A Highly Efficacious Lymphocyte Chemoattractant, Stromal Cell-derived Factor 1 (SDF-1)

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Summary

Chemotactic factors are postulated to direct emigration of lymphocytes from the blood stream into sites of inflammation. Members of a family of chemotactic cytokines, termed chemokines, have been shown to attract lymphocytes but efficacy, i.e., the maximal percentage of attracted cells, has been low. We have identified a highly efficacious lymphocyte chemotactic activity in the supernatants of the murine bone marrow stroma cell line MS-5 which attracts 10-fold more lymphocytes in vitro than currently described lymphocyte chemoattractants. Purification of this chemotactic activity revealed identity to stromal cell-derived factor 1 (SDF-1). SDF-1 acts on lymphocytes and monocytes but not neutrophils in vitro and is both a highly efficacious and highly potent mononuclear cell attractant in vivo. In addition, SDF-1 induces intracellular actin polymerization in lymphocytes, a process that is thought to be a prerequisite for cell motility. Since SDF-1 is expressed constitutively in a broad range of tissues it may have a role in immune surveillance and in basal extravasation of lymphocytes and monocytes rather than in inflammation.

Lymphocytes function as the reservoir of immunological memory. In contrast to neutrophils and monocytes, lymphocytes may leave tissues through lymphatics, emigrate from the blood stream into lymphoid and non-lymphoid tissues, and recirculate multiple times during their life history (for review see reference 1). Chemoattractants are postulated to help direct lymphocyte traffic into sites of inflammation and into lymphoid and non-lymphoid tissues during recirculation (1, 2). The best lymphocyte chemoattractants identified to date are members of a family of small, 60 to 80 amino acid chemotactic cytokines termed chemokines. The chemokines can be divided into two subfamilies, the CXC- and the CC-chemokines. The members of each subfamily are more homologous to one another than to members of the other subfamily, and differ in the spacing of the first two cysteines. The two subfamilies differ in their chromosomal localization and cluster on chromosome 4 (CXC-chemokines) and chromosome 17 (CC-chemokines), respectively. While CXC-chemokines, e.g., interleukin 8 (IL-8), act mostly on neutrophils, members of the CC-chemokines, e.g., monocyte chemoattractant protein 1 (MCP-1)[1], regulated on activation normal T cell expressed and secreted (RANTES), and macrophage inflammatory protein 1α (MIP-1α), have been shown to exert function on monocytes, eosinophils and lymphocytes (2, 3). However, the efficacy, i.e., the percent of attracted cells, of lymphocyte chemoattractants described so far is low, whereas chemokines active on monocytes, neutrophils, and eosinophils are highly efficacious (2).

The bone marrow not only produces precursor and immature lymphocytes, but also harbors mature T lymphocytes. These give rise to graft versus host disease and are an important problem in bone marrow transplantation. To understand what drives recirculation of mature lymphocytes into the bone marrow we screened supernatants from bone marrow stromal cells for chemotactic activity. We subsequently identified a lymphocyte chemoattractant produced by bone marrow stromal cells and purified it to homogeneity. It is highly efficacious, attracting greater than 10-fold more lymphocytes than currently described chemoattractants. It stimulates actin polymerization, is active on monocytes and not on neutrophils in vitro, and in vivo is both a highly efficacious and highly potent mononuclear cell attractant. Its sequence shows identity with stromal cell-derived factor (SDF-1)(4–6), previously known to augment B cell proliferation. SDF-1 shows equidistant sequence homology to CC- and CXC-chemokines and strong conservation in evolution that suggest it as a primordial chemokine. SDF-1 by contrast to most chemokines is constitutively expressed in a broad range of tissues (4–6)

[1]*Abbreviations used in this paper:* MCP-1, monocyte chemoattractant protein 1; MIP-1α, macrophage inflammatory protein 1α; RANTES, regulated on activation normal T cell expressed and secreted; SDF, stromal cell-derived factor.

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and therefore may have a role in immune surveillance rather than in inflammation.

Materials and Methods

Three-Step Purification of a Lymphocyte Chemoattractant. Adherent MS-5 murine bone marrow stromal cells (7) were cultured in expanded surface roller bottles (model 25285-1700; Corning, Corning, NY) in 150 ml Iscove's modified Dulbecco's medium with 10% FCS and 2×10^{-5}M 2-mercaptoethanol until they reached confluence. The medium was replaced with 250 ml X-VIVO serum-free medium (BioWhittaker, Walkersville, MD) containing 10 mM Hepes. Supernatants were collected and replaced with fresh medium every 5 d for up to five times. Conditioned medium (1,000 ml) was passed through a 0.22-μm filter and applied to a 5-ml-HiTrap heparin column (Pharmacia Biotech, Uppsala, Sweden) on a Pharmacia FPLC system using FPLC director 1.03 software. The column was washed with 25 ml of 10 mM sodium phosphate, pH 7.3, and eluted with 50 ml of a linear gradient of 0.4–2.0 M NaCl in 10 mM sodium phosphate, pH 7.3. Active material eluted at 0.8–0.9 M NaCl (14 ml) was pooled and diluted with 10 mM sodium phosphate, pH 7.3, to 0.15 M NaCl, and applied to a 1 ml SP Sepharose HP cation exchange column (Pharmacia Biotech, Uppsala, Sweden) equilibrated with the same buffer, washed with 5 ml of the same buffer, and eluted with 40 ml of a linear gradient of 0.1–1.0 M NaCl in 10 mM sodium phosphate, pH 7.3. Active material that eluted at ~0.52 M NaCl (2 ml) was applied to a 4.6 × 150 mm Vydac C4 reverse-phase HPLC column (Vydac, Hesperia, CA), equilibrated with 0.1% trifluoroacetic acid and eluted with a 60 ml 0–80% acetonitrile gradient in 0.1% trifluoroacetic acid. 2-ml fractions were lyophilized and resuspended in 100 μl of 10 mM sodium phosphate, pH 7.3, 150 mM NaCl for chemotaxis assays or in 10 mM sodium phosphate, pH 7.3, for NH_2-terminal amino acid sequencing (190 pmol), amino acid composition, and mass spectrometry. Purified protein was submitted to automated Edman microsequencing on an Applied Biosystems 494A protein sequencer (Foster City, CA) using the manufacturer's recommended protocol (8). Molecular weight was determined by microcapillary HPLC/electrospray ionization/tandem mass spectrometry on a Finnigan TSQ7000 triple quadrupole mass spectrometer (San Jose, CA) and by matrix-assisted laser desorption mass spectrometry on a Finnigan Lasermat 2000 (Hemel, England) as described previously (9). Protein concentrations were determined by BCA protein assay (Pierce Chem. Co., Rockford, IL).

Cell Preparation and Chemotaxis Assay. Human PBL were obtained from healthy donors by erythrocyte sedimentation with 6% Dextran T500 followed by Ficoll-Histopaque-1077 (Sigma Chem. Co., St. Louis, MO) separation. Monocytes were removed by two 30-min steps of plastic adherence (10). Human neutrophils were prepared as described (11). Human PBL (5×10^5) in 100 μl RPMI-1640 medium, 0.25% human serum albumin (HSA) were added to the top chamber of a 6.5-mm diameter, 5-μm pore polycarbonate Transwell™ culture insert (Costar, Cambridge, MA) and incubated in duplicate with the indicated concentrations of purified material or dilutions of fractions from purification in the bottom chamber for 3 h. Fractions from the heparin, SP Sepharose, and HPLC columns were diluted 2-fold, 10- to 50-fold, and 40- to 1,000-fold, respectively, before assay. The chemotaxis assays across bare filters and endothelial monolayers cultured on filters were carried out as described previously (10, 11), except cells that transmigrated into the lower chamber were vigorously suspended and counted with a FACScan® (Becton Dickinson, San Jose, CA) for 20 s at 60 μl/min, gating on the forward and side scatter of the cell type of interest. A 1:20 dilution of input PBL was similarly counted. For human monocytes, peripheral blood mononuclear cells (5×10^5), i.e., the cells from Ficoll-Histopaque before monocyte depletion, were migrated for 2 h through 5-μm pore polycarbonate filters and were scatter-gated on monocytes to exclude lymphocytes. Human neutrophils (5×10^5) were migrated for 1.5 h through 3-μm pore polycarbonate filters. Murine spleen and bone marrow cells were obtained from 129 mice and erythrocytes were lysed in NH_4Cl-buffer for 4 min on ice. Spleen cells and bone marrow cells were migrated for 3 h and 1.5 h and scatter-gated on lymphocytes and neutrophils, respectively. To effectively remove neutrophils and monocytes from plastic in the lower chamber a final concentration of 5 mM EDTA was added to each well and cells were resuspended vigorously.

Chemokines. MCP-1, RANTES, MIP-1α, IL-1α, IL2, IL-3, IL-4, IL-6, and IL-8 were obtained from Genzyme (Cambridge, MA) and lymphotactin from Peprotec (Rocky Hill, NJ). Synthetic lymphotactin was a kind gift of Dr. Ian Clark-Lewis (Honson Centre for Cancer Research, Adelaide, Australia). Murine MCP-1/JE was a kind gift of Dr. Catherine Ernst (Dana Farber Cancer Institute, Boston, MA).

Actin Polymerization Assay. Actin polymerization was tested as described before (12). Briefly, human PBL (1.25×10^6/ml) in L15 medium at 37°C were incubated with the indicated chemokines at concentrations that were shown to be optimal in chemotaxis experiments or PMA at 100 ng/ml. At the indicated time points, cells (400 μl) were added to 100 μl of a solution at 37°C containing 4×10^{-7} M FITC-labeled phalloidin, 0.5 mg/ml l-α-lysophosphatidylcholine (both Sigma) and 18% formaldehyde in PBS. The fixed cells were subjected to fluorescence flow cytometry on a FACScan® (Becton-Dickinson, San Jose, CA) and the mean relative fluorescence of each sample determined. All data points are plotted relative to the mean relative fluorescence of the sample before addition of chemoattractant.

Biologic Activity of SDF-1 In Vivo. C57Bl/6 mice were injected subcutaneously at a marked spot on the abdomen with 100 ng SDF-1 or murine MCP-1/JE in 20 μl sterile PBS or PBS alone. Injected protein solution was shown to contain <0.004 ng/ml LPS by the limulus assay (Sigma). Mice were sacrificed 18 h after injection, injection sites were excised and snap-frozen 5-μm cryosections were subjected to hematoxylin and eosin staining and for immunohistochemistry, sections were pretreated with avidin/biotin blocking reagent (Vector Labs., Burlingame, CA), stained with mAbs to Thy-1 (1:10; PharMingen, San Diego, CA) or MOMA-2 (1:10; Biosource International, Camarillo, CA) and developed with the avidin-biotin-HRP method (DAKO, Carpinteria, CA). Sections were counterstained with hematoxylin. No cellular infiltrate was detected in PBS injection sites that were sectioned in their entirety. The sections are representative of three experiments.

Phylogenetic Analysis. The mature amino acid sequences of known chemokines were aligned using the clustal method with a PAM250 residue weight table and default settings of gap penalties for pairwise and multiple alignments of 3 and 10, respectively (Megalign; DNASTAR, Madison, WI). The GenBank accession numbers or reference for sequences used from top to bottom were M31626, Z48479, M57440, X72308 (13), P80075, X77603, J04467, M23502, M23503, D00044, M23477, M77747, M21121,

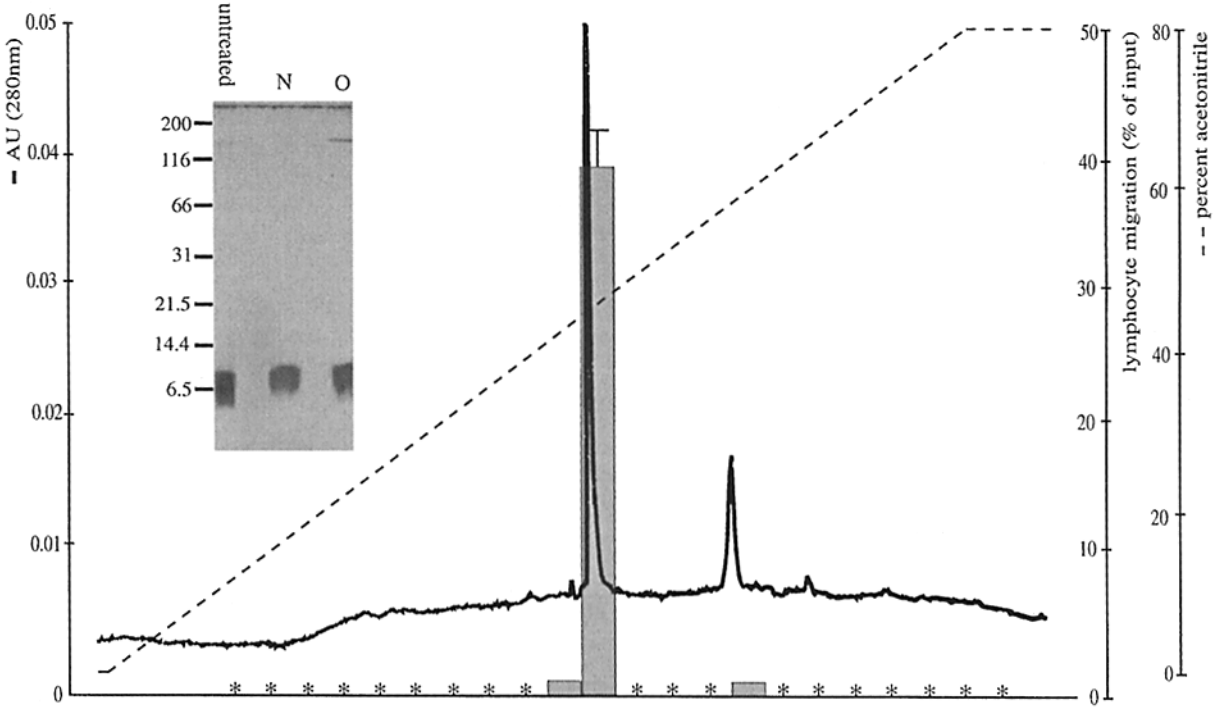

Figure 1. HPLC purification and SDS-PAGE of a stromal-derived lymphocyte chemoattractant. Reverse-phase HPLC on a Vydac C4 column was the last purification step. Fractions were diluted 40-fold for the chemotaxis assay, and the background migration of 4.5% was subtracted. Purified lymphocyte chemoattractant, fraction 21 from the HPLC column (0.5 μg) was subjected to SDS 15% PAGE and silver staining after no treatment or after treatment with *N*-glycanase (*N*) or *O*-glycanase (*O*) from Genzyme according to the manufacturer's instructions. The mass of standards is shown next to the gel. An asterisk indicates fractions with chemotactic activity equal to or below background.

U15607, U23377, U23772, M57506, M17957, J03561, M36820, M36821, J04596, M54995, X78686, M25897, M15254, Y00787, M57439, X02530, X72755, D21072, and U16752.

Results and Discussion

Purification of a Highly Efficacious Lymphocyte Chemoattractant. In peripheral blood lymphocyte chemotaxis assays, serum-free conditioned media of three murine bone marrow stromal cell lines, MS-5 (7), PA-6 (14), and ST-2 (15), consistently attracted 20–50% of the input lymphocytes, whereas supernatants from NIH 3T3 fibroblasts showed little activity. The chemotactic activity was purified 1,310-fold from MS-5 conditioned medium by sequential heparin, cation exchange, and reversed-phase HPLC chromatography (Table 1). The chemoattractant activity coeluted with a single protein peak in reversed-phase HPLC and migrated as a single band in SDS-PAGE of 8 kD (Fig. 1). We consistently obtained 80–90 μg of purified protein from 1,000 ml of conditioned medium. The NH_2-terminal sequence of the material was KPVSLSYRXPXRFFE, with smaller amounts of a sequence lacking the first five residues of the above sequence, SYRXPXRFFESHIA. This sequence, and the amino acid composition, showed identity with stromal cell-derived factor-1 (SDF-1), a chemokine identified by signal sequence trap cloning (4) and as a factor that augments IL-7-stimulated pre-B cell growth (5). Activity of SDF-1 in chemotaxis assays has not previously

Table 1. *Purification of a Stromal-derived Lymphocyte Chemoattractant*

	Volume	Chemotactic activity	Amount of protein	Specific activity	Purification	Yield
	ml	*U*	*mg*	*U/mg*	*-fold*	*%*
MS-5 supernatant	1000	4000	2720	1.47	1	100
Heparin HiTrap	72.3	1446	3.11	465	316	36.2
SP Sepharose HP	4	160	0.236	678	461	4.0
RP-HPLC	0.2	160	0.083	1930	1310	4.0

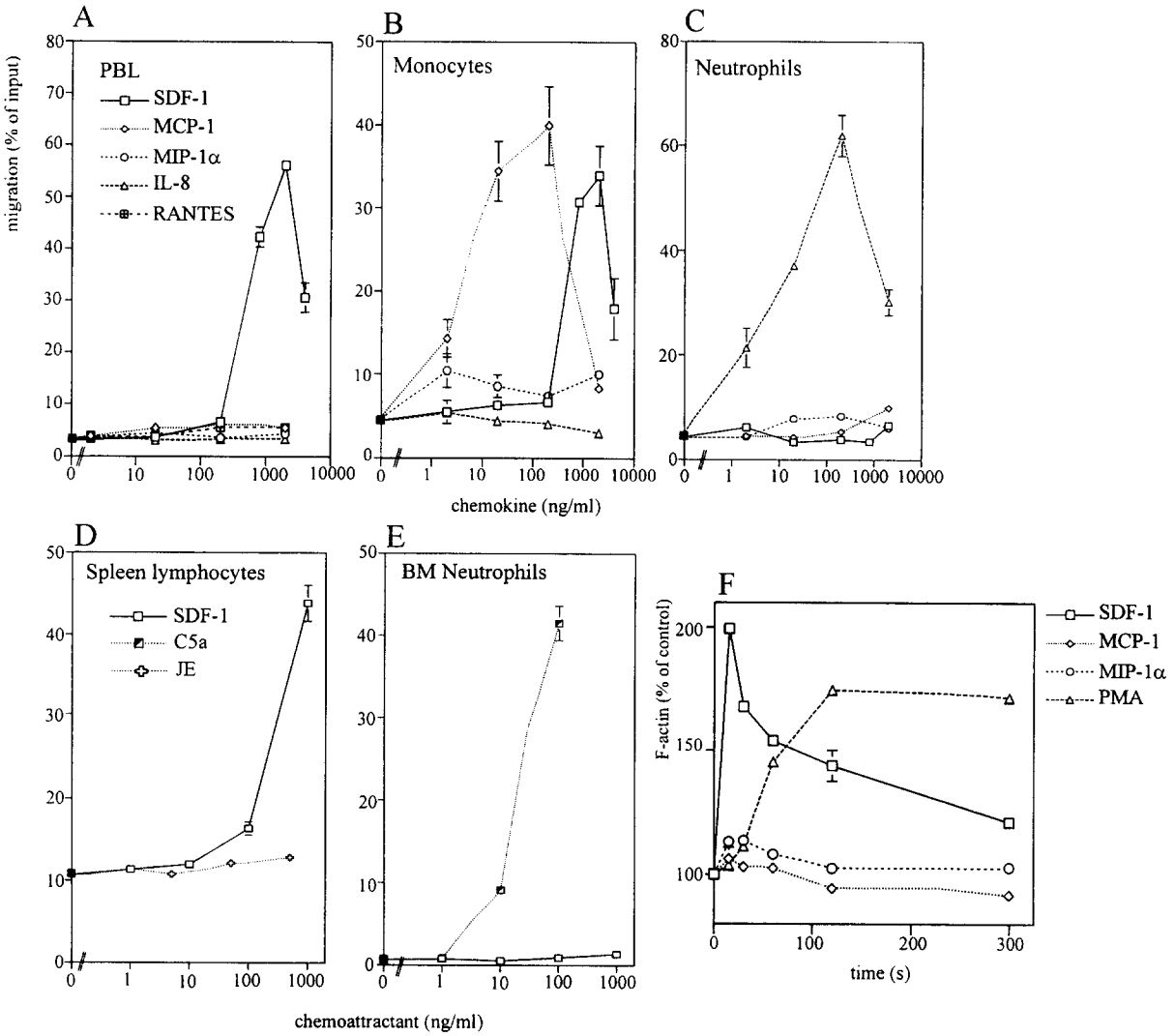

Figure 2. Chemotactic activity of SDF-1 on human and murine leukocyte subtypes and effect on actin polymerization. (*A–E*) The indicated human (*A–C*) or murine (*D–E*) leukocyte subtypes were subjected to chemotaxis through 5-μm pore (*A*, *B*, and *D*) or 3-μm pore (*C* and *E*) Transwell™ filters to the indicated chemoattractant in the lower chamber and the % of input cells that accumulated in the lower chamber was measured. The bars and symbols show duplicates and mean for one representative experiment of three. (*F*) Actin polymerization in human PBL. SDF-1 (1,000 ng/ml), huMCP-1 (100 ng/ml), huMIP-1a (100 ng/ml), and PMA (100 ng/ml) at the indicated optimal concentrations were added to PBL at time 0 and intracellular F-actin content in PBL at the indicated times was measured with FITC-phalloidin in permeabilized cells.

been reported. Two alternatively spliced forms of SDF-1 differ in the COOH-terminal four amino acid residues (4). Electrospray and matrix-assisted laser desorption mass spectrometry gave a mass of 7847.1 ± 0.4 daltons consistent with that of 7849.2 daltons predicted for SDF-1α beginning with the KPVSL sequence and with the COOH-terminal lysine residue removed by processing. SDF-1 is not glycosylated, as shown by its mass and lack of effect of *N*-glycanase or *O*-glycanase (Fig. 1), and lacks predicted glycosylation sites.

SDF-1 Efficiently Attracts Lymphocytes and Monocytes In Vitro. Purified SDF-1 was a highly efficacious lymphocyte chemoattractant, attracting 70% of human peripheral blood lymphocytes in filter chemotaxis assays at optimal concentrations of about 1 μg/ml (Fig. 2 *A*). Consistent with the high percentage of emigrating cells, the proportions of $CD4^+$, $CD8^+$, $CD26^+$, $CD45RA^+$, and $CD45RO^+$ T lymphocyte subsets were similar in the starting and emigrated populations by immunofluorescent flow cytometry. The proportions in the starting and emigrating populations were for CD4 47 and 48%, for CD8 24 and 28%, for CD26 64 and 66%, for CD45RA 42 and 44%, and for CD45RO 40 and 43%, respectively. SDF-1 was also an efficacious chemoattractant and showed a similar dose-response for murine lymphocytes (Fig. 2 *D*) and human monocytes (Fig. 2 *B*), but was not active on human or murine neutrophils (Fig. 2, *C* and *E*). Chemotaxis to SDF-1 was dependent on a $G_{\alpha i}$-coupled receptor. Pretreatment of PBL with 50 ng/ml of pertussis toxin inhibited migration to 1 μg/ml of SDF-1 of 85 ± 2.9% of input lymphocytes to background levels of 3.2 ± 0.7%.

We compared SDF-1 to other lymphocyte chemoattrac-

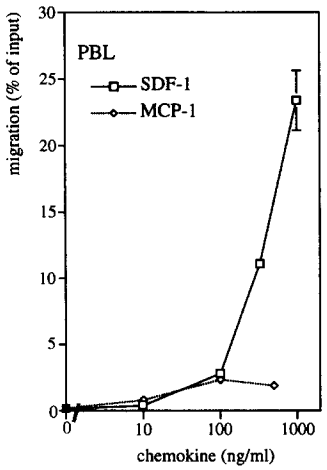

Figure 3. Transendothelial chemotaxis of human PBL to SDF-1 and MCP-1. Human umbilical vein endothelial cells (HUVEC) cultured on 8-μm pore Transwells™ were used in a chemotaxis assay with human PBL. One representative experiment of three is shown. Error bars indicate the range of duplicates.

tants in a range of concentrations previously reported to be most active; the greatest migratory response to each and the concentration giving the greatest response are shown in Table 2. MCP-1, RANTES, and MIP-1α consistently induced migration over background, and were more potent than SDF-1, i.e., active at lower concentrations, but were substantially less effective, attracting only 2–4% of the input cells. Furthermore, the chemotactic index, the ratio of specific to background migration is only 1.3- to 2.3-fold for MCP-1, RANTES, and MIP-1α in filter chemotaxis assays. Fig. 2 *A* visualizes this difference in efficacy between SDF-1 and proinflammatory chemokines over a broad range of chemokine concentrations. Two different sources of lymphotactin showed little activity in our assay, as did different interleukins (Table 2), basic fibroblast growth factor, leukemia inhibitory factor, transforming growth factor-β, platelet-derived growth factor AA, and granulocyte monocyte-colony stimulating factor (data not shown).

SDF-1 was also a highly effective transendothelial chemoattractant. It attracted 20–30% of input PBL (Fig. 3), whereas MCP-1, RANTES, and MIP-1α attract 1–3% of input lymphocytes across endothelium (Fig. 3) and (3, 10, 11). The background in transendothelial chemotaxis assays (Fig. 3) is markedly lower than in filter chemotaxis assays (Fig. 2 *A*), as previously noted (10). Checkerboard titrations demonstrated that SDF-1 stimulated chemotaxis rather than random migration in both transendothelial (Table 3) and bare filter (not shown) chemotaxis assays.

SDF-1 Induces Actin Polymerization in Lymphocytes. To monitor intracellular actin reorganization, a process that is thought to be a prerequisite for cell movement, we assayed actin polymerization (12). SDF-1 (1,000 ng/ml) induced a transient twofold increase in intracellular filamentous-actin within 15 s in human peripheral lymphocytes (Fig. 2 *F*). Actin polymerization in neutrophils is stimulated by IL-8, fMLP, C5a, and LTB4 with the same kinetics (16, 17) but to our knowledge an increase in F-actin has not been previously observed with chemokines in lymphocytes. Consistent with our findings in the chemotaxis assay, the other lymphocyte chemoattractants stimulated little actin polymerization.

Table 2. *Chemotaxis of Human Peripheral Blood Lymphocytes to Optimal Concentrations of SDF-1, Interleukins and Previously Described Lymphocyte Chemoattractants*

	Migrated lymphocytes above background	Chemotactic index
	% of input ± SD	*± SD**
SDF-1 (1,000 ng/ml)	69.2 ± 8.8	20.3 ± 2.1
hu MCP-1 (100 ng/ml)	3.4 ± 0.7	2.3 ± 0.4
mu MCP-1/JE (50 ng/ml)	1.9 ± 0.4	1.6 ± 0.1
hu RANTES (100 ng/ml)	2.5 ± 0.8	1.5 ± 0.2
hu MIP-1α (10 ng/ml)	2.7 ± 0.2	1.3 ± 0.3
hu lymphotactin (100 ng/ml)	0.4 ± 0.1	1.1 ± 0.1
hu IL-1α (10 ng/ml)	−1.4 ± 0.9	0.7 ± 0.1
hu IL-2 (10 U/ml)	−0.7 ± 1.3	0.9 ± 0.2
hu IL-3 (100 U/ml)	−0.3 ± 1.6	1.0 ± 0.3
hu IL-4 (30 U/ml)	−0.8 ± 1.3	0.8 ± 0.1
hu IL-6 (50 ng/ml)	−1.1 ± 1.0	0.9 ± 0.2
hu IL-8 (10 ng/ml)	0.4 ± 1.1	1.1 ± 0.2

*Chemokines and interleukins were assayed over a 1,000-fold concentration range and results at the optimal concentration are shown. Results are mean and SD of three independent experiments. Chemotactic index was defined as the ratio between the percentage of cells migrated to sample and to medium alone.

SDF-1 Is an Efficacious and Potent Chemoattractant for Mononuclear Cells In Vivo. Subcutaneous injection of 100 ng of purified SDF-1 into the skin of C57Bl/6 mice elicited a dense infiltrate of mononuclear cells in the subcutaneous tissue throughout a substantial surrounding area (Fig. 4, *B* and *C*). Multiple sections failed to reveal any infiltrate at PBS injection sites (Fig. 4 *A*). Injection of 100 ng of murine MCP-1 elicited an infiltrate of far fewer cells that were localized to the point of injection, and contained both monocytes and neutrophils (Fig. 4 *F*), in agreement with a

Table 3. *Checkerboard Assay of SDF-1 in Transendothelial Chemotaxis**

SDF-1 concentration in lower chamber	SDF-1 concentration (ng/ml) in top chamber			
	1,000	330	100	0
ng/ml	*Transendothelial migration (%)*			
1,000	0.9 ± 0.2	2.7 ± 0.1	14.2 ± 0.3	23.4 ± 2.3
330	0.2 ± 0.0	0.2 ± 0.0	1.4 ± 0.1	11.1 ± 0.2
100	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	2.8 ± 0.1
0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

*Results are representative of three experiments and are expressed as percentage of input lymphocytes ± range of duplicates.

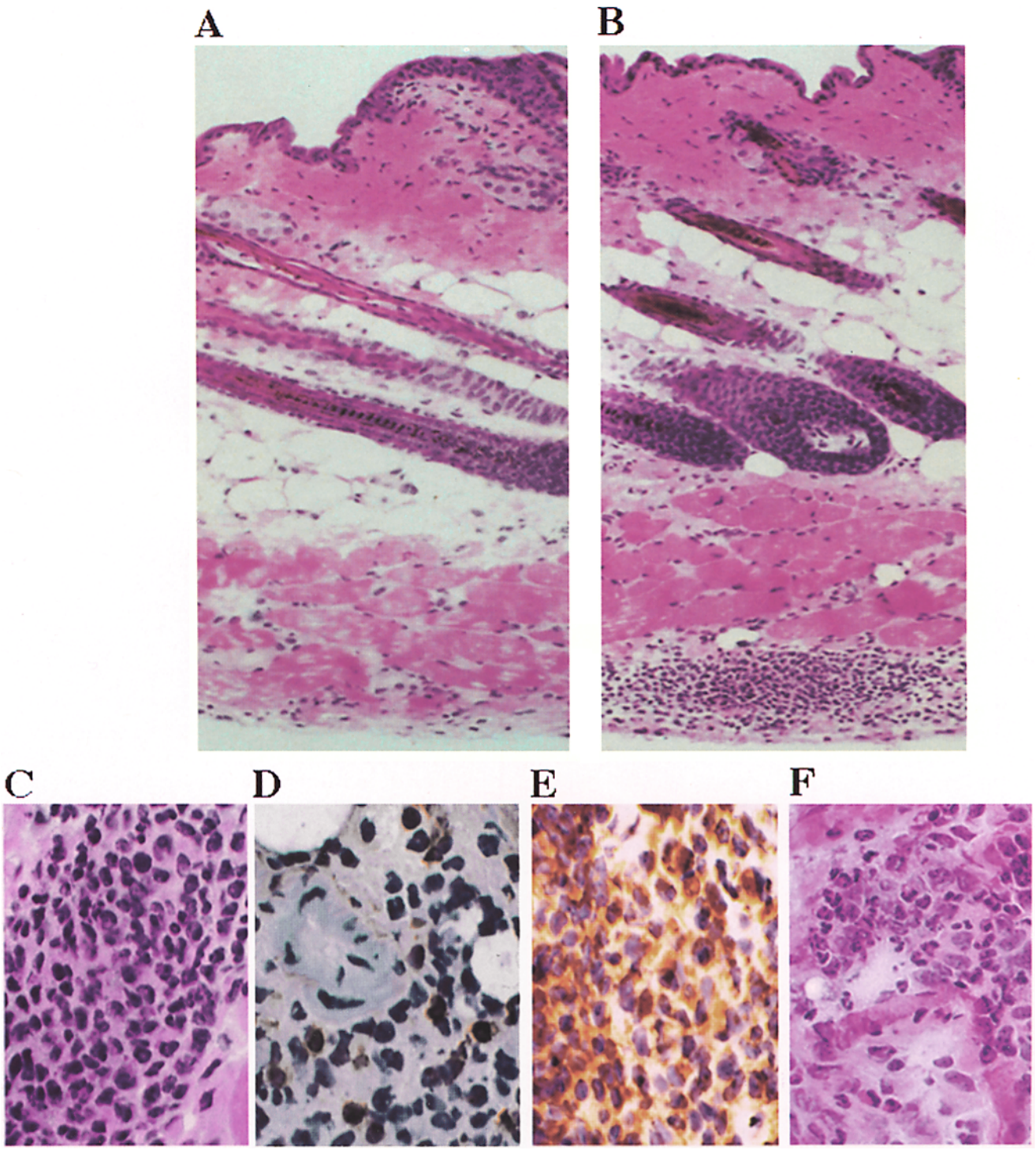

Figure 4. Activity of SDF-1 and MCP-1 in vivo. (*A*) Skin at the subcutaneous injection site of 20 μl PBS (10× magnification). (*B*) Skin at the subcutaneous injection site of 100 ng SDF-1 in 20 μl PBS (10× magnification). A dense cellular infiltrate is visible in the subcutaneous tissue below the panniculus carnosus. (*C–E*) 100× magnification of cellular infiltrate to 100 ng SDF-1. (*C*) Hematoxylin and eosin staining. (*D*) Immunohistochemical staining with mAb Thy-1 (19) as a marker for T cells. About 10% of the mononuclear cells were found to be Thy-1 positive. (*E*) Immunohistochemical staining with mAb MOMA-2 (20) as a marker for monocytes/macrophages. The majority of mononuclear cells were MOMA-2 positive. (*F*) Skin at the subcutaneous injection site of 100 ng murine MCP-1/JE in 20 μl PBS, 100× magnification, hematoxylin and eosin staining.

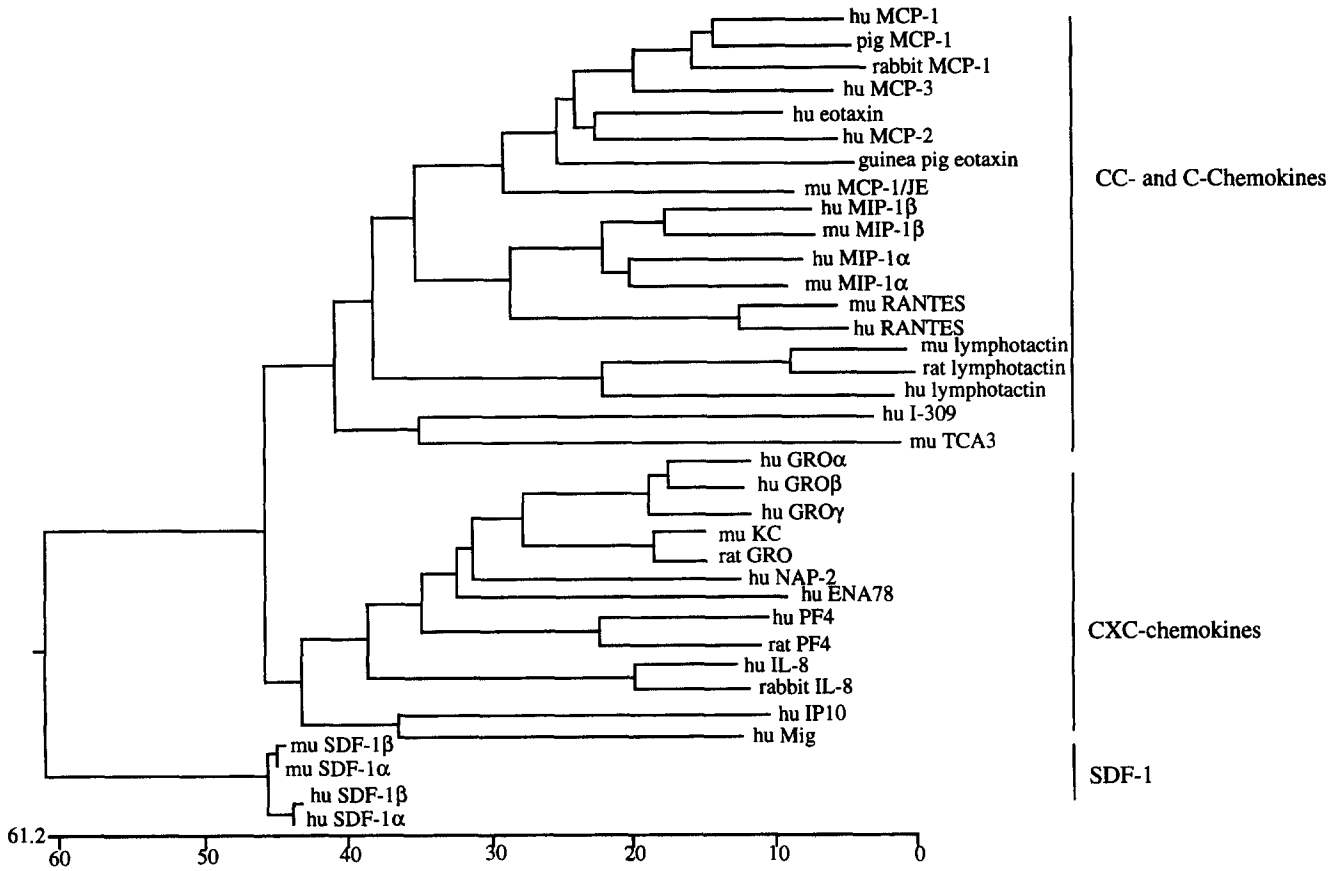

Figure 5. A phylogenetic tree of chemokines. The mature amino acid sequences of known chemokines in Swiss-Prot were aligned using the clustal method with a PAM250 residue weight table and default settings of gap penalties for pairwise and multiple alignments of 3 and 10, respectively (Megalign; DNASTAR, Madison, WI). Distances to branch points are proportional to amino acid sequence divergence from predicted ancestral sequences. The clustal alignment method grouped the CC-chemokines and the C-chemokine lymphotactin together in the same subfamily. Comparing mature human protein sequences, SDF-1α is 21 ± 4% and 27 ± 3% (mean ± SD) identical to CC- and CXC-chemokines, respectively; for comparison, MCP-1 is 49 ± 19% and 17 ± 2% identical and IL-8 is 19 ± 3% and 35 ± 7% identical to other human CC- and CXC-chemokine subfamily members, respectively.

previous report (18). Less than 1% of the cells infiltrating to SDF-1 were neutrophils. Immunohistochemistry with mAb to Thy-1 (19) revealed the presence of up to 10% lymphocytes (Fig. 4 *D*) among a majority of monocytes/macrophages as demonstrated by staining with mAb MOMA-2 (20; Fig. 4 *E*).

We have shown that SDF-1 exhibits unparalleled efficacy as a lymphocyte chemoattractant in vitro and effectively attracts mononuclear cells in vivo. It attracts ~10-fold more lymphocytes than previously described chemoattractants, and achieves an efficacy comparable only to that seen previously with attractants of neutrophils, eosinophils, and monocytes (2). In vitro SDF-1 is less potent, i.e., requires higher concentrations for maximal activity, than MCP-1, RANTES, and MIP-1α. However, in vivo SDF-1 is both a highly potent and highly efficacious chemoattractant for attraction of mononuclear cells. The number of cells infiltrating to 100 ng SDF-1 is far greater than to 100 ng MCP-1 in parallel experiments, and greater than seen in previous in vivo studies to all doses of MCP-1, MCP-2, MCP-3, and RANTES (18, 21–23). It is intriguing to observe the high percentage of monocytes in the infiltrate although SDF-1 attracts a similar percentage of lymphocytes and monocytes with similar dose-response in vitro (Fig. 2, *A* and *B*). This finding may be due to the higher migratory capability of monocytes compared to lymphocytes that has been observed in different in vivo models (24). Issekutz and Issekutz show that skin injections of zymosan-activated serum (ZAS), TNF-α, IFN-γ, or LPS in rats induce consistently more than fivefold greater monocyte than lymphocyte accumulation at all time points tested (24). This is comparable to the ratio of monocyte to lymphocyte emigration seen here with SDF-1 in vivo. Greater expression of adhesion molecules, greater motility, or a lower frequency of exit from the site after entry, may favor greater accumulation of monocytes than lymphocytes in vivo, even when the strength of the chemoattractive stimulus is comparable.

The in vivo potency of SDF-1 is remarkable. SDF-1 is highly basic with a predicted pI of 10.9 compared to 9.8 and 9.9 for IL-8 and MCP-1, respectively. SDF-1 binds heparin with higher affinity than IL-8 or MCP-1 in that elution from heparin Sepharose requires 800–900 mM NaCl, whereas IL-8 and MCP-1 elute at ~500 mM NaCl (3, 25). Strong binding to glycosaminoglycans may present

SDF-1 in an effective manner or help retain it at sites of production, and may in part explain its observed potency in vivo.

The evolutionary and sequence homology properties of SDF-1 are unique among chemokines. First, the human homologue of SDF-1 (26) has a single conservative Ile to Val substitution compared to the murine SDF-1 studied here. This strong conservation (99% identity) is unparalleled among chemokines; for comparison MCP-1 and RANTES are 60 and 83% identical between mouse and human. Second, based on amino acid sequence homologies, SDF-1 is almost equally related/distant from both CC- and CXC-chemokines (Fig. 5 and legend). This coincides with the finding that the human SDF-1 gene is localized on chromosome 10 while all other known CXC- and CC-chemokines cluster on chromosomes 4 and 17, respectively (26). Alignment of the mature amino acid sequences of known chemokines generates a phylogenetic tree that confirms that the CC- and CXC-chemokines belong to separate subfamilies, and suggests that SDF-1 belongs to neither and forms an outgroup. SDF-1 is predicted among currently known chemokines to have diverged the least from a primordial chemokine ancestor; this is consistent with the high conservation of SDF-1 between species. Because of both its functional and predicted phylogenetic distinctions from CC- and CXC-chemokines, SDF-1 is thus far a unique chemokine; whether it represents the first member of a new chemokine subfamily remains to be determined.

SDF-1 is constitutively produced by bone marrow stromal cells, and therefore could be involved in trafficking of lymphocytes and monocytes to this site. Bone marrow stromal cells produce it in large quantities, with 0.8 μg/ml recovered from cultured cells. Whether SDF-1 plays a special role in bone marrow, or whether production is higher to compensate for rapid loss through the fenestrated endothelium of bone marrow, remains to be determined. If SDF-1 is important in attracting mature lymphocytes to bone marrow, antagonism of this function before harvest of bone marrow for transplantation could be clinically beneficial.

SDF-1 may be of wider importance. SDF-1 mRNA is constitutively expressed in every tissue thus far examined, including heart, liver, lung, brain, muscle, spleen, and kidney (4, 6, 27), although protein expression has not yet been characterized. Most other chemokines are only expressed in response to proinflammatory stimuli, and are thought to regulate the emigration and activity of leukocytes at inflammatory sites. By contrast, expression of SDF-1 is unaltered by inflammatory stimuli (27). Normal trafficking of lymphocytes through tissues during immune surveillance requires G protein-coupled receptors (1) but the identity of the receptors on lymphocytes and the ligand(s) remain uncharacterized. In the absence of inflammation blood monocytes constantly replace mononuclear phagocytes in solid tissues maintaining a steady state by emigrating from the blood stream and differentiating into macrophages. SDF-1 has a tissue distribution that suggests it as a candidate for a function in lymphocyte recirculation, and in basal recruitment of monocytes in normal replenishment and turnover of tissue mononuclear phagocytes, rather than in inflammation.

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