RAPID COMMUNICATION

A CD4-Independent Interaction of Human Immunodeficiency Virus-1 gp120 With CXCR4 Induces Their Cointernalization, Cell Signaling, and T-Cell Chemotaxis

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The gp120 envelope glycoprotein of human immunodeficiency virus-1 (HIV-1) interacts with the CXCR4 chemokine receptor, but it is not known whether gp120 activates CXCR4-mediated signaling cascades in the same manner as its natural ligand, SDF1α. We assessed the effects of wild-type gp120 and a mutant gp120 that interacts with CXCR4 but not CD4 on CD4⁺/CXCR4⁺ and CD4⁻/CXCR4⁻ cells, respectively. Under both experimental conditions, the interaction of CXCR4 and gp120 resulted in their CD4-independent cointernalization. Both molecules were translocated into early endosomes, whereas neither protein could be detected in late endosomes. Binding of gp120 to CXCR4 resulted in a CD4-independent phosphorylation of Pyk2 and an induction of chemotactic activity, demonstrating that this interaction has functional consequences. Interestingly, however, whereas SDF1α activated the ERK/MAP kinase pathway, this cascade was not induced by gp120. Together, these results suggest that the pathologies of HIV-1 infection may be modulated by the distinct signal transduction pathway mediated by gp120 upon its interaction with CXCR4.

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ENTRY OF HUMAN immunodeficiency virus (HIV) into target cells requires binding to CD4, as well as to one of several coreceptors, which have been identified as chemokine receptors belonging to the G-coupled seven transmembrane protein family. The tropism of HIV depends on the usage of the chemokine receptor; CCR5 is primarily used by macrophagetropic (M-tropic) HIV strains, whereas lymphotrophic (T-tropic) HIV strains primarily use CXCR4 as a coreceptor. However, dual tropic HIV-1 strains have been described that interact with either type of chemokine receptor. This has led to a novel classification that identifies HIV-1 strains based solely on their coreceptor usage.

Upon interaction with their receptor, stromal-derived factor 1α (SDF1α), and regulation-upon-activation, normal T expressed and secreted (RANTES), the natural ligands of CXCR4 and CCR5, respectively, are able to trigger signaling cascades, resulting in chemotaxis. In addition, a number of chemokines, including RANTES, macrophage inflammatory protein 1α (MIP1α), MIP1β, and SDF1α, are able to inhibit HIV-1 infection after binding to their respective receptors. Indeed, it has been demonstrated that interaction of SDF1α with CXCR4 leads to a rapid downmodulation of this receptor, suggesting that this phenomenon might be responsible, at least in part, for the ability of chemokines and possibly other ligands of chemokine receptors to inhibit HIV-1 infection.

The existence of a tri-molecular complex formed between CD4, gp120, and CXCR4 at the cell surface has been reported. Although the sequence of events leading to its formation has not yet been well characterized, it has been proposed that the binding of the HIV surface envelope gp120 to CD4 induces conformational changes, resulting in a high-affinity interaction with the coreceptors. The interaction of gp120 with CD4 results in the endocytosis of the gp120-CD4 complex and the concurrent stimulation of signaling molecules normally activated through the CD4 receptor. However, some HIV strains can infect cells via a CD4-independent pathway, and it has recently been shown that gp120 can interact directly with CXCR4. Binding of the HIV envelope to one of its coreceptors has been reported to induce the activation of the ERK/MAP kinase pathway, as well as the phosphorylation of the tyrosine kinase Pyk2. However, these studies did not exclude the involvement of CD4 in the CXCR4 or CCR5-mediated signal transduction cascade. It is therefore still unclear whether gp120 can mimic its natural receptor ligand, SDF1α, and induce a CD4-independent signaling pathway upon binding to CXCR4.

We have previously demonstrated that a recombinant gp120 protein (gp120 ΔcHX1), which is deleted of its amphipathic α helix-1, is no longer able to bind CD4, but retains its capacity to interact with CXCR4. Using this gp120 mutant as well as a CD4⁺/CXCR4⁺ cell line, we have analyzed the cellular localization of CXCR4 after interaction with gp120 and addressed the question of whether the presence of CD4 is required for gp120/CXCR4-mediated signal transduction. It is demonstrated here, for the first time, that the CD4-independent interaction of HIV-1 gp120 with its coreceptor induces the cointernalization of these two proteins, Pyk2 phosphorylation, and T-cell chemotaxis.

MATERIALS AND METHODS

Cells and culture conditions. CD4⁺/CHO-K1 and CD4⁺ Jurkat cell lines were obtained from the American Type Culture Collection (ATCC;
HIV-1 gp120 INDUCES CELL SIGNALING VIA CXCR4

Rockville, MD) and grown in RPMI 1640 supplemented with 10% fetal calf serum (FCS; Biomedia, Toulouse, France). The CHO-K1 cell line, transfected with a CXCR4 expression vector (a generous gift of Marc Parmentier, Euroscreen Co, Bruxelles, Belgium), was grown in HamF12 medium (Life Technologies, Cergy Pontoise, France) supplemented with 10% FCS and 400 µg/mL of G418. The human Th2 clone CB-328 was generated using cloning and culture conditions as previously described and was grown in Yssel's medium (Irvine Scientific, Santa Ana, CA) supplemented with 1% AB human serum. Cell surface expression of CXCR4 on the latter cells was induced by the addition of 100 µM of recombinant interleukin-4 (IL-4) in the culture medium for at least 1 week before their use. Antibodies and reagents. The following antibodies were used: the anti-CD4 monoclonal antibodies (MoAbs) OKT4a (Ortho Diagnostic Systems, Ortho-Mune, Raritan, NJ) and ST4 (a kind gift of Dr Pierre Gros, SANOFI Recherche, Montpellier, France), the anti-CD3 UCHT1 MoAb (PharMingen, La Jolla, CA), the anti-CXCR4 12G5 MoAb6,39 (kindly provided by James Hoxie, University of Pennsylvania, Philadelphia, PA), as well as the fluorescein isothiocyanate (FITC)- and biotin-linked to bromacetyl-sepharose. Anti-gp120 antiserum, was produced in our laboratory after immunization with a recombinant HIV-1 IIB gp120 (purchased at Intracel Corp, Cambridge, MA). An FITC-conjugated anti-LAMP1 HA43 MoAb (anti-CD107) and human transferrin coupled to Texas Red (both from Molecular Probes Europe BV, Leiden, The Netherlands) were used for confocal microscopy studies. The anti-CD4 MAPK polyclonal Ab (Promega, Madison, WI) and anti-ERK-2 MoAb (Santa Cruz Laboratories, Santa Cruz, CA), anti-Pyk2 MoAb (Transduction Laboratories, Lexington, KY), anti-phosphotyrosine 4G10 MoAb (Upstate Biotechnology, Lake Placid, NY), and horseradish peroxidase (HRP)-conjugated goat antirabbit or antimouse secondary Abs (Amersham, Arlington Heights, IL) were used in signal transduction experiments. Stromal derived factor-1α chemokine (SDF1α) was purchased from R&D Systems. Recombinant SU-gp120 proteins. Recombinant monomeric gp120 wild-type (wt) and gp120 ΔαHx1 proteins were produced in baculovirus and purified as previously described. Briefly, a 1,414-bp fragment encoding gp120 wt (from amino acid V12 to V481) was amplified by polymerase chain reaction (PCR), using the plasmid pHXB2R (carrying the complete genome of a clone of HIV-1 IIB) as a template. The mutant gp120 ΔαHx1 protein was generated by a deletion of 26 amino-acid residues, corresponding to the putative α helix–1 from the C1 region of gp120 wt. The soluble gp120 wt and gp120 ΔαHx1 proteins were produced in insect SF9 cells infected by the respective recombinant baculovirus. Cell supernatants were collected 6 days postinfection and gp120 wt or gp120 ΔαHx1 was concentrated and immunopurified with the anti-gp120 D7324 Ab (Aalto, Dublin, Ireland) linked to bromacetyl-sepharose.

Immunofluorescence and flow cytometry analysis. Two hundred thousand T cells were resuspended in 50 µL of phosphate-buffered saline (PBS), supplemented with 3% bovine serum albumin (BSA) and 0.02% NaN3, in the presence of the relevant MoAbs. After 1 hour of incubation with agitation at 4°C, the cells were washed twice in PBS-0.3% BSA-0.02% NaN3 and resuspended in PBS-0.3% BSA-0.02% NaN3 in the presence of a 1/50 dilution of FITC-conjugated goat-antimouse Ab (Caltag, Burlingame, CA). After an additional 1 hour of incubation with agitation at 4°C, the cells were washed three times as described above, resuspended in PBS, and analyzed by single-color flow cytometry using a FACSABCort (Becton Dickinson, San Jose, CA) and LYSIS II software. Each datum point represents the acquisition of 10,000 gated events.

Internalization assays. Confocal microscopy studies were performed on transfected CHO-K1 cells and the T-cell clone CB-828 (2 × 106 cells/mL). Cells were preincubated for 1 minute at 37°C in an acid buffer (pH 3.0), consisting of 50 mmol/L glycine and 100 mmol/L NaCl, to remove any cell-bound proteins. To exclude a possible role of the CD4 in the different cellular responses induced by gp120, two different experimental procedures were used: either CD4+/CXCR4+ cells (CB-828) were incubated with the mutant gp120 ΔαHx1 or CD4+/CXCR4+ cells (CHO-K1) were incubated with gp120 wt. After washing with PBS-3% BSA, the appropriate cell line was incubated for 1 hour at 37°C with one of the gp120 proteins or SDF1α in PBS-3% BSA. Cells were then washed, fixed in PBS-3.7% paraformaldehyde for 20 minutes, washed again, incubated for 15 minutes in PBS-0.1 mol/L glycine to quench free aldehydes, and permeabilized by incubation with 0.05% saponine in PBS-0.2% BSA. Cells were incubated for 45 minutes at room temperature with either the anti-CXCR4 12G5 MoAb or the anti-gp120 110-4 MoAb, washed, and subsequently incubated with an FITC- or Texas Red conjugated goat antimouse IgG antibody (Caltag).

To identify the compartment in which gp120 proteins and CXCR4 were translocated after their internalization, their localization was monitored together with that of transferrin and LAMP1, markers of early and late endosomes, respectively. For this purpose, transferrin was coupled to Texas Red, and LAMP1 was detected with an FITC-conjugated anti–CD107 MoAb (anti-CD107) MoAb. Before staining with iron-loaded human transferrin, cells were incubated in serum-free RPMI medium for 24 hours at 37°C. Cells were slide-mounted in Mowiol and analyzed by confocal microscopy. Simultaneous double-fluorescence acquisition was performed at wavelengths of 488 and 588 nm. The images were assembled and printed directly using Adobe Photoshop software (Adobe Systems Inc, San Jose, CA).

Cell stimulations and immunoblot analysis. Before stimulation, CD4+ Jurkat cells were cultured for 24 hours in RPMI-1640 medium, supplemented with 0.5% FCS. For analysis of Pyk2 phosphorylation, 106 cells were resuspended in 100 µL of serum-free RPMI 1640 medium and incubated at 37°C for 15 minutes before stimulation with either 100 µg/mL anti-CD3 UCHT-1 MoAb, 10 µg/mL of each of the gp120 proteins, 25 mmol/L SDF1α, or 10 µg/mL of the anti-CD4 ST4 MoAb for different time periods. For analysis of ERK MAPK phosphorylation, 106 cells were preincubated on ice for 60 minutes in the presence of each of the above-mentioned reagents and then transferred to 37°C for the indicated times. Cells were lysed in a 1% NP40 buffer and postnuclear supernatants were immunoprecipitated for 1 hour at 4°C with a polyclonal anti-Pyk2 Ab, followed by collection on protein-A sepharose beads (Pharmacia, Uppsala, Sweden). Immune-precipitates or whole cell lysates were boiled, resolved on an 8.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, and transferred electrophoretically to nitrocellulose membranes. Membranes were incubated for 30 minutes in TBS (150 mmol/L NaCl, 20 mmol/L Tris, pH 7.5) containing 5% BSA and 0.1% Tween 20 and then incubated from 1 hour with either the anti-ß-actin or anti–P-tyr 4G10 MoAb or an anti-active MAPK Ab that recognizes the dual phosphorylated T183/Y185 form of ERK1/2 (Promega). Blots were washed in TBS containing 0.1% Tween 20 and incubated with HRP-conjugated goat antirabbit or antimouse secondary antibodies. Immuno-reactive proteins were visualized using the enhanced chemiluminescence assay (Amersham, Bucks, UK). For reblotting with the anti-Pyk2 or the anti-ERK2 MoAbs, filters were stripped as previously reported.

T-cell chemotaxis. Migration of CXCR4-expressing human T cells, in response to the chemotactic activity of SDF1α, gp120 wt, or gp120 ΔαHx1 proteins, was analyzed in Chemo Tx-96 Disposable Chambers with a 5-µm pore size that separate the upper and lower compartments (Neuro Probe Inc, Gaithersburg, MD), using the method described by Bacon and Schall. Expression of cell surface CXCR4 was assessed by flow cytometry before the experiment. Twenty nine microliters of Yssel’s medium 1% human serum, containing increasing concentrations of either SDF1α, gp120 wt, or gp120 ΔαHx1 (0.1 mmol/L, 1 mmol/L, 10 mmol/L, 100 mmol/L, and 1 mmol/L) were added to the lower wells. Fifty thousand T cells were transferred directly in triplicate on the filter sample sites (upper compartment) in a final volume of 25 µL. After 1
hour of incubation at 37°C in a 5% CO₂ incubator, cells that migrated through the filter were collected in the lower compartment, resuspended in culture medium, and counted through 10 power fields of a Malassez hemocytometer. Spontaneous cell migration was determined in the presence of medium alone or after the addition of SDF1α to both upper and lower compartments. The number of spontaneously migrating cells was subtracted from the total number of cells present in the lower compartment after the addition of a CXCR4-ligand to determine the actual extent of migration. Results are expressed as the ratio of migrating cells/total number of cells × 100%.

RESULTS

CD4-independent internalization of cell-surface CXCR4 after interaction with a gp120 mutant protein. Interaction of CXCR4 with its natural ligand, SDF1α, results in internalization of the receptor-ligand complex from the surface of T cells. To analyze whether cell surface expression of CXCR4 changes after binding to gp120, CXCR4-expressing T cells were stimulated with a recombinant wild-type gp120 protein and the expression of CXCR4 was analyzed by confocal laser scanning microscopy. As shown in Fig 1, incubation of the human T-cell clone CB-828 with similar concentrations of either gp120 (Fig 1B) or SDF1α (Fig 1D) for 1 hour at 37°C resulted in capping of CXCR4 at the cell surface and internalization of the receptor. To determine whether the gp120-induced internalization of CXCR4 was dependent on an interaction with CD4, the effect of gp120 ΔαHX1, a mutant gp120 envelope protein that binds to CXCR4 but not to CD4, was assessed (Fig 1C). Similar to the effects observed with the wild-type protein, incubation of T cells with gp120 ΔαHX1 resulted in an internalization of CXCR4, indicating that the gp120-mediated internalization of CXCR4 can occur in the absence of a gp120-CD4 interaction. Furthermore, we find that, upon incubation with gp120 proteins, there was a decrease of 35% in the level of CXCR4 receptor on the cell surface due to CXCR4 internalization (data not shown).

gp120 and CXCR4 cointernalize in early endosomes after the CD4-independent interaction of gp120 with CXCR4. To assess whether gp120 and CXCR4 cointernalized in a CD4-independent manner, the CD4⁻/CXCR4⁺ CHO-K1 cell line was incubated with gp120 wt protein for 1 hour at 37°C and the cellular localization of the CXCR4-gp120 complex was analyzed (Fig 2C). Although a residual amount of gp120 could still be detected at the surface of the cells, a significant level of CXCR4 and gp120 proteins were colocalized within the cells,
demonstrating that internalization as well as colocalization occurs independently of CD4. It is important to note that the colocalization of the gp120-CXCR4 complex can be observed only in cells in which endosomes are present in the microscopic confocal laser section.

We next determined the subcellular localization of internalized CXCR4 and gp120 molecules, by comparing the localization of each of these proteins with that of transferrin, a protein that is internalized and transported to early endosomes upon endocytosis, and CD107 (LAMP1), a lysosome-associated membrane protein that is present exclusively in late endosomes and primary lysosomes. After incubation of CD4−/CXCR4− CHO-K1 cells with gp120, both CXCR4 (Fig 3A, B, and C) and gp120 (Fig 3D) colocalized with transferrin, as detected by confocal laser microscopy. Similarly, binding of SDF1α to CXCR4, used as a positive control, resulted in a colocalization of CXCR4 and transferrin, after endocytosis (Fig 3B). In contrast, neither gp120 nor CXCR4 could be detected in late endosomes, as shown by the absence of colocalization of either protein with LAMP1 (Fig 4). Taken together, these results indicate that, after interaction of gp120 with CXCR4, the resulting receptor-ligand complex is rapidly internalized and translocated to early, but not late endosomes.

Pyk2, but not the ERK/MAP kinase pathway, is activated by the binding of gp120 to CXCR4, on CD4+ T cells. Interaction of CXCR4 with either SDF1α or gp120 triggers signaling cas-
cades, involving the phosphorylation of the Pyk2 protein tyrosine kinase and the activation of the ERK/MAPK pathway.\textsuperscript{33,34} However, it is not clear whether binding of gp120 to CXCR4 can activate these pathways in a CD4-independent manner. As shown in Fig 5, stimulation of CD4\textsuperscript{+}/CXCR4\textsuperscript{+} Jurkat cells with gp120 wt, as well as with gp120 ΔαHX1 proteins, resulted in the phosphorylation of Pyk2, albeit to a lower extent than that observed upon stimulation with SDF1α. Additionally, as expected, Pyk2 phosphorylation was induced by engagement of the TCR/CD3 complex signaling cascade (Fig 5).

We next assessed the ability of gp120 to activate the ERK/MAPK pathway in CD4\textsuperscript{+}/CXCR4\textsuperscript{+} Jurkat cells by mea-

Fig 3. CD4-independent internalization of CXCR4 and gp120 in early endosomes. CD4\textsuperscript{+}/CXCR4\textsuperscript{+} CHO-K1 cells were incubated in medium alone (A), in medium with 10 μg/mL of SDF1α (B), or in medium with 10 μg/mL of gp120 wt (C and D) in the presence of 125 μg/mL of Texas Red-conjugated transferrin for 1 hour at 37°C. After fixation and permeabilization, cells were stained with the FITC-conjugated anti-CXCR4 12G5 MoAb (A, B, and C) or the anti-gp120 110-4 MoAb and an FITC-conjugated antimouse IgG (D). The intracellular localizations of CXCR4 and transferrin (A, B, and C) or gp120 and transferrin (D) were analyzed by confocal microscopy. Yellow spots are indicative of the colocalization of transferrin (red) with either CXCR4 (green) or gp120 (green) in early endosomes.

Fig 4. Absence of internalized CXCR4-gp120 complex in late endosomes. CD4\textsuperscript{+}/CXCR4\textsuperscript{+} CHO-K1 cells were incubated in the presence of either 10 μg/mL of gp120 wt (A and D), 10 μg/mL of gp120 ΔαHX1 (B and E), or medium alone (C and F) for 1 hour at 37°C. After fixation and permeabilization, the intracellular presence of CXCR4 (red; A, B, and C), gp120 (red; D, E, and F), and LAMP1 (green) was analyzed by confocal microscopy. Cells were stained with either the biotin-conjugated anti-CXCR4 12G5 MoAb followed by staining with Texas Red-conjugated streptavidin (A, B, and C) or a rabbit anti-gp120 antiserum followed by staining with a Texas Red-conjugated antirabbit IgG (D, E, and F). Coexpression of LAMP1 was assessed after staining of the cells with 10 μL of the anti-CD107 FITC-conjugated MoAb.
suring the dual phosphorylation of the ERK1 and ERK2 kinases on residues T183 and Y185. Stimulation of cells with either SDF1α or an anti-CXCR4 MoAb resulted in a weak, but significant, phosphorylation of both ERK1 and ERK2 as compared with engagement of the CD3 complex that resulted in a dramatic phosphorylation of these two proteins. Because the kinetics of ERK phosphorylation differed after CD3 and CXCR4 stimulation with maximal response at 3 and 15 minutes, respectively, the abilities of gp120 wt and gp120 ΔαHX1 to induce ERK phosphorylation was assessed after both 5 and 15 minutes of stimulation at 37°C. However, irrespective of the conditions, neither gp120 wt nor gp120 ΔαHX1 was able to induce the phosphorylation of ERK1/ERK2. The lack of a functional CD4 receptor on the Jurkat cells used in this study was confirmed by the inability of an anti-CD4 MoAb to induce the phosphorylation of ERK1/ERK2 (Fig 6), whereas both proteins were phosphorylated in CD4+ T cells after stimulation with this MoAb (data not shown). Collectively, these data demonstrate that the interaction of gp120 with CXCR4, in the absence of CD4, results in a signaling cascade, which involves the phosphorylation of Pyk2, but does not induce the activation of the ERK/MAPK pathway.

gp120 induces a chemotactic response in T cells in a CD4-independent manner. The demonstration that gp120 can induce a CXCR4-mediated signaling response prompted us to investigate whether this interaction, like the binding of SDF1α to its receptor, would result in chemotaxis. Stimulation of CXCR4+ Th2 cells with either the wild-type or mutant gp120 protein resulted in a similar dose-dependent chemotactic response, showing typical bell-shape curves (Fig 7). The maximal chemotactic responses induced by both gp120 wt and gp120 ΔαHX1 proteins was observed at a concentration of 100 nmol/L, with a migration of approximately 22% of all cells in a representative experiment. Because equivalent results were observed with the two gp120 proteins, these data indicate that
the interaction of gp120 with CD4 does not modulate the capacity of gp120 to mediate a chemotactic response through the CXCR4 receptor. A typical bell-shaped chemotactic response was also induced by SDF1α, with a maximum migration of 72% observed after the addition of 10 nmol/L SDF1α. Although the SDF1α-induced migration was higher than that observed with gp120 proteins, our results indicate that the gp120 protein of the T-tropic HIV-1 IIIB strain is able to induce a significant CD4-independent chemotactic response in human T cells.

**DISCUSSION**

In the present work, we demonstrated that the surface (SU) subunit of the gp120 from the T-tropic HIV-1 IIIB strain induced CD4-independent cellular responses after binding to CXCR4, the fusogenic coreceptor of HIV-1. Indeed, SDF1α, the natural ligand of the CXCR4 chemokine receptor, as well as gp120 both induced cell migration. To assess the importance of the gp120-CXCR4 interaction in the absence of the CD4 receptor, the experiments in the present work were performed using a gp120 ΔαHX1 mutant that has been previously shown to interact with CXCR4 but not CD4. Additionally, equivalent results were obtained when the effects of wild-type gp120 were assessed on CD4+/CXCR4- cells.

Confocal laser scanning analysis demonstrated that the CXCR4 receptor was internalized after interaction with gp120 in a CD4-independent manner. gp120 was internalized and colocalized with CXCR4 within early endosomes, as shown by the colocalization of both proteins with an early endosome specific marker, transferrin. Our finding that neither CXCR4 nor gp120 was present within late endosomes suggests that CXCR4 may be recycled and re-expressed at the cell surface. In fact, we have observed that CXCR4 is rapidly recycled (within 30 minutes) after its gp120- or SDF1α-induced internalization (data not shown). Additionally, other groups have shown that, after the rapid downmodulation of CXCR4 upon binding of SDF1α, there is a subsequent re-expression of CXCR4 at the cell surface. The internalization of CXCR4 does not seem to be required for productive HIV-1 infection, because HIV-1 can infect cells with a CXCR4 deletion that inhibits its internalization. However, the presence of CXCR4 at the cell surface may be important for HIV-1 infection, because its downmodulation after interaction with SDF1α results in a decrease in HIV infection. In this context, the interaction of soluble gp120, shed from infected cells, with CXCR4 may inhibit HIV-1 infection, by both downmodulating CXCR4 at the cell surface and competing for receptor binding. Additionally, downmodulation of surface CD4 molecules after association with the HIV-1 envelope has been found to decrease the efficiency of subsequent HIV-1 infection.

The evolution and enhancement of lymphocyte infection as well as the subsequent physiopathological effects on HIV-1 infected lymphocytes likely result from several CXCR4-specific biological phenomena. First, at least in some instances, there is an increase in CXCR4 expression at the cell surface after T-cell activation. Specifically, IL-4 treatment has been shown to result in an increase in CXCR4 expression on Th2 cells, allowing them to be more efficiently infected by HIV-1. Second, we now show that gp120 can itself induce a chemotactic response in Th2 cells. Thus, the presence of soluble T-tropic gp120 during the late phase of HIV infection may participate in the recruitment (through chemotaxis) of uninfected cells to the lymph nodes. This, in turn, would increase the possibility that these cells would be infected by HIV-1 and accelerate the development of acquired immunodeficiency syndrome (AIDS). Of note, this hypothesis has been previously suggested for the M-tropic HIV-1 viruses in which chemotaxis of macrophages is induced by the interaction of gp160 with the CCR5 chemokine receptor.

Several groups have shown that the natural ligand of CXCR4, SDF1α, as well as gp120 triggers activation of the ERK/MAP kinase pathway and Pyk2 phosphorylation. However, in the context of the previous studies, the ability of gp120 to trigger a specific signal through CXCR4 could not be ascertained, because signals could also be transduced through the CD4 receptor. Phosphorylation of the Pyk2 protein tyrosine kinase is known to be induced by ligand binding to G-protein-coupled receptors, and its activation is clearly dependent on changes in osmolality induced by the mobilization of Ca2+ from internal stores. In addition, Pyk2 can function as an upstream mediator of the ERK/MAPK and/or JUN/MAPK signaling pathways. In the present work, we demonstrate that the binding of HIV-1 IIIB gp120 to CXCR4 triggers Pyk2 phosphorylation in a CD4-independent manner. These data are supported by previous work showing that gp120-induced phosphorylation of Pyk2 in CD4+ cells is partially inhibited by the anti-CXCR4 12G5 MoAb. Interestingly, however, we did not observe any phosphorylation of ERK1/2 MAPK after stimulation with gp120, even though SDF1α clearly induced ERK1/2 phosphorylation in a CD4-independent manner. Thus, gp120 and SDF1α appear to differentially activate CXCR4-mediated signaling cascades. This is the first demonstration that interaction of different CXCR4 ligands induces distinct transduction pathways.
The ability of HIV-1 IIIB gp120 to bind the CXCR4 chemokine receptor and induce Pyk2 phosphorylation and chemotaxis suggests that this association plays a major role in the activation status of the cells and may contribute to the evolution of HIV infection. Additionally, the physiological changes induced by the binding of gp120 to CXCR4 on target cells may induce alterations in intracellular structures that favor HIV infection through cell-to-cell contact.\textsuperscript{48,49} An understanding of the processes involved in SU binding and fusion of HIV envelopes will allow the development of new strategies to better control HIV infection.

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