JOURNAL OF VIROLOGY, Mar. 1993, p. 1461–1471
0022-538X/93/031461-11$02.00/0

Vol. 67, No. 3

# Two Mechanisms of Soluble CD4 (sCD4)-Mediated Inhibition of Human Immunodeficiency Virus Type 1 (HIV-1) Infectivity and Their Relation to Primary HIV-1 Isolates with Reduced Sensitivity to sCD4

SHERRY L. ORLOFF,[1]* M. SUSAN KENNEDY,[1] ALEXIA A. BELPERRON,[2] PAUL J. MADDON,[2] AND J. STEVEN MCDOUGAL[1]

*Immunology Branch, Division of HIV/AIDS, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333,[1] and Progenics Pharmaceuticals, Inc., Tarrytown, New York 10591[2]*

Received 28 September 1992/Accepted 11 December 1992

**Two assays for measuring inhibition of human immunodeficiency virus type 1 (HIV-1) infection by soluble CD4 (sCD4) are described. Experiments in which sCD4, HIV-1, and cell concentrations and sequence of combination, noninfectious/infectious particle ratio, and temperature were varied produced results that support the conclusion that sCD4 inhibits HIV-1 infection by two mechanisms: reversible blockage of receptor binding and irreversible inactivation of infectivity. Fresh isolates obtained from HIV-1-infected persons were tested in both assays and found to be more resistant to both mechanisms of sCD4-mediated inhibition than multiply passaged laboratory strains. Binding studies revealed similar affinities for sCD4 in detergent lysates of sensitive and resistant strains at both 4 and 37°C. The avidity of intact virions for sCD4 was lower at 4 than at 37°C, and in the presence of excess sCD4, less sCD4 was bound at 4 than at 37°C. The avidity differences were similar for fresh isolates and laboratory strains. However, fresh isolates were more resistant to sCD4-induced shedding of envelope glycoprotein gp120 from intact virions than was the laboratory strain. Relative resistance to sCD4 by certain isolates does not represent a lower intrinsic affinity of their envelope for sCD4 or a lower capacity for sCD4 binding. Rather, an event that occurs after binding may account for the differences. This postbinding event or feature may be determined by regions of the envelope outside the CD4 binding site.**

Binding of the outer envelope protein, gp120, of human immunodeficiency virus type 1 (HIV-1) to its cellular receptor, CD4, is a high-affinity interaction and a prime determinant of efficient infection. Although much is known about the isolated molecules with respect to the affinity of interaction and the binding sites, considerable uncertainty remains about interactions that occur when these molecules are organized on viral and cellular surfaces. The multivalent interaction would be expected to dramatically amplify the avidity of interaction. Also, the interaction may result in secondary changes (conformation, mobility, transduction of allosteric alterations with other proteins, or intracellular signalling), but it is not clear whether these are requisites for infection. Binding of gp120 to cellular CD4 interferes with certain normal signaling functions (25) and results in a conformation change in CD4 that can be detected serologically (5). However, CD4-associated signal transduction is not required for penetration (35), and the conformational change may be concomitant with rather than a requisite for infection. Nevertheless, the integrity of regions of CD4 not directly involved in binding may be required for successful infection (4, 5, 11, 36, 40).

Similarly, soluble forms of CD4 (sCD4) have been used to probe changes in gp120 on viral particles or on the surface of HIV-1-infected cells. The noncovalent association of gp120 with the transmembrane protein, gp41, can be disrupted by sCD4 (2, 10, 18, 31), and certain epitopes of both gp120 and gp41, normally cryptic on intact particles or cells, become accessible to antibody (36). Compared with laboratory strains of HIV-1, fresh isolates obtained from patients tend to be less sensitive to the neutralizing effect of sCD4 (6), yet they infect by a CD4-dependent pathway and have seemingly similar intrinsic affinities of their gp120s for CD4 (3, 24, 29, 41). These observations constitute strong evidence that the function of the gp120-CD4 interaction is much more than simply to focus virus on the cell membrane. Important secondary interactions must be occurring, but whether they involve secondary bonds between CD4 and gp120, conformational changes, or secondary interactions with other cellular or viral proteins remains to be determined.

In this study, we present two assays that separate two modes by which sCD4 blocks HIV-1 infectivity: reversible blockage of receptor binding and irreversible inactivation of infectivity. Resistant isolates and sensitive strains were tested. Separate measurements of intrinsic affinity and whole-virion avidity were made. Virions were probed for changes in covalent structure (proteolysis) and for dissociation of envelope subsequent to sCD4 binding.

## MATERIALS AND METHODS

**Reagents and viral stocks.** The LAV-1 and HTLV-IIIB prototype strains of HIV-1 were propagated in phytohemagglutinin (PHA)-stimulated normal peripheral blood lymphocytes (PBL) (22). Primary or first-passage HIV-1 isolates were obtained by coculture of lymphocytes from HIV-seropositive men with PHA-stimulated PBL (22). The HIV-1 infectious clones NL4-3 and JR-CSF were expressed by transfection in RG or A3.01 cells, and virus stocks were derived from a single passage in PHA-stimulated PBL (1, 34). sCD4 was provided by Progenics Pharmaceuticals, Inc. (Tarrytown, N.Y.).

**Microculture infectivity (50% infective dose [$ID_{50}$]) assay.**

* Corresponding author.

This assay was performed as previously described (19). Briefly, PHA-stimulated PBL were incubated with dilutions of HIV inoculum overnight at 37°C. Cells were washed by centrifugation, resuspended in medium, and dispensed into microculture wells ($10^5$ cells per well, 10 cultures per dilution). Eight and twelve days later, supernatants were monitored for viral replication by an antigen capture assay (19). The tissue culture $ID_{50}$ ($TCID_{50}$) is the reciprocal of the dilution that results in 50% positive cultures.

**Neutralization assay.** The microculture infectivity assay for productive viral replication was used. HIV (100 $TCID_{50}$) was mixed with graded doses of sCD4 at room temperature for 15 min. The mixtures were added to PHA-stimulated lymphocytes and incubated at 37°C overnight. (In some experiments, incubation was at 0 or 37°C for 4 h.) The cells were washed, plated in microculture, and monitored for viral replication as described above. In the usual assay, sCD4 is washed away after the initial incubation of HIV with the cells. Experiments in which sCD4 was added after the initial overnight incubation or was present throughout the incubation and culture period were also performed. The input virus inoculum of 100 $TCID_{50}$ was determined by prior titration. In all experiments, the titers of inoculum were determined in the same run, and the actual input ranged from 50 to 700 $TCID_{50}$.

**HIV inactivation assay.** An HIV preparation was combined with sCD4 (50, 100, or 200 μg/ml) and incubated for 2 h at 37°C. Serial 10-fold dilutions were made and added to PHA-stimulated PBL for an overnight incubation at 37°C. The cells were washed, plated in microculture, and monitored for viral replication as described above. In control cultures, HIV and sCD4 were preincubated at 0 instead of 37°C. As another control, HIV and sCD4 were preincubated in separate tubes at 37°C, dilutions were made, and the dilutions were combined just before addition to cells. Thus, the experimental and control cultures had identical amounts of HIV and sCD4 but differed in the opportunity for interaction at 37°C before exposure to cells.

**sCD4-gp120 enzyme-linked immunosorbent assay (ELISA).** HIV culture supernatant lysates (200 μl) containing 0.1% Triton X-100 were incubated in duplicate with various concentrations of sCD4 (0.3 to 352 nM) at 37 or 4°C for 2 h in a final volume of 250 μl. The mixtures were added to microtiter wells that had been coated with an affinity-purified sheep anti-HIV-1 gp120 (1.0 μg per well; International Enzymes, Fallbrook, Calif.), and blocked with 250 μl of 2% nonfat dry milk in phosphate-buffered saline (PBS). The plates were incubated for another 4 h at the respective temperatures. The sheep anti-gp120 reagent was generated by immunization with a peptide construct corresponding to amino acids 497 to 511 of the gp120 protein, and the reagent does not interfere with sCD4 binding (our data and reference 25). After being washed three times with cold (4°C) PBS containing 0.1% Triton X-100, 100 μl of polyclonal rabbit anti-CD4 reagent (1:1,000 dilution; a gift from Ray Sweet, Smith Kline, King of Prussia, Pa.) was added to each well, and incubation was continued overnight at 4°C. Wells were washed, and 100 μl of horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (1:2,000 dilution; Bio-Rad, Richmond, Calif.) was added for 4 h at 4°C. After washing, 200 μl of substrate solution (0.1% 1,2-phenylenediamine, 0.006% $H_2O_2$) was added; color reactions were stopped with the addition of 50 μl of 8 N $H_2SO_4$, and the $A_{490}$ was determined. After the initial binding to the wells, buffers, reagents, and incubations were kept at 4°C in order to minimize loss or elution of sCD4. (Less than 10% of the original amount is lost after 24 h at 4°C.) Optimal concentrations of reagents were predetermined by comparison of assay results with viral lysates containing 88 nM sCD4 and lysates without sCD4. The $A_{490}$ ranged from 0.050 to 0.125 for lysates without sCD4 or for sCD4 without lysate when the blank was reagent control (buffer substituted for lysate and/or sCD4). The maximal $A_{490}$ ranged from 0.775 to 1.000. Detection of bound gp120 (rather than sCD4) was equivalent at 4 and 37°C in the presence or absence of sCD4. The average difference in $A_{490}$ between duplicates was 0.056; this tended to be greater at the midpoint of the binding curves and smaller in the plateau regions. Affinity of the LAV isolate has been measured on four separate occasions, and the values ranged from 0.3 to 1.9 nM.

The same assay with some modification was performed for detecting sCD4 binding to whole virions. Virus preparations were ultracentrifuged (145,000 × $g$ at $R_{max}$ for 30 min; Beckman SW60 rotor, 38,500 rpm) to remove free gp120. The pellets were gently resuspended to about half the original volume in RPMI medium containing 10% fetal calf serum. A small portion was lysed with 0.1% Triton X-100 (to which the highest concentration of sCD4, 352 nM, would be added). The preparations were run through the procedure as described above with one modification. After the preparations were incubated on the anti-gp120-coated plates and unbound sCD4 was washed away, 0.1% Triton X-i00 was added. Incubation was continued for another 2 h at 4°C, the plates were washed, and the procedure was continued as described above.

**sCD4-induced dissociation of gp120 from virions.** The fractionation of sCD4-incubated virions was performed exactly as described by Moore et al. (31) with two modifications: the S-1000 minicolumns were equilibrated with RPMI–10% fetal calf serum, and single-drop fractions (approximately 37 μl) were collected into microtiter wells containing 63 μl of medium. Virus preparations were preincubated for 2 h at 37°C with or without 200 μg of sCD4 per ml in a final volume of 100 μl. Fractions were monitored for infectivity titer and p24 and gp120 content. (Every fourth fraction was tested by one of these assays, leaving one set of fractions for making a pool.) The p24 content was determined by using the HIV-1 Core Profile ELISA (Dupont, NEN Research Products, North Billerica, Mass.). The gp120 assay was the capture ELISA described above, except that a horseradish peroxidase-conjugated, polyclonal anti-HIV reagent was substituted for the CD4-detecting reagents. No interference by sCD4 was detected in this assay, as verified by the fact that assay of virus preparations in the presence and absence of sCD4 resulted in the same $A_{490}$ readings. The p24 levels and infectivity titers peaked in the void volume whereas gp120 levels were bimodal. The void volume fractions were pooled, and p24 and gp120 measurements were performed. The registered p24 contents of the void volume peaks for the sCD4-incubated and the respective control virion preparations were the same. The gp120 content of the void volume pool was taken as a measure of virion-associated gp120, and reduction in the ratio of gp120 to p24 was the measure of sCD4-induced dissociation of gp120 from virions.

**Radioimmunoprecipitation.** HIV (LAV strain)-infected C8166 cells were pulse-labeled with [$^{35}$S]cysteine and [$^{35}$S] methionine, and extracellular virus was harvested as described previously (20). $^{35}$S-HIV was incubated with 100 μg of sCD4 per ml for 2 h at 37 or 4°C, transferred to ice, and lysed with detergent buffer. Immunoprecipitations were performed with polyclonal human anti-HIV immunoglobulin G (20) or a sheep anti-gp120 antibody (courtesy of M. Phelan, AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Dis-

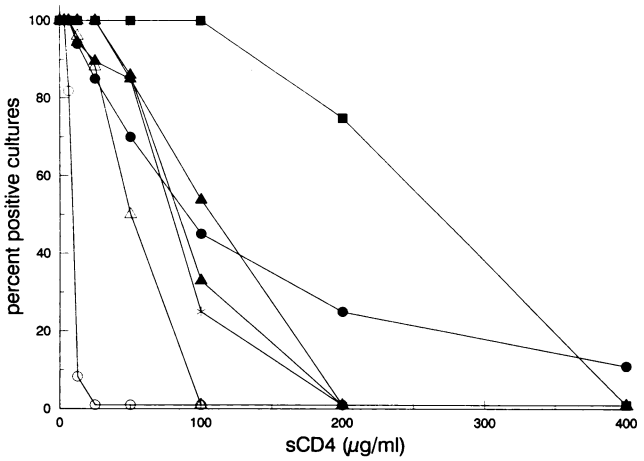


FIG. 1. Neutralization by sCD4. Laboratory strains and primary or first-passage isolates of HIV-1 (approximately 100 $TCID_{50}$) were incubated with cells and sCD4 for 18 h. The cells were washed and plated in microculture (10 cultures per datum point), and viral replication was monitored. The strains tested were LAV (○) and first-passage or primary isolates 1 (●); 2 (▲); 3 (*); 4 (▲); 5 (■); and 6 (△).

eases, Bethesda, Md.). The immunoprecipitation and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) procedures were performed as previously described (20).

## RESULTS

**Neutralization.** In the neutralization assay, 100 $TCID_{50}$ of HIV is mixed with graded doses of sCD4, and the mixtures are added to PHA-stimulated PBL for an overnight incubation. The cells are washed and plated in microculture ($10^5$ cells per well); viral replication is monitored by antigen capture assay 8 and 12 days later. Primary isolates from HIV-infected persons were less sensitive to sCD4 neutralization than were our reference isolates, $HIV_{LAV}$ (Fig. 1), $HIV_{NL4-3}$, and $HIV_{HTLV-IIIB}$ (data not shown). The LAV strain has been tested multiple times in this assay. The $I_{50}$ (the concentration of sCD4 required for 50% inhibition of infectivity) ranged from 2 to 13 μg/ml. Five fresh isolates with relative resistance to sCD4 ($I_{50}$ > 35 μg/ml) were run on at least two occasions, and the paired $I_{50}$s differed from each other by 10 to 22 μg/ml.

Parameters of sCD4 neutralization such as timing of sCD4 addition, HIV input, cell dose, and infectious/noninfectious particle ratios were examined by using the LAV reference strain (Fig. 2 and 3). In the usual neutralization assay (constant HIV inoculum, variable sCD4 dose), there was little difference in neutralization whether sCD4 was present only during the incubation phase or present throughout the culture period. Addition of sCD4 after overnight incubation of HIV with cells resulted in much less neutralization (Fig. 2a). In the converse assay, a constant amount of sCD4 (10 μg/ml) was incubated with serial dilutions of HIV. Again, infectivity was reduced by about the same amount whether sCD4 was present only during the initial incubation with HIV or throughout the culture period. Delayed addition of sCD4 reduced infectivity somewhat (Fig. 2b); an approximately 1-log-unit reduction was the usual effect in this type of assay (7). Presumably, at the limiting HIV input, subsequent viral spread in the culture is required to register above the threshold of the assay.

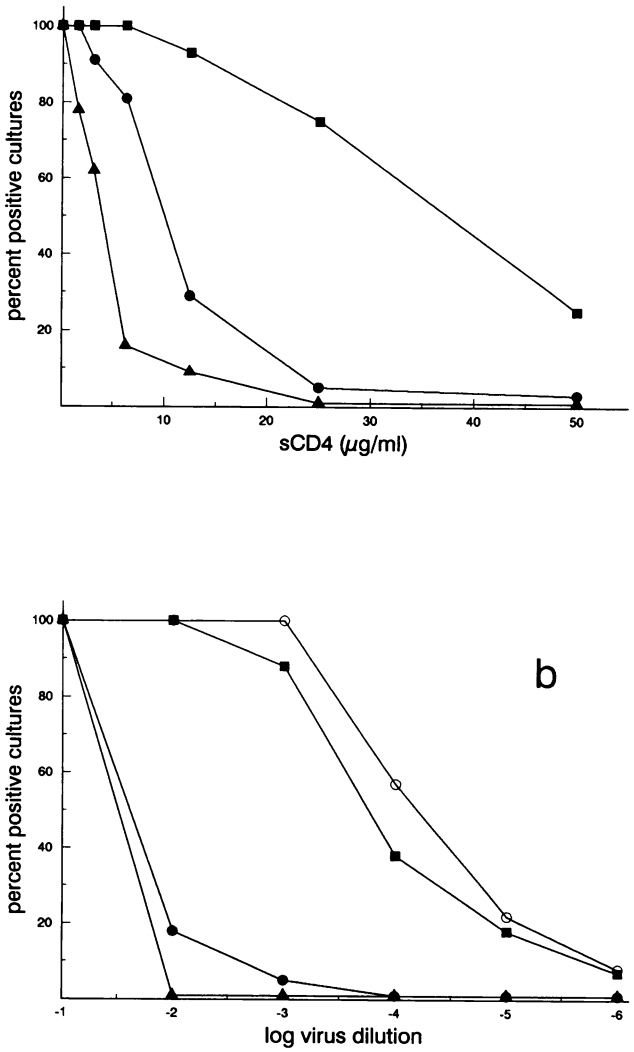


FIG. 2. Timing of sCD4 addition. HIV-1 (LAV strain) was incubated with cells for 18 h. The cells were washed and plated in microculture, and viral replication was monitored. sCD4 was present during the initial incubation with virus (●), was added to the microcultures after the initial incubation (■), or was present during both incubation and culture (▲). (a) Various doses of sCD4 and a constant amount of HIV-1 (100 $TCID_{50}$) were used. (b) A constant amount of sCD4 (10 μg/ml) was tested with serial dilutions of HIV-1. Titration of HIV-1 in the absence of sCD4 is also shown (○).

The doses of sCD4 required for 50% reduction of infectivity ($I_{50}$s) at different levels of cell and virus input were compared (Fig. 3). The $I_{50}$ rose with increasing cell dose (Fig. 3a) but was not influenced strongly by input virus dose, except at very high doses (Fig. 3b). An HIV inoculum that had been heat inactivated at 56°C for 30 min was mixed with the same unheated inoculum (100 $TCID_{50}$) in ratios of 10, 100, and 1,000 to 1. The heated inoculum was noninfectious, as has been reported previously (21), but did retain full CD4-binding capacity (23). The inhibitory capacity of sCD4 was not greatly influenced by the presence of excess noninfectious virus (Fig. 3c). We have also compared HIV inocula that were harvested early (5 days) and late (13 days) after

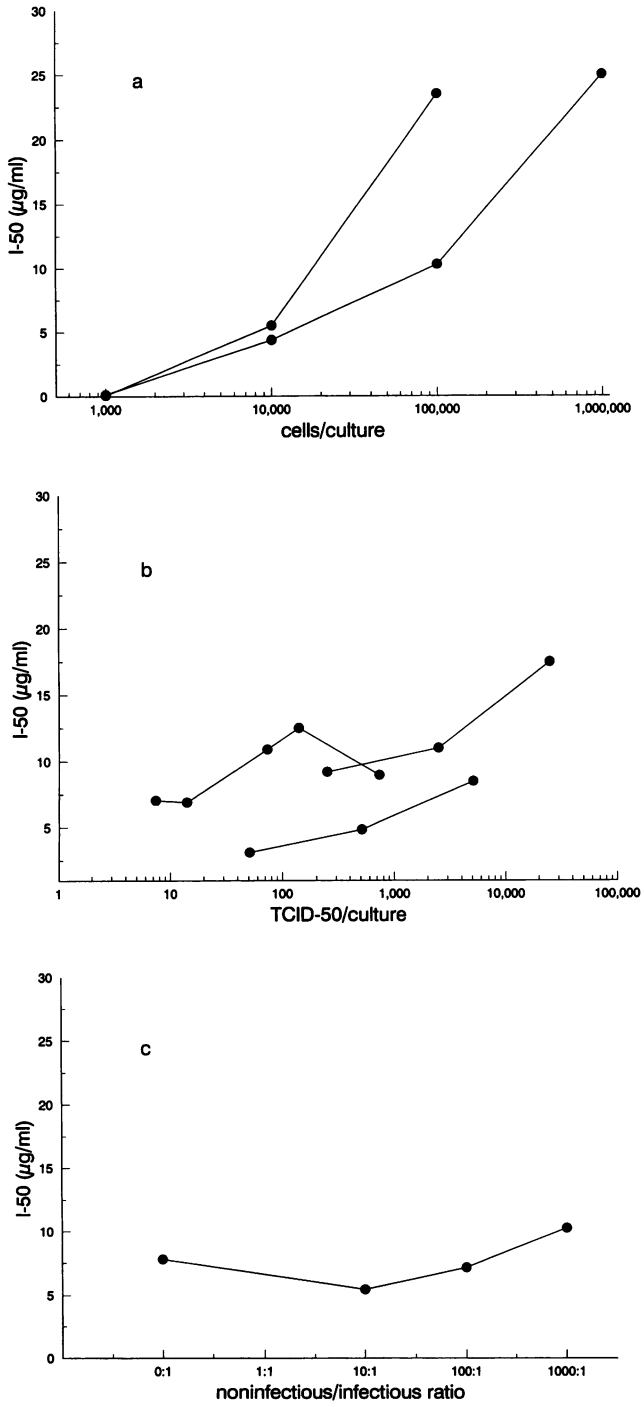


FIG. 3. Influence of cell number, virus concentration, and ratio of infectious to noninfectious particles on sCD4 neutralizing capacity. The dose of sCD4 required to inhibit infectivity by 50% ($I_{50}$) was determined with different numbers of cells and constant input virus (100 $TCID_{50}$) (a), with different amounts of input virus and constant numbers of cells ($10^5$ per culture) (b), or with different ratios of heat-inactivated to native HIV-1 (100 $TCID_{50}$) (c). The separate curves represent different experiments.

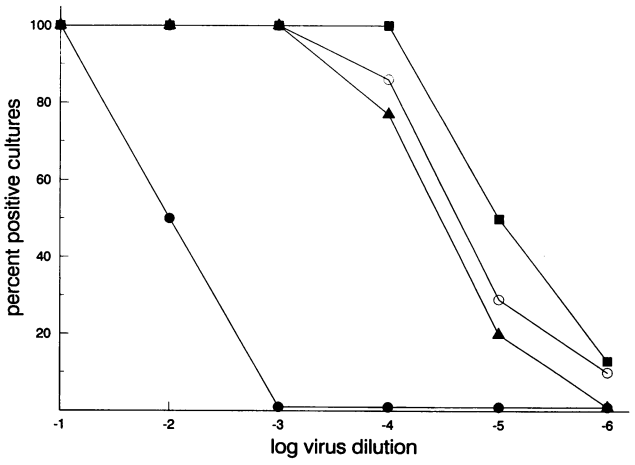


FIG. 4. Inactivation of HIV-1 by sCD4. An HIV-$1_{LAV}$ inoculum was incubated with sCD4 (50 μg/ml) for 2 h at 37°C (●) or at 0°C (○); serial 10-fold dilutions were made and added to cells in microculture for detection of viral replication. Alternatively, HIV-1 and sCD4 were held at 37°C in separate tubes and separate dilutions were made and mixed together just before addition to cell culture (■). Titration of HIV-1 incubated at 37°C in the absence of sCD4 is also shown (▲).

acute infection of PBL. Presumably, the two preparations would differ in the ratio of infection-competent to noncompetent particles (17). When the preparations were tested at the same infectivity (100 $TCID_{50}$), there was no substantial difference in sCD4 inhibition: the $I_{50}$ for the early harvest was 4.8 μg/ml, and that for the late harvest was 6.9 μg/ml.

**Inactivation.** An HIV inoculum (LAV strain) was mixed with sCD4 (50 μg/ml) and incubated for 2 h at 37°C. Serial 10-fold dilutions were made and added to cells; viral replication was monitored to determine the $ID_{50}$ titer (Fig. 4). Two control titrations were performed. In one, HIV and sCD4 were preincubated together at 0 instead of 37°C. In the other, HIV and sCD4 were preincubated in separate tubes and separate dilutions were made and combined just before addition to cells. Thus, the inactivation and control cultures contained identical amounts of HIV and sCD4 but differed in the opportunity for interaction at 37°C before addition to cells. A considerable reduction in titer occurred under these conditions (Fig. 4); we attribute this reduction to an inactivation of HIV that occurs at 37°C. The optimal dose of sCD4 required for inactivation was greater than that required in the neutralization assay, and a preincubation time at 37°C of 60 min was required for optimal inactivation (Fig. 5).

Primary or first-passage isolates were relatively resistant to inactivation by sCD4. Six isolates were tested for inactivation by sCD4 (200 μg/ml). Log reductions in titer of 0 to 0.64 (average, 0.27) were observed, in contrast to the titer of the LAV strain, which was consistently reduced by 3 log units (1,000-fold). Examples of sCD4 inactivation of four first-passage isolates are shown in Fig. 6. Prolonged incubation of one primary isolate or the LAV strain at 0°C for 6 h did not result in any greater inactivation than did the 2-h incubation.

**Radioimmunoprecipitation analysis of proteins from sCD4-incubated virions.** To determine whether the conditions of sCD4 incubation that result in inactivation cause a change in the covalent structure of HIV-1 proteins (proteolysis or covalent cross-linking), internally radiolabeled virions were incubated with sCD4 at 37 and 4°C. The preparations were

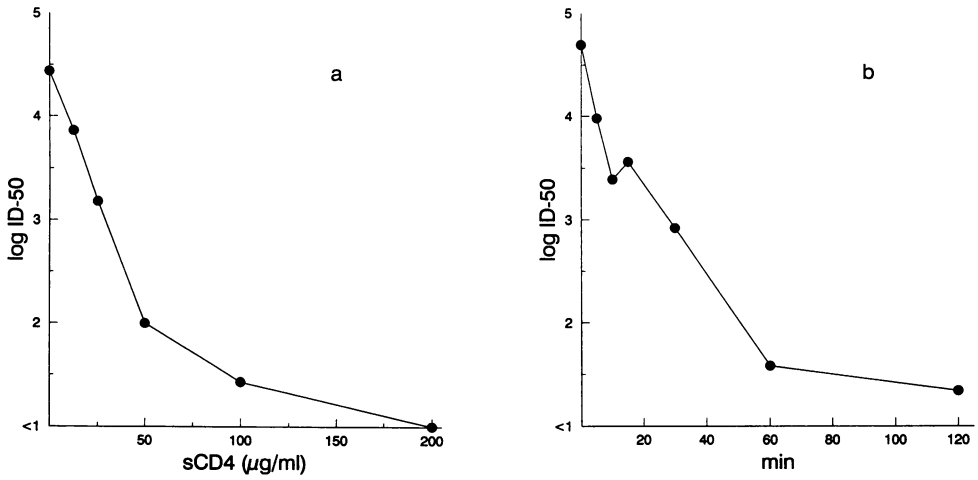


FIG. 5. Inactivation of HIV-1 by sCD4. HIV-1 and sCD4 were preincubated at 37°C and diluted for determination of infectivity titer ($ID_{50}$) in microculture. (a) Preincubation was for 2 h with the indicated concentrations of sCD4. (b) Preincubation was for the indicated times with 50 μg of sCD4 per ml. The zero concentration-time value is the $ID_{50}$ titer of the separately diluted control performed with 200 μg of sCD4 per ml (a) or at the 2-h time point (b).

lysed with detergent, and viral proteins were immunoprecipitated with polyclonal anti-HIV antibodies or polyclonal anti-gp120 antibodies. SDS-PAGE analysis of dissociated immunoprecipitates under reducing or nonreducing conditions failed to reveal a change in banding intensity or fragmentation or a molecular weight shift of the viral proteins (Fig. 7). (The small band above gp120 in Fig. 7a, lane 2, was not reproducible.)

**Contribution of inactivation to neutralization.** The optimal concentrations of sCD4 in the neutralization and inactivation assays were different, suggesting that the two assays measure separate phenomena (Fig. 1 and 5). Since inactivation does not occur or is very slow at 0°C, it was possible to determine the contribution, if any, of inactivation to blocking infectivity in the neutralization assay. HIV (100 $TCID_{50}$) and graded doses of sCD4 were incubated with cells at 0 or 37°C, and the sCD4 $I_{50}$ was determined (Fig. 8a). Alternatively, the HIV-sCD4 mixtures were preincubated for 2 h at 37 or 0°C and then incubated with cells at 0°C (Fig. 8b) or 37°C (Fig. 8c). Neutralization was somewhat more efficient at 37 than at 0°C, but the shift in $I_{50}$ was not dramatic under any of these conditions. Similar results were obtained with a first-passage isolate (data not shown). Assuming that neutralization at 0°C is due solely to reversible blockage of receptor binding and that the increment in neutralization at 37°C is caused by superimposed inactivation, inactivation appears to contribute relatively little to the results in the dynamic range of the neutralization curves. This is consistent with our finding that the optimal dose range of sCD4 for inactivation is higher than that required for neutralization.

**Propagation of primary isolates through cell lines.** Two primary isolates were passed 20 times through PHA-activated PBL. Recovered titers became progressively higher, but HIV from the 20th passage had the same relative resistance to sCD4 neutralization as HIV from the original and first passage. All first-passage isolates readily infected human monocytes purified by adherence (32) as well as nonadherent normal T-cell populations. We were unable to infect the T-cell lines CEM and H9 with the 1st-passage (or 20th-passage) isolates. However, the T-cell line C8166 was readily infected by primary isolates. Primary isolates passaged through the C8166 cell line became more sensitive to sCD4. For example, the $I_{50}$ of a primary isolate in the neutralization assay was 77 and 7 μg/ml before and after passage in C8166, respectively. In the inactivation assay, prepassage virus titer was reduced by 0.31 log unit (2-fold), whereas postpassage titer was reduced by >3.69 log units (4,898-fold).

**Binding and avidity measurements.** The relative avidities of primary isolates for sCD4 were compared by using an ELISA. Detergent lysates of HIV strains were incubated in solution with graded doses of sCD4. Envelope-sCD4 complexes were captured with solid-phase anti-gp120 antibodies, and bound sCD4 was detected with anti-CD4 reagents. The concentration of sCD4 required for half-maximal binding was taken as a measurement of relative affinities. Although not strictly a measurement of affinity (because precise measurements of bound and free gp120-sCD4 are not possible), the procedure is operationally the same as that done in formal Scatchard analysis. Essentially, when gp120 is half-saturated with sCD4, a numerator and a denominator term in the equation for the equilibrium constant $K_d$ cancel out, leaving free sCD4 concentration equal to $K_d$. At half-saturation, free sCD4 concentration equals input sCD4 concentration minus one-half the gp120 concentration (assuming 1:1 binding). If gp120 content is the same in all systems (verified by similar levels of maximal binding), input sCD4 concentration bears a constant relationship to free sCD4 concentration (and therefore to $K_d$). For practical purposes, variation in gp120 content will have little influence because the input sCD4 concentration exceeds the gp120 concentration by several orders of magnitude at half-saturation. Of course, it is also assumed that equilibrium is reached, that any errors are systematic, and that deviations affect all gp120 systems equally. Given this, comparison of sCD4 concentration required for half-maximal binding is a tentative but reasonable means of comparing affinities. This type of system has been used by Moore et al. (27, 29, 30) to measure CD4-gp120 affinity.

Relative affinity measurements of the LAV isolate and three primary isolates were performed at 37 and 4°C. Incubation of sCD4 with viral lysates at the respective tempera-

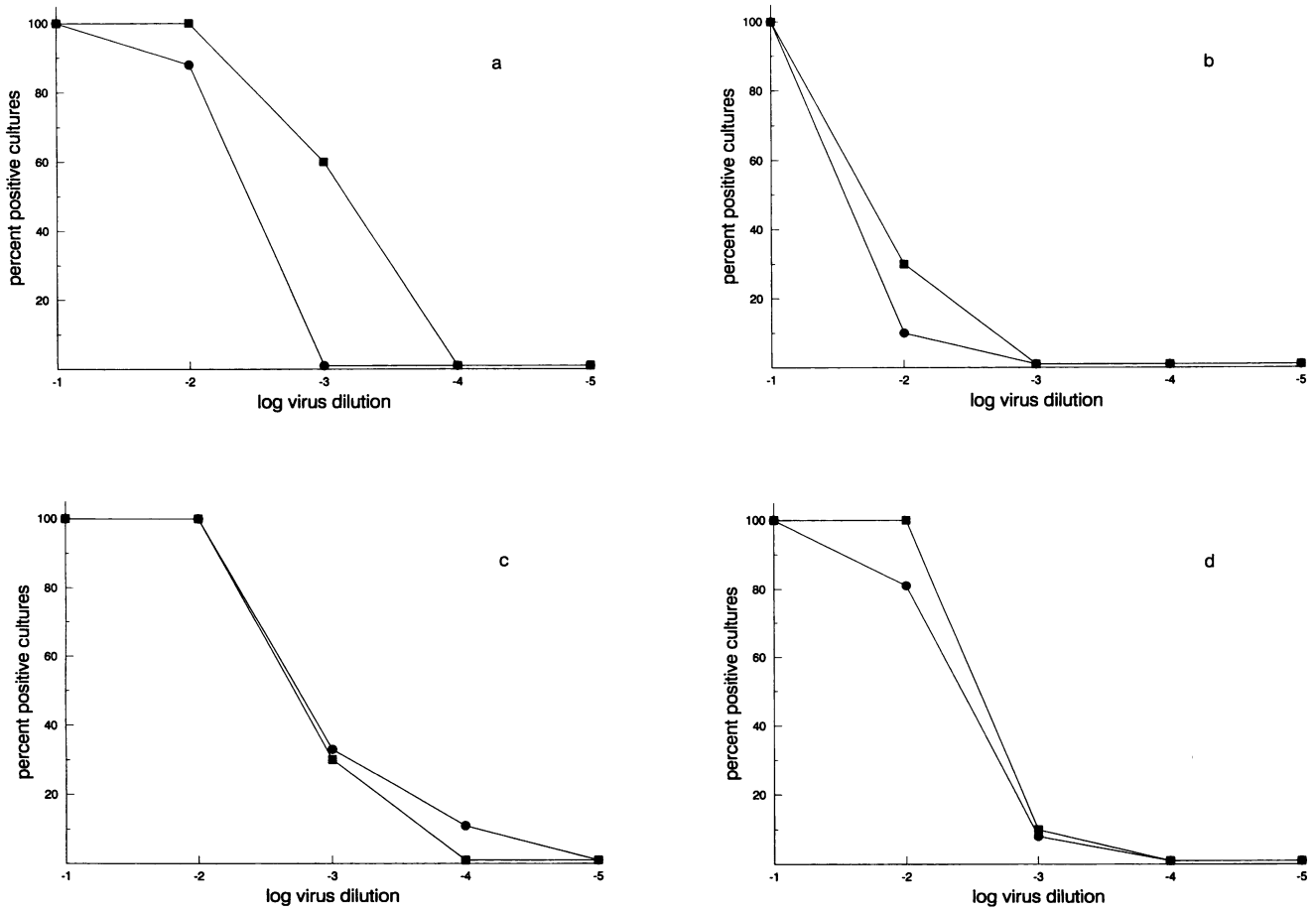


FIG. 6. Inactivation of fresh HIV-1 isolates by sCD4. Four fresh or first-passage isolates of HIV-1 were preincubated with 200 μg of sCD4 per ml for 2 h at 37°C, and viral titrations were performed (●). The titer is compared with that of cultures for which the isolate and sCD4 were separately incubated and diluted and then combined just before addition to microculture (■). (a) Strain 1; (b) strain 3; (c) strain 2; (d) strain 4. For a comparison with the LAV strain of HIV-1, see Fig. 4.

tures was for 6 h: 2 h in solution and 4 h while the solutions were in the gp120-capture wells. Thereafter, the procedure was performed at 4°C. No substantial differences in affinity between the strains or at either temperature were detected (Table 1). The $K_d$ was 0.2 to 0.7 nM for all isolates. The same binding assay was performed with whole virions suspended in solution after removal of free gp120 by ultracentrifugation. The binding reaction occurred in solution in the absence of detergent; virions were captured with solid-phase gp120 antibodies, and detergent was added after removal of unbound sCD4 to complete the ELISA for detection of bound sCD4. Under these conditions, the concentration of sCD4 required for half-maximal binding at 4°C was higher than that at 37°C (Table 1). Furthermore, the plateau level of binding (apparent saturation) for whole virions was lower at 4 than at 37°C. Maximum binding of whole virions at 4°C, expressed as a percentage of the binding observed with detergent lysates of the same preparations that were assayed in parallel, is shown in Table 1. The apparent incomplete saturation at 4°C is not due to incomplete capture of sCD4-incubated virions compared with lysates in the ELISA because detection of captured gp120 (rather than bound sCD4) was equivalent for whole virions or lysates at 4 or 37°C.

**sCD4-induced dissociation of gp120 from virions.** HIV-1 preparations were fractionated on S-1000 columns after a 2-h incubation at 37°C with or without sCD4. The infectivity titer and p24 levels peaked in the void volume, whereas gp120 levels were bimodal. The gp120 content of the void volume pool was taken as a measure of virion-associated gp120, and reduction in the ratio of gp120 to p24 was the measure of sCD4-induced dissociation of gp120 from virions. We opted for this method of calculation rather than trying to quantify the second gp120 peak, as this allows an internal control for virion content (p24 levels). Furthermore, the second gp120 peak tended to be spread out, and pools tended to register below the useful range of our gp120 assay (especially when primary isolates were tested). In multiple experiments, sCD4 induced the dissociation of 64 to 80% of virion-associated gp120 from the LAV strain but of only 6 to 26% from primary isolates.

## DISCUSSION

Two mechanisms by which sCD4 blocks HIV-1 infection are apparent: reversible blockage of receptor binding and irreversible inactivation of virion infectivity. For reasons that follow, each mechanism predominates in one of the two assays we performed. Both assays measure blocking of

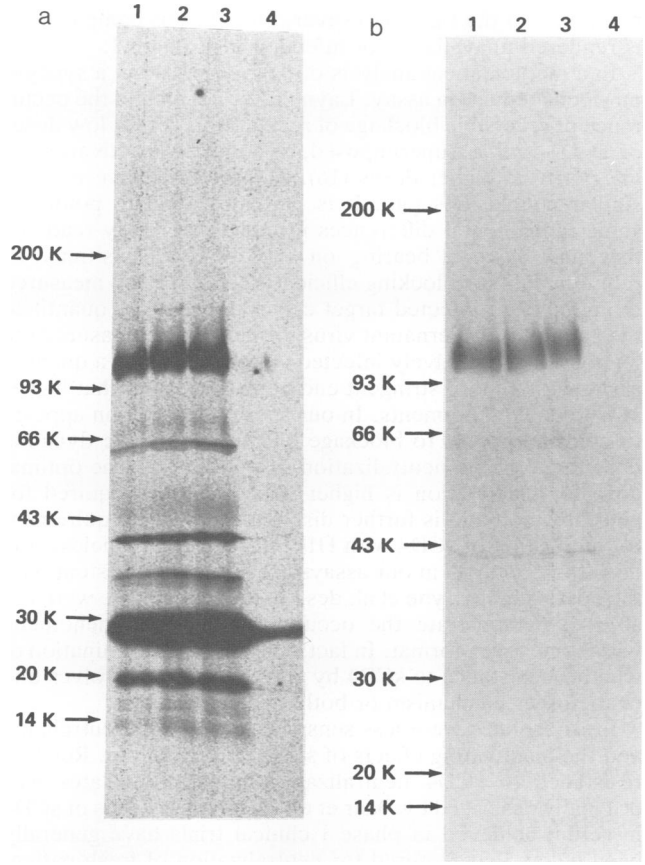


FIG. 7. Radioimmunoprecipitation analysis of sCD4-incubated, $^{35}$S-labeled HIV-1. [$^{35}$S]methionine- and [$^{35}$S]cysteine-labeled extracellular virus (LAV strain) was incubated with sCD4 (100 μg/ml) at 37 or 4°C for 2 h. The preparations were lysed with detergent, and immunoprecipitates were analyzed by SDS-PAGE under nonreducing (data not shown) and reducing conditions. (a) HIV-1 proteins were immunoprecipitated with polyclonal anti-HIV-1 antibodies. (b) Proteins were immunoprecipitated with polyclonal anti-gp120 antibodies. Lanes 1, HIV-1 incubated at 0°C; lanes 2, HIV-1 incubated at 37°C; lanes 3, HIV-1 incubated at 37°C without sCD4; lanes 4, nonimmune (control) immunoprecipitate.

infectivity but differ procedurally. In the neutralization assay, graded doses of sCD4 and a constant amount of HIV (100 $TCID_{50}$) are incubated with cells. In the inactivation assay, undiluted HIV is preincubated at 37°C with sCD4, and an infectivity titration of the mixture is performed.

The quantity of sCD4 required for a given amount of neutralization ($I_{50}$) depends on the target cell concentration, whereas parameters such as virus input and infectious/noninfectious particle ratio had less influence on the $I_{50}$ (Fig. 3). When conceptualized in terms of equilibrium, two reactions that compete for available virions can be considered:

Reaction 1

$$\text{virus} + \text{cellular CD4} \rightleftharpoons \text{virus-cellular CD4}$$

$$K_d = \frac{[\text{virus}]\,[\text{cellular CD4}]}{[\text{virus-cellular CD4}]}$$

Reaction 2

$$\text{virus} + \text{sCD4} \rightleftharpoons \text{virus-sCD4}$$

$$K_d = \frac{[\text{virus}]\,[\text{sCD4}]}{[\text{virus-sCD4}]}$$

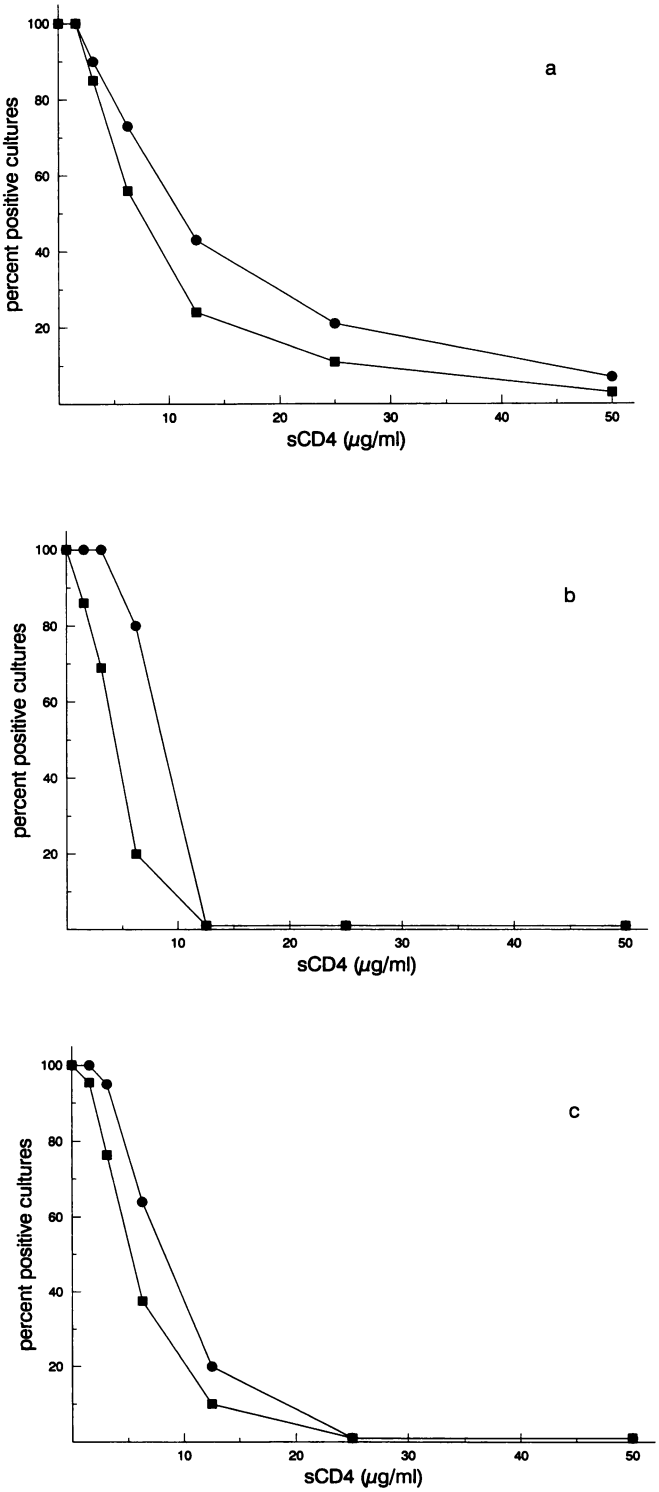


FIG. 8. Neutralization at 0 and 37°C. Levels of neutralization at 0°C (●) or 37°C (■) of a constant HIV-1 inoculum (100 $TCID_{50}$ of the LAV strain) by sCD4 are compared. (a) HIV and sCD4 were mixed and immediately incubated with cells at 0 or 37°C for 4 h before washing and plating in microculture. (b) HIV-sCD4 mixtures were preincubated at 0 or 37°C for 2 h and then incubated with cells for 4 h at 0°C. (c) HIV-sCD4 mixtures were preincubated at 0 or 37°C for 2 h and then incubated with cells for 4 h at 37°C.

TABLE 1. sCD4–HIV-1 binding measurements

| HIV-1 isolate | Affinity ($K_d$) (nM) of: | | | | sCD4 binding capacity (%)[a] | | sCD4-induced shedding of gp120 (%) |
|---|---|---|---|---|---|---|---|
| | Viral lysates | | Whole virions | | | | |
| | 4°C | 37°C | 4°C | 37°C | 4°C | 37°C | |
| LAV | 0.5 | 0.7 | 39.4 | 6.5 | 65 | 99 | 80 |
| Primary 1 | 0.7 | 0.7 | 42.7 | 2.0 | 68 | 98 | 7 |
| Primary 2 | 0.4 | 0.4 | 35.1 | 18.9 | 41 | 78 | 26 |
| Primary 3 | 0.2 | 0.4 | | 2.0 | | 98 | 6 |

[a] (Whole virion/viral lysate) × 100.

The dissociation constants ($K_d$) do not solely reflect the intrinsic affinity of the gp120-CD4 reaction, which would be expected to be the same in each reaction. Rather, the biological affinity or operational avidity may be influenced by positive or negative cooperativity in binding and by the potential for multipoint attachment. The relative difference in $K_d$s determines the $I_{50}$, and this difference may not be the same for different isolates. Given that the $K_d$s are fixed but may not be the same, the relative amounts of reaction products depend on the relative concentrations of cellular CD4 and sCD4; thus, the $I_{50}$ rises with increasing cell dose (Fig. 3a). On the other hand, virus concentration has the same effect on both reactions. Thus, the $I_{50}$ is relatively independent of input virus concentration (Fig. 3b) as long as virus input does not exceed available cellular CD4 and sCD4. Infection (the assay readout) can be considered an irreversible consequence of reaction 1, but does not necessarily drive the reaction to the right. In kinetic terms, the probability that an input virion (or a virion that has just dissociated from CD4) will bind to cellular CD4 or sCD4 is a function of their respective concentrations. In our assay, an $I_{50}$ of 5 μg/ml corresponds to a molar ratio of 1,000,000 molecules of sCD4 per molecule of cellular CD4 (assuming the cell culture is 60% CD4 T cells containing 40,000 molecules of CD4 per cell). The high ratio reflects the necessity of keeping the probability of "infectious hits" during the overnight incubation of virus with cells to near zero or at least below the threshold of detection by the assay. Thus, the neutralization assay performs as anticipated from considerations of reversible receptor-ligand interaction.

In the inactivation assay, undiluted HIV is preincubated with sCD4 at 37°C and an infectivity titration of the mixture is performed (Fig. 4 and 5). The reduction in titer cannot be explained on the basis of reversible blockage of receptor binding or neutralization that occurs in the culture. With dilution, the amount of sCD4 actually present in the cultures is orders of magnitude lower than that required in the neutralization assay (especially at the end point of the assay). If the titer reduction is caused by reversible blockage of receptor binding, the sample that was preincubated at 0°C should register similar results. With sCD4 in excess, binding to purified gp120 should reach a plateau within 2 h at 0°C (8, 9, 14, 18, 22, 27, 28, 35), and the difference between sCD4 dose requirements at 0°C and those at 37°C in the neutralization assay is small (Fig. 8). Also, with dilution of the sample preincubated at 37°C, there is ample time in the cultures during the overnight incubation with cells for dissociation of preformed gp120-sCD4 complexes (8, 9, 14, 26, 28). Inactivation of virus by sCD4 does not necessarily drive reaction 2 to the right. However, virus in this compartment is rendered unavailable for infection in reaction 1.

In a mathematical analysis of dose response in a syncytium-focus reduction assay, Layne et al. postulated the occurrence of reversible blockage of receptor binding at low doses of sCD4 and a superimposed, synergistic inactivation of infectivity at higher doses (16). Moore et al. have reached similar conclusions (30). It is perhaps useful to point out some fundamental differences in infectivity assay readouts that may have a bearing on the relative sensitivity or requirements for blocking efficiency. Layne et al. measured the number of infected target cells; Moore et al. quantified the amount of supernatant virus produced; we measured the number of productively infected cultures. Ours is a quantile assay with a more stringent end point and somewhat higher sCD4 dose requirements. In our assay, inactivation appears to contribute little to blockage of infection in the dynamic dose range of the neutralization assay (Fig. 8). The optimal dose for inactivation is higher than the dose required for neutralization and is further distinguished by a requirement for incubation of sCD4 with HIV at 37°C. Nevertheless, the dose requirements in our assays are entirely consistent with the postulate of Layne et al. despite the fact that we were not able to demonstrate the occurrence of both phenomena within one assay format. In fact, this allows determination of whether resistance to sCD4 by certain isolates involves one or the other mechanism or both mechanisms.

Fresh isolates were less sensitive to both the neutralizing and the inactivating effects of sCD4 (Fig. 1 and 6). Relative resistance to sCD4 neutralization by fresh isolates was originally described by Daar et al. (6), and the levels of sCD4 in serum achieved in phase I clinical trials have generally been below that required for neutralization of fresh patient isolates in vitro (15, 37). Higher doses of sCD4 can be tolerated without toxicity, but whether this will be effective or practical remains to be determined. It has been postulated that a difference in intrinsic affinity of gp120 for CD4 accounts for the relative resistance. This postulate is supported by analogy with HIV-2, for which intrinsic affinity of envelope for CD4 is lower than that of HIV-1 and higher concentrations of sCD4 are required for neutralization (14, 18, 27). It is also supported by experiments with mutant gp120 molecules generated by site-directed mutagenesis or by in vitro selection in the presence of sCD4. Mutants with lower affinity for CD4 generally (but not always) require more sCD4 for inhibition (24, 39). However, several groups have examined gp120 from selected strains of HIV-1 known to be relatively resistant or sensitive to sCD4 and found similar (high) affinities (3, 29, 41). Superficially, it does not seem reasonable that intrinsic affinity could account for the difference. A lower affinity for sCD4 would simply be balanced by less-efficient binding to cellular CD4. Nevertheless, a change in intrinsic affinity may well affect the operational $K_d$s of reactions 1 and 2 quite differently. For instance, binding of virions to cellular CD4 may proceed in a zipper-like manner by virtue of multipoint attachment, providing a competitive advantage for reaction 1 over reaction 2. This relative advantage (that is, the difference in the two $K_d$s) may be greater for isolates with lower affinity. In kinetic terms, more-frequent dissociation of sCD4 from low-affinity virions would require a higher ratio of sCD4 to cellular CD4 to maintain a low probability of infectious hits.

Like other investigators (3, 29, 41), we found no substantial difference in intrinsic affinity of gp120 for sCD4 when we measured affinity in detergent lysates of primary or laboratory strains at either 4 or 37°C (Table 1). However, whole-

virion avidity at 37°C was somewhat less than that of viral lysates and was reduced even more at 4°C. Moreover, the sCD4 binding capacity of whole virions at 4°C was less than that at 37°C. Whether the incomplete saturation of virions during a 6-h incubation at 4°C represents an absolute or a relative block remains to be determined. Similar differences in avidity of sCD4 for gp120 (when gp120 is organized on viral or cellular membranes) have also been reported by Moore et al. and Dimitrov et al. for somewhat different systems (8, 9, 28–30). If full saturation of gp120 sites on the virion were required for inactivation of HIV by sCD4, this would explain the lack of inactivation at 0°C and the finding that optimal inactivation at 37°C requires concentrations of sCD4 substantially in excess of the $K_d$. However, it does not explain the relative resistance of certain strains to sCD4 since both LAV and primary isolates were similar with respect to temperature-dependent binding properties.

The most obvious explanation for the apparent temperature dependence of sCD4 binding to whole virions is that a certain amount of flexibility and mobility of gp120 is required for efficient accommodation of sCD4 by the virion. Evidence for a shift in organization of gp120 on virions induced by sCD4 has been obtained by using serologic techniques. At 37°C, sCD4 induces shedding of gp120 from virions (31), exposing epitopes of the transmembrane protein (gp41) (36). The exposure of gp41 epitopes occurs at 4°C as well (36), and this is not simply a result of gp120 shedding, which occurs very slowly at 4°C (8, 9, 28–31). At both 4 and 37°C, binding of sCD4 causes regions of gp120, particularly the V3 region, a principal neutralizing epitope that is not involved in CD4 binding, to become more accessible to antibody (36). Conversely, similar changes have been described for CD4 subsequent to gp120 binding (5). For binding assays in which soluble gp120 binding to cell surface CD4 is measured, a temperature-dependent shift in cellular CD4 may be required to fully accommodate soluble gp120 (26, 39).

Moore et al. described sCD4-induced shedding of gp120 from cell-free virions at 37°C (31), and a similar phenomenon has been described for HIV-infected cells (2, 8–10, 18). Moore et al. demonstrated that shedding, loss of infectivity, and an avidity transition from 4 to 37°C were parallel phenomena (28–30). The inactivation we observe may be caused by shedding of gp120 from virions. The dose and temperature requirements are identical to those described by Moore et al. Therefore, the difference between fresh and laboratory isolates may relate to relative differences between strains in the potential for shedding (Table 1). However, this does not explain the resistance observed in our neutralization assay, in which inactivation contributed little to blocking in the dynamic range of the neutralization curves (Fig. 8). Fresh isolates maintain resistance to sCD4 when the incubation phase of the neutralization assay is performed at 0°C. However, a binding or postbinding event that occurs at both 4 and 37°C (and presumably is a prerequisite for shedding at 37°C) may be responsible for resistance to neutralization and inactivation in our assays.

All of our fresh or first-passage isolates readily infected normal monocytes and T cells, but we could not achieve infection of the T-cell lines CEM and H9. This biologic phenotype was maintained through multiple passages in PBL and is similar to that described by several groups as "monocytotropic," "slow-low," or "early" isolates. Several groups have mapped the "macrophage-tropic" phenotype to the V3 region of the envelope (13, 34, 38, 42), and recently, resistance to sCD4 has been mapped to this region as well, by using the same recombinant viruses (12, 33). The V3 region must mediate some secondary event that is required for infection, and this function is susceptible to interruption by V3-specific neutralizing antibodies. However, the precise role of the V3 region in infection and the mechanism by which it mediates sCD4 resistance remain completely obscure. Although we could not infect the T-cell lines H9 and CEM with our primary isolates and sCD4 resistance was maintained through 20 passages in PBL, infection of the T-cell line C8166 did result in high-titer progeny virus that had become sensitive to sCD4. Turner et al. (41) recovered sCD4-sensitive isolates by passage of relatively resistant stocks in C8166. No sequence changes in the V3 region or changes in CD4 affinity were found (41).

"Biologic affinities" have correlated very nicely with direct measurements of affinities, and some very cogent interpretations of the neutralization and inactivation (shedding) phenomena have been put forward, especially by Layne, Moore, and their respective colleagues (16, 29, 30). Realizing that to extrapolate from direct measurements of binding to results of infectivity assays with a remote readout is difficult, we conservatively interpret our data as follows. Parameters of the neutralization assay are consistent with reversible blockage of receptor binding. Inactivation of HIV by sCD4 has a dependence on temperature and concentration that parallels the saturation binding properties of whole virions; that is, binding is less efficient and less complete at 4 than at 37°C. The affinity-avidity properties of gp120 and virion-associated gp120 are similar for sCD4-resistant and -sensitive strains. However, resistant strains shed less gp120 as a consequence of sCD4 binding. This may explain resistance to inactivation, but it is difficult to invoke differential shedding as an explanation for resistance in the neutralization assay. Neutralization for both sensitive and resistant strains occurs in a dose range of sCD4 that causes minimal inactivation or shedding. However, a binding or postbinding event that occurs at both 4 and 37°C and that is a fundamental prerequisite both for neutralization and for inactivation and shedding may be responsible. Possibilities include secondary interactions of sCD4 with gp120 (induced fit or secondary intensification of the bond); intra- or intermolecular conformation changes in gp120, the V3 region, or CD4; and mobility changes of gp120 in the viral membrane.

## REFERENCES


1. **Adachi, A., H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, and M. A. Martin.** 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J. Virol. **59**:284–291.
2. **Berger, E. A., J. D. Lifson, and L. E. Eiden.** 1991. Stimulation of glycoprotein gp120 dissociation from the envelope glycoprotein complex of human immunodeficiency virus type 1 by soluble CD4 and CD4 peptide derivatives: implications for the role of the complementarity-determining region 3-like region in membrane fusion. Proc. Natl. Acad. Sci. USA **88**:8082–8086.
3. **Brighty, D. W., M. Rosenberg, I. S. Y. Chen, and M. Ivey-Hoyle.** 1991. Envelope proteins from clinical isolates of human immunodeficiency virus type 1 that are refractory to neutralization by soluble CD4 possess high affinity for the CD4 receptor. Proc. Natl. Acad. Sci. USA **88**:7802–7805.
4. **Camerini, D., and B. Seed.** 1990. A CD4 domain important for HIV-mediated syncytium formation lies outside the virus binding site. Cell **60**:747–754.
5. **Celada, F., C. Cambiaggi, J. Maccari, S. Burastero, T. Gregory, T. Patzer, J. Porter, C. McDanal, and T. Matthews.** 1990. Antibody raised against soluble CD4-gp120 complex recognizes the CD4 moiety and blocks membrane fusion without inhibiting

CD4-gp120 binding. J. Exp. Med. **172:**1143–1150.
6. **Daar, E. S., X. Li, T. Moudgil, and D. D. Ho.** 1990. High concentrations of recombinant soluble CD4 are required to neutralize primary human immunodeficiency virus type 1 isolates. Proc. Natl. Acad. Sci. USA **87:**6574–6578.
7. **Deen, K. C., J. S. McDougal, R. Inacker, G. Folena-Wasserman, J. Arthos, J. Rosenberg, P. J. Maddon, R. Axel, and R. W. Sweet.** 1988. A soluble form of CD4(T4) protein inhibits AIDS virus infection. Nature (London) **331:**82–84.
8. **Dimitrov, D. S., K. Hillman, J. Manischewitz, R. Blumenthal, and H. Golding.** 1992. Kinetics of soluble CD4 binding to cells expressing human immunodeficiency virus type 1 envelope glycoprotein. J. Virol. **66:**132–138.
9. **Dimitrov, D. S., R. L. Willey, M. A. Martin, and R. Blumenthal.** 1992. Kinetics of HIV-1 interactions with sCD4 and CD4+ cells: implications for inhibition of virus infection and initial steps of virus entry into cells. Virology **187:**398–406.
10. **Hart, T. K., R. Kirsh, H. Ellens, R. W. Sweet, D. M. Lambert, S. R. Petteway, J. Leary, and P. J. Bugelsky.** 1991. Binding of soluble CD4 proteins to human immunodeficiency virus type 1 and infected cells induces the release of envelope glycoprotein gp120. Proc. Natl. Acad. Sci. USA **88:**2189–2193.
11. **Healey, D., L. Dianda, J. Moore, J. S. McDougal, M. J. Moore, P. Estess, D. Buck, P. D. Kwong, P. C. L. Beverley, and Q. J. Sattentau.** 1990. Novel anti-CD4 monoclonal antibodies separate human immunodeficiency virus infection and fusion of CD4+ cells from virus binding. J. Exp. Med. **172:**1233–1242.
12. **Hwang, S. S., T. J. Boyle, H. K. Lyerly, and B. R. Cullen.** 1992. Identification of envelope V3 loop as the major determinant of CD4 neutralization sensitivity of HIV-1. Science **257:**535–537.
13. **Hwang, S. S., T. J. Boyle, K. Lyerly, and B. R. Cullen.** 1991. Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. Science **253:**71–74.
14. **Ivey-Hoyle, M., J. S. Culp, M. A. Chaikin, B. D. Hellmig, T. J. Matthews, R. W. Sweet, and M. Rosenberg.** 1991. Envelope glycoproteins from biologically diverse isolates of immunodeficiency viruses have widely different affinities for CD4. Proc. Natl. Acad. Sci. USA **88:**512–516.
15. **Kahn, J. O., J. D. Allen, T. L. Hodges, L. D. Kaplan, C. J. Arri, H. F. Fitch, A. E. Izu, J. Mordenti, S. A. Sherwin, J. E. Groopman, and P. A. Volberding.** 1990. The safety and pharmacokinetics of recombinant soluble CD4 (rCD4) in subjects with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. A phase I study. Ann. Intern. Med. **112:**254–261.
16. **Layne, S. P., M. J. Merges, M. Dembo, J. L. Spouge, and P. Nara.** 1990. HIV requires multiple gp120 molecules for CD4-mediated infection. Nature (London) **346:**277–279.
17. **Layne, S. P., M. J. Merges, J. L. Spouge, M. Dembo, and P. L. Nara.** 1991. Blocking of human immunodeficiency virus infection depends on cell density and viral stock age. J. Virol. **65:**3293–3300.
18. **Looney, D. J., S. Hayashi, M. Nicklas, R. R. Redfield, S. Broder, F. Wong-Staal, and H. Mitsuya.** 1990. Differences in the interaction of HIV-1 and HIV-2 with CD4. J. Acquired Immune Defic. Syndr. **3:**649–657.
19. **McDougal, J. S., S. P. Cort, M. S. Kennedy, C. D. Cabridilla, P. M. Feorino, D. P. Francis, D. Hicks, V. S. Kalyanaraman, and L. S. Martin.** 1985. Immunoassay for the detection and quantitation of infectious human retrovirus, lymphadenopathy-associated virus (LAV). J. Immunol. Methods **76:**171–183.
20. **McDougal, J. S., M. S. Kennedy, J. M. Sligh, S. P. Cort, A. Mawle, and J. K. A. Nicholson.** 1986. Binding of HTLV-III/LAV to $T4^+$ cells by a complex of the 110K viral protein and the T4 molecule. Science **231:**382–385.
21. **McDougal, J. S., L. S. Martin, S. P. Cort, M. Mozen, C. M. Heldebrant, and B. L. Evatt.** 1985. Thermal inactivation of acquired immunodeficiency syndrome virus. Human T lymphocytotropic virus-III/lymphadenopathy-associated virus, with special reference to antihemophilic factor. J. Clin. Invest. **76:**875–877.
22. **McDougal, J. S., A. Mawle, S. P. Cort, J. K. A. Nicholson, G. D. Cross, J. A. Scheppler-Campbell, D. Hicks, and J. Sligh.** 1986. Cellular tropism of the human retrovirus HTLV-III/LAV. I. Role of T cell activation and expression of the T4 antigen. J. Immunol. **135:**3151–3162.
23. **McDougal, J. S., J. K. A. Nicholson, G. D. Cross, S. P. Cort, M. S. Kennedy, and A. C. Mawle.** 1986. Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule: conformation dependence, epitope mapping, antibody inhibition, and potential for idiotypic mimicry. J. Immunol. **137:**2937–2944.
24. **McKeating, J., P. Balfe, P. Clapham, and R. A. Weiss.** 1991. Recombinant CD4-selected human immunodeficiency virus type 1 variants with reduced affinity for CD4 and increased cell fusion capacity. J. Virol. **65:**4777–4785.
25. **Mittler, R. S., and M. K. Hoffman.** 1989. Synergism between HIV gp120 and gp120-specific antibody in blocking human T cell activation. Science **245:**1380–1382.
26. **Moebius, U., L. K. Clayton, S. Abraham, S. C. Harrison, and E. L. Reinherz.** 1992. The human immunodeficiency virus gp120 binding site on CD4: delineation by quantitative equilibrium and kinetic binding studies of mutants in conjunction with a high-resolution CD4 atomic structure. J. Exp. Med. **176:**507–517.
27. **Moore, J.** 1990. Simple methods for monitoring HIV-1 and HIV-2 gp120 binding to soluble CD4 by enzyme-linked immunosorbant assay: HIV-2 has 25-fold lower affinity than HIV-1 for soluble CD4. AIDS **4:**297–305.
28. **Moore, J. P., and P. J. Klasse.** 1992. Thermodynamic and kinetic analysis of sCD4 binding to HIV-1 virions and gp120 dissociation. AIDS Res. Hum. Retroviruses **8:**443–450.
29. **Moore, J. P., J. A. McKeating, Y. Huang, A. Ashkenazi, and D. D. Ho.** 1992. Virions of primary human immunodeficiency virus type 1 isolates resistant to soluble CD4 (sCD4) neutralization differ in sCD4 binding and glycoprotein gp120 retention from sCD4-sensitive isolates. J. Virol. **66:**235–243.
30. **Moore, J. P., J. A. McKeating, W. A. Norton, and Q. J. Sattentau.** 1991. Direct measurement of soluble CD4 binding to human immunodeficiency virus type 1 virions: gp120 dissociation and its implications for virus-cell binding and fusion reactions and their neutralization by soluble CD4. J. Virol. **65:**1133–1140.
31. **Moore, J. P., J. A. McKeating, R. A. Weiss, and Q. J. Sattentau.** 1990. Dissociation of gp120 from HIV-1 virions induced by soluble CD4. Science **250:**1139–1142.
32. **Nicholson, J. K. A., G. D. Cross, G. S. Callaway, and J. S. McDougal.** 1986. In vitro infection of human monocytes with human T lymphotropic virus type III/lymphadenopathy associated virus (HTLV-III/LAV) J. Immunol. **137:**323–329.
33. **O'Brien, W. A., I. S. Y. Chen, D. D. Ho, and E. S. Daar.** 1992. Mapping genetic determinants of human immunodeficiency virus type 1 resistance to soluble CD4. J. Virol. **66:**3125–3130.
34. **O'Brien, W. A., Y. Koyanagi, A. Namazie, J.-Q. Zhao, and I. S. Y. Chen.** 1990. HIV-1 tropism for mononuclear phagocytes can be determined by regions of gp120 outside the CD4-binding domain. Nature (London) **348:**69–73.
35. **Orloff, G. M., S. L. Orloff, M. S. Kennedy, P. J. Maddon, and J. S. McDougal.** 1991. Penetration of CD4 cells by HIV-1. The CD4 receptor does not internalize with HIV and CD4-related signal transduction events are not required for entry. J. Immunol. **146:**2578–2586.
36. **Sattentau, Q. J., and J. P. Moore.** 1991. Conformational changes induced in the human immunodeficiency virus envelope glycoprotein by soluble CD4 binding. J. Exp. Med. **174:**407–415.
37. **Schooley, R. T., T. C. Merrigan, P. Gaut, M. S. Hirsch, M. Holodniy, T. Flynn, S. Liu, R. E. Byington, S. Henochowicz, E. Gubish, D. Spriggs, D. Kufe, J. Schindler, A. Dawson, D. Thomas, D. G. Hanson, B. Letwin, T. Liu, J. Gulinello, S. Kennedy, R. Fisher, and D. D. Ho.** 1990. Recombinant soluble CD4 therapy in patients with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. A phase I-II escalating dosage trial. Ann. Intern. Med. **112:**247–253.
38. **Shioda, T., J. A. Levy, and C. Cheng-Mayer.** 1991. Macrophage and T cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp120 gene. Nature (London) **349:**167–172.

39. **Thali, M., U. Olshevsky, C. Furman, D. Gabuzda, J. Li, and J. Sodroski.** 1991. Effects of changes in gp120-CD4 binding affinity on human immunodeficiency virus type 1 envelope glycoprotein function and soluble CD4 sensitivity. J. Virol. **65:**5007–5012.
40. **Truneh, A., D. Buck, D. R. Cassatt, R. Jusxcak, S. Kassis, S. Ryu, D. Healey, R. Sweet, and Q. J. Sattentau.** 1991. A region in domain 1 of CD4 distinct from the primary gp120 binding site is involved in HIV infection and virus-mediated fusion. J. Biol. Chem. **266:**5942–5948.
41. **Turner, S., R. Tizard, J. DeMarinis, R. B. Pepinsky, J. Zullo, R. Schooley, and R. Fisher.** 1992. Resistance of primary isolates of human immunodeficiency virus type 1 to neutralization by soluble CD4 is not due to lower affinity with the viral envelope glycoprotein gp120. Proc. Natl. Acad. Sci. USA **89:**1335–1339.
42. **Westervelt, P., H. E. Gendelman, and L. Ratner.** 1991. Identification of a determinant within the human immunodeficiency virus 1 surface envelope glycoprotein critical for productive infection of primary monocytes. Proc. Natl. Acad. Sci. USA **88:**3097–3101.