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Peripheral Blood-Derived CD34⁺ Progenitor Cells: CXC Chemokine Receptor 4 and CC Chemokine Receptor 5 Expression and Infection by HIV

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The present study demonstrates cell surface expression of both CXC chemokine receptor 4 (CXCR4) and CC chemokine receptor 5 (CCR5), major coreceptors for T cell-tropic and macrophage-tropic strains of HIV, respectively, on CD34⁺ progenitor cells derived from the peripheral blood. CD34⁺ progenitor cells were susceptible to infection by diverse strains of HIV, and infection could be sustained for prolonged periods in vitro. HIV entry into CD34⁺ progenitor cells could be modulated by soluble CD4, HIV gp120 third variable loop neutralizing mAb and the cognate ligands for the CXCR4 and CCR5 HIV coreceptors. This study suggests that a significant proportion of the circulating progenitor cell pool may serve as a reservoir for HIV that is capable of trafficking the virus to diverse anatomic compartments. Furthermore, the infection and ultimate destruction of these progenitor cells may explain in part the defective lymphopoiesis in certain HIV-infected individuals despite effective control of virus replication during highly active antiretroviral therapy. The Journal of Immunology, 1998, 161: 4169–4176.

The establishment of productive HIV infection in CD34⁺ progenitor cells and the ability of these cells to serve as an in vivo reservoir have been controversial. A number of studies in HIV-infected individuals have failed to detect productively infected CD34⁺ progenitor cells from bone marrow (1–5); however, other studies have shown that rare infection of CD34⁺ progenitor cells can occur (6–8) and may be more prevalent in a subset of HIV-infected patients with advanced disease (9). HIV infection in vitro has been reported in highly purified bone marrow-derived CD34⁺ cells (10) and in CD34⁺ progenitor cells that coexpress CD4 (11). In this regard, the CD4 molecule that binds with high affinity to HIV gp120 is expressed on a minor population of CD34⁺ progenitor cells (11–14). Infection of host cells by HIV is determined by viral envelope binding to the CD4 molecule (15, 16) together with binding to a chemokine coreceptor such as CXCR4² or CCR5, which facilitate fusion and entry of T cell (T)-tropic and macrophage (M)-tropic HIV strains, respectively (17–23). T-tropic strains of HIV-1 have been shown to infect cultures of purified CD34⁺ progenitor cells in vitro, suggesting the presence of an HIV coreceptor similar or identical with CXCR4 (24–27). Expression of CXCR4 and CCR5 mRNA in granulocyte CSF-mobilized progenitor cells has been recently reported (28); however, surface expression of CXCR4 and CCR5 chemokine receptors on CD34⁺ progenitor cells obtained from either medullary or extramedullary sites has not been demonstrated. Furthermore, susceptibility to HIV infection in peripheral blood-derived CD34⁺ progenitor cells has not been well characterized, nor has the role of these coreceptors in progenitor cell infection by HIV-1 been defined. Peripheral blood-derived CD34⁺ progenitor cells are capable of seeding extramedullary sites of lymphopoiesis (29–31) and, if infected, may serve to disseminate HIV into diverse anatomic sites. Defining the susceptibility to HIV infection specifically in peripheral blood-derived CD34⁺ progenitor cells is relevant not only to understanding the pathogenesis of HIV disease but also to addressing the potential for immune reconstitution. We therefore investigated the expression of the HIV coreceptors CXCR4 and CCR5 on peripheral blood-derived CD34⁺ progenitor cells and determined the susceptibility of these cells to infection by different strains of HIV.

Materials and Methods

Cytokines and chemokines

Recombinant human IL-3 and IL-6 (Stem Cell Technologies, Vancouver, Canada) were used at a final concentration of 25 ng/ml. Recombinant human stem cell factor (Sigma, St. Louis, MO) was used at a final concentration of 100 ng/ml. RANTES and macrophage inflammatory protein-1β (MIP-1β; PeproTech, Rocky Hill, NJ) were used at a final concentration of 200 ng/ml. Stromal-derived factor-1α (SDF-1α) (a gift from Upstate Biotechnology, Lake Placid, NY) was used at 1 μg/ml.

HIV viral isolates

Viral isolates used for infection of progenitor cells included pellet-purified HIV-1 strains NL4.3 (AIDS Research and Reference Reagent Program), IIIB (Advanced Biotechnologies, Columbia, MD), Ba-L (Advanced Biotechnologies), and MN (Advanced Biotechnologies) and JR-FL, JR-csf HIV-1 supernatants (AIDS Research and Reference Reagent Program). Infections were performed at a multiplicity of infection of 0.005 using viral particle count for pellet purified strains.

Hemopoietic progenitor cell isolation

PBMC were obtained by Ficoll-Hypaque density centrifugation. T cells were depleted using neuraminidase (Sigma)-treated SRBC agglutination.

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2 Abbreviations used in this paper: CXCR4, CXC chemokine receptor 4; CCR5, CC chemokine receptor 5; T-tropic, T cell-tropic; M-tropic, macrophage-tropic; MIF, macrophage inflammatory protein; SDF, stromal-derived factor; PE, phycoerythrin; LTR, long terminal repeat; V3, third variable loop; sCD4, soluble CD4.

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followed by a second Ficoll-Hypaque centrifugation. Monocytes and macrophages were depleted by overnight adherence on plastic flasks at 37°C in a 5% CO₂ incubator. The remaining progenitor cell-enriched fraction was positively immunoselected for CD34+ cells by passage over a magnetic column (CD34 isolation kit, Miltenyi Biotec, Sunnyvale, CA). CD34+ cells were placed in DMEM/Isco’s medium at a 1:1 ratio supplemented with 10% FCS, 5 μg/ml bovine insulin (Sigma), 0.8 mM sodium pyruvate (Life Technologies, Grand Island, NY), 2 μM glutamine, 7 mM HEPES (Life Technologies), 50 U/ml penicillin and 50 μg/ml streptomycin (Life Technologies), and cytokines, including recombinant human IL-3 and IL-6 (Stem Cell Technologies), each at a final concentration of 25 ng/ml and recombinant human stem cell factor (Sigma) at a final concentration of 100 ng/ml (complete medium). Cultures were maintained with biweekly 50% replenishment of complete medium and were incubated at 37°C in a fully humidified atmosphere containing 5% CO₂.

**Phenotypic analysis of progenitor cells**

Aliquots of cells were subjected to three-channel fluorometric analysis 12 to 24 h following the final progenitor cell purification to allow detection of the CD34 mAb used for selection. The expression of cell surface Ag was determined by flow cytometry using a Coulter Elite ELITE (Coulter, Hialeah, FL). The cells were washed, resuspended in PBS containing 10% FCS, and mixed with optimal concentrations of mAb. A panel of mAbs was used to assess progenitor cell purity; this panel included CD3 (clone HIT3a), CD4 (clone Q4120), CD8 (clone Leu 2a), CD14 (clone Leu M3), CD16 (clone Leu 11c), CD20 (clone Leu 16), CD33 (clone Leu M9), CD34 (clone HPCA-2), CD35 (clone E11), CD38 (clone Leu 17), CD45 (clone HLE-1), CD56 (clone Leu 19), CD57 (clone cv1.1/hnk), and CD90w (clone SE10; Becton Dickinson, San Jose, CA). A range of 5 × 10⁶ to 1 × 10⁷ events were collected per sample. CD34+ progenitor cells stained with murine keyhole limpet hemocyanin mAb conjugated to IgG1 FITC and IgG1 phycoerythrin (PE) were used to set the positive gate.

**RT assay**

Progenitor cell cultures were maintained up to 40 days in vitro following infection with different strains of HIV-1. Progenitor cell supernatants were harvested at serial intervals and assessed for HIV replication by RT assay (32, 33).

**Semiquantitative PCR assay for HIV-1 early LTR transcripts**

Aliquots of purified CD34+ progenitor cells (5–10 × 10⁶ cells/tube) were pretreated for 30 min with a panel of chemokines or receptor antagonists in parallel sets that were subsequently infected with different strains of HIV-1 pretreated with RNAse-free DNase (Boehringer Mannheim, Indianapolis, IN) or sCD4. Cells were incubated at 37°C in 5% CO₂, and serial harvests were collected at 0, 8, and 18 h postinfection. Cells were washed three times with PBS and pelletted before freezing at −80°C. Pellets were thawed and resuspended in lysis buffer (0.6 mg/ml proteinase K (Life Technologies, St. Louis, MO)). Samples were denatured at 94°C for 5 min, then hybridized at 56°C for 10 min with a probe labeled internal LTR probe (Boehringer Mannheim, Indianapolis, IN) before use in colocalization studies with CD34+ mAb. Non-specific fluorescence was determined by staining with an irrelevant murine isotype-specific control or a secondary mAb alone.

**Laser scanning confocal fluorescence microscopy**

Laser scanning confocal fluorescence microscopy was performed using a Zeiss LSM 410 scanning laser confocal microscope system (Zeiss, Thornwood, NY) built around a Zeiss 135 Axiovert inverted scope fitted with an Omnicon argon/krypton dual gas laser set to emit laser lines at 488, 568, and/or 647 nm. Oregon green and FITC images were recorded using a 488-nm argon excitation and broad band pass emission filter of 515 to 525 nm. PE images were recorded with a 488/568-nm argon excitation and a broad band pass emission filter of 580 to 640 nm. Brightness and contrast were set on appropriate positive control samples to obtain a full 8-bit gray scale rendering of each image. Negative controls were subsequently recorded at these same settings. Images were obtained using either a Zeiss ×40 Achromplan 0.60 Korr Ph2 objective or a Zeiss ×63 oil/NA = 1.25 Neofluor objective. Image processing with signal filtering and digital contrast enhancement were performed using Zeiss LSM 410 (version 3.8) software.

**Statistical analysis**

Comparison of mean values was performed using the Mann-Whitney U test.

**Results**

**CXCR4, CCR5, and CD4 surface expression on CD34+ progenitor cells**

PBMC obtained from normal donors (n = 7) and enriched for CD34+ progenitor cells demonstrated surface expression of CXCR4 and CCR5 (Fig. 1, top panel). CXCR4 and CCR5 expression was found on 78 ± 13 and 62.5 ± 23%, respectively, of the CD34+ CD38+ progenitor cells derived from peripheral blood (Table I). CD34+ progenitor cells that coexpressed CD4 (19.3 ±

**FIGURE 1.** Phenotypic expression of CXCR4 and CCR5 on peripheral blood-derived CD34+ CD38+ and CD34+ CD4+ progenitor cells. Surface expression of CXCR4 and CCR5 receptors in CD34+ CD38+ progenitor cells (top panel) and CD34+ CD4+ progenitor cells (lower panel) as demonstrated by FACS in PBMC following Ficoll-Hypaque centrifugation and a single round of CD34-positive immunoselection. Further depletion was not performed for this analysis.
9.9%; data not shown) demonstrated a similar percentage expression of CXCR4 and CCR5 (Fig. 1, lower panel) as did CD34\(^+\)CD38\(^-\) progenitor cells.

**Primitive progenitor cells express CXCR4 and CCR5**

The CD4 molecule is expressed on a minor population of CD34\(^+\) progenitor cells (Fig. 1) (11–13). Progenitor cells that lack lineage-specific markers and coexpress CD4 and CD90 (Thy-1) phenotypically define primitive totipotent and multipotent (prelymphoid) CD34\(^+\) progenitor subsets (4, 29, 31). CXCR4 and CCR5 expression was higher in CD34\(^+\) progenitor cells that coexpressed CD90 (Thy-1) than in CD34\(^+\)CD38\(^-\) lineage-committed progenitor cells. CXCR4 expression in the CD34\(^+\)CD90\(^-\) (Thy-1) subset was 96 ± 4\% compared with 78 ± 13\% among CD34\(^+\)CD38\(^-\) progenitor cells.

**FIGURE 2.** Immunocytochemistry using laser scanning confocal microscopy. Photomicrographs of purified CD34\(^+\) progenitor cells taken with a Zeiss \(\times 63\) oil/NA1.25 Neofluor objective. a, Progenitor cell stained with anti-CD34-PE (red). b, The same progenitor cell stained with anti-CCR5 mAb (clone 5C7)-Oregon green (green). c, Colocalization of the CCR5 receptors (green) and CD34 sialomucin receptor (red) is visualized in gold (spectral overlay of red plus green = gold). The CCR5 chemokine receptor displays distinct hemopoietic vesicle caveola-like morphology noted by the arrow. Zoom, \(\times 8\). d, Progenitor cell stained with anti-CD34-PE (red). e, Progenitor cell stained with anti-CXCR4 mAb (clone 12G5)-Oregon green (green). f, Colocalization of the CXCR4 receptors (green) and CD34 sialomucin receptor (red) is visualized in gold. Zoom, \(\times 4\). g, The majority of CD34\(^+\) progenitor cells express both CCR5 (green) and CXCR4 (red), which appear as gold when colocalized. h, The distribution of chemokine receptor expression is variable for each coreceptor among CD34\(^+\) progenitor cells. Zoom, \(\times 3\).
HIV replication, as determined by mean RT activity (Fig. 3, A), increases over time and is independent of the infection process (Table I). CCR5 expression in the CD34+ CD90+ (Thy-1) subset was 100 ± 0% compared with 62 ± 23% among CD34+ CD38+ progenitor cells (p = 0.01; Table I).

**Immunohistochemistry for detection of surface expression of CCR5 and CXCR4 coreceptors**

The CD34 sialomucin receptor is one of several adhesins involved in the intra- and extramural homing of progenitor cells into distinct microenvironments (35–37). Chemokines function similarly in their ability to direct leukocyte migration (38). Confocal microscopy was performed to analyze the distribution of chemokine receptors among CD34+ progenitor cells. High power photomicrographs (Fig. 2) illustrate the discrete distribution of CD34 and chemokine receptor staining on an individual progenitor cell (Fig. 2, a–c). A progenitor cell stained with anti-CD34-PE (Fig. 2a, red staining) and anti-CCR5 mAb-Oregon green (Fig. 2b, green staining) demonstrates the expression of CCR5 localized to hemopoietic vesicles that were organized singly or in clusters at the plasma membrane (39, 40) (Fig. 2c, arrow). CCR5 expression appeared diffusely colocalized, focally colocalized, or independent of CD34. Discrete areas of CD34 and CCR5 colocalization were visualized along ruffled folds of the cell membrane (Fig. 2c, red plus green = gold). In contrast, a progenitor cell stained with anti-CD34-PE (Fig. 2d, red staining) and anti-CXCR4 mAb-Oregon green (Fig. 2e, green staining) demonstrates that CXCR4 expression was distributed basolaterally and exhibited less colocalization with CD34 (Fig. 2f, red plus green = gold). Photomicrographs (Fig. 2, g, low power, and h, zoom 3) illustrate CXCR4 and CCR5 receptor expression among CD34+ progenitor cells. The majority of progenitor cells expressed both receptors (Fig. 2g, visualized as CXCR4 (red), CCR5 (green), or both receptors colocalized (gold)); however, there was a heterogeneous distribution (Fig. 2h) among subsets within the progenitor cell population appreciable as different densities of each coreceptor.

**Peripheral blood-derived progenitor cells can be infected in vitro by diverse strains of HIV-1**

Peripheral blood-derived CD34+ progenitor cells were cultured in the presence of M-tropic (BaL, JR-fl, JR-csf), T-tropic (NL4.3), T cell line-adapted (IIIB), or dual tropic (MN) HIV-1 viral strains and maintained in vitro for up to 40 days. FACS analysis performed on cells harvested after 1 wk of in vitro culture with different strains of HIV demonstrated that cultures exposed to virus maintained a lower mean percentage of CD34+ expressing cells compared with control cultures (data not shown).

Highly purified progenitor cells with <1% contamination by CD3+, CD16+, or CD57+ cells (n = 5) all demonstrated active HIV replication, as determined by mean RT activity (Fig. 3, A and B). RT activity could be detected as early as day 6 in both cell suspension and adherent cell supernatants (data not shown). RT activity was detected throughout the in vitro culture period (>30 days) for all HIV-1 strains. Similar results were obtained in five independent experiments.

**HIV entry in CD34+ progenitor cells can be modulated by anti-gp120 neutralizing mAb**

HIV entry is dependent not only on surface expression of CD4, but also on the expression of certain chemokine coreceptors, such as CXCR4 and CCR5 (17–23). The third variable loop (V3) of HIV gp120 facilitates coreceptor binding, potentially through multiple conformational domains (41–43). To address whether HIV-1 infection in progenitor cells was dependent on the V3 region of gp120, two different neutralizing V3 loop mAb were employed in neutralization studies. M-tropic (BaL) and several T-tropic HIV-1 strains were pretreated with either neutralizing mAb 178.1 or mAb F1.9B that bind to epitopes within the V3 loop, but that do not interfere with HIV gp120 binding to the CD4 receptor (44–46). The mAb 178.1 specifically binds the V3 loop of T-tropic strains of HIV and pretreatment with mAb 178.1 inhibits the infection of Tropic strains in mature CD4+ T cells (46). In contrast, mAb

**FIGURE 4.** Neutralization of infection of CD34+ progenitor cells and mature CD4+ T cells by anti-HIV gp120 V3 loop mAb. Pretreatment of HIV by anti-gp120 V3 loop mAb reduced HIV-1 early viral transcripts in CD34+ progenitor cells and mature CD4+ T cells, F1.9B mAb reduced BaL entry and early LTR transcripts, and 178.1 mAb reduced NL4.3 entry and early LTR transcripts into progenitor cells and mature T cells.
shown). Mature CD4 chemokine receptor reduced the level of early HIV-1 transcripts. The sCD4 cells can be modulated by CC chemokines and SDF-1 or inhibited with these receptors by their respective ligands. To address whether density, chemokine coreceptor expression, or the modulation of progenitor cells due to differences in CD4 receptor strains of HIV; however, a viral phenotypic preference may pre-

The V3 loop of HIV-1 gp-120 of HIV-1 is a key determinant for

FIGURE 5. HIV entry and early LTR transcripts in CD34+ progenitor cells can be modulated by CC chemokines and SDF-1 or inhibited with sCD4. Pretreatment of progenitor cells with the cognate ligand for each chemokine receptor reduced the level of early HIV-1 transcripts. The sCD4 reduced the level of early HIV-1 transcripts in CD34+ progenitor cells.

F1.9B has been shown to bind specifically to the V3 loop of M-tropic strains of HIV (44), and pretreatment with mAb F1.9B inhibits the infection of M-tropic strains in mature CD4+ T cells. Mature CD4+ T cells pretreated with neutralizing mAb F1.9B and exposed to the M-tropic BaL strain demonstrated a >80% reduction in the level of early HIV-1 LTR transcripts compared with T cells exposed to BaL, with no mAb pretreatment or with pretreatment with an isotype control mAb (Fig. 4). Pretreatment of BaL with neutralizing mAb 178.1 had no significant effect on the level of BaL early LTR transcripts in mature CD4+ T cells (data not shown). Mature CD4+ T cells exposed to the T-tropic NL4.3 strain pretreated with neutralizing mAb 178.1 demonstrated a >90% reduction in the level of early HIV-1 LTR transcripts compared with T cells exposed to NL4.3 receiving no mAb pretreatment or after pretreatment with an isotype control mAb (Fig. 4). Pretreatment of NL4.3 with neutralizing mAb F1.9B had no significant effect on the level of NL4.3 early LTR transcripts in mature CD4+ T cells (data not shown). Isoytype-specific mAb were included to evaluate potential effects due to Fc binding on progenitor cells. Progenitor cells exposed to M-tropic BaL or T-tropic strains pretreated with neutralizing mAb F1.9B or mAb 178.1, respectively, demonstrated a significant reduction in the level of early HIV-1 LTR transcripts compared with CD34+ progenitor cells exposed to virus pretreated with an isotype control mAb or exposed to virus alone (Fig. 4). The observed decrease in early HIV LTR transcripts in the presence of isotype-specific mAb is consistent with the expression of Fc receptors on progenitor cells (29, 31).

HIV entry in CD34+ progenitor cells can be modulated by sCD4, SDF-1α, and CC chemokines

The V3 loop of HIV-1 gp-120 of HIV-1 is a key determinant for defining host cell tropism (41–43, 47, 48). Cells that express CD4, CXCR4, and CCR5 may be permissive for viral entry by diverse strains of HIV; however, a viral phenotypic preference may predominate in progenitor cells due to differences in CD4 receptor density, chemokine coreceptor expression, or the modulation of these receptors by their respective ligands. To address whether HIV infection of CD34+ progenitor cells required the presence of cell surface CD4 molecules, inhibition studies with sCD4 were performed. Pretreatment of M-tropic BaL and T-tropic NL4.3 HIV strains with sCD4 inhibited viral entry into CD34+ progenitor cells more efficiently compared with mature T cells (Fig. 5).

To address whether T-tropic HIV strains use the CXCR4 chemokine receptor as a coreceptor for viral entry into CD34+ progenitor cells, SDF-1α, the natural ligand for CXCR4, was added to progenitor cell cultures to prevent binding by HIV gp120 (49–52). The mean channel CXCR4 density on progenitor cells is less than that on mature T cells (M. Ruiz, M. Ostrowski, and A. Kinter, unpublished observations); therefore, a suboptimal dose of SDF-1α was used. Progenitor cells treated with 1 μg/ml of SDF-1α before exposure to the T-tropic NL4.3 strain demonstrated a 70% reduction in the level of early LTR transcripts (Fig. 5) compared with that in cells not receiving SDF-1α pretreatment (Fig. 5). In contrast, treatment of mature T cells with 1 μg/ml SDF-1α resulted in only a 10% reduction in NL4.3 early LTR transcripts compared with that in untreated cells (Fig. 5).

Infection by HIV strains that use CCR5 as a coreceptor for viral entry may be inhibited in the presence of CCR5 agonists due to competition for receptor occupancy or receptor down-modulation (53). To address whether M-tropic HIV strains use the CCR5 chemokine receptor as a coreceptor for viral entry into progenitor cells, progenitor cells and mature CD4+ T cells derived from the same donor were pretreated with RANTES or MIP-1β (19, 23, 42). Mature T cells pretreated with RANTES demonstrated a 54% reduction in the level of early HIV-1 LTR transcripts compared with that in untreated controls when exposed to the M-tropic BaL strain; progenitor cells pretreated with RANTES and exposed to the M-tropic BaL strain demonstrated an 81% reduction in the level of early HIV-1 LTR transcripts compared with that in untreated controls when exposed to the M-tropic BaL strain. Similarly, progenitor cells pretreated with MIP-1β and exposed to the M-tropic BaL strain demonstrated a 94% reduction in the level of early HIV-1 LTR transcripts compared with that in untreated controls (Fig. 5).

Discussion

Different chemokine receptors may predominate in specific tissue microenvironments; however, the majority of HIV-1 isolates efficiently use CXCR4 and/or CCR5 as primary entry coreceptors; alternative chemokine receptors may be used less efficiently. The present study was designed to determine whether CD34+ progenitor cells express the same chemokine receptors that are known to facilitate the fusion and entry of HIV-1 into mature CD4+ T cells. The present study demonstrates the expression of chemokine receptors CXCR4 and CCR5, the primary T-tropic and M-tropic HIV-1 coreceptors, respectively, on peripheral blood derived-CD34+ progenitor cells analyzed immediately following isolation. The majority of these CD34+ progenitor cells expressed both CXCR4 and CCR5, although different patterns of coreceptor expression could be appreciated by FACS and confocal microscopy. Peripheral blood-derived CD34+ progenitor cells were capable of sustaining a prolonged productive infection by diverse strains of HIV-1. HIV entry into progenitor cells could be modulated by soluble CD4 and HIV gp120 V3 loop neutralizing mAb, demonstrating that infection was dependent on CD4, a coreceptor, and the V3 loop of the HIV envelope in a manner similar to HIV infection of mature mononuclear cells. The utilization of CXCR4 and CCR5 as entry cofactors by T-tropic and M-tropic HIV strains was demonstrated by a reduction of viral entry into progenitor cells in the presence of the cognate ligand for each chemokine coreceptor.
These data suggest that the circulating CD34+ progenitor cell population may be infected in vivo and may serve as a dynamic reservoir for HIV that is capable of disseminating virus to diverse anatomic sites.

The susceptibility of host cells to infection by HIV is determined not only by surface expression of the CD4 molecule, but also by the surface expression of various chemokine receptors that are required for fusion and entry of virus into its target cells (15–23). Deichmann et al. (28) have recently demonstrated the presence of mRNA for both CXCR4 and CCR5 in granulocyte CSF-mobilized CD34+ progenitor cells; however, cell surface expression of CXCR4 and CCR5 on CD34+ progenitor cells has not been previously determined. The present study demonstrates cell surface expression of CXCR4 and CCR5 on peripheral blood-derived CD34+ progenitor cells. A lower percentage of CD34+ CD38− lineage-committed cells express both coreceptors compared with the multipotent CD34+ CD90 (Thy-1)+ progenitor cells as determined by FACS analysis, suggesting that coreceptor expression may vary during differentiation. The high percentage of HIV coreceptor expression in the multipotent CD34+ CD90 (Thy-1)+ progenitor subset may result in an increased susceptibility to HIV infection. In this regard, select CD4+ subsets are altered in the bone marrow during the course of HIV infection; the proportion of nonlineage-committed primitive progenitor cells (CD34+ CD38− CD4+ CD90+/−) is significantly decreased, while the proportion of lineage-committed (CD34+ CD38+ CD4+) progenitor cells is maintained (4). Direct infection of the primitive progenitor compartment, which represents a minor percentage (0.01%) of bone marrow cells, is difficult to detect. Depletion of primitive progenitors observed in later stages of HIV disease may represent a virally induced alteration in progenitor cell differentiation (54–63) or may be due to exhaustion of prelymphoid progenitors that are mobilized to sites of extramedullary lymphopoiesis.

Morphologically, we observed that the distribution of each coreceptor on CD34+ progenitor cells was distinct, as demonstrated by confocal microscopy; this may indicate a differentiation-dependent or lineage-specific effect on expression. Colocalization of CXCR4 and CCR5 could be appreciated to varying degrees among the progenitor cell population. These data demonstrate that chemokine receptor expression may be dependent not only on the progenitor cell source as derived from the medullary (64) (M. Ruiz, unpublished observations) or vascular compartment, but also on the state of maturation and/or lineage commitment of the progenitor cells.

In this study, efficient viral entry occurred in mature CD4+ T cells and in CD34+ progenitor cells. The expression of a sufficient density of CD4 molecules on the host cell surface in addition to recruitable CXCR4 or CCR5 coreceptors are important variables that define the efficiency of viral fusion and entry into host cells. A minor population (range, 10–40%) of CD34+ progenitor cells express a low density of surface CD4 molecules, equivalent to 5% of the number of CD4 molecules found on mature T cells (this report and Refs. 11–13). A recent report (64) demonstrated that all CD34+ bone marrow progenitors express CD4 mRNA as well as CXCR4 and CCR5 mRNAs. The precise stoichiometry between CD4 and chemokine receptors that is necessary for the establishment of productive infection at the single cell level is not known. In this regard, it has been shown that infection of mature CD4+ T cells by M-tropic strains of HIV-1 is dependent on the density of CD4 molecules on the cell surface. Cells that express low or high amounts of CD4 are equally infectable when CCR5 concentrations are above threshold levels for maximal infection; however, infection becomes dependent on coreceptor expression levels when CD4 expression is low. Therefore, a high chemokine receptor density can compensate for low surface expression of CD4 in mediating HIV-1 infection. In contrast, infection of mature CD4+ T cells by dual tropic and T-tropic strains of HIV-1 is less dependent on the density of CD4 molecules on the surface of the cell (65). The selective expression of chemokine receptors and their local surface membrane association with CD4 receptors in CD34+ progenitor cells may influence the susceptibility of certain CD34+ subpopulations to HIV-1 infection in a strain-specific manner. In this study we have demonstrated that the majority of CD34+ progenitor cells derived from peripheral blood express both CXCR4 and CCR5 receptors and are susceptible to infection by M-tropic, dual tropic, T-tropic, and T cell laboratory-adapted strains of HIV and produced very high levels of virus (>10,000 cpn/μl mean RT activity). Unlike highly activated mature CD4+ T cells, CD34+ progenitor cells produced high levels of virus for a prolonged period of culture (>30 days), suggesting that progenitor cells may serve as a relatively stable source of HIV once a productive infection becomes established.

In the present study the primary targets for HIV infection appear to be the CD34+ CD4+ progenitor cells, since treatment with sCD4 effectively suppressed entry of all HIV-1 strains; however, the potential infection of CD34+ CD4− progenitor cells in vivo cannot be excluded. Abs directed against either the M- or T-tropic specific envelope V3 epitopes effectively inhibited HIV-1 infection in mature CD4+ T cells and in CD34+ progenitor cells, indicating that the interaction of these V3 epitopes with CD4/CCR5 or CD4/CXCR4 on progenitor cells is similar in both cell populations.

It has previously been demonstrated that the CCR5 ligands MIP-1α, MIP-1β, and RANTES, and the CXCR4 ligand SDF-1 potentially suppress M- and T-tropic HIV entry and replication, respectively, in mature CD4+ T cells; however, the effects of chemokines on viral entry or replication in cells expressing low levels of CD4, as in dendritic cells or macrophages, is less evident (66). The effect of chemokines on HIV infection of CD34+ progenitor cells, which also express low levels of CD4, had not been previously delineated. HIV-1 entry and early replication events (early LTR transcripts) of the M-tropic BaL strain were sensitive to inhibition by the CCR5 ligands, RANTES and MIP-1β, in CD34+ progenitor cells and in mature CD4+ T cells. However, early replication events of the T-tropic NL4.3 strain in CD34+ progenitor cells appeared to be more sensitive to inhibition with low concentrations of the CXCR4 ligand, SDF-1, compared with mature CD4+ T cells. The basis of the increased sensitivity to SDF-1-mediated inhibition may be due to an altered stoichiometry between CD4 and CXCR4 receptors, due perhaps to the low density of CD4 molecules on CD34+ progenitor cells. Alternatively, cell type-specific differences in the glycosylation of chemokine receptors may alter ligand or HIV binding characteristics (67).

Circulating CD34+ cells represent a distinct progenitor pool responsible for seeding extramedullary sites of lymphopoiesis. Progenitor cell infection with HIV may effect long term functional consequences within extramedullary sites of lymphopoiesis. Cells within extramedullary sites of lymphopoiesis that express low levels of CD4, such as accessory cells and mesenchymal cells, are susceptible to infection by HIV-1 and, in turn, are capable of transmitting the virus to immature cells of the lymphoid and myeloid lineages (14, 68). Extramedullary lymphopoiesis is dependent on an intact mesenchymal environment that is functionally capable of accommodating immigrating multipotent progenitor cells originating from the bone marrow. The observed failure to normalize the CD4+ T cell count and the apparent failure to restore the TCR-Vβ repertoire in individuals with HIV, even those undergoing highly active antiretroviral therapy (69, 70), may be due to irreversible effects of HIV within the progenitor cell pool itself or within the
progenitor cell microenvironment, either proximally within the bone marrow (4, 14) or distally within extramedullary sites of lymphopoiesis (5, 54, 69). Thus, infection of progenitor cells may have important consequences with regard to the potential for spontaneous immunologic reconstitution following adequate suppression of HIV replication by highly active antiretroviral therapy.

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