The Paramyxoviruses Simian Virus 5 and Mumps Virus Recruit Host Cell CD46 To Evade Complement-Mediated Neutralization

John B. Johnson,1 Ken Grant,2 and Griffith D. Parks1*

Departments of Microbiology and Immunology1 and Pathology,2 Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157-1064

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The complement system is a critical component of the innate immune response that all animal viruses must face during natural infections. Our previous results have shown that treatment of the paramyxovirus simian virus 5 (SV5) with human serum results in deposition of complement C3-derived polypeptides on virion particles. Here, we show that the virion-associated C3 component includes the inactive form iC3b, suggesting that SV5 may have mechanisms to evade the host complement system. Electron microscopy, gradient centrifugation, and Western blot analysis indicated that purified SV5 virions derived from human A549 cells contained CD46, a plasma membrane-expressed regulator of complement that acts as a cofactor for cleavage and inactivation of C3b into iC3b. In vitro cleavage assays with purified complement components showed that SV5 virions had C3b cofactor activity, resulting in specific factor I-mediated cleavage of C3b into inactive iC3b. SV5 particles generated in CHO cells, which do not express CD46, did not have cofactor activity. Conversely, virions derived from a CHO cell line that was engineered to overexpress human CD46 contained elevated levels of virion-associated CD46 and displayed enhanced C3b cofactor activity. In comparison with C3b, purified SV5 virions had very low cofactor activity against C4b, consistent with the known preference of CD46 for C3b versus C4b. Similar results were obtained for the closely related mumps virus (MuV), except that MuV particles derived from CHO-CD46 cells had higher C4b cofactor activity than SV5 virions. In neutralization assays with human serum, SV5 and MuV containing CD46 showed slower kinetics and more resistance to neutralization than SV5 and MuV that lacked CD46. Our results support a model in which the rubulaviruses SV5 and MuV incorporate cell surface complement inhibitors into progeny virions as a mechanism to limit complement-mediated neutralization.

The complement system constitutes a complex group of both soluble and cell-associated proteins that together form an integral part of the innate host defense against pathogens (reviewed in references 7, 9, 11, and 31). Complement can serve to link innate and adaptive immunity through a large number of activities, including recognition of viruses, direct neutralization of infectivity, recruitment and stimulation of leukocytes, opsonization by immune cells, and activation of T- and B-cell responses (9, 11, 27). Complement activation and the ability of viruses to counteract complement can play important roles in viral pathogenesis, as well as the design of more effective vaccines and therapeutic vectors (6, 9, 17, 36, 43). The overall goal of the work described here was to determine the mechanism by which the paramyxoviruses simian virus 5 (SV5) and mumps virus (MuV) limit activation of complement pathways.

The complement cascade can be initiated through three main pathways: the classical pathway, the lectin pathway, and the alternative pathway (11, 40). These three pathways converge on a central component, C3, which is activated by cleavage into C3a and C3b. C3a serves as an anaphylatoxin to promote inflammation. C3b can bind covalently to viral components to aid in opsonization and phagocytosis. In addition, C3b can associate with other factors, such as factor B, to form the C3 convertase (e.g., C3bBb), and this function to amplify the initially deposited C3b signal by further cleavage of C3 molecules in a feedback loop. Likewise, C4 can be activated by cleavage into the anaphylatoxin C4a and the C4b fragment, which links the classical and lectin pathways with the alternative pathway. The association of C3b with components further downstream, such as C6 through C9, can lead to formation of the membrane attack complex, which is capable of lysing virus particles or infected cells (reviewed in references 7, 11, and 28).

The complement system needs to be highly regulated to prevent inappropriate activation and potential damage to normal cells and healthy tissues (3). Self-regulation of complement pathways involves the highly concerted actions of a family of soluble and cell-associated proteins called regulators of complement activation (RCA). RCA proteins can limit inappropriate complement activation through two major mechanisms: (i) by accelerating the disassociation of C3 or C5 convertase or (ii) by acting as a cofactor to promote proteolytic cleavage of C3b or C4b by the complement protease factor I. Examples of RCA proteins are factor H, CD46, complement receptor 1 (CR1, or CD35), and C4 binding protein (14, 19, 24, 45).

CD46, or membrane cofactor protein, is an integral membrane RCA protein that is expressed on a wide range of tissues and cell types (32). CD46 is an N- and O-linked glycosylated protein expressed at the plasma membrane as multiple isoforms that are derived from alternative splicing (32, 33, 39, 41). CD46 selectively binds to both C3b and C4b on cell surfaces,
where it acts as a cofactor to promote efficient cleavage by complement protease factor I (44; reviewed in references 5 and 32). For C3b, CD46 and factor I combine to mediate inactivation to iC3b, and this is a major mechanism for limiting the amplification of low basal levels of C3b that arise from spontaneous alternative-pathway activation. CD46 also serves as a cofactor for factor I-mediated cleavage of C4b into C4c and C4d, but this cofactor activity is less efficient than that seen for C3b cleavage (35, 45).

Viruses have evolved a number of mechanisms to inhibit or to delay the neutralizing effects of complement (9, 16). Large DNA viruses have coding capacities that allow them to encode a variety of mimics of host cell RCA proteins, and these viral homologs often function to inactivate complement components by supplying cofactor activity or by accelerating the decay of convertases (reviewed in references 2, 7, 29, and 31). For example, herpesvirus saimiri expresses a complement control protein that inhibits the C3 convertase (20). As an alternative mechanism to counteract complement, a number of enveloped DNA viruses and retroviruses have been shown to recruit cell-associated RCA proteins into budding particles (15, 47, 48). Examples of this are vaccinia virus and human immunodeficiency virus type 1, which incorporate CD55, CD59, and CD46 into progeny virions (16, 42, 48).

In contrast to retroviruses and large DNA viruses, mechanisms that are employed to limit or evade host cell complement pathways have not been described for the paramyxovirus family of negative-strand RNA viruses (30). It has been known for many years that complement is an important factor in paramyxovirus neutralization (18, 23, 34, 49, 50). For example, the closely related paramyxoviruses SV5 and MuV preferentially activate the complement alternative pathway in vitro, and this activation can contribute to the efficiency of neutralization by human serum (23, 26). These findings raised the question of whether negative-strand RNA viruses have mechanisms to limit activation and/or amplification of the complement cascade.

We have previously shown that treatment of SV5 and MuV particles with normal human serum led to deposition of C3-derived components on virions, but the virion-associated C3 molecules had properties of the inactive form, iC3b, and not the intact C3b (26). In this study, we tested the hypothesis that SV5 and MuV incorporate host cell RCA proteins into budding virions as a mechanism to limit complement activity through inactivation of C3b. CD46 was found associated with purified SV5 and MuV virions that were derived from cells expressing CD46, and these particles displayed C3b cofactor activity in vitro. Consistent with this inactivation of C3b, CD46-containing virus was more resistant to in vitro neutralization by human serum than virus derived from CD46-deficient cells. Our results support a model in which these closely related paramyxoviruses incorporate at least one cell surface RCA protein into progeny virions as a mechanism to evade complement-mediated neutralization.

**MATERIALS AND METHODS**

Cells and viruses. A549 and CV1 monolayer cultures were grown in Dulbecco modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum, 100 μg/ml streptomycin, and 200 mM L-glutamine. Chinese hamster ovary (CHO) cells that overexpress the CYT2 isoform of human CD46 (CHO-CD46) and the control drug-resistant CHO cells were kindly provided by Denis Gerlier (21) and were maintained in Dulbecco modified Eagle medium supplemented with 4.5 g/liter glucose, 10 mM HEPES, pH 7.2, 10 μg/ml gentamicin, L-glutamine, 1% nonessential amino acid, and 6% fetal bovine serum. Recombinant wild-type SV5 (strain W3A) or the Enders strain of MuV (ATCC VR-1379) was grown in A549, CHO-CD46, or control CHO cells in the presence of heat-inactivated serum. The virus was purified by sucrose gradient centrifugation and titrated as previously described (26).

**RESULTS**

iC3b cleavage products and cellular CD46 are associated with purified SV5 virions. C3b is composed of an alpha chain and a beta chain. In the presence of factor I and a cofactor, such as CD46, the alpha chain is cleaved into 67- and 43-kDa fragments, and the drug-resistant CHO cells were kindly provided by Denis Gerlier (21) and were maintained in Dulbecco modified Eagle medium supplemented with 4.5 g/liter glucose, 10 mM HEPES, pH 7.2, 10 μg/ml gentamicin, L-glutamine, 1% nonessential amino acid, and 6% fetal bovine serum. Recombinant wild-type SV5 (strain W3A) or the Enders strain of MuV (ATCC VR-1379) was grown in A549, CHO-CD46, or control CHO cells in the presence of heat-inactivated serum. The virus was purified by sucrose gradient centrifugation and titrated as previously described (26).

**Ultracentrifugation and Western blotting.** Sucrose gradient-purified particles alone or particles that had been incubated with normal human serum (NHS) at a ratio of 1:1 (vol/vol) were layered on top of 15 to 60% sucrose gradients and subjected to ultracentrifugation as described previously (26, 37). Fractions collected from the bottom of the tube (250 μl) were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by Western blotting with an antibody against the SV5 P protein (26) or with a polyclonal goat antibody against human C3 (MP Biomedics, Cappell, CA) at 1:1,000 dilution. For CD46, gradient fractions were concentrated by trichloroacetic acid precipitation before analysis by Western blotting with rabbit anti-human CD46 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:500. SV5-infected A549 cell lysates were used as an electrophoretic marker for the position of CD46. The blots were treated with Super Signal West Pico chemiluminescent substrate (Thermo Scientific), and the signal was detected by exposing the blots to film.

**Complement reagents.** NHS was collected, processed as described previously (26), and stored at −80°C in small aliquots. The results were consistent among sera from multiple donors. The purified complement proteins C3b, C4b, factor H, and factor I were from Complement Technologies (Tyler, TX) and were used in standard assays as described previously (8). Soluble CR1 was a kind gift from Henry Marsh (Celldex Therapeutics, Needham, MA).

**Electron microscopy.** Sucrose gradient-purified SV5 or MuV particles (10 μl; ≈106 PFU/ml) that had been generated in either A549, CHO, or CHO-CD46 cells were analyzed for the presence of CD46 by adsorption on carbon-coated 200-mesh gold grids (Electron Microscopy Sciences, PA) and incubated at room temperature in a humidified chamber for 5 min. The grids were blocked with phosphate-buffered saline (PBS) containing 1% bovine serum albumin, and the adsorbed virions were probed with a mouse anti-human CD46 monoclonal antibody (R&D Systems, MN) at a dilution of 1 μg/10 μl and incubated for 12-24 h. The labeled particles were subjected to negative staining with 2% phosphotungstic acid (pH 6.6) and analyzed with a Philips TEM400 transmission electron microscope as described previously (26).

**Factor I cofactor activity assay.** In vitro cofactor activity was assayed as described previously (8). SV5 or MuV particles generated in either A549, CHO, or CHO-CD46 cells were purified by sucrose gradient centrifugation, and the protein concentrations were determined by bicinchoninic acid assay. Seven micrograms of viral particles was incubated with 3 μl of either C3b or C4b, along with 100 ng of factor I in a total volume of 20 μl. Incubation was in PBS (pH 7.4) at 37°C for the times indicated in the figure legends. Reactions were terminated by adding 5 μl of SDS-PAGE sample buffer containing mercaptoethanol and boiling them. C3b reaction products were analyzed on 9% SDS-PAGE gels, while C4b reaction products were analyzed on 10% gels. The gels were stained with Gelcode Blue Stain reagent (Thermo Scientific, IL) to visualize the proteins. In some cases, reaction products were analyzed by Western blotting with polyclonal goat anti-human C3 at a dilution of 1:1,000. Factor H, which is known to have cofactor activity for cleavage of C3b into iC3b, served as a control for the C3b assay. Soluble CR1, which is known to act as a cofactor for promoting cleavage of C4b into C4c and C4d, was used for the C4b assay.

**Virus neutralization assay.** Time- or concentration-dependent neutralization assays were carried out as described previously (26). One hundred PFU of SV5 or MuV grown in either CHO or CHO-CD46 cells was treated at 37°C with various concentrations of NHS for 1 h or with dilutions of NHS for various times. After incubation, viral titers were determined by plaque assays as described previously (26). The reported results were the averages of six reactions, with the significance of data points calculated using Student’s t test.
fragments, but the C3b beta chain remains intact (1). To determine the form of C3 that was associated with serum-treated SV5, purified virions produced in A549 cells were left untreated or were treated for 1 h with NHS as a source of complement. Samples were centrifuged on 15 to 60% sucrose gradients, and fractions were collected and analyzed for the positions of virions by Western blotting with antiserum specific for the SV5 P protein. As shown in Fig. 1A, untreated SV5 virions sedimented with a peak in fractions 10 to 12, while NHS-treated virions sedimented further down the gradient to fractions 7 to 9 as described previously (26). The more rapid sedimentation of NHS-treated SV5 particles was dependent on the presence of C3 (data not shown). When fractions from the NHS-treated SV5 sample were probed with antibodies to C3, two main C3 fragments the size of iC3b proteins were detected that cosedimented to the same peak position as SV5 virions (Fig. 1A, bottom). Compared to purified C3b and iC3b marker standards (Fig. 1B), the C3 proteins that cosedimented with SV5 particles did not show an intact C3b alpha chain but instead showed a polypeptide profile matching that of the beta chain of C3b and the 43-kDa fragment that is produced when C3b is cleaved to iC3b (1). These data demonstrate that serum-treated SV5 particles are associated with iC3b, the inactivated form of C3b.

The above-mentioned finding of iC3b cleavage products co-sedimenting with NHS-treated virion particles raised the hypothesis that an RCA protein was associated with SV5, with CD46 being the most likely candidate. In the presence of NHS, CD46 would promote factor I-mediated cleavage to produce iC3b. In support of this hypothesis, Fig. 1C shows that the same gradient fractions from NHS-treated virus that contained cleaved C3b also contained cellular CD46. Using immunogold electron microscopy, purified SV5 particles showed strong labeling for CD46 (Fig. 1D). Together, these data indicate that the C3 protein associated with serum-treated SV5 virions is the inactivated iC3b form and that the cellular cofactor CD46 is associated with purified SV5 particles.

Purified SV5 virions display cofactor activity that promotes factor I-mediated cleavage of C3b into iC3b. To directly test the hypothesis that SV5 virions had C3b cofactor activity, SV5 was grown in A549 cells and purified in the absence of NHS. Factor I-mediated cleavage of C3b was reconstituted in vitro using purified SV5 virions and purified commercially available components as described previously (8). The appearance of iC3b cleavage products was monitored by SDS-PAGE and Coomassie blue staining. As shown in the positive control samples in Fig. 2A, the C3b alpha chain was cleaved into 67-kDa and 43-kDa fragments when incubated with purified factor I and factor H (compare lanes 1 and 3). When SV5 virions were tested for cofactor activity by themselves, C3b cleavage was not detected (lane 4). However, incubation of SV5 particles with factor I and C3b resulted in a time-dependent disappearance of the C3b alpha chain and corresponding

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**FIG. 1.** CD46 and iC3b are associated with purified SV5 particles. (A) Gradient sedimentation. Purified SV5 was incubated alone or with NHS for 1 h and then analyzed by centrifugation through 15 to 60% sucrose gradients. Fractions were collected and analyzed for viral proteins by Western blotting with antiserum specific for the SV5 P protein (α-P) (top and middle) or for C3-related proteins (α-C3) (bottom). (B and C) Virus-associated iC3b and CD46. Fractions 7 to 9 from the SV5 plus NHS gradient shown in panel A were pooled and analyzed by Western blotting for C3 proteins (B) or CD46 (C). The markers were purified C3b and iC3b (B) or a lysate from SV5-infected A549 cells (C). The positions of the alpha and beta chains of C3b and the 67-kDa and 43-kDa fragments of iC3b and CD46 are indicated. (D) Electron microscopy (EM) analysis. Sucrose gradient-purified virus was treated with anti-CD46 antibody, followed by 12-nm colloidal gold goat anti-mouse antibody (middle and bottom) or with secondary antibody (29) alone (top). The samples were analyzed by EM at a magnification of ×55,000.
CHO cells express very low to undetectable levels of CD46, and no detectable CD46 was found in purified virions derived from CHO cells. In contrast, CHO-CD46 cells express high levels of CD46 (Fig. 3A), and SV5 virus purified from CHO-CD46 cells had abundant CD46. Using immunogold electron microscopy, purified virions from CHO-CD46 cells showed high labeling for CD46 around the outside edges of particles (Fig. 3B), whereas virus from control CHO cells gave only background labeling.

CD46 in virions derived from CHO, CHO-CD46, and A549 cells were compared by Western blotting. As shown in Fig. 3C, abundant CD46 was detected in virions from CHO-CD46 cells, but not from CHO cells. In the case of virions from A549 cells, the level of CD46 was greatly reduced compared to virions from CHO-CD46 cells. In addition, there was a clear difference in the isoform that was associated with SV5 particles from A549 cells compared to CHO-CD46 cells, consistent with the CHO-CD46 cell line stably expressing the CYT2 form from a plasmid (21).

To determine if virus grown in CHO-CD46 cells was associated with inactivated C3b, purified virus was incubated with NHS as a source of complement for 1 h and then sedimented on sucrose gradients as described for Fig. 1 above. Peak fractions containing virus were pooled and analyzed for C3b fragments. By comparison with marker lanes in Fig. 3D, the C3 protein fragments that coloaddent with SV5 virions (fractions 7 to 9) were found to be the iC3b fragments (43- and 67-kDa proteins) and not the intact C3b proteins.

The above-mentioned results predicted that SV5 derived from the CHO-CD46 cells would have increased C3b cofactor activity compared to virus from the CD46-deficient CHO cell line. To test this, factor I-mediated cleavage of C3b was reconstituted in vitro using purified commercially available components, and the reactions were monitored by SDS-PAGE and Coomassie blue staining. As shown in Fig. 4A, purified SV5 particles derived from CHO-CD46 cells by themselves did not promote C3b cleavage (lane 4). Incubation of SV5 particles with factor I and C3b resulted in a rapid time-dependent disappearance of both the 43- and 67-kDa fragments (compare lanes 4 with lanes 9 and 10).

The above-mentioned results were supported by Western blot analysis of reaction products (Fig. 2B), which demonstrated the loss of the alpha' fragment and the appearance of the 43- and 67-kDa proteins in the case of the positive control sample, which included C3b, factor I, and factor H (lane 3). C3b alpha' cleavage was also seen in the case of SV5 virions incubated with C3b and factor I (lane 5). In the case of the SV5 samples, C3b cleavage produced both a 43- and a 46-kDa fragment, as reported elsewhere (1). These data provide direct evidence that purified SV5 particles contain a functional C3b cofactor that promotes C3b cleavage and are consistent with the association of CD46 with SV5 virions (Fig. 1C and D).

Increasing the level of virion-associated CD46 leads to increased C3b cofactor activity. To further test the hypothesis that virion-associated CD46 contributes to C3b cofactor activity, SV5 was grown and purified from CHO cells, which are deficient in CD46, or in a CHO cell line that was engineered to overexpress the CYT2 isoform of human CD46 (CHO-CD46) (21). Virions from these two cell lines had similar gradient sedimentation profiles (data not shown). As shown in Fig. 3A,
ing infectivity was determined by plaque assay. As shown in Fig. 5A, virus derived from both cell lines was effectively neutralized at serum dilutions of 1:10 and 1:20, since few if any plaques could be detected in these samples. However, at dilutions of 1:40 and 1:60, virus grown in CHO-CD46 cells was not neutralized as efficiently as SV5 from CHO cells. The number of plaques was significantly higher at these two dilutions ($P < 0.001$) for SV5 grown in CHO-CD46 cells than for control CHO cells.

SV5 containing CD46 was also neutralized at a lower rate than virus deficient in CD46. This is evident in the time course of neutralization shown in Fig. 5B, where the number of plaques was significantly higher at these two dilutions ($P < 0.001$) for SV5 grown in CHO-CD46 cells than for control CHO cells.

SV5 virions have low C4b cofactor activity. CD46 can function as a cofactor for factor I-mediated cleavage of the C4b alpha’ chain into a 25-kDa fragment and C4d. To determine if purified SV5 particles have C4b cofactor activity, C4b cleavage assays were reconstituted in vitro from purified commercially available components and assayed by SDS-PAGE and Coomassie blue staining. As shown in the positive control lane 3 of Fig. 6, the C4b alpha’ chain was efficiently cleaved into C4d and the 25-kDa fragments when incubated with purified factor I and soluble CR1. Addition of factor I and C4b to purified SV5 derived from CHO-CD46 cells resulted in a very low level of C4b cleavage, which is most clearly evident in Fig. 6 from the appearance of low levels of C4d (lane 8). SV5 from CHO control cells did not have detectable C4b cofactor activity (Fig. 6B). These data indicated that SV5 particles have only very low levels of C4b cofactor activity, even when associated with large amounts of CD46 due to growth in CHO-CD46 cells.

**MuV particles containing CD46 inactivate C3b and C4b and are resistant to complement-mediated neutralization.** To determine if the above-mentioned results with SV5 extended to other related paramyxoviruses, MuV was grown in control CHO cells or CHO-CD46 cells. As shown in the Western blot in Fig. 7A, purified MuV was associated with CD46 when grown in CHO-CD46 cells but not in control CHO cells. Similarly, immunogold electron microscopy of MuV particles showed CD46 labeling of virions from CHO-CD46 cells, but not from CHO cells (Fig. 7B). As shown in Fig. 7B, a large fraction of MuV particles displayed a concentration of immu-
nogold labeling on one side, but the significance of this is not known (see Discussion).

To determine if MuV particles had C3b or C4b cofactor activity, factor I-mediated cleavage reaction mixtures were reconstituted in vitro using purified commercially available components, and the reactions were monitored by SDS-PAGE and Coomassie blue staining. As shown in Fig. 7C, purified MuV from CHO-CD46 cells had efficient C3b cofactor activity (lane 5), which is most evident in the disappearance of the C3b-alpha chain and the corresponding appearance of the 43-kDa fragment. MuV from control CHO cells lacked cofactor activity (data not shown). In contrast to SV5 particles, MuV particles containing CD46 had high C4b cofactor activity. This is most evident in the time course shown in Fig. 7D, where there is substantial loss of the C4b-alpha chain and appearance of the 25-kDa fragment as early as 2 h after incubation. This contrasts with the low C4b activity associated with SV5 virions (Fig. 6A), even after 24 h of incubation.

Similar to the results with SV5 (Fig. 5), MuV derived from CHO-CD46 cells was more resistant to in vitro neutralization by human serum than virus derived from the control CHO cells. This is evident in the results from a time course of in vitro neutralization by sera from two donors shown in Fig. 8.

### DISCUSSION

Complement is an important mediator of the innate response to paramyxovirus infections. A number of paramyxoviruses have been shown to be neutralized by complement through either the classical or alternative pathway (18, 23, 34, 49, 50). However, to our knowledge, no previous study has addressed the mechanisms by which paramyxoviruses can counteract or limit the effects of complement. This study was initiated by our previous finding that C3 protein fragments are associated with SV5 and MuV virions after exposure to NHS in vitro (26). However, the virion-associated C3 fragments were the inactive iC3b species that results from cleavage of C3b by factor I in combination with a cofactor, such as CD46 or factor H. This finding raised the hypothesis tested here that SV5 and MuV virions contain cofactor activity that promotes the inactivation of complement pathways. As described below, our results support a model in which the closely related paramyxoviruses SV5 and MuV recruit the cellular RCA protein CD46 from the plasma membrane during the budding process. Together with factor I derived from serum, these CD46-enriched virions mediated cleavage of C3b and C4b into their inactive forms.

Our results are consistent with the proposal that SV5 and MuV incorporate CD46 into progeny virions, as evidenced by
coseedmentation on sucrose gradients, immunogold electron microscopy, and different levels of CD46 in virions grown in cells that differ in CD46 expression. Preliminary data (not shown) indicate that our findings of SV5- and MuV-associated CD46 also extend to a third rubulavirus, human parainfluenza virus type 2. The signals that direct some membrane proteins into budding paramyxovirus particles or exclude other cell surface proteins are not completely understood. For CD46, different cell types express distinct isoforms through differential splicing (39, 41), and this results in CD46 molecules that differ in two protein domains: a serine- and threonine-rich ectodomain segment, which is likely to be heavily glycosylated, and the length of the cytoplasmic C-terminal tail (32). In our studies, we have used either A549 cells, which express one predominant isoform (Fig. 1C and 3C), or a stable CHO cell line engineered to overexpress the CYT2 isoform from a transfected plasmid. Since the length and sequence of the CD46 cytoplasmic domain could influence incorporation into budding SV5 or MuV particles, it is not known whether budding SV5 or MuV particles preferentially incorporate a particular CD46 isoform. It is notable that in our electron microscopy studies most SV5 and MuV particles derived from CHO-CD46 cells contained an enrichment of CD46 on one face of spherical particles (Fig. 1D and 7B). In the case of filamentous particles, CD46 staining was predominantly on one tip of the particle (not shown). Further studies are required to determine if this reflects a selective enrichment of CD46 at sites that initiate budding.

![Graph A](image1.png)

**FIG. 5.** Reduced efficiency and rate of in vitro neutralization of SV5 derived from CHO-CD46 cells. (A) Effect of serum dilution. One hundred PFU of SV5 grown in CHO-CD46 cells or control CHO cells was incubated for 1 h with PBS (left) or with the indicated dilutions of NHS. The remaining infectious titers were determined by plaque assays. (B) Time course of neutralization. One hundred PFU of SV5 grown in CHO-CD46 cells or control CHO cells was incubated for the indicated times with a 1/40 dilution of NHS. The remaining infectious titers were determined by plaque assays. For both panels A and B, the results are the averages of six reactions, with the error bars representing standard deviations. *, P < 0.001, and #, P < 0.01 comparing corresponding values from CHO and CHO-CD46.

![Graph B](image2.png)

**FIG. 6.** SV5 virions have low C4b cofactor activity. Purified SV5 derived from CHO-CD46 cells (A) or from control CHO cells (B) was incubated with the indicated combinations of C4b and factor I. As a positive control, purified C4b was incubated with factor I and sCR1. The cleavage of the C4b alpha’ chain into C4d and 25-kDa fragments was monitored by SDS-PAGE and Coomassie blue staining. (A) Lanes 1 to 4 are samples from a 24-h incubation, while lanes 5 to 8 were incubated for 8, 12, 16, and 24 h, respectively. The arrow indicates the position of the C4d cleavage product. (B) Samples from a 24-h incubation. The positions of C4b components and the C4d cleavage products are indicated.
In our in vitro assays using purified complement components, both SV5 and MuV particles had cofactor activity needed for cleavage of C3b to the inactive iC3b. Cofactor activity against C4b was also seen, but it was less efficient than that seen for C3b cleavage, as described previously (35, 45). Interestingly, C4b cofactor activity associated with MuV particles was much more efficient than that seen with SV5 particles. One explanation for this difference could be that CD46 in

![FIG. 7. MuV particles containing CD46 inactivate complement. (A) Western blotting. Purified MuV derived from control CHO cells or CHO-CD46 cells was analyzed by Western blotting for CD46 or for viral P protein. (B) Electron microscopy (EM) analysis. Sucrose gradient-purified MuV was treated with anti-CD46 antibody, followed by 12-nm colloidal gold goat anti-mouse antibody. Samples were analyzed by EM at a magnification of ×55,000. (C) C3b cofactor activity. Purified MuV grown in CHO-CD46 cells was assayed for C3b cofactor activity as described in the legend to Fig. 2. The cleavage of the alpha' chain of C3b into the 67- and 43-kDa products was monitored by SDS-PAGE and Coomassie blue staining. Lanes 1 to 3 are controls showing C3b-alpha' cleavage with factor I and factor H. (D) C4b cofactor activity. Purified MuV derived from CHO-CD46 cells was assayed for C4b cofactor activity by incubation for the indicated times at 37°C as described in the legend to Fig. 4.

In our in vitro assays using purified complement components, both SV5 and MuV particles had cofactor activity needed for cleavage of C3b to the inactive iC3b. Cofactor activity against C4b was also seen, but it was less efficient than that seen for C3b cleavage, as described previously (35, 45). Interestingly, C4b cofactor activity associated with MuV particles was much more efficient than that seen with SV5 particles. One explanation for this difference could be that CD46 in

![FIG. 8. Reduced efficiency and rate of in vitro neutralization of MuV containing CD46. One hundred PFU of MuV grown in CHO-CD46 cells or control CHO cells was incubated for the indicated times with a 1:60 (donor no. 1) or 1:20 (donor no. 2) dilution of NHS. The remaining infectivity was determined by plaque assays. The results are the averages of six reactions, with the error bars representing standard deviations. *, P < 0.001, and #, P < 0.01 comparing MuV from CHO and CHO-CD46 cells.

![FIG. 8. Reduced efficiency and rate of in vitro neutralization of MuV containing CD46. One hundred PFU of MuV grown in CHO-CD46 cells or control CHO cells was incubated for the indicated times with a 1:60 (donor no. 1) or 1:20 (donor no. 2) dilution of NHS. The remaining infectivity was determined by plaque assays. The results are the averages of six reactions, with the error bars representing standard deviations. *, P < 0.001, and #, P < 0.01 comparing MuV from CHO and CHO-CD46 cells.

MuV is more concentrated or in a different conformation that allows a higher preference for inactivation of both C3b and C4b. Alternatively, MuV may have mechanisms to recruit additional complement inhibitors, and this could promote more effective cofactor activity against C4b.

It is not clear whether SV5 and MuV recruit other cellular RCA proteins or inhibitors of complement in addition to CD46. A recent study by Shaw et al. (46) showed that influenza virus can incorporate the inhibitory protein CD59, which acts downstream at the assembly of the membrane attack complex, but CD46 was not found in purified virions. This raises the interesting possibility that enveloped RNA viruses may have selective incorporation of host inhibitors of complement depending on the particular pathway activated by an individual virus type or sites of budding at the plasma membrane. Consistent with this hypothesis, influenza virus can activate the lectin or classical pathway (22, 25) and does not incorporate CD46 (46), which acts preferentially on the alternative pathway (35, 45). Conversely, SV5 activates the alternative pathway, incorporates at least CD46, and has low activity against the classical and lectin pathway factor C4b. It has recently been shown that the flavivirus West Nile virus evades complement activation by recruiting the soluble factor H from serum (13). This finding raises the possibility that SV5 and MuV may also recruit soluble serum-associated RCA proteins, in addition to membrane-bound factors, such as CD46.

It is important to note that the effect of SV5- and MuV-associated cofactor activity on neutralization was not absolute but instead resulted in a delay in the kinetics of neutralization and a decrease in the efficiency of inactivation (Fig. 5). We interpret this as showing that the potency of complement activities eventually overcomes any viral inhibitory mechanisms, and the virus-associated RCA proteins act only to delay, but not completely block, neutralization. A similar proposal has been made for human immunodeficiency virus, and it is thought that delaying neutralization confers an advantage for virus growth (4). All of our data have been obtained using human serum, a conventional approach that takes advantage of the classical and lectin pathways. Parainfluenza viruses, such as SV5, are typically restricted to the respiratory tract (30) and are generally shielded from the high concentrations of complement in serum. It is known that complement levels and pathways in the respiratory tract differ significantly from those in serum (10). Thus, it is possible that virus-associated RCA proteins will be more potent factors in the inhibition of virus neutralization when examined in the context of immunity specific for the respiratory tract.

CD46 is expressed on the surfaces of nearly all human cells, but the levels of expression can vary between different cell types or tissues (32). SV5 does not induce a global shutdown of cellular transcription or translation (38), raising the possibility that the level of CD46 incorporation into budding SV5 particles is determined by the constitutive level in a particular cell type. CD46 and other cell RCA proteins can function to inactivate complement at the cell surface. Since SV5 establishes a largely noncytopathic infection of human epithelial cells (12), constitutive expression of RCA proteins could contribute to establishing noncytopathic persistent infections.

In summary, our results demonstrate that the paramyxoviruses SV5 and MuV activate the alternative complement pathway (26) but also incorporate the cellular RCA protein CD46 to decrease the efficiency of virus neutralization. This is consistent with the general principle that viruses cannot avoid activation of complement but instead limit the effects of this activation by employing mechanisms that target a downstream inhibitory step in the complement cascade (9, 16).

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