Neutralization of Human Immunodeficiency Virus Type 1 by Complement Occurs by Viral Lysis

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The ability of complement to inactivate human immunodeficiency virus (HIV) in the presence of specific antibody was evaluated. HIV was treated with complement and/or antibody, and then its titer was determined on the CD4⁺ H9 cell line. While complement alone had no effect on the HIV titer, complement plus subneutralizing levels of antibody resulted in titer reductions. Complement sources deficient in membrane attack component C5 or C8 did not inactivate antibody-treated HIV, suggesting that neutralization occurred via lysis. This possibility was investigated by assessing release of reverse transcriptase (RT) from the virion. Antibody plus complement, but neither reagent alone, released RT from HIV in a dose-dependent manner. Release of RT did not occur with C5- or C8-deficient sera, also indicating a requirement for membrane attack components. These studies show that complement can neutralize HIV via the classical complement pathway and that this neutralization occurs via C5b-9-mediated viral lysis. Thus, complement may play a major role in resistance to disease by lysing HIV and preventing infection of Fc- and complement receptor-positive cells, as well as CD4⁺ cells.

The complement system can neutralize viruses by several mechanisms (7, 12). Virus-specific antibody can activate either the classical or alternative complement pathway. Antibody-independent activation of the alternative pathway can also occur. Some animal retroviruses have been found to activate the classical complement pathway without antibody by direct binding of C1q to viral proteins, resulting in virus destruction (2, 6, 27). However, the human retroviruses human immunodeficiency virus (HIV) (1) and human T-cell lymphotropic virus type I (HTLV-I) (1, 15) are not destroyed by complement in this manner.

The ability of complement to neutralize HIV in the presence of specific antibody has not been reported. Complement, if activated by antibody, can neutralize viruses by at least two mechanisms, i.e., direct lysis and physical interference with infection by deposition of complement proteins on the virus surface (12). Evaluation of potential HIV neutralization by antibody and complement is important in view of reports that antibody or complement may actually, under some conditions, mediate or enhance the ability of HIV to infect cells that bear Fc or complement receptors (11, 14, 19, 22, 23, 25). Thus, if complement does not lyse HIV in the presence of antibody, even in the presence of antibody capable of neutralizing infection of CD4⁺ cells, the virus could remain infectious for both complement receptor- and Fc receptor-positive cells.

The goal of this study was to determine whether complement can inactivate HIV in the presence of antibody. Complement-mediated neutralization was found, and the mechanism by which it occurred was investigated.

MATERIALS AND METHODS

Cell and virus culture. The H9 T-cell line was used to propagate the HTLV-III_{RF} strain of HIV (21). The HTLV-III_{MN} isolate was contributed by Robert Gallo and obtained from the AIDS Research and Reference Reagent Program,

National Institutes of Health (Rockville, Md.). The HIV₇₆ primary isolate of HIV was obtained from peripheral blood mononuclear cells of an asymptomatic person (designated 76) by standard coculture methods and then propagated on H9 cells (24).

Infected H9 cells were grown in RPMI 1640 culture medium containing 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Whittaker MA Bioproducts, Walkersville, Md.) and supplemented with 10% heat-inactivated (HI) fetal bovine serum (Hyclone, Logan, Utah) and gentamicin (50 µg/ml). Infection by HIV was routinely verified by testing cells for HIV antigen by enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, Ill.). Virus preparations used in titration assays were produced by overnight incubation of washed cells infected 7 days before at approximately 1×10^6 to 2×10^6 cells per ml in fresh culture medium. Cell-free supernatants were collected after centrifugation, filtered (0.45-µm pore size), aliquoted, and stored at -70°C. For reverse transcriptase (RT) experiments, cell-free supernatants from infected cells were filtered and used immediately. All cell lines and virus stocks were periodically checked for mycoplasma contamination by using a commercial kit (Gen-Probe, San Diego, Calif.) capable of detecting a wide range of mycoplasma types.

Serum sources. Sera used as sources of complement or antibody were first tested by enzyme-linked immunosorbent assay (Abbott) for antibody to HIV. Antibody-positive (HIV⁺) sera were also tested by Western immunoblot (Du Pont Co., Wilmington, Del.) for reactivity to HIV proteins. The serum used as a complement source was collected from an HIV antibody-negative AB⁺ donor, aliquoted, and frozen at -70°C until use. The C8- and C2-deficient sera were obtained from patients with documented deficiencies. C5-depleted serum was purchased from Quidel (San Diego, Calif.).

For neutralization and RT release studies, sera from 10 HIV antibody-positive persons were pooled to provide a

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reagent that would contain antibodies reactive with a wide variety of HIV isolates. The sera used for the pool were positive by Western blot for all major HIV proteins, including p17, p24, p31, gp41, p51, p55, p66, gp120, and gp160. Positive sera were also tested for antibody to HTLV-I by enzyme-linked immunosorbent assay (Abbott). Only sera negative for HTLV-I were used. In some experiments, serum from patient 76 was also used as an antibody source. All sera used as antibody sources were HI (56°C, 30 min).

Anti-RT antibody was removed from the pooled sera for RT experiments only. Aliquots of serum (500 μ l) were passed over columns of recombinant RT (0.5 mg) (provided by Ian Bathurst, Chiron, Emeryville, Calif.) coupled to cyanogen bromide-activated Sepharose (Sigma Chemical Co., St. Louis, Mo.).

Virus titration. Undiluted HI pooled sera or HI 76 serum neutralized both HTLV-III_{RF} and HIV₇₆ significantly, so that the effect of complement could not be assessed accurately. Therefore, in neutralization assays, a 1:54 final dilution of both antibody sources was used. Dilutions of antibody and complement were made in serum-free RPMI 1640 medium. Equal volumes of virus, antibody, and complement were mixed (300- μ l final volume) and incubated at 37°C for 30 min. Immediately after incubation, 0.1 ml of undiluted or serially diluted treated virus was added directly to microcultures of H9 cells. The H9 cells were cultured for 7 days with feeding every other day. Cultures were assessed for HIV antigen by enzyme-linked immunosorbent assay (Abbott), and 50% tissue culture infective doses (expressed as log₁₀ virus titers per 0.1 ml) were calculated by the method of Reed and Muench. In some experiments, positive cultures were detected by RT activity.

RT assay. Incubation of virus with complement and antibody to measure RT release was done essentially as described above, except that the final incubation volume was 60 μ l and pooled sera (RT absorbed) were used at a 1:12 final dilution. The RT assay is an adaption (28) of a previously described method (10). Briefly, 10 μ l of culture supernatant was mixed with 50 μ l of an RT reaction mixture containing a template primer of (A)_n (5 μ g/ml) and (dT)₁₂₋₁₈ (1.57 μ g/ml) (both from Pharmacia LKB Biotechnology, Piscataway, N.J.) in 50 mM Tris (pH 7.8)–7.5 mM KCl–2 mM dithiothreitol–5 mM MgCl₂–0.5 μ Ci of [³²P]dTTP (400 Ci/mmol). To determine the detergent-releasable counts, the above-described buffer was made to contain 0.05% Nonidet P-40. Following incubation for 90 min at 37°C, 10 μ l of the reaction mixture was spotted onto DEAE ion-exchange paper (Whatman International Ltd., Maidstone, England) and washed four to six times in 2 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate) to remove unincorporated [³²P]dTTP. Papers were counted in a scintillation counter. Percent maximal release was calculated by the following formula: (experimental cpm – untreated virus cpm)/(maximal cpm – untreated cpm).

RESULTS

Neutralization of HIV by complement. While complement alone has been found to neutralize many viruses, Banapour et al. (1) have demonstrated that complement alone has no effect on HIV. In the present study, the infectivities of two HIV isolates, HTLV-III_{RF} and HIV₇₆, were unchanged by treatment with complement, confirming the results of Banapour et al. (1) (Fig. 1; data not shown).

Since essentially all infected patients develop antibody to HIV, the combination of antiserum plus complement was

FIG. 1. Dose dependency of reduction in HIV titer by complement (C) plus antibody (Ab). The log₁₀ reduction in HIV titer over the control (medium) treatment was determined for complement-only, antibody-only (pooled antibody, 1:54), or complement-plus-antibody treatment of HTLV-III_{RF}. The mean $\pm$ the standard error of the mean of four experiments is shown. *, $P \leq 0.05$ compared with antibody-only treatment (Student *t* test). Complement-only treatment at all dilutions and no-virus treatment were not significantly different.

tested for the ability to neutralize HIV. Pooled sera used as a source of anti-HIV antibody was HI to destroy complement activity and diluted to have a low neutralizing effect. When virus was treated with pooled sera alone, a low level of neutralization was observed (Fig. 1). In contrast, addition of complement, at final dilutions of 1:3 and 1:12, to pooled sera resulted in significant decreases in HIV titer (Fig. 1). While some HIV neutralization was noted with complement at a final dilution of 1:48 plus antibody, it was not significantly greater than that observed with antibody treatment alone. Complement treatment alone did not significantly affect HIV titer (Fig. 1).

The ability of complement at a dilution of 1:12 to neutralize two virus isolates in combination with pooled sera or antiserum from one person was tested. Complement plus antibody (pooled or antibody from patient 76) reduced HTLV-III_{RF} titers by an average of 88% compared with antibody treatment alone (Fig. 2). Complement plus antiserum from patient 76 reduced HIV₇₆ titers by 80% over antibody treatment alone, while complement and pooled antisera reduced HIV₇₆ titers by 47% over treatment with pooled antisera alone (Fig. 2). In two of the experiments shown in Fig. 2, HI of the complement source destroyed the neutralizing activity (data not shown).

It was possible that antibody-plus-complement treatment neutralized either by lysis or by deposition of complement proteins on the surface of HIV, which would interfere with cell binding or entry of the virus. To determine which mechanism(s) is important for complement-plus-antibody-mediated neutralization of HIV, virus was treated with pooled antisera along with fully active, C5-depleted or C8-deficient complement and the level of neutralization was compared with the level of neutralization caused by treatment with HI complement. The C5 and C8 complement components are part of the membrane attack complex of complement which is necessary for pore formation and viral lysis. Thus, C5-depleted and C8-deficient sera do not mediate viral lysis but do allow deposition of components C1q, C4, C2, and C3 on the virus surface, which for herpes simplex virus, vaccinia virus, or vesicular stomatitis virus is

FIG. 2. Reduction in titer of HIV isolates by complement (C) in the presence of anti-HIV sera. HTLV-III_{RF} or HIV₇₆ was treated with either pooled or 76 antibody alone or antibody with complement (1:12 final dilution). The titer of the virus was determined, and the percent reduction caused by antibody-plus-complement treatment over antibody-only treatment is shown (average of four experiments). Complement-only treatment had no effect on titer in these experiments.

sufficient for neutralization (8, 17). While pooled sera along with normal complement neutralized HIV, no neutralization with C5-depleted or C8-deficient complement was detected (Table 1). These results indicated that the terminal attack components of complement were necessary for neutralization, which in turn suggests that neutralization was due to lysis of HIV virions.

Release of RT by antibody and complement. Intact virions do not express RT activity until the virus envelope is disrupted. If complement-and-antibody treatment results in virion lysis, RT activity should be released, as with animal retroviruses (2, 6, 27). Initial experiments to detect RT release demonstrated a complicating factor; i.e., sera from HIV-infected persons contained anti-RT antibody which inhibited detection of RT (data not shown), as also reported by others (16). To avoid this problem, the anti-RT antibody was removed from sera by passage over an RT affinity column before use. No RT-inhibitory activity was detected in the resulting antisera (data not shown).

Incubation of HTLV-III_{RF} with antibody plus complement at dilutions of 1:3 to 1:27 resulted in release of RT (Fig. 3), while antibody plus complement at higher dilutions (1:81 and 1:243) did not result in measurable RT release. In contrast, treatment of virus with complement alone or antibody alone did not result in detectable release of RT (Fig. 3).

The ability of complement to release RT from diverse isolates of HIV was also evaluated. Pooled antibody in conjunction with complement released RT from HTLV-

FIG. 3. Release of HIV RT by complement (C) and antibody (Ab) treatment. Mean RT activity ($\pm$ the standard deviation) of HTLV-III_{RF} is expressed as a percentage of the detergent-releasable counts per minute of untreated virus. Detergent lysis of virus gave 14,095 cpm in this experiment. Data representative of three experiments are shown.

III_{MN} and HIV₇₆ in addition to HTLV-III_{RF}, while complement or antibody alone did not induce release (Table 2).

The mechanism by which complement released RT was also assessed by using sources of complement deficient in or depleted of one complement component. While fully active complement and antibody induced RT release, sera deficient in C2 did not, indicating that RT release was induced by the classical pathway (Table 3). As in the neutralization experiments, sera deficient in C5 or C8 were not able to cause RT release (Table 3). Mixing of the deficient sera restored RT-releasing activity (Table 3). In two experiments, addition of subphysiologic, limiting amounts of highly purified C2, C5, or C8 to the appropriate deficient sera also partially restored RT-releasing activity (8.8, 5.9, and 6.2% specific RT release, respectively; data not shown).

DISCUSSION

This study shows that complement combined with antibody can inactivate HIV. The requirement for antibody, together with the observation that C2-deficient serum plus antibody does not release RT, indicates that HIV neutralization occurred by the classical complement pathway. This mechanism of neutralization is similar to that shown for many other enveloped viruses (12).

This study also provides evidence that the mechanism of neutralization is viral lysis by showing that the membrane attack complex of complement is required and that RT is released during incubation with complement. Some animal

TABLE 1. Requirement of terminal complement components for HIV neutralization^a

Virus treatment	Log ₁₀ HIV titer/0.1 ml
Antibody-HI normal complement	2.7
Antibody-normal complement	1.0
Antibody-C5-depleted complement	3.0
Antibody-C8-deficient complement	2.7

^a Pooled antibody was used at a final dilution of 1:54. Each complement source was used at a final dilution of 1:3. Data representative of two experiments are shown.

TABLE 2. Complement and antibody induction of RT release from diverse HIV isolates

HIV isolate	% RT activity ^a	
	Complement only	Complement-antibody
HTLV-III _{RF}	0.3 $\pm$ 0.1	18.7 $\pm$ 2.1
HTLV-III _{MN}	0.0 $\pm$ 0.0	15.8 $\pm$ 1.4
HIV ₇₆	0.8 $\pm$ 0.3	21.8 $\pm$ 0.5

^a Mean percent maximal release $\pm$ the standard deviation is shown. The data are representative of two experiments. With antibody only, all values were 0.0 $\pm$ 0.0.

TABLE 3. Requirement of C2 and terminal complement components for antibody-mediated RT release

Serum used as complement source ^a	No. of expts	% of maximal detergent-releasable RT activity		
		Antibody only	Complement only	Antibody-complement
Normal human	6	0.1	0.7	25.3
C2 deficient ^b	3	0.3	0.1	0.0
C5 depleted	3	0.3	0.7	0.2
C8 deficient	3	0.3	0.0	1.0
C2 deficient and C5 depleted	1	0.8	0.3	25.0
C2 and C8 deficient	1	0.8	0.7	13.0

^a Complement was used at a 1:12 final dilution, except for C5-deficient serum, which was used at a 1:3 final dilution in two experiments.

^b C8- and C2-deficient sera were obtained from patients with documented deficiencies. C5-depleted serum was purchased from Quidel (San Diego, Calif.).

retroviruses have also been shown to be susceptible to complement-mediated lysis and neutralization, although the mechanism of complement activation was antibody independent (2, 6, 27). The human HTLV-I retrovirus has been shown by Hoshino et al. (15) to be resistant to lysis by complement with or without the presence of anti-HTLV-I antibody. Thus, the current study is the first description of susceptibility of a human retrovirus to complement-mediated lysis.

Many recent studies have shown that immunoglobulin G-coated HIV can infect cells after binding to Fc receptors (14, 19, 25). Thus, anti-HIV antibody that is neutralizing for Fc receptor-negative cells may actually enhance infection of Fc receptor-positive cells. Monocytes and macrophages are Fc receptor-positive cells that are possible *in vivo* targets of immunoglobulin G-enhanced infection (3). However, the current study suggests that lysis of HIV by complement subsequent to HIV and immunoglobulin G interaction may abrogate Fc receptor-mediated enhancement of infection.

Robinson et al. have reported that complement deposition on HIV may also enhance infection of complement receptor-bearing cells (22, 23). However, these studies have not reported the effect of complement at concentrations higher than a 1:40 final dilution. The present study showed no significant neutralization of HIV at a 1:48 dilution of complement. Additionally, no lysis of HIV was observed at final complement dilutions of >1:27 (Fig. 3). Therefore, it seems likely that at higher concentrations of complement, complement-mediated enhancement of HIV infection may disappear because of neutralization. Preliminary experiments performed in this laboratory support this contention (G.T.S., unpublished data).

This study suggests that complement in conjunction with antibody in the blood of HIV-infected individuals may lyse HIV and destroy infectivity. However, the presence of infectious virus in plasma from HIV-infected persons has been reported (5, 9, 13). Virus titers were found to be 10-fold (5) to 100-fold (13) higher in symptomatic persons than in asymptomatic persons. There are several possible mechanisms by which plasma virus could escape complement lysis. Infectious virus found in plasma may be resistant to complement lysis because of low levels of complement components during infection. Significant depletion of classical pathway components and increases in complement activation products have, in fact, been reported in patients with acquired immunodeficiency syndrome (18, 20, 26). This

activation and depletion may be, to a large extent, due to antibody complexed to HIV or HIV antigens (4). Alternatively, virus present in plasma could represent complement-resistant virus due to mutated envelope proteins. Thus, complement-activating anti-HIV antibodies present in plasma may be unable to bind to, and thus lyse, HIV isolates from plasma of the same individual. The virus found in plasma may, however, represent complement-sensitive virus that was recently released by cells and which is in the process of being lysed. In fact, free HIV antigen which may represent lysed HIV is found in these patients (5, 13). Further studies in which the sensitivities of virus from plasma and other viral isolates to complement lysis are compared and which attempt to correlate complement levels and viremia are needed.

In conclusion, the results of this study show that complement can have significant effects on HIV infectivity. These findings emphasize the importance of considering the contribution of complement to HIV neutralization in studies which address HIV-antibody interaction.

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