JOURNAL OF VIROLOGY, Jan. 1996, p. 255–263 Vol. 70, No. 1
0022-538X/96/$04.00+0

Receptor (CD46) Modulation and Complement-Mediated Lysis of Uninfected Cells after Contact with Measles Virus-Infected Cells

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Received 26 June 1995/Accepted 4 October 1995

Recently, it has been observed that the infection of human target cells with certain measles virus (MV) strains leads to the downregulation of the major MV receptor CD46. Here we report that CD46 downregulation can be rapidly induced in uninfected cells after surface contact with MV particles or MV-infected cells. Receptor modulation is detectable after 30 min of cocultivation of uninfected cells with MV-infected cells and is complete after 2 to 4 h, a time after which newly synthesized MV hemagglutinin (MV-H) cannot be detected in freshly infected target cells. This contact-mediated receptor modulation is also induced by recombinant MV-H expressed by vaccinia virus and is inhibitable with antibodies against CD46 and MV-H. By titrating the effect with MV Edmonston strain-infected cells, a significant contact-mediated CD46 modulation was detectable up to a ratio of 1 infected to 64 uninfected cells. As a result of CD46 downregulation, an increased susceptibility of uninfected cells for complement-mediated lysis was observed. This phenomenon, however, is MV strain dependent, as observed for the downregulation of CD46 after MV infection. These data suggest that in acute measles or following measles vaccination, uninfected cells might also be destroyed by complement after contacting an MV-infected cell.

As a highly contagious agent, measles virus (MV) is among the most widespread human pathogens, causing a well-characterized clinical disease that normally occurs early in childhood. Associated with the pronounced tropism of MV for lymphocytes and macrophages during the acute infection, a characteristic lymphopenia and immunosuppression which impair important immune functions so that the establishment and the outcome of superinfections are favored is observed (3, 4, 10, 13, 27–29, 48). In vitro, the proliferative responses of T cells are depressed for more than 6 weeks after the onset of the rash in the absence of infectious MV (13). The immunosuppressive effect is the major cause for the high rate of mortality associated with MV infections in Third World countries.

Recently, the major MV receptor on human cells, CD46 (7, 30), and an associated molecule, moesin (8, 9, 37), have been identified. The normal function of CD46 is to prevent the deposition of the complement components C3b and C4b on host cells and subsequent lysis of cells (20, 21). It has been demonstrated that glycosylated CD46 directly binds MV hemagglutinin (MV-H) (24) with its first and second short consensus repeat (SCR) domains, a site distinct from the complement C3 binding site (25, 26). The infection of target cells with certain MV strains causes the downregulation of CD46 from the cell surface (31, 36, 39), which renders these cells susceptible to complement lysis, as demonstrated in tissue culture experiments (41). The observed downregulation of CD46 after infection of cells is MV strain dependent (36, 39). Of 34 MV strains investigated so far, 19 led to the downregulation of CD46 and 15 did not. Strains modulating CD46 were all vaccine strains as well as a number of wild-type isolates (36).

Although the proportion of peripheral blood mononuclear cells (PBMC) which are found to be infected during acute measles is low (40), MV infection causes marked lymphopenia and long-lasting suppression of certain immunological functions of the host's remaining lymphocytes (3, 4, 27). Little is known about the mechanisms leading to the elimination of lymphocytes and the immunosuppression. Recently, apoptosis has been described as a cause of death of MV-infected cells (11). Furthermore, MV-infected and irradiated PBMC, which produce small amounts of infectious MV but express MV-H and fusion protein on their surface, cause a significant inhibition of T cell proliferation when added to fresh autologous PBMC (35, 51). Anti-MV antibodies and a peptide blocking MV-mediated fusion can reverse the inhibition of the T-cell proliferation in vitro (22, 51). These data suggest that cell surface contact with MV-infected cells might impair the function of uninfected lymphocytes. Since MV-H-expressing infected cells interact with uninfected cells via the major MV receptor CD46, this interaction might induce the observed biological effects in the target cells.

Therefore, we investigated early events of cell surface changes of uninfected cells following contact with MV particles or MV-infected cells. We found that modulation of CD46 is induced on uninfected cells shortly after contact with MV-H on viral particles or infected cells but not after treatment with supernatant containing small amounts of virus from persistently infected cells. By this contact-mediated downregulation of CD46, a single infected cell can rapidly induce CD46 modulation on many uninfected cells and render these cells susceptible to complement-mediated lysis. Thus, a greater proportion of cells than are infected might be eliminated by complement-mediated lysis.

MATERIALS AND METHODS

Propagation of cells. The human cell lines U937 (promyelocytic-monocytic), BJAB (B-cell type), Molt4 (T-cell type), U251 (astrocytoma), IMR-32 (neuroblastoma), HeLa cells, and Vero (African green monkey kidney) cells were cultured in minimal essential medium (MEM) containing 10% fetal calf serum

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(FCS) and additives as described before (38). Cell lines U937-p, BJAB-p, Molt4-p, U251-p, and IMR-32-p, persistently infected with MV Edmonston (MV-Edm), and cell line BJAB-pWTF, persistently infected with MV-WTF, were established in our laboratory (38, 42). More than 99% of the cells in such cultures are infected. The persistently infected cells express high amounts of MV-H and MV fusion protein (MV-F) on their surfaces and produce approximately 10^2 PFU of infectious MV per ml.

Primary human PBMC were isolated from the blood of healthy donors by Ficoll-Hypaque (Pharmacia) gradient centrifugation as described before (41). Macrophages were separated by adherence to a plastic dish during a 1-h incubation at 37°C. Nonadherent cells are referred to as peripheral blood lymphocytes (PBL). PBL were infected with MV-Edm or MV-WTF at a multiplicity of infection (MOI) of 0.1 for 1 h at 37°C, washed, and cultured in RPMI medium containing 10% FCS and 2.5 μg of phytohemagglutinin (Sigma) per ml.

MV strains. Experiments were carried out with the following MV strains: (i) vaccine strain MV-Edm, (ii) wild-type isolates which modulate CD46 after infection (Y22 and Halle), and (iii) wild-type isolates which do not modulate CD46 after infection of target cells (WTF, R118, and EBT). The biological properties of these MV strains and sequence relationships have been described (34, 36, 39).

The viruses were propagated as described before (39). Briefly, most of the MV strains were grown on Vero cells in MEM containing 5% FCS, and the lymphotropic strains were grown on human BJAB cells in the same medium. Cells were infected at an MOI of 0.01 at 37°C and incubated at 33°C for 3 to 5 days, depending on the optimal titer of infectious MV produced. MV was harvested by partially removing the medium, one cycle of freeze/thawing, and centrifugation at 200 × *g* for 10 min to remove cell debris and stored at −70°C.

Antibodies. The mouse monoclonal antibody (MAb) 13/42, recognizing the first SCR of CD46; the anti-MV-H MAb L77; the anti-MV-F protein MAb A504; the anti-MV nucleocapsid (MV-N) MAb F227; the anti-major histocompatibility complex (MHC) class I MAb W6/32; and polyclonal rabbit anti-MV serum were produced in our laboratory (37). The MAbs were purified over protein G affinity columns. The MAb GB24, recognizing the fourth SCR of CD46, was a gift of G. Yeh, CytoMed Inc. Polyclonal anti-CD46 antibodies were a gift from F. Wild, Lyon, France. Secondary goat anti-mouse and swine anti-rabbit antibodies conjugated to fluorescein isothiocyanate for the flow cytometric analyses were obtained from Dako.

Expression of recombinant MV-H and MV-F by vaccinia virus. Vaccinia virus recombinants encoding MV-H or MV-F proteins (VV-H and VV-F, respectively) were a kind gift from T. F. Wild, Lyon, France (49, 50). HeLa cells were seeded into six-well plates and infected with VV-H or VV-F at an MOI of 0.1 for 24 h at 37°C. Infected cells were then detached from the plastic dish by pipetting and pipetted into cultures of uninfected HeLa cells at a ratio of 1:1. After 2 h at 37°C, cells were detached by treatment with calcium- and magnesium-free phosphate-buffered saline (PBS) and fixed with 3.7% paraformaldehyde in PBS prior to analysis by flow cytometry.

Flow cytometry. Flow cytometric analyses were performed as described before (38). Briefly, 2×10^5 cells were fixed for 10 min with 3.7% paraformaldehyde containing PBS and then incubated for 45 min on ice with 2 μg of MAb in 200 μl of FACS buffer (PBS containing 0.4% bovine serum albumin and 0.02% sodium azide). Cells were washed twice in FACS buffer and incubated with 200 μl of a 1:100 dilution of fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Dako) on ice for a further 45 min. After three washes with FACS buffer, flow cytometric analysis was performed on a FACScan (Becton Dickinson).

Complement cytotoxicity assay. For labeling, target cells were incubated with ^{51}Cr (100 μCi/10^6 cells) for 1 h at 37°C, washed, and placed in 96-well plates at a density of 10^5/50 μl per well as described before (41). Unlabeled U937-p cells (50 μl per well) were added in different concentrations, and cells were incubated for 4 h at 37°C. Then, 100 μl of human serum without antibodies against MV was added per well and incubated at 37°C for 3 h. From each well, 100 μl of supernatant was harvested, and the radioactivity was measured in a gamma counter. The specific ^{51}Cr release was calculated as described before (41).

Inhibition of pH-dependent endocytosis. Uninfected and infected U937 cells were treated with NH_4Cl (10 mM; Sigma), monensin (10 μM; Calbiochem), nigericin (1 μM; Calbiochem), or chloroquin (100 mM; Calbiochem) for 30 min at 37°C and then mixed for detection of contact-mediated downregulation of CD46 for 4 h at 37°C.

RESULTS

Infection-mediated downregulation of CD46 is biphasic. Investigating the kinetics of CD46 modulation, we found a clear dependency of the velocity of the downregulation on the amount of input virus. With U937 cells, 70% downregulation of CD46 was achieved with an MOI of 0.1 after 48 h, with an MOI of 1.0 after 24 h, and with an MOI of 5.0 after 12 h (Fig. 1). It is striking that after just 1 and 2 h of infection, considerable CD46 modulation is detected. With lower MOIs, the curve of CD46 expression is clearly biphasic, leading to an initial CD46 modulation after 1 and 2 h of infection, when no newly synthesized MV proteins are present, and after 24 to 48 h, when newly synthesized MV-H is present on the surface of the infected cells. The initial downregulation of CD46 is most likely due to the direct interaction of the cellular receptors with the envelope proteins of the input virus. Therefore, we hypothesized that the direct interaction of an uninfected cell with a persistently infected cell expressing large amounts of the viral glycoproteins on the surface may lead to a very rapid modulation of CD46.

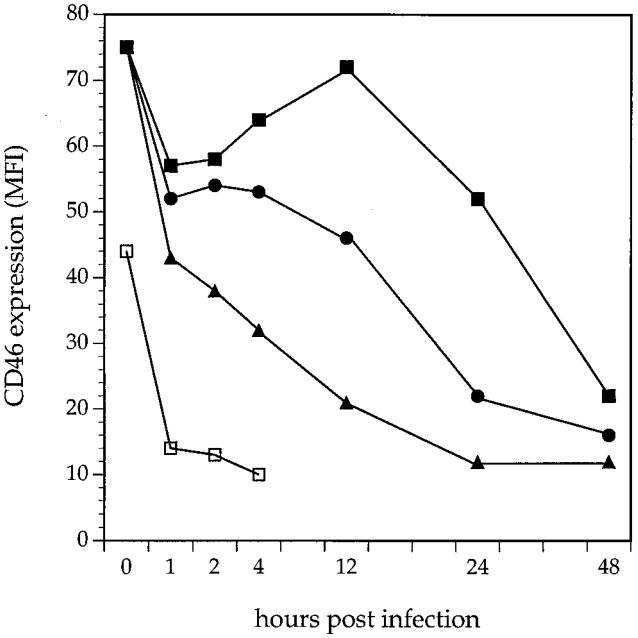

FIG. 1. Kinetics of downregulation of CD46 from the surface of U937 cells after infection with MV-Edm and after contact with persistently infected U937-p cells. U937 cells were infected with MV-Edm at MOIs of 0.1 (■), 1.0 (●), and 5.0 (▲) or mixed with U937-p cells at a ratio of 1:1 (□) and incubated at 37°C for various times. Cells were fixed, and the expression of CD46 on the cell surface (mean fluorescence intensity [MFI]) was determined by flow cytometry with MAb 13/42.

Cell surface contact of MV-infected cells with uninfected cells leads to downregulation of CD46. In order to study the direct effect of cells expressing MV envelope proteins on uninfected cells, we cocultivated persistently MV-Edm infected U937-p cells, which express high levels of MV-H and MV-F protein and very little CD46 on their surface, with uninfected U937 cells (8, 41). To study the kinetics of CD46 modulation, uninfected and infected cells were mixed at a ratio of 1:1, incubated at 37°C for various times, fixed, and analyzed for surface expression of CD46 by flow cytometry (Fig. 2). As controls, the infected and uninfected cells were first fixed separately and then mixed, and the expression of CD46 was determined (Fig. 2C). After just 1 h of cocultivation of cells at 37°C, a drastic reduction in surface CD46 expression on uninfected cells was observed (Fig. 2F), which was complete after 4 h of cocultivation (Fig. 2H and Fig. 1, open symbols). The steepest decrease in CD46 occurred between 0.5 and 1 h after contact.

As further control, cells were stained with antibodies against MV-N and secondary antibodies to determine the ratio of uninfected to infected cells in the cultures. The expression of MHC class I molecules and other surface molecules such as CD55 was not altered during infection-mediated (39, 41) and contact-mediated CD46 modulation (not shown).

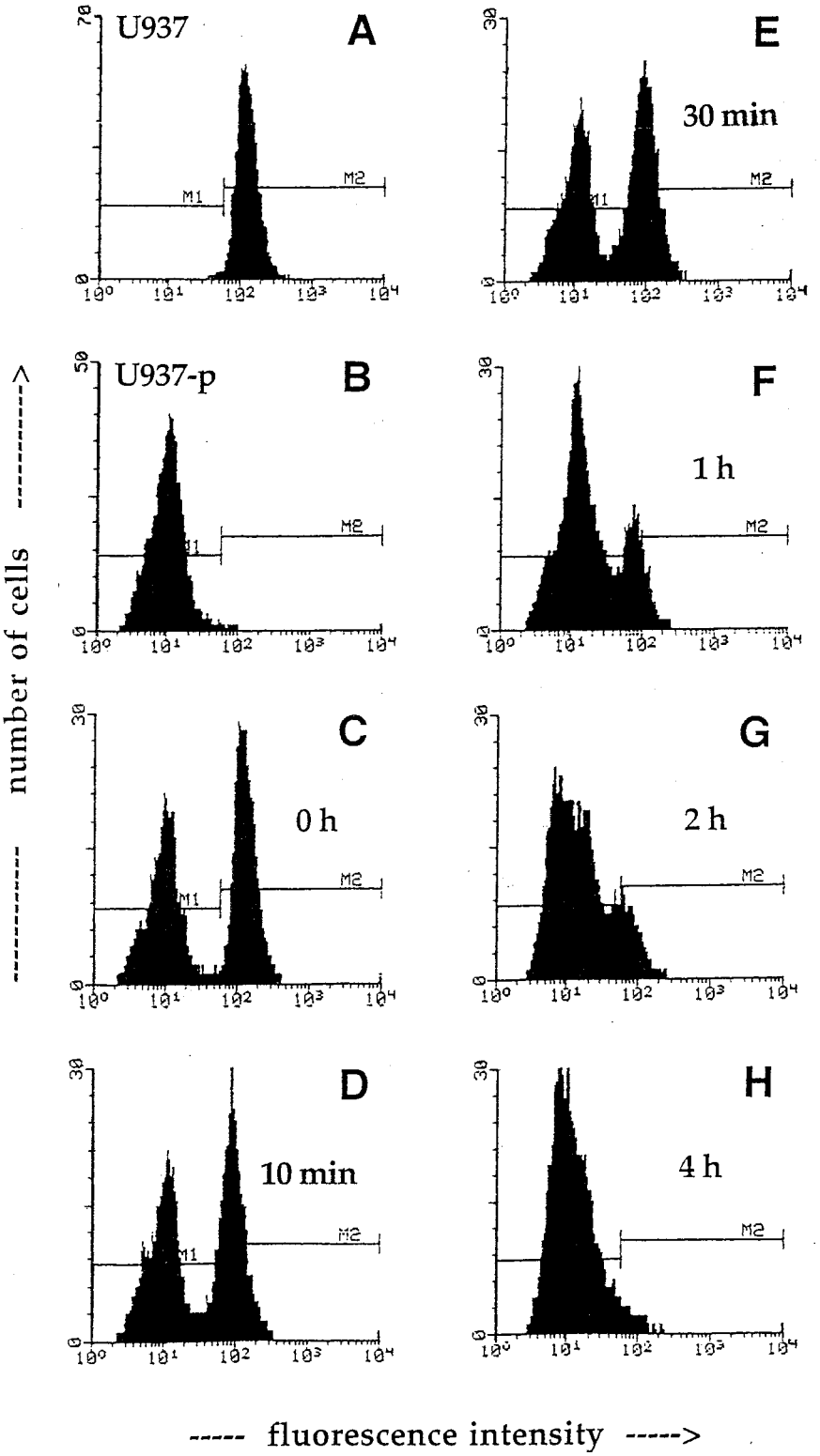

FIG. 2. Flow cytometric analysis of the downregulation of CD46 from the cell surface of uninfected U937 cells after contact with persistently MV-Edm infected U937-p cells. (A) Uninfected U937 cells express CD46 on their surfaces (99% CD46-positive cells in marker region M2); (B) persistently infected U937-p cells express little CD46 on their surfaces (98% CD46-negative cells in marker region M1). Cells were mixed at a ratio of 1:1 and incubated at 37°C for 0 h (C), 10 min (D), 30 min (E), 1 h (F), 2 h (G), or 4 h (H) prior to processing for CD46 surface staining. After 4 h, the signal of the CD46-positive uninfected cells disappeared completely.

TABLE 1. Modulation of CD46 expression from the surface of various uninfected cell lines after contact with infected cells

Cell lines[a] (cell type)	Mean CD46 fluorescence intensity[b]		% Reduction in CD46 fluorescence by contact
	0 h	2 h	
U937 + U937-pEdm (monocyte)	66.93	35.76	47
BJAB + BJAB-pEdm (B cell)	65.27	26.18	60
Molt4 + Molt4-pEdm (T cell)	58.46	40.65	28
U251 + U251-pEdm (astrocyte)	145.63	91.28	37
IMR32 + IMR32-pEdm (neuronal)	86.19	41.66	52
BJAB + BJAB-pWTF (B cell)	73.00	76.26	−4

[a] Uninfected and persistently MV-Edm or MV-WTF infected cell lines were mixed in a ratio of 1:1 for 2 h of contact at 37°C or fixed before mixing on ice as a control (0 h of contact).

[b] The mean fluorescence intensity of CD46 on the surface of cells at 0 and 2 h of contact was determined by flow cytometry with MAb 13/42.

Since incubation of infected with uninfected cells leads to the infection of cells after longer incubation times and then the contact-mediated modulation of CD46 cannot be separated from that following infection with intracellular synthesis of viral proteins, all contact experiments in this study were carried out within 2 to 4 h. Since input MV as well as MV envelope protein-expressing cells led to the rapid downregulation of CD46, this phenomenon is most likely mediated by direct surface contact. This interpretation is supported by the finding that the extent of CD46 modulation after cocultivation of cells depends on the cell density in the culture: with 10^4 cells per ml, there was no detectable effect on CD46 expression (not shown), but with 4×10^5 cells per ml, the full effect was measurable after 2 to 4 h (Fig. 2). The supernatant of persistently infected U937-p cells, which contained approximately 10^2 PFU of infectious MV per ml, had no effect on CD46 expression within this short time.

The phenomenon of contact-mediated CD46 modulation was observed by flow cytometry with different antibodies against CD46, including those recognizing the first SCR domain of CD46, such as MAb 13/42; the fourth SCR domain, such as MAb GB24; and polyclonal antibodies (see below). Furthermore, contact-mediated CD46 modulation was observed with different homologous pairs of persistently MV-Edm infected cell lines and uninfected cells of monocytic (U937), lymphocytic (BJAB and Molt4), and other origins (HeLa, U251, and IMR-32) (Table 1), as well as with heterologous pairs of these cell lines (not shown). Similar results were obtained with primarily infected cell lines expressing MV-H on their surfaces (see below).

Inhibition of contact-mediated downregulation of CD46 by antibodies against MV-H and CD46. The specificity of the cell contact-mediated CD46 modulation was investigated with antibodies against MV-H, CD46, MV-F, and MHC class I (Fig. 3 and Table 2). The cells were treated for 30 min with antibodies prior to mixing of uninfected and infected cells and incubation for 2 h at 37°C. When uninfected U937 cells were treated with

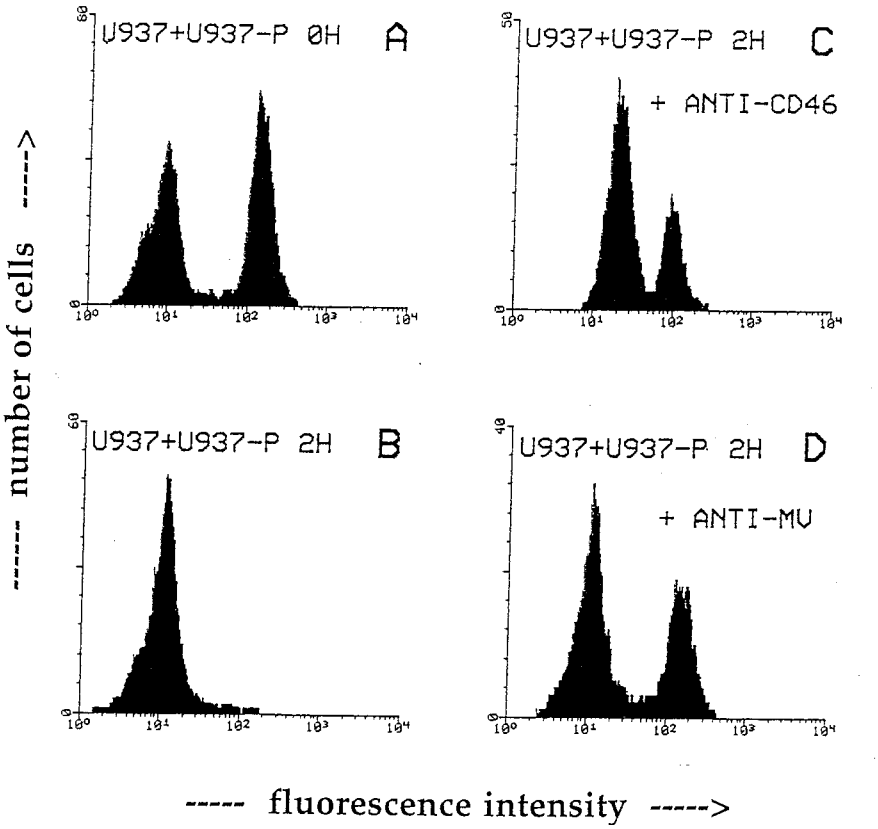

FIG. 3. Inhibition of CD46 modulation with antibodies against CD46 and MV-H. (A) U937 and U937-p cells were mixed at a ratio of 1:1 and fixed and (B) incubated without antibodies for 2 h as a control. (C) U937 cells were treated with MAb 13/42 against CD46 and then mixed with U937-p cells in the presence of the same antibody for 2 h. (D) U937-p cells were treated with polyclonal anti-MV antiserum for 30 min and then mixed with uninfected U937 cells and incubated in the presence of the antiserum for 2 h. The flow cytometric analysis was done with MAb 13/42 against CD46.

TABLE 2. Inhibition of CD46 modulation by antibodies against CD46 and MV-H[a]

Cell line pairs (x + y)	Antibody	Mean CD46 fluorescence intensity[b]	CD46 expression (% of control)	% Modulation of CD46[c]
U937 (fixed) + U937-p (fixed) (control)	None	66.93	100	0
U937 + U937-p	None	35.76	53	100
U937 + U937-p	α-CD46	64.73	97	7
U937 + U937-p	α-MV-F	34.62	51	104
U937 + U937-p	α-MHC	40.21	60	86
U937-p + U937	α-MV-H	66.28	99	2
U937-p + U937	α-MV	65.47	98	5
U937-p + U937	α-MV-F	30.14	45	118
U937-p + U937	α-MHC	41.17	62	83

[a] The antibodies used for pretreatment were MAb 13/42, recognizing CD46; A504, recognizing MV-F; MAb W6/32, recognizing MHC class I; MAb L77, recognizing MV-H; and rabbit polyclonal anti-MV antibodies. The first cell line (x) of each pair was treated with antibodies for 30 min at room temperature before uninfected and infected cells were mixed and incubated for 2 h at 37°C.

[b] The mean fluorescence intensity of CD46 expression was determined by flow cytometry with MAb 13/42 or rabbit polyclonal anti-CD46.

[c] The percent modulation of CD46 for cocultivation of cell line pairs x and y was calculated as: % modulation = 100 − [(mfi of [x + y] − mfi of [U937 + U937-p])/(mfi of [U937 (fixed) + U937-p (fixed)] − mfi of [U937 + U937-p])] × 100. mfi, mean fluorescence intensity.

antibodies against CD46 or persistently infected U937-p cells were treated with antibodies against MV-H or polyclonal antibodies to MV, the contact-mediated downregulation of CD46 was considerably inhibited, by 93, 98, and 95%, i.e., reduced to 7, 2, and 5%, respectively (Table 2). In contrast, antibodies against MV-F did not reduce and antibodies to MHC class I reduced the modulation slightly, by only 14 and 17%, respectively. These results suggest that the direct interaction of MV-H on the surface of infected cells with CD46 on the surface of uninfected cells leads to CD46 modulation on uninfected cells.

CD46 downregulation after contact with cells expressing recombinant MV-H. To exclude any influence of infectious MV or MV-induced surface proteins on the persistently infected cells, we studied the effect of recombinant MV-H and MV-F proteins expressed by vaccinia virus (VV-H and VV-F). HeLa cells were infected with VV-H or VV-F for 24 h, then mixed with uninfected HeLa cells (1:1), cocultivated for 2 h at 37°C, and fixed with 3.7% formaldehyde, and the expression of CD46 was determined by flow cytometry. CD46 was modulated from the surface of uninfected cells after contact with MV-H-expressing cells by approximately 65% (Fig. 4, lane 4) and slightly modulated after contact with MV-F-expressing cells (Fig. 4, lane 3).

As controls, the infected and uninfected cells were first fixed separately and then mixed, and the expression of CD46 was determined by flow cytometry (Fig. 4, lanes 1 and 2). As additional controls, the modulation of CD46 on the VV-H- and VV-F-infected cells and uninfected cells is compared in Fig. 4, lanes 5 to 7. This experiment shows that 2 h of contact with recombinant MV-H-expressing cells is sufficient to downregulate CD46 from the surface of uninfected cells.

MV strain differences in contact-mediated downregulation. The previous finding that CD46 downregulation after infection of target cells is MV strain dependent (39) led us to study this aspect in contact-mediated CD46 modulation. We used a selection of MV vaccine strains (group I), wild-type strains which lead to the downregulation of CD46 after infection of cells (group II), and wild-type strains lacking the capacity to downregulate CD46 (group III) (39). Experiments were done with MV-Edm (group I), Y22 and Halle (group II), and MV-WTF, R118, and EBT (group III). For modulation experiments, human BJAB cells were infected for 48 h and then mixed with uninfected cells for 4 h. The expression of MV-H on the surface of infected cells was controlled by flow cytometry. With all virus strains tested, results similar to those obtained following infection of cells were obtained for contact-mediated downregulation of CD46. MV-Edm, Y22, and Halle led to contact-mediated downregulation of CD46, whereas WTF, R118, and EBT did not. We also used BJAB cells persistently infected with MV-WTF, which express very high levels of MV-H on their surfaces. Contact of such cells with uninfected BJAB cells did not lead to the downregulation of CD46 (Table 1), indicating that this phenomenon cannot be influenced by increasing the amount of MV-H protein expressed on infected cells.

In addition, we investigated contact-mediated CD46 modulation on primary PBL with strains MV-Edm and MV-WTF (Fig. 5). As controls, the expression of CD46 after infection of PBL with these strains for 48 h is shown. As shown in Fig. 5A and D, infection with MV-Edm leads to CD46 downregulation, whereas infection with MV-WTF does not. Similar results were obtained after contact of infected PBL with uninfected PBL (1:1); only contact with MV-Edm-infected cells led to CD46 modulation (Fig. 5C), whereas contact with MV-WTF-infected cells did not (Fig. 5F). Thus, the H protein of the different MV

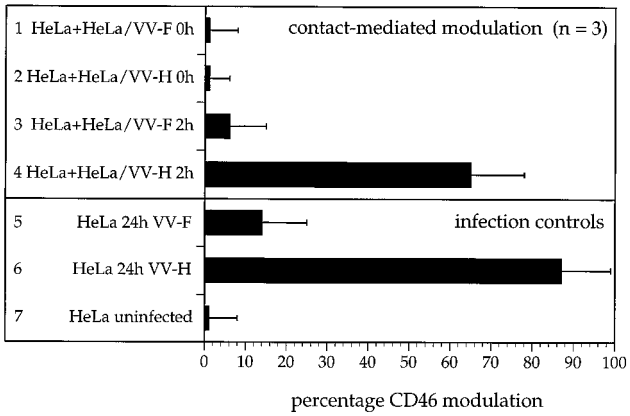

FIG. 4. Analysis of the capacity of vaccinia virus recombinants expressing MV-H and MV-F to downregulate CD46 after contact with uninfected cells. HeLa cells were infected with VV-H or VV-F for 24 h and mixed 1:1 with uninfected HeLa cells for 0 h (lanes 1 and 2) and 2 h (lanes 3 and 4). To calculate the percent specific CD46 modulation, the mean value of the fluorescence intensity of 0-h mixed cells was taken as 0% modulation. The contact of VV-H-infected with uninfected cells led to approximately 62% modulation of CD46 in the culture (lane 4). Further controls are the 24-h VV-F-infected cells (lane 5), 24-h VV-H-infected cells (lane 6), and uninfected HeLa cells. For these three lanes, the mean values of the fluorescence intensity of CD46 on uninfected cells were taken as 0% modulation. VV-H infection led to approximately 88% downregulation of CD46 (lane 6).

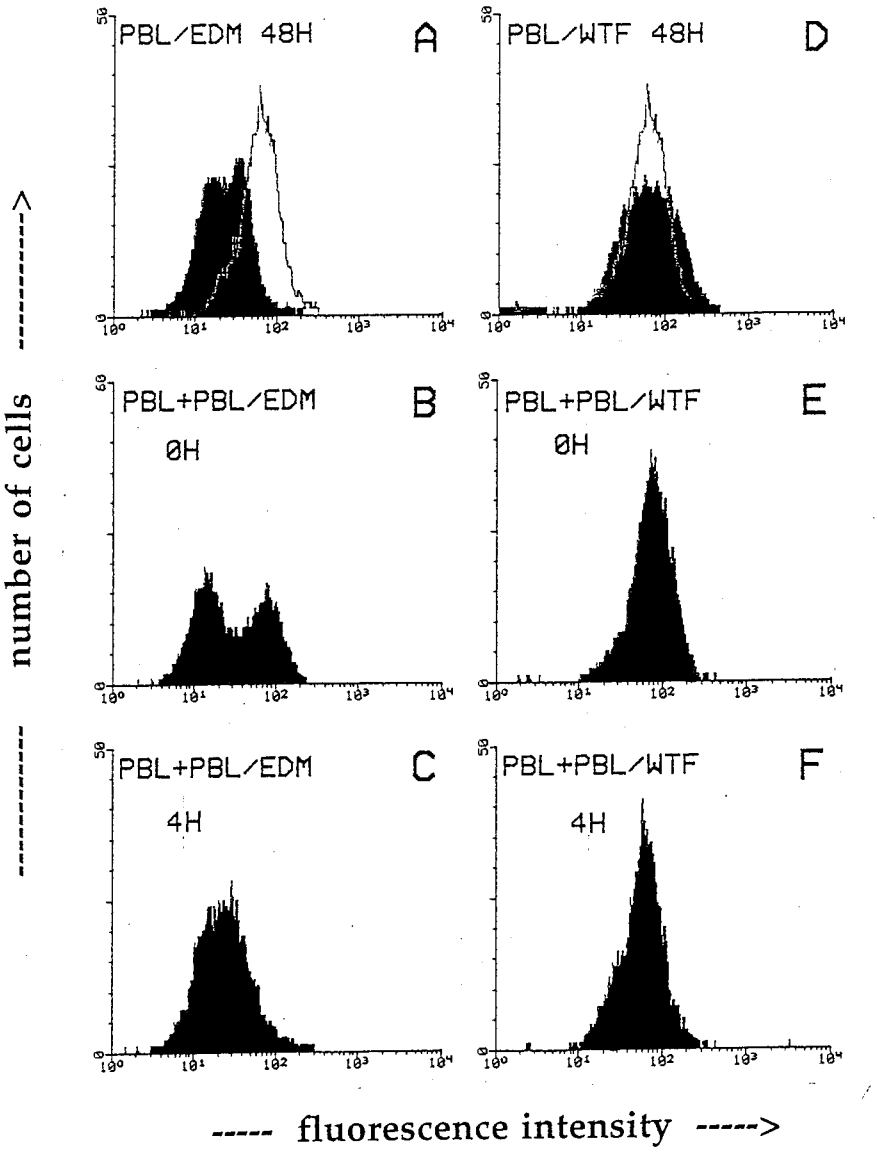

FIG. 5. Analysis of contact-mediated CD46 downregulation on uninfected PBL by MV-Edm- and MV-WTF-infected PBL. (A) The fluorescence intensity of CD46 on uninfected PBL (open curve) is shown in comparison to 48-h MV-Edm-infected PBL (solid curve) as a control. (B and C) Uninfected PBL were mixed with MV-Edm-infected cells for 0 h (B) and 4 h (C). The contact led to a downregulation of CD46 from the surface of uninfected PBL. (D) In contrast, infection with MV-WTF does not lead to a reduction in fluorescence intensity (compare open and solid curves). (E and F) Also after contact of infected with uninfected PBL for 0 and 4 h, no downregulation of CD46 was observed.

strains determines the downregulation of CD46 regardless of whether this results from infection of cells or cell-cell contact.

A single infected cell affects many uninfected cells. In the experiments described so far, we mixed infected and uninfected cells at a ratio of 1:1. Since in acute measles only a small proportion of PBL are infected but a marked lymphopenia is observed (3), we determined how many uninfected cells can be induced to downregulate CD46 by a single infected cell. To titrate the effect, we used U937 and persistently MV-Edm infected U937-p cells at ratios of 1:1 to 256:1. Two series of cell mixtures with ratios of 1:1 to 256:1 were set up, one being cocultured at 37°C for 4 h and one being fixed first and then mixed as the 0-h contact control. Specific CD46 modulation was determined by subtracting the fluorescence intensities of 4-h-incubated cocultures from those of control cells (0-h contact) for each cell ratio and expressing the data as percent CD46 modulation (Fig. 6). As further controls, uninfected cells with 0% modulation and persistently infected cells with approximately 75% modulation are shown. Significant contact-mediated CD46 modulation could be detected by flow cytometry at ratios of up to 64 uninfected to 1 infected cell (Fig. 6). This result clearly demonstrates that many uninfected cells can be affected within a short time by interactions with MV-infected cells.

Complement-mediated lysis of uninfected cells after contact with MV-infected cells. Recently, we have shown that the downregulation of CD46 from the cell surface has dramatic consequences for the viability of cells in the presence of complement (41). Since the modulation of CD46 is complete after 4 h of contact of uninfected with infected cells, we determined the susceptibility to complement-mediated lysis of uninfected cells after short contact with infected cells. Uninfected U937

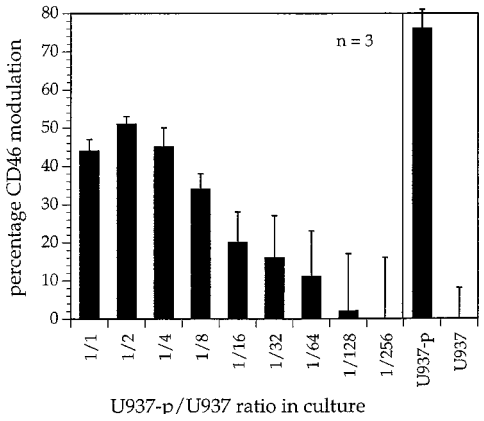

FIG. 6. Titration of the cell ratio leading to contact-mediated CD46 modulation. Persistently MV-Edm infected U937-p cells were mixed with uninfected U937 cells at ratios of 1:1 to 1:256 and incubated for 0 h (control series) and 4 h at 37°C. The mean fluorescence intensities of the 4-h-incubated cultures were subtracted from those of the 0-h-incubated control cultures for each cell ratio. These differences reflect specific contact-mediated downregulation of CD46. The signal of uninfected cells was taken as 100% CD46 expression, or 0% modulation. The data are presented as percent modulation of CD46 dependent on the cell ratios. Maximal modulation was found with a cell ratio of 1:2 (infected to uninfected).

cells were labeled with ^{51}Cr and cocultured with unlabeled persistently infected U937-p cells for 4 h; subsequently, human serum that did not contain antibodies against MV was added as a source of complement for 3 h. Up to 20% of contacted uninfected cells are specifically lysed, as shown in Fig. 7. Note that the complement added to these cultures is lysing both unlabeled infected (50% of the cells) and labeled uninfected cells which modulated CD46, but only the ^{51}Cr release of the labeled uninfected cells is measured. Therefore, 10 to 20% specific lysis of uninfected cells is a high and significant effect, indicating that contact-mediated CD46 modulation can lead to complement-mediated lysis of uninfected cells.

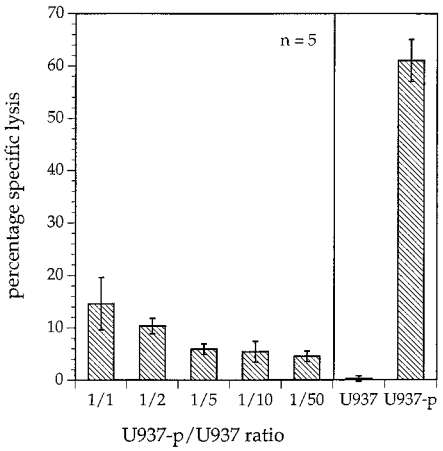

FIG. 7. Complement-mediated lysis of cells after contact with infected cells. Uninfected U937 cells were labeled with ^{51}Cr and cocultivated with persistently MV-Edm infected U937-p cells for 4 h prior to addition of human serum that did not contain antibodies against MV for 3 h. The U937-p/U937 cell ratio was varied between 1:1 and 1:50 as indicated. At most, 20% of the labeled uninfected U937 cells were lysed by complement after contact with infected cells (1:1). As controls, complement-dependent lysis within 3 h for ^{51}Cr-labeled U937 cells alone (no specific lysis) and labeled U937-p cells alone (approximately 60% specific lysis) was determined.

Slight fixation of cells and inhibitors of pH-dependent endocytosis do not inhibit CD46 modulation. As a first step towards investigation of the mechanism of CD46 downregulation, we tested the effect of slight fixation and of inhibitors of endocytosis on contact-mediated downregulation. When uninfected or infected cells were slightly fixed for 5 min with 3.7% paraformaldehyde (either one population of cells or both partners) and then mixed and incubated for 4 h at 37°C, the downregulation of CD46 from the surface of uninfected cells was not inhibited. With inhibitors of pH-dependent endocytosis such as NH_4Cl, monensin, nigericin, and chloroquin used at concentrations which inhibit the uptake of vesicular stomatitis virus by U937 cells, contact-mediated downregulation of CD46 by MV-H was not inhibited. Therefore, further experiments are required to investigate the mechanism of contact-mediated CD46 modulation.

DISCUSSION

The observed downregulation of CD46 from the cell surface is not the result of a masking of the recognition sites of anti-CD46 antibodies by MV-H, since different anti-CD46 MAbs recognizing various domains of CD46 as well as polyclonal antibodies to CD46, which bind to sites distinct from the sites with which MV-H interacts, led to similar results. Several findings suggest that the direct protein-protein contact of MV-H with CD46 is necessary for receptor modulation: the effect is dependent on the amount of input virus or on the cell density in the culture, and recombinant MV-H is sufficient to induce and specific antibodies against MV-H and CD46 inhibit CD46 modulation. This contact-mediated CD46 modulation is quite effective, since under our experimental conditions, a single infected cell could lead to downregulation of CD46 on up to 64 uninfected cells.

The contact of an MV-infected cell with uninfected cells may lead to cell fusion and the formation of giant cells, a process which could also be accompanied by CD46 modulation. However, for several reasons, it seems unlikely that complete cell-cell fusion occurred under the experimental conditions that we were using: (i) a single infected cell affected many uninfected cells, (ii) in the flow cytometric analyses, an alteration of the size and granularity of the cell populations was not observed, and (iii) cell numbers were stable during the short incubation times. Therefore, there is clearly one mechanism by which CD46 modulation can be induced from without, mediated via surface interaction of MV-H on the infected cell with CD46 on uninfected cells. Few data are available on the fate of the CD46 molecule after downregulation. Results obtained by immunofluorescence assays suggest that CD46 is taken up by the infected cell (31), and flow cytometric data suggest that the total amount of CD46 per cell stays constant after downregulation (37). Since inhibitors of the pH-dependent endocytic pathway did not inhibit CD46 modulation, the mechanism of CD46 downregulation is still unclear. Further investigation will show whether the downregulation of CD46 mediated via newly synthesized MV-H from within also requires the surface expression of MV-H and interaction with CD46 at the cell surface or is caused by a different mechanism.

The phenomenon of receptor modulation after infection of target cells has also been observed with other viruses, such as human immunodeficiency virus (HIV) (6, 15, 19) and human herpesvirus 7 (23). Infection with both of these viruses leads to downregulation of CD4. HIV receptor modulation is supposed to facilitate the release of infectious viral particles from the cell surface and to prevent the superinfection of cells (43). This CD4 modulation seems to be an important factor supporting

the survival of HIV, since during evolution this virus has developed three gene products associated with the downregulation of the receptor: the viral envelope protein gp160 retracts CD4 in the endoplasmic reticulum of the infected cell by blocking its movement and maturation (17, 43); the viral Nef protein induces the internalization of CD4, resulting in its degradation in lysosomes (1, 14); and the Vpu protein induces the degradation of CD4 in the endoplasmic reticulum (33, 47). In the case of MV, not all hemagglutinins of MV strains developed receptor-modulating capacity, which might mean that this property is not necessary for the survival of the virus.

The interaction of a downregulating MV-H with CD46 is sufficient to mediate the observed effects. In contrast to the mechanisms of action of Vpu and Nef, the cytoplasmic and transmembrane domains of CD46 are not required for downregulation from the cell surface (12, 46). For MV, it is not known whether CD46 is retained by MV-H in the endoplasmic reticulum and subsequently degraded. Whereas a retention mechanism could well lead to the downregulation of CD46 from within after infection of cells with MV, contact-mediated downregulation of CD46 requires the induction of a different mechanism. It resembles the antibody-induced downregulation of CD4 from the surface of T lymphocytes (5). Although the modulation of CD4 can be induced by phorbol ester, the induction of protein kinase C is not required for the MAb-induced modulation of CD4 and CD3 (32, 45). In the case of the interaction of MV-H with CD46 leading to contact-mediated modulation of CD46, it remains to be investigated whether a signal transduction pathway is involved.

The downregulation of CD46 from the cell surface of uninfected cells is accompanied by an effective lysis of the cell by the complement system (41). If this occurs in vivo, it is not advantageous for the survival of the virus but rather a factor in the attenuation of the MV infection, since it limits the spread of virus. This interpretation is supported by animal experiments. Albrecht and coworkers (2) have shown in experimental infections of marmosets that the MV-Edm and MV strain Moraten, which both downregulate CD46 in our hands, are less virulent than MV strain JM, which does not lead to the downregulation of CD46 (36). In this study, infection with the vaccine strain Moraten did not kill the animals, whereas wild-type isolate JM caused death in four of four animals. Antigenic differences between vaccine strains and wild-type MV strains such as JM which reflect sequence differences between these viruses have been detected (44). It is interesting that CD46 is used as a receptor by all MV strains tested so far, but downregulation of CD46, although based on the interaction of the same molecules, is different (39). These results suggest that two sites of interaction between CD46 and MV-H might exist, influencing separately binding and downregulation. Since a single MV-infected cell can cause CD46 modulation on the surface of many uninfected cells, this mechanism, in addition to others such as apoptosis (11), could well influence the pathogenicity in patients with acute measles. The cause-and-effect relationship between the capacity of a particular H protein to cause receptor downregulation and the virulence of an MV strain awaits further investigation.

ACKNOWLEDGMENTS

We thank S. Löffler and K. Pech for technical assistance.

We thank the Deutsche Forschungsgemeinschaft, the Bundesministerium für Forschung und Technologie, the Doktor Robert Pfleger-Stiftung, Bamberg, and the World Health Organization, Geneva, for financial support.

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