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Expression of Chemokine Receptors CXCR4 and CCR5 in HIV-1-Infected and Uninfected Individuals

Mario A. Ostrowski,1* Shawn J. Justement,* Andrew Catanzaro,* Claire A. Hallahan,* Linda A. Ehler,* Stephanie B. Mizell,* Princy N. Kumar,† Jo Ann Mican,* Tae-Wook Chun,* and Anthony S. Fauci*

The chemokine receptors CXCR4 and CCR5 have been identified as major coreceptors for HIV-1 entry into CD4+ T cells. The majority of primary HIV-1 isolates in early disease use CCR5 as a coreceptor, whereas during disease progression with the emergence of syncytium-inducing viruses, CXCR4 is also used. We performed a cross-sectional study in which we evaluated the expression of two HIV-1 coreceptors, CCR5 and CXCR4, in whole blood samples taken from HIV-1-infected and uninfected individuals. We demonstrate that CXCR4 on CD4+ and CD8+ T cells, and CD14+ monocytes is significantly down-regulated, and CCR5 expression on CD4+ T cells is up-regulated in HIV-infected individuals compared with uninfected controls. Coreceptor expression correlated with the level of cellular activation in vivo in both HIV-infected and uninfected individuals, with CXCR4 being expressed predominantly on quiescent (HLA-DR-+) T cells and CCR5 being expressed predominantly on activated (HLA-DR+) T cells. Lower expression of CXCR4 and higher expression of CCR5 on CD4+ T cells correlated with advancing disease. In addition, a tendency for greater activation of CXCR4+CD4+ T cells in patients with advanced disease was observed. Patients who harbored syncytium-inducing viruses, however, could not be distinguished from those who harbored nonsyncytium-inducing viruses based on the level of CD4+ T cell activation or chemokine receptor expression.


Recent evidence indicates that HIV-1 can use a number of chemokine receptors to enter CD4+ T cells (1–6). The β-chemokine receptor CCR5 is of major importance in the pathogenesis of HIV-1 disease, as usage of this receptor by primary HIV-1 strains appears to be critical in the establishment of persistent infection (7–10). The α-chemokine receptor, CXCR4, is most likely also of importance since usage of CXCR4 by HIV-1 isolates often evolves during disease progression associated with the emergence of syncytium-inducing (SI)2 variants (11–14). Recent studies have (15, 16) demonstrated a relatively reciprocal relationship between CCR5 and CXCR4 expression on PBMCs taken from normal individuals. CXCR4 was expressed predominantly on CD26low, CD45RA+, CD45RO+ T cells, indicating a naïve unactivated phenotype; and CCR5 was expressed on CD26high, CD45RA−, CD45RO+, CD25+, CD69+, CD95+ T cells, a phenotype consistent with previously activated memory cells. Cellular activation is critical for productive infection in vitro and in vivo (17–20). Thus, it can be postulated that the major targets for initial infection by HIV-1 are those CD4+ T cells that express an appropriate coreceptor and are activated, i.e., CCR5-expressing memory cells. In addition, HIV-1-infected individuals manifest persistent CD4+ and CD8+ T cell activation, which provides an optimal environment for continuous viral replication (21–24).

In the present study, we have examined the expression of CCR5 and CXCR4 on fresh whole blood samples taken from HIV-1-infected and uninfected individuals. In addition, we had the opportunity to temporally follow HIV-1 coreceptor expression in one individual who was experiencing acute, symptomatic HIV-1 seroconversion. We compared CXCR4 and CCR5 expression in the context of cellular activation markers and stage of disease. Our findings indicate that the heightened state of cellular activation in HIV-1-infected individuals is associated with CCR5 up-regulation and CXCR4 down-regulation on CD4+ T cells, and that this milieu may favor the propagation of macrophage-tropic (CCR5-using) viruses. In those patients with SI viruses, we could not find significant differences in the levels of cellular activation, nor expression of CXCR4 and CCR5 on CD4+ T cells in comparison with those patients harboring only nonsyncytium-inducing (NSI) viruses.

Materials and Methods

Abs and reagents

A mAb to CXCR4 (12G5) was a generous gift from Dr. James Hoxie (25). A mAb to CCR5 (5C7) was kindly provided by Dr. Charles MacKay (16). All of the following (FITC, phycoerythrin (PE), PerCP) conjugated mAbs were obtained from Becton Dickinson (San Jose, CA): anti-mouse IgG1, and IgG2a isotype controls, anti-CD45, anti-CD14, anti-CD4, anti-CD8, anti-HLA-DR, and anti-CD3. An unconjugated mouse polyclonal isotype IgG was obtained from Sigma (St. Louis, MO). A goat anti-mouse F(ab’)2 light and γ-chain human IgG-adsorbed FITC was obtained from Biosource (Camarillo, CA).

Patients and control subjects

Twenty-five HIV-1-infected adults and ten control volunteers were recruited from the Washington D.C. area. Fifty-two percent of the HIV-1-infected subjects were receiving highly active antiretroviral treatment that included a protease inhibitor. Most patients were asymptomatic. Patient 22

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2 Abbreviations used in this paper: SI, syncytium-inducing; CI, confidence intervals; NSI, nonsyncytia-inducing; PE, phycoerythrin; PerCP, peridinin chlorophyll protein.


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presented with an acute seroconversion illness; he is a 47-yr-old homosexual male who presented with fever, rash, pharyngitis, severe malaise, and diarrhea 2 wk after a high risk exposure. Patient 3 is a recent asymptomatic seroconverter (HIV-infected <1 yr). Patient 23 is being treated for chronic bacterial sinusitis.

Blood was drawn by venipuncture from each subject in EDTA tubes (Becton Dickinson) for FACS analysis, CBC, plasma viral load, and absolute CD4+ T cell count. All samples for FACS analysis were prepared within 2 h of the draw.

Whole blood surface staining and FACS analysis

For cytofluorometric analyses in this study, we used the following Ab combinations: 1) CD45 (FITC) and CD14 (PE); 2) CD4 (FITC) and CD8 (PE); 3) CD4 (FITC) and CD8 (FITC) or CD48 (FITC) and HLA-DR (PE) and CD3 (PerCP); 4) 12G5 or SC7 (FITC) and CD4 (PE) and CD3 (PerCP); 5) 12G5 or SC7 (FITC) and CD8 (PE) and CD3 (PerCP); 6) 12G5 or SC7 (FITC) and CD14 (PE); and 7) 12G5 or SC7 (FITC) and HLA-DR (PE) and CD4 (PerCP).

For each stain, 100 μl of whole blood was washed in PBS/1% FCS/0.02% sodium azide. For un conjugated Abs, blood was incubated with either unconjugated isotype, 12G5, or SC7 for 30 min on ice at final concentrations of 1, 8, and 5 μg/ml, respectively. Blood was washed and then incubated with a 1/50 dilution of a FITC goat anti-mouse Ig F(ab’2) for 15 min. Blood was washed again, and finally, the appropriate directly conjugated mAb(s) was added. The blood was washed, RBCs were lysed, and cells were fixed and suspended in 1% paraformaldehyde in PBS. Three-color flow-cytometric analysis was performed on a dual-laser Epics-C Coulter flow cytometer (Hialeah, FL). At least 5000 events were counted for each stain. For subset analysis, lymphocyte or monocyte gates as well as gates uniquely identifying CD3 or CD14 cells were applied. For example, CXCR4 expression of CD4+ T cells was obtained after gating for lymphocytes based on forward and side scatter, and then gating on CD3+ positive cells.

Plasma viremia quantitation

Plasma viremia was quantitated using branched DNA methodology (Chiron, Emeryville, Ca). All plasma viremia quantitations from patient 22 were obtained using quantitative reverse-transcriptase PCR (J. Lifson et al., unpublished data).

Virus isolation and phenotyping

PBMCs from HIV-infected patients were depleted of CD8+ T cells by magnetic beads (Dynal, Oslo, Norway) and cultured in complete medium (RPMI 1640, 2% penicillin-streptomycin, 2% t-glutamine; BioWhittaker, Walkersville, MD) in the presence of heat-inactivated 10% FCS at 37°C, irradiated feeder cells (HIV-uninfected blood donor), anti-CD3 Ab (mouse ascites, 1/4000 dilution), and IL-2 (20 U/ml; Boehringer Mannheim, Mannheim, Germany) at 37°C in a 5% CO2 atmosphere. Three-day-old CD8-depleted, anti-CD3 Ab-stimulated blasts from HIV-negative donors were added to the cultures on the following day and 1 wk later. Reverse-transcriptase activity was monitored at day 14 and was performed as previously described (17). Cultured virus was phenotyped using an MT2 cell assay. A total of 50 μl of culture supernatant was added to 0.1 × 10⁶ MT2 cells (National Institutes of Health AIDS Research and Reference Reagent Program) in 96-well plates. The assay was performed in quadruplicate, and was monitored for the presence of syncytia over a 2-wk period.

CCR5 genotype analysis

CCR5 genotype was determined according to previously described methods (9).

Statistical analysis

Geometric means were determined for log-normally distributed variables. Means are reported with 1 SE. Comparisons of means were done using analysis of variance; for log-normally distributed residuals, data were logged for analysis. Comparison of CXCR4 expression in CD4+ monocytes that had skewed distributions was done using the Wilcoxon 2-sample test, and the medians are reported. Spearman rank correlations were used as a measure of association between percentage of CD4 and other variables. HLA-DR expression of CXCR4+ CD4+ T cells and CCR5+CXCR4+ T cells were compared by the paired t test. The Bonferroni method of adjusting p values for multiple testing was used.

Results

Expression of CXCR4 and CCR5 on leukocyte subsets in HIV+ and HIV− individuals

Clinical and laboratory data on 25 HIV-1-infected patients in various stages of disease and on 10 uninfected control subjects are summarized in Tables I and II, respectively. FACS analysis of PBMCs within fresh whole blood specimens was performed. As expected, the level of cellular activation as measured by HLA-DR expression in both CD4+ and CD8+ T cells was significantly greater in HIV-infected patients than in HIV-uninfected control subjects (for CD4+ T cells, geometric means of 18.5% (95% CI: 14.6, 23.3) and 3.9% (95% CI: 2.9, 5.2), p = 0.03; for CD8+ T cells, geometric means of 39.3% (95% CI: 33.6, 46) and 8.9% (95% CI: 5.9, 13.6), p < 0.001, HIV-infected and HIV-uninfected individuals, respectively) (Fig. 1). CXCR4 expression in both CD4+ and CD8+ T cells and CD14+ monocytes was reduced significantly when compared with HIV-uninfected controls (Fig. 2) (for CD4+ T cells, arithmetic means of 22.8% (95% CI: 18, 27.6) and 61.2% (95% CI: 53.1, 70).
and 64% (95% CI: 52.9, 75.2), p = 0.03; for CD8+ T cells, geometric means of 10.7% (95% CI: 7.5, 15.3) and 61.1% (95% CI: 51.6, 72.4), p < 0.001; for monocytes, medians 8.9 and 68.5%, p = 0.001, for HIV-infected and HIV-uninfected individuals, respectively). Conversely, CCR5 expression on CD4+ T cells of HIV-infected individuals was increased significantly when compared with HIV-uninfected controls (Fig. 3) (arithmetic means of 13.2% (95% CI: 10.3, 16.1) expression vs 6.5% (95% CI: 4.1, 8.9), p = 0.03, in HIV-infected and HIV-uninfected individuals, respectively). However, CCR5 expression of CD8+ T cells was not significantly different between the HIV-infected and HIV-uninfected groups (arithmetic means of 28.2% (95% CI: 22.1, 34.2) vs 21.2% (95% CI: 12.7, 29.9), p > 0.5, respectively). CCR5 expression on monocytes was undetectable to low (<5%) in both study groups (data not shown). Of note was the large degree of interindividual variability of CXCR4 and CCR5 expression on lymphocytes and monocytes in both populations. The variability in CCR5 expression within the HIV-infected group could not be explained entirely by CCR5 genotype. For example, CD4+ T cells from patients 14, 16, and 24, who are heterozygotes for the 32-bp deletion of CCR5 (Δ32CCR5), showed levels of CCR5 expression of 2.7, 17, and 13.1%, respectively, which as a group were not significantly different from the mean level of CCR5 expression among CCR5 wild-type patients. The one Δ32CCR5-heterozygous HIV-uninfected control subject, however, did demonstrate the lowest levels of CCR5 expression of the control group, i.e., 1.7% of CD4+ T cells, and 0.7% of CD8+ T cells. The level of CXCR4 and CCR5 expression was stable for asymptomatic individuals who were sampled repeatedly and were followed over a 1-mo period (data not shown). A typical example of co-receptor expression in an uninfected individual is shown in Figure 4A. The most dramatic changes in co-receptor expression in vivo were observed in patient 22, who presented with an acute seroconversion illness (Fig. 4B). Both CD8+ and CD4+ T cells showed high levels of activation, as reflected by HLA-DR expression of 84 and 28%, respectively. These changes were associated with marked down-regulation of CXCR4 expression in CD8+ and CD4+ T cells (1.2 and 10%, respectively), and up-regulation of CCR5 (76 and 29%, respectively). Sampling of this patient over a 5-wk period (Fig. 5) showed a decrease in CCR5 expression on CD4+ T cells (and CD8+ T cells, data not shown) that paralleled resolution of symptoms, decrease in plasma viremia, and return of CD4+ T cell count to a new baseline. Low levels of CXCR4 expression, however, continued over the study period.

Correlation of activation state and CXCR4/CCR5 expression

We examined the levels of HLA-DR expression on CXCR4+ and CCR5-expressing CD4+ T cells (Fig. 6). Among both HIV-infected and HIV-uninfected individuals, CXCR4+CD4+ T cells had significantly lower levels of HLA-DR expression when compared with CCR5+CD4+ T cells (geometric means of 12.5% (95% CI: 7.8, 20.1) and 51.1% (95% CI: 42.1, 60.1), respectively, p < 0.001 for HIV-infected group; geometric means of 4.8% (95% CI: 3.3, 7) and 27.9% (95% CI: 22.8, 32.9), respectively, p < 0.001 for HIV-uninfected group). A representative patient (1) is shown in Figure 7, in which 38% of CCR5+CD4+ T cells also expressed HLA-DR, whereas only 5% of CXCR4+CD4+ T cells were also HLA-DR+. In addition, the level of CCR5 expression in the entire
group of patients (HIV-infected and uninfected) positively correlated with the activation state of the CD4⁺ T cells, as reflected by HLA-DR expression, whereas the level of CXCR4 expression negatively correlated with HLA-DR on CD4⁺ T cells (data not shown).

Correlation of CXCR4 and CCR5 expression with disease stage and viral phenotype

The degree of CD4⁺ T cell activation as measured by HLA-DR expression directly correlated with advanced disease \((r = 0.65, p = 0.001)\) (Fig. 8A). In addition, there was a significant correlation between disease stage and CXCR4 expression of CD4⁺ T cells, with healthier patients expressing more CXCR4 \((r = 0.45, p = 0.05)\) (Fig. 8B). A significant correlation was observed between CCR5 expression of CD4⁺ T cells and disease stage, with higher levels expressed on more advanced patients \((p = 0.5, p = 0.02)\) (Fig. 8C). The degree of activation of CCR5⁺CD4⁺ T cells correlated with disease stage, with more advanced patients having a greater proportion of CCR5⁺CD4⁺ T cells expressing HLA-DR \((r = -0.60, p = 0.04)\) (Fig. 8D). A similar trend of borderline statistical significance was observed with CXCR4⁺CD4⁺ T cells in which a higher proportion expressed HLA-DR in patients with more advanced disease \((r = -0.54, p = 0.09)\) (Fig. 8E). In particular, patients 23 and 25 showed 53.5 and 83.2% of CXCR4⁺CD4⁺ cells to be activated. However, this amount of activation was not observed in all late stage patients (for example, patient 24). Similarly, a correlation between levels of plasma viremia and degree of down-regulation of CXCR4 and up-regulation of CCR5 was observed in the untreated patients (data not shown).

The level of chemokine receptor expression in our HIV-infected patients was evaluated in relationship to virus phenotype. We identified six HIV-infected patients who had SI viruses isolated in culture (see Tables I and II). We did not observe statistically significant differences in the levels of CXCR4, CCR5, or HLA-DR expression in those patients with SI viruses as compared with those patients in which we could only isolate NSI viruses (data not shown).
Discussion

The chemokine receptors CCR5 and CXCR4 play important roles in the immunopathogenesis of HIV-1 infection (26). Early in infection, macrophage-tropic (generally NSI) viruses predominate even in individuals exposed to mixtures of SI and NSI viruses (12, 14, 27). In addition, individual homozygotes for Δ32CCR5 are resistant to HIV-1 infection (7–9, 28–30), with few exceptions (7, 31, 32). Thus, usage of CCR5 by HIV-1 appears to be critical for the maintenance of a successful infection in susceptible individuals. Primary isolates of HIV-1 that use CXCR4, although rapidly growing in vitro, emerge later in the course of infection in approximately 50% of infected individuals (33). The selective forces preventing the emergence of CXCR4-using viruses early in HIV-1 infection are unknown. We evaluated the levels of CCR5 and CXCR4 expression in HIV-1-infected and uninfected individuals to identify trends that may help explain the predominance of macrophage-tropic over T cell line-tropic viruses, particularly during the earlier stages of disease. Using three-color flow cytometry of whole blood specimens, we found that HIV-1-infected individuals had a significantly greater percentage of CCR5-expressing CD4+ T cells compared with normal controls, and marked down-regulation of CXCR4 on their CD4+ and CD8+ T cells, and CD14+ monocytes. These changes were directly correlated with the activation state of the cell, with CXCR4+ being expressed predominantly on quiescent (HLA-DR−) cells and CCR5 being expressed predominantly on activated (HLA-DR+) cells. We could most dramatically demonstrate this finding in patient 22, who experienced an acute HIV syndrome and had both the highest levels of in vivo CCR5 expression (70–80% of CD8+ T cells, and 30–40% of CD4+ T cells) and lowest levels of CXCR4 expression (10% of CD4+ T cells, and 0.3–1% of CD8+ T cells) of the entire group. In addition, the level of activation of CD4+ T cells, the down-modulation of CXCR4, and the up-regulation of CCR5 correlated with advancing disease, which further supports the reciprocal role that CXCR4 and CCR5 play in cellular activation (15, 16).

It is unclear whether the observed changes in coreceptor expression in HIV infection are specific to HIV infection and replication or whether they are purely a secondary consequence of broad immune activation. CXCR4 and CCR5 expression have been shown to be differentially regulated in vitro depending on the activation stimulus (16). PHA was shown to down-modulate CCR5 and up-regulate CXCR4, whereas a more physiologic stimulus, such as prolonged culture in IL-2 with or without anti-CD3 stimulation, was shown to enhance CCR5 and diminish CXCR4 expression.

![Figure 8](http://www.jimmunol.org/)

**FIGURE 8.** Correlation between stage of HIV-1 disease, as described by percentage of CD4 (horizontal axis), and A, CD4+ T cell activation ($r = -0.65$, $p = 0.001$), B, CXCR4 expression on CD4+ T cells ($r = 0.45$, $p = 0.05$), C, CCR5 expression on CD4+ T cells ($r = -0.50$, $p = 0.02$), D, activation of CCR5+CD4+ T cells ($r = -0.60$, $p = 0.04$), and E, activation of CXCR4+CD4+ T cells ($r = -0.54$, $p = 0.09$).
We have similarly studied patients with other forms of chronic immune activation, such as asymptomatic chronic hepatitis C virus infection, and have observed up-regulation of CCR5, but normal to increased CXCR4 expression in CD4⁺ T cells (data not shown). Thus, our finding of decreased CXCR4 expression during acute and chronic HIV infection suggests that these changes may not be a secondary consequence of broad immune activation, but may be relatively specific to HIV infection. Further evaluation of individuals with other diseases will be necessary to fully evaluate the spectrum of chemokine receptor expression related to various forms of acute and chronic activation.

We found the level of coreceptor expression to be stable within asymptomatic individual HIV-infected and uninfected individuals when tested over multiple time points over a 2-mo period. However, a wide range of variability was noted among individuals, even among the three HIV-infected individuals who were heterozygous for the Δ32CCR5 allele, as similarly reported by Wu et al. in HIV-1-uninfected individuals (15). This implies that surface expression of CCR5 and CXCR4 among individuals is complex and most likely regulated by multiple factors.

Bleul and others (16) have shown that CXCR4 is expressed predominantly on naive T cells, and CCR5 on previously activated memory cells. The dual expression of CCR5 and HLA-DR in CD4⁺ T cells of HIV-infected as well as uninfected individuals in our study is consistent with CCR5 being expressed on a subpopulation of memory T cells at a later stage of activation (i.e., CD69⁺, HLA-DR⁺). Thus, the increase in the state of activation of CD4⁺ T cells in HIV-infected individuals would enhance the efficiency of virus replication in general, while the increased expression of CCR5 together with little or no CXCR4 expression on this population of activated CD4⁺ T cells would theoretically favor the replication of macrophage-tropic as opposed to T cell line-tropic virus. In contrast, naive CD4⁺ T cells express abundant CXCR4, which would theoretically favor entry of T cell line-tropic HIV-1; however, recent studies have demonstrated that the relatively quiescent metabolic state of these cells does not support efficient viral replication (34, 35).

The mechanisms responsible for the emergence of SI (CXCR4-using) viruses during HIV disease are unknown (11, 33). It is possible that the level of CXCR4 and CCR5 expression on activated CD4⁺ cells may influence coreceptor usage by HIV-1. For example, a failure to down-regulate CXCR4 on activated CD4⁺ T cells may favor usage of this receptor by HIV. It is noteworthy that in our study the proportion of CXCR4⁺, CD4⁺ T cells with an activated phenotype (HLA-DR⁺) tended to increase in some patients with advanced disease. Thus, the possibility that a significant number of activated cells dually expressing both CXCR4 and CCR5 could favor conditions for a broader range of coreceptor usage by HIV-1. We, however, could not distinguish patients harboring SI viruses from those with only NSI viruses based on the levels of CXCR4, CCR5, or HLA-DR on CD4⁺ T cells. In this regard, the relatively small number of patients studied may have precluded a

### Table I. Clinical characteristics of HIV-infected individuals

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<th>Patient no.</th>
<th>Clinical</th>
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<th>CD4%</th>
<th>CD4/µl</th>
<th>RX</th>
<th>HIV-1 Viremia (copies/ml)</th>
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<td>11,000,000 NSI</td>
<td>SI</td>
</tr>
<tr>
<td>23</td>
<td>INFX</td>
<td>WT</td>
<td>9.8</td>
<td>31</td>
<td>HAART</td>
<td>&lt;500 SI</td>
<td>SI</td>
</tr>
<tr>
<td>24</td>
<td>ASX</td>
<td>HZ</td>
<td>4.9</td>
<td>21</td>
<td>HAART</td>
<td>267,400 SI</td>
<td>SI</td>
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<td>WT</td>
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<td>59</td>
<td>HAART</td>
<td>507,000 NSI</td>
<td>SI</td>
</tr>
</tbody>
</table>

* ASX, asymptomatic; ASX REC, asymptomatic recently infected; ACUTE SX SERO, acute asymptomatic seroconversion illness INFX, chronic bacterial sinusitis in pt 23; WT, wild type; HZ, heterozygote; HAART, highly active antiretroviral therapy; NA, not available; NSI, nonsyncytia inducing; SI, syncytia-inducing by MT2 assay.
sufficient analysis of this question. In addition, longitudinal studies of HIV-infected patients will likely be necessary to definitively show whether changes in coreceptor expression within a given individual during the course of HIV infection correlate with a change in viral phenotype in vivo.

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References