probes according to Manie et al. (2000).

**Virion infectivity determination.** The cells were infected with MV (m.o.i. = 1) for 2 h at 37 °C. Non-adsorbed virus was removed and cells were incubated overnight at 37 °C in complete culture medium. One or 2 days post-infection (p.i.), the percentage of cells expressing the MV H protein was determined by flow cytometry, as described above.

**sCD46–C4bp binding assay.** The binding of 10 µg/ml of sCD46–C4bp to 2 × 10^5 MV-infected B95 cells was performed as detailed previously (Christiansen et al., 2000a). The results were expressed as percentage binding to MV Hallé-infected B95 cells as a function of H protein expression level measured in parallel by cytometry using anti-H cl55 antibody.

**Results**

**Ability of CD46CD[55–46] to bind MV**

The CD46CD[55–46] protein was found to be stably expressed on the cell surface of CHO cells as shown by its reactivity with both the anti-CD46 (Fig. 2, Table 1) and anti-CD55 antibodies (Table 1). CHO.CD46CD[55–46] cells were able to bind a much higher amount of MV than CHO cells (Fig. 2). Comparison of MV-binding ability of CHO.CD46 and CHO.CD46CD[55–46] cells revealed that the latter, which expressed a lower amount of virus binding site as detected by labelling with the anti-SCR1 MCI20.6 antibody, was more efficient than the former. Such an increase in MV-binding ability of an elongated receptor is in agreement with a previous finding using a CD46/CD4 chimeric receptor (Buchholz et al., 1996; Devaux et al., 1997). In contrast and as expected, the expression of CD46 or CD46CD[55–46] protein did not result in a significantly enhanced binding of CD46-non-using MV Ma93F strain. B95 cells, which expressed CD150 and a truncated SCR1− CD46 (Erlenhoef et al., 2001; Hsu et al., 1998; Iwata et al., 1995; Murikami et al., 1998), bound CD46-using Hallé and CD46-non-using Ma93F MV with similar, albeit low, efficiency. This poor MV-binding ability of CD150 receptors was also noticed on human CD150-expressing rodent cell lines (unpublished data) and is in agreement with data reported by others (Tatsuo et al., 2000b).

**Inability of CD46CD[55–46] to mediate MV-induced cell–cell fusion**

When tested as a ‘receptor’ partner in a cell–cell fusion assay, the CD46CD[55–46] molecule was unable to mediate fusion with a cell partner expressing the MV H and F proteins derived from a MV strain able to bind to CD46 (CD46-using MV) (Table 1). As expected, cell–cell fusion was observed between CHO.CD46 cells and cells expressing MV H and F derived from a CD46-using MV strain, but not with CD46-non-using MV-infected cells. Likewise, B95 cells, which express CD150 and very little full-length CD46 but a large amount of SCR1− truncated CD46 (Erlenhoef et al., 2001; Hsu et al., 1998; Iwata et al., 1995; Murikami et al., 1998), reacted with SCR3/4-specific GB24 but not with SCR1-specific MCI20.6 anti-CD46 antibody, and, as expected, were able to fuse with both CD46-non-using and CD46-using MV-infected cells.

**Inability of CD46CD[55–46] to mediate MV infection**

Stable expression of the CD46CD[55–46] protein was unable to mediate efficient MV replication as shown by the very low expression of MV H at the cell surface 48 h p.i. (Fig. 3), not significantly different to that observed on infected CHO cells. In contrast, MV H was expressed at a significant level after infection of CHO.CD46. Moreover, this infection induced the down-regulation of CD46, whereas the CD46CD[55–46] expression was slightly but not significantly increased.

![Fig. 2. Ability of the CD46CD[55–46] chimera to bind to MV. CHO, CHO.CD46, CHO.CD46CD[55–46] and B95 cells were analysed for cell surface expression of receptor using MCI-20.6 anti-SCR1 antibody and for ability to bind CD46-using Hallé or CD46-non-using Ma93F MV strain revealed with MV H-specific antibody by flow cytometry.](image-url)
Table 1. Cell surface expression of MV cellular receptors and their ability to mediate MV-induced cell–cell fusion

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Mean fluorescence after labelling with antibody recognizing:</th>
<th>Fusion with cells expressing H and F from MV strain*</th>
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<tbody>
<tr>
<td></td>
<td>None</td>
<td>CD150</td>
</tr>
<tr>
<td>B95</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>CHO</td>
<td>2</td>
<td>ND†</td>
</tr>
<tr>
<td>CHO.CD46</td>
<td>5</td>
<td>ND</td>
</tr>
<tr>
<td>CHO.CD46CD[55–46]</td>
<td>3</td>
<td>ND</td>
</tr>
</tbody>
</table>

* Fusion efficiency was determined either by visual observation of syncytia or by quantitative fusion assay or both; the MV H and F expressing cell partner was B95 cells infected with MV (Hallé, Ma93F or Lys-1 strain) or HeLa cells infected with recombinant vaccinia virus encoding H and F derived from MV Hallé.
† nd, Not determined.

CD46CD[55–46] inhibits both CD46- and CD150-mediated infection by CD46-using MV

CD46CD[55–46] was co-expressed with either the CD46 or CD150 functional cellular receptor by transient expression in the CD46+ human fibroblastic cell line 293-EBNA or in the CD150+ simian B95 cells. The expression of CD46 and CD46CD[55–46] was verified by Western blot (Fig. 4) and by flow cytometry (Fig. 5a, c). The surface expression of the chimeric molecule on 293-EBNA cells was shown by a small shift to the right of the anti-CD46 labelling, which also recognized endogenous CD46 (Fig. 5a). After infection with the CD46-using MV Hallé strain, syncytia were readily observed with both 293 and B95 cells (Fig. 6c, g) but not when the cells expressed the CD46CD[55–46] molecule (Fig. 6d, h). In contrast, syncytia induced by the CD46-non-using MV Lys-1 strain was not inhibited when B95 cells expressed the CD46CD[55–46] molecule (Fig. 6, compare i and j). These results were confirmed by the strong reduction in H expression (~ 90%) on cells expressing the chimeric receptor after infection with CD46-using MV Hallé strain (Fig. 5b, d) but not after infection with the CD46-non-using MV Lys-1 strain (Fig. 5e). Taken together, these data reveal that the chimeric CD46CD[55–46] receptor has a dominant negative function as it can inhibit the infection of CD46+ 293-EBNA cells and CD150+ B95 cells by CD46-using MV strain.

Inhibition of MV-mediated cell–cell fusion using soluble sCD46–C4bp and correlation with inhibition of MV infection by chimeric CD46CD[55–46] according to MV strain

To get an insight into the possible mechanism underlying the inhibitory effect of the chimeric CD46CD[55–46] receptor,
Fig. 5. Reduction in cell surface expression of H protein on transfected 293-EBNA (a, b) and B95 (c, d, e) cells induced by the expression of CD46CD[55–46] (a, c, light grey histograms) after infection with the CD46-using MV Hallé strain (b and d, respectively, light grey histograms), but not after infection with the CD46-non-using MV Lys-1 strain (c, light grey histograms). CD46 expression (a, c) and H expression (b, d, e) on control non-transfected cells are shown by dark grey histograms. Note that on in (e), the two histograms fully overlap.

Discussion

By analogy with the long CD46/CD4 protein composed of the four SCR domains of CD46 fused to the four Ig-like domains of the CD4 molecule (Buchholz et al., 1996), the CD46CD[55–46] chimeric protein was generated with eight SCRs with SCR1 and SCR2 of CD46, which harbour the functional MV binding site (Buchholz et al., 1997; Casanovas et al., 1999; Iwata et al., 1995; Manchester et al., 1995, 1997; Mumenthaler et al., 1997), being located at the N-terminus. These two chimeric proteins are expected to have a similar size, ~22 nm for the long CD46/CD4 protein and ~20 nm for CD46CD[55–46], according to previous modelling and 3-D structures of two and four SCR lengths (Buchholz et al., 1996; Casanovas et al., 1999; Mumenthaler et al., 1997). The CD46CD[55–46] was unable to mediate the MV H- and F-induced cell-to-cell fusion. One could speculate that this is due to increased protein length and hence moving the MV binding site away from the membrane. In addition, this CD46CD[55–46] protein exerted a dominant negative effect in inhibiting the fusion mediated by MV H interaction with wild-type CD46. This result is in agreement with the inhibitory effect of the long CD46/CD4 receptor on the fusion mediated by a short CD46/CD4 chimeric protein made of CD46 SCR1–SCR2 and the fourth CD4 Ig-like domain (Buchholz et al., 1996).

To address the specificity of our observations, the activity of CD46CD[55–46] was also tested on MV entry mediated by the so far ‘universal’ MV receptor, CD150. Simian B95 cells were chosen because they express only a limited amount of functional CD46 receptor. Indeed, although an mRNA encod-
Fig. 6. Syncytium formation within 293-EBNA cells (a–d) and B95 cells (e–j) infected by CD46-using MV Hallé strain for 24 h (m.o.i. = 1) (c, d, g, h) is inhibited upon expression of chimeric CD46CD[55–46] molecule (d, h) whereas syncytium formation within B95 cells infected by CD46-non-using MV Lys-1 strain (i, j) is not inhibited upon expression of CD46CD[55–46] (j). Magnification × 10. n.i., Not infected.
After infection with CD46-using MV, we noticed that while the syncytium formation was almost fully inhibited (see Fig. 6), the level of cell surface H expression was strongly but not completely inhibited. In particular, whereas ~ 82% of B95 cells expressed a significant level of CD46CD[55–46], up to ~ 52% expressed a detectable level of H (see Fig. 5). Similar results were obtained when the expression of the F glycoprotein was analysed (not shown). This suggests that cell–cell fusion is more sensitive to receptor-mediated inhibition than virus infection, as observed with other soluble virus entry inhibitors (Christiansen et al., 2000a).

When studying the CD46/CD4 chimeric receptors, the dominant negative interference of a long fusion-incompetent receptor over a short functional receptor, even at an unfavourable molar ratio, argued for the existence of a MV fusion complex (Buchholz et al., 1996). In particular, one could speculate whether the presence of a fusion-incompetent long receptor among several functional homologous receptors involved in a single MV fusion complex can disable the whole molecular scaffold. The ability of a fusion-incompetent receptor made from the CD46 backbone to inhibit MV-induced fusion of the short structurally unrelated CD150 receptor made of two Ig-like domains, together with inhibitory activity of the soluble sCD46–C4bp, suggest another inhibitory mechanism. A long receptor (estimated to be > 20 nm) is likely to be more accessible from the outside of the cells than a short one such as CD46 (~ 10 nm) or CD150 (~ 6 nm), and can sequester any cell surface-contacting MV H glycoproteins. This interaction could lead to an irreversible conformational inactivation of the companion F molecule, as suggested by the potent MV inhibitory activity of the soluble sCD46–C4bp (Christiansen et al., 2000a). This effect would be amplified if a single CD46-based receptor could interact sequentially with several MV H–F complexes.

The efficient inhibitory effect of CD46CD[55–46] on the CD150-mediated MV infection and the higher MV-binding efficiency of CD46-based molecules suggests that, when a CD46-using MV strain infects human cells expressing both CD46 and CD150 receptors, i.e. activated B and T cells, dendritic cells and memory T cells, a competition for receptor usage may occur. Such competition could play a role in the attenuation process of live attenuated MV vaccine. Similarly, engineered H protein for retargeting to a new cellular receptor without abrogation of the binding to the natural receptor as recently described (Hammond et al., 2001; Schneider et al., 2000) may lead to competition for receptor usage.

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