Biochemical and Biophysical Research Communications **264,** 268–275 (1999)
Article ID bbrc.1999.1517, available online at http://www.idealibrary.com on IDEAL®

Interaction of Measles Virus (Hallé Strain) with CD46: Evidence That a Common Binding Site on CD46 Facilitates Both CD46 Downregulation and MV Infection

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Received September 7, 1999

CD46 acts as a cellular receptor for vaccine strains of measles virus (MV). The MV/CD46 interaction—mediated by the MV attachment glycoprotein, the hemagglutinin (H)—not only facilitates infection but also induces CD46 downregulation. A conflict of opinion exists as to whether a single MVH binding site on CD46, or two separate sites, facilitates the two phenomena. To investigate this conundrum we first tested and compared a panel of CD46-specific monoclonal antibodies (mAbs) for their capacity to block both processes. One (mAb 13/42) abrogated both MV fusion and CD46 downregulation. Mutation of an amino acid (arg59 in the SCR1 of CD46) essential for the epitope of mAb 13/42 resulted in the abrogation of both CD46 downregulation and viral fusion. This strongly suggests that the same MV binding site on CD46 is responsible for both CD46 downregulation and MV infection.

CD46 or membrane cofactor protein (MCP) was recently shown (7, 20) to serve as a cellular receptor for Hallé and Edmonston, two vaccine strains of measles virus (MV), an enveloped negative-sense RNA virus that is a member of the *Morbillivirus* genus of the *Paramyxoviridae* family. A member of the regulators of complement activation (RCA) protein family, CD46 is ubiquitously present on primate cells, protecting them from complement lysis by binding complement cascade components C3b and C4b and by acting as a cofactor for their proteolytic inactivation by serine protease complement factor I. Members of the structurally-related RCA protein family are type-I membrane proteins which possess a repeated motif of some 60 amino acids called a "short consensus repeat" (SCR) in the extracellular domain. CD46 contains four contiguous SCRs but the number varies between different RCA family members (16).

MV contains two glycoproteins in the virion envelope: the hemagglutinin (H) and the fusion protein (F). The primary function of the MVH is attachment to the cellular receptor whereas MVF mediates viral fusion (29). However, it has been shown that the presence of MVH is required for fusion to take place (26) and that the two glycoproteins are associated in the viral membrane via part of the cysteine-rich region on the MVF (28).

To identify CD46 as a cellular receptor of MV, Naniche *et al.* (19) generated a monoclonal antibody (MCI20.6) that blocked HeLa cell fusion induced by the Hallé strain of MV. The cellular membrane protein recognized by mAb MCI20.6—subsequently shown to be CD46 (20)—is downregulated from the HeLa cell surface upon infection by the Hallé strain. CD46 downregulation requires only the expression of MVH to occur (19).

Our previous results (15) demonstrated that the mutation of two amino acids in the Hallé H protein abrogated not only CD46 downregulation but also the hemadsorption of simian erythrocytes and viral fusion. All three phenomena implicate an interaction with CD46 but is the binding of MVH to CD46 that induces its downregulation equivalent to that which facilitates infection? Reports that certain wild-type strains of MV appear to use CD46 as receptor without the molecule being downregulated (2, 24) suggests that separate MVH binding sites on CD46 are responsible for CD46 downregulation and MV infection. Furthermore, it has recently been reported that CD46 is downregulated upon rinderpest virus (RPV) infection (8). As CD46 does not appear to act as a receptor for RPV this suggests that CD46 downregulation occurs independently of receptor usage.

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0006-291X/99 $30.00

To investigate whether CD46 downregulation and MV infection are the result of MVH binding to two separate sites on CD46, we first tested and compared a panel of CD46-specific mAbs for their capacity to block both phenomena. If separate sites on CD46 are involved, it could be expected that anti-CD46 mAbs would either have an effect on fusion or CD46 downregulation. If, on the other hand, a common binding site is the basis for both phenomena then it should be possible to find an anti-CD46 mAb capable of blocking both processes. We found that one of the mAbs (13/42) not only inhibited CD46 downregulation but also blocked fusion. mAb 13/42 also blocked the binding of both the MV virion and a soluble version of MVH to CD46. A preliminary mapping of the epitope of mAb 13/42 identified an arginine residue (arg59) in the SCR1 of CD46 as being crucial for the binding of this antibody to CD46. When this arginine was mutated to serine both viral fusion and CD46 downregulation were abrogated. This argues that the same MV binding site on CD46 is responsible for both infection and CD46 downregulation.

MATERIALS AND METHODS

Cells. RK13 (rabbit kidney) and BJAB/P3 (EBV-infected Burkitt lymphoma B cells) cells were grown in RPMI (Gibco BRL) supplemented with 10% fetal calf serum, 10 mM Hepes, 2 mM L-glutamine and 50 μg/ml of penicillin/streptomycin. HeLa, Ltk$^-$ (murine fibroblast, Ltk-MVH and Ltk-CD46 cells were grown in DMEM supplemented as for RPMI medium.

Virus stocks. Wild-type vaccinia virus (Copenhagen strain) and recombinant vaccinia virus encoding MV Hallé H (VV-H), Hallé H + Hallé F (VV-HF) or CD46 (VV-CD46) were propagated on Vero cells. Clarified supernatants were used as the virus stocks. MV Hallé propagated on Vero cells was purified by ammonium sulfate precipitation followed by ultracentrifugation on sucrose gradients.

Antibodies. E8/5 and E12/14 are in-house anti-CD46 mAbs which were selected from hybridomas—derived from BALB/c mice immunized with vv-CD46—by screening for supernatants positive in immunofluorescence with HeLa cells. Ascites from anti-CD46 hybridomas E8/5, E12/14, MCI20.6 (20), and 13/42 (22) were produced in BALB/c mice. E8/5, MCI20.6 and 13/42 ascites were purified by caprilic acid precipitation and conjugated to fluorescein isothiocyanate (FITC).

Concentrations of immunoglobulin (Ig) in ascites and in purified mAbs were determined by ELISA using IgG1 or IgG2a coating and with IgG1 and IgG2a standards (Sigma) as described previously (6). CD46-specificity was controlled on Ltk-CD46 and BJAB/P3 cells by ELISA using intact cells as previously described (5).

Titrations were performed by flow cytometry analysis after incubation of BJAB/P3 cells with serial dilutions of ascites at 4°C, followed by incubation with anti-mouse IgG-FITC. For competition assays, BJAB/P3 cells were incubated simultaneously with a saturating concentration of ascites (10 μg/ml) and FITC-conjugated E8/5, MCI20.6 or 13/42. Flow cytometry analysis was performed using a FACScan (Becton–Dickinson). mAb M177 which is specific for SCR2 of CD46 was a kind gift from T. Seya.

Construction of CD46 mutants. Site-directed mutagenesis was made on the CD46 cDNA (a kind gift from B. Rossi) for introducing both amino acid substitutions and endonuclease restriction sites as previously described (28). All mutants generated were sequenced by the method of Sanger *et al.* (21). Vaccinia recombinants expressing the various mutants were constructed as described previously (15). Mutant proteins were tested for expression by immunoprecipitation with the IgG2a E8/5 mAb and by FACScan analysis or immunofluorescence staining.

Inhibition of MVH/F-induced syncytia. Inhibition of cell–cell fusion was assayed on HeLa cells seeded in 96-well plates, infected with VV-H/F at 0.1 pfu/cell in medium containing 1/4 serial dilutions of antibody. Cells were fixed in formol the day after infection and colored with bromophenol blue. Syncytia number and size were assessed using an inverted light microscope.

Binding of sH (Hallé strain). ^{35}S-labeled sH was produced as previously described (17). Briefly, 10^6 RK13 cells, infected at 0.1 pfu/cell with VV-H, were radiolabeled, 6 h postinfection, with 10 μCi of ^{35}S-TRAN (ICN) in cysteine- and methionine-free medium. Supernatant was collected after an overnight incubation at 37°C, clarified, then either used immediately for binding studies or kept at −70°C. Binding was performed overnight on a confluent monolayer of 10^6 HeLa cells or on Ltk$^-$ cells infected with VV wt (0.1 PFU/cell) and transfected with pgpt-CD46 constructs 2 h later. In the case of Ltk$^-$ cells, sH-containing medium was added 30 h following transfection. Following two washes in cold PBS, cells were lysed in RIPA buffer and bound sH was monitored by immunoprecipitation of the cell lysate with anti-MVH mAb Cl.55 (9) followed by SDS–PAGE analysis under reducing conditions.

Inhibition of MV binding. HeLa cells (2×10^5) were incubated with 4 μl of anti-CD46 ascites on ice for 30 min, then 20 μl of purified MV (Hallé) was added and left on ice for a further hour. After three washes in FACS buffer (PBS/1% BSA/0.1% azide) the cells were analyzed in a FACScan analysis using biotinylated anti-MVH C155 mAb (9) followed by streptavidin–FITC.

Inhibition of MV infection. HeLa cells (2×10^5) were incubated simultaneously with 4 μl of anti-CD46 ascites and 0.1 pfu/cell of purified MV (Hallé) for 1 h at 37°C. After two washes in 5% SVF-DMEM, cells were cultured for 2 days and FACScan analysis was then performed as described for the MV binding assay.

CD46 downregulation assay. MVH-induced downregulation of CD46 was measured using two systems. In the first, BJAB/P3 cells were infected with VV-H or VV-wt as a control. In the second, we used a slightly modified version of the coculture system described by Krantic *et al.* (13). Briefly, 2×10^5 BJAB/P3 cells were first incubated with 4 μl of anti-CD46 ascites for 30 min at 20°C and then put onto 10^5 adherent Ltk-MVH or Ltk$^-$ cells. Immunofluorescence staining with GAM-FITC was performed at 4°C after 2 h of coculture. Cells were then analyzed by flow cytometry and, as the mean fluorescence intensity (mfi) varies from one mAb to another, calculations were made for each mAb. CD46 downregulation was calculated as the difference from 100% of the rate between the mfi with VV-H-infected or Ltk-MVH cells and the control mfi with VV-wt or Ltk$^-$ cells (the control mfi with GAM-FITC alone being equivalent for the two cell types).

For testing the effect of the R59S mutation on CD46 downregulation, a vaccinia recombinant expressing the CD46 R59S mutation (VV-46R59S) and a vaccinia recombinant expressing unmutated CD46 (VV-CD46) were used to infect Ltk$^-$ cells overnight. The next day, the VV-infected Ltk$^-$ cells were added to Ltk-H cells in the presence of 50 μg/ml cyclohexamide for 1 h. Following removal of the Ltk-H cells the VV-infected Ltk$^-$ cells were stained with either mAb 13/42 or mAb 10/88 then a FITC-labeled anti-mouse IgG antibody and subjected to FACScan analysis.

Immunofluorescence. 15 h after infection cells were collected, washed in PBS and distributed on immunofluorescence slides (Amilabo, France). Dried cells were fixed in acetone for 5 min at −20°C before staining was performed as described for cytometry. Stained cells were observed using a UV light microscope.

TABLE 1

Specificity, Isotype, and Concentration of Anti-CD46 mAbs Ascite Fluids

mAb	SCR specificity[a]	Isotype	IgG concentration (mg/ml)[b]
E8/5	1	IgG2a	11.1
E12/14	1	IgG1	6.7
MCI20.6	1	IgG1	10.3
13/42	1	IgG1	0.35
10/88	4	IgG1	3.4

[a] SCR specificity determined using CD46 deletion mutant expressing either SCR1 or SCR1 + 4.

[b] IgG concentration determined by ELISA with standards of known concentration.

RESULTS

Preliminary characterization of the panel of SCR1-specific anti-CD46 mAbs. Isotype and immunoglobulin (Ig) concentration were determined by ELISA for the five ascites fluids of the four SCR1-specific mAbs (E8/5, E12/14, MCI20.6, and 13/42) plus the SCR4-specific mAb 10/88 (Table 1). We then verified that the avidities of the mAbs were comparable by measuring the relative affinities for CD46. CD46-binding titrations were carried out on BJAB/P3 cells and binding was measured for serial dilutions of ascites by cytometric analysis. Titration curves drawn versus Ig concentrations represent the relative affinities of the mAbs. The five mAbs had similar affinities although that of E8/5 was slightly higher than the others (Fig. 1A).

The specificity of the mAbs for the different SCR domains of CD46 was determined by the use of CD46 deletion mutants (Fayolle and Buckland, unpublished results). RK13 cell surface expression of the mutant proteins analyzed by flow cytometry showed that deletion of SCR domains 2, 3, and 4 did not impair the binding of E8/5, E12/14, MCI20.6 and 13/42, indicating that these mAbs are specific for SCR1. A second mutant in which SCR2 and SCR3 are deleted was recognized by all five mAbs including 10/88 confirming that the 10/88 epitope maps to SCR4 (results summarized in Table 1).

Competitive binding assays were performed in order to assess the interdependence of the four epitopes mapping to SCR1. FITC-conjugated E8/5, MCI20.6 and 13/42 were used in the presence of excess nonlabeled mAb (determined from the titration curves, Fig. 1A). As a negative control, competitions carried out with SCR4-specific mAb 10/88 did not lead to the inhibition of binding of any of the SCR1-specific mAbs. However, when combinations of SCR1-specific mAbs were made, different levels of inhibition were detected (Fig. 1B). Individual SCR1-specific mAbs from the panel inhibited or interfered with the binding of each of the other mAbs indicating that their epitopes overlap at least partially.

To determine whether these mAbs recognize conformational or linear epitopes we tested their capacity to stain the CD46 protein in Western blots. We found that only E8/5 was positive (results not shown) indicating that the epitope of this mAb is linear whereas the epitopes of E12/14, MCI20.6 and 13/42 are conformational.

MVH-induced downregulation of CD46 is inhibited specifically by mAb 13/42. We studied the efficacy of the different anti-CD46 mAbs to block the downregulation of CD46 in two systems. Downregulation was either induced by infection of BJAB/P3 cells with VV-H (Fig. 2A) or by coculture of BJAB/P3 cells with Ltk-H cells (Fig. 2B). BJAB/P3 cells were incubated with the different mAbs from the panel and then infected with VV-H or coincubated with Ltk-MVH+ cells. mAb 13/42 was the only antibody which completely blocked CD46 downregulation in the two systems (Figs. 2A and 2B). The others did so with less than 10% efficiency.

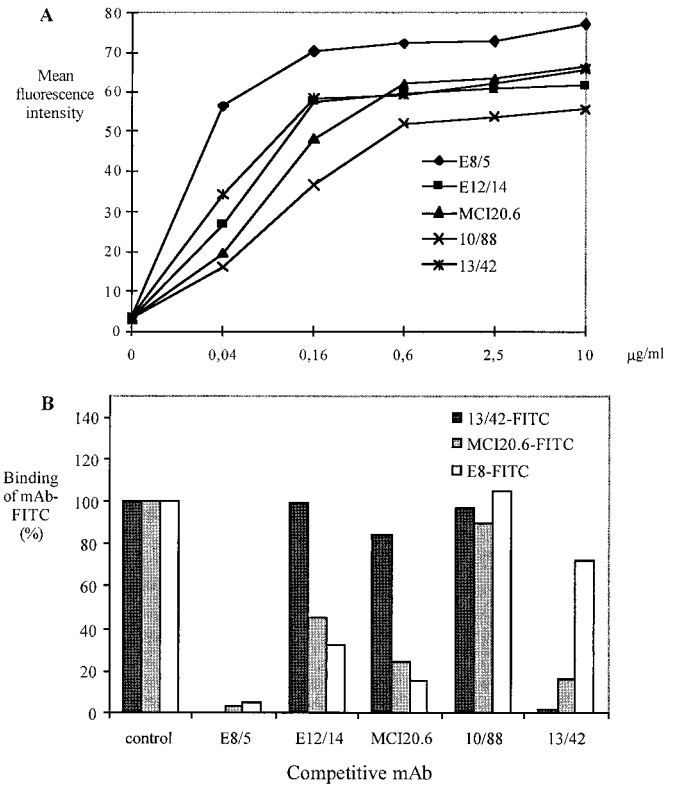

FIG. 1. Characterization of SCR1-specific anti-CD46 mAbs. (A) Relative affinities measured by flow cytometry using serial dilutions of ascites fluids on BJAB/P3 cells followed by GAM-FITC labeling. The mean fluorescence intensity representing the quantity of bound mAb is plotted versus the quantity of antibody incubated with the cells. (B) Competitive binding assays. BJAB/P3 cells were simultaneously incubated with 10 μg of unlabeled mAb and FITC-labeled mAbs. Binding of labeled antibody is represented as a percentage of the control binding minus unlabeled antibody.

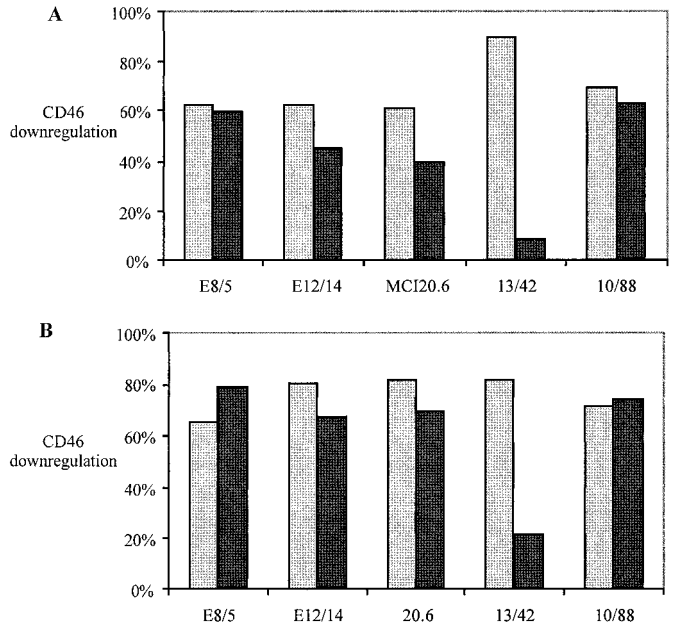

FIG. 2. Inhibition of CD46 downregulation. (A) BJAB/P3 cells were infected overnight with VV-H or wild-type VV in control medium (gray bars) or in the presence of anti-CD46 mAbs (black bars). After three washes, cells were analyzed by flow cytometry with GAM-FITC. (B) BJAB/P3 cells were cultured for 1 h on Ltk-MVH or Ltk$^-$ monolayers in control medium (gray bars) or in the presence of anti-CD46 mAbs (black bars). Nonadherent BJAB/P3 cells were collected and analyzed by flow cytometry using GAM-FITC, after three washes. Expression rate of CD46 is calculated by dividing the mfi of VV-H (A) or Ltk-H (B) by the wild-type VV (A) or the Ltk$^-$ (B) mfi. Downregulation is calculated by the difference from 100% of the expression rate.

mAb 13/42 significantly blocks the binding of the MV virion to CD46. Using FACScan analysis, the panel of anti-CD46 mAbs were tested for their capacity to block the binding of purified MV (Hallé strain) to HeLa cells at 4°C. No significant inhibition of MV binding could be detected for the E8/5 or E12/14 anti-CD46 mAbs. mAb MCI20.6 blocked MV binding by 37% but with mAb 13/42 inhibition at 75% was almost total (Table 2).

TABLE 2

Inhibition of MV Binding, MV Infection, and H/F-Induced Syncytia Formation by Anti-CD46 mAbs in HeLa Cells

mAb	MV binding inhibition (%)	MV infection inhibition (%)	H/F fusion inhibition	
			Maximum %	Inhibiting titer (ng/ml)
E8/5	12	31	50	20
E12/14	20	30	30	40
MCI20.6	38	45	20	80
13/42	76	66	100	80
10/88	15	15	0	—

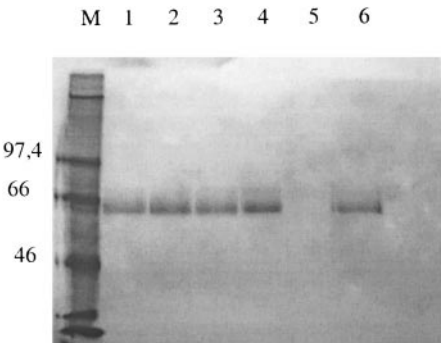

FIG. 3. Inhibition of sH binding. ^{35}S TRAN-labeled sH was incubated overnight at 4°C on 10^6 HeLa cells in the absence (1) or in the presence of 10 μl of anti-CD46 ascites fluids E8/5 (2), E12/14 (3), MCI20.6 (4), 13/42 (5) and 10/88 (6). Cell-bound sH was immunoprecipitated with anti-MVH mAb Cl.55 and analyzed in reducing conditions by SDS–PAGE.

mAb 13/42 completely inhibits the binding of soluble MVH to CD46. We used a soluble version of MVH (sH) to confirm the above results. Binding of ^{35}S-labeled dimeric sH (Hallé strain) to CD46 was studied on HeLa cells in the presence of the different mAbs. Examination of the immunoprecipitation of cell-bound sH (Fig. 3) shows that only mAb 13/42 is able to block the binding of sH to the HeLa cell surface. A protein concentration of 3.5 μg/ml for 10^6 cells was sufficient to achieve complete inhibition of binding: no significant effect could be detected for the other SCR1-specific mAbs E8/5, E12/14 and MCI20.6 or the SCR4-specific mAb 10/88, even at a protein concentration of 35 μg/ml.

mAb 13/42 completely inhibits MVH/F-induced fusion. The MV fusion-inhibiting properties of the different anti-CD46 mAbs were analyzed using HeLa cells infected with the VV double recombinant (27) expressing both MVH and MVF (Hallé strain). Serial dilutions of the mAbs showed that all the anti-SCR1 mAbs (E8/5, E12/14, MCI20.6 and 13/42) inhibit MVH/F-induced fusion to some degree (see Table 2) but complete inhibition was only observed with 13/42. Anti-SCR4 mAb 10/88 could not block syncytia formation even at the highest concentration of 20 μg/ml.

We also studied the capacity of the different mAbs to block MV infection. HeLa cells were infected with MV (Hallé strain) in the presence and absence of the mAbs and the infection rate calculated as the number of cells positive for MV—measured using flow cytometry analysis as for the MV binding assay—after 2 days of culture. We found that all SCR1-specific mAbs could partially block MV (Hallé) infection of HeLa cells but the highest rate of inhibition (66%) was achieved with mAb 13/42.

Binding of mAb 13/42 to SCR1 of CD46 is abrogated by the mutation Arg59Ser. As the epitope of mAb 13/42 on SCR1 of CD46 could be equivalent to the MV

TABLE 3

Immunofluorescence Staining of CD46 Point Mutants Expressed by Transfection in Ltk⁻ Cells

	E8/5	E12/14	MCI20.6	13/42	10/88	M177[a]
CD46	+	+	+	+	+	+
R59S CD46	+	+	+	−	+	+
R82S CD46	+	+	+	+	+	+
R96S CD46	+	+	+	+	+	+
R103S CD46	+	+	+	+	+	−

[a] SCR2-specific anti-CD46 mAb.

binding site, we decided to try to map its location by means of site-directed mutagenesis. For several reasons, as a preliminary epitope mapping strategy, we decided to particularly target arginine residues. We mutated arginines R59 and R82 in SCR1 as well as R96 which is present in the SCR1-2 linker region and R103 the sole arginine in SCR2 to serine. The R59S, R82S, R96S, and R103S CD46 mutant proteins were expressed from the vaccinia expression plasmid pgpt in both Ltk⁻ and RK13 cells and staining for immunofluorescence with the anti-SCR1 mAbs plus anti-SCR4 and anti-SCR2 mAbs as positive and negative controls respectively. Both the R82S and the R96S CD46 mutants stained positive with all of the mAbs in the panel. The R103S mutant was negative with M177 as expected but was positive with all the other mAbs. The R59S CD46 mutant however, was positive for all mAbs from the panel with the exception of 13/42: in both Ltk⁻ (Table 3) and RK13 cells (not shown) the R59S mutant was not stained by 13/42 (see also lower panels of Fig. 4B).

CD46 mutant R59S is negative for fusion. We then tested the capacity of all four CD46 SCR1/2 arginine mutants to support fusion induced by a vaccinia virus recombinant expressing both MVH and MVF (27). Following infection with the double recombinant VV-HF, Ltk⁻ cells were transfected with pgpt constructions expressing the R59S, R82S, R96S, and R103S mutants and then examined for the presence of syncytia after 20 h of culture at 37°C. Although syncytia were very evident in cells transfected with the R82S, R96S, and R103S mutants (not shown) and were also present as expected, in the case of unmutated CD46 (Fig. 4A, left panel). However, no syncytia were present in the cells transfected with the R59S mutant (Fig. 4A, right panel) which resembled those transfected with the empty vector (not shown).

CD46 mutant R59S does not downregulate. Finally, we investigated whether the mutation R59S has an effect on CD46 downregulation induced by MVH. A vaccinia recombinant expressing the CD46 R59S mutation (VV-CD46R59S) and a vaccinia recombinant expressing unmutated CD46 (VV-CD46) were used to infect Ltk⁻ cells. The capacity of Ltk-H cells to induce CD46 downregulation in the two sets of VV-infected Ltk⁻ cells was followed by FACScan analysis using two anti-CD46 mAbs (Fig. 4B). In accordance with the immunofluorescence results, although the unmutated CD46 (in the absence of Ltk-H cells) is stained by both 10/88 and 13/42 anti-CD46 mAbs, in the case of the CD46 R59S mutant (in the presence of Ltk-H cells), mAb 10/88 is positive but mAb 13/42 is negative. In the Ltk⁻ cells infected with VV-46 the CD46 protein was clearly downregulated following contact with the Ltk-H cells (Fig. 4B—compare upper left panel with upper right panel) but the cell surface expression of the CD46 R59S mutant (Fig. 4B—compare lower left panel with lower right panel) remained constant.

DISCUSSION

For Hallé—a vaccine strain of MV—both infection and CD46 downregulation are initiated by the binding of MVH to CD46 (Nan/Nan). Although CD46 can act as a receptor for vaccine strains there is evidence that wild-type strains use another as yet unknown lymphocyte-specific molecule (4, 11). However, this hypothesis does not enjoy universal acceptance. Reports that anti-CD46 antibodies substantially block the replication of the Hallé strain (4) and that CD46 downregulation is a phenotypic marker of vaccine strains (lecout) have been matched by other studies (22, 24) showing that whereas all MV strains—vaccine and wild-type—are inhibited by anti-CD46 antibodies, not all downregulate CD46. For Schneider-Schaulies *et al.* (24) such results suggested the existence of two sites of interaction between CD46 and MVH, influencing separately binding and CD46 downregulation. Support for this hypothesis was recently given by the report that although CD46 is not a receptor for rinderpest virus (RPV) it is nevertheless downregulated upon RPV infection (8).

Thus, we have used the characterization of anti-CD46 monoclonal antibodies (mAbs) to investigate whether MV infection and CD46 downregulation are induced by two separate MVH binding sites on CD46 or a common site. Our rationale was that if the same MVH binding site facilitates both MV infection and CD46 downregulation then a mAb that prevents the MVH binding should inhibit both phenomena. On the other hand, should two sites exist it should be possible to find mAbs that block either infection or CD46 downregulation but not both. Our starting point was thus to screen a panel of anti-CD46 mAbs for those which not only inhibited MV fusion but which also inhibited CD46 downregulation.

Our preliminary characterization of the anti-CD46 mAbs in the panel showed not only that E8/5, E12/14, MCI20.6, and 13/42 are SCR1-specific but—through competitive binding assays—that their epitopes over-

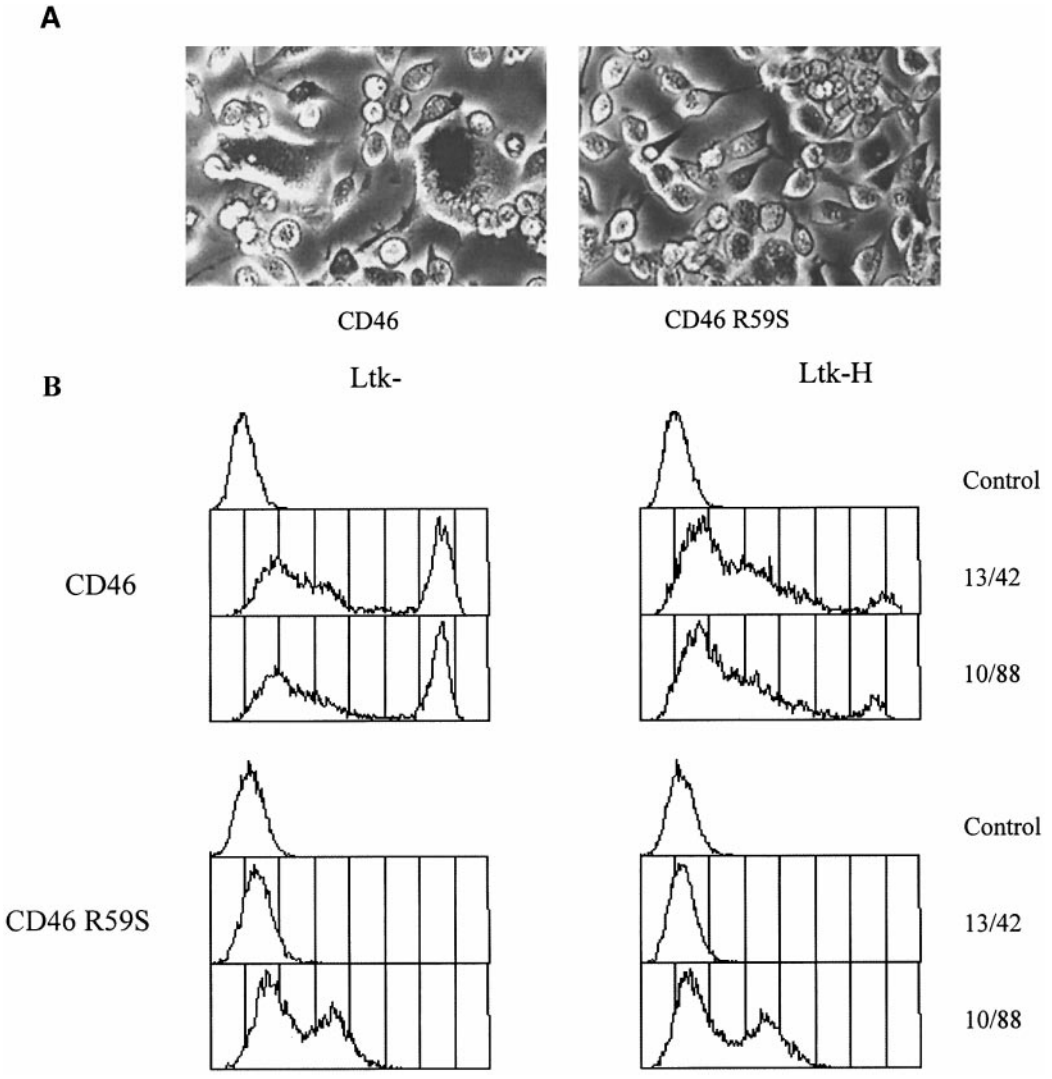

FIG. 4. Effect of the R59S mutation on fusion and CD46 downregulation. (A) Fusion test on CD46 mutants. Ltk$^-$ cells were infected with VV-H/F and then transfected with pgpt-CD46 or pgpt-CD46R59S. The cells were observed for the presence or absence of syncytia 20 h posttransfection using an inverted light microscope. (B) Ltk$^-$ cells were infected with VV-CD46 or VV-CD46R59S. 16 h after infection, cells were detached using PBS-EDTA, cyclohexamide-treated and cocultured on monolayers of Ltk-H cells or Ltk$^-$ cells for 1 h. VV-infected Ltk$^-$ cells were collected and analyzed for CD46 expression by flow cytometry using 10/88 or 13/42 mAbs.

lap. Furthermore, mAbs E12/14, MCI20.6 and 13/42 were negative in Western blot, whereas E8/5 was positive. This suggests that the linear epitope of E8/5 is part of the E12/14, MCI20.6 and 13/42 conformational epitopes: a particular domain on the SCR1 appears to be a common target for all four antibodies.

Downregulation of CD46 during MV infection was first described by Naniche *et al.* (19). The same group further showed that CD46 downregulation was dependant upon MVH-CD46 interaction as it could be inhibited, albeit partially, by both an anti-MVH mAb and the anti-CD46 mAb MCI20.6 (13). It is clear that mAb MCI20.6 does not prevent the binding of MVH completely because CD46 downregulation—which is reliant upon MVH binding—still occurs in the presence of MCI20.6 (19). We found that of the SCR1-specific anti-CD46 mAbs in our panel—which included MCI20.6—only 13/42 had the capacity to significantly block CD46 downregulation.

Our interpretation of these results is that the epitope of mAb 13/42 is closer to the MV binding site than the epitopes of the others, so that the binding of MVH is more efficiently blocked by this antibody. By the same token, viral fusion, and binding of the MV virion and sH to CD46 were inhibited to some degree by all of the SCR1-specific mAbs but only 13/42 was capable of complete inhibition. Taken together these results strongly suggest that the MVH/CD46 interaction that leads to CD46 downregulation is equivalent to that which leads to infection: when mAb 13/42 occupies its epitope on

CD46, the binding of MVH is effectively prevented with the consequence that both CD46 downregulation and MV infection are abrogated.

As a preliminary strategy for mapping the epitope of mAb 13/42, we decided in the first instance to particularly target arginine residues. There were several reasons for this. First, arginine is the amino acid most commonly found in B epitopes (12). Second, arginine residues had already been identified as participating in the MVH/CD46 interaction by two groups who—investigating the participation of residues on CD46 in the interaction with MVH—had generated point mutations in CD46 by use of scanning mutagenesis (3, 10). Although both groups identified several amino acids as participating in the interaction, comparison of their results reveals that two arginine residues—R59 in SCR12 and R103 in SCR2—are heavily implicated in both studies. In particular, Buchholz *et al.* found that the mutation R59A resulted in a loss of binding for mAb B97. Consequently, these authors suggested that a MVH binding site is close to R59. Moreover, Hsu *et al.* found that the double mutation ER58/59AA inhibited MVH binding by up to 80% (10). Third, we were influenced by the observation made by Lebon *et al.* more than twenty years ago, that 1M arginine disrupts the hemadsorption of African green monkey erythrocytes (which in contrast to human erythrocytes possess CD46 on their surface) to MV-infected cells (14). This suggests that the MV/CD46 interaction is electrostatic in nature and introduces the possibility that arginine residues on the surface of one or the other of the two protagonists contributes to the association. As the residues on MVH glycoproteins coming from vaccine strains that have been identified to participate in CD46 binding (1, 11, 15) do not include arginine, this points the finger at CD46.

Preliminary targeting of the SCR1 arginines turned out to be a serendipitous choice as we found that mAb 13/42 failed to stain the R59S mutant of CD46 whereas it was stained by all the other anti-SCR-1 mAbs in the panel. Additional evidence that R59 is important in the MVH/CD46 interaction has been provided in a recent publication which makes use of natural variations between CD46 proteins (18). Murakami *et al.* show that the CAM vaccine strain of MV can bind human, Vero and B95a forms of CD46. This means that residues important for the interaction with MVH should be conserved between the three CD46 proteins. An inspection of the primary sequences from these three CD46 proteins reveals that R59 is one of only four residues (the others are E45, E58, and P73) from the large number of amino acids identified as potentially participating in the MVH/CD46 interaction (3, 10). Interestingly, the other arginines in SCR1—R82 and R96—are also conserved between the three CD46 proteins. However, as SCR2 residue 103 in the B95a protein is proline rather than arginine this would appear to dramatically reduce the possibility that there is an important role for R103—and SCR2—in the MVH/CD46 interaction.

If R59 is an essential part of the epitope of an anti-CD46 mAb that blocks both MV-induced fusion and CD46 downregulation then mutating this residue should have an effect on both phenomena. In agreement with this, we found that fusion occurred with CD46 mutants R82S, R96S and R103 but this was not the case for R59S. Continuing this logic, if the R59S mutant cannot bind MVH and CD46 downregulation is dependent upon MVH binding, it should remain at the cell surface. We found that this was also the case.

Although MVH and mAb 13/42 appear to have a common binding site on CD46 it is only the former which triggers downregulation. One explanation for this could be that CD46 downregulation is a complex and specific process that requires more than a primary binding step. Primary binding could induce conformational changes in the MVH leading to a secondary binding step required for downregulation of CD46 to occur. Alternatively, if downregulation were to be dependant upon subsequent aggregation of the binding ligand then the incapacity of mAb 13/42 to induce the process could be related to the mAb's valency.

In conclusion, we have mapped an amino acid (R59) on CD46 which is an essential part of the epitope of an anti-CD46 mAb (13/42) that inhibits two phenomena that are facilitated by MVH binding: MV infection and CD46 downregulation. Furthermore, mutation of R59 to serine results in the abrogation of both MV-induced fusion and CD46 downregulation. Take together, these results strongly suggest that a single MVH binding site—of which R59 is a crucial component—in the SCR1 of CD46, facilitates both MV infection and CD46 downregulation.

ACKNOWLEDGMENTS

We thank J. Schneider-Schaulies for mAbs 13/42 and 10/88, D. Gerlier for mAb MCI20.6, T. Seya for M177, H. Stunnenberg for vaccinia virus expression plasmids, and B. Rossi for CD46 cDNA. We also thank Chantal Bella for FACScan analysis, Agnés Vallier for valuable advice on ELISA, and Evelyne Manet (ENSL) for reading the manuscript. V.L. was supported by a grant from the "Association Recherche et Partage." R.B. is a CNRS scientist.

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