Human Immunodeficiency Virus Type 1 Membrane Fusion Mediated by a Laboratory-Adapted Strain and a Primary Isolate Analyzed by Resonance Energy Transfer

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Previous studies of human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein-mediated membrane fusion have focused on laboratory-adapted T-lymphotropic strains of the virus. The goal of this study was to characterize membrane fusion mediated by a primary HIV-1 isolate in comparison with a laboratory-adapted strain. To this end, a new fusion assay was developed on the basis of the principle of resonance energy transfer, using HeLa cells stably transfected with gp120/gp41 from the T-lymphotropic isolate HIV-1_{LAI} or the macrophage-tropic primary isolate HIV-1_{JR-FL}. These cells fused with CD4⁺ target cell lines with a tropism mirroring that of infection by the two viruses. Of particular note, HeLa cells expressing HIV-1_{JR-FL} gp120/gp41 fused only with PM1 cells, a clonal derivative of HUT 78, and not with other T-cell or macrophage cell lines. These results demonstrate that the envelope glycoproteins of these strains play a major role in mediating viral tropism. Despite significant differences exhibited by HIV-1_{JR-FL} and HIV-1_{LAI} in terms of tropism and sensitivity to neutralization by CD4-based proteins, the present study found that membrane fusion mediated by the envelope glycoproteins of these viruses had remarkably similar properties. In particular, the degree and kinetics of membrane fusion were similar, fusion occurred at neutral pH and was dependent on the presence of divalent cations. Inhibition of HIV-1_{JR-FL} envelope glycoprotein-mediated membrane fusion by soluble CD4 and CD4-IgG2 occurred at concentrations similar to those required to neutralize this virus. Interestingly, higher concentrations of these agents were required to inhibit HIV-1_{LAI} envelope glycoprotein-mediated membrane fusion, in contrast to the greater sensitivity of HIV-1_{LAI} virions to neutralization by soluble CD4 and CD4-IgG2. This finding suggests that the mechanisms of fusion inhibition and neutralization of HIV-1 are distinct.

Following the binding of human immunodeficiency virus type 1 (HIV-1) gp120/gp41 to the cell surface receptor human CD4, a domain of gp41 mediates fusion of the viral and target cell membranes, resulting in the introduction of the viral capsid into the target cell cytoplasm (15). Cells expressing HIV-1 gp120/gp41 also fuse with CD4-expressing cells, leading to the formation of multinucleated giant cells, or syncytia. The initial events in syncytium formation are analogous to the attachment and fusion stages of viral entry (9). First, the cell membranes connect at localized sites; this connection is a rapid and reversible event. Later, the cells fuse irreversibly to form syncytia.

To date, real-time studies of HIV-1 envelope glycoprotein-mediated membrane fusion have been performed with strains of HIV-1 that have been extensively propagated in transformed human T-cell lines. While reporter gene assays have been used successfully to analyze the tropism of primary HIV-1 isolates (3), these have limited utility for analysis of the mechanisms and properties of the membrane fusion process. In order to analyze and compare membrane fusion mediated by the envelope glycoproteins of primary HIV-1 isolates and laboratory-adapted strains, we have developed a new resonance energy transfer (RET)-based fusion assay. This assay is modeled on methods designed to study membrane fusion mediated

by mutants of herpes simplex virus type 1 (HSV-1) or cell fusion induced by polyethylene glycol (14, 32).

The RET assay measures HIV-1 envelope glycoprotein-mediated membrane fusion. This fluorescence-based technique involves labeling one fusion partner (an HIV-1 gp120/gp41-expressing cell line) with fluorescein octadecyl ester (F18; Molecular Probes, Eugene, Oreg.) and the other fusion partner (a CD4-expressing cell line) with octadecyl rhodamine (R18; Molecular Probes). These probes consist of fluorescent molecules conjugated to saturated hydrocarbon chains, 18 carbons long, which spontaneously insert into cell plasma membranes (14). They do not inhibit cellular replication or fusion efficiency (32). The fluorochromes are chosen such that the emission spectrum of one (F18) overlaps the excitation spectrum of the second (R18). Fusion results in the close association of the dyes in the plasma membrane, and thus transfer of the energy generated by F18 excitation to R18 is followed by emission at the R18 spectrum.

Briefly, F18 (5 mg/ml in ethanol) was diluted 1:1,000 in complete tissue culture medium containing 10% fetal bovine serum and adjusted such that the A_{506} was 0.34. R18 (10 mg/ml in EtOH) was similarly diluted such that the A_{565} was 0.52. Cells were incubated overnight in the fluorescent dye-containing culture medium. Fluorochrome-labeled adherent cells were removed from culture flasks by treatment with 0.5 mM EDTA and washed several times in culture medium containing 10% fetal bovine serum. HeLa-env cells (2×10^4) were plated with an equal number of CD4-expressing cells per well in a 96

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TABLE 1. HIV-1 envelope glycoprotein-mediated membrane fusion determined by RET

R18-labeled cells	% RET for fusion with F18-labeled cells ^a				
	HeLa-env _{LAI}		HeLa-env _{JR-FL}		HeLa
	Alone	With OKT4A ^b	Alone	With OKT4A	
HeLa-CD4	10.7 ± 4.4	0.8 ± 0.7	1.0	ND ^c	0.5 ± 0.4
C8166	14.2 ± 1.6	1.3 ± 1.1	2.3 ± 0.4	ND	1.3 ± 0.9
Sup-T1	18.7 ± 1.0	0	0	ND	0
HUT 78	8.2 ± 1.6	0.9 ± 0.6	1.0 ± 1.6	ND	1.0 ± 1.6
PM1	5.0 ± 3.9	1.0 ± 0.6	10.2 ± 3.7	1.1 ± 0.6	0.6 ± 0.7
CHO-CD4 ^d	0.3	ND	0.3	ND	0.2
U87MG-CD4	0.7 ± 0.4	ND	0.6 ± 0.6	ND	1.2 ± 1.1

^a The data are the means ± standard deviations for at least three independent assays, unless otherwise stated.

^b At the initiation of culture, 0.3 µg of MAb per ml was added.

^c ND, not determined.

^d The results are the means for two assays.

well plate in a final volume of 200 µl and incubated for 4 h at 37°C. Controls included wells containing each cell line alone. Following three washes in phosphate-buffered saline (PBS), fluorescence was measured with a Cytofluor plate reader (PerSeptive Biosystems, Framingham, Minn.).

The emission values, X , Y , and Z , were recorded for the following cell combinations. A, HeLa-env cells and CD4-expressing cells; B, HeLa-env cells alone; and C, CD4-expressing cells alone. The following filter combinations were used. X , excitation at 450 nm and emission at 530 nm; Y , excitation at 530 nm and emission at 590 nm; and Z , excitation at 450 nm and emission at 590 nm. For example, A_Z is the measurement obtained by using cell combination A and filter combination Z. The output from the fluorescence plate reader was used to calculate percent RET.

The excitation and emission spectra of F18 and R18 are broad; therefore, when each dye is excited at 450 nm, there is a background emission of energy at 590 nm (B_Z and C_Z). Since this background, or spillover, fluorescence occurs in the absence of RET, the following calculation is used to correct for the spillover. F_{spill} and R_{spill} represent the F18 (B_Z/B_X) and R18 (C_Z/C_Y) spillover coefficients which we have empirically determined to be 0.52 and 0.03, respectively.

$$\%RET = 100 \times \frac{A_Z - (A_X \times F_{\text{spill}}) - (A_Y \times R_{\text{spill}})}{A_Y}$$

The data are expressed as the percent RET, which is derived from a comparison of the RET value to the maximum R18 emission obtained by direct excitation of R18 at 530 nm (A_Y). Only a fraction of the maximum R18 emission is expected to be achieved via RET. This assumption was confirmed by double-labeling cells with both F18 and R18 and calculating the resultant percent RET. When HeLa, PM1 (obtained from R. Gallo and P. Lusso, National Institutes of Health [NIH], Bethesda, Md.) (19), or C8166 (obtained from R. Weiss, Institute of Cancer Research, London, England) cells, were double-labeled, RET values ranging from 15 to 20% were achieved. Accordingly, the largest theoretical RET value expected following fusion of cells would be in the range of 15 to 20%. Indeed, RET values in this range were often observed, indicating that highly efficient mixing of the plasma membranes and dyes occurred in the membrane fusion experiments.

When F18-labeled HeLa-env_{LAI} cells (11) were incubated with R18-labeled HeLa-CD4 cells (20) for 4 h, RET values of

approximately 11% were obtained (Table 1). In contrast, minimal RET levels (0.5%) were observed when F18-labeled HeLa cells were used instead of HeLa-env_{LAI} cells (Table 1). When different ratios of F18-HeLa-env_{LAI} and F18-HeLa cells were incubated with R18-HeLa-CD4 cells and the total number of F18-labeled cells was held constant, the level of RET was directly proportional to the number of input R18-HeLa-env_{LAI} cells (data not shown), indicating that RET is proportional to the number of fusogenic cells present. Moreover, RET was detectable above background when only 10% of the input F18-labeled cells were fusogenic.

The monoclonal antibody (MAb) OKT4A, which inhibits the binding of HIV-1 gp120 to CD4 (21), abrogated RET in cocultures of HeLa-env_{LAI} and CD4⁺ cells (Table 1). No inhibition was observed with the control MAb OKT4 (data not shown). Several T-lymphoblastoid cells which are known to be susceptible to T-cell tropic strains of HIV-1 also specifically fused with the HeLa-env_{LAI} cells (Table 1). HIV-1 is known to bind to, but not infect, rodent cells expressing human CD4; likewise, cells expressing HIV-1 gp120/gp41 will not fuse with rodent cells expressing human CD4 (20). When the R18-labeled CD4⁺ Chinese hamster ovary transfectant (CHO-CD4; Progenics) was incubated with F18-labeled HeLa-env_{LAI} cells, no RET was detected. Similarly, HeLa-env_{LAI} cells did not fuse with the glioblastoma CD4 transfectant U87.MG-CD4 (obtained from P. Clapham, Institute of Cancer Research), which is one of the few CD4⁺ human cell lines refractory to infection or fusion by HIV-1 (5, 6) (Table 1). Thus, conditions which allow cell-to-cell binding in the absence of membrane fusion did not result in RET. Taken together, these results demonstrate that with the RET assay, real membrane fusion events are determined and not the spontaneous transfer of fluorescent dyes between membranes in close proximity.

RET assay as a model system to investigate HIV-host cell tropism. In contrast to T-cell-tropic laboratory-adapted strains, macrophage-tropic primary isolates of HIV-1 do not infect CD4⁺ T-cell lines and often exhibit reduced or no syncytium formation. In order to investigate membrane fusion mediated by a macrophage-tropic primary isolate of HIV-1, HeLa cells stably expressing the envelope glycoprotein from the macrophage-tropic primary isolate HIV-1_{JR-FL} were generated (designated HeLa-env_{JR-FL}). The HIV-1_{LAI} env gene was excised from the plasmid pMA243 (11) and the HIV-1_{JR-FL} env gene was inserted by the splicing by overlap extension technique. The HIV-1_{JR-FL} env gene was amplified from the plasmid pUCFL112-1 (provided by I. S. Y. Chen, University of California at Los Angeles) (17). The resultant plasmid, designated JR-FL-pMA243, was sequenced by the dideoxy method and introduced into HeLa cells by the lipofectin (Gibco BRL) method. HeLa-env_{JR-FL} transfectants were selected in methotrexate (Sigma) and cloned twice by limiting dilution. Flow cytometric analysis with a MAb to the CD4 binding site on gp120, F105 (NIH AIDS Research and Reference Reagent Program) (26), indicated that HeLa-env_{JR-FL} and HeLa-env_{LAI} cells expressed comparable levels of HIV-1 envelope glycoprotein at the cell surface (Fig. 1). Next, the two cell lines were surface labeled with biotin, solubilized, and immunoprecipitated with F105 (26), a polyclonal sheep antibody (6205) to the carboxy terminus of gp120 (International Enzymes, Fallbrook, Calif.) (27), or CD4-IgG2 (Progenics) (1) by published procedures (13, 18, 22). The amount of gp120 in the precipitates was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Western blotting (immunoblotting) and incubation with streptavidin-horseradish peroxidase and then detected by the enhanced chemiluminescence system (Amersham Life Sciences, Arlington Heights,

FIG. 1. Surface expression of HIV-1 envelope glycoprotein in HeLa transfectants. Cells were removed from culture flasks by treatment with 0.5 mM EDTA and washed in culture medium. HeLa (A), HeLa-env_{LAI} (B), and HeLa-env_{JR-FL} cells (C) were stained with 2 μ g of F105 or 2 μ g of isotype control antibody (clg) (Sigma) for 15 min at 4°C and washed three times in PBS containing 0.05% NaN₃. Next, cells were incubated in phycoerythrin-conjugated goat anti-human immunoglobulin (Southern Biotechnology Associates, Birmingham, Ala.), washed, and fixed in 0.2% paraformaldehyde. Samples were analyzed on a FACScan flow cytometer (Becton Dickinson, San Jose, Calif.). Fluorescence intensity is shown on the x axis (four decade log scale), and the relative number of cells is indicated on the y axis.

III.) (data not shown). These analyses indicated that the levels of gp120 expression on the surfaces of the two cell lines are similar, with differences in the levels detected of no more than twofold.

Background levels of RET, indicating the absence of membrane fusion, were obtained when HeLa-env_{JR-FL} cells were mixed with HeLa-CD4 cells and most CD4⁺ T-cell lines (C8166, HUT 78 and Sup-T1) (NIH AIDS Research and Reference Reagent Program) (Table 1). Similar results were obtained when HeLa-env_{JR-FL} cells were mixed with the T:B hybrid CEM $\times$ 174 (28, 30) (NIH AIDS Research and Reference Reagent Program) or the macrophage cell lines U937 or phorbol myristate acetate-treated THP-1 (American Type Culture Collection) (30) (data not shown). The recently described CD4⁺ T-cell line PM1 is the only cell line permissive to infection by both macrophage-tropic and T-cell-tropic HIV-1 isolates, including HIV-1_{JR-FL} (2, 19). PM1 was derived as a direct clone of the HUT 78 T-cell line, selected on the basis of infectibility by the macrophage-tropic isolate HIV-1_{BaL} (19). PM1 cells fused substantially with HeLa-env_{JR-FL} cells (10% RET) (Table 1) but not with HeLa cells (0.6% RET) (Table 1). Membrane fusion between PM1 and HeLa-env_{JR-FL} was completely inhibited in the presence of OKT4A (Table 1). Similar to that with HeLa-env_{LAI}, no membrane fusion was observed between HeLa-env_{JR-FL} and CHO-CD4 or U87MG-CD4 (Table 1).

In RET analysis, the tropism of membrane fusion mediated by the HIV-1_{LAI} and HIV-1_{JR-FL} envelope glycoproteins mirrored that of the respective viruses. This observation is consistent with the concept that the envelope glycoprotein is a major determinant of HIV-1 tropism (3, 16, 24, 29). Furthermore, the results obtained with combinations of HeLa-env_{JR-FL} and PM1 cells demonstrate that membrane fusion mediated by gp120/gp41 from a macrophage-tropic primary HIV-1 isolate occurs at neutral pH.

Characterization of HIV-1 envelope glycoprotein-mediated membrane fusion by RET. Calcium ions are known to be required for the fusion of biological membranes. Dimitrov et al. established that HIV-1_{IIB} envelope glycoprotein-mediated membrane fusion and syncytium formation require the presence of calcium ions, whereas the binding of gp120 to CD4 is calcium independent (8). The RET generated by fusion between HeLa-env_{LAI} or HeLa-env_{JR-FL} and CD4⁺ target cells decreased by more than 50% in the presence of concentrations greater than 2.25 mM EDTA, a chelator of divalent cations (data not shown). These experiments demonstrate that, as with laboratory-adapted strains, membrane fusion mediated by the envelope glycoprotein of a macrophage-tropic primary isolate of HIV-1 is dependent on the presence of divalent cations.

The kinetics of membrane fusion were examined by the RET assay. Specific membrane fusion was first detected by the RET

assay at 90 min and increased up to 4 h, with similar results being obtained with HeLa-env_{LAI} and HeLa-env_{JR-FL} (Fig. 2). Beyond 4 h, there was no further increase in the percentage of specific RET (data not shown). These results are consistent with previous reports of fusion mediated by a laboratory-adapted strain of HIV-1 (9) and demonstrate that the rates of fusion mediated by gp120/gp41 from a laboratory-adapted strain and a primary isolate of HIV-1 are similar.

Syncytium formation. The tropism of HIV-1_{LAI} and HIV-1_{JR-FL} envelope glycoproteins in the RET assay was mirrored by the development of syncytia in cocultures of HeLa-env cells with CD4⁺ target cell lines (data not shown). For example, syncytia were observed in cocultures of HeLa-env_{JR-FL} with PM1 cells but not with C8166 or HeLa-CD4 cells. While syncytium formation between HeLa-env_{LAI} cells and HeLa-CD4 or C8166 was apparent at 4 h, there was a substantial increase in both the number and size of multinucleated cells by 24 h. In contrast, membrane fusion was maximal after 4 h in the RET assay (Fig. 2). This delay between membrane fusion and visible syncytia has previously been observed with cells expressing gp120/gp41 from the laboratory-adapted strain HIV-1_{IIB} (9, 12). In the present study, a similar delay was found between membrane fusion and visible syncytium formation in cocultures of HeLa-env_{JR-FL} and PM1 cells. While membrane fusion was maximal at 4 h (Fig. 2), few syncytia were noted at this time point, although many were evident at 24 h (data not shown).

FIG. 2. Time course of HIV-1_{LAI} and HIV-1_{JR-FL} envelope glycoprotein-mediated membrane fusion by the RET assay. The rate of membrane fusion between HeLa-env_{LAI} and HeLa-CD4 (■) or C8166 (●) and HeLa-env_{JR-FL} and PM1 (◆) was determined by the RET assay. Cells were mixed, and the percent RET was determined at various intervals thereafter. Nonspecific RET, defined as the percent RET generated when HeLa cells were mixed with HeLa-CD4 (□), C8166 (○), or PM1 (◇) cells, was also evaluated at each time point.

TABLE 2. Inhibition of RET by CD4-based proteins^a

F18-labeled cells	R18-labeled cells	IC ₅₀ (μg/ml)	
		sCD4	CD4-IgG2
HeLa-env _{JR-FL}	PM1	30.5	1.2
HeLa-env _{LAI}	PM1	38.9	7.2
	C8166	54.5	17.0
	HeLa-CD4	88.3	26.6

^a Inhibitors were added simultaneously with the cells at the initiation of the 4-h incubation. Six twofold dilutions of sCD4 and CD4-IgG2 were added at concentrations ranging from 200 to 6.25 μg/ml and 42 to 1.2 μg/ml, respectively. The difference in potency of sCD4 and CD4-IgG2 is fourfold greater on the basis of molarity than on the basis of mass, since the molecular masses of the proteins are 46 and 200 kDa, respectively. Data are the means for at least three independent experiments which were run in duplicate.

Inhibition of membrane fusion determined by RET assay.

Antibodies to CD4 and the HIV-1 envelope glycoprotein inhibit membrane fusion. For example, OKT4A exhibited similar levels of inhibition of RET mediated by the primary isolate, HIV-1_{JR-FL} (50% inhibitory concentration [IC₅₀], 15.7 ng/ml), and the laboratory-adapted strain HIV-1_{LAI} (IC₅₀, 11.7 ng/ml). The human MAb 2F5, which recognizes a conserved region of gp41 (4), inhibited membrane fusion mediated both by HIV-1_{LAI} and HIV-1_{JR-FL} with an IC₅₀ of approximately 50 μg/ml.

CD4-IgG2 is a CD4-Ig fusion protein in which the variable regions of both the heavy and light chains of human IgG2 have been replaced by the N-terminal domains of CD4 (1). This heterotetramer potentially neutralizes laboratory-adapted strains and primary isolates of HIV-1 (1, 31). CD4-IgG2 substantially inhibited HIV-1 envelope glycoprotein-mediated membrane fusion detected by RET (Table 2). A comparison of the IC₅₀ values demonstrates that membrane fusion between HeLa-env_{JR-FL} and PM1 cells was more sensitive to inhibition by CD4-IgG2 than was fusion between HeLa-env_{LAI} and PM1, HeLa-CD4, or C8166. Although a less potent inhibitor than CD4-IgG2, soluble CD4 (sCD4) (Progenics) also inhibited HIV-1 envelope glycoprotein-mediated membrane fusion (Table 2). However, the HeLa-env_{JR-FL} and HeLa-env_{LAI} assays exhibited smaller differences in sensitivity to inhibition by sCD4 than by CD4-IgG2.

In contrast to these results, the results of previous studies have shown that HIV-1_{LAI} is approximately 1,500-fold more sensitive than HIV-1_{JR-FL} to neutralization by sCD4 and 15-fold more sensitive to CD4-IgG2 (1, 7). It has been demonstrated that differences between the sensitivities of primary and laboratory-adapted HIV-1 strains to neutralization by CD4-based molecules may result from either differences in affinity of CD4 for the membrane-associated oligomeric envelope glycoprotein or differences in dissociation of gp120 from gp41 by CD4-based molecules (23, 25). Analysis by flow cytometry in-

dicates that CD4-IgG2 bound more readily to HeLa-env_{JR-FL} cells than to HeLa-env_{LAI} cells (Fig. 3). This was true over a wide range of concentrations (0.063 to 32 μg/ml), even though these cells express similar levels of surface gp120 (Fig. 1). Previous reports have demonstrated that under the incubation conditions used for flow cytometry in the present study (15 min at 4°C), CD4-based molecules induce minimal shedding of gp120 from laboratory-adapted strains of HIV-1 (23). Therefore, these results suggest that CD4-IgG2 binds more avidly to the oligomeric envelope glycoprotein of HIV-1_{JR-FL} than to that of HIV-1_{LAI}.

Dimitrov et al. reported a similar discrepancy in the potency of sCD4 in neutralization and cell fusion assays for the laboratory-adapted strain HIV-1_{IIB} (10) and suggested that dissociation of gp120 from HIV-1_{IIB} virions may have a more important role in virus neutralization than in the inhibition of cell membrane fusion (10). Other studies have found that CD4-based proteins are much less effective at dissociating gp120 from primary isolates of HIV-1 than from laboratory-adapted strains at 37°C (23, 25). This may explain why, in contrast to laboratory-adapted HIV-1 strains, the IC₅₀ values for inhibition of HIV-1_{JR-FL} envelope glycoprotein-mediated membrane fusion by CD4-based proteins determined by the RET assay (e.g., with CD4-IgG2, IC₅₀ of 1.2 μg/ml) are similar to those previously reported in neutralization studies with this isolate (with CD4-IgG2, IC₅₀ of 3.5 μg/ml) (1).

The RET assay permits real-time determinations of membrane fusion mediated by gp120/gp41 from a primary isolate and a laboratory-adapted strain of HIV-1. We have shown that the fusogenicity of cells expressing the envelope glycoproteins of these viruses mimics the tropism of the viruses, indicating that gp120/gp41 is a major determinant of tropism. Presumably, PM1 cells are permissible for fusion with HeLa-env_{JR-FL} cells because the PM1 clone expresses one or more fusion accessory molecules not present in the parental HUT 78 line. However, a phenotypic analysis of these cell lines did not indicate any difference in expression of a panel of 15 leukocyte surface markers (CD3⁺, CD4⁺, and CD26⁺) but not others (CD2⁺ and CD7⁺), and both cells display some macrophage markers (CD11c⁺ and CD33⁺) (data not shown). The RET assay provides a valuable system for the further analysis of the determinants of HIV-1 tropism, which is currently underway.

Despite significant differences exhibited by HIV-1_{JR-FL} and HIV-1_{LAI} in terms of tropism and sensitivity to neutralization by CD4-based proteins, in the present study membrane fusion mediated by the envelope glycoproteins of these viruses showed remarkably similar properties. In particular, the degree and kinetics of membrane fusion were similar for the laboratory-adapted strain and the primary isolate. Membrane fusion mediated by both isolates occurred at neutral pH and was dependent on the presence of divalent cations. Moreover,

FIG. 3. CD4-IgG2 binding to HeLa-env transfectants. HeLa (A), HeLa-env_{LAI} (B), and HeLa-env_{JR-FL} (C) cells were incubated with 20 ng of CD4-IgG2 or 20 ng of human IgG2 (cIg) followed by phycoerythrin-conjugated goat anti-human immunoglobulin. Samples were analyzed on a FACScan flow cytometer. Fluorescence intensity is shown on the x axis (four decade log scale), and the relative number of cells is indicated on the y axis. The data are representative of at least three assays.

both isolates exhibited similar levels of sensitivity to inhibition by MAbs directed against CD4 and gp41.

Finally, it was demonstrated that the relative levels of sensitivity of membrane fusion mediated by the two HIV-1 strains to inhibition by CD4-based proteins did not reflect the greater sensitivity of HIV-1_{LAI} than of HIV-1_{JR-FL} to neutralization by these agents, indicating that the mechanisms of membrane fusion inhibition and neutralization of HIV-1 are distinct.

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