Complement Activation by Human Monoclonal Antibodies to Human Immunodeficiency Virus

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It has been shown that the incubation of human immunodeficiency virus (HIV) with polyclonal antibodies from HIV-infected persons and complement results in complement-mediated neutralization due, at least in part, to virolysis. The current study was performed to determine whether any of a panel of 16 human monoclonal antibodies to HIV could activate complement and, if so, which determinants of the HIV envelope could serve as targets for antibody-dependent complement-mediated effects. Human monoclonal antibodies directed to the third variable region (V3 region) of HIV_{MN} gp120 induced C3 deposition on infected cells and virolysis of free virus. Antibodies to two other sites on HIV_{MN} gp120 and two sites on gp41 induced few or no complement-mediated effects. Similarly, only anti-V3 antibodies efficiently caused complement-mediated effects on the HIV_{IIB} isolate. In general, the level of C3 deposition on infected cells paralleled the relative level of bound monoclonal antibodies. As expected, pooled polyclonal antibodies from infected persons were much more efficient than monoclonal antibodies inducing C3 deposition per unit of bound immunoglobulin. Treatment of virus or infected cells with soluble CD4 resulted in increases in anti-gp41 antibody-mediated virolysis and C3 deposition but decreases in anti-V3 antibody-mediated virolysis and C3 deposition. In general, virolysis of HIV was more sensitive as an indicator of complement-mediated effects than infected-cell surface C3 deposition, suggesting the absence of or reduced expression of functional complement control proteins on the surface of free virus. Thus, this study shows that human monoclonal antibodies to the V3 region of gp120 are most efficient in causing virolysis of free virus and C3 deposition on infected cells. Elution of gp120 with soluble CD4 exposes epitopes on gp41 that can also bind antibody, resulting in virolysis and C3 deposition. These findings establish a serologically defined model system for the further study of the interaction of complement and HIV.

Complement activation by antibody-antigen complexes is an important biological effector mechanism that is dependent on a number of factors, including the accessibility of antibody binding sites, epitope number, epitope density, and isotype of the activating antibody. Persons infected with human immunodeficiency virus (HIV) produce specific antibodies that have been shown to activate the classical complement pathway *in vitro* on free HIV and HIV-infected cells (19, 20).

Several lines of evidence indicate that complement is activated *in vivo* during both the asymptomatic and the symptomatic phases of HIV infection. Thus, decreased levels of complement receptors are found on several cell types, including erythrocytes (4, 9, 10, 21), and complement activation products are found in blood (3, 14, 17). Also, there is evidence that persons deficient in complement component C4 may progress more quickly to AIDS than persons with an intact complement system (2, 8).

It is possible that complement, if activated by HIV *in vivo*, has several beneficial effects, such as lysis of free virus and clearance of infected cells (18). However, some studies have also shown that complement may contribute to deleterious effects, such as enhancement of infection of certain cells (11, 15, 18).

The development of human monoclonal antibodies (huM

Abs) to HIV has allowed delineation of the biological activities of antibodies of various specificities. For example, anti-gp41 and anti-gp120 huMAbs can mediate antibody-dependent cellular cytotoxicity (12, 23), anti-gp120 huMAbs can cause direct neutralization (reviewed in reference 1), and anti-gp41 huMAbs can have immunotoxin activity (22). Therefore, to clarify which huMAbs play an important role in complement activation, we analyzed a panel of huMAbs for the ability to induce C3 deposition on infected cells and to cause complement-mediated lysis of free virus. Since it appears that complement can contribute to anti-HIV immunity, characterization of the interaction of antibody with HIV epitopes, resulting in complement activation, could contribute to the rational design of vaccines or the therapeutic use of polyclonal or monoclonal antibody preparations.

MATERIALS AND METHODS

Viruses and cells. The HIV_{IIB} and HIV_{MN} isolates of HIV type 1 were obtained from the AIDS Research and Reference Reagent Program (contributed by Robert Gallo), National Institutes of Health (Rockville, Md.). The H9 T-cell line, a subclone of the HUT 78 line (5), was used to propagate virus. Cells were grown in RPMI 1640 culture medium containing 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) (Whittaker Bioproducts, Walkersville, Md.) and supplemented with 10% heat-inactivated fetal bovine serum (Hyclone, Logan, Utah) and gen-

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TABLE 1. Characteristics of the huMAbs used in this study

Cell line	Isotype, subclass, and light-chain type	Specificity (peptide region)
71-31	IgG1(λ)	p24
240-D	IgG1(κ)	gp41 (I) ^a
246-D	IgG1(κ)	gp41 (I)
50-69	IgG2(κ)	gp41 (I)
167-D	IgG1(λ)	gp41 (II) ^b
98-6	IgG2(κ)	gp41 (II)
257-D	IgG1(λ)	gp120 (V3, MN)
268-D	IgG1(λ)	gp120 (V3, MN)
412-D	IgG1(κ)	gp120 (V3, MN)
419-D	IgG1(λ)	gp120 (V3, MN)
311-11-D	IgG1(λ)	gp120 (V3, MN)
447-52-D	IgG3(λ)	gp120 (V3, MN, IIIB)
694-D	IgG1	gp120 (V3, IIIB, MN)
559/64-D	IgG1(κ)	gp120 (CD4bd) ^c
448-D	IgG1(λ)	gp120 (CD4bd)
450-D	IgG1(λ)	gp120 (C terminus)

^a gp41 (I), reactivity to gp41 cluster I (amino acids 579 to 604).

^b gp41 (II), reactivity to gp41 cluster II (amino acids 644 to 663).

^c CD4bd, CD4 binding domain of gp120.

tamicin (50 μ g/ml). All experiments with infected cells and virus supernatants were performed within 3 weeks after infection of freshly thawed, uninfected aliquots of H9 cell stocks. All cell lines and virus stocks were found negative for mycoplasmas by a commercial detection system (Gen-Probe, San Diego, Calif.).

Antibodies and complement. The isotypes and specificities of the huMAbs used in this study are summarized in Table 1. The methods used for the production and screening of these huMAbs have been described (6, 7, 25). The pool of sera used as a polyclonal antibody source has also been described (19, 20) and was used at a 1:10 final dilution. The serum used as a human complement source was collected from an HIV antibody- and human T-cell leukemia virus antibody-negative AB⁺ donor, divided into aliquots, and frozen at -70°C until use.

Deposition of C3 on infected cells. HIV-infected or uninfected cells were washed twice with serum-free RPMI 1640 medium containing 25 mM HEPES (serum-free medium) and adjusted to 2×10^6 cells per ml. The cell suspension (200 μ l) was pelleted in tubes, and the pellets were resuspended with 50 μ l of polyclonal or monoclonal antibody diluted in serum-free medium. Cells were incubated in the presence of antibody for 30 min on ice. In some experiments, before incubation with antibody, cells were preincubated with soluble CD4 (sCD4) at 50 μ g/ml (final concentration) (a kind gift of Ray Sweet, Smith Kline & French Laboratories, Philadelphia, Pa.) for 1 h at 37°C and then washed.

After incubation with antibody, cells were washed once in serum-free medium and resuspended with 100 μ l of a 1:20 dilution of complement in serum-free medium, and the cell suspension was incubated for 30 min at 37°C. After complement treatment, cells were washed twice in Mg²⁺- and Ca²⁺-free Dulbecco's phosphate-buffered saline containing 0.1% gelatin and 0.1% sodium azide (PBS-azide). Cells were then stained for 30 min on ice with fluorescein isothiocyanate (FITC)-labeled F(ab')₂ goat anti-human C3 (Organon-Teknika-Cappel, West Chester, Pa.) diluted 1:200 in PBS-azide. After being stained, cells were washed in PBS-azide and fixed with 1% paraformaldehyde.

In some experiments, for amplification of complement

activation, cells were incubated with 0.1 ml of rabbit anti-human immunoglobulin G (IgG) (Organon-Teknika-Cappel) (100 μ g/ml) after incubation with huMAbs but before the addition of complement.

For assessment of the levels of monoclonal or polyclonal antibody bound to infected cells, cells were washed and then stained with a 1:80 dilution (in PBS-azide) of FITC-labeled F(ab')₂ goat anti-human immunoglobulin (Ig) (catalog number 4203; TAGO, Burlingame, Calif.) immediately after the antibody incubation step. Cells were washed and then fixed.

The green fluorescence of cells stained for C3 or Ig was analyzed with an Epics C flow cytometer (Coulter, Hialeah, Fla.). The fluorescence of cells is given as the mean channel intensity of fluorescence of 5×10^3 cells, as measured by logarithmic amplification, except as noted.

Release of RT from HIV by complement. Virus preparations used in virolysis experiments were produced by overnight culturing of washed, HIV-infected H9 cells at 2×10^6 cells per ml in fresh culture medium. Cell supernatants were collected after low-speed centrifugation. One-milliliter volumes of the virus supernatants were centrifuged at $15,000 \times g$ in a microcentrifuge for 2 h at 4°C, after which the virus was resuspended in 100 μ l of serum-free medium.

The virus was mixed with antibodies and/or complement to yield a final volume of 60 μ l. The final dilution of complement was 1:15, a dilution that has been shown to result in optimal reverse transcriptase (RT) release (20). The mixture was incubated at 37°C for 1 h, and the level of RT that was released was measured. The RT assay was an adaption of a previously described method (24), except that detergent was added to the RT buffer only to measure the maximal releasable activity. In some experiments, the virus was preincubated with CD4 (50 μ g/ml) before the addition of antibody and complement. Percent RT release was calculated by the following formula: (experimental cpm [without detergent] - untreated virus cpm [background]) / (experimental cpm [with detergent] - untreated virus cpm), where cpm is counts per minute.

RESULTS

Mediation by huMAbs of deposition of complement component C3 on HIV-infected cells. Experiments were performed to determine whether a variety of huMAbs to HIV envelope proteins gp41 and gp120 could activate complement on HIV-infected cells. Cells infected with HIV_{MN} were incubated with huMAbs, and complement activation was assessed by measuring the binding of C3 to the cell surface. Two huMAbs, 257-D and 268-D, which have been demonstrated to react with the third variable region of gp120 (V3 region), induced increased levels of C3 binding on the infected cells (Fig. 1A). The levels of bound C3 obtained when the highest concentrations of either of these two anti-V3 huMAbs were used were only slightly lower than the levels induced by a pool of sera obtained from infected donors and used at a 1:10 dilution. In contrast, huMAbs to the CD4-binding region of gp120 (Fig. 1A) as well as a huMAb to an immunodominant region of gp120 and a huMAb to p24 (data not shown) induced little if any complement activation. None of the huMAbs induced C3 deposition on uninfected cells (data not shown).

Similar experiments were also performed to assess complement activation by huMAbs that react with gp41. These huMAbs react with two separate regions of gp41, amino acids 590 to 600 and amino acids 644 to 663 (25). While some low-level C3 deposition was noted (Fig. 1B), the anti-gp41

FIG. 1. Complement activation by huMAbs on HIV_{MN}-infected cells. HIV_{MN}-infected cells were incubated with huMAbs at the concentrations shown plus complement. Activation was assessed by a flow cytometric determination of C3 levels on cells after staining with FITC-labeled F(ab')₂ goat anti-human C3. Antibody specificities are shown in parentheses. (A) Anti-gp120 huMAbs (representative of three experiments). (B) Anti-gp41 huMAbs (representative of three experiments). CD4bd is as defined in Table 1, footnote c. Ab, antibody.

huMAbs induced less C3 deposition than the anti-V3 huMAbs.

There are at least two possible explanations for the lack of efficient complement activation by the non-anti-V3 huMAbs. First, the huMAbs may have bound to the infected cells, but either the number of antibodies bound or the orientation of the antibodies may have been inadequate for efficient complement activation. Second, it is possible that the epitopes for those huMAbs were not exposed at the surface of the infected cells. To determine the more likely of the two explanations, we reacted cells with the huMAbs, washed them, and incubated them with rabbit anti-human IgG before incubating them with complement. This treatment substantially increased C3 deposition from that achieved with sub-optimal levels (8 μ g/ml) of anti-V3 huMAb 257-D to greater than that achieved with optimal levels (25 μ g/ml) (Fig. 2). The addition of the secondary layer of rabbit antibody increased C3 deposition with two anti-gp41 huMAbs and huMAb 559/64-D, which reacts with gp120 at the CD4 binding site, indicating that the epitopes for these huMAbs were indeed present to some extent on the surface of infected cells (Fig. 2). In contrast, increased C3 deposition was not observed with 448-D (specific for the CD4 binding site of gp120) or 450-D (specific for the C-terminal immunodominant domain of gp120), indicating that the epitopes for these huMAbs were not exposed at levels high enough to be detected. The largest increases in C3 deposition due to the rabbit antibody were observed with several V3-specific huMAbs that displayed low affinities (412-D, 419-D, and 311-11-D) (5a). These huMAbs, when used alone, resulted in relatively low levels of complement activation, compared

FIG. 2. Amplification of huMAb activation of complement on HIV_{MN}-infected cells. HIV_{MN}-infected cells were incubated with huMAbs alone (mAb alone) or with huMAbs and then rabbit anti-human IgG (mAb + 2nd Ab). Antibody specificities are shown in parentheses. After the cells were washed, complement was added and C3 deposition on the cells was assessed as described in the legend to Fig. 1. The final concentration of all huMAbs was 25 μ g/ml, except as follows: 257-D (lo), 8 μ g/ml; 71-31, 12 μ g/ml; and 240-D, 14 μ g/ml. The data are representative of two experiments, with a standard deviation of <10% of the mean. hi, high; c-term., C terminus. CD4bd is as defined in Table 1, footnote c.

with that achieved with 257-D. As expected, the addition of the secondary layer of rabbit antibody did not increase baseline C3 deposition with anti-p24 huMAb 71-31, since p24 is not expressed on the surface of infected cells.

To further explore the relationship between the binding of the huMAbs and C3 deposition, we stained separate aliquots of HIV_{MN}-infected cells with each huMAb at a high concentration (25 μ g/ml, except for 257-D and 268-D, which were used at several dilutions). One aliquot was then used to assess the level of Ig bound to the cells (x axis), while the other was used to assess C3 deposition with the huMAb (y axis). By analyzing and plotting the data in this way, we could easily visualize the relationship between binding and C3 deposition efficiency.

The pooled sera mediated high levels of C3 deposition, with relatively little Ig being detected on the cell surface (Fig. 3). In contrast, although much more Ig was bound to infected cells when the anti-V3 huMAbs were used, equivalent or slightly lower levels of C3 were deposited with these huMAbs than with the pooled sera.

While the other huMAbs induced C3 deposition less efficiently than the anti-V3 huMAbs, this method of plotting revealed discernible differences between some of the huMAbs. For example, one of the largest differences was found with 559/64-D, which binds to gp120 in the CD4 binding region. This huMAb bound to infected cells at detectable levels but activated little or no complement. In contrast, anti-gp41 huMAb 240-D bound even less to infected cells than 559/64-D but induced C3 deposition more efficiently.

Effect of CD4 treatment on complement activation by huMAbs. In the experiments described above, the anti-gp41 huMAbs caused relatively less C3 deposition than the anti-V3 huMAbs. To determine whether the relevant epitopes were sequestered by gp120, we pretreated HIV-infected cells with sCD4. Treatment with sCD4 has been shown to cause the dissociation of gp120 from some strains of the virus (13, 16), resulting in the exposure of certain gp41 epitopes. Strain HIV_{IIB} was compared with strain HIV_{MN}, since the dissociation of gp120 has been reported to be pronounced in strain HIV_{IIB} (13).

FIG. 3. Relationship of binding to complement activation by huMAbs. HIV_{MN}-infected cells were incubated with huMAbs at 25 μ g/ml, except for 240-D, which was used at 14 μ g/ml, while the pool of sera was used at a 1:10 dilution. In addition, the two anti-V3 huMAbs, 257-D and 268-D, were used at 1.5, 3.1, 6.3, 12.5, and 25 μ g/ml. Antibody specificities are shown in parentheses. After incubation with the antibodies, the cells were split into two tubes. In one tube, Ig binding was assessed by flow cytometry after staining with FITC-labeled F(ab')₂ goat anti-human Ig. The level of complement activated by the huMAbs was evaluated in the other tube by the method described in the legend to Fig. 1. The level of Ig binding was then plotted against the level of C3 binding. The data are the mean $\pm$ standard deviation for three experiments. CD4bd is as defined in Table 1, footnote c.

The anti-gp41 huMAbs (246-D, 167-D, 98-6, and 50-69) did not induce C3 deposition on HIV_{IIB}-infected cells, while the anti-V3 huMAb, 694-D, did (Fig. 4A). After treatment with sCD4, however, pronounced increases in complement activation were noted with several of the anti-gp41 huMAbs. The increased complement activation due to sCD4 was paralleled by increases in the amounts of the anti-gp41 huMAbs bound to the infected cells (Fig. 4B). Although both gp41 site I and gp41 site II determinants were exposed by sCD4 treatment, as determined by increases in Ig binding (Fig. 4B), only the two site I huMAbs, 246-D and 50-69, caused significantly increased C3 deposition. These two antibodies caused similar complement activation on the cells even though the 246-D isotype is IgG1 and the 50-69 isotype is IgG2. Interestingly, although a larger quantity of the anti-gp41 huMAbs with sCD4 treatment than of the anti-V3 huMAb 694-D with no sCD4 treatment was bound to HIV_{IIB}-infected cells, less C3 deposition was induced by the anti-gp41 huMAbs than by 694-D.

When HIV_{MN}-infected cells were used in these experiments, increases in the binding of the anti-gp41 huMAbs could also be detected with sCD4 treatment (Fig. 5B), although the increases were less dramatic than those observed when HIV_{IIB}-infected cells were treated with sCD4. Moreover, essentially no increases in complement activation due to the anti-gp41 huMAbs were noted after treatment with sCD4 (Fig. 5A). Decreases in C3 deposition mediated by, and binding of, anti-V3 huMAb 268-D were observed after sCD4 treatment, consistent with the possible dissociation of gp120 (Fig. 5).

In these experiments, treatment of HIV_{MN}- or HIV_{IIB}-infected cells with sCD4 did not decrease either C3 deposition or Ig binding mediated by the pooled sera (Fig. 4 and 5), suggesting that both anti-gp120 and anti-gp41 antibodies are present in patient sera and are capable of contributing to HIV-specific complement activation.

Complement-mediated virolysis induced by huMAbs. Pre-

FIG. 4. Effect of sCD4 treatment of HIV_{IIB}-infected cells on complement activation and binding mediated by huMAbs. HIV_{IIB}-infected cells were preincubated with or without sCD4 (50 μ g/ml). Complement activation (A) or Ig binding (B) mediated by the huMAbs was then assessed as described in the legend to Fig. 3. Antibody specificities are shown in parentheses. huMAbs were used at 25 μ g/ml, except that 240-D was used at 14 μ g/ml. The data are the mean $\pm$ standard deviation linear fluorescence for four separate experiments. The data from this experiment and the one shown in Fig. 5 are displayed as linear fluorescence to allow us to statistically analyze the data more accurately and visualize more easily the magnitude of the differences. An asterisk indicates a significant ($P < 0.05$; analysis of variance) difference between a huMAb and the control (both with no sCD4). A section sign indicates a significant difference between a huMAb without sCD4 and the same huMAb with sCD4. ND, not determined. CD4bd is as defined in Table 1, footnote c.

vious studies showed that free virus can be disrupted by the membrane attack complex of complement in the presence of HIV-specific antibody, as measured by the release of RT from the virions (20). Experiments were performed to determine whether the huMAbs assessed in this study could also mediate complement-dependent virolysis.

Preliminary experiments with 257-D showed that 4 μ g/ml resulted in maximal RT release (data not shown); therefore, this concentration was used in all of the following huMAb experiments. Anti-V3 huMAbs reactive with HIV_{IIB} and HIV_{MN} caused 12 to 20% release of RT from virus preparations (Fig. 6). In a previous study, this level of RT release

FIG. 5. Effect of sCD4 treatment of HIV_{MN}-infected cells on complement activation and binding mediated by huMAbs. See the legend to Fig. 4 for details.

was associated with a ca. 10-fold reduction in virus titer (20). Interestingly, anti-gp41 huMAb 50-69 was capable of causing RT release from native HIV_{IIB} that was nearly equivalent to the release caused by the anti-V3 huMAbs. The anti-gp120 huMAbs that bound at the CD4 binding domain did not cause the lysis of either virus isolate (data not shown).

Preincubation of virus preparations with sCD4 caused significant decreases in RT release mediated by the anti-V3 huMAbs but caused increases in RT release mediated by the anti-gp41 huMAbs. Complement alone did not mediate lysis of the virions (Fig. 6). The huMAbs alone or heat-inactivated complement in combination with any of the huMAbs also did not mediate virolysis (data not shown).

DISCUSSION

During HIV infection, complement activation by specific antibodies may provide and augment important antiviral effector mechanisms, such as direct virolysis of HIV and increased susceptibility of HIV-infected cells to killing by natural killer cells (reviewed in reference 18). This study shows that huMAbs to HIV can activate complement both on HIV-infected cells and on free virus and thus establishes a serologically defined model system for the further study of

FIG. 6. Direct lysis of HIV by anti-gp120 and anti-gp41 huMAbs. Preparations of HIV_{IIB} virus (A) or HIV_{MN} virus (B) were treated with huMAbs plus complement for 1 h. Antibody specificities are shown in parentheses. The level of RT released by the treatment was measured and expressed as a percentage of RT released by detergent. The mean $\pm$ standard deviation for four separate experiments is shown. An asterisk indicates a significant ($P < 0.05$; analysis of variance) difference between a huMAb and the control (both with no sCD4). A section sign indicates a significant difference between a huMAb without sCD4 and the same huMAb with sCD4. mAb, monoclonal antibody.

the interaction of complement and HIV. However, while complement has been clearly shown in this study and in others (11, 15, 18–20) to have substantial effects in vitro, no studies have yet been performed to determine whether similar effects may occur in vivo, so the clinical implications of this study are unclear at this time.

In this study, the anti-V3 antibodies caused complement-mediated effects on both HIV_{IIB} and HIV_{MN}, while the anti-gp41 huMAbs affected native HIV_{IIB} only. These results are consistent with reports that mature HIV_{IIB} virions lose gp120 in vitro by spontaneous shedding, thus exposing gp41 epitopes (13, 16). Treatment of cells or virus with sCD4 also provided access for complement activation by the anti-gp41 huMAbs on both HIV_{IIB} and HIV_{MN}, although the effects were more pronounced with HIV_{IIB}. These data suggest that epitopes capable of activating complement must have an appropriate critical density to allow bridging of C1q to at least two different IgG molecules.

While the activation of complement on free virus or infected cells was easily demonstrated with antibodies to the V3 region of gp120, antibodies to the other regions of gp120 were relatively inefficient at activating complement on virus

in its native form. There was little binding of the huMAbs to the non-V3 regions, indicating that the V3 region is relatively accessible for huMAb binding, compared with the other regions of gp120.

Interestingly, the anti-gp41 huMAbs appeared to be more efficient at activating complement on free virus than on infected cells. For example, while none of the anti-gp41 huMAbs significantly activated complement on HIV_{MN}-infected cells, either before or after sCD4 treatment (Fig. 5A), both 167-D and 50-69 caused significant lysis of free virus after sCD4 treatment (Fig. 6B). Similar results were found with HIV_{IIIB} and HIV_{IIIB}-infected cells, and 50-69 caused significant lysis of untreated free virus (Fig. 6A) but not activation on infected cells (Fig. 4A). These apparent differences in sensitivity could be explained in a number of ways. One intriguing explanation could be that some of the complement control proteins, such as CD59 or decay-accelerating factor (CD55), may not be incorporated into the virion during budding; this lack of incorporation not only would increase active C3 deposition but also would make formation of the membrane attack complex (C5b-9) more efficient on the virion than on the infected cells. Alternatively, gp120 may be shed more readily by the free virion than by the infected cells.

In this study, differences in isotype did not appear to have an effect on either virolysis or C3 deposition. For example, anti-V3 huMAbs 447-52-D (IgG3) and 694-D (IgG1) acted similarly when bound to HIV_{IIIB}. Also, anti-gp41 (site I-specific) huMAbs 50-69 (IgG2) activated complement in a manner similar to that of either 240-D or 246-D (IgG1).

This study also demonstrated the use of complement-mediated virolysis as a method for determining the accessibility of epitopes on the free virus surface. Further mapping of surface epitopes could be performed with either antisera to peptides or human sera absorbed with peptides.

In conclusion, this study showed that huMAbs to the V3 region of gp120 are most efficient in inducing complement-mediated effects on both free virus and infected cells but that elution of gp120 with sCD4 exposes epitopes on gp41 that can then also bind antibody and result in complement activation. These findings establish a serologically defined model system for the further study of the interaction of complement and HIV.

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