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Expression of CXCR4 in Eosinophils: Functional Analyses and Cytokine-Mediated Regulation

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We examined the expression of transcripts of a panel of chemokine receptors in human eosinophils and found intense constitutive expression of CXCR4 mRNA. Although surface CXCR4 protein was hardly detectable in the peripheral blood or freshly isolated eosinophils, surface expression of CXCR4 became gradually apparent during incubation at 37°C. In contrast, the level of CCR3 expression was virtually unchanged during the incubation. Stromal cell-derived factor-1α (SDF-1α), the natural ligand of CXCR4, elicited an apparent Ca²⁺ influx in these cells and induced a strong migratory response comparable to that by eotaxin. The surface expression of CXCR4 in eosinophils was up-regulated by IFN-γ, TNF-α, and TGF-β while it was down-regulated by IL-4 and eosinophil-directed hemopoietins such as IL-5. The CXCR4 expression did not always parallel the apoptotic changes in cytokine-treated eosinophils. In contrast to IL-4 and IFN-γ, IL-5 potently reduced the level of CXCR4 mRNA. It seems unlikely that CXCR4 is fundamentally involved in the pathogenesis of allergic disorders by inducing the migration of eosinophils toward inflammatory sites, because a Th2-dominant state down-regulates eosinophil CXCR4 expression. However, CXCR4 may affect the size of the mobilizable pool by holding eosinophils at noninflamed tissues. Th2-dominant state may favor the liberation of eosinophils by down-regulating CXCR4 expression. The interplay between CXCR4 and SDF-1α in eosinophils potentially plays an important role in the accumulation of these cells at the allergic inflammatory sites. The Journal of Immunology, 2000, 164: 5935–5943.

M assive accumulation of eosinophils is a characteristic aspect of inflammation associated with allergic diseases. Historically, eosinophils had long been assumed to play an anti-inflammatory role in allergic disorders by virtue of their antagonizing effects on mast cell-derived mediators such as histamine. However, it has become apparent that the eosinophils involved in these conditions are highly destructive: eosinophil-derived mediators, especially various cationic proteins, contribute to the tissue damage associated with allergic diseases. Along with the progression of allergic reactions, eosinophils migrate from the blood compartment to inflamed tissues and function as allergic inflammatory cells (1). The processes involved in tissue eosinophilia consist of a complex interplay of various pathways and are not fully understood. However, several chemoattractants generated at inflammatory sites potentially play a pivotal role in the recruitment of eosinophils in humans as well as in animals (2).

Chemotactic cytokines, termed chemokines, are now recognized as essential participants in the sequence of events by which circulating leukocytes migrate toward inflammatory sites. Chemokines are divided into two major subfamilies based on the sequence of arranged cysteine groups: the CXC subfamily and the CC subfamily. Two minor subfamilies, i.e., the CX3C and the C chemokines, are also categorized. To date, ~40 chemokines have been identified, and 15 chemokine receptors, i.e., five CXC chemokine receptors (CXCRs), eight CCRs, one CX3CR, and one XCR, have been cloned. It has been reported that eosinophils express CCR3, and ligands of CCR3 such as eotaxin induce strong migration of eosinophils (3, 4). Expression of CCR1 (5), and under certain circumstances expression of CXCR2 (6), have also been reported in eosinophils. The expression and function of these receptors have been extensively investigated. In contrast, there is little information regarding the expression and function of other chemokine receptors in eosinophils.

In this study, we have examined the expression of transcripts of a panel of chemokine receptors in human eosinophils and found intense constitutive expression of CXCR4 mRNA as well as CCR3 mRNA. Here, we demonstrate that surface expression of CXCR4 is inducible in eosinophils and that stromal cell-derived factor-1α (SDF-1α)3 elicits strong eosinophil migration comparable to that induced by eotaxin. The effects of cytokines on eosinophil CXCR4 expression have also been investigated.

Materials and Methods

Reagents and mAbs

The following reagents were purchased as indicated: recombinant human SDF-1α, eotaxin, IL-4, and TGF-β1 (PeproTech, London, U.K.); IFN-γ (Shionogi Pharmaceutical, Osaka, Japan); TNF-α (Dainippon Pharmaceutical, Osaka, Japan); C5a, cytochalasin B, and cycloheximide (Sigma, St. Louis, MO); ionomycin (Seikagaku, Tokyo, Japan); pertussis toxin (PTX) (Calbiochem-Behring, La Jolla, CA); anti-Fas mAb (CH-11, subclass IgM)

3 Abbreviations used in this paper: SDF-1α, stromal cell-derived factor-1α; PTX, pertussis toxin; MOPS, molecules of equivalent soluble fluorochrome units; EPO, eosinophil peroxidase; OPD, o-phenylenediamine dihydrochloride, EDN, eosinophil-derived neurotoxin.

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and mouse IgM A1 with irrelevant specificity (Medical and Biological Laboratories, Nagoya, Japan and Organon Teknika, West Chester, PA, respectively). Anti-CCR3 mAb (7) was provided by Dr. H. Kawasaki (Institute of Medical Science, University of Tokyo, Tokyo, Japan). IL-3 and IL-5 were kindly donated by Kirin Brewery (Tokyo, Japan) and Suntory (Osaka, Japan), respectively. Anti-CCR3 mAb (7) was provided by Dr. H. Kawasaki (Institute of Medical Science, University of Tokyo, Tokyo, Japan). IL-3 and IL-5 were kindly donated by Kirin Brewery (Tokyo, Japan) and Suntory (Osaka, Japan), respectively.

**Eosinophil separation and culture conditions**

Eosinophils were separated from normal volunteers who had no history of allergy, as previously described (6). In brief, buffy coat cells were obtained from venous blood by dextran T300 sedimentation. Eosinophils were isolated by Percoll (1.088 g/ml; Pharmacia, Uppsala, Sweden) density centrifugation. Unless Percoll separation achieved purity of 90%, the eosinophils were further purified by negative selection using anti-CD16-bound micromagnetic beads (Miltenyi BioTech, Bergisch-Gladbach, Germany) and a magnetic-activated cell sorter column (Miltenyi BioTech) as the second step (9). After this negative selection, the mean eosinophil purity was consistently >99%, and the viability was consistently >95%. Eosinophils (0.5–1.0 x 10⁶) were cultured in RPMI 1640 (Life Technologies, Minneapolis, MN) and anti-CD16-PE (Coulter) at 10⁵ cells/ml for 45 h at 37°C. During flow cytometry procedures, contaminating neutrophils were discriminated on the basis of their different fluorescence properties.

**Flow cytometry of isolated eosinophils**

Isolated eosinophils were washed in PBS supplemented with 3% FCS and 0.1% NaCl, and then incubated with anti-CXCR4 mAb (12G5; Pharmingen, San Diego, CA) at 10 µg/ml for 60 min at 4°C. An isotype-matched mouse IgG2a with irrelevant specificity (UPC 10; Sigma) was used as a negative control. After washing, the cells were stained with FITC-labeled goat F(ab)₂ against mouse IgG (Jackson ImmunoResearch, West Grove, PA) at 7 µg/ml for 30 min at 4°C. During flow cytometry procedures, contaminating neutrophils were discriminated on the basis of their different fluorescence properties.

**Flow cytometry of eosinophils in whole blood**

FACS analysis of eosinophils in whole venous blood was performed as previously described (11). In brief, blood was anti-coagulated with EDTA, and an equal volume of FACS buffer (PBS with 3% FCS and 0.1% NaN₃) was added. Cells were stained with anti-CXCR4-FITC (12G5; R&D Systems, Minneapolis, MN) and anti-CD16-PE (Coulter) at 10 µg/ml for 45 min at 4°C. An isotype-matched mouse IgG2a-FITC (R&D Systems) and
mouse IgG1-PE (Immunotech, Marseille, France) with irrelevant specificity were used as negative controls. Contaminating erythrocytes were eliminated with a lysis buffer (Ortho Diagnostic Systems, Tokyo, Japan). Granulocytes were discriminated on the basis of different forward/side scatter properties, and electronic gates were set on CD16-negative cells to identify eosinophils.

**Measurement of intracellular calcium concentration**

Purified eosinophils (purity, >99%) were resuspended in HBSS with Ca\(^{2+}\) and Mg\(^{2+}\) (Life Technologies) and 2% BSA at a cell density of 2.0 × 10\(^6\)/ml. Fura-2 AM (Dojindo, Tokyo, Japan) was added at a final concentration of 2 μM. After incubation for 20 min, excess dye was removed by centrifugation, and the cells were resuspended in a buffer containing 119 mM NaCl, 5 mM KCl, 1 mM CaCl\(_2\), 1 mM MgCl\(_2\), 0.03% human serum albumin, and 25 mM PIPES, pH 7.4, at a concentration of 1.6 × 10\(^6\) cell/ml. Ca\(^{2+}\) influx was measured using excitation at 340 and 380 nm on a Hitachi F-2500 fluorescence spectrometer (Hitachi, Tokyo, Japan). Calibration was performed using 0.1% Triton X-100 for total Ca\(^{2+}\) release and 10 mM EGTA to chelate free Ca\(^{2+}\).

**Chemotaxis assay of eosinophils**

Eosinophil migration was measured using a 96-well multiwell Boyden chamber (Neuroprobe, Bethesda, MD) and a 10-μm-thick polycarbonate membrane filter with pores of 5 μm in diameter (Neuroprobe), as previously described (8). Aliquots of 362 μl of triplicate samples were transferred into the lower wells, while 200 μl of a cell suspension that contained 1.5 × 10\(^6\) eosinophils was introduced into each well of the top compartment. After incubation for 90 min, the eosinophil peroxidase (EPO) activity of cells at the bottom of wells was determined with 200 μl of 0.05 M Tris-HCl, pH 8.0, containing 0.1% (v/v) Triton X-100, 0.1 mM o-phenylenediamine dihydrochloride (OPD; Sigma) and 50 mM hydrogen peroxide. The OD was read at 490/570 nm in an ELISA reader (Model 550; Bio-Rad, Hercules, CA). Data were analyzed with the Microplate Manager III program (Bio-Rad), and the numbers of migrated eosinophils were calculated based on a standard curve established with varying known numbers of eosinophils.

**Eosinophil degranulation**

Freshly isolated eosinophils (purity, >99%) were cultured for 24 h with or without IFN-γ (10 ng/ml). These cells were pretreated with 5 μg/ml of cytochalasin B for 5 min and then stimulated with SDF-1α (333 ng/ml) or C5a (5 × 10\(^{-9}\) M) for 5 h. The level of eosinophil-derived neurotoxin (EDN) was measured with an EDN ELISA kit (Medical and Biological Laboratories).

**Analysis of apoptotic cells**

Differential analysis of apoptotic and necrotic cells was performed using a MEBCYTO apoptosis kit (Medical and Biological Laboratories). Apoptotic cells were quantitatively determined by their ability to bind annexin V and exclude propidium iodide. Cells stained with propidium iodide were considered to be necrotic cells. In some experiments, eosinophils were double-stained with anti-CXCR4 mAb (12G5, 10 μg/ml) followed by a second step reaction Ab (PE-conjugated goat F(ab')\(_2\); against mouse IgG Fc; Beckman Coulter, Tokyo, Japan) and FITC-conjugated annexin V.

**Statistics**

Unless otherwise noted, all data are expressed as the mean ± SEM, and differences between values were compared by the paired t test.

**Results**

**Expression of CXCR4 in eosinophils**

Eosinophils were highly purified by means of Percoll density gradient centrifugation followed by negative selection using anti-CD16 microbeads to apparent homogeneity. Expression of transcripts of 15 chemokine receptors was investigated by RT-PCR, and Fig. 1 shows the results of a representative experiment from among four different donors. Although CXCR2 and CCR1 transcripts were weakly detected in some specimens, eosinophils from all of the donors strongly expressed both CXCR4 and CCR3 transcripts. Having observed the consistent expression of CXCR4 mRNA, we next studied the expression of the CXCR4 protein in eosinophils. Unexpectedly, surface expression of CXCR4 was hardly detectable when Percoll-separated eosinophils were immediately stained with anti-CXCR4 mAb. Although no significant expression of CXCR4 was induced when incubated at 4°C (Fig. 2A), these eosinophils started to express CXCR4 during incubation at 37°C. Apparent surface expression of CXCR4 was observed within 4 h of incubation at 37°C, and the level of expression increased linearly up to 24 h of incubation (Figs. 2, B and C). It should be mentioned that the level of CCR3 expression was virtually unchanged during the incubation at 37°C (Fig. 2D).

To determine whether the Percoll separation steps modulate the expression of CXCR4 in eosinophils, expression of CXCR4 was examined in whole blood eosinophils in place of Percoll-separated eosinophils. As shown in Fig. 3, eosinophils in fresh whole blood expressed no significant amount of CXCR4 on their surface. However, as observed with Percoll-separated eosinophils, expression of eosinophil CXCR4 in whole blood eosinophils became evident after incubation for 24 h at 37°C (Fig. 3C). These results indicate that the purification step did not modulate the expression of CXCR4, and we therefore used Percoll-separated eosinophils in the following experiments.

**CXCR4-mediated eosinophil activation**

In the next series of experiments, we investigated whether eosinophil CXCR4 is functionally activated by a specific ligand for CXCR4, SDF-1α. As shown in Fig. 2, freshly isolated eosinophils did not express significant amounts of CXCR4 on their surface, but SDF-1α elicited a small but apparent Ca\(^{2+}\) influx in these cells (Fig. 4A). In cells incubated for 24 h at 37°C, much stronger influx of Ca\(^{2+}\) was observed (Fig. 4C). The magnitude of Ca\(^{2+}\) influx elicited by SDF-1α was comparable to that induced by eotaxin (Fig. 4D). Furthermore, SDF-1α-induced Ca\(^{2+}\) influx was completely attenuated by treatment with PTX (Fig. 4E), indicating the involvement of G proteins of the G\(_i\) class in the signal transduction pathways. To determine whether CXCR4 expressed in eosinophils has functional relevance, we tested the migration-inducing ability of SDF-1α. As shown in Fig. 5, SDF-1α was capable of inducing a migratory response in eosinophils; although SDF-1α did not induce significant migration in freshly isolated cells, eosinophils incubated for 24 h at 37°C exhibited a migratory response toward SDF-1α in a dose-dependent manner. Apparent migration was observed at 33 ng/ml of SDF-1α, and much stronger migration was observed at 333 ng/ml of SDF-1α. It should be noted that SDF-1α-induced eosinophil chemotaxis as strongly as eotaxin, which is known as the most potent eosinophil chemoattractant. Furthermore, the migration induced by SDF-1α was chemotactic rather than chemokinetic: when the same concentration of SDF-1α was added to both the upper and lower wells, the migration was significantly reduced (data not shown). No significant decrease in

**FIGURE 1.** mRNA expression of chemokine receptors in human eosinophils. Eosinophils were purified by means of Percoll density gradient centrifugation followed by negative selection using anti-CD16 microbeads (purity, >99%), and RT-PCR was performed as described in Materials and Methods. The PCR products were electrophoresed and then visualized with ethidium bromide. A representative example of four separate experiments is shown, and the other experiments all showed similar results. BA; β-actin.
granulation was noted in cells that had migrated in response to SDF-1α compared with freshly isolated or 24-h-cultured eosinophils (Fig. 6). In fact, in vitro experiments showed that CXCR4 was not involved in the process leading to degranulation: SDF-1α failed to induce significant release of EDN from eosinophils cultured for 24 h with or without IFN-γ or from freshly isolated cells (Fig. 7).

Modulation of surface CXCR4 expression in eosinophils by cytokines

In the next series of experiments, we examined the effects of cytokines on CXCR4 expression by eosinophils during 24 h of incubation. As shown in Table II, TGF-β1, IFN-γ, and TNF-α upregulated the expression of CXCR4. In contrast, eosinophil-directed hemopoietins, i.e., IL-3, IL-5, and GM-CSF, almost completely attenuated the surface expression of CXCR4. In addition, IL-4 also drastically down-regulated the CXCR4 expression (Table II and Fig. 8A). As shown in Fig. 8B, as small as a femtomolar level of IL-5 was sufficient to inhibit the CXCR4 expression. Half-maximal inhibition was observed at a concentration of ~100 fM of IL-5 with dose-dependent inhibition seen between 0.1 fM and 10 pM. For IL-4, half-maximal inhibition was observed at a concentration of ~1 pM with dose-dependent inhibition seen between 10 fM and 10 pM. On a molar basis, IL-5 was 10-fold more potent than IL-4. Furthermore, delayed addition of IL-5 or IL-4 effectively down-regulated CXCR4 expression. When eosinophils were cultured for 24 h without addition of any factors and then treated with IL-5 or IL-4, the level of CXCR4 expression in the
eosinophils was decreased at 6 and 24 h after the addition of each cytokine (data not shown).

Substantial evidence has shown that isolated eosinophils rapidly undergo apoptosis. In fact, when apoptotic cells were quantita-

FIGURE 4. SDF-1α caused calcium influx to eosinophils. Calcium influx in highly purified eosinophils (purity, >99%) just after purification (A and B) and eosinophils cultured for 24 h at 37°C without (C and D) or with (E) further treatment with PTX. E, Cultured cells were treated with PTX at 100 nM for 2 h at 37°C, and then stimulated sequentially with SDF-1α and ionomycin. The data shown are representative of two independent analyses from different donors, each showing similar results. The concentration of chemokines and ionomycin were 400 ng/ml and 1 μM, respectively.

FIGURE 5. SDF-1α induced migration of eosinophils. The migration-inducing activity of SDF-1α and eotaxin was analyzed using eosinophils just after purification (purity, 96.3 ± 1.3%) and eosinophils cultured for 24 h at 37°C. The data are expressed as the percentage of total cells introduced into each of the upper wells (mean ± SEM, n = 4). The effects of the concentration of chemokines were analyzed by the two-way ANOVA test. When this test indicated a significant differences between concentrations (indicated as ++, p < 0.01), Fisher’s protected least significant difference (PLSD) test was used to compare individual groups. **, p < 0.01 vs values of eosinophil migration in control buffer at the same time point.

FIGURE 6. Photomicrographs of eosinophils that were freshly isolated (A), 24-h-cultured (B), and migrated in response to SDF-1α (C). The photos are representatives of two different experiments and another showed similar results. Magnification, ×600.

FIGURE 5. SDF-1α induced migration of eosinophils. The migration-inducing activity of SDF-1α and eotaxin was analyzed using eosinophils just after purification (purity, 96.3 ± 1.3%) and eosinophils cultured for 24 h at 37°C. The data are expressed as the percentage of total cells introduced into each of the upper wells (mean ± SEM, n = 4). The effects of the concentration of chemokines were analyzed by the two-way ANOVA test. When this test indicated a significant differences between concentrations (indicated as ++, p < 0.01), Fisher’s protected least significant difference (PLSD) test was used to compare individual groups. **, p < 0.01 vs values of eosinophil migration in control buffer at the same time point.

FIGURE 6. Photomicrographs of eosinophils that were freshly isolated (A), 24-h-cultured (B), and migrated in response to SDF-1α (C). The photos are representatives of two different experiments and another showed similar results. Magnification, ×600.

Conversely, IFN-γ significantly reduced the number of apoptotic cells.
iodide-negative cells are defined as apoptotic, and propidium iodide-positive cells are defined as necrotic. Data are expressed as the mean with FITC-conjugated annexin V and propidium iodide. Annexin V-negative and propidium iodide-negative cells are defined as alive. Annexin V-positive and propidium

number (5 each cytokine was 10 ng/ml, except for TNF-α).

6 incubated in culture medium alone or incubated with control IgM (mean

7 duplicate experiments. *, p < 0.05 vs values of EDN release in control buffer at the same time point.

cells, while it failed to suppress and actually up-regulated the eosinophil CXCR4 expression (Table II). Finally, treatment of eosinophils with anti-Fas mAb slightly but significantly decreased the number of living cells, whereas it failed to up-regulate CXCR4 expression (Table II).

Modulation of CXCR4 mRNA expression in eosinophils by cytokines

The surface expression of CXCR4 by eosinophils was significantly but partially inhibited by treatment with a protein synthesis inhibitor, cycloheximide (MESF: 86.9 ± 3.0% and 59.5 ± 7.7% of inhibition for 4-h and 24-h-cultured cells, respectively, n = 3), indicating that de novo protein synthesis is involved in the process leading to the surface expression of CXCR4. In the last series of experiments, we studied cytokine-mediated regulation of CXCR4 expression at the mRNA level. When we examined the expression of CXCR4 mRNA by RT-PCR amplification, we observed that treatment with IL-5, but not IL-4, apparently decreased the level of CXCR4 mRNA, although both cytokines suppressed surface protein expression of CXCR4. In addition, CXCR4 mRNA remained at the baseline level in eosinophils cultured with IFN-γ. In contrast, none of these cytokines had any effect on the level of CCR3 mRNA (Fig. 10).

Discussion

In this study, we have demonstrated the functional expression of CXCR4 in human eosinophils. Among the 15 chemokine receptors, intense expression of CXCR4 as well as CCR3 transcripts was always detected in freshly isolated eosinophils (Fig. 1). While we were preparing this manuscript, Chelucci et al. reported the surface expression of CXCR4 in the eosinophil lineage during the culture of purified hemopoietic progenitors (12), although they did not examine the functional relevance of CXCR4 in these cells. In contrast to those cultured eosinophils, we observed that surface CXCR4 protein was hardly detected in freshly isolated eosinophils (Fig. 2). However, surface CXCR4 expression gradually became apparent during incubation at 37°C. Because surface CXCR4 in cultured eosinophils was widely variable, it is of importance to clarify the control mechanisms of eosinophil CXCR4 expression. In this study, we observed that several cytokines exerted reciprocal and divergent effects on eosinophil CXCR4 expression (Table II). The patterns of cytokine-mediated regulation of CXCR4 expression in eosinophils seem to be distinct from those in other types of leukocytes such as T lymphocytes. In T lymphocytes, IL-4 up-regulates (13–15) and IFN-γ down-regulates (16) CXCR4 expression, while both cytokines exerted completely opposite effects on eosinophils.

Studies of time course and various conditions for CXCR4 expression raised a possibility that the expression of CXCR4 is related to apoptotic changes. IL-5, IL-3, and GM-CSF, all of which are known to rescue eosinophils from apoptosis (17–19), completely attenuated CXCR4 expression in eosinophils. Furthermore, the concentration of IL-5 necessary for the inhibition of CXCR4 induction matched the reported range required for inhibition of eosinophil apoptosis (20) (Fig. 5B). Consequently, it would be reasonable and even attractive to speculate that CXCR4 is preferentially induced in eosinophils undergoing apoptosis, and that these cells are then eliminated from the circulation by the interplay between CXCR4 and ubiquitously expressed SDF-1α. However, the level of eosinophil CXCR4 expression did not always parallel

![Image](http://www.jimmunol.org/DownloadedfrombyguestonJuly25,2017)
the apoptotic changes (Table II). IL-4, which is devoid of anti-apoptotic effects, almost completely attenuated CXCR4 expression (Table II). On the contrary, IFN-γ, which is known to block eosinophil apoptosis (Ref. 20 and Table II), efficiently up-regulated CXCR4 expression. It might be possible that each cytokine may regulate eosinophil CXCR4 expression via different pathways or mechanisms. However, these discrepancies observed between apoptotic changes and CXCR4 expression in cytokine-treated eosinophils, together with the direct determination of CXCR4 expression in nonapoptotic populations (Fig. 9), strongly suggest that eosinophil CXCR4 expression cannot be explained merely as a consequence of apoptotic changes in these cells.

Spontaneous sustained increase in CXCR4 expression similar to our present findings has also been reported for monocytes and lymphocytes (21, 22). In these cells, surface expression of CXCR4 seems not to be regulated at the mRNA level but mainly at the protein level, such as receptor trafficking between the intracellular space and cell surface (23). In the present study, despite the constitutive expression of CXCR4 mRNA (Fig. 1), freshly isolated eosinophils did not express significant amounts of CXCR4 protein on their surface (Fig. 2). Furthermore, spontaneous sustained increase in CXCR4 expression was reversibly down-regulated by delayed addition of IL-4 and IL-5. These findings strongly suggest that surface CXCR4 expression in eosinophils is regulated, at least in part, at the level of receptor trafficking. However, the expression of surface CXCR4 dose not seem to be regulated merely at the level of posttranscription. The process leading to the expression of CXCR4 in eosinophils was partly inhibited by cycloheximide, indicating that the sustained induction of surface CXCR4 expression involves a cycloheximide-sensitive component. Although a direct relationship between the level of mRNA and the amount of surface protein was not always observed in cytokine-mediated alteration of CXCR4 expression, IL-5 apparently down-regulated the level of both mRNA and surface protein of CXCR4 (Table II and Fig. 10). Taken together, it seems plausible that surface CXCR4 expression in eosinophils is modulated not only at the level of posttranscription but also at least in part at the level of transcription.

The most striking findings of our study are that surface expression of CXCR4 in eosinophils has functional relevance, and that SDF-1α induced strong eosinophil migration comparable to that
transcriptional regulation of SDF-1 of tissues (26, 29), and there has been little evidence demonstrating complicated in eosinophil influx observed during ongoing allergic re-
kines (28). This inducible tissue-specific eotaxin expression is im-
mediated migration is not the primary mechanism of the “selec-
tive migration of eosinophils toward inflammatory sites. Firstly, in con-
volved in the pathogenesis of allergic disorders by inducing the
accumulation of eosinophils has been established in vivo as well as in
vitro. In contrast, the in vivo role of CXCR4/SDF-1α-mediated chemotaxis is yet to be established and remains totally speculative,
but it seems unlikely that CXCR4/SDF-1α is fundamentally in-
volved in the pathogenesis of allergic disorders by inducing the
migration of eosinophils toward inflammatory sites. Firstly, in con-
trast to CCR3, the expression of which is restricted only to eosin-
ophils (4) and basophils (24, 25), CXCR4 is expressed in various
white blood cells (reviewed in Ref. 26), indicating that CXCR4-
mediated migration is not the primary mechanism of the “selective”
accumulation of eosinophils observed in allergic inflammation.
Secondly, transcription of eotaxin mRNA is induced by in
vivo Ag challenge (27) and in vitro stimulation with certain cyto-
kines (28). This inducible tissue-specific eotaxin expression is im-
plicated in eosinophil influx observed during ongoing allergic re-
actions. However, SDF-1α is constitutively expressed in a variety of
tissues (26, 29), and there has been little evidence demonstrating transcriptional regulation of SDF-1α. Finally, allergic diseases ex-
hibit polarized Th2 responses that are closely implicated in the
onset and outcome of these disorders (30). However, the expres-
sion of CXCR4 in eosinophils is down-regulated by IL-4 and IL-5
and up-regulated by IFN-γ, indicating that a Th1-dominant state
rather than a Th2-dominant state favors eosinophil CXCR4 ex-
pression. Thus, in view of Th1/Th2 governance in allergic inflam-
mation, it seems unlikely that eosinophil CXCR4 mediates a major
mechanism of migration of eosinophils in allergic diseases.

However, CXCR4 in eosinophils might be indirectly involved in
the exacerbation of eosinophilic inflammation. A substantial body
of evidence indicates that CXCR4 regulates myelopoiesis and lym-
phopoiesis in a stage-specific fashion: CXCR4 is preferentially
expressed in immature progenitors in the bone marrow or thymus
during the maturation stage (31–35), and SDF-1α retains these
cells via CXCR4 to prevent their liberation from these tissues into
the circulation. Thus, CXCR4 in immature leukocytes potentially
serves as an anchor for these cells by inhibiting their movement
rather than inducing their migration. A similar situation can be
imagined for CXCR4 in mature eosinophils: eosinophil CXCR4
may affect the size of the mobilizable pool, and anchorage of ma-
ture eosinophils by SDF-1α may be crucial for retaining eosino-
phils in noninflamed tissues. Th2-dominant state may favor the
liberation of eosinophils by down-regulating CXCR4 expression,
which in turn would permit enhanced accumulation of eosinophils
at allergic inflammatory sites by eosinophil-active chemokines
such as eotaxin. The presence of IL-5 in the serum, which is often
observed in patients with allergic and helminthic disorders, may
increase the distribution of eosinophils to inflamed tissues.

In summary, we have demonstrated that functional expression
of CXCR4 is inducible in eosinophils, and that SDF-1α elicits strong
migration comparable to that induced by eotaxin. Th2 cytokines
such as IL-4 and IL-5 drastically inhibited the expression of
CXCR4. The interplay between CXCR4 and SDF-1α may affect the
distribution and migration of eosinophils, thus indicating that
CXCR4 in eosinophils might represent an important mechanism in
diseases in which eosinophils play pathogenic roles.

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