Differential receptor usage by measles virus strains

Roman Bartz,1 Ruth Firsching,1 Bert Rima,2 Volker ter Meulen1 and Jürgen Schneider-Schaulies1

1 Institut für Virologie und Immunobiologie, Versbacher Str. 7, D-97078 Würzburg, Germany
2 School of Biology and Biochemistry, The Queen’s University of Belfast, Belfast BT9 7BL, UK

Recently, we demonstrated that infection of cells with all measles virus (MV) strains tested was inhibited by antibodies against CD46, although not all strains caused downregulation of the MV receptor CD46 from the surface of human cells. We now show that infection of cells with MV strain WTFb, a variant of wild-type isolate WTF which has been isolated and propagated on human BJAB cells, is not inhibited by antibodies against CD46. In contrast, infection of cells with the closely related strain WTFv, a Vero cell-adapted variant of WTF, is inhibited by antibodies against CD46. This observation led us to investigate the interaction of these viruses and the vaccine strain Edmonston (Edm) with CD46 and target cells. Cellular receptors with high affinity binding for WTFb are present on BJAB cells, but not on transfected CD46-expressing CHO cells. In contrast to the Edm strain, virus particles and solubilized envelope glycoproteins of WTFb have a very limited binding capacity to CD46. Furthermore, we show that recombinant soluble CD46 either does not bind, or binds very weakly, to WTFb glycoproteins expressed on the cell surface. Our findings indicate that wild-type MV strain WTFb and vaccine strain Edm use different binding sites on human cells. In addition, the results suggest that MV strains may alternatively use CD46 and an unknown molecule as receptors, and that the degree of usage of both receptors may be MV strain-specific.

Introduction

Measles virus (MV), a member of the genus Morbillivirus, family Paramyxoviridae, is among the most widespread human pathogens. The virus is monotypic (Rota et al., 1992) and was first isolated by Enders & Peebles (1954) by using primary human and rhesus monkey kidney cell cultures. One of the isolates, the Edmonston strain (Edm), has been passaged in many laboratories using monkey kidney cell lines such as Vero and CV-1 cells and has been used as a standard strain of MV ever since. Recently, CD46 was identified as the major MV receptor on human cells by different approaches using vaccine or closely related strains which were propagated on Vero cells (Dörig et al., 1993; Manchester et al., 1994, 1995; Naniche et al., 1993a, b; Schneider-Schaulies et al., 1995a). The natural function of CD46 is to act as a cofactor for plasma protease factor I and to protect cells from complement mediated lysis (Liszewski et al., 1991). It has been shown that different isoforms containing the short consensus repeats (SCR) 1 and 2 of CD46 can serve as receptor for MV vaccine strains (Buchholz et al., 1996, 1997; Manchester et al., 1994, 1997; Varior-Krishnan et al., 1994), and that N-glycosylation of SCR2 is essential for its function as an MV receptor (Maisner et al., 1996). Soluble CD46 binds to soluble viral haemagglutinin of a vaccine strain and can also block infection of cells with MV (Devaux et al., 1996; Seya et al., 1995). MV vaccine strains and a proportion of wild-type isolates lead to downregulation of CD46 from the cell surface after infection of cells or after contact of infected cells with uninfected cells (Krantic et al., 1996; Schneider-Schaulies et al., 1995 b, c, 1996; Schnorr et al., 1995). However, a number of MV wild-type strains, most of which were isolated by cultivation on monkey or human B cell lines such as B95-8 or BJAB, lack the capacity to downregulate CD46 (Schneider-Schaulies et al., 1995 b, c). Several of these strains show a clear tropism for lymphoid cells and a reduced capacity to replicate in Vero cells.

For several years it has been known that fresh MV isolates can be easily propagated on B cell lines, whereas propagation on Vero cells needs multiple passages for adaptation of the virus (Kobune et al., 1990). It has also been reported that wild-type isolates do not interact with monkey red blood cells in the haemagglutination assay unless they have been adapted to Vero cells after approximately 20 passages on these cells.
(Kobune et al., 1990; Shibahara et al., 1994). Nucleotide sequencing showed that two of these strains had the amino acid exchange Asn → Tyr at position 481 in their haemagglutinins (Shibahara et al., 1994). Recently, the haemagglutinin of Vero cell-adapted MV strain WTFv, a variant of the original isolate WTF, was found to have two expressed amino acid differences compared with the original BJAB-isolated WTF haemagglutinin, namely Thr instead of Ile at position 192, and Gly instead of Ser at position 546 (Rima et al., 1997). Interestingly, the same amino acid exchange at position 546 was observed in Vero cell-adapted MV strain DLv in comparison with lymphotropic isolate DLb (Rima et al., 1997). These results indicate that this single amino acid strongly influences the capacity of MV-haemagglutinin (MV-H) to interact with the Vero cell CD46 homologue.

The use of vaccinia virus recombinants expressing MV-H of Edm and the related strain Halle has shown that expression of MV-H alone is sufficient for downregulation of CD46 (Naniche et al., 1993b; Schneider-Schaulies et al., 1995b). Two amino acids of the haemagglutinin, Val-451 and Tyr-481, were identified as being mainly responsible for the downregulating capacity of strains Edm and Halle (Bartz et al., 1996; Lecouturier et al., 1996). In addition, mutation of these two amino acids leads to abrogation of haemadsorption and HeLa cell fusion activity of the H-protein (Lecouturier et al., 1996). In the two CD46 non-downregulating strains analysed, WTF and Ma93F, the amino acids at positions 451 and 481 are Glu instead of Val, and Asn instead of Tyr. Thus, the phenotype of interaction of a particular MV-H with CD46 is governed by few amino acids, which vary group-specifically between haemagglutinins of vaccine strains, CD46 downregulating wild-type viruses and non-downregulating MV strains (Bartz et al., 1996).

In an earlier study, we analysed the capacity of anti-CD46 antibodies to inhibit infection of cells with various MV strains, and found that infection with all MV strains tested, including four CD46 non-downregulating MV isolates, was inhibited by antibodies against CD46 (Schneider-Schaulies et al., 1995b). These results suggested that non-downregulating MV strains may use CD46 as cellular receptor, and that the interaction with CD46 may not be strong enough to lead to downregulation of CD46.

The differential capacities of MV strains to downregulate CD46, and the fact that some CD46-negative mouse cell lines and primary mouse B lymphocytes are susceptible to infection with MV wild-type and vaccine strains in the absence of CD46 (Dunster et al., 1995; Horvat et al., 1996; Rager-Zisman et al., 1984; Yanagi et al., 1994) led us to investigate whether some MV strains may use alternative receptor molecule(s), assuming that the lower the affinity of a particular MV strain for CD46, the more a strain may use this alternative receptor(s). In order to investigate this phenomenon, we determined the receptor usage of wild-type strain WTFb propagated in BJAB cells, in comparison with Vero cell-adapted variant WTFv and vaccine strain Edm.

### Methods

**Antibodies, cells and viruses.** MAbs L77, Nc32 and K83 (anti-
MV-H, Liebert et al., 1994); 13/42 (anti-CD46 SCR1, Schneider-Schaulies et al., 1995a); B97 (anti-CD46 SCR1, Buchholz et al., 1997); and 10/88 (anti-CD46 SCR3/4) were produced and purified over protein G-Sepharose in our laboratory. The rabbit polyclonal anti-MV-H and anti-MV-F sera raised against conserved peptides in the cytoplasmic parts of the molecules (Buchholz et al., 1996; Hu et al., 1995) were a gift of R. Cattaneo, Zürich, Switzerland. The FITC-conjugated rabbit anti-mouse Ig and rabbit anti-human IgC antibodies were purchased from DAKO.

The Epstein–Barr virus negative human lymphoblastoid B cell line BJAB (Menezes et al., 1975), and the lines BJAB-pEdm and BJAB-pWTv persistently infected with MV-Edm and WTg (a kind gift of J.-J. Schnorr, Würzburg, Germany), were cultured in RPMI 1640 medium containing 5% FCS, HeLa, CHO and CD46 transfected CHO-H5, CHO-5.3, CHO-3.0 (a kind gift of B. Loveland, Heidelberg, Australia; Loveland et al., 1993) and CHO-1-IV/3-4 (a kind gift of C. Buchholz, Zürich, Switzerland; Buchholz et al., 1996) were cultured in MEM medium containing 5% FCS; CHO-3.6 in the presence of HT, and CHO-1-IV/3-4 in the presence of G418.

MV-Sp strain WTF was isolated in 1990 using human B cells (Schneider-Schaulies et al., 1995b). After propagation for more than 30 passages on BJAB cells, this strain is called WTg. WTg was also adapted to grow on Vero cells; it was passaged more than 30 times on Vero cells and named WTg. MV-Edm was propagated on Vero cells. For virus production, cells were infected at an m.o.i. of 0.01 and virus was harvested when maximum giant cell formation was observed by one cycle of freezing–thawing and twice pelleting cell debris by centrifugation. Supernatants were stored at −80 °C.

**Infection inhibition assay.** The infection inhibition assay was essentially carried out as described previously (Schneider-Schaulies et al., 1995b). Briefly, 1 × 10⁵ BJAB cells were incubated with 10 µg/100 µl MAb for 45 min at 4 °C before infection at an m.o.i. of 0.1 for 60 min at 37 °C. After washing the cell with PBS, half the samples were treated with an acidic glycine buffer (8 mM glycine, 140 mM NaCl, 0.1% BSA, pH 2.5) for 4 min at 4 °C. Cells were then washed with PBS and incubated in medium for 48 h at 37 °C. For analysis of infection by flow cytometry, cells were fixed and permeabilized with 3% paraformaldehyde, 0.25% Triton X-100 in PBS and stained with a human anti-MV hyperimmune serum and FITC-conjugated anti-human antibodies. The reduction of median fluorescence intensity (m.f.i.) in comparison with mock-treated cells was determined and expressed as percentage of reduction of m.f.i.

**Virus–cell binding assay.** Similar m.o.i. values or amounts of proteins for virus preparations of Edm and WTg were used in the virus–cell binding assays. The m.o.i. values were determined according to infection of Edm on Vero cells and WTg on BJAB cells; the amounts of viral glycoprotein were determined by Western blot using rabbit polyclonal sera against the cytoplasmic domain of H and F. 2 × 10⁵ BJAB, HeLa, CHO and CHO-5.3 cells in 100 µl PBS were incubated at 4 °C for 1 h with virus at a given m.o.i. or amount of protein, washed with FACS buffer (PBS without Ca²⁺ and Mg²⁺, containing 0.4% BSA, 0.02% NaN₃), and stained with anti-MV-H MAb L77 and FITC-conjugated goat anti-mouse antibodies, as described (Schneider-Schaulies et al., 1995a). Bound virus was determined by analysis with a FACScan (Becton Dickinson).

**Preparation of soluble and solubilized CD46 and binding assay.** Two kinds of recombinantly expressed CD46 proteins were used. CHO cell-expressed soluble CD46 (sCD46, BC-isofrom) purified over a CL-4B Sepharose column was kindly provided by G. Yeh (Cytomed) and has been described previously (Devaux et al., 1996). For expression in the
baculovirus system, a CD46-encoding cDNA was generated from HeLa cell RNA by RT–PCR using primers 5′ GACGCTATCGAATACTATACATTGAGGCTTCCCG 3′ (upper primer containing a Clal cloning site and the atg start codon) and 5′ CGAGCTAATTCGAGCCTTCGCTCAGCTTCGTTGCT 3′ (lower primer containing an EcoRI site), and cloned into pBlueScript (SK). The CD46-BC2 isoform was confirmed by sequencing and subcloned into the SnaI site of baculovirus transfer vector pAcCL29.1. After co-transfection with genomic AcRP23lacZ virus DNA, CD46 recombinant viruses were selected and plaque purified. The Spodoptera frugiperda (Sf9) insect cell-expressed CD46 (Sf9-CD46) was used for preparation of solubilized membrane proteins generated from CD46-expressing Sf9 cells 48 h after infection with a recombinant baculovirus, as described for preparation of solubilized viral glycoproteins and binding assay (see below). Expression of CD46 was confirmed by immunofluorescence and Western blotting.

For the binding assay, 1 × 10⁶ BJAB cells were incubated with 10 µg sCD46 or Sf9-CD46 in PBS containing 0.1% CHAPS for various times, or various concentrations of CD46 were incubated for 45 min at 4 °C. After one wash with FACS buffer cells were stained with anti-MV-H MAb L77 or Nc32 and anti-mouse FITC and analysed by flow cytometry. Binding was calculated as percentage reduction of m.f.i. compared with mock-treated cells.

### Preparation of solubilized viral glycoproteins
Persistently infected BJAB cells were resuspended in HEPES buffer [25 mM HEPES, 150 mM NaCl, 10% sucrose, 2.5 mM EGTA, 1% proteinase inhibitors (Boehringer Mannheim) pH 7.4], and lysed by adding 10 vols hypertonic HEPES buffer (25 mM HEPES, 2.5 mM EGTA, 1% proteinase inhibitors pH 7.4) for 10 min on ice, followed by shearing the cells through a 21 gauge needle and sonification. The nuclei were removed by centrifugation, and membrane proteins were pelleted by centrifugation at 100 000 g, solubilized with 4% CHAPS in PBS and dialysed against 0.1% CHAPS in PBS. The protein concentration was then adjusted to 0.5 mg/ml, the haemagglutination activity of the 0.1% CHAPS-containing MV glycoprotein extracts was measured using monkey red blood cells, and the amount of MV-H and F proteins was analysed by Western blot.

### Scanning electron microscopy (SEM)
Adherent cells were grown in Falcon cell culture inserts with a pore size of 0.45 µm. After incubation with suspension cells, the cell monolayers on the membranes were washed with PBS for 5 min and then fixed with 2.5% glutaraldehyde in 50 mM sodium cacodylate buffer overnight at 4 °C. A secondary fixation was carried out with 1% osmium tetroxide pH 7.4 for 60 min at 4 °C. Then the inserts were warmed to room temperature and dehydrated in graded dilutions of ethanol. The dehydrated membranes were then incubated for 5 min in hexamethyldisilazane and air-dried in a fume hood for 30 min. The membranes were removed with a scalpel from the cell culture insert and mounted on an SEM stub. The samples were then coated with gold and viewed in a electron microscope (Zeiss) at 15 kV.

### Results

#### Infection of cells with MV-WTFb is not inhibited by antibodies against CD46

In order to find out whether all MV strains (especially those which do not lead to downregulation of CD46) can use CD46 as cellular receptor, we looked for MV strains for which the infection process was not inhibited by antibodies against CD46. In an earlier study (Schneider-Schaullies et al., 1995 b) we demonstrated that the anti-CD46 MAb 13/42 inhibited infection of cells with all strains tested, including wild-type isolates AB, DL, DF and WTF, provided that virus-exposed cells were washed with an acidic glycine buffer to destroy residual attached virus on the cell surface. In the present study, we tested strain WTFb, a variant of WTF which had been propagated for more than 30 passages on BJAB cells, and WTFv, a Vero cell-adapted variant of WTF propagated for more than 30 passages on Vero cells. We analysed the capacity of various antibodies, directed against the first (MAb 13/42 and B97) or third/fourth (MAb 10/88) SCR domains of CD46, to inhibit infection of BJAB cells with WTFb, WTFv and Edm. Cells were incubated with or without MAb prior to infection with MV, and washed with acidic glycine buffer or left unwashed. Infection of BJAB cells with Edm was inhibited by MAb 13/42 by 55% without the glycine wash, and by 84% after a glycine wash (Fig. 1). Similar percentages of inhibition were found with WTFb without (46%) or with (74%) the glycine wash. In contrast, infection with WTFb was not inhibited in the absence of the glycine wash, and slightly inhibited (approximately 14%) in the presence of the glycine wash. Similar results were obtained with MAb B97, directed against SCR1 of CD46, whereas MAb 10/88, recognizing SCR3/4 of CD46, did not inhibit infection of BJAB cells with any of the MV strains (Fig. 1).

Since WTFb and WTFv were quite different in their behaviour concerning inhibition by antibodies against CD46, we measured their capacity to downregulate CD46 from the surface of BJAB cells. Interestingly, neither WTFb and WTFv
Fig. 2. Downregulation of CD46 after infection of BJAB cells with Edm, but not WTFv and WTFb. Uninfected (grey lines) BJAB cells and BJAB cells infected with the three MV strains (black lines; m.o.i. = 1·0; 48 h) were fixed and stained with anti-CD46 MAb 13/42 (A, B, C) and as control of infection with anti-MV-H MAb L77 (D, E, F) and secondary antibodies. The m.f.i. of CD46 was reduced after infection with Edm by approximately 50% (A), but not after infection with WTFv and WTFb (B, C).

Fig. 3. Competition with antibody L77 of binding of soluble CD46 to viral haemagglutinins expressed on the surface of persistently infected BJAB cells. Binding of MAb L77 to MV-H on the surface of persistently infected BJAB cells was measured by flow cytometry. (A) Binding of L77 to MV-Edm infected cells (BJAB-pEdm) was reduced in the presence of soluble CD46 (10 µg/1 × 10⁵ cells/100 µl), whereas sCD46 did not reduce the signal detected for binding of MAb L77 to cells persistently infected with WTFb (BJAB-pWTF). (B) The percentage reduction of L77 binding is shown with 2, 5 and 10 µg/1 × 10⁵ cells/100 µl of solubilized Sf9-CD46 and CHO-derived sCD46, respectively. (C) The percentage reduction of L77 binding to the surface of BJAB-pEdm and BJAB-pWTF cells with solubilized Sf9-CD46 and CHO-derived sCD46 (10 µg/100 µl each) is shown after 2, 5 and 15 min. After 15 min Sf9-CD46 reduced MV Edm-H interaction with MAb L77 by 48% and sCD46 by 54%, whereas interaction of WTF-H with MAb L77 was only slightly inhibited by soluble CD46.

led to downregulation of CD46, whereas after infection with MV-Edm CD46 was downregulated by approximately 50% (Fig. 2). These results, combined with those in Fig. 1, indicate that the Vero cell-adapted variant of WTF, WTFv, used CD46 as receptor but did not downregulate CD46. This finding is similar to those described earlier for wild-type isolates AB, DL,
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Fig. 4. Binding of solubilized viral glycoproteins (H/F) to CD46 on the surface of CHO cells. (A) Pre-incubation of CHO-5.3 cells with solubilized glycoproteins from Edm reduced the CD46 signal (MAb 13/42) drastically, whereas no difference was detected by flow cytometry after incubation of cells with WTFb glycoproteins. (B) The percentage reduction of binding of MV glycoproteins (percentage reduction of the control CD46 signal) is given for CHO cells transfected with various forms of CD46, as indicated in (C). CHO-3.6 cells do not contain a functional SCR2 and therefore do not bind Edm glycoproteins. CHO-5.3 and CHO-1H5 cells express natural forms of CD46, as indicated. CHO-I-IV/3-4 cells express SCR1–4 of CD46 and the immunoglobulin domains 3–4 and transmembrane parts of CD4.

DF and WTF (Schneider-Schaulies et al., 1995b). In contrast, the BJAB cell-passaged variant WTFb seemed not, or only very weakly (Fig. 1), to interact with CD46 as receptor.

Binding capacity of soluble CD46 to viral glycoproteins

To assess whether CD46 can bind to viral glycoproteins of strain WTFb, we first investigated the binding capacities of soluble forms of CD46 to viral envelope proteins in their natural conformation on the cell membrane of persistently infected cells. We determined the competition of soluble CD46 with the MV-H-specific MAb L77 for binding to viral haemagglutinins on the surface of BJAB cells persistently infected with Edm or WTFb (BJAB-pEdm and BJAB-pWTF, respectively). Both of these cell lines are characterized by a very high surface expression of MV envelope glycoproteins H and F, with their respective strain-specific capacities to downregulate CD46 (not shown). Recombinant CD46 was either expressed in Sf9 cells using the baculovirus system (Sf9-CD46) and solubilized as membrane protein preparations, or expressed in CHO cells as a recombinant soluble form (sCD46) and purified on a Sepharose CL-4B column. Both Sf9-CD46 and sCD46 blocked surface accessibility to MV-H with MAb L77 on BJAB-pEdm cells, whereas no competition was detected on BJAB-pWTF cells (Fig. 3A). The interaction was dependent on the concentration of CD46 (Fig. 3B) and on time (Fig. 3C). L77 reactivity on BJAB-pEdm cells was blocked by up to 56%, whereas there was hardly any effect on the fluorescence signal using BJAB-pWTF cells. Similar percentages of inhibition were obtained with the neutralizing anti-MV-H antibody Nc32, recognizing an epitope different from MAb L77 on MV-H (Liebert et al., 1994). These results indicated that the binding capacity of soluble or solubilized CD46 to MV glycoproteins expressed on the surface of persistently infected cells is MV strain-specific.

Binding capacity of soluble viral glycoproteins to CD46

To verify these observations in a second set of experiments, we used solubilized viral glycoprotein preparations of strains Edm and WTFb prepared from persistently infected cells, and measured their binding capacities to the surface of various CD46-positive and CD46-negative cells, where CD46 is expressed in its native form. We found very limited specific binding of WTFb envelope glycoproteins to CD46-positive cells, while Edm envelope glycoproteins bound well to cells expressing various native forms of CD46 in their membranes (Fig. 4). Efficient binding of Edm glycoproteins H/F to these cells was dependent on the presence of domains SCR1/2 of CD46, a finding which is in agreement with earlier studies, using CD46 chimera, concerning binding of MV-Edm (Buchholz et al., 1996) and infectivity of cells with MV-Edm (Manchester et al., 1994).

Virus particles of WTFb bind with high affinity to cells, but not to CD46

To determine whether WTFb can bind to receptors on susceptible cells (BJAB), intermediate susceptible cells (HeLa)
Fig. 5. Attachment of MV particles of strains WTFb and Edm to BJAB and HeLa cells. (A) Similar m.o.i.'s of viral particles, as indicated, were bound to the cells, and binding was quantified by flow cytometry with an antibody against MV-H. The FACS signals of WTFb and Edm (as indicated; MAb L77) bound to BJAB and HeLa cells are shown in comparison with signals of cells in the absence of virus (control, black line). (B) The amount of glycoproteins in two independent preparations of Edm (Edm 1/2) and WTFb (WTFb 1/2) was controlled by Western blot using polyclonal sera against MV-H and -F. The WTFb preparations were prediluted 4-fold to achieve similar signals to Edm in the dilution series blotted. (C) Overlays of FACS signals of viral glycoprotein (MAb L77) corrected for the amounts of viral glycoprotein reveal a lower affinity of WTFb glycoprotein for cells in comparison with Edm glycoprotein.
or non-susceptible cells (CHO), we analysed the binding capacities of virus particles of MV strains WTFb and Edm to the cell surfaces of these cells. Virus particles of both strains attached well to the two human (CD46-positive) cell lines BJAB and HeLa (Fig. 5 A). Large amounts of WTFb and Edm bound to the surface of BJAB cells, whereas the binding capacity of HeLa cells was lower for WTFb than for Edm, and saturation was reached earlier with WTFb than with Edm. This indicates that in contrast to BJAB cells, HeLa cells have only a limited number of receptors for WTFb. Since CD46 expression on HeLa cells is higher than on BJAB cells (not shown), and since the number of binding sites for WTFb and Edm on HeLa cells is different, the data indicate that the binding capacity of WTFb to cells is not correlated with the level of CD46 expression.

As the m.o.i. values (infectious units) of a virus preparation do not necessarily reflect the amount of viral glycoprotein in the preparations, binding studies using similar concentrations of viral glycoprotein may be more suitable. We therefore controlled the amount of viral glycoproteins present in the preparations of Edm and WTFb by Western blot with antisera against the cytoplasmic domains of haemagglutinin and fusion protein (Fig. 5B). Dilutions of the virus preparations revealed that WTFb contained approximately four times more glycoprotein than preparations of Edm. For comparison with data obtained with similar m.o.i. values, we now overlaid the FACS signals of similar amounts of glycoprotein in the virus–cell binding assay (Fig. 5C). This experiment shows that the binding capacity of WTFb glycoproteins is generally lower than the capacity of Edm glycoproteins to bind to the cell surface of human cells.

To measure the influence of CD46 on the binding capacity of both viruses to the surface of cells, CD46-negative and CD46-positive CHO cells were used. On the surface of the CD46-negative CHO cells, only a background level of binding of both virus strains was detected (Fig. 6). Expression of CD46 on transfected CHO-5.3 cells increased binding of Edm drastically, while binding of WTFb remained unchanged. Even with high levels of WTFb (m.o.i. = 16), no increase in CD46-specific binding was detected (not shown). Thus, in contrast to binding of Edm virus to cells, there was no CD46-specific binding of WTFb particles to CHO-5.3 cells.

**Binding of persistently infected BJAB cells to CD46-positive and CD46-negative cell monolayers**

In order to investigate by a further method the capacity of WTFb glycoproteins to bind to a cell surface expressing CD46 or unknown receptors, a cell–cell adhesion assay was used. BJAB cells persistently infected with WTFb or Edm were overlaid on monolayers of HeLa, CHO-5.3 and CHO cells. After extensive washing, cells were fixed and processed for SEM (Fig. 7). WTFb-infected cells (BJAB-pWTF) bound to HeLa cell monolayers, but significantly less to CD46-positive CHO-5.3 and CD46-negative CHO cells (Fig. 7 A, cf. B, C). In
Fig. 7. Adhesion of BJAB cells persistently infected with WTFb or Edm to HeLa, and CD46-positive and CD46-negative CHO cells. BJAB-pWTF and BJAB-pEdm cells were overlaid on monolayers of HeLa (A, D), CHO-5.3 (B, E) and CHO (C, F) cells, respectively, washed extensively and processed for SEM. Uninfected BJAB cells were incubated on a HeLa cell monolayer as control (G). The magnification in panels A–G is indicated by the bar in G (100 µm). Enlargements of BJAB-pWTF and BJAB-pEdm cells binding to a HeLa cell monolayer are shown in H and I (bar in H represents 5 µm).

contrast, expression of CD46 on CHO cells greatly improved binding of Edm-infected cells (BJAB-pEdm) to the monolayer (Fig. 7E, cf. F). The affinity of the binding reaction is reflected by the numbers of persistently infected cells adhering to the monolayers. The formation of strongly adhering microvilli between MV-H/F expressing and receptor bearing cells was visualized at higher magnification (Fig. 7H, I). Under the same conditions, few uninfected BJAB cells bound to monolayers of HeLa (Fig. 7G) or CHO cells (not shown). These results indicate that WTFb glycoproteins interact with receptors on HeLa cells, but not with CD46 expressed by CHO cells.

Discussion

In this study, we characterized the CD46 binding properties of the closely related MV strains WTFb and WTFv, and the vaccine strain Edm. Strains WTFb and WTFv, selected by passages on BJAB and Vero cells, respectively, might have been selected from pre-existing variants in the original virus isolate, or might have emerged as variants with point mutations originating during the process of adaptation in tissue culture. Whereas infection of human BJAB cells with Edm and WTFv was inhibited by antibodies against the first SCR domain of
CD46, these antibodies had no effect on infection of cells with WTFb. Also, an antibody against the SCR3/4 domains of CD46 did not inhibit infection of cells with WTFb. The results of these studies do not exclude the possibility that WTFb may bind to a binding site on CD46 different from that for Edm, with which the antibodies used do not interfere. For MV vaccine strains, the binding sites to CD46 have been mapped on SCR1/2 (Buchholz et al., 1997; Manchester et al., 1997; Hsu et al., 1997). Since B95 cells express a CD46 molecule which has a deletion of SCR1, as found in New World monkeys (Hsu et al., 1997), and are easily infected with WTFb (not shown) and other MV wild-type strains (Kobune et al., 1990), this hypothetical binding site must be independent of SCR1. Furthermore, our binding studies of soluble viral glycoproteins and virus particles indicate that binding of WTFb to cells is independent of CD46 (SCR1–4, STP BC and C) on CHO cells. We cannot exclude the possibility that an unknown cell type-specific co-receptor could modify the binding activity of CD46, so that a new binding site for WTFb not identical with the binding site for Edm is exposed on susceptible cells. However, since HeLa cells also express binding sites for WTFb, and since the number of binding sites is different from the number of binding sites for Edm (Fig. 5A), this possibility is unlikely.

WTFb virus particles, and persistently WTFb-infected BJAB cells, bind with good affinity to the cell surface of BJAB and HeLa cells, suggesting the presence of an unknown receptor for MV on these cells. Furthermore, the lack of a complete inhibition of infection with antibodies to CD46 suggests that the different MV strains may use CD46 and the unknown receptor as alternative receptors in varying ratios. This hypothesis is supported by our finding that WTFb may have a residual weak capacity to interact with CD46 (Figs 1 and 7).

A small number of amino acid exchanges is sufficient to alter the phenotype of MV (Bartz et al., 1996; Lecouturier et al., 1996; Rima et al., 1997; Shibahara et al., 1994). These findings may reflect the flexibility of the virus to become adapted to certain cell types in vivo, and support the hypothesis of an alternative receptor usage. In addition to alternative receptors mediating primary binding to cells, WTFb and other MV wild-type isolates may require additional specific cofactor(s) not present on Vero cells but present on lymphoid cells such as BJAB. The presence or absence of MV strain-specific cofactors may determine the lymphotropism of a proportion of the MV strains, and could explain the fact that lymphotropism and CD46 interaction (downregulation) correlate for most, but not all, MV strains (Schneider-Schaulies et al., 1995b, c).

The differential usage of alternative receptors may have important consequences for virulence, pathogenesis and MV-induced immunosuppression. Interestingly, proliferative inhibition of T lymphocytes by MV in vitro is dependent on direct contact between virus particles and infected cells presenting the viral envelope proteins and the target cells, but seems to be independent of CD46, since CD46-negative rat lymphocytes are also inhibited (Schlender et al., 1996). Therefore, a receptor-mediated mechanism is likely to be involved in MV-induced inhibition of proliferation and might well be mediated by the unknown alternative receptor(s).

Downregulation of CD46 by MV vaccine strains and other strains with high binding capacity to CD46 could contribute to attenuation of these strains by leading to an increased susceptibility of infected cells to complement lysis (Schneider-Schaulies et al., 1995b; Schnorr et al., 1995). The same mechanism could probably lead to selection against such viruses in vivo. Differential usage of alternative receptors might also be responsible for variations in virulence between circulating wild-type MV strains. Within the eight groups of MV distributed all over the world (Rima et al., 1995, 1997; Rota et al., 1992) there also exist wild-type isolates which have been isolated and propagated on lymphoid cells, but nevertheless downregulate CD46 (Schneider-Schaulies et al., 1995b, c). On the other hand, there are also exceptions, strains which have been grown on Vero cells and do not downregulate CD46 from the surface of human cells (Schneider-Schaulies et al., 1995b, c). With respect to these findings, it is tempting to speculate that MV strains might use both CD46 and an alternative molecule, in varying ratios, as cellular receptors.

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