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CXCR4 Expression during Lymphopoiesis: Implications for Human Immunodeficiency Virus Type 1 Infection of the Thymus

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Human immunodeficiency virus type 1 (HIV-1) infection of the human thymus results in depletion of CD4-bearing thymocytes. This depletion is initially manifested in the immature $CD4^+/CD8^+$ thymocyte subset. To determine cellular factors involved in HIV infection in the thymus, we examined the expression of the recently identified viral coreceptor, CXCR4, on fresh human thymocytes and on human cells from SCID-hu (Thy/Liv) mice. CXCR4 is a member of the chemokine receptor family which is required along with CD4 for entry into the cell of syncytium-inducing (SI) HIV-1 strains. Our analyses show that CXCR4 expression is modulated during T-lymphoid differentiation such that immature thymocytes display an increased frequency and higher surface density of the coreceptor than do more mature cells. In addition, using an SI strain of HIV-1 which directs expression of a reporter protein on the surface of infected cells, we have found that the immature $CD4^+/CD8^+$ thymocytes that express the highest levels of both CD4 and CXCR4 are the cells that are preferentially infected and depleted by the virus in vitro. Thus, high levels of both primary receptor and coreceptor may allow efficient infection of the thymus by certain HIV-1 strains. This in part may explain the rapid disease progression seen in some HIV-infected children, where the thymus is actively involved in the production of new T lymphocytes.

Recent studies have identified several members of the chemokine receptor family that function as coreceptors with CD4 to allow entry of various strains of human immunodeficiency virus type 1 (HIV-1) into the cell (3, 6, 11–14, 17, 27). Coexpression of the chemokine receptor CXCR4 with CD4 has been shown to allow infection by syncytium-inducing (SI), T-cell-tropic (T-tropic) isolates (6, 17, 27), and coexpression of the chemokine receptor CCR5 with CD4 allows entry of non-SI, macrophage tropic (M-tropic) viruses (3, 11–14). Other chemokine receptors that appear to allow infection with various strains of HIV-1, notably strains with dual T- and M-tropic phenotypes, have also been identified (11, 13, 35). The relationship between coreceptor expression in human lymphoid organs and viral pathogenesis has not yet been elucidated.

Coexpression of a chemokine coreceptor with CD4 on different types of cells in lymphoid organs could render them more susceptible to infection by HIV-1. HIV-1-induced depletion in the human thymus and in human thymic implants in SCID-hu mice initially occurs in the less mature thymocyte population that expresses both CD4 and CD8 (CD4CD8 double-positive [DP] cells) and subsequently is manifested in the more mature $CD4^+$ $CD8^-$ (CD4 single-positive [SP]) cells (2, 9, 33, 39). The kinetics of viral replication and the ability to induce CD4 cell depletion in the SCID-hu mouse and in vitro, however, vary according to phenotypic characteristics of different viral isolates (20, 39). Viral isolates with an SI, T-tropic phenotype appear to be more pathogenic, replicate to higher titer, and result in more severe $CD4^+$ cell depletion than non-SI, M-tropic viral isolates (20, 22, 24). However, the cellular factors that contribute to this association between increasing pathogenic potential and the SI phenotype are not characterized. Cellular permissivity of infection, viral replication kinetics, and susceptibility to HIV-1-induced depletion of thymocytes are all factors potentially affected by differential expression of these coreceptors.

The purpose of this study was to examine if productive infection of thymocytes is directly related to the expression and/or modulation of second-receptor expression. We examined the relationship between CXCR4 expression on thymocytes representing different stages of lymphoid development and HIV-1 infection. We found that CXCR4 is expressed in higher levels and on more cells in immature thymocyte populations than in mature populations in the thymus. Moreover, proviral DNA localization and expression of a T-tropic, SI molecular clone of HIV-1 in thymocyte subsets correlated with CXCR4 expression. We conclude that the greater levels of expression of the HIV-1 coreceptor CXCR4 in these less mature $CD4^+$ thymocyte populations, in particular the CD4CD8 DP population and the populations that express low levels of CD3 and CD5 on their cell surface, contributes to the increased susceptibility to infection and depletion by T-tropic, SI HIV-1 isolates. Our studies demonstrate that HIV-1 second-receptor expression is modulated in vivo as much as eightfold, suggesting that this modulation plays an important role in the tropism and pathogenesis of HIV-1 infection in the human thymus.

MATERIALS AND METHODS

Virus stocks. HIV-1_{NL4-3}, HIV-$1_{NL-\Delta nef}$, and HIV-1_{NL-thy} have been previously described (1, 19, 21, 32), and viral stocks were obtained by electroporation of plasmid containing infectious cloned DNA (30 μg) into CEM cells followed by coculture with uninfected CEM cells. $p24^{gag}$ quantitation in virally infected culture supernatants was performed by enzyme-linked immunosorbent assay

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(Coulter, Hialeah, Fla.). Determination of infectious units per milliliter of virus stocks was achieved by infection of CEM cells followed by PCR for proviral sequences 24 h postinfection (41). Values were calculated by comparison to standard curves of known amounts of both HIV-1 plasmid DNA and human genomic DNA, using an Ambis (San Diego, Calif.) radioanalytic imager. Our data showed that 1 ng of p24 is approximately equivalent to 125 infectious units.

SCID-hu mice. C.B. 17 mice homozygous for the SCID (severe combined immunodeficiency) defect were bred and housed at the University of California at Los Angeles as described previously (19). Fetal tissue between 18 and 23 weeks of gestational age was used in the construction of the SCID-hu (Thy/Liv) mouse as previously described (2, 29, 30). Circulating blood from SCID-hu mice was obtained through retro-orbital bleeding under anesthesia 4 to 6 weeks following implantation, as approved by the UCLA Animal Research Committee. Mice were then sacrificed, and the Thy/Liv implants were removed. Thymocytes were subsequently teased away from the stromal elements and analyzed.

In vitro thymocyte cell culture. Fetal thymus ranging from 18 to 22 weeks of gestational age was obtained from Advanced Bioscience Resources (Alameda, Calif.) or Anatomical Gift Foundation (Woodbine, Ga.). Single-cell suspensions of minced tissue were depleted of adherent cells by nylon wool purification and infected with HIV-1 as described previously (39, 40). Twenty million cells per ml of Iscove's modified Dulbecco medium (Irvine Scientific, Santa Ana, Calif.) containing 1.1 mg of bovine serum albumin per ml, 85 μg of transferrin per ml, 2 mM glutamine, and 25 mg of penicillin-streptomycin (Irvine Scientific) per ml were then cultured in the presence of 20 U of human recombinant interleukin-2 (IL-2) per ml and 20 ng of recombinant human IL-4 (R&D Systems, Inc., Minneapolis, Minn.) per ml for up to 18 days as described previously (40).

Flow cytometry and FACS. Fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, or peridinin chlorophyll protein (PerCP)-conjugated monoclonal antibodies specific for human CD3, CD4, and CD8 were obtained from Becton Dickinson (San Jose, Calif.). Conjugated monoclonal antibodies specific for human CD5 (FITC) and mouse Thy1.2 (allophycocyanin (APC) were obtained from Pharmingen (San Diego, Calif.) and Caltag (South San Francisco, Calif.), respectively. Cells were stained as instructed by the manufacturers. Fluorescence-activated cell sorting (FACS) lysing solution (Becton Dickinson) was used to remove erythrocytes from stained SCID-hu peripheral blood. CXCR4 monoclonal antibody 12G5 from James Hoxie (15) was obtained through the AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases. Cells stained for CXCR4 were counterstained with goat anti-mouse immunoglobulin G2a conjugated with either FITC or PE (Pharmingen). Immunophenotypic analysis was performed with the Cellquest program (Becton Dickinson). Live cells were gated by using forward versus side scatter. Thirty thousand events were acquired for thymocytes, and the number of events acquired for SCID-hu peripheral blood was limited to the total number of cell recovered (more than 50,000 events). Mouse isotype antibodies were used as a negative control for the gating of those cells staining negative for a cell surface marker. As a positive control for 12G5 staining, CXCR4 expression on unstimulated peripheral blood lymphocytes (PBL) from two different donors was analyzed, and 91 and 80% of the cells expressed CXCR4 at relative fluorescent intensities of 79 and 81, respectively (not shown). FACS was performed as described previously (2, 18), using a FACSTAR^{Plus} (Becton Dickinson).

Quantitative PCR. Cells to be subjected to quantitative PCR were pelleted and resuspended in urea lysis buffer (4.7 M urea, 1.3% sodium dodecyl sulfate, 0.23 M NaCl, 0.67 mM Tris-HCl [pH 8.0]). DNA was purified by phenol and chloroform extractions followed by ethanol precipitation. Quantitative PCR was performed on purified DNA samples, using primers specific for HIV-1 sequences (AA55 and M667) and human beta-globin sequences (41). One primer from each pair was end labeled with ^{32}P as described previously (41). Following 25 cycles of PCR, samples were resolved on a 6% polyacrylamide gel, and quantitation was performed by value comparison to standard curves of known amounts of both HIV-1 and human genomic DNA, using an Ambis radioanalytic imager. The percentage of DNA in each described subset was calculated as follows (23): percentage of proviral DNA in subset = (amount of proviral DNA in subset × (percentage of subset in total culture)/total amount of proviral DNA in culture.

RESULTS

HIV-1 proviral DNA distribution and expression in thymocyte subsets. We have previously shown that early after HIV-1_{NL4-3} infection of Thy/Liv implants in the SCID-hu mouse, prior to CD4 cell depletion, proviral DNA can be found localized primarily in the CD4CD8 DP subset (2). In addition, proviral DNA distribution in HIV-1-infected fetal thymocytes cultured in the presence of cytokines in vitro was similar to that observed in vivo (23). HIV-1 infection and expression in this in vitro system occur primarily in the CD4CD8 DP population early after infection, as assessed by quantitative PCR and reporter gene expression as well as by intracellular staining for $p24^{gag}$ (23, 39). In the present study, proviral DNA distribution in cultured fetal thymocytes infected with HIV-1_{NL4-3} or an isolate containing a deletion of the nef gene (HIV-$1_{NL-\Delta nef}$) was examined. HIV-1 proviral DNA was found primarily in the CD4CD8 DP population within 1 week following infection (Fig. 1A). There is also detectable proviral DNA in the CD4-negative populations within the first week postinfection. Pro-

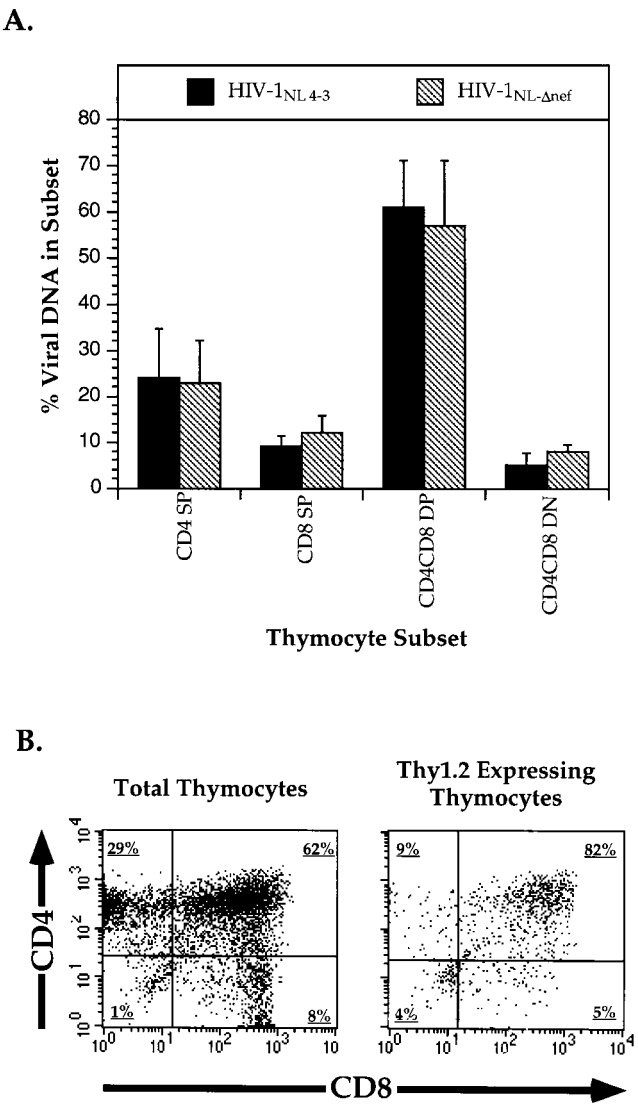

FIG. 1. HIV-1 infection of thymocytes cultured in vitro. (A) Early HIV-1 proviral DNA distribution in thymocyte cultures in vitro. Nylon wool-purified fetal thymocytes were infected with HIV-1_{NL4-3} or HIV-$1_{NL-\Delta nef}$ and cultured in the presence of IL-2 and IL-4 for 1 week. Thymocytes from infected cultures were stained for CD4 and CD8 expression and sorted by FACS into the respective populations. Postsort analysis revealed that these populations were greater than 99.8% pure. DNA from the sorted populations was isolated, and quantitative PCR for HIV-1 and cellular sequences was performed. Quantitation was done and the percentage of proviral DNA in each subset was calculated as described in Materials and Methods. The graph represents the average of three experiments. (B) HIV-1_{NL-thy} expression in in vitro-infected thymocytes. Ten days after infection with HIV-1_{NL-thy}, thymocytes cultured in vitro were stained by using monoclonal antibodies specific for CD4 (PE), CD8 (FITC), and mouse Thy1.2 (APC). Total thymocyte staining for CD4 and CD8 is represented in the left panel. Thy1.2 expression at this time point was seen in 7% of total thymocytes. The phenotype of thymocytes expressing Thy1.2 as shown in the panel on the right was determined by gating on Thy1.2-positive cells followed by subsequent analysis for CD4 and CD8 expression. Percentages of thymocytes residing in each quadrant are indicated.

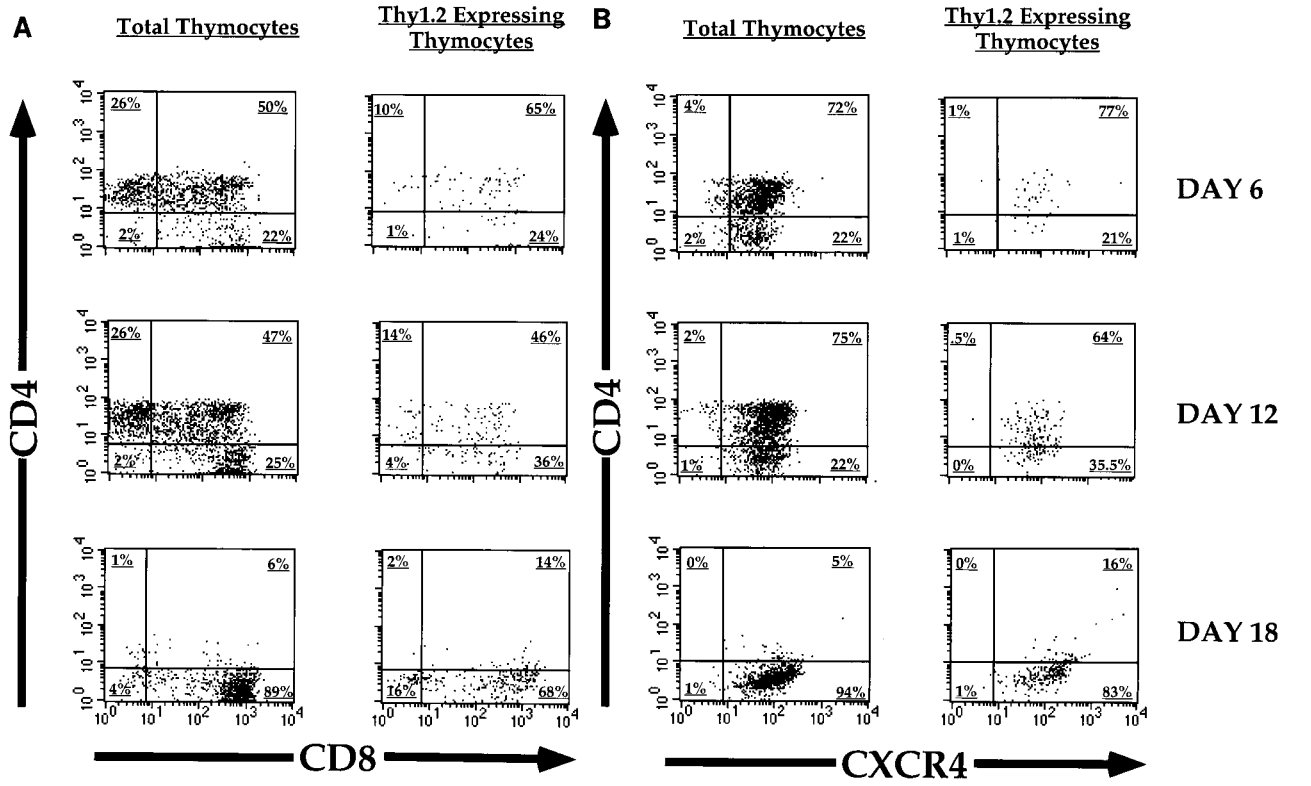

FIG. 2. CXCR4 and HIV-1 expression in infected thymocytes cultured in vitro. Nylon wool-purified thymocytes were infected with HIV-$1_{NL\text{-}thy}$ at a multiplicity of infection of 0.001 and cultured in the presence of IL-2 and IL-4 for 2 weeks. Cells were removed at days 6, 12, and 18 and subjected to three-color immunophenotypic analysis using antibody combinations including CD4 (FITC), Thy1.2 (APC), and either CXCR4 (goat anti-mouse immunoglobulin G2a) or CD8 conjugated with PE. (A) CD4 and CD8 expression. Total thymocytes expressing CD4 and CD8 are shown in the left panels, and the CD4 and CD8 distributions of only those cells expressing Thy1.2, as determined by gating, are shown in the right panels. Percentages of cells in each quadrant are indicated. (B) CD4 and CXCR4 expression. Total thymocytes expressing CD4 and CXCR4 are shown in the left panels, and the CD4 and CXCR4 profiles of only those cells expressing Thy1.2, as determined by gating, are shown in the right panels. Percentages of cells in each quadrant are indicated.

viral DNA localizes to the CD4-negative subsets primarily through infection of a less mature, CD4-positive cell type which further differentiates into either a CD8 SP or $CD4^-$ $CD8^-$ (CD4CD8 double-negative [DN]) population (23).

Since deletion of the nef gene does not result in different proviral distribution patterns compared with the wild type, we examined viral expression in these populations with that of a molecular clone of HIV-$1_{NL4\text{-}3}$ containing the murine Thy1.2 gene within the nef open reading frame (HIV-$1_{NL\text{-}thy}$) (32). The use of this virus allows cell surface expression analysis for a virally encoded reporter protein. Purified thymocytes were infected with HIV-$1_{NL\text{-}thy}$ and cultured in the presence of IL-2 and IL-4. These two cytokines are present in the normal thymus, where they function in the differentiation and proliferation of thymocytes and have a synergistic effect in promoting HIV-1 replication in this in vitro system (18, 37, 38, 40). Cells were removed 10 days after infection and analyzed by flow cytometry for CD4, CD8, and Thy1.2 expression. Expression of Thy1.2 indicated that at this time the virus was primarily expressed in the CD4CD8 DP population, particularly those cells expressing high levels of CD4 (Fig. 1B). These results indicate that during the first few rounds of replication, an SI, T-tropic isolate of HIV-1 is found preferentially localized and expressed in the relatively immature CD4CD8 DP population.

CXCR4 and HIV-1 expression in infected thymocytes. To examine T-tropic, SI HIV-1 infection and expression in thymocytes in relation to cellular expression of the coreceptor CXCR4, thymocytes were infected with HIV-$1_{NL\text{-}thy}$, cultured, and subsequently phenotypically examined at 6, 12, and 18 days after infection (Fig. 2). Early after infection (day 6), overall viral expression was low, with 3.7% of total thymocytes expressing the Thy1.2 marker. The greatest percentage of expression was found to occur in the CD4CD8 DP population, where 65% of those cells expressing HIV-1 are found at this time (Fig. 2A). When examined for CXCR4 expression, essentially all cells expressing Thy1.2 also expressed CXCR4, and of these, the vast majority (77%) were $CD4^+$ (Fig. 2B). Thus, early in infection following the initial few rounds of replication, virus is expressed primarily in $CD4^+$/$CXCR4^+$ cells. Specifically, Thy1.2 expression is most apparent in those cells staining for higher levels of cell surface expression of both CD4 and CD8 (Fig. 1B). Later in infection (day 12), the percentage of total thymocytes expressing Thy1.2 increased to 4.5%, with 46% of Thy1.2-positive cells found in CD4CD8 DP cells, 36% found in CD8 SP cells, 14% found in CD4 SP cells, and 4% found in CD4CD8 DN cells. Following further depletion of CD4-bearing cells (day 18), the percentage of thymocytes expressing Thy1.2 was 6.3%; of these, the majority (84%) of expression was found in the CD4-negative populations, with 68% of Thy1.2-positive cells in the CD8 SP population and 16% in the CD4CD8 DN population. We have previously shown that the increase in expression of Thy1.2 in the CD4-negative population is due to infection of a less mature CD4-positive cell type which further differentiates into a CD4-negative cell type that continues to express virus (23). Thus, through differentiation, infected thymocytes may lose the pri-

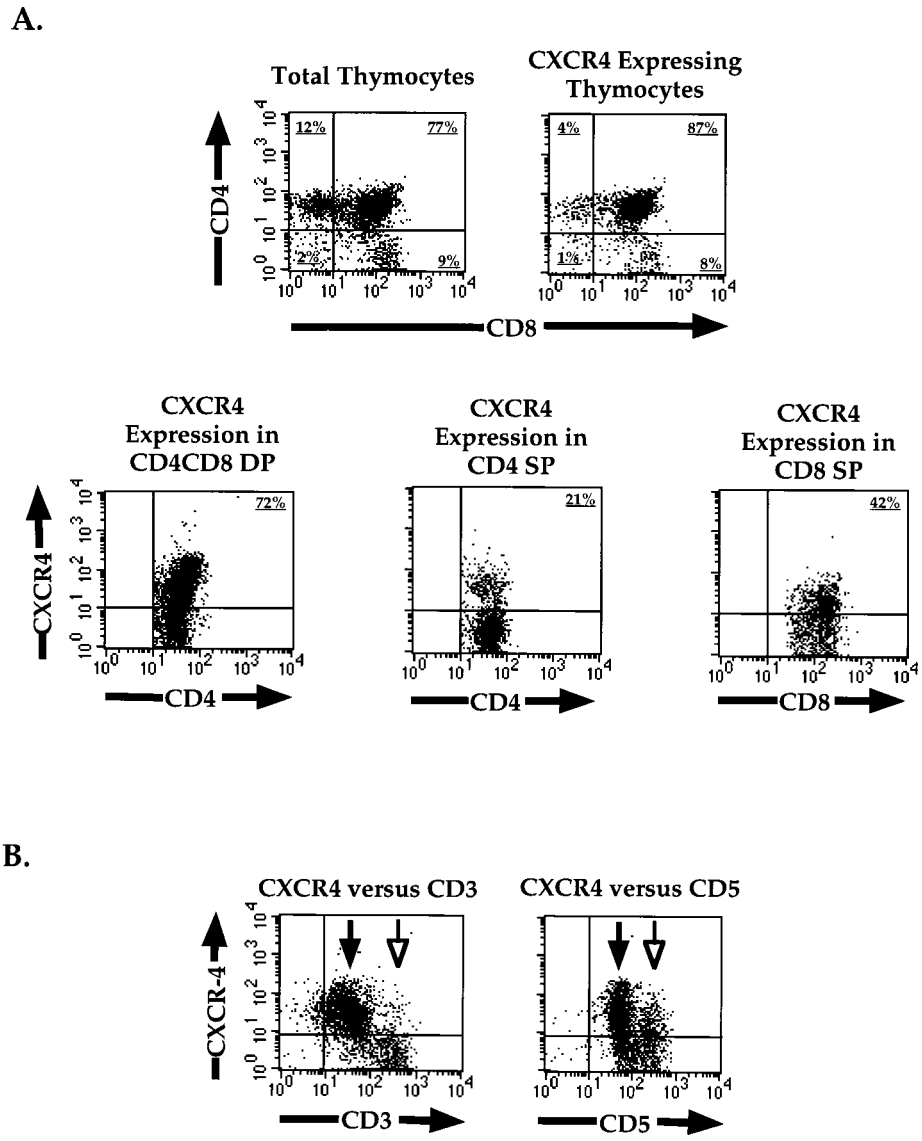

FIG. 3. CXCR4 expression in fresh human fetal thymocytes. Freshly obtained fetal thymus was analyzed for expression of CXCR4 and markers of different stages of lymphopoiesis: CD3, CD4, CD5, and CD8. (A) CD4 (PerCP), CD8 (FITC), and CXCR4 (PE) expression in the fetal thymus. Thymocytes were simultaneously stained for all three markers. Total thymocytes staining for CD4 and CD8 are represented in the left panel. Thymocytes expressing CXCR4 were gated and analyzed for CD4 and CD8 expression and are represented in the right panel. Percentages of CD4 and CD8 expression in total thymocytes are indicated within the quadrants on the left panel. Percentages of total thymocytes expressing CXCR4 in each quadrant are shown in the right panel. The lower panels illustrate CXCR4 expression on only the CD4CD8 DP, CD4 SP, and CD8 SP populations which were isolated by gating the respective populations. Note that the mean fluorescence intensity of CD4 in this staining is less than that of Fig. 1B due to the use of different fluorescent conjugates. (B) CD3 (FITC), CD5 (FITC), and CXCR4 (PE) expression in the fetal thymus. Thymocytes stained for CD3 and CXCR4 expression are shown in the left panel, and thymocytes stained for CD5 and CXCR4 expression are shown in the right panel. Closed arrows denote those thymocytes expressing lower density levels of CD3 or CD5 ($CD3^{lo}$ $CD5^{lo}$), and open arrows denote those thymocytes expressing higher density levels of CD3 and CD5 ($CD3^{hi}$ $CD5^{hi}$).

mary HIV-1 receptor. In contrast, the data in Fig. 2 indicate that the coreceptor is not lost during the differentiation process from CD4CD8 DP cells to CD8 SP cells. Virtually all of the Thy1.2-expressing cells expressed CXCR4.

CXCR4 expression at various stages of lymphopoiesis in the human thymus. Since activation of lymphocytes is associated with increased expression of CXCR4 (8, 10), we further characterized CXCR4 expression on fresh human fetal thymocytes in the absence of cytokine stimulation. Fresh fetal thymocytes were initially examined by flow cytometry for CXCR4 coexpression with cell surface markers indicative of different stages of T-cell development. The process of thymopoiesis is characterized by changes in cell surface expression of various cell surface markers. During T-cell development, CD5 is expressed early in the thymus, followed by expression of CD4 and subsequently CD8. CD3 is increasingly expressed in greater amounts following CD4 and CD8 expression. The CD4CD8 DP cell that is $CD3^+$ and $CD5^+$ undergoes further differentiation to become either a $CD4^+$ $CD8^-$, a $CD4^-$ $CD8^+$, or less frequently a CD4CD8 DN cell (4, 36). The density of both CD3 and CD5 on the surface of the cell increases as the thymocyte matures, which is reflected in the relative fluorescence intensity of the marker when analyzed by flow cytometry (36). A $CD3^{hi}$ cell has approximately six- to ninefold-greater

TABLE 1. CXCR4 expression on mature and immature fetal thymocytes[a]

Thymocyte population	% of cells expressing CXCR4		
	Thymus 1	Thymus 2	Thymus 3
Total	60 (33)	68 (27)	72 (34)
$CD3^{lo}$	70 (36)	83 (39)	85 (33)
$CD5^{lo}$	65 (34)	76 (32)	80 (33)
CD4CD8 DP	66 (36)	77 (34)	80 (38)
$CD3^{hi}$	24 (23)	34 (26)	18 (24)
$CD5^{hi}$	30 (22)	39 (21)	27 (20)
CD4 SP	23 (42)	25 (28)	26 (34)
CD8 SP	45 (25)	52 (19)	49 (22)

[a] Immature thymocytes are identified by immunophenotypic expression of various markers ($CD3^{lo}$, $CD5^{lo}$, and CD4CD8 DP); mature thymocytes are denoted by their differential expression of these same markers ($CD3^{hi}$, $CD5^{hi}$, CD4 SP, and CD8 SP). The geometric mean of fluorescence intensity of CXCR4 expression in each total population is given in parentheses.

cell surface expression of the marker than a $CD3^{lo}$ cell, and a $CD5^{hi}$ cell has approximately four- to sixfold-greater expression of the marker than a $CD5^{lo}$ cell, as determined by relative fluorescence intensity (Fig. 3B). Thus, the $CD3^{hi}$ and $CD5^{hi}$ cells are more mature than the $CD3^{lo}$ or $CD5^{lo}$ cells.

We analyzed thymocytes from three different human fetal thymuses for expression of CXCR4, CD3, CD4, CD5, and CD8. When expression of CD4 and CD8 was analyzed in relation to CXCR4 expression, CXCR4 expression was found to be overrepresented in CD4CD8 DP cells and underrepresented in CD4 SP cells (Fig. 3A). CXCR4 was found to be expressed on a greater percentage of cells in the immature thymocyte subsets ($CD3^{lo}$, $CD5^{lo}$, and CD4CD8 DP) than the more mature subsets ($CD3^{hi}$, $CD5^{hi}$, CD4 SP, and CD8 SP) (Fig. 3; Table 1). CXCR4 is also expressed approximately three- to eightfold more densely on these immature cells, as determined by relative fluorescence intensity (Table 1). The differential expression of CXCR4 in thymocyte subsets representing successive stages of development suggests regulated expression of this gene in a maturation-dependent fashion.

CXCR4 expression on thymus-derived PBL in the SCID-hu mouse. Expression of CXCR4 appears to be differentially expressed through thymopoiesis. To determine if this pattern is followed in the peripheral blood, we examined human PBL in the circulation of SCID-hu (Thy/Liv) mice. It has been previously shown that the few circulating human PBL in the SCID-hu mouse phenotypically and functionally resemble those found in umbilical cord blood or the peripheral blood of neonates (reference 26 and our unpublished observations). Nearly all of these circulating PBL express human CD3 and CD45, respond to mitogenic or allogeneic cell stimulation, and express either CD4 or CD8 in percentages similar to that in adult peripheral blood. The vast majority of these cells express CD45RA, indicative of a naive T cell.

Human PBL from SCID-hu mice were examined for expression of CXCR4 by flow cytometry in $CD45^+$ and $CD4^+$ populations and compared to CXCR4 expression on human thymocytes from Thy/Liv implants in the same mouse. In studies not shown, we found that the $CD45^+/CD4^-$ population is a CD8 SP population. Since recovery of these human cells was low, we restricted our staining to CD4 and CD45 for analysis of CXCR4 expression on these cells. CXCR4 expression in the Thy/Liv implant was similar to that found in freshly isolated human thymocytes (Fig. 4A). The percentage of CD4 SP cells expressing CXCR4 was 30%, whereas 79% of CD8 SP cells expressed the coreceptor. CXCR4 expression on human PBL from the peripheral blood in the same mouse was similar to that seen on mature cells in the Thy/Liv implant, with 31% of the $CD45^+/CD4^+$ population and 85% of the $CD45^+/CD4^-$ cells expressing the T-tropic HIV-1 coreceptor (Fig. 4B). Thus, the percentage of cells expressing CXCR4 in human PBL in the circulation of SCID-hu mice was similar to that of the mature thymocyte subsets in the Thy/Liv implant of the same mouse. We did, however, detect a difference in mean fluorescence intensity of CXCR4 on these peripheral cells, suggesting differential regulation of expression in the periphery. CD8 SP cells in the thymus and periphery appear to have similar intensities of expression, whereas CD4 SP cells in the periphery appear to have somewhat lower levels of expression than do mature thymocytes. Levels of CXCR4 on CD4CD8 DP and CD8 SP thymocytes from human fetal thymus and SCID-hu mice were similar. However, expression of CXCR4 was somewhat higher on CD4 SP thymocytes from SCID-hu mice than in human fetal thymus cells. The reasons for this are unclear. Taken together, these results suggest that the thymus in children may be a major target for HIV-1 infection in part because of the relatively high level of CXCR4 on immature cells.

DISCUSSION

HIV-1 infection of the human thymus results in depletion of cells that express CD4. The aim of this study was to address whether productive infection of thymocytes is related to modulation of second-receptor expression during T-cell maturation. These studies were facilitated by the availability of a CXCR4-specific monoclonal antibody and the use of a CXCR4-tropic infectious molecular clone, $HIV\text{-}1_{NL\text{-}thy}$, which allowed direct examination of expression of HIV-1 and CXCR4. While we have not observed changes in proviral distribution between nef-deleted and wild-type viruses (Fig. 1 and reference 23), the use of a nef-deleted virus here could possibly alter viral distribution and expression in ways not yet detected. It would be of interest to examine expression of HIV strains bearing a marker gene and expressing nef. However, the use of $HIV\text{-}1_{NL\text{-}thy}$ has been well characterized in vitro and allows direct examination of a virally encoded marker gene in this in vitro system (23). We have determined that CD4 cell depletion induced by a T-tropic, SI HIV-1 isolate occurs primarily in immature thymocyte subsets that express both CD4 and a greater number and intensity of the CXCR4 coreceptor than do more mature thymocyte subsets. HIV-1 proviral DNA sequences and viral gene expression colocalize to the $CXCR4^{hi}$ subsets which are subsequently depleted. Thus, the greater coexpression of CD4 and CXCR4 in the less mature thymocytes is associated with increased susceptibility to viral infection and viral cytopathicity.

CXCR4 is expressed on a variety of other human cell types, including brain, spleen, and lung tissue (7, 16, 28, 31). The natural ligand for CXCR4 is stromal cell-derived factor 1, which stimulates calcium release and chemotaxis in transfected cells and hence may play a role in cellular activation and motility (7, 31). The differential expression of CXCR4 during thymopoiesis seen in our studies could implicate a role for the receptor in T-lymphoid differentiation. The lesser expression of CXCR4 on a mature CD4 SP thymocyte could partially explain why the depletion of this population during $HIV\text{-}1_{NL4\text{-}3}$ infection of the Thy/Liv implant in the SCID-hu mouse is kinetically slower than that of the CD4CD8 DP population.

CD4CD8 DP thymocytes constitute the proliferating subset in the thymus (25) and also express the highest levels of CXCR4. This activated state would also render these cells

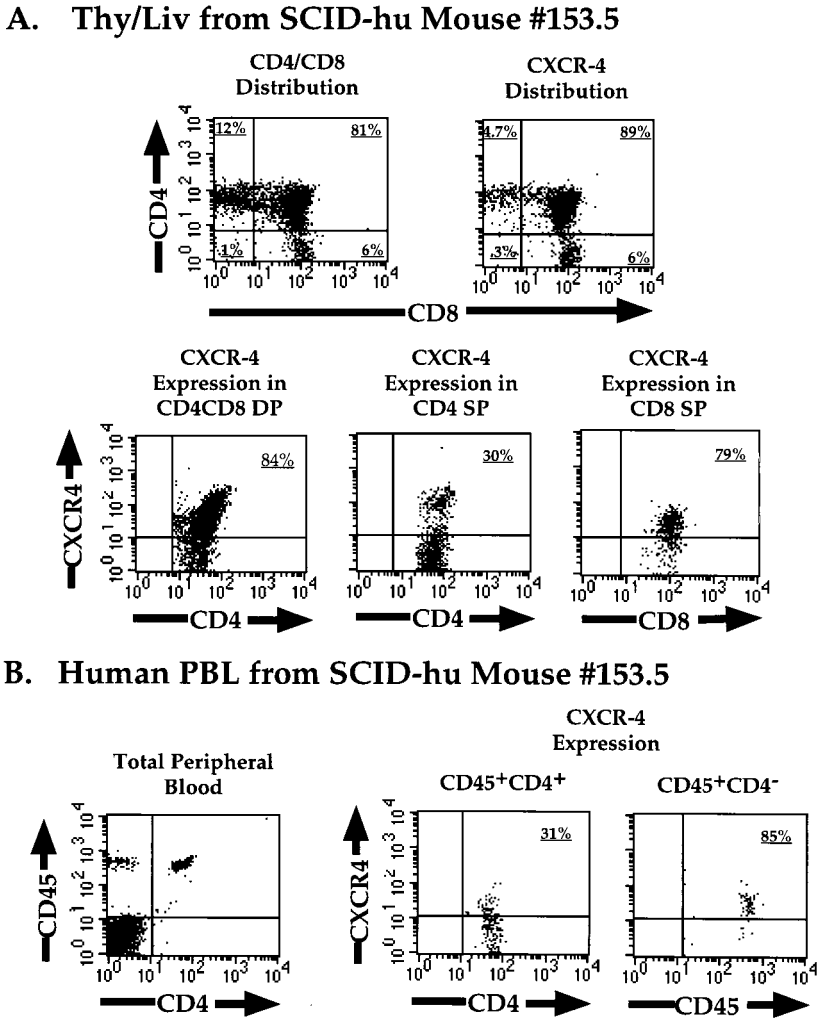

FIG. 4. CXCR4 expression in Thy/Liv implants and human PBL from the SCID-hu mouse. (A) Expression of CXCR4 in Thy/Liv implants from the SCID-hu mouse. Thy/Liv implants were removed from a SCID-hu mouse 6 months following transplantation and immunophenotyped by three-color analysis using antibodies specific for CD4 (FITC), CD8 (PerCP), and CXCR4 (PE). The upper left panel illustrates CD4 and CD8 staining on total thymocytes. The upper right panel illustrates CD4 and CD8 staining of only those thymocytes expressing CXCR4. Percentages of cells in each population are indicated. The lower panels illustrate CXCR4 expression on only the CD4CD8 DP, CD4 SP, and CD8 SP populations which were isolated by gating the respective populations. The mean fluorescence intensity for CXCR4 expression in CD4CD8 DP cells was 28, the mean intensity in CD4 SP cells was 29, and the mean intensity in CD8 SP cells was 26. (B) CXCR4 expression in human PBL from the SCID-hu mouse. Circulating blood from the same SCID-hu mouse examined in panel A was obtained from the retro-orbital sinus. Whole blood was immunophenotyped for CD4 (FITC), CD45 (PerCP), and CXCR4 (PE) expression. Percentages of cells in each population expressing CXCR4 are indicated. The left panel illustrates staining for CD45 and CD4. The middle panel illustrates CXCR4 expression on only the $CD45^+$ $CD4^+$ cells, as assessed by back gating on this population. The right panel shows CXCR4 expression on the $CD45^+$ $CD4^-$ population, which is equivalent to CD8 SP circulating human cells. The mean fluorescence intensity of CXCR4 expression in the $CD45^+$ $CD4^+$ population was 10, and the intensity of the CXCR4 expression in the $CD45^+$ $CD4^-$ population was 37. Data similar to those shown in panels A and B were obtained from a second SCID-hu mouse (not shown).

capable of both reverse transcription and long terminal repeat-mediated gene expression (41, 42). Thus, multiple factors appear to render the CD4CD8 DP subset more permissive than other thymocyte subsets for HIV-1 infection and replication. Chemokines appear to be involved in lymphocyte activation and in the control of cellular proliferation (5, 34). Thus, expression of CXCR4 and other chemokine receptors appears to be regulated according to cellular activation, proliferation, and differentiation events. This differential regulation of expression appears to have profound effects on the tropism and pathogenesis of T-tropic, SI HIV-1 in the human thymus.

Our findings provide new insight into HIV-1-induced pathogenosis in the thymus and the cellular factors involved in HIV-1 tropism and infection. Our results further suggest that cells that express both CD4 and CXCR4 are directly depleted by the virus due to infection and expression in these subsets in contrast to undergoing an indirect method of cell death. This knowledge of the modulation of CXCR4 expression by as much as eightfold on thymocytes at different stages of differentiation allows increased understanding of the pathogenesis of HIV-1 and the development of therapeutic strategies. Those therapeutic strategies that inhibit viral interaction with second receptors could specifically rescue T-cell development at early stages by not allowing virus entry into the cells most susceptible to infection.

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