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Interaction between Virion-Bound Host Intercellular Adhesion Molecule-1 and the High-Affinity State of Lymphocyte Function-Associated Antigen-1 on Target Cells Renders R5 and X4 Isolates of Human Immunodeficiency Virus Type 1 More Refractory to Neutralization

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The oligomeric nature of the viral envelope proteins has been partly held responsible for the observed differences in neutralization sensitivity between primary and laboratory-adapted strains of human immunodeficiency virus type 1 (HIV-1). However, recent evidence suggests that host factors can also modify the sensitivity of HIV-1 particles to neutralization. Having previously demonstrated that the acquisition of host-encoded intercellular adhesion molecule (ICAM)-1 proteins by newly formed viruses has a functional significance for the life cycle of HIV-1, we investigated whether the acquisition of host-derived ICAM-1 by HIV-1 could affect the virus sensitivity to neutralization. In this study, we have first shown that the physical presence of host cell membrane ICAM-1 on HIV-1 was not modifying virus sensitivity to neutralization by either two different anti-gp120 monoclonal antibodies (0.5β and 4.8D) or soluble CD4. However, the ability of the F105 anti-gp120 monoclonal antibody (specific for the CD4-binding site) to neutralize ICAM-1-bearing virions was diminished when target cells were pretreated with an lymphocyte function-associated antigen-1 (LFA-1)-activating antibody. Interestingly, ICAM-1/POS progeny viruses were found to be slightly more resistant to neutralization by individual human sera in target cells expressing a low-affinity form of LFA-1 than viruses devoid of host-encoded ICAM-1 proteins. This resistance was markedly enhanced when target cells expressed an activated LFA-1 form on their surface. These results suggest that the interaction between virally embedded host ICAM-1 and target cell surface LFA-1 should be considered a factor modulating neutralization sensitivity of HIV-1 by human sera from HIV-1-infected individuals. © 2000 Academic Press

INTRODUCTION

Like other retroviruses, human immunodeficiency virus type 1 (HIV-1) buds out from the host cell through the cytoplasmic membrane. During this budding process, the viral lipid membrane is taken directly from the plasma membrane. It is also at this step that nascent virions acquire their external (gp120) and transmembrane (gp41) envelope glycoproteins, both of which are targeted to the cell surface by the secretory pathway (Hunter, 1994). In addition to these virally encoded Env glycoproteins, HIV-1 also incorporates a wide array of constituents of cellular origin (reviewed in Tremblay *et al.,* 1998). Among these are the major histocompatibility complex class II (MHC-II) molecules (HLA-DR, -DP, and -DQ determinants), β_2-microglobulin, CD44, CD55, CD59, as well as lymphocyte function-associated antigen-1 (LFA-1) and ICAM-1 adhesion molecules (Benkirane *et al.,* 1994; Cantin *et al.,* 1996; Castilletti *et al.,* 1995; Fais *et al.,* 1995; Henderson *et al.,* 1987; Hoxie *et al.,* 1987; Lee Guo and Hildreth.,1995; Saifuddin *et al.,* 1995).

Over the past few years, these molecules have been found to retain their biological functions after incorporation on the surface of budding viral particles. For example, HIV-1-incorporated CD55 and CD59 complement control proteins have been reported to protect virions against complement-mediated virolysis (Saifuddin *et al.,* 1995). Cellular HLA-DR embedded in HIV-1 has been shown to efficiently present superantigens to T-cells (Rossio *et al.,* 1995). Some HIV-1-acquired cell adhesion molecules have also been shown to be functionally important at the level of the viral life cycle. Incorporation of CD44 from activated cells in the envelope of budding HIV-1 helps the virus to attach to cells bearing hyaluronic acid, the natural ligand for CD44, on their surface (Lee Guo and Hildreth, 1995). Increased incorporation of host HLA-DR and ICAM-1, after stimulation of producer cells by γ-IFN, enhances HIV-1 infectivity for CD4-negative cells (Castilletti *et al.,* 1995). The addition of anti-LFA-1 antibodies to neutralizing sera has been demonstrated to potentiate their blocking activity against infection by cell-free viruses, thus suggesting a role for LFA-1 in HIV-1 infection (Gomez and Hildreth, 1995). Recently, we developed a transient transfection and expression sys-

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tem to produce virus particles differing only by the absence or the presence of a specific cellular molecule in their envelope. With this system, we determined that the acquisition of host HLA-DR1 and ICAM-1 molecules by HIV-1 is associated with an increase in viral infectivity ranging from 1.6- to 2.3-fold and from 5- to 10-fold, respectively (Cantin *et al.,* 1997; Fortin *et al.,* 1997). Using a similar technical approach, Rizzuto and Sodroski (1997) corroborated our findings with virion-bound host ICAM-1 proteins.

The first step in the HIV-1 replicative cycle is the attachment to the target cell. This stage is achieved through the interaction between the viral gp120 protein and the cell surface CD4 protein (Dalgleish *et al.,* 1984; Klatzmann *et al.,* 1984). Such an interaction then creates a high-affinity binding site for the appropriate chemokine receptor (Lapham *et al.,* 1996; Trkola *et al.,* 1996), acting as a necessary cofactor (Alkhatib *et al.,* 1996; Choe *et al.,* 1996; Deng *et al.,* 1996; Doranz *et al.,* 1996; Dragic *et al.,* 1996; Feng *et al.,* 1996). Ultimately, through a conformational change, the gp41 fusion peptide is exposed, an event necessary to lead to the fusion between viral and cellular membranes (Binley and Moore, 1997; Weissenhorn *et al.,* 1997). Virion-acquired ICAM-1 molecules are thought to act at the early steps of the attachment of the virus by increasing the avidity of the virus–cell conjugate and, thereby, complements of the CD4–gp120 interaction (Fortin *et al.,* 1997). The crucial role played by the interaction between virion-bound ICAM-1 and cell surface LFA-1 in the process of virus infection is further indicated by the demonstration that target cells expressing LFA-1 with a high-affinity conformational state for its counterreceptor ICAM-1 are more susceptible to infection with ICAM-1-bearing HIV-1 particles (Fortin *et al.,* 1998).

It is now well established that primary isolates of HIV-1 are more resistant to neutralization induced by anti-HIV-1 antibodies and soluble CD4 (sCD4) than laboratory-adapted strains (Matthews, 1994; Moore *et al.,* 1995). However, the precise reason for this difference is not well understood. Some studies have reported that the affinity of gp120 for sCD4 was from 10 to 30 times lower for primary isolates of HIV-1 than for laboratory strains (Moore *et al.,* 1992), whereas others have suggested that the level of gp120 per virion was much higher on primary viral isolates (Karlsson *et al.,* 1996). However, it should be noted that this issue is still controversial as some investigators have demonstrated that increased envelope spike density and stability are not responsible for the higher level of neutralization resistance shown by primary isolates of HIV-1 (Karlsson *et al.,* 1996). From these studies, it can be postulated that the intrinsic characteristics of gp120 from primary and laboratory HIV-1 isolates must be in part responsible for the observed differences in neutralization sensitivity. However, it is quite possible that the nature of host-derived proteins embedded in newly formed progeny viruses might also represent a key factor in the observed change in neutralization sensitivity. This is reminiscent of the fact that most laboratory viral isolates are expanded in T-lymphoid cell lines, whereas virus stocks of HIV-1 clinical strains are mainly produced in mitogen-stimulated primary mononuclear cells.

With the help of our recently described transient transfection and expression system (Fortin *et al.,* 1997), we have produced isogenic virus particles bearing or not bearing host-derived ICAM-1 on their surfaces to evaluate the role of this adhesion molecule in the neutralization sensitivity of HIV-1. Using a panel of gp120-directed monoclonal antibodies, sCD4, and individual sera, we found that the acquisition of cellular ICAM-1 by nascent HIV-1 particles is a factor affecting sensitivity to neutralization. Moreover, for the first time, we have determined that neutralization sensitivity of ICAM-1-bearing virions is even more significantly altered by the conformational state of LFA-1 that is expressed on the surface of target cells.

RESULTS

Elaboration of a reporter gene-based system to evaluate neutralization sensitivity of HIV-1

We previously reported that virion-embedded host ICAM-1 can interact with its counterreceptor LFA-1 on the surface of the target cell and that this association resulted in an increase in HIV-1 infectivity (Fortin *et al.,* 1997). We thus wondered whether this additional interaction could render these virions less sensitive to neutralization. Our primary objective was to develop a reporter gene-based neutralization assay. The efficiency and sensitivity of luciferase-based assays to monitor single-round infection event are widely accepted. We have already demonstrated that HIV-1 infection of 1G5, a cell line containing an HIV-1 LTR-driven luciferase gene, leads to a dose-dependent increase in reporter gene activity 48 h after virus infection (Fortin *et al.,* 1997). In this system, the production of the viral Tat protein by *de novo* virus infection mediates a rapid and strong upregulation of HIV-1 LTR-driven luciferase activity, allowing the quantitative evaluation of single-cycle infection events. We also demonstrated that infection of PM1 cells with recombinant luciferase-encoding viruses culminates in a strong increase in luciferase activity at 72 h postinfection (Fortin *et al.,* 1998; Paquette *et al.,* 1998).

To evaluate the efficiency of this system to measure virus neutralization sensitivity, we first used serum from an HIV-1-positive individual to neutralize HIV-1_{NL4-3} particles bearing or not bearing host ICAM-1 proteins on their surfaces (termed ICAM-1/POS and ICAM-1/NEG, respectively). For this purpose, standardized amounts of ICAM-1/NEG and ICAM-1/POS virions were incubated with increasing concentrations of a human serum before incubation with 1G5 cells. Virus infection were next mon-

itored by measuring luciferase activity from 1G5 cells. As depicted in Fig. 1A, higher concentrations of sera resulted in a gradual decrease in the level of HIV-1 infection, as determined by HIV-1 LTR-dependent luciferase activity. Similar results were obtained when PM1 cells were infected with ICAM-1/NEG or ICAM-1/POS macrophage-tropic viruses (Fig. 1B). In agreement with our previous reports, ICAM-1/POS virions were still more infectious than their ICAM-1/NEG counterparts (Fortin *et al.,* 1997; Fortin *et al.,* 1998). The results from this set of experiments indicate that the present reporter gene-based system is appropriate to measure neutralization sensitivity of HIV-1. This system was found to be sensitive, reproducible, and rapid, requiring no more than 48 to 72 h for completion.

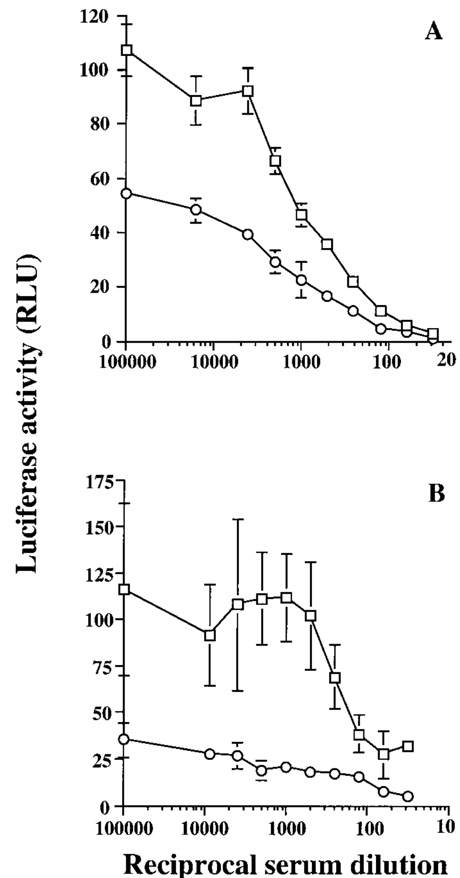

FIG. 1. Infection with ICAM-1/NEG and ICAM-1/POS virions is neutralized by an individual sera. Similar amounts of ICAM-1/NEG (○) and ICAM-1/POS (□) T-tropic (strain NL4-3) or macrophage-tropic (luciferase-encoding viruses pseudotyped with JR-FL envelope) strains of HIV-1 (10 ng of p24) were first incubated with increasing concentrations of a human sera. These T- and macrophage-tropic viral strains were then used to infect 1G5 (A) and PM1 (B) cells, respectively. After an incubation period at 37°C of 48 h for 1G5 and 72 h for PM1, cells were next lysed and luciferase activity was monitored. Results are the mean ± SD for triplicate samples and are representative of three independent experiments.

Neutralization sensitivity of virions bearing host ICAM-1 proteins is influenced by the nature of the neutralizing monoclonal antibody and the LFA-1 activation state

Initially, ICAM-1/NEG and ICAM-1/POS virus stocks were treated with increasing concentrations of various anti-gp120 antibodies and sCD4 and compared in terms of susceptibility to neutralization. The incorporation of ICAM-1 on HIV-1 particles did not affect the sensitivity to neutralization by 0.5β and 4.8D, two anti-gp120 monoclonal antibodies (Figs. 2A and 2B). We also tested sCD4 because it is considered to be one of the most potent inhibitor of laboratory-adapted strains of HIV-1 (Clapham *et al.,* 1989; Deen *et al.,* 1988). Again, a comparable sensitivity to sCD4-mediated neutralization was noticed for progeny viruses bearing or not bearing host-encoded ICAM-1 proteins on their surfaces (Fig. 2C). Altogether, these data suggest that the sensitivity of HIV-1 particles to neutralization by anti-gp120 monoclonal antibodies tested and sCD4 is not altered by the incorporation of host cell constituents such as ICAM-1.

Previous studies have elegantly shown that LFA-1 can switch from a low- to a high-affinity conformational state for its counterreceptor ICAM-1, a conformational change that is induced on T-cell activation through the T-cell receptor (Dustin and Springer, 1989). On the basis of our recent observation indicating that expression of the high-affinity conformational state of LFA-1 on the surface of target cells rendered these cells more susceptible to infection by ICAM-1/POS virions (Fortin *et al.,* 1998), we were next interested in determining whether the conformational state of LFA-1 could affect neutralization sensitivity of ICAM-1/POS progeny viruses. To attain this goal, before incubation with HIV-1 particles, 1G5 cells were treated with NKI-L16, an antibody known to induce a high-affinity LFA-1 conformational state for ICAM-1 (Keizer *et al.,* 1988). It should be mentioned that although LFA-1 has signaling properties, the treatment of 1G5 cells with NKI-L16 has no effect on HIV-1 LTR-driven luciferase activity in 1G5 cells (data not shown; Paquette *et al.,* 1998). Neutralization sensitivities of isogenic ICAM-1/NEG and ICAM-1/POS viruses to 0.5β, 4.8D, and sCD4 were comparable even in the presence of the activated form of LFA-1 on the surface of target 1G5 cells (Figs. 2D–2F).

We next tested F105, a monoclonal antibody directed against a conformational epitope of gp120 mapping to the CD4-binding site (Posner *et al.,* 1993). Similar sensitivity to neutralization by F105 was observed when infection was performed with either ICAM-1/NEG or ICAM-1/POS virus preparations (Fig. 3A). However, when 1G5 cells bearing the activated form of LFA-1 were used as targets, ICAM-1/POS progeny viruses were found to be significantly more resistant to neutralization by F105 than were ICAM-1/NEG viruses (Fig. 3B). To explore the role

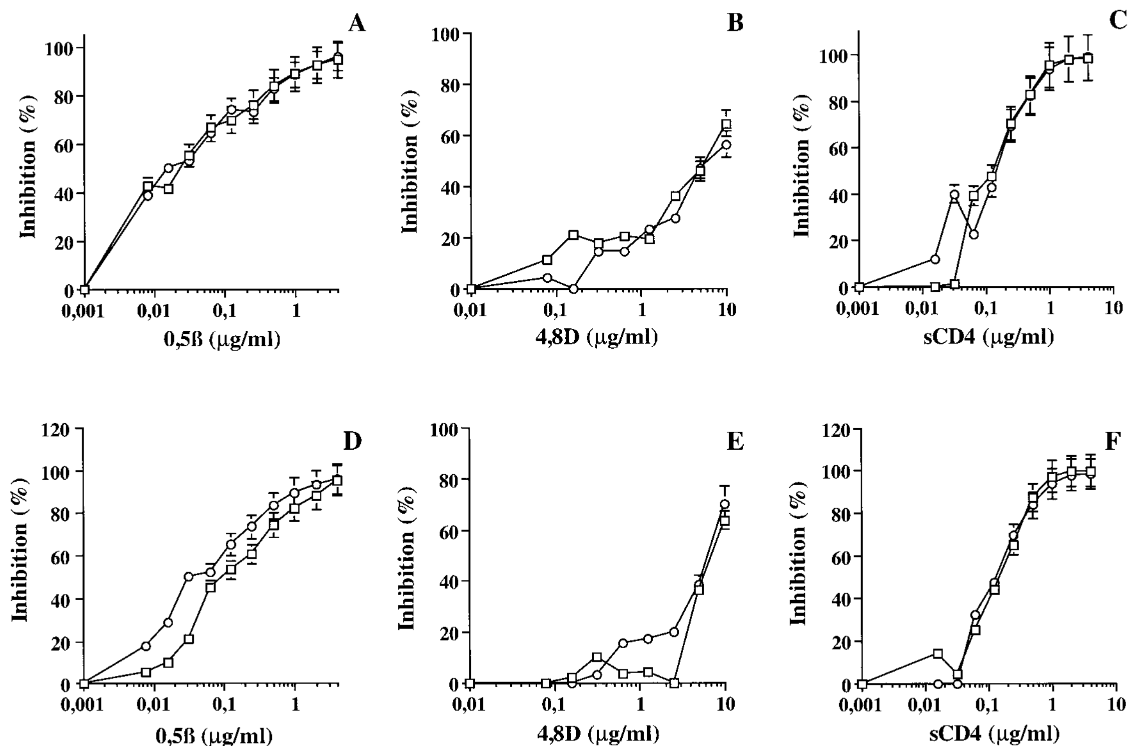

FIG. 2. Effect of the LFA-1 activation state on neutralization of ICAM-1/NEG and ICAM-1/POS viruses by monoclonal anti-gp120 antibodies and sCD4. Identical amounts of ICAM-1/NEG (○) and ICAM-1/POS (□) viruses (10 ng of p24) were first treated with increasing concentrations of 0.5β (A and D), 4.8D (B and E), and sCD4 (C and F) for 30 min at 37°C in 96-well plates. In parallel, 1G5 cells were either left untreated (A–C) or treated with NKI-L16 at 1 μg/ml (D–F) for 30 min at 37°C. 1G5 cells (10^5) were next added to each well, and plates were incubated for an additional 48 h at 37°C. Cells were then lysed, and luciferase activity was monitored. Inhibition of virus infection was calculated with the following formula: percentage of inhibition = [1 − (treated virus/untreated virus) × 100%]. Results shown are the mean ± SD for triplicate samples and are representative of three independent experiments.

of the interaction between virus-embedded ICAM-1 and high-affinity LFA-1 in the observed differences in sensitivity to neutralization, an anti-ICAM-1 antibody was added to the virus–cell mixture during the incubation period with F105. As depicted in Fig. 3C, a similar pattern of neutralization by F105 for both ICAM-1/NEG and ICAM-1/POS virus preparations was observed after blocking of the ICAM-1/LFA-1 interaction. These data indicate that the reduced sensitivity to neutralization conferred by virally embedded host ICAM-1 is dependent on both the LFA-1 activation state and the viral epitope(s) recognized by the neutralizing agent.

The LFA-1-activated state on target cells also influences the neutralization sensitivity of ICAM-1/POS virions to sera from HIV-1-infected persons

To more closely parallel physiological conditions, we next compared the sensitivity of ICAM-1/NEG and ICAM-1/POS virus stocks to neutralization by sera from HIV-1-infected individuals. 1G5 cells expressing or not the high-affinity conformational LFA-1 state for ICAM-1 were again used as targets in these experiments. Individual sera from three HIV-1-infected individuals at various stages of the disease were used for this series of experiments (Table 1). When using sera from HIV-1-infected individuals and target 1G5 cells expressing LFA-1 in the low-affinity state for ICAM-1, ICAM-1/POS viruses were almost as sensitive to neutralization as ICAM-1/NEG viruses (Figs. 4A–4C). However, surface expression of the activated form of LFA-1 on 1G5 cells, by means of treatment with NKI-L16, rendered ICAM-1/POS HIV-1 particles markedly more resistant to neutralization by sera from HIV-1-infected persons than ICAM-1/NEG viruses (Figs. 4D–4F). For example, serum from patient P017 when diluted at 1:256 could inhibit infection with ICAM-1/NEG virions by 73%, whereas infectivity of ICAM-1/POS HIV-1 particles was decreased by 8% (Fig. 4F). The serum neutralization curves indicates that 3- to 13-fold higher sera concentrations were required to achieve a 50% neutralization (IC_{50}) of ICAM-1/POS viruses compared with the IC_{50} value for ICAM-1/NEG virus stocks (Table 1). The sum of these experiments underscores the importance of virally acquired host proteins such as ICAM-1 in HIV-1 sensitivity to neutralization by sera of HIV-1-infected individuals. It also provides information on the influence of the target cell with regard to neutralization sensitivity of progeny virions carrying host-encoded molecules on their surfaces.

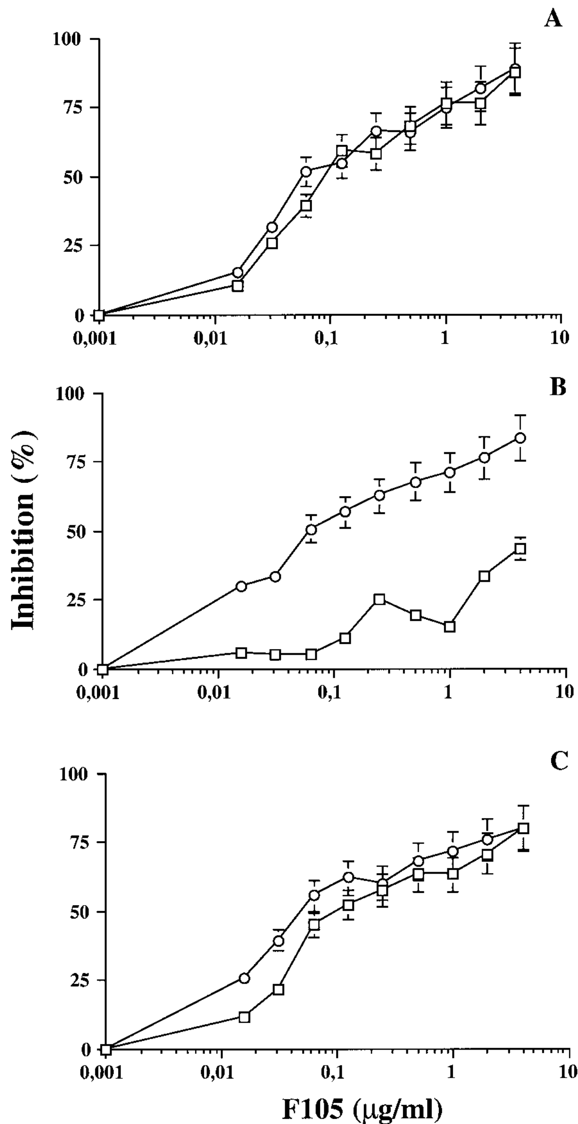

FIG. 3. The increased resistance to neutralization of ICAM-1/POS virions with target cells expressing the activated LFA-1 form is inhibited by treatment with an anti-ICAM-1 antibody. Identical amounts of ICAM-1/NEG (○) and ICAM-1/POS (□) viruses (10 ng of p24) were first treated with increasing concentrations of F105 for 30 min at 37°C in 96-well plates in the absence (A and B) or the presence (C) of an anti-ICAM-1 antibody (clone RR1/1.1.1 at 20 μg/ml). In parallel, 1G5 were either left untreated (A) or were treated with NKI-L16 at 1 μg/ml (B and C) for 30 min at 37°C. Thereafter, 1G5 cells were added to each well, and plates were incubated for an additional 48 h at 37°C. Cells were then lysed, and luciferase activity was monitored. Inhibition of virus infection was calculated as described in Fig. 2. Results are the mean ± SD for triplicate samples and are representative of three independent experiments.

ICAM-1 incorporation can also affect the neutralization sensitivity of a primary, macrophage-tropic strain of HIV-1

To determine that the observed effect of virion-embedded ICAM-1 on neutralization sensitivity was neither cell-type specific nor restricted to T-tropic HIV-1 isolates (X4), our next set of experiments was performed with an established CCR5-positive human T lymphoid cell line (i.e., PM1) and virions pseudotyped with the envelope proteins from the primary macrophage-tropic JR-FL viral strain. Virus preparations bearing or not host-derived ICAM-1 proteins on their surfaces were incubated with PM1, a cell line susceptible to infection with both T- and macrophage-tropic viruses, in the presence of the human serum from patient P054. Interestingly, ICAM-1/POS virions were found to be more resistant to neutralization than ICAM-1-free progeny viruses even when cells were expressing the low-affinity form of LFA-1 on the surface of PM1 cells (Fig. 5A). The sensitivity to neutralization of ICAM-1/POS virions bearing the macrophage-tropic JR-FL envelope proteins was even more greatly reduced by induction of the LFA-1 high-affinity state on the surface of PM1 cells (Fig. 5B). These results indicate that the increased resistance to neutralization conferred by the combined action of virally acquired host ICAM-1 and cell surface-activated LFA-1 is a phenomenon that is not restricted to viruses of a particular tropism.

DISCUSSION

Multifactorial parameters are governing sensitivity of HIV-1 to neutralization, and the exact nature of these factors is still ill defined. In the present study, our primary goal was to determine whether the acquisition of host-derived molecules by progeny viruses can modulate the neutralization sensitivity of HIV-1. This investigation was prompted by our previous reports showing that incorporation of cellular ICAM-1 in newly formed virions was associated with an increase in viral infectivity and that T-cells expressing the high-affinity form of LFA-1 on their surfaces were more susceptible to infection by ICAM-1-bearing HIV-1 particles (Fortin *et al.,* 1997, 1998). This last observation is of physiological relevance considering that the activated form of LFA-1 is most likely expressed on the surface of a fairly high proportion of lymph node-

TABLE 1

Characteristics of Sera Donors and Neutralization Sensitivity of ICAM-1/NEG and ICAM-1/POS Virus Stocks when 1G5 Cells Expressing the Activated LFA-1 Form Are Used as Targets

Patient	Age (yr)	CD4 (cells/mm^3)	Therapy	Resistance ratio[a]
P032	46	632	AZT-ddC	3.3
P054	39	238	AZT-ddC-Indinavir	6.7
P017	33	8	AZT-ddC-Indinavir	13

[a] Relative resistance ratios of studied virus preparations to sera neutralization in 1G5 cells expressing the high-affinity LFA-1 form were calculated as [(IC_{50} ICAM-1/POS)/(IC_{50} ICAM-1/NEG)]. The 50% neutralization titers (IC_{50}) were defined as the sera dilution at which there was a 50% reduction in luciferase activity compared with uninfected cells and were calculated from the neutralization curves.

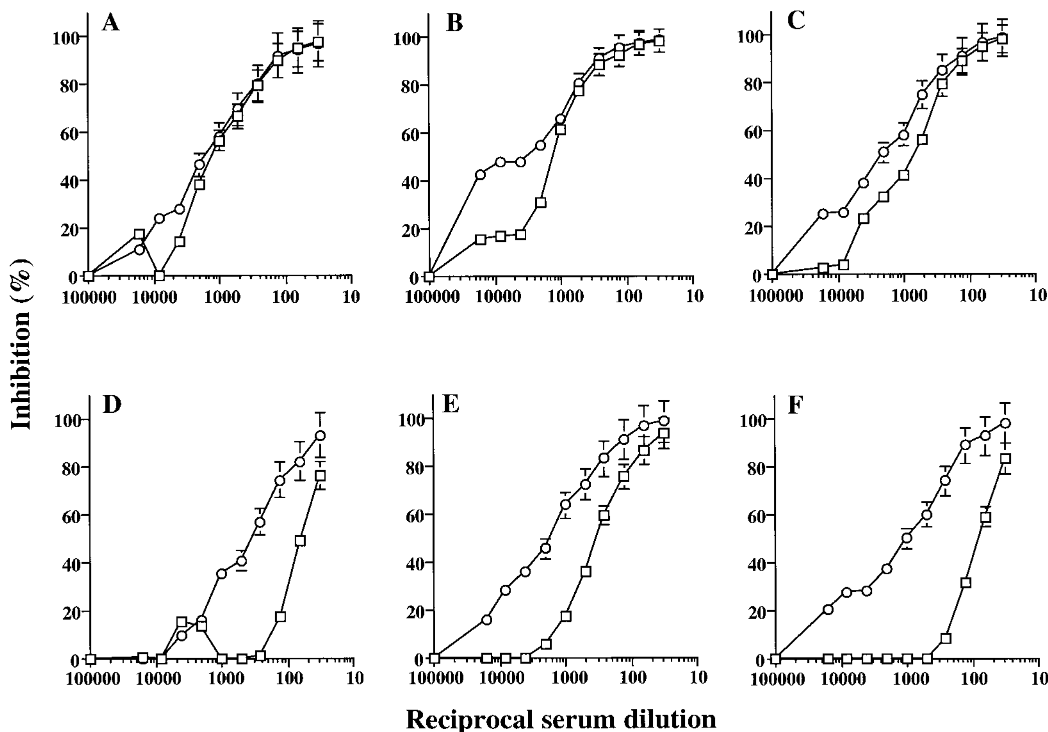

FIG. 4. Sensitivity to neutralization by sera from HIV-1-infected individuals is diminished by the acquisition of host-encoded ICAM-1 proteins by newly formed HIV-1 particles. Similar amounts of ICAM-1/NEG (○) and ICAM-1/POS (□) progeny virions (10 ng of p24) were incubated for 30 min at 37°C with twofold serial dilution of sera from three HIV-1-infected subjects (P054, panels A and D; P032, panels B and E; P017, panels C and F). In these experiments, 1G5 target cells were either left untreated (A–C) or were pretreated with 1 μg/ml NKI-L16 for 30 min at 37°C (D–F). Cells were next added to the virus–sera mixture, and infection was allowed to proceed for an additional 48 h at 37°C. Cells were lysed, and luciferase activity was monitored. Inhibition of virus infection was evaluated as described in the legend to Fig. 2. Results are the mean ± SD for triplicate samples and are representative of three independent experiments.

associated T lymphocytes because antigenic stimulation, a process known to induce the high-affinity state of LFA-1 for ICAM-1, is taking place in such anatomical sites (Parrot and Wilkinson, 1981).

The use of susceptible cells containing a reporter gene (secreted alkaline phosphatase) under the control of the regulatory elements of HIV-1 (LTR) has been recently shown to be an interesting alternative approach to assess neutralization sensitivity of simian immunodeficiency viruses (Means *et al.,* 1997). The 1G5 cell line, a Jurkat derivative stably transfected with two copies of a construct containing the luciferase reporter gene under the control of the HIV-1_{SF2} LTR (Aguilar-Cordova *et al.,* 1994), was used as a target to develop a novel neutralization assay. We were confident that this cell line would be appropriate to assess neutralization based on the notion that infection of 1G5 cells with HIV-1 permits to monitor single-cycle virus infection events (Fortin *et al.,* 1997, 1998). However, it should be noted that this assay is restricted to the use of T-cell line-adapted X4 strains of HIV-1 given that 1G5 cells do not express CCR5 on their surface (Berger *et al.,* 1998). Therefore, to measure the neutralization sensitivity of macrophage-tropic (R5) strains of HIV-1, we also tested PM1 cells in a neutralization assay using recombinant luciferase-encoding progeny viruses pseudotyped with the JR-FL envelope. These novel neutralization assays were found to be highly sensitive, reproducible, and rapid, allowing the complete assay to be carried out within a time frame of 48–72 h (Fig. 1).

It has been reported that ICAM-1-dependent cellular adhesion diminishes the potency of antibodies known to abrogate HIV-1-mediated syncytium formation (Berman and Nakamura, 1994). We were thus wondering whether the incorporation of host cell membrane ICAM-1 proteins in progeny HIV-1 particles could also attenuate the overall potency of neutralizing agents such as sCD4 and anti-gp120 antibodies (monoclonal and polyclonal). Our initial experiments indicated that ICAM-1-bearing progeny viruses were not less sensitive to neutralization by sCD4 and three monoclonal anti-gp120 antibodies (i.e., F105, 0.5β, and 4.8D) when target cells were expressing LFA-1 molecules in a low-affinity state for ICAM-1 (Figs. 2 and 3A). Our observations hence do not support a previous study that has shown that ICAM-1-bearing HIV-1 particles are slightly less susceptible to neutralization by F105 than isogenic virions devoid of host-encoded ICAM-1 (Rizzuto and Sodroski, 1997). We believe that such a discrepancy might be related to the use of a different cellular target in the neutralization assay.

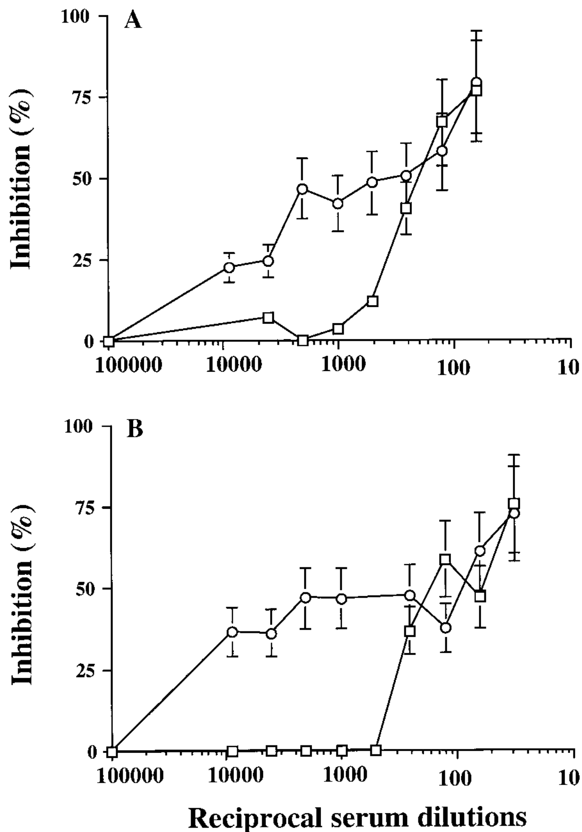

FIG. 5. Diminished sensitivity to neutralization is also observed using ICAM-1-bearing viruses pseudotyped with macrophage-tropic envelope proteins. Similar amounts of ICAM-1/NEG (○) and ICAM-1/POS (□) luciferase-encoding progeny virions pseudotyped with the envelope protein from JR-FL (5 ng of p24) were incubated for 30 min at 37°C with twofold serial dilutions of sera from P054. PM1 target cells were either left untreated (A) or pretreated with 1 μg/ml NKI-L16 for 30 min at 37°C (B). Cells were next added to the virus–sera mixture, and infection was allowed to proceed for an additional 72 h at 37°C. Cells were lysed, and luciferase activity was monitored. Inhibition of virus infection was evaluated as described in the legend to Fig. 2. Results are the mean ± SD for triplicate samples and are representative of three independent experiments.

A novel aspect of this work is the role played by the conformational state of LFA-1 with regard to neutralization sensitivity of ICAM-1-bearing HIV-1 particles. This parameter was studied based on our previous data indicating that expression of the high-affinity LFA-1 form significantly enhanced the susceptibility of target cells to infection by ICAM-1/POS virions without affecting the infection by ICAM-1/NEG virions (Fortin *et al.,* 1998). It is quite clear from our results that surface expression of the activated LFA-1 form on target cells reduces the sensitivity to neutralization of ICAM-1/POS viruses but not of isogenic ICAM-1/NEG HIV-1 particles. It should be noted that such a higher resistance depends on the type of neutralizing agents used in the assay because a diminished neutralization sensitivity is observed with F105 but not with sCD4 and the other monoclonal anti-gp120 antibodies (i.e., 0.5β and 4.8D) (Figs. 2 and 3). The underlying mechanism or mechanisms responsible for this observation are unclear but might be related to the epitope specificity (linear versus conformational), the binding affinity, and/or the mode of neutralization by the studied inhibiting agents. F105 recognizes a conformational epitope of gp120 (Posner *et al.,* 1993), and 0.5β binds to a linear determinant of the V3 loop (Matsushita *et al.,* 1988). The mechanism of action of F105 relies on its ability to directly block the binding of HIV-1 to CD4-expressing cells, whereas 0.5β inhibits at a postbinding step. On the other hand, 4.8D and sCD4, which are not affected by the activated state of LFA-1, neutralize virus infectivity by inducing a shedding of gp120 (Ugolini *et al.,* 1997). In the presence of target cells bearing activated LFA-1 molecules, the increased resistance to neutralization is solely due to the interaction between LFA-1 and ICAM-1, as abrogation of this interaction by pretreatment of the virions with a blocking anti-ICAM-1 antibody rendered ICAM-1/POS viruses as sensitive as ICAM-1/NEG virions to neutralization (Fig. 3C).

Even though the observed differences were not significant, ICAM-1/POS progeny viruses were found to be harder to neutralize by sera from HIV-1-infected individuals than virus preparations devoid of host-derived ICAM-1 when using 1G5 cells expressing a low-affinity form of LFA-1 (Figs. 4A–4C). The degree of neutralizability of ICAM-1/POS virions by human serum samples was more dramatically modified when using 1G5 target cells expressing the activated LFA-1 form (Figs. 4D–4F). The resistance ratios [(IC_{50} ICAM-1/POS)/(IC_{50} ICAM-1/NEG)] that were obtained when using 1G5 cells bearing the activated LFA-1 form varied between the different studied sera of the HIV-1-infected individuals (Table 1). The precise reasons for this observation are not clear at the moment, and further study is warranted to shed light onto this matter.

In sera from HIV-1-infected patients, most of the neutralizing activity directed against T-cell line-adapted strains (e.g., HIV-1_{NL4-3}) is targeted against two specific regions of gp120: the CD4 binding site (CD4bs) and the V3 loop (Ugolini *et al.,* 1997). Based on these observations and our data, it is tempting to speculate that antibodies specific for CD4bs and V3 domain will be more affected in their capacity to neutralize virus infection in the presence of secondary interactions occurring between virally embedded host ICAM-1 and the high-affinity conformational state of LFA-1 expressed on the surface of target cells. This postulate is based on the obtained results with F105 and sera from seropositive patients that demonstrated that the neutralizing ability of these agents is more sensitive to the activated state of LFA-1 than for both 4.8D and sCD4, two agents known to mediate shedding of gp120. The strong additional interaction between virally acquired host ICAM-1 proteins and the activated LFA-1 form probably helps the virus to

attach to the cell despite the presence of antibodies that reduce the efficacy of the initial contact between gp120 and CD4.

We have also been able to demonstrate that the effect of the ICAM-1/LFA-1 interaction on neutralization sensitivity by human sera was observed with viral entities bearing either T- (X4) or macrophage-tropic (R5) envelope proteins. This is in agreement with recent studies that have established that sera-mediated neutralization of HIV-1 is independent of the coreceptor use (LaCasse *et al.,* 1998; Montefiori *et al.,* 1998; Trkola *et al.,* 1998). This observation is highly relevant due to the proposed crucial role played by HIV-1 R5 strains in sexual transmission and establishment of infection (Cornelissen *et al.,* 1995; Veenstra *et al.,* 1995).

We and others have reported that depending on the infected cell type, the specific nature of host-derived molecules acquired by budding HIV-1 particles varies (Bastiani *et al.,* 1997; Cantin *et al.,* 1996; Frank *et al.,* 1996). More importantly, it has been demonstrated that the sensitivity of low-passage clinical and laboratory strains of HIV-1 to neutralization by human sera is influenced by the cell type in which the progeny virions are propagated (Sawyer *et al.,* 1994). Overall, these studies and the present observations led us to postulate that the neutralization sensitivity of HIV-1 is indeed modified to some extent by host cell constituents acquired by nascent viruses.

In summary, we have demonstrated that the physical presence of host-derived ICAM-1 proteins on HIV-1 differently modulates neutralization sensitivity of HIV-1 particles, depending on both the type of inhibitory agents used and the conformational state of LFA-1 on the target cells. In light of the data that we have gathered with polyclonal sera form HIV-1-infected persons, it seems reasonable to postulate that infection with ICAM-1-bearing HIV-1 particles is harder to inhibit and that the neutralization sensitivity of such virions is markedly diminished if target cells are expressing a high-affinity form of LFA-1 for ICAM-1 on their surface. The T-cell receptor-mediated activation of LFA-1 is a more transient event than the NKI-L16-induced LFA-1 activation used in the present work. However, in HIV-1-infected individuals, the very high proportion of activated T-lymphocytes in secondary lymphoid organs (Pantaleo *et al.,* 1993a, 1993b) leads us to propose that the modulation of virus neutralization sensitivity by the ICAM-1/LFA-1 interaction might play a role in the HIV-1 immunopathogenesis. Our data further emphasize the role of host factors in the pathogenesis of HIV-1 infection and could also help in the design of new vaccination strategies, as recently stressed by Hioe *et al.* (1998), particularly after taking into account the effect of the activated state of LFA-1 relatively to the type of antibodies used to mediate neutralization.

MATERIALS AND METHODS

Cells and plasmids

The 1G5 T-cell line, a Jurkat E6–1 derivative that harbors two stably integrated constructs constituted of the luciferase reporter gene under the control of the HIV-1_{SF2} LTR element (Aguilar-Cordova *et al.,* 1994), was obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health). PM1, a clonal derivative of HUT 78 (Lusso *et al.,* 1995), was kindly provided by Dr. P. Lusso (The San Rafael Scientific Institute, Milan, Italy). These cells were maintained in complete culture medium made of RPMI 1640 supplemented with 10% FBS (Hyclone Laboratories, Logan, UT), 2 mM glutamine, 100 U/ml penicillin G, and 100 μg/ml streptomycin. We have also used human embryonic kidney 293T cells, which express the simian virus 40 large T antigen (Pear *et al.,* 1993). This cell line was maintained in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin G, and 100 μg/ml streptomycin and was kindly provided by Dr. Warner C. Greene (The J. Gladstone Institutes, San Francisco, CA). Flow cytometric analysis revealed that 1G5 and PM1 cells express high levels of LFA-1 on their surface, whereas 293T cells are negative for ICAM-1 (data not shown).

pNL4-3 is a full-length infectious molecular clone of HIV-1 (Adachi *et al.,* 1986). This vector has been provided by the AIDS Repository Reagent Program. pNL4-3-Luc-E^-R^+, which is derived from pNL4-3, contains a frameshift mutation in the *env* gene and has the *nef* gene replaced by the *luciferase* reporter gene. pcDNA-1/JR-FL *env* vector codes for the envelope proteins from the macrophage-tropic (R5) JR-FL viral strain (Deng *et al.,* 1996). Both plasmids are kind gifts from Dr. N. Landau (The Salk Institute for Biological Studies, La Jolla, CA). pCD1.8 is an eukaryotic expression vector containing the entire human ICAM-1 cDNA and was obtained from Dr. Timothy A. Springer (The Center for Blood Research, Boston, MA) (Staunton *et al.,* 1988).

Antibodies, human serum samples, and sCD4

The anti-ICAM-1 antibody (anti-CD54) RR1/1.1.1 has been previously shown to inhibit ICAM-1/LFA-1 interaction (Marlin and Springer, 1987) and was kindly provided by Dr. Robert Rothlein (Boehringer Ingelheim, Ridgefields, CN) (Rothlein *et al.,* 1986). The LFA-1-activating antibody NKI-L16 (anti-CD11a) was obtained from Dr. Carl C. Figdor (University Hospital Nijmegen, The Netherlands) (Keizer *et al.,* 1988). The monoclonal antibody 0.5β mapping to amino acids 308–331 from HIV-1_{IIIB} gp120 (Matsushita *et al.,* 1988), 4.8D, a human monoclonal antibody specific for a conformational epitope of gp120 (Thali *et al.,* 1993), and F105, another human monoclonal antibody directed against a conformational

epitope of gp120 mapping to the CD4 binding site (Posner *et al.,* 1993), were obtained from Dr. Shuzo Matsushita, James Robinson, and Marshall Posner, respectively, through the AIDS Repository Reagent Program. Individual heat-inactivated sera from three HIV-1-infected subjects at different stages of the disease were also used in this study. Serum samples were obtained from HIV-1-infected persons followed at the HIV/AIDS Clinic of the Centre Hospitalier de l'Université Laval (Quebec City, Canada). Informed consent was obtained from each individual before their blood was collected. All serum samples were heat inactivated at 56°C for 30 min before use. Recombinant sCD4 was also obtained through the AIDS Repository Reagent Program from Dr. Ray Sweet (SmithKline Beecham) (Arthos *et al.,* 1989).

Virus stocks

Viral particles differing only by the absence or the presence of host-derived ICAM-1 proteins on their surfaces were produced by $CaPO_4$ transfection of pNL4-3 (10 μg) or, for pseudotyped virions, pNL4-3-Luc-E^-R^+(5 μg) with pcDNA-1/JR-FL(5 μg), as described previously (Fortin *et al.,* 1997; Paquette *et al.,* 1998). These transfections were done in the absence or the presence of 5 μg of the ICAM-1 expression vector to produce ICAM-1/NEG or ICAM-1/POS virus particles, respectively. Virus stocks were normalized for virion content using a commercial assay for the major viral core protein p24 (Organon Teknika, Durham, NC).

Neutralization assay

Fixed amounts of ICAM-1/NEG and ICAM-1/POS virus stocks (5–10 ng of p24) were either left untreated or incubated in the presence of increasing concentrations of individual sera, monoclonal anti-gp120 antibodies, or sCD4. These incubations were performed at 37°C in 96-well plates in a final volume of 100 μl for 30 min. In some experiments, target cells were either left untreated or were treated with the LFA-1-activating antibody NKI-L16 at 1 μg/ml for 30 min at 37°C. After this incubation period, 100 μl of the cell suspension (1×10^6 cells/ml for 1G5, 5×10^5 cells/ml for PM1) was added to each well, and the plates were incubated at 37°C before lysis (48 h for 1G5 and 72 h for PM1). The level of virus infection was quantified by monitoring virus-encoded luciferase activity in the infected cells as described previously (Fortin *et al.,* 1997).

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