Expression of Stromal-Derived Factor-1 Is Decreased by IL-1 and TNF and in Dermal Wound Healing[1]

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Stromal-derived factor-1 (SDF-1) is a CXC chemokine that is believed to be constitutively expressed by stromal cells of numerous tissues. In this report, we demonstrate that dermal fibroblasts and vessels of noninflamed tissues express SDF-1. Unexpectedly, we found that expression of SDF-1 is regulated by inflammation. Expression of SDF-1 by primary cultures of human gingival fibroblasts is potently inhibited by activated macrophages via secretion of IL-1α and TNF-α. Levels of SDF-1 mRNA also decrease in acutely inflamed mouse dermal wounds. We propose that SDF-1 functions as a homeostatic regulator of tissue remodeling, whose expression stabilizes existing dermal architecture. *The Journal of Immunology,* 2001, 166: 5749–5755.

Stromal cell-derived factor 1 (SDF-1)[3] is a CXC chemokine that attracts hemopoietic progenitor cells (1, 2), lymphocytes (3, 4), and monocytes (5) in vitro and is required for fetal B lymphopoiesis and myelopoiesis in vivo (6–8). It is highly conserved between species; human and mouse SDF-1α are 99% identical (9), making SDF-1 the most highly conserved chemokine described to date. Two alternate splice variants of the *sdf1* gene give rise to SDF-1α and SDF-1β, which are identical, except that SDF-1β contains an additional four amino acid residues in the carboxyl terminus (9). There appear to be no differences between SDF-1α and SDF-1β in regulated expression or function.

Unlike other CXC chemokines, SDF-1 is basally expressed in numerous tissues (9, 10), and expression is not increased in experimental allergic encephalomyelitis (11). Therefore, it has been assumed that SDF-1 is a constitutively expressed chemokine. *Sdf1* is unusual in that it is located on human chromosome 10, whereas all other known CXC chemokines are located on chromosome 4 (9). The promoter of *sdf1* contains several CpG islands, a transcription factor binding motif commonly observed in housekeeping genes (9). Binding motifs for the transcription factors NF-κB and AP-1 have not been found in a 19-kb *sdf1* genomic clone (9).

In contrast, binding sites for NF-κB and AP-1 exist in the promoters of other CXC chemokine genes, and these transcription factors induce expression of these chemokines in response to inflammatory stimuli.

CXC chemokines and SDF-1 also regulate mesenchymal cell activities. Several chemokines that contain an amino-terminal Glu-Leu-Arg (ELR) motif, such as IL-8, are angiogenic, whereas several CXC chemokines that lack the ELR motif, such as IFN-γ-inducible protein-10 (IP-10), are angiostatic (12). SDF-1 is a non-ELR, CXC chemokine and is unique in that it has both angiogenic and angiostatic properties. SDF-1 is chemotactic for endothelial cell lines in vitro, and these cells express its receptor, CXCR4 (13–16). SDF-1 is angiogenic ex vivo in aortic strips and in vivo in the dermis (16). Conversely, SDF-1 has no effect in the corneal micropocket assay, whereas IL-8, vascular endothelial growth factor, and basic fibroblast growth factor are angiogenic (12, 17). Furthermore, SDF-1 and other non-ELR, CXC chemokines block the angiogenic effect of IL-8, vascular endothelial growth factor, and basic fibroblast growth factor in this system, suggesting that non-ELR-chemokines can have a dominant inhibitory effect on angiogenesis (12, 17). SDF-1 and CXCR4 also regulate fetal vascular development. Targeted disruption of *sdf1* or the gene for CXCR4 results in perinatal death, aberrant vascularization of the gastrointestinal tract and kidney, and thin-walled vessels that are susceptible to hemorrhage (7, 18).

Herein we demonstrate a novel modality of chemokine regulation: down-regulation in response to inflammation. We describe the expression of SDF-1 by stromal cells and the vasculature of skin and observe that this expression is inhibited during dermal wound healing. We demonstrate that IL-1 and TNF-α inhibit the expression of SDF-1 by human fibroblasts in vitro. Therefore, we believe that SDF-1 is a homeostatic molecule whose expression maintains normal dermal function.

Materials and Methods

Chemokines, cytokines, and Abs

Synthetic full-length, human SDF-1α (residues 1–67) and inactive, truncated SDF-1α (residues 6–67), were gifts from I. Clark-Lewis (University of British Columbia, Vancouver, Canada). Cytokines, ELISAs for IL-1 and TNF, IL-1R antagonist (IL-1Ra), soluble TNF receptor 1 Fc chimera (TNF-R1/Fc), and blocking Abs against SDF-1 were purchased from R&D Systems (Minneapolis, MN). LPS, PMA, and ionomycin were purchased from Sigma (St. Louis, MO). Anti-human CXCR4 mAbs were prepared

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within the laboratory. Myeloma IgG1 was purified from supernatants of the X63 mouse myeloma and used as a control IgG.

Human tissue and primary cell culture

Healthy noninflamed abdominal skin biopsies were obtained from the Department of Obstetrics and Gynecology, Center for Reproductive Biology, University of Edinburgh (Edinburgh, U.K.), and gingival biopsies were obtained from the Department of Periodontology, Eastman Dental Center (Rochester, NY). Primary cultures of skin and gingival keratinocytes were obtained from the Harvard Skin Disease Research Center (Boston, MA). Primary dermal microvasculature endothelial cells, and aortic and coronary smooth muscle cells were obtained from Clonetics (Walkersville, MD). For assays, confluent fibroblast monolayers were dissociated with trypsin-EDTA, washed three times with PBS, and resuspended to 5×10^4 cells/ml in DMEM/10% FBS/50 μg/ml L-glutamine/50 U/ml gentamicin (medium A), and 100 μl was added per well of a flat-bottom, 96-well, tissue culture-treated dish (Corning-Costar, Corning, NY). Cells were incubated at 37°C in 10% CO_2 for 3 days. Medium was then replaced with 100 μl of medium A containing monocyte supernatants, IL-1, TNF, etc., at the indicated concentrations. Supernatants were removed 3 days later, stored at 4°C for further analysis, and replaced with medium A containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to measure cellular respiration (reflecting viability and proliferation) within these cultures. All primary cultures of fibroblasts were genotyped for SDF1–3′A, a polymorphism of *sdf1* (19). IL-1α and TNF-α regulated SDF-1 production by primary cultures of homozygous and heterozygous fibroblasts with similar magnitude.

Immunohistochemistry of tissue sections

Human and mouse tissue sections were snap-frozen in OCT compound (Sakura Finetek USA, Torrance, CA). Serial cryostat sections were stained in hematoxylin and eosin for histological evaluation or were fixed in acetone or 4% neutral buffered formalin for immunohistochemical staining with Abs directed against SDF-1α (rabbit polyclonal; Torrey Pines Biolabs, San Diego, CA), mouse CXCR4 (goat polyclonal; Santa Cruz Biotechnology, Santa Cruz, CA), and mouse CD31 (rat monoclonal; BD PharMingen, San Diego, CA). Some sections were stained with the macrophage marker CD68 (mouse mAb KP1; Dako, Carpenteria, CA) and myeloperoxidase. Detection was accomplished using the appropriate biotinylated secondary Ab and the Elite Vectastain avidin-biotin-HRP reagent followed by the chromogenic substrate diaminobenzidine tetrahydrochloride (Vector, Burlingame, CA). Sections were counterstained with hematoxylin.

Immunohistochemistry of acetone-fixed, short term, primary stromal cultures employed a rabbit polyclonal Ab directed against SDF-1α (Torrey Pines Biolabs). Healthy noninflamed abdominal skin biopsies were obtained from the Department of Obstetrics and Gynecology, Center for Reproductive Biology, University of Edinburgh. Detection was performed

with the appropriate biotinylated secondary Ab and the Elite Vectastain avidin-biotin-HRP reagent followed by the substrate 3-amino-9-ethylcarbazole

ELISA detection of SDF-1

Linbro/Titertek, 96-well, flat-bottom plates (Flow Laboratories, McLean, VA) were coated with 100 μl of a 1 μg/ml solution of affinity-purified, goat anti-human anti-SDF-1 polyclonal Ab (R&D Systems) at 4°C for 24 h. Plates were blocked by adding 200 μl of blocking buffer (PBS, 1% BSA, 5% sucrose, and 0.05 sodium azide (pH 7.4)) for 1 h at 22°C. Plates were washed three times with wash buffer (PBS and 0.05% Tween 20 (pH 7.4)). SDF-1α and SDF-1β standards and fibroblast culture supernatants were added in 100-μl volumes, and the plates were incubated for 2 h at 22°C. Plates were washed three times with wash buffer. Next, 100 μl of a 1 μg/ml solution of biotinylated, goat anti-human SDF-1β polyclonal Ab (R&D Systems) was added. Plates were incubated for 2 h at 22°C and were washed three times with wash buffer. Biotin-avidin-HRP conjugate (Vector; 100 μl/well) was added, and plates were incubated at 22°C for 20 min. Plates were washed six times, and 100 μl of 3,3′,5,5′-tetramethylbenzidine substrate (Calbiochem, La Jolla, CA) was added per well. The reaction was stopped by adding 50 μl of 0.5 mM sulfuric acid, and OD was determined at a primary wavelength of 450 nm and a correction wavelength of 540 nm. This ELISA is sensitive to 25 μg/ml for SDF-1α and 100 pg/ml for SDF-1β. An ELISA for IL-8 was purchased from R&D Systems and was conducted following the manufacturer's protocol.

Bioassay detection of SDF-1

Bioassays employed chemotaxis of the human B acute lymphoblastic leukemia line, Nalm-6, that responds equally well to SDF-1α and SDF-1β, but does not respond to the CXC chemokines IL-8, neutrophil-activating protein 2, monokine induced by IFN-γ, and IP-10; the CC chemokines hemofiltrate CC chemokine 1, monocyte chemoattractant protein-1 (MCP-1), MIP-1α, MIP-3α (exodus-1), MIP-3β (ELC), RANTES, and 6Ckine (secondary lymphoid tissue chemokine) the C chemokine, lymphotactin; C5a; or SDF-1α_{6-67} (4). Chemotaxis was measured with the ChemoTx system (Neuro Probe, Cabin John, MD) using 3.25-mm diameter, 5-μm pore size polycarbonate membranes. Supernatants from primary fibroblast cultures or SDF-1 standards were placed in the lower chamber of a 96-well plate, and 2.5×10^5 Nalm-6 cells in 25 μl of medium A were added to the upper side of the membrane. The plate was placed into a humidified, 37°C incubator for 3 h. Cells in the lower chamber were vigorously resuspended and analyzed by forward vs side scatter using a FACScan (BD Biosciences, San Jose, CA), and viable cells were counted for 30 s at 60 μl/min. To determine the percentage of viable input cells that transmigrated to the lower chamber, dilutions of the input cell populations were counted by the same method.
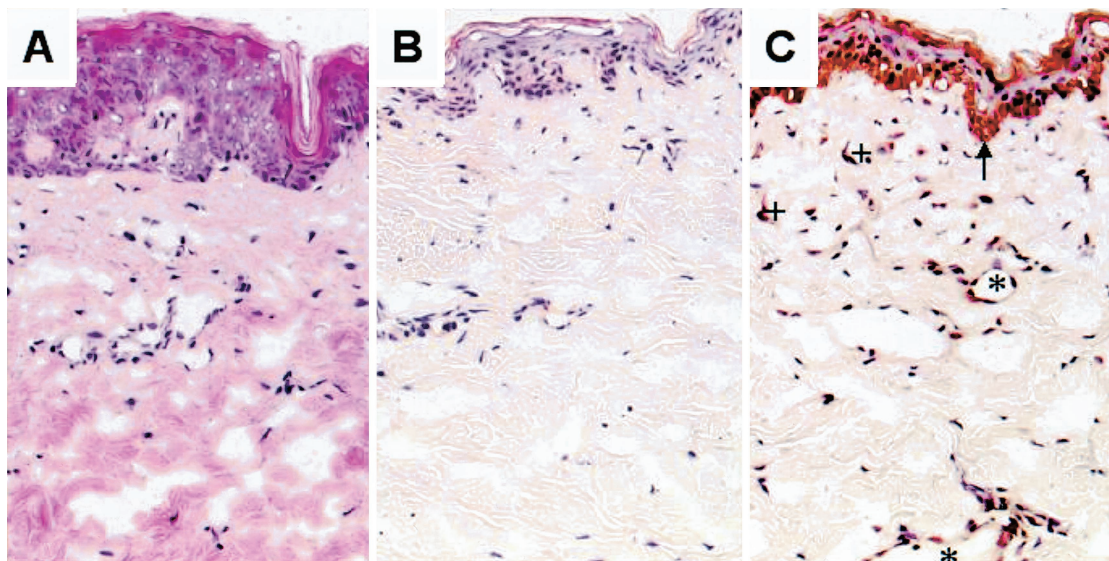

FIGURE 1. Keratinocytes, stromal cells, and vessels of human skin express SDF-1. Sections of noninflamed human abdominal skin were stained with hematoxylin and eosin (*A*), control rabbit Ig (*B*), or anti-SDF-1 Ig (*C*). The arrow, plus, and star symbols highlight specific immunostaining of keratinocytes, dermal stromal cells, and vessels, respectively.

RNase protection for mRNA encoding SDF-1

Fibroblasts were cultured in medium A in 75-cm^2 flasks (Corning-Costar). Medium was changed every 3 days, and upon reaching confluence, fresh medium A containing IL-1α or TNF-α was added to monolayers, and the cultures were incubated at 37°C in 5% CO$_2$ for 3 days. Monolayers were harvested using trypsin, washed, and counted. Cells were lysed in TRIzol (Life Technologies, Grand Island, NY), and total RNA was isolated according to the manufacturer's instructions.

The cDNAs for human IL-8, MCP-1, lymphotactin, RANTES, IP-10, MIP-1α, MIP-1β, I-309, L32, and GAPDH were obtained as the hCK-5 RiboQuant Human Cytokine MultiProbe Template Set (BD PharMingen). To construct a cDNA for human SDF-1α, oligonucleotide primers that exclusively bind to the 3′ untranslated region of SDF-1α mRNA and include a T7 transcriptional start site (sense, 5′-GCCTTAACCATGAGGACCAG-3′; antisense, 5′-GGATCCTAATACGACTCACTATAGGGACCAACGTGC ACAGGTACAGG-3′) were used to amplify cDNA containing the SDF-1 mRNA from human fibroblasts by RT-PCR. Similar oligonucleotide primers specific to the 3′ untranslated region of SDF-1β mRNA (sense, 5′-GC AAAAGGACTCTCAGGACC-3′; antisense, 5′-GGATCCTAATACGACT CACTATAGGGAGACATTGGTAGAGGAGGACGCTGGCTTTG-3′) were used to amplify an SDF-1β template. For riboprobe synthesis, the two SDF-1 cDNAs were included with a GAPDH template (Ambion, Austin, TX) or the hCK-5 template set, using [α-^{33}P]UTP (NEN, Boston, MA) and an in vitro transcription kit (BD PharMingen), following the manufacturer's instructions. Probes were hybridized to total RNA from human gingival fibroblasts, and RNase digestion was conducted using the RiboQuant RNase protection kit (BD PharMingen) according to the manufacturer's instructions. The protected fragments were resolved in a denaturing, 6% poly-acrylamide sequencing gel.

Wounding of mouse tissues

Female B6129F2/J mice, 6–8 wk old (The Jackson Laboratory, Bar Harbor, ME), were weighed and anesthetized i.p. with the appropriate dose of ketamine HCl (Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (Phoenix Pharmaceutical, St. Joseph, MO) solution (0.15 ml of xylazine, 0.15 ml of ketamine HCl, and 0.7 ml of PBS). Hair was removed from the dorsal surface using Nair lotion (Carter Products, New York, NY), and this area was swabbed with ethanol. The hairless area was drawn away from the mouse with forceps, and a 2-mm full thickness dermal punch biopsy (Sklar Instruments, West Chester, PA) was removed from both exposed epidermal surfaces. One to 10 days after wounding, a lethal dose of xylazine/ketamine HCl solution was administered i.p. Wounds were photographed, and wound areas were measured and then surgically excised. Excised wounds were then processed for semiquantitative RT-PCR or immunohistologic analysis. Housekeeping genes were employed to control for differences in the amount of RNA collected between healthy biopsies and wound tissue.

Semiquantitative RT-PCR analyses of mRNA encoding SDF-1

Inflamed wound tissue was removed from the surrounding normal tissue via dissection under a microscope. Wound tissue was homogenized in TRIzol (Life Technologies), RNA was isolated according to the manufacturer's instructions, and the concentration of total RNA was determined by spectrophotometry (OD$_{260}$). A Perkin-Elmer 9600 Thermocycler (Foster City, CA) was employed to detect mRNA encoding SDF-1 and the housekeeping GAPDH by RT-PCR. One microgram of total RNA was added to diethylpyrocarbonate-treated water containing 1 μg of oligo(dT) (Roche, Indianapolis, IN); 0.2 mM dATP, dCTP, dGTP, and dTTP (Roche); 5.0 mM DTT, 50 U of avian myeloblastosis virus reverse transcriptase; and
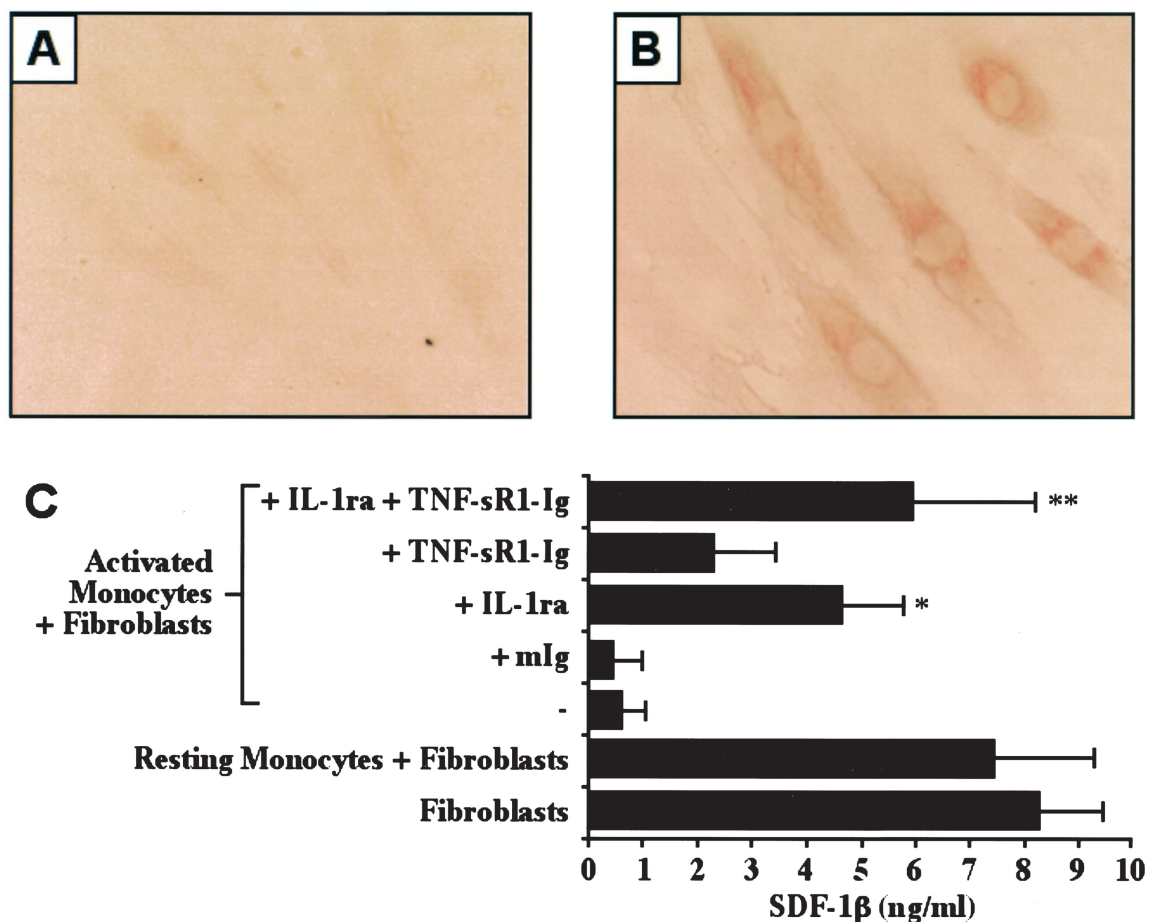

FIGURE 2. Expression of SDF-1 by fibroblasts is inhibited by activated monocytes via IL-1 and TNF. *A* and *B*, Immunohistochemistry of primary cultures of human gingival fibroblasts incubated with control rabbit Ig (*A*) or anti-SDF-1 Abs (*B*) demonstrated cytoplasmic staining of a majority of the cultured cells. *C*, Human gingival fibroblasts were cultured with supernatants from unstimulated or LPS-activated monocytes, and SDF-1 levels in fibroblast supernatants were measured by ELISA. Data are presented as the mean of three independent experiments that assayed three different fibroblast donors and three different monocyte donors. *, $p < 0.05$; **, $p < 0.01$.
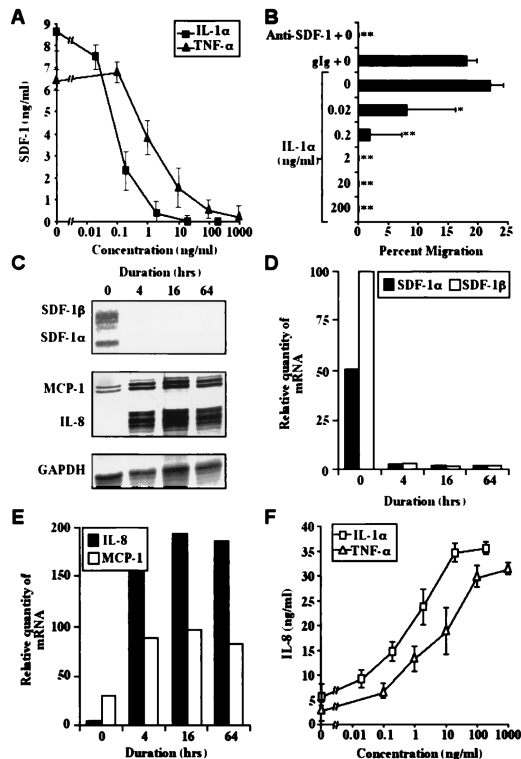
FIGURE 3. Recombinant IL-1α and TNF-α potently inhibit SDF-1 production by human fibroblasts while increasing the expression of IL-8 and MCP-1. *A* and *B*, Primary cultures of human gingival fibroblasts were stimulated with rIL-1α or rTNF-α, and the amount of SDF-1 in culture supernatants was measured by ELISA (*A*) and by a Nalm-6 chemotaxis bioassay (*B*). *, $p < 0.05$; **, $p < 0.01$ (compared with untreated controls). *C–E*, Human gingival fibroblasts were stimulated with IL-1α (1 μg/ml) for the indicated period, and levels of mRNA encoding SDF-1α, SDF-1β, IL-8, MCP-1, and GAPDH were determined by RNase protection assay (*C*). The intensities of the mRNA for GAPDH, SDF-1α, and SDF-1β (*D*) or IL-8 and MCP-1 (*E*) were measured, and values for GAPDH were used to normalize for differences in the total amount of RNA analyzed at the indicated time points. *F*, Primary cultures of human dermal fibroblasts were stimulated with rIL-1α or rTNF-α, and the amount of IL-8 in culture supernatants was measured by ELISA. Data in *A*, *B*, and *F* are presented as the mean of three independent experiments that assayed three different fibroblast donors. Error bars exhibit the SD of the values. Data in *C*, *D*, and *E* are from three independent experiments.

avian myeloblastosis virus buffer (Roche). Reactions were incubated at 50°C for 30 min and then at 94°C for 5 min and stored at −20°C. An aliquot of the RT reaction was added to PCR buffer (Roche); 0.2 mM dATP, dCTP, dGTP, and dTTP (Roche); 1 mM oligonucleotide primers to the shared coding region of SDF-1α and SDF-1β (sense, 5′-AGTGACGG TAAACCAGTCAG-3′; antisense, 5′-CTTTCTCCAGGTACTCTTGG-3′); and 1 mM oligonucleotide primers to GAPDH (sense, 5′-ATGGTGAAGGT CGGTGTGAACG-3′; antisense, 5′-GTTGTCATGGATGACCTTGGCC-3′), and 2 units of Taq DNA polymerase (Roche). Reactions were initially denatured at 94°C for 2 min and run for 40 cycles of PCR amplification (94°C for 0.5 min, 60°C for 0.5 min, and 72°C for 1.0 min), with a final extension at 72°C for 7 min. For each RT-PCR, a parallel reverse transcriptase-negative control reaction was performed. Aliquots of each reaction were removed at the indicated cycles and were subjected to gel electrophoresis in a 2% agarose gel containing ethidium bromide.

Results

SDF-1 is expressed by fibroblasts and vascular elements of human skin

Cells that produce SDF-1 in vivo were identified in cryostat sections of uninflamed human abdominal skin (Fig. 1). Compared with control serum (Fig. 1*B*), specific immunostaining was ob-

served in the stratum basale of the epidermis (Fig. 1*C*). Specific immunostaining was also observed of stromal cells and vessels in the papillary and reticular layers of the dermis (Fig. 1*C*).

Activated monocytes inhibit production of SDF-1 via secretion of IL-1 and TNF

To examine the production of SDF-1 under better defined conditions, we dissociated skin and gingival biopsies and established primary fibroblast cultures. Immunohistochemistry with anti-SDF-1 Abs yielded specific immunostaining within a subset (60%) of fibroblasts in these monolayers, and the subcellular distribution of immunostain was consistent with staining of the endoplasmic reticulum (Fig. 2, *A* and *B*). We developed an ELISA for SDF-1 and found that primary cultures of skin and gingival fibroblasts produced relatively high amounts of SDF-1 (1.0–10 ng/ml), whereas similar cultures of microvascular smooth muscle and endothelial cells produced less SDF-1 (0.1–1.0 ng/ml; data not shown). We did not detect production of SDF-1 by cultures of primary skin and gingival keratinocytes, the human oral epidermoid carcinoma line KB, the human embryonic palatal mesenchymal cell line HEPM, or the human oral squamous cell carcinoma line SCC-25 (data not shown).

Injured and inflamed skin contains neutrophils, macrophages, and T cells in the dermis, in close proximity to fibroblasts. To examine the effect of inflammation on SDF-1 production, human monocytes were isolated from peripheral blood and cultured with and without LPS. Supernatants from these cultures were added to cultures of primary human gingival fibroblasts. Supernatants from LPS-activated monocytes inhibited secretion of SDF-1β by fibroblasts ($p < 0.01$), whereas supernatants from unstimulated monocytes were not inhibitory (Fig. 2*C*). IL-1 and TNF are primary secretory mediators of activated monocytes. ELISAs of supernatants from monocyte cultures detected 3 ng/ml of IL-1 and 2 ng/ml of TNF in LPS-activated cultures, whereas at least 200-fold lower concentrations existed in resting supernatants (data not shown). Preincubation of activated monocyte supernatants with IL-1Ra significantly ($p < 0.05$) restored secretion of SDF-1β in a dose-dependent manner (Fig. 2*C* and data not shown). Addition of a dimeric soluble TNF receptor (TNF-R1/Fc) to activated monocyte supernatants partially restored SDF-1β production (Fig. 2*C*). Addition of IL-1Ra and TNF-R1/Fc to activated monocyte supernatants restored more SDF-1β production than IL-1Ra alone (Fig. 2*C*).

Recombinant IL-1α and TNF-α inhibit SDF-1 production and increase IL-8 expression by human fibroblasts

IL-1α and TNF-α were directly tested for their ability to inhibit the secretion of SDF-1β by primary cultures of human fibroblasts. Each cytokine could completely inhibit the secretion of SDF-1β (Fig. 3*A*). IL-1α was an extremely potent inhibitor, exhibiting an IC$_{50}$ of 0.05 ng/ml, whereas TNF-α was less potent, with an IC$_{50}$ of 1.0 ng/ml (Fig. 3*A*). Nalm-6 pro-B cells, which Chemotax to SDF-1α and SDF-1β, but not to any other chemokine tested (4), were used to bioassay for secretion of SDF-1 (Fig. 3*B*). Nalm-6 migrated to primary fibroblast supernatants, and chemotaxis was completely inhibited by an Ab to SDF-1 (Fig. 3*B*). Stimulating fibroblast cultures with IL-1α abolishes this chemotactic activity with an IC$_{50}$ of 0.01 ng/ml (Fig. 3*B*), and TNF-α inhibited chemotaxis with an IC$_{50}$ of 0.5 ng/ml (data not shown). Lastly, GM-CSF, G-CSF, IL-3, IL-6, IL-7, IL-8, IL-10, IFN-γ, stem cell factor, PMA, and ionomycin did not stimulate or inhibit the production of SDF-1 protein as measured by ELISAs and bioassays (data not shown).

We established an RNase protection assay to determine whether mRNA encoding SDF-1α and SDF-1β were regulated by IL-1 and TNF. High levels of SDF-1α and SDF-1β message were detected
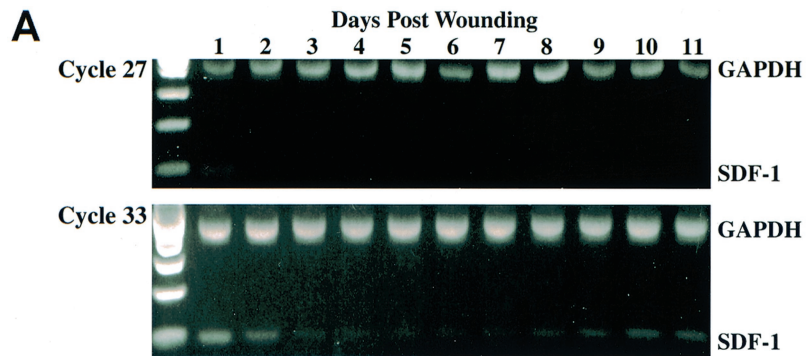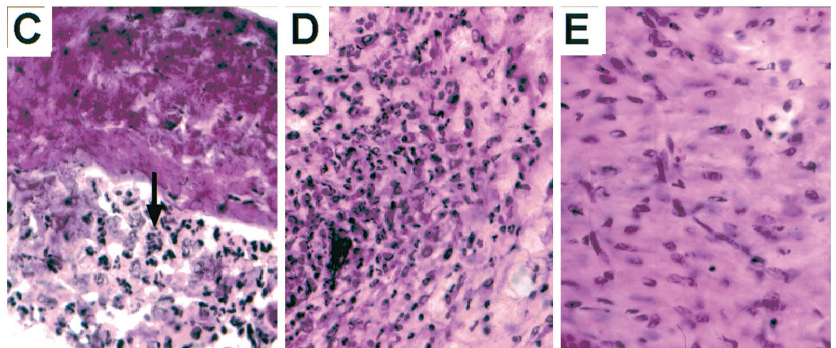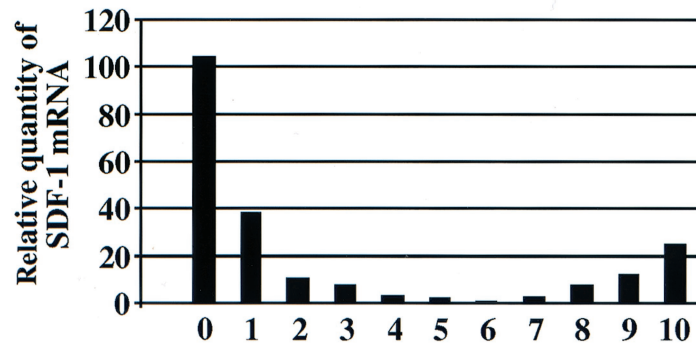
FIGURE 4. Levels of mRNA encoding SDF-1 are regulated in dermal wound healing. Dermal punch biopsies were removed from the dorsum of mice to create wounds (day 0), and wound tissue was excised on the indicated day thereafter. *A*, RT-PCR analysis of SDF-1 and GAPDH message in RNA from wounded tissue. *B*, Densitometry values for GAPDH were employed to normalize for differences in the amount of total cDNA at the indicated time points. *C–E*, Hematoxylin staining of wound sections exhibiting accumulation of polymorphonuclear cells (*arrow*) and fibrin on day 2 (*C*), polymorphonuclear leukocytes and macrophages on day 5 (*D*), and granulation tissue with minimal inflammatory cells on day 8 (*E*).

in unstimulated fibroblast cultures (Fig. 3*C*). Within 4 h of stimulation by IL-1α (Fig. 3*C*) or TNF-α (data not shown), levels of SDF-1α and SDF-1β mRNA decreased 16- and 23-fold, respectively, and remained reduced for at least 64 h after simulation (Fig. 3, *C* and *D*). In contrast, stimulation with IL-1α increased levels of mRNA encoding IL-8 and MCP-1 (Fig. 3, *C* and *E*) within 4 h of stimulation and had no effect on level of the housekeeping mRNA for GAPDH. TNF-α also increased IL-8 and MCP-1 message levels (data not shown). An ELISA specific for IL-8 confirmed that IL-1α and TNF-α stimulated the secretion of IL-8 protein, with EC$_{50}$ values of 0.2 and 2.0 ng/ml, respectively (Fig. 3*F*).

SDF-1 mRNA decreases in healing dermal wounds

Mouse dermal wound healing was employed to determine whether acute inflammation inhibits the expression of SDF-1 in vivo. Full thickness 2-mm dermal punch biopsies were removed from the dorsum of anesthetized mice (time zero) and at 24-h intervals thereafter. We detected less SDF-1 mRNA in day 1 tissue relative to fresh biopsies (day 0; Fig. 4, *A* and *B*) and progressively less SDF-1 mRNA until day 6 (Fig. 4*B*). This decline coincided with the period of acute inflammation within the wound, characterized by accumulation of polymorphonuclear cells by day 2 (Fig. 4*C*) and macrophages by days 4–5 (Fig. 4*D*). In contrast, we detected

progressively more SDF-1 mRNA from day 6 to day 10 (Fig. 4*B*), which coincided with reduced inflammatory infiltrate and maximal formation of granulation tissue (Fig. 4*E*). By contrast, the amounts of mRNA encoding MIP-2 and MCP-1 increased from days 1 through 10 (data not shown). Furthermore, there was no consistent change in the level GAPDH mRNA over the same time period (Fig. 4*A*).

Discussion

Numerous healthy tissues basally express mRNA encoding SDF-1 (9, 10), and, therefore, it has been assumed that expression of SDF-1 is not regulated. Furthermore, CpG transcription factor binding motifs (typical of housekeeping genes) are present in the *sdf1* gene, and comparable motifs for inflammation-associated transcription factors, such as NF-κB and AP-1, have not been identified in a 19-kb genomic clone (9). We found that primary fibroblast cultures grown from dermal biopsies produced SDF-1 and that production was inhibited by supernatants from LPS-stimulated, but not unstimulated, monocytes. Experiments with IL-1 and TNF receptor antagonists demonstrated that IL-1 accounted for most of this inhibitory activity, and that the remainder was attributable to TNF. Furthermore, purified recombinant IL-1α or TNF-α could each completely inhibit the production of SDF-1. IL-1α was

more potent than TNF-α; these two cytokines had EC$_{50}$ values of 0.02 and 1.0 ng/ml, respectively. These EC$_{50}$ values are similar to those reported for stimulation of IL-8 production by dermal fibroblasts (20), as verified here. IL-1α decreased mRNA levels of SDF-1 within 4 h, and inhibition was maintained for at least 144 h. Fibroblasts stimulated in this manner were viable, and production of IL-8 and MCP-1 was increased for the duration of these experiments. Curiously, IL-1 and TNF-α activate NF-κB and AP-1, and these transcription factors mediate many proinflammatory activities. The biochemical mechanism by which IL-1 and TNF regulate the expression of SDF-1 remains unknown. Other inflammatory cytokines, including IFN-γ, IL-6, and IL-8, did not regulate the production of SDF-1 by human dermal fibroblasts. LPS exerted a slight inhibitory effect at later time points (48 and 72 h; data not shown), which was probably a secondary effect due to IL-1 production by fibroblasts and autocrine signaling. Recently, we found that another pathway can also trigger down-regulation of SDF production: ligation of CD40 on fibroblasts by CD40 ligand on activated platelets (our unpublished observations).

We found that SDF-1 is expressed by stromal cells and vessels in skin. RT-PCR analysis of wounded mouse skin gave results consistent with the studies of cells cultivated in vitro. From days 0 to 6 after wounding, we detected progressively less SDF-1 in skin, a period during which polymorphonuclear leukocytes and then macrophages were predominant in the wound. These findings suggest that expression of SDF-1 is also regulated in vivo, during wound healing in skin.

Monocytes promote wound healing by resolving the inflammatory phase and initiating repair processes, such as formation of granulation tissue. IL-1 and TNF mediate these macrophage functions (21). This provides a possible connection between our observations of down-regulation of SDF-1 production by fibroblasts in vitro by LPS-stimulated monocyte supernatants, IL-1 and TNF, and the function of monocytes in vivo in wound healing. Because SDF-1 is a monocyte chemoattractant in vivo (5), it could increase infiltration by monocytes and prolong inflammation. Two other cell types that predominate in wound tissue also express CXCR4, T lymphocytes (22, 23) and endothelial cells (13–15). We found that stimulation with IL-1α and TNF-α shifts the type of CXC chemokines being produced by human fibroblasts from the non-ELR-containing chemokine SDF-1 to the ELR-containing chemokine IL-8. This observation could have implications for the differing roles of ELR- and non-ELR-containing chemokines in the regulation of angiogenesis (12).

Acknowledgments

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