Cutting Edge: IL-4 Induces Functional Cell-Surface Expression of CXCR4 on Human T Cells

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References

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Here we report that IL-4 specifically enhances cell surface expression of CXCR4 on resting peripheral and cord blood T cells. Whereas polarized Th2 clones express variable levels of CXCR4, expression of this receptor is undetectable on polarized Th1 clones but can be induced on the latter cells as well, following short-term culture in the presence of IL-4. The IL-4-induced CXCR4 is functional since interaction with its ligand, stromal-derived factor (SDF)-1, activates the p42 MAP-kinase ERK-2. In addition, although CXCR4 expression is down-regulated following stimulation of T cells and T cell clones via CD28 or CD3 and CD2 cell surface molecules, respectively, it is re-induced by IL-4. These data indicate an important role for IL-4 in rendering CD4+ T cells susceptible to infection with HIV via CXCR4, as well as in promoting SDF-1-induced migration of these cells. The Journal of Immunology, 1998, 160: 4153–4157.

Cutting Edge: IL-4 Induces Functional Cell-Surface Expression of CXCR4 on Human T Cells

Patrick Jourdan,* Claire Abbal,2* Nelly Nora,2† Toshiyuki Hori,8 Takashi Uchiyama,8 Jean-Pierre Vendrell,‡ Jean Bousquet,* Naomi Taylor,† Jérôme Pène,* and Hans Yssel3*

Cutting edge refers to research that presents a new, unexpected finding or insight into a scientific problem. In this case, the article reports a significant observation about the role of interleukin-4 (IL-4) in enhancing cell surface expression of the chemokine receptor CXCR4 on human T cells. This finding is important because it suggests that IL-4 can modulate the susceptibility of T cells to HIV infection via CXCR4, potentially opening new avenues for the development of therapeutic strategies against HIV. The article is published in *The Journal of Immunology* in 1998, volume 160, pages 4153–4157.

**Materials and Methods**

**Cells**

CD4+ T cells were purified by negative selection (purity > 95%) from peripheral and cord blood mononuclear cell preparations, using a mixture of isotype-matched mAbs specific for B cells, monocytes, NK cells, NK cells, CD8+ T cells and erythrocytes, and IgG-coated magnetic beads (Stem Cell Technologies, Vancouver, Canada), according to the manufacturer’s instructions. Cloned T cell lines were generated by using stimulation, cloning, and culture procedures that have been described previously (16). All cultures and experiments were conducted in Yssel’s medium (Ref. 17; Irvine Scientific, Santa Ana, CA), supplemented with 1% human AB serum.

**mAbs, immunofluorescence, and flow cytometry**

Anti-human biotinylated anti-CXCR4 mAb IVR-7 (IgG1; Ref. 7), biotinylated isotype-matched control mAb, phycoerthrin-conjugated streptavidin and FITC-conjugated anti-CD25 mAb (Becton Dickinson, San Jose, CA) were used for flow cytometry. Immunofluorescence staining techniques have been described previously (7), and cells were analyzed by using a FACScalibur flow cytometer (Becton Dickinson).

**Cell culture and stimulation conditions**

One million purified peripheral blood T cells, cord blood T cells, or T cell clones were stimulated with bead-immobilized anti-CD3 (SPV-T3b) and anti-CD28 mAbs (B-T3; Diaclone, Besançon, France) at a ratio of beads/T cells of 5:1, or stimulated with a mitogenic combination of anti-CD2 and anti-CD28 mAbs (B-T3; Diaclone, Besançon, France) at a ratio of beads/T cells of 5:1, or stimulated with a mitogenic combination of anti-CD2 and 39Cl·5 and 6F10.3, kindly provided by Dr. D. Olive, Institut National de la Sante et de la Recherche Medicale U454, 371 Avenue Doyen Gaston Giraud, 34295 Montpellier, Cedex 5, France. E-mail address: yssel@montp.inserm.fr

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*Abbreviations used in this paper: SDF-1, stromal-derived factor-1; MAPK, MAP kinase.*
FIGURE 1. IL-4 enhances cell surface CXCR4 expression on peripheral blood and cord blood T cells. CXCR4 expression was analyzed by flow cytometry on peripheral blood (A) or cord blood (B) T cells immediately following isolation or following culture of the cells for 72 h in medium supplemented with either 2 ng/ml rIL-2, 0.5 pg/ml rIL-12, or 5 ng/ml rIL-4, as indicated. Cells stained with isotype-matched control mAb are depicted in grey histograms. Representative data of three (cord blood) and six (peripheral blood) independent experiments are shown, respectively.

Results and Discussion

Freshly isolated peripheral blood T cells express low levels of cell surface CXCR4 that are strongly enhanced following incubation of the cells in medium only (Fig. 1A). This spontaneous induction of CXCR4 expression was observed after 6 h of culture in medium containing either human or FCS, as well as in serum-free medium (data not shown) and lasted for as long as the cells could be maintained in culture. It has recently been reported that freshly isolated Langerhans cells contain intracellular CXCR4 protein that is rapidly transported to the cell surface during in vitro culture (20), and the observed spontaneous induction of CXCR4 on T cells might be due to a similar mechanism. The addition of rIL-2 had no effect on the spontaneous induction of CXCR4 expression on cultured T cells. However, addition of rIL-4 to cultures of peripheral blood T cells resulted in a further increase in CXCR4 expression, with maximal levels of expression observed after 48–76 h of incubation (Fig. 1A). Similar results were obtained using purified CD44+ cord blood T cells where rIL-4, but not rIL-2, strongly enhanced cell surface expression of CXCR4. In addition, incubation of cord blood T cells with rIL-12, which has a strong IFN-γ-enhancing effect on these cells, did not affect CXCR4 expression (Fig. 1B). The effect of rIL-4 was tested at a concentration range between 0.5 and 500 ng/ml, and optimal induction of CXCR4 expression was observed at concentrations of 5 ng/ml rIL-4.

All five Th2, but not Th1, clones included in this study expressed CXCR4 at the cell surface (Fig. 2), albeit at variable levels. Although these results suggest that CXCR4 might discriminate between Th cell subsets with different cytokine production profiles, CXCR4 expression on Th2 clones is likely to be the result of

FIGURE 2. Cell surface CXCR4 expression on human Th1 and Th2 clones. Cell surface expression of CXCR4 was analyzed by flow cytometry on cord blood-derived Th1 and Th2 clones cultured in the presence of rIL-2 (normal histograms) or rIL-2 and rIL-4 (bold histograms) for 72 h. Cells stained with isotype-matched control mAb are depicted in grey histograms.
induction by endogenously produced IL-4. Indeed, incubation of cord blood-derived (Fig. 2) and peripheral blood-derived (data not shown) Th1 clones with rIL-4 for 72 to 96 h also resulted in surface expression of CXCR4, at levels comparable to those on Th2 clones. Fourteen of 15 Th1 clones tested could be induced to express CXCR4 following incubation in the presence of rIL-4 (data not shown). The latter results suggest that the induction of CXCR4 is not due to the outgrowth of a small subpopulation of CXCR4-expressing cells. However, it remains to be determined whether the IL-4-mediated effects are due to de novo synthesis of CXCR4 or relocation of preexisting receptor to the cell surface.

Carroll et al. (15) have reported that stimulation of peripheral blood T cell with immobilized anti-CD3 and CD28 mAbs resulted in enhanced expression of CXCR4 mRNA after 72 h of culture. In view of this observation, it was initially not clear why the Th1 clones used in this study, which were generated from peripheral and cord blood T cells following in vitro activation and differentiation, did not express CXCR4. Moreover, IL-4-induced expression of CXCR4 on T cell clones was found to be down-regulated following each restimulation of these cells with allogeneic PBMC and phytohemagglutinin that was used to maintain the cells in culture (data not shown). We therefore studied the combined effects of IL-4 and activation via CD2, CD3, and CD28 molecules on CXCR4 expression. As described above, CD4+ T cells, cultured for short periods of time in medium only, expressed enhanced surface levels of CXCR4 (Figs. 1 and 3). Surprisingly, 24 h following stimulation of the same cells with immobilized anti-CD3/anti-CD28 mAbs or with a combination of mitogenic anti-CD2 and anti-CD28 mAbs, CXCR4 surface expression was significantly diminished, although high expression levels of CD25, a marker of cell activation, were induced (Fig. 3A). Moreover, CXCR4 expression on the surface of a Th1 clone grown in the presence of rIL-4 was quickly lost following stimulation of these cells via CD2 or CD3 and CD28 molecules, respectively (Fig. 3B). Surface expression of CXCR4 did not change following continued culture of the cells in the presence of exogenous rIL-2. However, addition of both rIL-2 and rIL-4 to cultures of activated T cells or T cell clones resulted in a gradual re-induction of high levels of CXCR4 expression that were again maximal between 72 and 96 h (data not shown).

In their study, Carroll et al. (15) reported increased CXCR4 mRNA in activated T cells and although cell surface expression of CXCR4 was not measured, T cells showed greater susceptibility to infection with a T-lymphotropic HIV strain following anti-CD3/CD28-mediated stimulation. Although we have no explanation for the discrepancy between CXCR4 transcription and CXCR4 cell surface expression, following activation of T cells, the observed increase in HIV infection of stimulated PBMC T cells could be due to an entry of HIV that is not restricted by CXCR4 expression. Moreover, our results are in agreement with those of Bleul et al. (14) who reported that CXCR4 is predominantly expressed on unactivated CD26low, CD45RA+, CD45RO+ T lymphocytes. Finally, antigen-specific stimulation of peripheral blood T cells was found to result in a down-regulation, rather than an induction of CXCR4 expression (our unpublished data).

As has been shown for many seven-transmembrane proteins, chemokine R signaling is dependent on coupling to Bordetella pertussis toxin-sensitive G proteins, resulting in downstream signaling events, including those mediated via the Ras/Raf/MAPK pathway (21, 22). To investigate whether IL-4-induced CXCR4 expression on T cells was functional, the ability of SDF-1 to activate the MAPK-signal transduction cascade was assessed. As shown in Fig. 4A, activated ERK-2 MAPK could be detected in the rIL-4-cultured, CXCR4-expressing, Th1 clone HY-243 following...
stimulation of the cells with 25 nM rSDF-1. Levels of activated ERK-2 MAPK were comparable to those induced following triggering of the TCR/CD3 complex on these cells with a cross-linked anti-CD3 mAb. Although equivalent activation of ERK-2 was observed upon CD3-engagement of the Th1 clone, cultured in the absence of rIL-4, stimulation of the MAPK pathway was not observed upon treatment of these cells with rSDF-1. Similar results were obtained with the cord blood-derived Th1 clone CB-214 (data not shown). The observed differences between T cell clones cultured in the absence or presence of rIL-4 were not due to changes in global levels of signaling proteins, because ERK-2 expression, as well as levels of the T cell-specific ZAP-70 protein tyrosine kinase, were equivalent under both culture conditions (Fig. 4, A and C). Although T cell clones, cultured in rIL-2 and rIL-4, constitutively expressed low levels of activated ERK-2 before activation, it is important to note that IL-4 itself is not able to activate ERK-2 (23). Taken together, it is shown here that interaction of CXCR4 with its ligand results in a rapid activation of the Ras/Raf/MAPK pathway on T lymphocytes. However, it remains to be determined whether the MAPK signaling cascade is also activated upon binding of HIV to CXCR4 and, if so, whether this activation pathway plays a role in HIV entry as well.

In view of the role of CXCR4 as a T-tropic coreceptor for HIV entry, we determined whether IL-4-mediated induction of CXCR4 expression on the cell surface results in a higher susceptibility to infection with HIV, as demonstrated by the intracellular presence of p24 viral protein. Th1 clones that had been cultured in the presence of rIL-2 alone, and which therefore did not express CXCR4 at the cell surface, were negative for the presence of intracellular p24, following coculture with the T-tropic strain HIV-1LA1 (Table I). By contrast, in a significant percentage of Th1 clones expressing IL-4-induced CXCR4, intracellular p24 protein could be detected intracellularly. These data, suggesting that Th2 cells, which dominate in IL-4-induced responses, might be more susceptible to infection with HIV than Th1 cells support the results of Maggi et al. (24), who reported that HIV replicates preferentially in Th2 and not in Th1 clones in vitro. Although the observed inability of HIV to replicate in Th1 cells is independent from its capacity to enter into the cell, our results might explain, at least in part, the decreased susceptibility of these cells to infection with certain strains of HIV.

Acknowledgments

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References


Table I. Human Th1 clones become susceptible to infection with HIV following IL-4-mediated up-regulation of CXCR4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Infected Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>2.0</td>
</tr>
<tr>
<td>IL-2+IL-4</td>
<td>0.7</td>
</tr>
<tr>
<td>CB-214</td>
<td>1.5</td>
</tr>
<tr>
<td>IL-2</td>
<td>1.2</td>
</tr>
<tr>
<td>CB-214</td>
<td>2.3</td>
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* Determined by intracellular staining for p24 gag protein at day 6 after infection with HIV-1a. Under these conditions, 20 and 50% of the T lymphoblastoid cell line CEM, used a positive control, will be infected.