

Chemokine stromal cell–derived factor 1 α activates basophils by means of CXCR4

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Background: The CXC chemokine receptor 4 (CXCR4) is predominantly expressed on inactivated naive T lymphocytes, B lymphocytes, dendritic cells, and endothelial cells. CXC chemokine stromal cell–derived factor 1 α (SDF-1 α) is the only known ligand for CXCR4. To date, the CXCR4 expression and function of SDF-1 α in basophils are unknown.

Objective: The purpose of this study was to investigate the expression of CXCR4 and functions of SDF-1 α in basophils and to characterize the role of the CXCR4-SDF-1 α receptor ligand pair in the allergic inflammation.

Methods: Basophil purification, flow cytometry, real-time quantitative RT-PCR assay, Northern blotting, intracellular free Ca²⁺ change, chemotaxis assay, and histamine release assay were used.

Results: CXCR4 is abundantly expressed on peripheral blood resting basophils (91%). Likewise, CXCR4 messenger (m)RNA is expressed in resting basophils (3.2×10^3 copies per 2×10^2 cells). The existence of CXCR4 mRNA was also confirmed in basophils by means of Northern blot analysis. SDF-1 α induces an increase in intracellular free Ca²⁺ in basophils. SDF-1 α activates basophils to chemotaxis (chemotactic index = 3.8) and histamine release (36% of total content) through CXCR4 on the cells. The chemokines SDF-1 α , eotaxin, RANTES, monocyte chemoattractant protein (MCP) 1, and macrophage inflammatory protein (MIP) 1 α have been demonstrated at different potencies in induction of chemotaxis (eotaxin > SDF-1 α > RANTES $\approx$ MCP-1 >> MIP-1 α) and histamine release (MCP-1 $\approx$ SDF-1 α > eotaxin > RANTES > MIP-1 α). The optimal concentration seen for SDF-1 α effects (chemotaxis and histamine release) on basophils was 100 ng/mL.

Conclusion: These results indicate that the CXCR4-SDF-1 α receptor ligand pair may be important for the recruitment and activation of the basophils, which is a characteristic effector cell of the allergic inflammation. (*J Allergy Clin Immunol* 2000;106:313-20.)

Key words: Chemokine, receptor, chemotaxis, histamine release, inflammation, basophils

The CXC chemokine receptor 4 (CXCR4), with the CXC chemokine stromal cell–derived factor 1 α (SDF-1 α) as the only known ligand, has been shown to be an important coreceptor for HIV-1 infection.¹ CXCR4 is predominantly expressed on the naive, inactivated, CD26^{low}CD45RA⁺CD45RO⁺ T-cell subset of peripheral blood lymphocytes,² B lymphocytes,³ dendritic cells,⁴ endothelial cells,⁵ and mature polyploid megakaryocytes.⁶ CXCR4 expression is rapidly upregulated on PBMCs during PHA stimulation and IL-2 priming, and cellular responsiveness to SDF-1 α is simultaneously increased.² SDF-1 α is a highly efficacious lymphocyte chemoattractant. It induces an increase in intracellular free Ca²⁺ ([Ca²⁺]_i) and chemotaxis in CXCR4-transfected cells and inhibits infection by CXCR4-dependent HIV virus.¹ To date, the CXCR4 expression has not been reported in basophils. In the present study we have documented that CXCR4 is abundantly expressed on human peripheral blood resting basophils. CXCR4 messenger (m)RNA expression in resting basophils has been detected by real-time quantitative RT-PCR and Northern blotting. SDF-1 α induces an increase in [Ca²⁺]_i in basophils. Furthermore, SDF-1 α activates basophils into chemotaxis and induces histamine release through CXCR4 on the cells, which is demonstrated by the fact that anti-CXCR4 mAb blocks SDF-1 α –induced chemotaxis and histamine release. Our results suggest that the CXCR4-SDF-1 α receptor ligand pair is important for the recruitment and activation of basophils.

METHODS

Basophil purification

As described elsewhere,⁷ 50 mL of EDTA-anticoagulated venous blood from healthy nonallergic subjects was immediately subjected to a discontinuous Percoll gradient to obtain basophil-enriched cells followed by depletion of contaminating cells by addition of Dynabeads M-450 (CD3, CD4, CD8, CD14, CD15, and CD19). The purity of basophils ranged from 85% to 95%, as determined by using the Alcian blue staining method. In some experiments, subsequent to the negative selection, a positive selection was applied by using Dynabeads M-450 coated with mouse anti-IgE receptor mAb (CRA-1, Cosmo BioCo Ltd) to obtain greater than 99% basophils.

Flow cytometry

As previously described,⁸ freshly isolated basophils were first incubated with a mouse anti-human CXCR4 mAb (clone number

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Abbreviations used

[Ca²⁺]_i: Intracellular free Ca²⁺
 CXCR: CXC chemokine receptor
 fMLP: N-formyl-Met-Leu-Phe
 MCNC: Migrating cells on negative control
 MCP: Monocyte chemoattractant protein
 MIP: Macrophage inflammatory protein
 SDF-1α: Chemokine stromal cell-derived factor 1α

44708.111, R&D Systems Europe Ltd) at 5 µg/mL or 5 µg/mL matched isotype mouse IgG_{2a} (Dako) in PBS containing 2% human pooled AB serum and 0.1% sodium azide for 20 minutes. The cells were then washed twice in staining buffer and resuspended in FITC-conjugated F(ab)₂ donkey anti-mouse mAb (1:250 vol/vol, Jackson ImmunoResearch Laboratories Inc) for 20 minutes, followed by washing twice in staining buffer. The cells were incubated with a rabbit anti-human IgE mAb (Dako)⁹ at 5 µg/mL in PBS containing 2% human pooled AB serum and 0.1% sodium azide for 20 minutes, followed by washing twice. Finally, the cells were labeled with phycoerythrin-conjugated goat anti-rabbit mAb (1:250 vol/vol, Sigma Chemical Co) for 20 minutes and washed twice in staining buffer. All procedures were carried out at 4°C. The analyses were performed with a flow cytometer (COULTER XL). In some experiments the stained cells were spun down on a slide and photographed under a conventional immunofluorescence microscope (400×).

Real-time quantitative RT-PCR assay

All real-time quantitative RT-PCR experiments were performed, as described elsewhere.^{10,11} Briefly, total RNA from peripheral resting basophils (2 × 10⁵ cells, purity >99%) was prepared by using Quick Prep Total RNA Extraction Kit (Pharmacia Biotech), and any potential contaminating chromosomal DNA was digested with DNase I according to the manufacturer's instructions. For reverse transcription, the RNA was reverse transcribed by using oligo (dT)₁₂₋₁₈ and Superscript II reverse transcriptase (Life Technologies), according to the manufacturer's instructions. Reverse transcription was performed for 60 minutes at 37°C, and any potential contaminating protein was denatured by incubation for 10 minutes at 95°C. Quantitative PCR was performed in special optical tubes in a 96-well microtiter plate (PE Applied Biosystems) format on an ABI PRISM 7700 Sequence Detector Systems (PE Applied Biosystems), according to the manufacturer's instructions. The following sequences of the specific primers for CXCR4 (DNA Technology) and β-actin (PE Applied Biosystems) were used: CXCR4 sense 5'-CAGCGGTACCATGGAGGG-3'; CXCR4 antisense, 5'-TTC-CTCGGTGTAGTTATCTGAAGTGT-3'; CXCR4-specific probe, 5'-ATCAGTTCATTCCTTTGCCCTCTTTTGCA-3'; β-actin sense, 5'-ATTGCCGACAGGATGCAGAA-3'; β-actin antisense, 5'-GCTGATCCACATCTGCTGGAA-3'; β-actin-specific probe, 5'-CAAGATCATTTGCTCCTCTGAGCGCA-3'.

These oligonucleotide sequences were designed by using software Primer Express 1.0 (PE Applied Biosystems). They span exon junctions to prevent amplification of genomic DNA. Taqman universal PCR master mix (PE Applied Biosystems) containing Passive Reference 1 (ROX) was used to normalize for non-PCR-related fluctuations in fluorescence signal. The reporter dye was 6-carboxy-fluorescein, and the quencher was 6-carboxy-tetramethyl-rhodamine. The standard DNA template with known amounts of molecules (1.0 × 10³, 2.0 × 10³, 4.0 × 10³, 1.0 × 10⁴, 2.0 × 10⁴, and 1.0 × 10⁵ per well) and no-template controls were used to create standard curves. All unknown complementary (c)DNAs were diluted to contain equal amounts of β-actin cDNA. The standards, no-tem-

plate controls, and unknown samples were added in a total volume of 50 µL per reaction. PCR retaining conditions were 2 minutes at 50°C and 10 minutes at 95°C and 40 cycles with 15 seconds at 95°C and 60 seconds at 60°C for each amplification. Potential PCR product contamination was digested by uracil-N-glycosylase because deoxythymidine triphosphate is substituted by deoxyuridine triphosphate.¹⁰ All PCR experiments were performed with a hot start. In the reaction system uracil-N-glycosylase and AmpliTaq Gold (PE Applied Biosystems) were applied according to the manufacturer's instructions.^{10,11} To analyze data of PCR products, two terms were used to express the results: ΔR_n, representing the normalized reporter signal minus the baseline signal established in the first few cycles PCR, and C_T (threshold cycle), representing the PCR cycle at which an increase in reporter fluorescence signal above a baseline can first be detected.

Northern blot analysis

As previously described,¹² total RNA from peripheral basophils or different cells as indicated was prepared by using Quick Prep Total RNA Extraction Kit (Pharmacia Biotech). Five micrograms of total RNA from each sample was electrophoresed under denaturing conditions, followed by blotting onto Nytran membranes (Schleicher & Schuell Inc) and cross-linked by using UV irradiation. cDNA probes were labeled with α-phosphorous 32 deoxycytidine triphosphate. CXCR4 cDNA probe was obtained by PCR amplification of the sequence mentioned above from total RNA of CD3⁺ T lymphocytes from umbilical blood. The membranes were hybridized overnight with 10⁶ cpm/mL of phosphorous 32-labeled probe, followed by intensively washing with 0.2× standard saline citrate (1× standard saline citrate = 0.15 mol/L NaCl and 0.015 mol/L sodium citrate, pH 7.0) and 0.1% SDS before being autoradiographed.

Intracellular free Ca²⁺ change

As previously described,¹³ approximately 50,000 purified basophils were loaded with fura 2-acetoxymethylester (2 µmol/L) at 37°C in a total volume of 1 mL of Pipes buffer. The cells were washed once with Pipes buffer. The fura 2-AM-loaded basophils were resuspended in Pipes buffer. Samples of 1-mL basophil suspensions were placed in a specially constructed chamber (Nunc). The chamber was placed at a digital imaging system consisting of Zeiss Axiovert 135 microscope, a light sensitive video camera (Genesys, DAGE MTI), and software from Universal Imaging Company. One hundred microliters of either SDF-1α or macrophage inflammatory protein (MIP) 1α (final reaction concentration, 50 ng/mL or 0.5 ng/mL) and ionophore (final reaction concentration, 0.1 nmol/L) was applied from the top of the chamber directly onto the basophil suspensions and gently mixed to ensure immediate and uniform exposure of the cells to the chemokine or reagent tested. The changes in [Ca²⁺]_i were determined by the gray values from a single cell or total cells and were converted into [Ca²⁺]_i by dividing fluorescence emissions measured after 340 and 380 nm excitation. The R_{max} value was 1.1, and the R_{min} value was 0.1. The dissociation constant for fura 2 was 224 nmol/L, and sf2/sb2 was 2.

Chemotaxis assay

The following human recombinant chemokines were studied: SDF-1α, eotaxin, RANTES, monocyte chemoattractant protein (MCP) 1, and MIP-1α (R&D Systems Europe Ltd). The chemotaxis assay was a 48-well microchamber (Neuro Probe) technique.¹⁴ Briefly, chemokines were diluted in RPMI-1640 with 0.5% BSA and placed in the lower wells (25 µL). Fifty microliters of the cell suspension at 1 × 10⁵ cells/mL was added to the upper well of the chamber, which was separated from the lower well by a 5-µm pore size, polycarbonate, polyvinylpyrrolidone-free membrane (Nucleo-

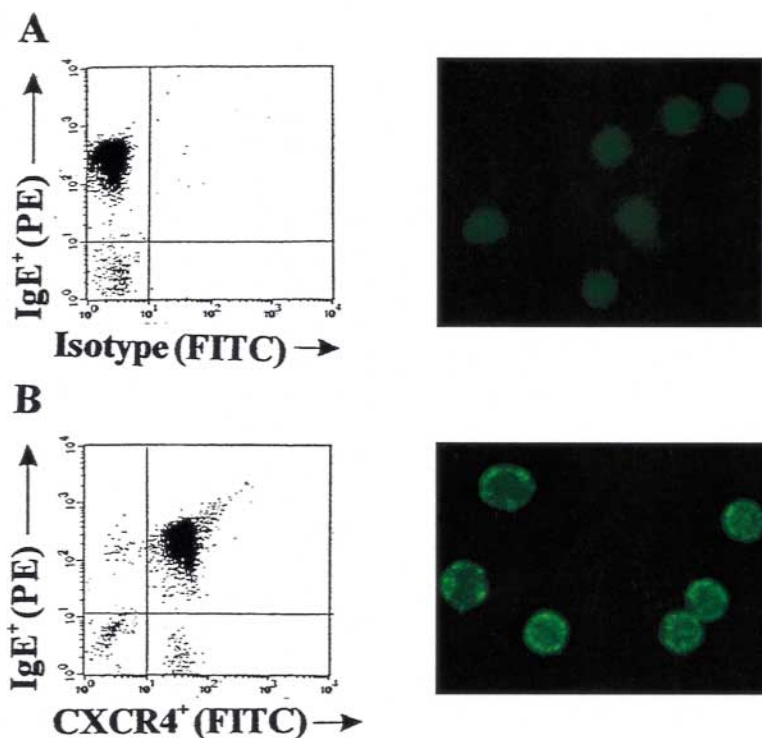


FIG 1. Double color flow cytometric analysis of the distribution of CXCR4 (**B**) on resting human basophils (*left side*), including observation under an immunofluorescence microscope (*right side*). The cells were freshly isolated from healthy nonallergic donors, as described in the "Methods" section. **A**, A matched isotype mouse IgG_{2a} negative control for CXCR4. The percentages of CXCR4⁺ cells are indicated in the "Results" section. The cells were stained as detailed in the "Methods" section. The pictures on the *right side* are observations under immunofluorescence microscope with FITC light. The data are from a single experiment, which is representative of 4 similar experiments performed.

atmosphere containing 5% CO₂, allowing the cells to migrate toward the chemokine indicated. Then the membrane was carefully removed, fixed in 70% methanol, and stained for 5 minutes in Coomassie brilliant blue. The cells that migrated and adhered to the lower surface of the membrane were counted by using a light microscope. Approximately 6% of basophils will migrate spontaneously (known as migrating cells on negative control [MCNC]),¹⁵ corresponding to between 250 and 400 cells. This may vary from day to day, but it varies very little within the same day's experiments. The chemokine-induced degranulation will not affect the chemotaxis assay because Coomassie brilliant blue stains nuclear protein, and only the number of migrating cells will be taken into account in the assay. The results were expressed as the chemotactic index, which is the ratio between the numbers of migrating cells in the sample and those in the medium control¹⁴ and with SD. For blocking tests of basophil chemotaxis toward SDF-1 α , the cells were preincubated with either anti-CXCR4 mAb (5 μ g/mL, R&D Systems Europe Ltd; clone number 12G5) or with isotype mouse IgG_{2a} (5 μ g/mL) for 60 minutes at room temperature before chemotaxis assay. In some experiments the cells were primed with IL-3 (10 ng/mL) for 10 minutes before chemotaxis assays.

Histamine release assay

The following human recombinant chemokines or reagents were studied in histamine release assays: SDF-1 α , eotaxin, RANTES, MCP-1, MIP-1 α , N-formyl-Met-Leu-Phe (fMLP; Sigma), and mouse anti-human IgE (Dako). The purified basophils were tested either freshly primed or IL-3 primed (10 ng/mL for 10 minutes). Histamine release was performed as described elsewhere.¹⁶ Briefly, the

cells were suspended at a concentration of 50,000 cells per milliliter in Pipes buffer, and aliquots of 25 μ L of purified basophils were added in glass fiber-coated microtiter plates (The RefLab), of which 25 μ L of stimulus had previously been added to each well (total reaction volume, 50 μ L). All stimulations were performed in duplicates, and the glass fiber-coated microtiter plates were incubated for 60 minutes at 37°C. During incubation, released histamine will adsorb to the glass fibers with high affinity and selectivity. After incubation, the plates were rinsed with deionized water, followed by another incubation with 175 μ L of 0.4% SDS per well for 30 minutes at 37°C to ensure a complete removal of interfering substances. Subsequently, the plates were rinsed again with deionized water. The histamine release was determined by using the *o*-phthaldialdehyde method and expressed in percentages of the total content of histamine, which was determined after cell lysis in an identical aliquot of the sample.¹⁶ Spontaneous histamine releases were less than 5% in all experiments.

RESULTS

CXCR4 is expressed on basophils

We examined the expression of CXCR4 on human peripheral resting basophils. The results from flow cytometric analyses document that in freshly isolated basophils there are 91.3% of CXCR4⁺ and surface-bound IgE⁺ cells,⁹ whereas CXCR4⁺ IgE⁻ cell fractions constitute less than 2% (Fig 1, *B*, *left*; contaminating lymphocytes morphologically determined under immunofluorescence microscope). There are more than 95% surface-bound IgE⁺ cells in both

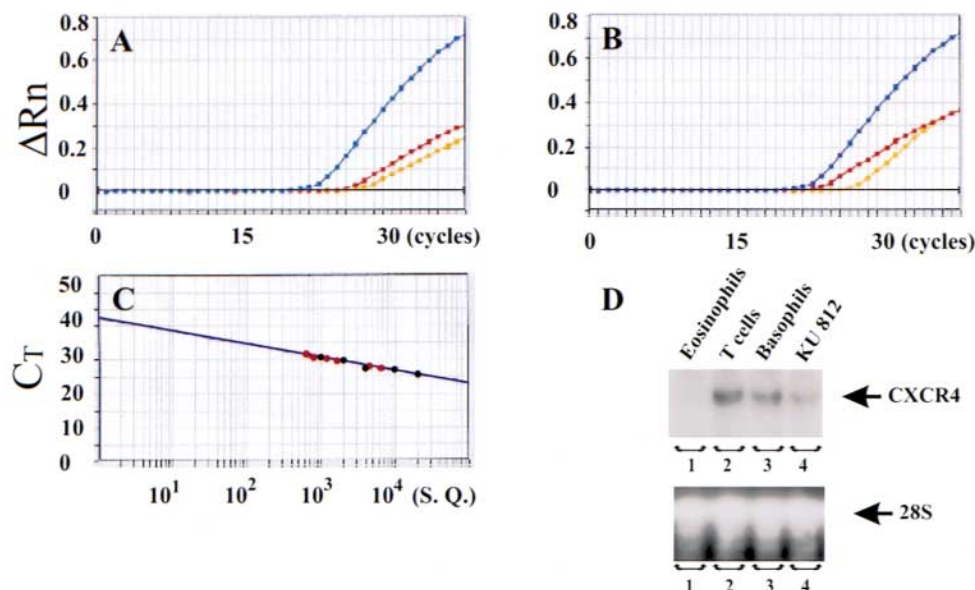


FIG 2. The plots of the real-time detection and amplification for CXCR4 in cDNA of resting basophils. **A**, Red plots represent the amplification for CXCR4 in cDNA samples; orange plots represent the amplification for CXCR4 in 10-fold diluted cDNA samples. Blue plots represent the amplification of standard DNA template (2.0×10^4 copies) with a housekeeping gene (β -actin). C_T s were 20.8 for standard DNA template, 25.4 for CXCR4 in cDNA samples, and 26.6 for CXCR4 in 10-fold diluted cDNA samples. **B**, Red plots represent the amplification for β -actin in cDNA samples; orange plots represent the amplification for β -actin in 10-fold diluted cDNA samples. Blue plots represent the amplification of standard DNA template (2.0×10^4 copies) with a housekeeping gene (β -actin). C_T s were 20.8 for standard DNA template, 22.5 for β -actin in cDNA samples, and 24.8 for β -actin in 10-fold diluted cDNA samples. **C**, The linear relationship between C_T and log starting quantity (SQ) of standard DNA template (black circles) or target (CXCR4 and β -actin) in cDNA samples (red circles). The plots are representatives of 4 similar experiments. **D**, CXCR4 mRNA Northern blot of different cells. Total RNA from different cells as indicated were isolated, electrophoresed, and blotted, as described in the "Methods" section. The hybridization signals for CXCR4 mRNA from different cells were shown in the upper panel. The 28S recombinant RNAs in the lower panel confirm comparable amounts of loaded total RNA.

the CXCR4 mAb and the matched IgG_{2a} isotype antibody-negative control (Fig 1, A and B, left), indicating the purity of isolated peripheral basophils in this experiment. A positive staining has been observed with an immunofluorescence microscope (Fig 1, B, right). The negative control was isotype mouse IgG_{2a} (Fig 1, A, right).

CXCR4 mRNA is expressed in basophils

The results in Fig 2, A, show that mRNA of CXCR4 has been detected in the human peripheral resting basophils (purity >99%). Compared with the amplification of the standard DNA template (2.0×10^4 copies) with a housekeeping gene (β -actin), there are approximately 3.2×10^3 copies of CXCR4 in the tested 5- μ L samples of original cDNA product from 2×10^5 resting basophils (red plots), and there are approximately 2.5×10^2 copies for CXCR4 in the tested 5- μ L 10-fold diluted samples of cDNA product from 2×10^5 resting basophils (orange plots). The results in Fig 2, B, show that mRNA of a housekeeping gene (β -actin) has been detected in samples of the human peripheral resting basophils. Compared with the amplification of standard DNA template (2.0×10^4 copies) with a housekeeping gene (β -actin), there are approximately 6.5×10^3 copies for β -actin in

the tested 5- μ L samples of original cDNA product from 2×10^5 resting basophils (red plots), and there are approximately 7×10^2 copies for β -actin in the tested 5- μ L 10-fold diluted samples of cDNA product from resting basophils (orange plots). In all experiments the correlation coefficients are around 0.94. Additionally, identical quantitative RT-PCR results have been obtained from experiments by using SYBR Green as a reporter (basophil purity >99%, data not shown). CXCR4 mRNA expression in basophils has also been confirmed by Northern blotting, as shown in Fig 2, D. In the upper panel basophils indeed expressed CXCR4 mRNA, whereas T lymphocytes abundantly express CXCR4 mRNA (as a positive control). CXCR4 mRNA is also weakly expressed in KU812 (basophilic cell line), whereas it is not seen in eosinophils. In the lower panel it is showing that comparable total RNA amounts from different cells have been added.

SDF-1 α induces increase in $[Ca^{2+}]_i$ in basophils

SDF-1 α induces an increase in $[Ca^{2+}]_i$ in basophils. There is an immediate increase in $[Ca^{2+}]_i$ on SDF-1 α (CXCR4 ligand) stimulation at a concentration of 50

ng/mL (Fig 3, B and D) but no increase in $[Ca^{2+}]_i$ at a lower dose (0.5 ng/mL; Fig 3, A). MIP-1 α , which is known to activate the basophils and has been used as a positive control (Fig 3, C; 50 ng/mL), where it induces less increase in $[Ca^{2+}]_i$ in purified basophils. A maximal increase in $[Ca^{2+}]_i$ is demonstrated by an addition of ionomycin after stimulation with SDF-1 α and MIP-1 α . In Fig 3, D, the changes of intercellular free Ca^{2+} in 9 individual basophils were recorded simultaneously after stimulation with SDF-1 α .

SDF-1 α induces chemotaxis in basophils through CXCR4

We have examined the abilities of SDF-1 α , eotaxin, RANTES, MCP-1, and MIP-1 α to induce basophil chemotaxis. SDF-1 α has induced significant basophil chemotactic migration. The results in Table I show that SDF-1 α induces chemotactic migration in freshly isolated basophils, yielding typical bell-shaped, dose-dependent, chemotaxis response curves. The optimal chemotactic concentration of SDF-1 α is at 100 ng/L. Eotaxin, RANTES, and MCP-1 have been used as positive controls that have also induced a stronger or similar basophil chemotactic migration (Table I). The different chemotactic potencies of the chemokines applied are as follows: eotaxin > SDF-1 α > RANTES $\approx$ MCP-1 >> MIP-1 α . Interestingly, IL-3 priming has not potentiated basophil chemotaxis induced by the chemokines applied. To confirm that the basophil chemotaxis we have observed is indeed induced by SDF-1 α through CXCR4, we have used anti-CXCR4 mAb to block the basophil chemotactic activity of SDF-1 α . The anti-CXCR4 mAb can completely block the SDF-1 α -induced chemotaxis of basophils (chemotactic index = 1.08 ± 0.06 and MCNC = 237 ± 59 at 100 ng/mL; $P < .001$, $n = 3$), whereas the isotype antibody has no blocking effect at all (chemotactic index = 3.18 ± 0.27 and MCNC = 203 ± 55 at 100 ng/mL; $P > .05$, $n = 3$). We have also used anti-CCR3 mAb (clone number 7B11, Leukocyte, Inc), which actually binds to basophils in the blocking test. The anti-CCR3 mAb cannot block SDF-1 α -induced basophil chemotaxis but completely blocks eotaxin-induced basophil chemotaxis (data not shown). These results demonstrate that SDF-1 α induces chemotaxis in basophils through the CXCR4. In our experimental system chemotactic indices of 1.8 to 2.0 are considered moderate, 2.1 to 3.0 are considered strong, and greater than 3.1 are considered very strong.^{15,17}

SDF-1 α induces histamine release in basophils through CXCR4

We have examined the abilities of SDF-1 α , eotaxin, RANTES, MCP-1, MIP-1 α , a-IgE, and fMLP to induce basophil histamine release. Interestingly, SDF-1 α has induced histamine release in purified basophils in a dose-dependent manner (Fig 4). The results in Fig 4 show that SDF-1 α induces histamine release in freshly isolated basophils, yielding typical bell-shaped, dose-dependent, response curves. The optimal release concentration of

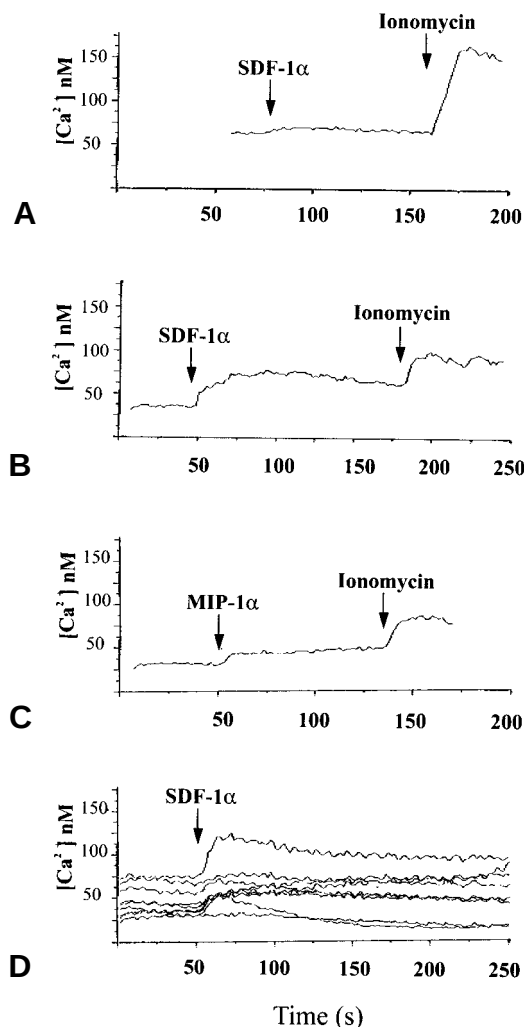


FIG 3. The changes of $[Ca^{2+}]_i$ in total human basophils or a single basophil after chemokine stimulation. The fura 2-loaded basophils were stimulated with 0.5 ng/mL SDF-1 α (A) or 50 ng/mL (B and D) and with 50 ng/mL MIP-1 α (C). Ionomycin was applied at a final concentration of 0.1 nmol/L. B and C, Averaged changes of $[Ca^{2+}]_i$ in the total cells. D, The changes of $[Ca^{2+}]_i$ in 9 individual basophils were recorded simultaneously after stimulation with SDF-1 α . The figures are representatives of 3 similar experiments.

SDF-1 α is at 100 ng/mL (36% of total histamine content). Eotaxin, RANTES, and MCP-1 have been used as positive controls, all of which have induced histamine release. SDF-1 α induces a significantly higher histamine release from basophils compared with MIP-1 α , which is in agreement with previous reports. As expected, the a-IgE and fMLP also induce histamine release from freshly isolated basophils. The different histamine release potencies of the chemokines applied are as follows: MCP-1 $\approx$ SDF-1 α > eotaxin > RANTES > MIP-1 α . Interestingly, IL-3 priming has potentiated histamine release from basophils induced by these chemokines applied with the exception of MIP-1 α . Anti-CXCR4 mAb (5 μ g/mL) completely inhibits SDF-1 α -induced histamine release in purified

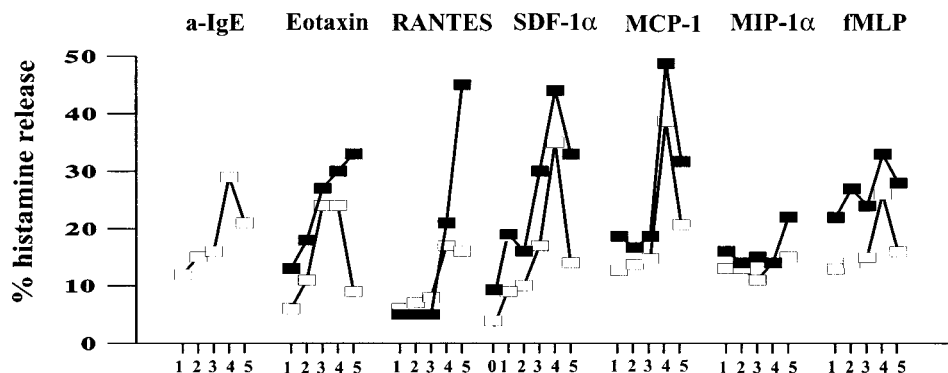


FIG 4. The histamine release of basophils induced by a-IgE, SDF-1 α , eotaxin, RANTES, MCP-1, MIP-1 α , and fMLP as indicated. The basophils were isolated from venous blood from healthy volunteers and incubated without (open squares) or with IL-3 (filled squares) at 10 ng/mL for 10 minutes before histamine release assay. The chemokines indicated in the figure were applied as indicated in the "Methods" section. The concentrations of chemokines or other reagents are indicated in the figure as 1, 2, 3, 4, and 5. They represent 12.5, 25, 50, 100, and 1000 ng/mL for chemokines (0-5 represent 5, 12.5, 25, 50, 100, and 1000 ng/mL for SDF-1 α); 4, 10, 40, 100, and 400 U/mL for a-IgE; and 10^{-10} , 10^{-9} , 10^{-8} , 10^{-7} , and 10^{-6} mol/L for fMLP, respectively. All illustrated data were determined as described in the "Methods" section and expressed as percentages based on triplicate determination of histamine release on each concentration of chemokine or other reagent. The data are from a single experiment, which is representative of 5 similar experiments performed. On average, SDF-1 α -induced (at 100 ng/mL) histamine releases of basophils are $20.6\% \pm 12.9\%$ in freshly isolated cells and $37.8\% \pm 18.7\%$ in IL-3-primed cells. The statistical data for 5 conducted experiments show that there is a statistically significant difference ($P < .02$) in freshly isolated basophil histamine release versus IL-3-primed basophil histamine release at optimal concentrations of each corresponding chemokine applied, except MIP-1 α . There is no statistically significant difference ($P > .05$) in freshly isolated basophil histamine release versus IL-3-primed basophil histamine release induced by MIP-1 α and fMLP.

TABLE I. Chemotaxis of untreated and IL-3-primed basophils toward chemokines

Chemokine [†]	Untreated basophils*					IL-3-primed basophils*				
	1 [‡]	10	100	1000	MCNC	1	10	100	1000	MCNC
SDF-1 α	$0.98 \pm 0.18^{\S}$	3.55 ± 0.34	3.8 ± 0.31	1.08 ± 0.12	327 ± 59	1.05 ± 0.13	3.66 ± 0.17	3.59 ± 0.32	1.29 ± 0.28	320 ± 60
Eotaxin	1.09 ± 0.17	4.39 ± 0.40	4.5 ± 0.62	1.14 ± 0.24	297 ± 48	1.18 ± 0.11	3.29 ± 0.46	4.41 ± 0.29	1.49 ± 0.26	262 ± 42
RANTES	1.12 ± 0.18	2.88 ± 0.21	3.04 ± 1.06	1.12 ± 0.12	297 ± 48	1.31 ± 0.28	2.92 ± 0.46	3.11 ± 0.29	1.19 ± 0.26	262 ± 42
MCP-1	1.05 ± 0.16	2.07 ± 0.37	2.81 ± 0.14	1.11 ± 0.12	327 ± 59	1.07 ± 0.08	2.05 ± 0.32	3.03 ± 0.11	1.12 ± 0.36	320 ± 60
MIP-1 α	1.06 ± 0.39	1.93 ± 0.18	1.97 ± 0.31	1.01 ± 0.26	297 ± 48	1.07 ± 0.30	1.85 ± 0.31	1.99 ± 0.28	1.03 ± 0.10	262 ± 42

*Basophils were isolated from venous blood from healthy volunteers and incubated with or without IL-3 (10 ng/mL) for 10 minutes before chemotaxis assay.

[†]Chemokines indicated in the table were applied as indicated in the "Methods" section.

[‡]Concentrations of chemokines were applied in nanograms per milliliter.

[§]All listed data were determined as described in the "Methods" section and expressed as chemotactic index $\pm$ SD, which was based on triplicate determination of chemotaxis on each concentration of chemoattractant. The listed data are from a single representative experiment of 5 performed. Among the 5 experiments, 100 ng/mL SDF-1 α produced an average chemotactic index of 4.12 ± 0.62 in freshly isolated cells and a chemotactic index of 3.39 ± 0.37 in IL-3-primed cells. The statistical analysis of 5 experiments shows that there was no statistically significant difference ($P > .05$) between freshly isolated basophil chemotaxis and IL-3-primed basophil chemotaxis in any concentration of the chemokines applied.

basophils (<5% of total histamine content), whereas anti-IgE-induced histamine releases have not been affected by anti-CXCR4 mAb (34% of total histamine content). Thus these results demonstrate that SDF-1 α induces histamine release in basophils through CXCR4. Additional experiments have demonstrated that SDF-1 α induces histamine release in dextran-sedimented leukocytes from normal subjects ($n = 12$, data not shown).

DISCUSSION

Several CC chemokines and their receptors have been considered to play an important role in allergic inflammation because of their abilities to activate

basophils.^{18,19} Human blood basophils express high numbers of the chemokine receptors CCR1, CCR2, and CCR3, which subsequently bind to their ligands MCP-1, MCP-3, MCP-4, eotaxin, and RANTES to activate the cells.²⁰⁻²⁴ So far, few studies have addressed CXC chemokine receptors and the functions of their ligands in human peripheral blood basophils. However, it has been demonstrated that human basophils express CXCR1 and CXCR2 and that IL-8 and growth-regulating oncogene induce activation of basophils.^{25,26} CXCR4 is broadly expressed in the cells of the immune system¹ and the cells in the central nervous system.²⁷ It can mediate migration of different resting or activated leukocytes and hematopoietic progenitors in response to its ligand SDF-

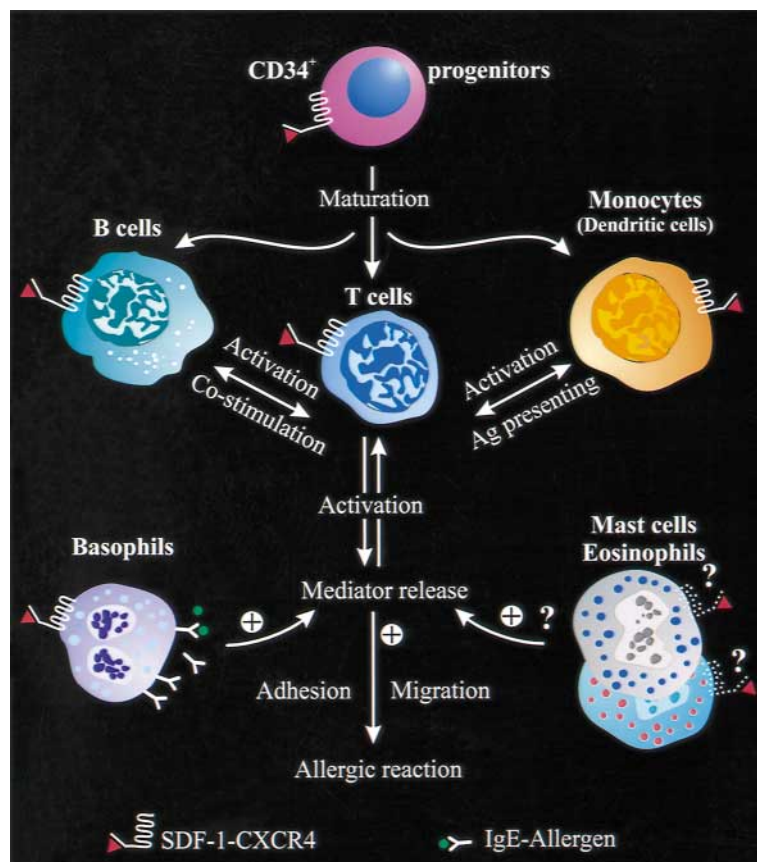


FIG 5. Schematic diagram to illustrate the interaction of the SDF-1 α -CXCR4 pair in various cell types.

1 α . It has been found that beyond T lymphocytes and monocytes, SDF-1 α also attracts naive and memory, but not germinal center, B lymphocytes.³ To our knowledge, it is the first report that human peripheral blood basophils express CXCR4, which has been here documented at both the protein and mRNA levels. We have also demonstrated that on binding to its receptor CXCR4 on basophils, SDF-1 α causes a rise of [Ca²⁺]_i influx, chemotactic migration, and histamine release. Thus the CXCR4-SDF-1 α receptor ligand pair may be important for the recruitment and activation of basophils, which are characteristic effector cells of allergic inflammation.

Basophils express high levels of C5a receptors, CCR2, CCR3, CXCR1, CXCR2, and platelet-activating factor receptors and lower levels of fMLP receptors. Chemoattractant receptors mediate different cellular responses unrelated to their expression levels in terms of leukocyte C4 formation and release.²⁸ It has been shown that CC chemokines activate basophil and eosinophil leukocytes with different selectivities and patterns of activity. The most effective are MCP-1, MCP-2, and MCP-3. RANTES is a weak stimulus of mediator release and a strong chemoattractant for basophils and eosinophils.²⁹ CCR3 is the major receptor for eotaxin, eotaxin 2,¹⁹ RANTES, and MCP-4 in human basophils. The characteristic effector cells of allergic inflammation, basophils

and eosinophils, are largely dependent on CCR3 for migration toward different chemokines into inflamed tissues.²¹ Natural human C5adesarg is a strong inducer of release of inflammatory mediators by human basophils, particularly in several cytokine-primed cells. It triggers basophil mediator release superior to that of other endogenous IgE-independent agonists, such as MCP-1, IL-8, C3a, and platelet-activating factor. Priming of basophils with either IL-3, IL-5, GM-CSF, or nerve growth factor enhances histamine release and conditions the cells to produce large amounts of leukotriene C4.³⁰ In the present study we have compared the different chemotactic potencies of chemokines. We have found that the applied chemokines can induce basophil chemotaxis with different potencies (eotaxin > SDF-1 α > RANTES $\equiv$ MCP-1 >> MIP-1 α). Interestingly, IL-3 priming has not affected the basophil chemotactic response to the chemokines applied. Moreover, we have also compared the histamine release of basophils induced by the chemokines applied, resulting in the following ranking of potencies: MCP-1 $\equiv$ SDF-1 α > eotaxin > RANTES > MIP-1 α . In contrast to chemotaxis experiments, IL-3 priming has potentiated histamine release from basophils induced by the chemokines applied (except MIP-1 α). Our results are in agreement with other studies demonstrating that chemokines activate basophil leukocytes

with different selectivities and patterns of activity.^{18-24,28-30} The novel findings about CXCR4 expression on basophils and SDF-1 α as an activator of basophils will draw certain attention, and therefore the considerations of the mechanisms of allergic basophil recruitment should include the possibility that the SDF-1 α -CXCR4 receptor ligand pair participates. A schematic diagram to illustrate SDF-1 α -CXCR4 receptor ligand pair interaction in various cell types, including those of the allergic response, was developed (Fig 5). It overall describes the main findings in SDF-1 α -CXCR4 pair studies so far. The findings of CXCR4 in HIV coentry and angiogenesis were not included to simplify the figure.

In conclusion, the SDF-1 α -CXCR4 pair seems to represent a new signal-activation system of the basophil. Interestingly, SDF-1 α has been described as a constitutive, rather than an inflammatory, chemokine,³¹ whereas we have here found that it induces chemotaxis and histamine release in basophils, processes that are usually associated with allergic reactions. This could indicate that the SDF-1 α CXCR4 pair is more important for inflammation than previously believed, that the basophil may participate in other biologic functions such as postinflammatory regenerative processes, or both.

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