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J Immunol 2000; 165:716-724; doi: 10.4049/jimmunol.165.2.716
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Cytokines and Cell Surface Molecules Independently Induce CXCR4 Expression on CD4⁺ CCR7⁺ Human Memory T Cells

Patrick Jourdan,* Jean-Pierre Vendrell,† Marie-France Huget,‡ Michel Segondy,* Jean Bousquet,* Jérôme Pène,* and Hans Yssel‡*

In the present study, we show that IL-2, IL-4, IL-7, and IL-15 are able to induce functional CXCR4 surface expression on resting in vitro-generated CD4⁺ T cells. Cytokine-mediated induction of CXCR4 expression was associated with an increase in CXCR4 transcription, enhanced stromal-derived factor-1-induced T cell migration in vitro, and increased susceptibility of these cells to infection with X4 strains of HIV-1. CXCR4 expression could also be induced through an alternative pathway, following coculture of these cells with CD40-activated, autologous, CD34⁺ progenitor-derived dendritic cells. Although these dendritic cells express transcripts for IL-7 and IL-15, addition of neutralizing anti-IL-7R and IL-15 mAbs did not block induction of CXCR4 expression. Indeed, dendritic cell-mediated up-regulation of CXCR4 expression was found to depend on CD40/CD154 and CD134/CD134L interactions. Whereas activated autologous dendritic cells induced the expression of both CXCR4 and CD25 on a portion of CCR7⁺ memory T cells, concomitant CD3-mediated activation of these cells further enhanced CD25 expression, but, in contrast, prevented induction of CXCR4 expression. This observation suggests that triggering of the CD134 and CD154 molecules, in contrast to TCR/CD3 complex-mediated stimulation, results in simultaneous T cell activation and CXCR4 expression. Taken together, these results show that common γ-chain-interacting cytokines as well as signals mediated via noncognate interactions between activated dendritic cells and memory T cells are involved in the up-regulation of CXCR4 expression.


CXCR4 is a chemokine receptor that, together with its natural ligand stromal-derived-factor-1 (SDF-1)¹ (1, 2), is involved in the regulation of leukocyte migration as well as other immunological and developmental processes (reviewed in Ref. 3). In addition, CXCR4 has been identified as a co-factor for HIV entry into T cells (4). We and others have shown previously that cell-surface expression of CXCR4 on T lymphocytes is strongly up-regulated by IL-4, rendering these cells susceptible to infection with certain strains of HIV-1 (5, 6). In contrast, T cell-expressed CXCR4 is strongly down-regulated, following TCR/CD3 complex-mediated activation, using either anti-CD3 mAbs (5, 7) or specific Ag (8). Together, these data suggest that the expression of CXCR4 is not only dependent on the presence of endogenous IL-4, but also on the activation state of the cells.

Recently, it has been shown that CD45RO⁺ memory T cells can be divided into two subpopulations, based on the expression of the chemokine receptor CCR7, which controls homing to secondary lymphoid organs (9). Thus, central memory T cells express CCR7 and are capable to migrate to lymphnodes and to interact with dendritic cells. In contrast, another subpopulation of CD45RO⁺ T cells, called effector memory T cells, is CCR7⁻, and these cells migrate into and exert their effector function at sites of inflammation (9). Whereas CXCR4 is present on most naive CD45RO⁺ T cells (10, 11), its expression is variable on central memory and effector memory T cells (9). Because IL-4 is mainly produced in response to inflammatory immune reactions, its presence alone cannot account for the constitutive expression of CXCR4 on memory T cells, and therefore it is likely that other, as yet to be determined, factors are involved in the regulation of CXCR4 on these cells as well.

It has been shown previously that IL-2 may also up-regulate CXCR4 expression (11), suggesting that signaling through the common γ (γc) chain that is shared by both IL-2R and IL-4R (12) may be involved in the induction of expression of this chemokine receptor. Thus, likely candidates are IL-7, IL-9, and IL-15, the receptors that share the γc chain. Importantly, IL-7, like SDF-1, is produced by stromal cells (13, 14) and acts synergistically with this chemokine in certain immune functions (2). In addition, IL-7 promotes HIV infection of T lymphocytes by X4 HIV strains that use CXCR4 as a coreceptor for viral entry (15, 16). IL-15 not only induces T cell chemotaxis (17), but is also ubiquitously present throughout the immune system.

Interestingly, transcripts for both IL-7 and IL-15, but not for IL-2, IL-4, and IL-9, have recently been shown to be expressed in vitro-generated, activated, dendritic cells, in the vitro counterparts of Langerhans cells (18). Dendritic cells are not only potent APCs in secondary lymphoid organs, but are also a primary target and a reservoir for HIV infection, in particular for X4 HIV-1 strains (19–22). Furthermore, activated dendritic cells express numerous cell-surface molecules, notably CD80, CD86, CD40, and

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Received for publication October 1, 1999. Accepted for publication April 26, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

*P.I. is supported by “Med-Bio-Med.”

[Abbreviations used in this paper: SDF-1, stromal-derived-factor-1; γc, common γ; h, human.]

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0022-1767/00/$02.00
CD134L, which following interaction with their respective ligands have been reported to be important in lymphocyte activation, differentiation, and effector function (23–26) and might therefore be implicated in the regulation of CXCXR4 expression as well.

In the present study, we have analyzed the role of cytokines that signal through the γc chain in the regulation of CXCXR4 expression using a population of CXCXR4+CCR7+ T cells generated in vitro from CD4+ cord blood T lymphocytes that are reminiscent of central memory T cells (9). In addition, because of the important role that dendritic cells play in the pathogenesis of HIV infection, we have investigated whether in vitro-generated dendritic cells can modulate the expression of CXCXR4 on in vitro-generated central memory T cells, either indirectly, via the production of cytokines, or directly via cell-cell interaction.

Materials and Methods

Cells

Peripheral or cord blood mononuclear cells were isolated from freshly collected, heparinized peripheral blood (Centre de Transfusion Sanguine, Montpellier, France) or cord blood (generously provided by the Department of Gynecology and Obstetrics, Centre Hospitalier Universitaire Arnaud de Villeneuve, Montpellier, France), respectively, by Ficoll-Hypaque density gradient centrifugation. CD4+ T cells were purified (purity >95%) by negative selection from mononuclear cell preparations, using mAb-coated magnetic beads (Stem Cell Technologies, Vancouver, Canada), according to the manufacturer’s instructions.

T cell clones were generated form purified CD4+ cord blood T cells, following stimulation with plate-bound anti-CD3 and anti-CD28 mAb (see under stimulation and culture conditions) and cloning by limiting dilution, as described previously (27).

Dendritic cells, reminiscent of Langerhans cells, were generated according to the method described by Caux et al. (28). Briefly, cord blood CD34+ hematopoietic progenitor cells were purified by negative selection using a mixture of isotype-matched mAb, specific for B cells, CD41, CD45, CD81 T cells, monocytes, NK cells, erythrocytes, and IgG-coated magnetic beads (Stem Cell Technologies). The isolated cells were 86–95% CD34+, as judged by staining with the anti-CD34+ mAb B-B4 (Diaclone Research, Besançon, France). CD34+ cells were cultured in the presence of stem cell factor (25 ng/ml), GM-CSF (100 ng/ml), and TNF-α (2.5 ng/ml) at a concentration of 2 × 105 cells/ml in a 75-cm2 flask. Cells were expanded at day 3 with medium containing fresh GM-CSF and TNF-α and maintained at 5 × 106 cells/ml until day 12 at which they were used in the experiments. The resulting population of dendritic cells were predominantly CD1a+, CD14+, CD83+, HLA-DR+.

Mouse fibroblasts, expressing human (h) CD154 (CD-40L), were a kind gift of Dr Carl Figdor, University of Nijmegen, The Netherlands, and the anti-CD154 mAb 131 and CD134 mAb ik1 (kindly provided by Dr. Toshiyuki Hori, University of Kyoto, Japan), and the anti-86 and CD80 mAbs, B-T7 and 104 (kind gifts of Drs. John Wijdenes, Diaclone Research and André van Aghoven, Immunotech, Marseille, France, respectively).

Stimulation and culture conditions

One million purified cord blood-derived CD4+ T lymphocytes/ml were stimulated with the plate-bound anti-CD3 mAb SPV-T3b (Ref. 29; 24-well tissue culture plates (Linbro, McLean, VA) were incubated with 10 μg/ml SPV-T3b in PBS for at least 24 h at 4°C, after which the plates were washed three times with PBS) and the anti-CD28 mAb B-T3 (1 μg/ml; a generous gift of Dr. John Wijdenes). After 7 days of culture in the presence of suboptimal concentrations (0.5 ng/ml) of IL-2, the cells were collected, washed, and stimulated with anti-CD3 and anti-CD28 mAbs, and used in experiments 7 days later. This procedure resulted in a down-regulation of CXCXR4 on 90–99% of the cells. These cells, which are CD45RO+ CCR7+ (Fig. 1), are referred to as central memory T cells (9). Cultures of central memory T cells (105 cells/ml) with cytokines or cocultures with autologous dendritic cells (104 cells/ml) were conducted in the presence or absence of irradiated (50 Gy) CD154-expressing mouse fibroblasts (25 × 106/ml), in 24-well culture plates (Linbro, McLean, VA). Alternatively, central memory T cells were incubated with culture supernatants of dendritic cells that had been stimulated for 3 days with irradiated CD154+ L cell transfectants. Neutralizing mAbs directed against IL-7R or IL-15, or against CD11a, CD18, CD40, CD80, CD86, CD134, and CD134L, were added at a final concentration of 10 μg/ml. Dendritic cells were cocultured with CD154+ mouse fibroblasts in 24-well Linbro plates in a final volume of 500 μl for 9 h, after which central memory cells and mAbs were added in a volume of 500 μl. After 3 days of culture with either recombiant cytokines, dendritic cells, or culture supernatants from activated dendritic cells, in the presence or absence of mAbs, central memory T cells were used in the various experimental read-out systems.

Immunofluorescence and flow cytometry

All immunofluorescence and flow cytometry procedures were conducted using the method of Lanier and Rickettenwald (30). Cell-surface expression of CXCXR4 was analyzed using the biotinylated anti-human CXCXR4 mAb 12GS, a biotinylated isotype-matched control mAb (PharMingen, San Diego, CA), and PE-conjugated streptavidin (Becton Dickinson, San Jose, CA). CCR7 expression was measured using the rat mAb 3D12 (IgG2a), a generous gift of Dr. Martin Lipp (Max-Delbrück-Center for Molecular Medicine, Berlin, Germany). Expression of CD45RO, CD62L, and CD25 was analyzed using APC-conjugated, PE-conjugated, and FITC-conjugated mAbs, respectively (Becton Dickinson). Immunofluorescence was analyzed on a FACScalibur flow cytometer using CellQuest software (Becton Dickinson).

Chemotaxis

Migration of T cells in response to stimulation with SDF-1α was analyzed, using the method described by Bacon and Schall (31). Briefly, 29 μl of Yssel’s medium (Ref. 32; purchased from Diaclone Research), supplemented with 1% human AB+ serum, containing different concentrations of human rSDF-1β, were added to the lower wells of a 96-well Chemotx-T9 disposable chamber with a filter sample site of 3.2 mm diameter and 5-μm pore size filters (Neuroprobe, Gaithersburg, MD). Forty thousand T cells, in a volume of 20 μl, were transferred directly in triplicate onto the filter sample sites. After 1 h of incubation in 5% CO2 at 37°C, cells that had migrated through the filter into the lower chamber were collected, resuspended in culture medium, and counted using a hemocytometer. Results are expressed as the ratio of (number of cells migrated in rSDF-1-containing medium − number of cells migrated in medium alone)/total number of cells used in the assay × 100%.

RNase protection assay

Total RNA was extracted from 5 × 106 central memory T cells that had been cultured for 6 h in the presence or absence of cytokines, using RNAzol B (Tel-Test, Friendswood, TX) and CD154 transcripts were detected by RNase protection assay using the multiprobe template set hcr-6, containing the DNA template for CXCXR4 and GAPDH and L32 as house keeping genes, according to the manufacturer’s standard protocol (PharMingen). The ratio of expression between CXCXR4 and those of GAPDH and L32, respectively, was determined by counting the radio activity (cpm) of the relevant bands on the autoradiographs, blotted onto paper filters, by liquid β-scintillation counting (Wallac, Turku, Finland).

Infection with and detection of HIV-1

CD4+ central memory T cells, were cultured in medium in the absence or presence of either rIL-2, rIL-4, rIL-7, or rIL-15 or with resting or CD40-activated dendritic cells, respectively, in 24-well culture plates in a final volume of 1 ml. After 3 days, viral isolates of the HIV-1 Tropic strains HIV-1MDK (33) or HIV-1LA (34) were added to the cultures. Thirty minutes after infection (with HIV-1, the CXCXR4-selective antagonist AMD3100 (Ref. 35; a kind gift of Dr. Dominique Schols, Rega Institute, Leuven, Belgium) was added at a final concentration of 10 μg/ml where indicated. After 5 days of culture, cells were harvested, washed extensively
with PBS, and fixed with paraformaldehyde. Frequencies of infected T cells were determined by analyzing the presence of the HIV-1 protein p24, detected by flow cytometry, using an intracellular staining technique as described (36) on a Coulter Epics flow cytometer (Coulter, Hialeah, FL).

Statistical analysis

The $\chi^2$ test was used to analyze the significance of differences in inhibitory activity of mAbs.

Results

In vitro generated CD45RO$^+$ CXCR4$^-$ memory T cells express CCR7 and are present in freshly isolated peripheral blood

Populations of CD4$^+$ CD45RO$^-$ cord blood T cells that had been stimulated twice in vitro with anti-CD3 and anti-CD28 mAbs were analyzed 7 days after the last stimulation for the expression of CD45RO, CXCR4, and CCR7 by flow cytometry. All cells were CD45RO$^+$ (Fig. 1A) and furthermore expressed CCR7 (Fig. 1B). As expected, following CD3-mediated activation, the expression of CXCR4 was strongly down-regulated with low expression remaining only on 1–10% of cells (Fig. 1C). Furthermore, all CD45RO$^+$ T cells expressed CD62L (Fig. 1D). Based on the expression of CCR7 and CD62L, the in vitro-generated anti-CD3- and anti-CD28 mAb-activated cord blood cells seem to be reminiscent of the recently described subpopulation of central memory T cells (9).

To determine whether a counterpart of the population of cultured CD45RO$^+$ CXCR4$^-$ CCR7$^+$ cells could be detected in vivo, freshly isolated peripheral blood lymphocytes were analyzed for the expression of these cell-surface molecules by immunostaining and three-color flow cytometry. CCR7 was strongly expressed on CD45RO$^+$ and CCR7 and are present in freshly isolated peripheral blood. Expression of CD45RO (A), CCR7 (B), CXCR4 (C), and CD62L (D) was analyzed on CD4$^+$ cord blood-derived memory T cells, as well as on freshly isolated PBMC (E and F) by flow cytometry. Expression of CCR7 (FITC) was measured by three-color analysis on PBMC that had been stained with PE-conjugated anti-CXCR4 and APC-conjugated anti-CD45RO mAb (G). Histograms from cells stained with isotype control mAb (dotted lines) are superimposed over histograms of cells stained with specific mAb. Data in E and F are displayed as dot plots of correlated FITC (x-axis) and APC (x-axis) (E) or PE (x-axis) and APC (x-axis) (F) fluorescence. Quadrant markers were positioned to include >98% of control Ig-stained cells in the lower left (not shown). Representative data of two experiments.

FIGURE 1. In vitro-generated CD45RO$^+$ CXCR4$^-$ memory T cells are CCR7$^+$ CD62L$^-$ and are present in freshly isolated peripheral blood. Expression of CD45RO (A), CCR7 (B), CXCR4 (C), and CD62L (D) was analyzed on CD4$^+$ cord blood-derived memory T cells, as well as on freshly isolated PBMC (E and F) by flow cytometry. Expression of CCR7 (FITC) was measured by three-color analysis on PBMC that had been stained with PE-conjugated anti-CXCR4 and APC-conjugated anti-CD45RO mAb (G). Histograms from cells stained with isotype control mAb (dotted lines) are superimposed over histograms of cells stained with specific mAb. Data in E and F are displayed as dot plots of correlated FITC (x-axis) and APC (x-axis) (E) or PE (x-axis) and APC (x-axis) (F) fluorescence. Quadrant markers were positioned to include >98% of control Ig-stained cells in the lower left (not shown). Representative data of two experiments.

Modulation of CXCR4 surface expression on central memory T cells by cytokines that bind to $\gamma_c$ chain-containing receptors

The capacity of cytokines that signal via the $\gamma_c$ chain to induce CXCR4 expression was determined by culturing central memory T cells in the presence of rIL-2, rIL-4, rIL-7, rIL-9, or rIL-15 for different periods of time and analyzing the cell-surface expression of CXCR4 by flow cytometry. As shown in Fig. 2, rIL-2, rIL-4, rIL-7, and rIL-15 induced CXCR4 expression to variable extent. Whereas rIL-4 and rIL-7 induced strong and comparable levels of CXCR4 expression, rIL-2 and rIL-15 were slightly less efficient. Higher concentrations of any of the cytokines did not further augment surface levels of CXCR4. Maximal CXCR4 expression was detected between 48 and 72 h after addition of the cytokines. In addition, all cytokines were able to induce a proliferative response in central memory T cells (results not shown). In contrast, rIL-9 was unable to induce the expression of CXCR4 (Fig. 2), even at concentrations as high as 100 ng/ml, nor did it induce a proliferative response in these cells (results not shown). Next, we investigated whether combinations of cytokines had additive or synergistic effects on the induction of CXCR4 expression. Culture of memory T cells in the presence of combinations of cytokines did not modulate the induction of CXCR4 expression, induced by each
cytokine-induced CXCR4 expression was furthermore demonstrated by the increased susceptibility of central memory T cells to infection with the X4 HIV strains HIV-1\textsubscript{NDRK} and HIV-1\textsubscript{LAI} (Fig. 5 and Table I). A 3-day culture of the latter cells in the presence of either rIL-2, rIL-4, rIL-7, or rIL-15, before infection with HIV-1\textsubscript{NDRK}, resulted in infection rates between 30 and 60% as demonstrated by the presence of intracellular p24, whereas about 7% of cells cultured in medium alone were infected with this virus (Fig. 5). The addition of the CXCR4-specific antagonist AMD3100 to cultures of T cells that had been precultured with rIL-2 or rIL-4 resulted in a total inhibition of infection by HIV-1\textsubscript{LAI}, demonstrating the direct involvement of CXCR4 in the cytokine-induced susceptibility of HIV-1 infection. The addition of AMD3100 inhibited HIV-1\textsubscript{NDRK} infection in rIL-2- and rIL-4-treated cells by about 80 and 60%, respectively (Table I), indicating that this strain might use chemokine receptors other than CXCR4 for entry into T cells. Taken together, these results demonstrate that cytokines induce the expression of a functional CXCR4 on central memory T cells.

Activated dendritic cells induce functional expression of CXCR4 on central memory T cells

In view of the effects of rIL-7 and rIL-15 in inducing CXCR4 expression, we analyzed whether CD34\textsuperscript{+} hemopoietic progenitor cell-derived dendritic cells, were able to up-regulate CXCR4 expression on T cells in a similar manner. To this purpose, central memory T cells were cocultured with autologous in vitro-differentiated dendritic cells, derived dendritic cells, were able to up-regulate CXCR4 expression on T cells in a similar manner. To this purpose, central memory T cells were cocultured with autologous in vitro-differentiated dendritic cells. In vitro-generated dendritic cells resulted in a specific migration of about 30% in the presence or absence of combinations of rIL-2, rIL-4, rIL-7, rIL-9, rIL-15, and GM-CSF (all used at a concentration of 10 ng/ml), for 3 days, after which the surface expression of CXCR4 was analyzed by flow cytometry using the biotinylated anti-CXCR4 mAb 12G5 (bold lines), a biotinylated control mAb (dotted lines), and PE-conjugated streptavidin.
HIV-1 NDK strain following coculture with activated dendritic cells, underscoring the notion that enhanced viral entry of X4 HIV strains may, at least in part, be mediated by dendritic cell-mediated induction of CXCR4.

Induction of CXCR4 on central memory T cells by activated dendritic cells involves CD40/CD154 and CD134/CD134L interactions

Although cocultures of central memory T cells with activated dendritic cells resulted in induction of CXCR4 expression, culture supernatants from the latter cells were ineffective (data not shown). In addition, neutralizing anti-IL-15 or IL-7R mAbs had no effect, indicating that dendritic cell-mediated induction of CXCR4 expression is unlikely to be due to endogenously produced cytokines. Therefore, the nature of the signal mediated by the interaction of activated dendritic cells and central memory T cells was investigated, using neutralizing mAbs directed against various costimulatory and adhesion molecules known to be expressed at the cell surface of either activated dendritic cells or T cells. For these experiments, dendritic cells were preactivated with CD154-expressing fibroblasts for 9 h, and subsequently added to central memory T cells, in the presence or absence of neutralizing mAbs. The addition of neutralizing mAbs directed at CD40 or CD134, as well as

FIGURE 3. Cytokine-induced surface expression of CXCR4 is transcriptionally regulated. One million CD4+ in vitro-generated central memory T cells were cultured for 6 h in the presence or absence of rIL-2, rIL-4, rIL-7, rIL-9, or rIL-15, and the expression of CXCR4 transcripts was determined by RNase protection assay (A). C+ and C− are positive and negative internal controls of the assay, respectively. Induction of CXCR4 transcripts is expressed as the ratio between expression of specific CXCR4 mRNA and GAPDH (■) or L32 mRNA (□), respectively (B). The results represent the mean ± SD of two independent experiments.

FIGURE 4. Central memory T cells, cultured with rIL-7 or rIL-15, migrate in response to rSDF-1. One million CD4+ in vitro-generated central memory T cells were cultured for 3 days in the presence of either medium alone, rIL-4, rIL-7, or rIL-15 (all used at 10 ng/ml), after which the cells were washed and analyzed for in vitro migration in response to stimulation with rSDF-1. The results represent the mean ± SD of triplicate values of two independent experiments.

FIGURE 5. Central memory T cells become susceptible to infection with HIV-1 following culture with rIL-2, rIL-4, rIL-7, or rIL-15. In vitro-generated CD4+ central memory T cells were cultured in absence or presence of rIL-2, rIL-4, rIL-7, or rIL-15 (all used at 10 ng/ml) for 3 days, washed, and infected with the HIV-1 strain HIV-1NDK. After 5 days of culture, the intracellular presence of p24 was measured by immunofluorescence. Histograms from cells stained with isotype control mAb (nearest to the y-axis) are superimposed over histograms of cells stained with the specific anti-p24 mAb KC57 and a FITC-conjugated goat-anti-mouse Ab.
expression of p24 was measured after 5 days of infection with the HIV-1 strains HIV-1 LAI or HIV-1 NDK by immunofluorescence, using the specific Ab KC57. Frequency of p24 expression in noninfected cells or in T cells stained with an isotype control Ab was <2%.

Activated dendritic cells were able to simultaneously activate central memory T cells and to induce CXCR4 expression, following a noncognate interaction. Interestingly, about 30% of the T cells that had up-regulated CXCR4, following interaction with CD40-activated dendritic cells, coexpressed CD25 (Fig. 8C), whereas the addition of exogenous rIL-4 or rIL-7 further enhanced CXCR4 expression (not shown). In contrast, however, the addition of an anti-CD3 mAb to T cell/activated dendritic cell cultures, irrespective of the presence of exogenous cytokines resulted in a rapid loss of expression of CXCR4 and a concomitant strong enhancement of CD25 expression (Fig. 8D). Taken together, these results suggest that CD40-stimulated dendritic cells are able to deliver an “activation” signal(s) to T cells, resulting in the up-regulation of CXCR4, as well as in the induction of CD25 expression, via a pathway that is functionally distinct from that induced by triggering of the TCR/CD3 complex.

Discussion

In this study, we show that the expression of CXCR4 at the surface of T cells is induced by cytokine-dependent signals, as well as via noncognate interactions between memory T cells and activated autologous dendritic cells. CXCR4 is mainly expressed on naïve CD45RO+ T cells and only on a fraction of freshly isolated memory T cells (37). To study the regulation of its expression on the latter type of cells, we have used in vitro-generated CD4+ CD45RO+, CXCR4+ CCR7+ T cells that are reminiscent of central memory T cells (9). A population of CXCR4+ and CCR7+ T cells could also be detected in freshly isolated peripheral blood, and it is hypothesized that the cultured T cells, described in this study, might be an in vitro counterpart of CCR7+ central memory T cells detected in vivo. The use of cultured memory T cells permitted us, at the same time, to avoid the rapid and strong increase in CXCR4 expression that is invariably observed on freshly isolated cord blood and peripheral blood T lymphocytes following their in vitro culture even in the absence of added cytokines (5). In addition, we have used autologous CD34+ hemopoietic progenitor cell-derived dendritic cells that are considered as a relevant counterpart for Langerhans cells for use in in vitro studies, because of their close functional and phenotypic resemblance to the Langerhans cells found in vivo (28).

The CXCR4 expression-inducing cytokines IL-2, IL-4, IL-7, and IL-15 all bind to the γ chain, suggesting that the JAK/STAT signaling pathway activated through their receptors is important for CXCR4 induction. Of note, rIL-9, the receptor of which also contains the γ chain, did not induce CXCR4 expression; however,

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a Intracellular presence of p24 was measured after 5 days of infection with the HIV-1 strains HIV-1 LAI or HIV-1 NDK, by immunofluorescence, using the specific Ab KC57. Frequency of p24 expression in noninfected cells or in T cells stained with an isotype control Ab was <2%.

b CD4+ central memory T cells were cultured in absence or presence of rIL-2, rIL-4, rIL-7, or rIL-15 (10 ng/ml) or resting or CD40-activated dendritic cells for 3 days, as described in Materials and Methods.

c AMD3100 (10 μg/ml) was added 30 min prior to the addition of HIV-1.

FIGURE 6. Activated dendritic cells induce CXCR4 expression on central memory T cells. One million in vitro-generated central memory T cells were cocultured with either 105 CD34+ hemopoietic progenitor cell-derived dendritic cells and 25 × 103 irradiated CD154+ mouse fibroblasts (A) or with dendritic cells (B) or fibroblasts only (C), and the cells were analyzed for CXCR4 surface expression by flow cytometry after 3 days of culture using the biotinylated anti-CXCR4 mAb 12G5 (bold lines), a biotinylated control mAb (dotted lines), and PE-conjugated streptavidin.
FIGURE 7. Induction of CXCR4 on central memory T cells by activated dendritic cells involves CD40/CD154 and CD134/CD134L interactions. In vitro-generated central memory T cells were cocultured with CD34⁺ hemopoietic progenitor cell-derived dendritic cells and CD154-expressing fibroblasts, as described in Materials and Methods, in the presence or absence of combinations of neutralizing mAbs directed at CD40, CD134, CD11a, CD18, CD80, and CD86. Expression of CXCR4 was analyzed by flow cytometry, using the biotinylated anti-CXCR4 mAb 12G5, in the absence (bold lines) or in the presence of mAbs (gray lines) and with a biotinylated control mAb (dotted lines) and PE-conjugated streptavidin.

Dendritic cells, derived from CD34⁺ hemopoietic progenitor cells and activated via CD40, have been shown to express transcripts for a large variety of cytokines, including IL-7 and IL-15, but not IL-2, IL-4, or IL-9 (16). However, it is not clear whether these cells secrete IL-7 and IL-15 protein. Our observation that culture supernatants of activated dendritic cells were unable to induce either CXCR4 expression or proliferative responses by effector memory T cells indicates that this particular subpopulation of dendritic cells does not secrete IL-7 and IL-15 at levels that are adequate for CXCR4 up-regulation. This was not due to a particular property of cord blood-derived T lymphocytes, because similar results were obtained using T cell clones generated from peripheral blood or human skin. The identity of other factors required for cytokine-induced CXCR4 up-regulation is currently being assessed.

as it also failed to induce T cell proliferation, it appears that IL-9 does not act on T lymphocytes (Ref. 38, 39, and this report). Cytokine-mediated induction of CXCR4 expression on memory T cells involves γς chain-mediated signaling, because its induction was inhibited, although to variable extent, in the presence of the inhibitor AG-490, which blocks signaling via JAK-3, a tyrosine kinase that binds specifically to the γς chain (P. Jourdan and H. Yssel, unpublished results).

It is of note that activation of this cytokine-mediated signal transduction pathway does not seem to be sufficient for the induction of CXCR4 surface expression. Specifically, rIL-2 and rIL-15 failed to induce CXCR4 expression in cord blood-derived T cell clones, reminiscent of the CD45RO⁺ CCR7⁻ effector memory T cells, described by Sallusto et al. (9), whereas they induced strong proliferative responses in these cells (data not shown). The observed proliferation indicates that, although the JAK/STAT pathway is involved in this process, the activation of cytoplasmic substrates following cytokine stimulation in these cells was not adequate for CXCR4 up-regulation. This was not due to a particular property of cord blood-derived T lymphocytes, because similar results were obtained using T cell clones generated from peripheral blood or human skin. The identity of other factors required for cytokine-induced CXCR4 up-regulation is currently being assessed.

![Image](326x187 to 517x387)

**Table II. Induction of CXCR4 by activated dendritic cells involves noncognate CD40/CD154 and CD134/CD134L-mediated interactions**

<table>
<thead>
<tr>
<th>Culture Conditions</th>
<th>Inhibition (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CD40</td>
<td>56.5 ± 5.8</td>
</tr>
<tr>
<td>Anti-CD134</td>
<td>59.5 ± 18.3</td>
</tr>
<tr>
<td>Anti-CD40/anti-CD134</td>
<td>82.3 ± 21.0</td>
</tr>
<tr>
<td>Anti-CD154</td>
<td>50.0 ± 7.7</td>
</tr>
<tr>
<td>Anti-CD134L</td>
<td>39.6 ± 6.3</td>
</tr>
<tr>
<td>Anti-CD154/anti-CD134L</td>
<td>79.5 ± 25.7</td>
</tr>
<tr>
<td>Anti-CD80/anti-CD86</td>
<td>27.6 ± 17.5</td>
</tr>
<tr>
<td>Anti-CD11a/anti-CD18</td>
<td>14.4 ± 8.6</td>
</tr>
</tbody>
</table>

*<sup>a</sup> In vitro-generated central memory T cells were cocultured with dendritic cells and CD154-expressing fibroblasts for 3 days in the presence or absence of neutralizing mAbs, as described in Materials and Methods, and expression of CXCR4 was determined by flow cytometry.

*<sup>b</sup> Percentage inhibition (mean ± SD) of CXCR4 expression. Expression of CXCR4 in the presence or absence of mAbs is shown in Fig. 6. Results are from three independent experiments. All values are statistically significant (p < 0.001), as determined by χ² test.

FIGURE 8. Noncognate signals mediated by activated dendritic cells have opposing effects on CXCR4 surface expression as compared with those induced by CD3 complex triggering. One million in vitro-generated central memory T cells were cultured in medium alone (A), with 10 μg/ml plate-bound anti-CD3 mAb and 1 μg/ml soluble anti-CD28 mAb (B), or cocultured with 10⁵ CD3⁴⁺ hemopoietic progenitor cell-derived dendritic cells and 25 × 10⁵ irradiated CD114⁺ mouse fibroblasts in the absence (C) or presence (D) of 1 μg/ml soluble anti-CD3 mAb. After 3 days of culture, CXCR4 and CD25 expression on the T cells was analyzed by flow cytometry using the biotinylated anti-human CXCR4 mAb 12G5, PE-conjugated streptavidin, and an FITC-conjugated anti-CD25 mAb. Data are represented as contour blots of correlated FITC and PE fluorescence (four decade log scale). Quadrant markers are positioned to included >98% of control Ig-stained cells in the lower left (not shown). Representative data of two independent experiments.
sufficient to induce biological activity. It has been suggested that the membrane-bound form of IL-15, rather than soluble IL-15, is biologically active (40). However, the lack of inhibitory activity of a neutralizing anti-IL-15 mAb added to cocultures of effector memory T cells and activated dendritic cells (data not shown) suggests that membrane-bound IL-15 does not play an important role in our culture system.

The importance of the interaction of dendritic cells and T cells in the transmission of HIV has been reported extensively in the literature (19, 21, 22, 41–43). In addition, the ability of dendritic cells to augment infection of T cells by HIV Tropic strains was found to be mediated, at least in part, by the interaction of CD40 and its ligand (44). However, the effect of CD40-mediated signaling on the expression of coreceptors for HIV has not been directly addressed in these studies. Here, we demonstrate that the increased susceptibility of T cells to infection with Tropic strains of HIV, induced by autologous activated dendritic cells, is associated with an up-regulation of CXCR4 expression. This is due, at least in part, to the interaction of CD40 and CD134 with their respective ligands. In addition, we find that CD40 and CD154 play a dual role in this process because the interaction of these molecules is involved in the activation of the dendritic cells, as well as in the propagation of a CXCR4 expression-inducing signal.

The required activation of dendritic cells in vivo involves activated T cells, which express high levels of CD154. CD134 is also expressed on activated T cells (25), whereas CD134L is expressed on dendritic cells following CD40-mediated activation (26). It is possible that central memory T cells express CD154 and CD134 on their surface, which, albeit undetectable by flow cytometry (data not shown), might be functionally active. Interaction of CD154 with CD40, constitutively expressed on dendritic cells, would then result in the up-regulation of other costimulatory molecules and their ligands. This reciprocal dialogue between activated dendritic cells and central memory T cells will lead to the induction of CXCR4 expression on the T cells. Because both central memory T cells and activated Langerhans cells are able to specially migrate to secondary lymph nodes, it is likely that this type of noncognate interactions take places in these sites.

In conclusion, regulation of CXCR4 expression on T cells involves a wide range of signals, including soluble factors such as IL-2, IL-4, IL-7, and IL-15, the receptors of which share the γc chain, cell-surface molecules like CD134 and CD154, as well as other unrelated stimuli such as those mediated by glucocorticoids (45). Further studies will be necessary to determine the precise role, under physiological conditions, of each of these CXCR4 regulatory stimuli on T cell migration and HIV infection.

Acknowledgments
We thank Drs. André van Aghoven, Christophe Caux, Carl Figdor, Pierre Garonne, Toshiyuki Hori, Martin Lipp, Sem Saedon, and John Wijdenes for their kind gifts of reagents; Vera Boulay for technical assistance; and Drs. Nelly Noraz and Naomi Taylor for critical reading of the manuscript and helpful suggestions. The generous supply of cord blood samples by Joelle Peltier and the staff of the Department of Obstetrics and Gynecology, Centre Hospitalier Universitaire Arnaud de Villemeuve, Montpellier, as well as the help of Dr. Frederica Sallusto is greatly acknowledged.

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42. Weissman, D., Y. Li, J. M. Orenstein, and A. S. Fauci. 1995. Both a precursor and a mature population of dendritic cells can bind HIV: however, only the mature population that expresses CD80 can pass infection to unstimulated CD4 T cells. J. Immunol. 155:4111.

