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Increased Adhesion as a Mechanism of Antibody-Dependent and Antibody-Independent Complement-Mediated Enhancement of Human Immunodeficiency Virus Infection

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Enhancement of human immunodeficiency virus (HIV) infection by complement alone or in conjunction with antibodies was studied experimentally and theoretically. Experimental studies showed that while HIV-positive sera neutralize HIV infection, the addition of fresh complement abrogated neutralization and could even cause enhancement. Enhancement was blocked by anti-complement receptor 2 antibodies, and infection under enhancing conditions could be blocked by soluble CD4. Antibody-dependent complement-mediated enhancement (C'ADE) was dependent on the alternative complement activation pathway, as factor B-deficient serum could enhance only after the addition of factor B. The observed enhancement was also antibody dependent, since the addition of antibodies increased the level of enhancement. Under C'ADE conditions, infection reached a plateau within 5 min and was not caused by activation of cells by factors in the human serum. On the contrary, preincubation of cells with complement decreased the level of enhancement. A theoretical model of HIV infection in vitro which exhibited similar enhancement in an antibody- and complement concentration-dependent way was developed. Model studies indicated that the enhanced infection process could be explained by the fact that virions, because of complement deposition on the surface, bind more efficiently to cells. The model also indicated that the saturation of the enhanced infection process seen after a few minutes could be caused by saturation of the complement receptors. The effect of neutralizing antibodies can thus be overcome by the enhancing effect of complement that facilitates the contact between gp120 and CD4. These studies demonstrate that the main features of the complement-dependent enhancement phenomenon can be understood in terms of a simple mathematical model.

Complement-mediated antibody-dependent enhancement (C'ADE) has been found in vitro both in sera from three of four volunteers vaccinated with recombinant vaccinia virus expressing human immunodeficiency virus type 1 (HIV-1) gp160 and boosted with baculovirus-derived recombinant gp160 (rgp160) (20) and in 25% of healthy adults vaccinated with HIV-1 rgp160 (8). It is therefore possible that C'ADE reduces or even reverses vaccine efficacy. HIV and HIV-infected cells either in conjunction with or independent of antibody can activate the complement cascade by the classical or the alternative pathway (7). The complement system is chronically activated throughout the infection in vivo, probably resulting in the reduced levels of complement found in HIV-infected patients by several groups (7, 24). C'ADE and complement-mediated antibody-independent enhancement (C'AIDE) may contribute to the in vivo pathogenesis of HIV infection. This hypothesis is supported by the findings that 10 to 50% of human $CD4^+$ T lymphocytes express complement receptor 2 (CR2) and that this fraction is reduced during HIV infection, suggesting that these $CD4^+$ $CR2^+$ T cells are selectively infected and killed either by direct cytopathic effects of HIV or by HIV-specific immunological mechanisms (14). Complement-coated cells have also been shown to be more sensitive to attack by natural killer cells (37). Although it has been reported in several instances (26, 34), viral neutralization by complement-mediated lysis is still controversial (7, 33).

It is generally believed that C'ADE requires complement receptors and CD4 receptors (30, 35), since C'ADE can be blocked by antibodies against CR2 (10, 31) and CR3 (28). Complement-mediated binding to target cells seems independent of CD4 (1, 21, 22), but infection is generally believed to require CD4 receptors (7, 10, 11, 22), although CD4-independent C'ADE infection of MT-2 cells has been reported (3, 7).

At least three fundamentally different hypotheses can be proposed to explain the observed enhancement phenomena. (i) Antibodies and/or complement may increase adhesion of HIV to complement receptor-bearing cells. C3 fragments deposited on either gp41 (29, 30) or other epitopes (13), classically (normally antibody dependently) or alternatively (normally antibody independently), could increase the binding of HIV to target cells possessing complement receptors (CR2 in the case of MT-2 cells) and thereby facilitate the contact between gp120 and cell-associated CD4, causing increased virus uptake (1) and provirus formation (15). (ii) Complement bound to virus may facilitate HIV-cell fusion. (iii) Complement deposited on HIV may have nonspecific stimulatory effects on target cells, such as increasing cellular metabolism and virus production. C3a and C5a, which stimulate many immune cells, are released during complement activation and could have such effects. Alternatively, cells may also be stimulated by CR2 cross-linking or CR2 and CD4 cross-linking caused by complement deposition on cells (15).

It is not trivial to predict the effect of antibodies and complement on HIV infection, since both have been shown to have

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neutralizing as well as enhancing effects. We have previously shown that antibody-dependent enhancement of HIV infection of U937 cells is well explained by a mathematical model based on a hypothesis of increased adhesion of antibody-coated virions to target cells (19). In this study we have investigated the mechanisms of complement and antibody-dependent enhancement of HIV IIIB infection of MT-2 cells experimentally and examined whether a mathematical model based on the observed phenomena shows similar infection kinetics and antibody and complement dose dependence.

MATERIALS AND METHODS

Cells and virus. The human T-lymphoblastoid cell line used in the experiments was MT-2, a human T-cell leukemia virus type I (HTLV-I)-transformed $CD4^+$ and $CR2^+$ cell line (repository reference ADP014 obtained from the Medical Research Council, MRC, London, England). Cells were cultured in RPMI 1640 containing 10% heat-inactivated fetal bovine serum, 100 IU of penicillin per ml, 20 μg of gentamicin per ml, and 100 IU of streptomycin (growth medium) at a density of 0.2×10^6 to 1.2×10^6 cells per ml, and maintained at 37°C and 5% CO_2. The HIV-1 laboratory strain HIV IIIB (obtained from R. C. Gallo, National Cancer Institute, Bethesda, Md.) was obtained as a supernatant from freshly infected H9 cells (25). Supernatants were harvested, filtered (pore size, 0.45 μm), aliquotted, and stored at −80° until use. The approximate 50% tissue culture infectious dose ($TCID_{50}$) of HIV IIIB was calculated by end point titration with MT-2 cells by the method of Reed and Muench (27). Cells (5×10^5) were inoculated with a fivefold dilution series of virus for 3 h and then extensively washed and cultured in quadruplicates of 5×10^4 cells. The HIV antigen concentrations in the supernatants were determined by HIV antigen enzyme-linked immunosorbent assay (ELISA) as described below.

ELISA for HIV antigen. Cell-free culture supernatants were examined for HIV antigen with a biotin-avidin potentiated double-antibody ELISA with human immunoglobulin G against HIV, as described previously (12, 23). Each plate included a dilution series of a standard HIV antigen preparation, and optical densities were expressed relative to this standard in arbitrary units.

Sera and reagents. Human serum used as a complement source (i.e., fresh human serum) was collected from an HIV-negative donor, aliquotted, and stored at −80°C until use. For neutralization, HIV IIIB neutralizing serum (anti-HIV serum) was obtained from an HIV-positive patient. Serum was heat inactivated at 56°C for 30 min, aliquotted, and stored at −20°C. Further, a serum sample was freshly frozen and stored at −80°C to be used as an autologous complement source. Factor B-deficient serum, factor B, and a standard serum were purchased from Sigma (catalog numbers C0535, C4909, and C2038, respectively). Monoclonal antibodies directed to CR2 (OKB7) and CD4 (OKT4) were purchased from Ortho Diagnostic Systems (Raritan, N.J.; catalog numbers 700720 and 700420, respectively). All antibodies were dialyzed and filtered (pore size, 0.22 μm) before use. Recombinant soluble CD4 (sCD4), produced in CHO cells, was obtained from Neosystem Laboratoire, Strassbourg, France (catalog number SC 925100).

Infection neutralization and enhancement assay. To determine whether enhancement occurred, a dilution series of anti-HIV serum was mixed with a dilution series of complement, and then 25 $TCID_{50}$ of HIV IIIB was added. After preincubation for 1 h at 37°C, 5×10^5 MT-2 cells in growth medium were added and inoculated for 3 h at 37°C. After an extensive washing, cells were resuspended in 2 ml of growth medium, and quadruplicates of 5×10^4 cells were transferred to a 96-well cell culture plate (Nunc, Roskilde, Denmark), and plates were incubated at 37°C in 5% CO_2 for 4 days. Supernatants were then harvested, and the HIV antigen concentration was measured.

Unless otherwise stated, experiments were performed at least twice with identical results. Means of quadruplicates were compared by a Student's *t* test (two-sided), allowing for unequal variances.

Model formulation. The model is based on an in vitro system in which a population of target cells is infected by a population of HIV virions. It is an extension of the model proposed by Lund et al. (19) for antibody-mediated enhancement. Target cells and HIV are present in a supernatant in concentrations M and V, respectively. It is assumed that the concentration of target cells, M, is constant. The concentration of live virions is assumed to decay exponentially at a per capita rate, k_n. The concentration of free live virions will also diminish because of adhesion to the target cells at rate k_aMV, thus giving rise to a population of cell-bound virions, the concentration of which we shall denote as B. k_a is the time-dependent association rate for the complex formation. The adhered virions will infect the cells at rate k_iB, where k_i is the infection rate per adhered virion. The concentration of cell-bound virions is also assumed to decrease because of inactivation of the adhered virus at rate k_nB. The concentration of infected cells, which we shall denote as I, rises at a rate equal to the infection by cell-bound virions. It is assumed that the multiplicity of infection is low. The above assumptions lead to the following equations:

$$\frac{dB}{dt} = k_aMV - k_nB - k_iB \tag{1}$$

$$\frac{dI}{dt} = k_iB \tag{2}$$

$$\frac{dV}{dt} = -k_aMV - k_nV \tag{3}$$

The rate of reaction, k_a, can be calculated as the product of the maximum rate constant for diffusion-limited aggregation, k_d, and the probability of reaction, P_a. $k_d = 4\pi(D_c + D_v)(r_c + r_v)$, where D and r are the diffusion constants and radii for cells (subscript c) and virus (subscript v), respectively (4). The diffusion constant for a particle with radius r is $D = kT/6\pi\eta r$, where k is the Boltzman constant, T is the absolute temperature, and η is the viscosity of the liquid in which the particle diffuses (4). As shown by Berg and Purcell (2), the probability (P_a) that a small molecule which has arrived at the surface of a cell will find a receptor to bind to can be estimated as $P_a = Nr_r/(Nr_r + \pi r_c)$. Here, N is the number of receptors on the cell surface to which the small molecule can bind, and r_r is the functional radius of these receptors, i.e., the radius within which the small molecule has to be in order to bind to the receptor. A similar result was obtained by Shoup and Szabo (32) by a different approach in which they used the result obtained by Collins and Kimball (5) that the forward reaction rate for a ligand to a partly reacting sphere (k_a) can be written as $k_a = k_d\kappa/(k_d + \kappa)$. By choosing the Collins-Kimball constant κ as $N4Ds$, where N is the number of receptors on the cell surface and $4Ds$ is the reaction rate of a point ligand to a single reactive circular site with radius s on an infinite unreactive surface, Shoup and Szabo immediately recover the result obtained by Berg and Purcell. To extend this finding to a virus only partly covered with ligands for receptors on the cell, the Collins-Kimball constant is redefined as

$$\kappa = 4D\sum_i f_iN_is_i \tag{4}$$

where f_i is the fraction of the virus covered with ligand for the receptor of type i of which there are N_i on the cell surface. We thus obtain

$$P_a = \frac{\sum_i(f_iN_is_i)}{\sum_i(f_iN_is_i) + \pi r_c} \tag{5}$$

In the case of HIV, the fraction of the surface that can bind to CD4 is

$$f_c = \frac{N_gr_g^2}{4r_v^2} \tag{6}$$

where N_g and r_g are the number and radius of gp120 molecules on the surface of an HIV virion, respectively. In this equation it is assumed that the binding of antibody to gp120 does not block the binding of gp120 to CD4. Correspondingly, the fraction of the viral surface that can bind to complement receptors on the cell surface is

$$f_k = \frac{N_kr_k^2\psi_k}{4r_v^2} \tag{7}$$

where N_k is the number of sites on the virus where complement can bind and r_k is the radius of the complement receptor-binding site on deposited complement. ψ_k is the fraction of complement-binding sites occupied. The probability of adhesion (P_a) for this two-receptor system is

$$P_a = \frac{f_cN_{CD}r_{CD} + f_kN_{CR}r_{CR}}{f_cN_{CD}r_{CD} + f_kN_{CR}r_{CR} + \pi r_c} \tag{8}$$

Here r_{CD} and r_{CR} are the radii of the CD4 and the complement receptors, respectively. Using a quasi-steady-state assumption, the numbers of free CD4 receptors (N_{CD}) and complement receptors (N_{CR}) are calculated as

$$N_{CD} = \max(0, N_{CDt} - \beta BN_g/M) \tag{9}$$

$$N_{CR} = \max(0, N_{CRt} - \beta BN_k\psi_k/M) \tag{10}$$

where N_{CDt} and N_{CRt} are the average number of CD4 and CR2 receptors on the cells, respectively, and $N_k\psi_k$ is the average number of complement molecules on each virion. In the above equation it is assumed that HIV binds to a cell with the ligands on a contact surface which is assumed to cover a fraction (β) of the virion surface.

Under the assumption of antibody excess, the fraction of gp120 molecules complexed with antibodies (ψ_a) can be calculated as (6, 19).

$$\psi_a = \frac{a}{1/k_m + a} \tag{11}$$

where a is the concentration of antibodies and k_m is the equilibrium constant for

TABLE 1. Parameters used in the simulations in this study

Parameter	Symbol	Value	Reference
Death rate for HIV	k_n	3.8×10^{-5}/s	18
Radius of HIV	r_v	5.0×10^{-8} m	
Radius of a lymphocyte	r_c	5×10^{-6} m	16
Absolute temperature	T	310 K (≈37°C)	
Viscosity for water at 37°C	η	6.9×10^{-4} Ns/m^2	36
Radius of a gp120 CD4-binding site	r_g	4×10^{-9} m	
Radius of a complement CR2-binding site	r_k	4×10^{-9} m	
Radius of a CD4 gp120-binding site	r_{CD}	4×10^{-9} m	
Radius of a CR2 complement-binding site	r_{CR}	4×10^{-9} m	
No. of CD4 molecules/cell	N_{CDt}	1×10^4	
No. of CR2 receptors/cell	N_{CRt}	1×10^4	
No. of gp120 molecules/virion	N_g	10	17
No. of complement-binding sites/virion	N_k	240	
Infection rate	k_{i0}	10^{-3}/s	
Affinity of antibodies	k_m	10^7 M^{-1}	
Antibody-independent complement-binding constant	k_1/k_-	1	
Antibody-dependent complement-binding constant	k_2/k_-	1,000	
Fraction of bound HIV virion in contact with cell	β	0.25	
Fraction of HIV virions which are infectious	γ	10^{-6}	17

the reaction. Similarly, the fraction of complement-binding sites occupied (ψ_k) is written

$$\psi_k = \frac{K}{k_-/(k_1 + k_2\psi_a) + K} \quad (12)$$

where K is the concentration of complement relative to the concentration of complement in normal human serum, and k_- is the dissociation rate for complement. The association rate is assumed to be a linear combination of an antibody-independent term (k_1) and an antibody-dependent one ($k_2\psi_a$). Furthermore, we assume that HIV infects the cells that it has adhered to at a per capita rate (k_i) proportional to the number of non-antibody-complexed gp120 molecules on the viral surface:

$$k_i = k_{i0}(1 - \psi_a)\gamma \quad (13)$$

Here k_{i0} is the infection rate for a virion with no gp120 complexed with antibodies, and it is assumed that a fraction ($\gamma = 10^{-6}$) is infectious. This value of γ lies in the range found by Layne et al. (17).

The parameters used in the simulations are listed in Table 1. Simulations of the model were performed on a 66 MHz, 486 PC with a five-stage Runge-Kutta formula pair, RKN(3,4) (9), implemented in Borland Pascal (c) to solve the set of coupled nonlinear equations.

TABLE 2. Effects of sera on infection of MT-2 cells with HIV-1 IIIB

Fresh serum (dilutions)	Infection[a] with the following dilutions of heat-inactivated serum:				
	0	0.001	0.01	0.02	0.1
0	1.00 ± 0.15	0.92 ± 0.11	0.25 ± 0.10	0.08 ± 0.03	0.04 ± 0.01
0.001	1.32 ± 0.20	1.68 ± 0.09	2.10 ± 0.38	1.63 ± 0.23	0.24 ± 0.13
0.01	1.15 ± 0.08	2.27 ± 0.31	2.08 ± 0.33	2.01 ± 0.30	1.25 ± 0.08
0.1	1.98 ± 0.23	2.43 ± 0.22	1.98 ± 0.22	1.79 ± 0.20	1.55 ± 0.05

[a] Results (in arbitrary units) are the means ± standard deviations of quadruplicate determinations normalized to values for a control culture to which no sera were added.

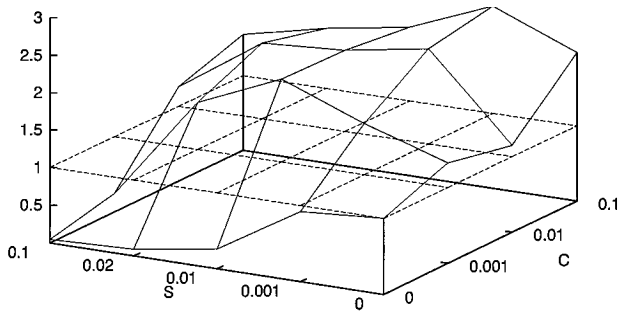

FIG. 1. Three-dimensional plot of the relative level of infection. Shown are the effects of dilution series of fresh human serum (C) and heat-inactivated serum from an HIV-1-infected patient (S) on infection of MT-2 cells with HIV-1 IIIB, from the data listed in Table 2. ——, S plus C; – – – –, control.

RESULTS

In vitro experiments. (i) Enhancement or inhibition depends on concentrations of antibodies and complement. The infection enhancement and inhibition assays were performed with MT-2 cells. HIV IIIB was preincubated with various concentrations of antibodies from heat-inactivated serum from an HIV-1-infected patient and complement from fresh serum from an uninfected donor. Table 2 and Fig. 1 show the effects of combinations of antibodies and complement on the infection. When no complement was added, a dose-dependent neutralization as a function of the antibody concentration was seen ($P < 0.01$ when the dilution of antibody was less than 1:1,000). When no antibody was added, a dose-dependent enhancement as a function of the complement concentration was seen ($P < 0.01$ for a dilution of 1:10). For combinations of antibody and complement, the highest enhancement was seen for antibodies in a dilution of 1:1,000 and complement in a dilution of 1:10.

(ii) Diluted complement from an HIV-1-infected patient can enhance infection. To evaluate whether fresh serum from an HIV-1-infected patient can enhance infection, combinations of fresh serum and heat-inactivated serum from the same HIV-1-infected patient were tested for enhancing and neutralizing effects. Table 3 and Fig. 2 show that when the dilution of serum (fresh plus heat inactivated) was lower than or equal to 1:10 or when only heat-inactivated serum was added, neutralization was seen; otherwise, enhancement was seen.

(iii) Enhancement can be blocked by heat inactivation of serum or by anti-CR2 antibodies. Enhancement is abrogated and antibody-mediated neutralization is regained during infection with heat-inactivated sera, as seen in Fig. 2. To evaluate the involvement of CR2, MT-2 cells were preincubated with the monoclonal anti-CR2 antibody OKB7 for 1 h, and then

TABLE 3. Combinations of fresh and heat-inactivated sera from an HIV-1-infected patient can inhibit or enhance infection of MT-2 cells with HIV-1 IIIB, depending on the concentrations

Fresh serum (dilutions)	Infection[a] with the following dilutions of heat-inactivated serum:				
	0	0.001	0.01	0.02	0.1
0	1.00 ± 0.11	0.86 ± 0.15	0.18 ± 0.05	0.11 ± 0.07	0.04 ± 0.01
0.001	1.94 ± 0.27	1.82 ± 0.11	1.70 ± 0.11	1.50 ± 0.09	0.21 ± 0.11
0.01	1.70 ± 0.19	1.96 ± 0.27	1.89 ± 0.22	1.77 ± 0.22	0.92 ± 0.16
0.1	0.10 ± 0.02	0.29 ± 0.17	0.14 ± 0.03	0.15 ± 0.05	0.18 ± 0.02

[a] Results (in arbitrary units) are the means ± standard deviations of quadruplicate determinations normalized to values for a control culture to which no sera were added.

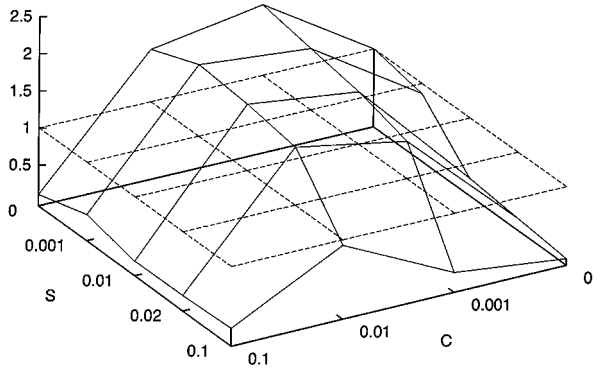

FIG. 2. Three-dimensional plot of the level of infection as a function of dilution series of antibodies (S) and complement (C), from the data listed in Table 3. Note that we have used a perspective different from that of Fig. 1. ——, S plus C; – – – –, control.

cells were incubated with HIV for 3 h. The enhancement found when HIV was preincubated with complement in a dilution of 1:100 and HIV-positive serum in a dilution of 1:1,000 relative to a control to which no serum was added was decreased 2.7-fold by preincubating the cells with anti-CR2 antibodies (OKB7), but not with an indifferent antibody (OKT4), indicating that enhancement of MT-2 cells by HIV-1 IIIB is complement mediated and requires CR2.

(iv) Infection under enhancing conditions can be blocked with sCD4. To delineate whether gp120 binding to CD4 is necessary for infection, we tested the effect on infection of preincubating HIV for 1 h with various dilutions of sCD4 together with complement in a dilution of 1:100 and/or antibodies in a dilution of 1:1,000, before the addition of cells for 3 h. Both with enhancing concentrations of antibodies and/or complement and with the control, a dose-dependent neutralizing effect was seen (Fig. 3). Thus, CR2 is not an alternative receptor which renders MT-2 cells permissive to HIV without interaction with the CD4 receptor.

(v) Enhancement is mediated via the alternative complement activation pathway. To evaluate whether enhancement occurred by the classical or the alternative pathway, HIV was preincubated with a dilution series of factor B-deficient serum which is deficient in the alternative complement activation pathway. Preincubating HIV with factor B-deficient serum (Sigma) gave no enhancement in the presence or absence of heat inactivated serum from an HIV-1-infected donor in a dilution of 1:1,000 (Fig. 4). Enhancement was seen after restoration of the alternative pathway by the addition of factor B ($P < 0.05$) during preincubation, and the enhancement was amplified in the presence of HIV-positive serum ($P < 0.05$). The addition of factor B to normal, fresh human serum did not alter the level of infection (data not shown).

(vi) Infection under C'ADE conditions increases the virus titer 27-fold. Preincubating HIV in a twofold dilution series with HIV-positive serum in a dilution of 1:1,000 and fresh human serum in a dilution of 1:100 for 1 h before the addition of cells for 3 h resulted in an end point virus titer 27-fold higher (titer = 1,024) than that in a control to which no serum was added (titer = 37).

(vii) Enhancement is not due to activation of cells by factors in serum. In order to examine whether enhancement could be due to serum factors activating the cells to become more susceptible to HIV infection or inclined to produce more virus, we preincubated the cells with heat-inactivated HIV-positive serum in a dilution of 1:1,000 and/or fresh human complement in a dilution of 1:100 for 30 min before a washing and subsequent incubation with virus for 3 h. These pretreatments did not alter the level of infection ($P > 0.3$ relative to the control to which no serum was added). In contrast, preincubation of virus with complement or serum plus complement for 3 h increased the level of infection significantly ($P < 0.01$), and inoculation in the presence of antibodies alone decreased the infection ($P < 0.05$) relative to that in a control to which no serum was added (Fig. 5).

(viii) Infection under C'ADE conditions reaches maximum level within 5 min after incubation. To determine the kinetics

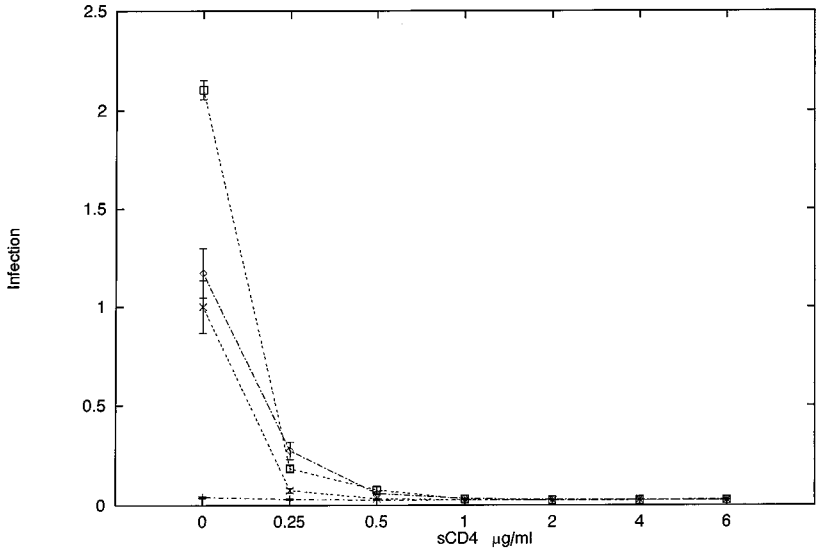

FIG. 3. sCD4 blocks infection of MT-2 cells with HIV-1 IIIB under enhancing conditions. Virus preparations were incubated with fresh serum from an HIV-negative donor in a dilution of 1:100 (□), heat-inactivated serum from an HIV-1-infected patient in a dilution of 1:100 (——), a combination of the two (◇), and no serum (×), together with a dilution series of sCD4. Results (in arbitrary units) are the means of quadruplicate determinations ± standard errors of the mean normalized to the values of the mock-treated control culture.

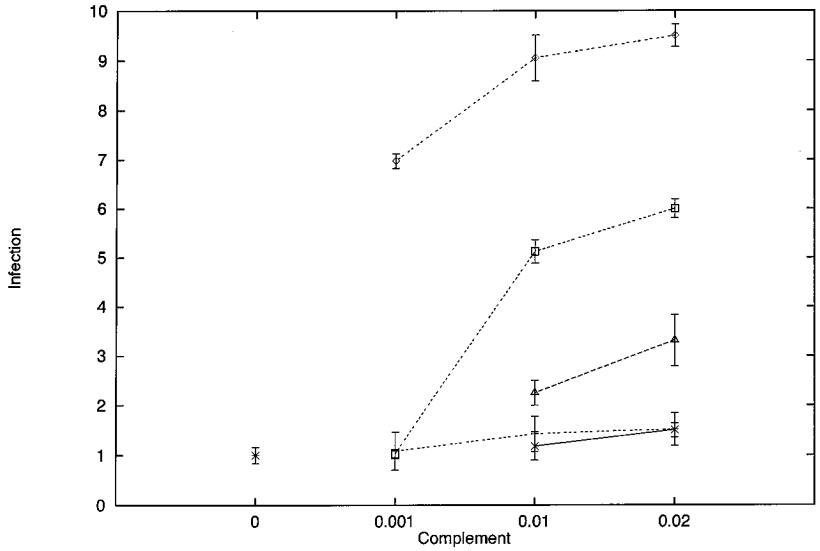

FIG. 4. Effects of factor B-deficient serum on infection. HIV was preincubated with various combinations of HIV-positive serum (S), fresh human serum from Sigma (C), factor B-deficient serum from Sigma (C b-def) and factor B (B). Shown are S + C (◇), S + C b-def (——), S + C b-def + B (□), C b-def (×), C b-def + B (△), and the control (complement = 0). Results (in arbitrary units) are the means of quadruplicate determinations ± standard errors of the mean normalized to the values of the mock-treated control culture.

of HIV infection, an assay in which HIV was allowed to bind to the MT-2 cells for 5 to 1,440 min before being washed was performed. Under C'ADE conditions, the infection reached a high level within 5 min and increased slowly, though not significantly ($P > 0.05$), thereafter (Fig. 6). The addition of sCD4 after 30 min did not lead to a significantly ($P > 0.05$) lower infection rate. Without HIV-positive serum and complement, the infection rate started to rise after 30 min and reached a maximum level after 1,080 min, and the infection was blocked when sCD4 was added after 30 min ($P < 0.05$). Calculation of the diffusion-limited aggregation rate (k_aM) shows that it takes approximately 30 min before half of the virions have encountered a cell. The saturation of the infection process seen under C'ADE conditions after only 5 min can therefore not be due to a major part of the HIV particles being bound to cells. We therefore wanted to determine whether the abrogation of the enhanced infection process could be due to complement saturating the complement receptors. Cells, virus, or both were preincubated with complement prior to incubation of cells with virus for 3 h. Table 4 shows that preincubation of cells for 30 min with fresh serum from an HIV-negative donor did not enhance, while virus preincubated for 1 h with the serum did. When virus was preincubated with complement, the addition of complement to cells resulted in a reduced level of enhancement, indicating that complement competed for complement receptors.

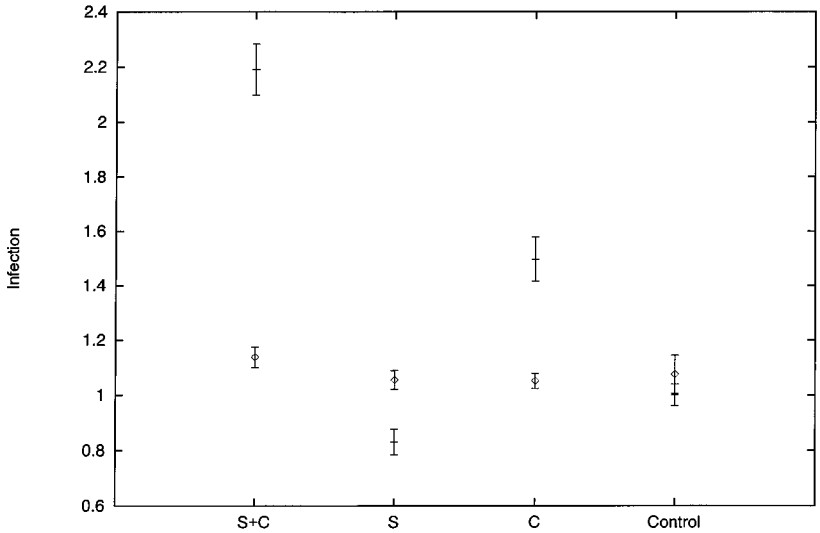

FIG. 5. Enhancement of HIV-1 IIIB infection of MT-2 cells is not due to activation of the cells. Incubating cells for 30 min with fresh human serum from an HIV-negative donor in a dilution of 1:100 (C) and/or heat-inactivated serum from an HIV-1-infected patient in a dilution of 1:1,000 (S) before the cells were washed and the subsequent addition of the virus to the cells for 3 h did not change the level of infection (◇). Incubation of virus with S and/or C for 1 h before the addition of the cells did change the level of infection (——). Results (in arbitrary units) are the means of quadruplicate determinations ± standard errors of the mean normalized to values of the mock-treated control culture.

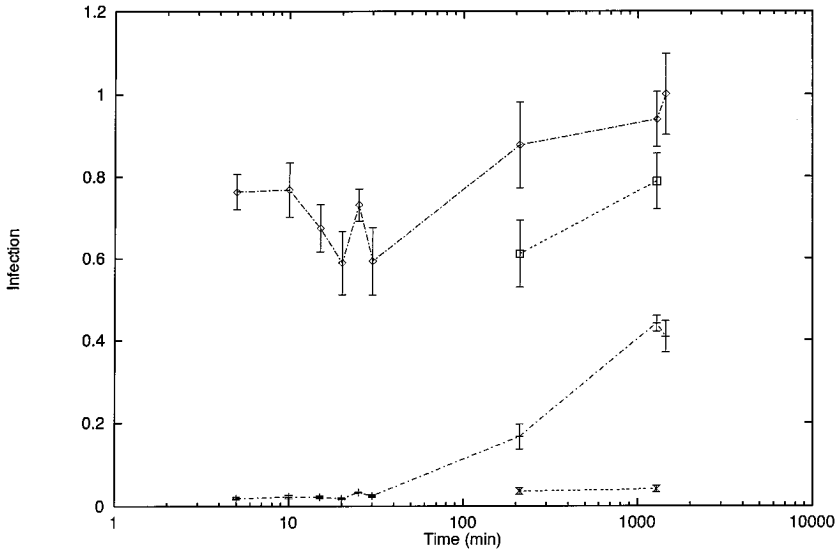

FIG. 6. Kinetics of HIV infection when HIV is incubated with cells for 5 to 1,440 min before being washed. Shown are the results for HIV preincubated for 1 h with serum from an HIV-negative donor in a dilution of 1:100 and serum from an HIV-infected patient in a dilution of 1:1,000 before the addition of cells (◇) and for a mock-treated control (——). Also shown are the effects of adding 2 μg of sCD4 to cells and HIV after 30 min of incubation in the cultures in which HIV was incubated with serum (□) and in the mock-treated control (×). Results (in arbitrary units) are the means of quadruplicate determinations ± standard errors of the mean normalized to the values of the serum-treated culture with a 1,440-min incubation time.

Model simulations. (i) The rapid saturation of the enhanced infection process may be due to saturation of complement receptors. Figure 7 shows a model simulation of the number of virions bound to cells under enhancing conditions and under control conditions as a function of the incubation time.

The reason for the faster reaction under enhancing conditions in the model simulation is that a larger fraction of the HIV surface is covered by complement than is covered by gp120. Saturation of the infection process occurs, since a major part of the complement receptors is occupied by HIV-associated complement.

(ii) The model qualitatively explains the experimental results. Figure 8 shows simulation results for the combined effects of antibodies and complement. It can be seen that the model captures the main features of the experimental data shown in Fig. 1 and Fig. 2. Note that the antibody titer giving the maximal enhancement increases as the complement is diluted, corresponding to the experimental results shown in Fig. 1. At high complement concentrations, less antibody is needed to obtain high-level saturation of HIV with complement. For the model to show enhancement when the concentration of antibodies is set to zero, we need to assume either that complement can bind to HIV in the absence of antibodies as in Fig. 8 or alternatively that the complement serum contains heterologous antibodies that can facilitate complement binding to HIV.

TABLE 4. Enhancement of HIV-1 IIIB infection[a]

Fresh serum (dilution) preincubated with		Infection
Cells	Virus	
0	0	1.00 ± 0.22
0	0.05	4.93 ± 1.04
0	0.1	7.54 ± 2.95
0.05	0	1.09 ± 0.27
0.05	0.05	3.63 ± 1.19
0.1	0	1.40 ± 0.29

[a] Cells were preincubated for 30 min with fresh serum from an HIV-negative human donor in dilutions resulting in a final dilution of 0.0, 0.05, and 0.1. Virus was preincubated for 1 h with the fresh serum in dilutions resulting in final dilutions of 0.0, 0.05, and 0.1. Virus was hereafter incubated with cells for 3 h. Results (in arbitrary units) are the means ± standard deviations of quadruplicate determinations normalized to the value of the mock-treated control culture.

DISCUSSION

Complement-mediated enhancement can be explained by at least three mechanisms: (i) antibody and/or complement may increase the binding of virus to target cells, (ii) they may increase the fusion of HIV with cells, or (iii) they may have nonspecific stimulatory effects on target cells. These mechanisms might also act to render otherwise inert virions infectious. When cells were preincubated with complement and then washed, we did not find any alteration of the level of infection, indicating that enhancement was not due to activation of the cells. Our results, indicating that the mechanism of enhancement is well explained by the increased adhesion to target cell CR2s after complement deposition on HIV, are in accordance with those of Montefiori et al. (21), who found that binding of HIV to $CR2^+$ MT-2 and Raji-3 cells increased after pretreatment with complement. Our finding that the infection was still sensitive to sCD4 is also in accordance with the results of Montefiori et al. (21), who could not infect Raji-3 cells which are $CD4^-$ $CR2^+$. Thus, complement might act to get a larger fraction of the virions into close proximity of target cell CD4 receptors, thus enabling them to infect. Adhesion of HIV to the cell surface via complement, Fc, or alternative receptors and specific binding to CD4 are therefore two separate processes, and infection is crucially dependent on the latter. Simulation studies showed that the observed complement and antibody dose dependences are well explained by a hypothesis involving increased adhesion of complement-coated virions to target cells. Our previous work with antibody-mediated Fc receptor-dependent enhancement (19) is also in accordance with this conclusion.

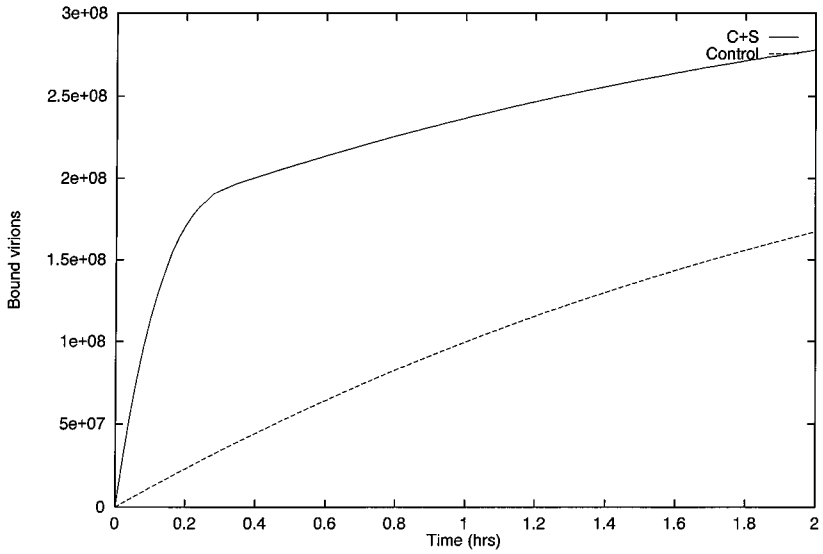

FIG. 7. Model of the kinetics of HIV infection. Shown are the amounts of HIV bound to cells (eX = 10^x) in a simulation with complement $K = 0.1$ and antibodies $a = 6.7 \times 10^{-9}$ M (0.001 mg/ml) (C+S) and in a simulation with $K = 0$ and $a = 0$ (Control).

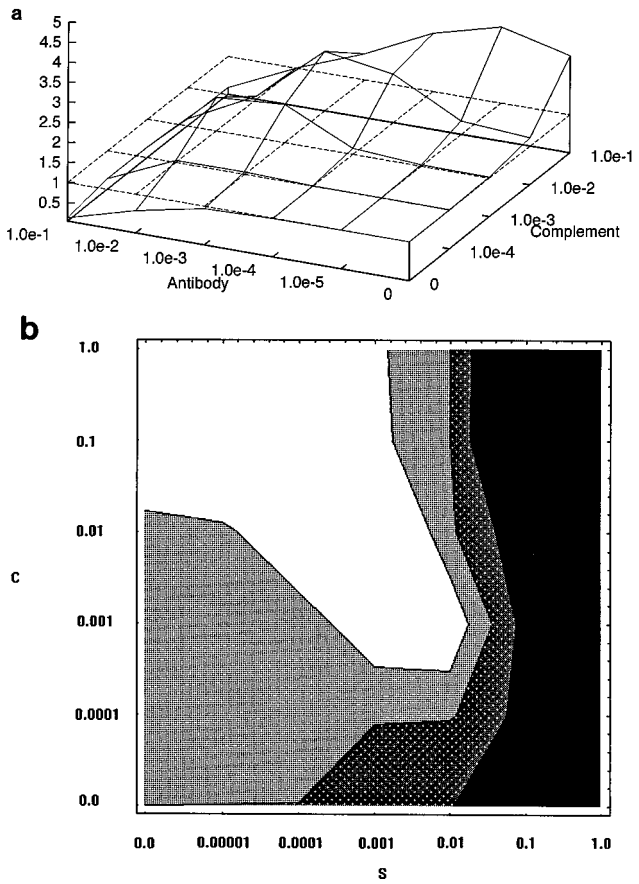

FIG. 8. Infection relative to a control with neither antibodies nor complement. Parameters are as described in the text. (a) Three-dimensional plot of the level of infection (eX = 10^x) as a function of the dilutions of a serum containing 1 mg of anti-HIV antibodies per ml and complement. (b) Contour plot of the infection as a function of the concentrations of antibodies (S) and complement (C). Infection, in arbitrary units: □, >1.5; ▒, 1.0 to 1.5; ▓, 0.5 to 1.0; ■, <0.5.

Enhancement of MT-2 cells with HIV IIIB was shown to be mediated via the alternative complement activation pathway and increased in the presence of heat-inactivated serum from an HIV-infected donor. We found that infection, under C'ADE conditions, reached a high level within minutes and did not increase significantly thereafter. Simulation studies showed that this could be explained by a large excess of HIV-complement complexes which rapidly saturate the complement receptors, thus saturating the enhanced infection process. This is possible even though we used a low $TCID_{50}$ relative to the number of complement receptors on the cells. Electron microscopy studies have shown that there are 10^4- to 10^7-fold more physical than infectious virus particles (17). Furthermore, in binding assays, Montefiori et al. (22) have shown that as much as 40% of the virus input was bound to the CR2-positive MT-2 cells in the presence of HIV-positive serum and fresh human serum. A peculiar consequence of this hypothesis is that even if all complement receptors are saturated, it is still only a small fraction of the cells that are infected, as the $TCID_{50}$ value under enhancing conditions was only 1,024, corresponding to an infection of approximately 0.2% of the cells.

The enhancement seen in the experiments in which no serum from an HIV-positive donor was added can be explained by antibody-independent activation of the alternative complement pathway (C'AIDE). Experiments showed that fresh sera from normal human donors in many cases enhance HIV infection, while heat-inactivated sera neutralize infection (unpublished data). C'ADE may have severe implications for vaccine development, since antibody raised against all synthetic peptides encompassing the whole gp120 and gp41 sequence seem able to produce variable degrees of C'ADE (13). Even antibodies raised against 10 of 21 strain-specific V3 loop peptides could enhance (13), thereby reducing the possibility of finding a non-C'ADE mediating epitope as a safe target for vaccine development.

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REFERENCES

1. **Bakker, J. L., H. S. L. M. Nottet, N. M. de Vos, L. de Graaf, J. A. G. Van Strijp, M. R. Visser, and J. Verhoef.** 1992. Antibodies and complement enhance binding and uptake of HIV-1 by human monocytes. AIDS **6:**35–41.
2. **Berg, H. C., and E. M. Purcell.** 1977. Physics of chemoreception. Biophys. J. **20:**193–219.
3. **Boyer, V., C. Desgranges, M.-A. Trabaud, E. Fischer, and M. D. Kazatchkine.** 1991. Complement mediates human immunodeficiency virus type 1 infection of a human T cell line in a CD4- and antibody-independent fashion. J. Exp. Med. **173:**1151–1158.
4. **Chandrasekhar, S.** 1943. Stochastic problems in physics and astronomy. Rev. Mod. Phys. **15:**1–89.
5. **Collins, F. C., and G. E. Kimball.** 1949. Diffusion-controlled reaction rates. J. Colloid Sci. **4:**425–437.
6. **Davis, B. D., R. Bulbecco, H. N. Eisen, and H. S. Ginsberg.** 1980. Microbiology. Harper & Row, Publishers, Inc., New York.
7. **Dierich, M. P., C. F. Ebenbichler, P. Marchang, G. Füst, N. M. Thielens, and G. J. Arlaud.** 1993. HIV and human complement: mechanisms of interaction and biological implication. Immunol. Today **14:**435–440.
8. **Dolin, R., B. S. Graham, S. B. Greenberg, C. O. Tacket, R. B. Belshe, K. Midthun, M. L. Clements, G. J. Gorse, B. W. Horgan, R. L. Atmar, D. T. Karzon, W. Bonnez, B. F. Fernie, D. C. Montefiori, D. M. Stabelin, G. E. Smith, W. C. Koff, and the NIAID Vaccine Clinical Trials Network.** 1991. The safety and immunogenicity of a human immunodeficiency virus type 1 (HIV-1) recombinant gp160 candidate vaccine in humans. Ann. Intern. Med. **114:**119–127.
9. **Enright, W. H., K. R. Jackson, S. P. Nørsett, and P. G. Thomsen.** 1986. Interpolants for Runge-Kutta formulas. ACM Trans. Math. Soft. **12:**193–218.
10. **Gras, G. S., and D. Dormont.** 1991. Antibody-dependent and antibody-independent complement-mediated enhancement of human immunodeficiency virus type 1 infection in a human Epstein-Barr virus-transformed B-lymphocytic cell line. J. Virol. **65:**541–545.
11. **Hansen, J. E. S., C. Nielsen, H. Clausen, L. R. Mathiesen, and J. O. Nielsen.** 1991. Effect of anti-carbohydrate antibodies on HIV infection in a monocytic cell line (U937). Antiviral Res. **16:**233–242.
12. **Hansen, J.-E. S., C. Nielsen, M. Arendrup, S. Olofssen, L. Mathiasen, J. O. Nielsen, and H. Clausen.** 1991. Broadly neutralizing antibodies targeted to mucin-type carbohydrate epitopes of human immunodeficiency virus. J. Virol. **65:**6461–6467.
13. **Jiang, S., K. Lin, and A. R. Neurath.** 1991. Enhancement of human immunodeficiency virus type 1 infection by antisera to peptides from the envelope glycoproteins gp120/gp41. J. Exp. Med. **174:**1557–1563.
14. **June, R. A., A. L. Landay, K. Stefanik, T. F. Lint, and G. T. Spear.** 1992. Phenotypic analysis of complement receptor 2+ T lymphocytes: reduced expression on CD4+ cells in HIV-infected persons. Immunology **75:**59–65.
15. **June, R. A., S. Z. Schade, M. J. Bankowski, M. Kuhns, A. McNamara, T. F. Lint, A. L. Landay, and G. T. Spear.** 1991. Complement and antibody mediate enhancement of HIV infection by increasing virus binding and provirus formation. AIDS **5:**269–274.
16. **Klein, J.** 1990. Immunology. Blackwell Scientific Publications, Boston.
17. **Layne, S. P., M. J. Merges, M. Dembo, J. L. Spouge, S. R. Conley, J. P. Moore, J. L. Raina, H. Renz, H. R. Gelderblom, and P. L. Nara.** 1992. Factors underlying spontaneous inactivation and susceptibility to neutralization of human immunodeficiency virus. Virology **189:**695–714.
18. **Layne, S. P., J. L. Spouge, and M. Dembo.** 1989. Quantifying the infectivity of human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **86:**4644–4648.
19. **Lund, O., J. Hansen, E. Mosekilde, J. O. Nielsen, and J.-E. S. Hansen.** 1993. A model of enhancement and inhibition of HIV infection of monocytes by antibodies against HIV. J. Biol. Phys. **19:**133–145.
20. **Montefiori, D. C., B. S. Graham, S. Kliks, P. F. Wright, and the NIAID AIDS Vaccine Clinical Trials Network.** 1992. Serum antibodies to HIV-1 in recombinant vaccinia virus recipients boosted with purified recombinant gp160. J. Clin. Immunol. **12:**429–439.
21. **Montefiori, D. C., K. Stewart, J. M. Ahearn, J. Zhou, and J. Zhou.** 1993. Complement-mediated binding of naturally glycosylated and glycosylation-modified human immunodeficiency virus type 1 to human CR2 (CD21). J. Virol. **67:**2699–2706.
22. **Montefiori, D. C., J. Zhou, and D. I. Shaff.** 1992. CD4-independent binding of HIV-1 to the B lymphocyte receptor CR2 (CD21) in the presence of complement and antibody. Clin. Exp. Immunol. **90:**383–389.
23. **Nielsen, I., M. Carsten, I. C. Bygbjerg, and B. F. Vestergaard.** 1987. Detection of HIV antigens in eluates from whole blood collected on filter paper. Lancet **i:**566–567.
24. **Perricone, R., L. Fontana, C. de Carolis, C. Carini, M. C. Sirianni, and F. Aiuti.** 1987. Evidence for activation of complement in patients with AIDS related complex (ARC) and/or lymphoadenopathy syndrome (LAS). Clin. Exp. Immunol. **70:**500–507.
25. **Popovic, M., M. G. Sarnagadharan, E. Read, and R. C. Gallo.** 1984. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science **224:**497–500.
26. **Posner, M. R., H. S. Elboim, T. Cannon, L. Cavacini, and T. Hideshima.** 1992. Functional activity of an HIV-1 neutralizing IgG human monoclonal antibody: ADCC and complement-mediated lysis. AIDS Res. Hum. Retroviruses **8:**553–558.
27. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27:**493–497.
28. **Reisinger, E. C., W. Vogetseder, D. Berzow, D. Köfler, G. Bitterlich, H. A. Lehr, H. Wachter, and M. P. Dierich.** 1990. Complement-mediated enhancement of HIV-1 infection of the monoblastoid cell line U937. AIDS **4:**961–965.
29. **Robinson, W. E., M. K. Gorny, J. Y. Xu, W. M. Mitchell, and S. Zolla-Pazner.** 1991. Two immunodominant domains of gp41 bind antibodies which enhance human immunodeficiency virus type 1 infection in vitro. J. Virol. **65:**4169–4176.
30. **Robinson, W. E., and W. M. Mitchell.** 1990. Neutralization and enhancement of in vitro and in vivo HIV and simian immunodeficiency virus infections. AIDS **4**(Suppl. 1)**:**151–162.
31. **Robinson, W. E., D. C. Montefiori, and W. M. Michell.** 1990. Complement-mediated antibody dependent enhancement of HIV-1 infection requires CD4 and complement receptors. Virology **175:**600–604.
32. **Shoup, D., and A. Szabo.** 1982. Role of diffusion in ligand binding to macromolecules and cell bound receptors. Biophys. J. **40:**33–39.
33. **Spear, G. T.** 1993. Interaction of non-antibody factors with HIV in plasma. AIDS **7:**1149–1157.
34. **Spear, G. T., S. L. Brenda, A. L. Landay, and T. F. Lint.** 1990. Neutralization of human immunodeficiency virus type 1 by complement occurs by viral lysis. J. Virol. **64:**5869–5873.
35. **Thieblemont, N., C. Delibrias, E. Fischer, L. Weiss, M. D. Kazatchkine, and N. Haeffner-Cavaillon.** 1993. Complement enhancement of HIV infection is mediated by complement receptors. Immunopharmacology **25:**87–93.
36. **Weast, R. C. (ed.).** 1982. CRC Handbook of chemistry and physics. CRC Press, Boca Raton, Fla.
37. **Yefenof, E., B. Asjo, and E. Klein.** 1991. Alternative complement pathway activation by HIV infected cells: C3 fixation does not lead to complement lysis but enhances NK sensitivity. Int. Immunol. **3:**395–401.