

Stem cell factor in nasal polyposis and allergic rhinitis: Increased expression by structural cells is suppressed by in vivo topical corticosteroids

Young-Ki Kim, MD, Noriaki Nakagawa, MD, Koichi Nakano, MD,
Irakly Sulakvelidze, MD, Jerry Dolovich, MD,[†] and Judah Denburg, MD
Hamilton, Ontario, Canada

Background: Mast cells are increased in nasal polyp (Np) and allergic rhinitis (AR) tissue and are suppressed by topical corticosteroid treatment. Stem cell factor (SCF), a mast cell growth and survival factor, may explain these phenomena.

Objective: We investigated structural cell gene expression and production of SCF in nasal tissues in patients who had received and who had not received in vivo intranasal corticosteroid therapy.

Methods: Northern blot analyses for messenger RNA and ELISA for biologically active SCF protein from cultured Np epithelial cells and fibroblasts were performed. Immunostaining for SCF in cultured and tissue nasal structural cells in the presence or absence of steroid treatment was also performed.

Results: We detected significant expression of SCF mRNA and protein by cultured Np epithelial cells and Np fibroblasts; Np fibroblast SCF supported the differentiation of mast cells in vitro. There were more immunoreactive SCF-positive Np epithelial cells in patients with AR than in control subjects (97.2 ± 2.8 vs $45.6 \pm 22.0\%$; $p < 0.0001$). SCF that could be immunostained was significantly diminished overall in Np structural cells in the group given in vivo steroid treatment, with a modest (trend to significant) effect on any given cell type analyzed. In vitro treatment with budesonide of SCF-producing fibroblasts demonstrated inhibition of unstimulated, primary Np fibroblasts but not of IL-1-stimulated fibroblasts or transformed cell lines.

Conclusions: Human Np and AR tissue structural cells express and produce increased SCF. Our in vitro studies suggest that intranasal steroids blunt SCF expression in Nps, an effect that may be responsible for a decrease in mast cells and symptoms. (*J Allergy Clin Immunol* 1997; 100:389-99.)

Key words: Nasal polyposis, allergic rhinitis, mast cells, stem cell factor, corticosteroids

Abbreviations used

AR:	Allergic rhinitis
BMMNCs:	Bone marrow nonadherent mononuclear cells
DPBS:	Dulbecco's phosphate-buffered saline
FBS:	Fetal bovine serum
HEPES:	N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid
Np:	Nasal polyp
NpEps:	Nasal polyp epithelial cells
NpFbs:	Nasal polyp fibroblasts
NpEpCM:	Nasal polyp epithelium conditioned medium
NpFbCM:	Nasal polyp fibroblast conditioned medium
SPB:	Sucrose phosphate buffer
PLP:	Periodate-lysine-paraformaldehyde
SCF:	Stem cell factor

Stem cell factor (SCF) was first recognized in mast cell-deficient mice because of a defect at the Steel locus gene.¹ The white-spotting (*W*) and Steel (*Sl*) loci in mice encode the transmembrane protein tyrosine kinase receptor, *c-kit*, and its ligand (also known as SCF), respectively.^{2,3} A defect in either locus in mice causes anemia, sterility, white spotting of the skin, and mast cell deficiency, suggesting that the *c-kit*/SCF signal transduction pathway is essential for the development of erythroid,⁴ mast cell, melanocyte, and germ cell lineages.⁵

SCF is a major growth and differentiation factor for human mast cells derived from progenitors in fetal liver,⁶ cord blood,⁷ and bone marrow.⁸⁻¹⁰ SCF also maintains mast cell survival by suppressing programmed cell death,¹¹ promotes chemotaxis of immature mast cells,¹² and enhances their expression of receptors for components of the extracellular matrix.¹³ SCF not only promotes proliferation of immature and mature mast cells, it can also influence mast cell maturation by altering mast cell phenotype and mediator content.¹⁴ SCF may also act as an adhesion molecule for mast cells, which express *c-kit* throughout their development.¹⁵ Although a number of other cytokines such as IL-3, IL-4, IL-9, IL-10, and nerve growth factor reportedly induce growth and differentiation of mouse mast cells, these cytokines do not act similarly on human mast cell differentiation.⁹

From the Departments of Medicine and Pediatrics, McMaster University, Hamilton, Ontario, Canada.

Supported by grants from the Medical Research Council of Canada and Astra Draco, Lund, Sweden.

Received for publication May 8, 1996; revised Apr. 29, 1997; accepted for publication Apr. 29, 1997.

[†]Deceased.

Reprint requests: Judah A. Denburg, MD, FRCPC, McMaster University Medical Centre, Department of Medicine, Room 3V46, 1200 Main St. West, Hamilton, Ontario, L8N 3Z5, Canada.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/1/82990

SCF is produced by epithelial cells,¹⁶ fibroblasts,^{17, 18} and endothelial cells.¹⁹ It can be expressed either in a soluble or membrane-bound form, generated by tissue-specific splicing;²⁰ both these forms are biologically active.¹ There is accumulating evidence that the *c-kit* signal transduction pathway is begun through intimate cell-to-cell contact between proliferating cells expressing the *c-kit* receptor and tissue-anchored stromal cells expressing the membrane-bound SCF.²¹

Because airway epithelial cells, fibroblasts, and endothelial cells constitutively or on stimulation with IL-1 or lipopolysaccharide provide a rich source of proinflammatory mediators and cytokines²² including IL-1,²³ IL-6,^{23, 24} IL-8,^{24, 25} endothelin,²⁶ granulocyte-macrophage colony-stimulating factor,^{24, 27, 28} transforming growth factor- β ,^{29, 30} fibronectin,³¹ eicosanoids,³² and SCF,^{28, 33} we examined SCF expression and production in nasal polyps (Nps) and allergic rhinitis (AR), conditions in which mast cells are abundant. Because it is not known whether SCF is produced by structural cells in inflamed nasal tissues such as Nps and mucosae of AR or whether SCF expression is different in AR when compared with normal nasal mucosa, we assessed the expression of SCF messenger RNA, the secretion of biologically active SCF protein, and nasal localization of immunoreactive SCF protein in various nasal structural cell populations. Finally, because glucocorticoids downregulate the production of numerous cytokines³⁴ and could thus be potent inhibitors of the mast cell infiltrates in AR and Nps, we also investigated the effect of the topical corticosteroid, budesonide, on expression of SCF in Nps.

METHODS

Subjects

Steroid-untreated Np tissues were obtained from 11 patients (8 men and 3 women aged 49.4 ± 12.1 years [mean $\pm$ SD]) who had not received steroid treatment 1 month before polypectomy. Steroid-treated Np tissues were obtained from 10 patients (10 men aged 48.3 ± 16.4 years) who had received topical steroid treatment for at least 4 weeks immediately before polypectomy. All patients were referred to the Ear, Nose and Throat Clinic at McMaster Medical Centre, Hamilton, Ontario for polypectomy, which was carried out after topical or general anesthesia had been induced. No patient had taken antibiotics during the month before surgery.

Nasal brushing specimens were obtained from 24 patients with seasonal AR who had severe symptoms during the grass pollen season and positive skin prick test responses to grass pollen allergens. Nasal brushings from 23 nonallergic healthy subjects who had no clinical history of respiratory infection for at least 1 month before the study were used as normal control specimens. The mean ages of patients with seasonal AR and control subjects were 33.0 ± 9.7 years and 42.2 ± 10.5 years, respectively ($p > 0.1$).

Human bone marrow-derived nonadherent mononuclear cells were obtained as pathologic waste from the sternum of two patients undergoing open heart surgery.

These studies conformed to guidelines for human experimentation and were approved by the Ethics Committees of

Chedoke-McMaster and Hamilton Civic Hospitals; when appropriate, subjects provided written informed consent.

Cell culture

Np epithelial cells (NpEps), Np fibroblasts (NpFbs), the human cervical carcinoma squamous epithelial cell line HeLa (American Type Tissue Culture, Rockville, Md.) and the human small cell lung carcinoma cell line NCI-H69 (American Type Culture Collection) were cultured as previously described³⁵ to prepare Northern blot analyses of SCF mRNA. Briefly, Nps obtained from patients undergoing nasal polypectomy were placed in Ham's F12 medium supplemented with 1% penicillin-streptomycin and 27 μ g/ml amphotericin B and immediately transported to the laboratory. Preparation of collagen-coated culture dishes and isolation of NpEps were done according to the method previously described. Isolated epithelial cells were plated on the collagen-coated wells (100,000 cells/well) with 2 ml of serum-free culture media supplemented with antibiotics, glutamine, and growth factors. To obtain NpFbs, some portions of the Np tissue were cut into pieces (0.3 mm²) and cultured with RPMI-1640 medium (Gibco, Grand Island, N.Y.) supplemented with heat-inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin, and 25 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) buffer.

The human cervical carcinoma squamous epithelial cell line HeLa was cultured in F12/Dulbecco's modified Eagle's medium. The human small cell lung carcinoma cell line NCI-H69 was cultured to confluence in RPMI-1640 medium supplemented with 1% penicillin-streptomycin, 1 mmol/L L-glutamine, and 10% FBS. These cells were used as positive controls for SCF mRNA and SCF protein expression. All cells were cultured in a 5% CO₂ humidified incubator at 37° C. Culture media were changed every 2 days until confluence was reached in 7 to 10 days. Supernatants were harvested after a further 48-hour incubation, sterilized through a Millipore filter (0.22 μ m; Millipore Corp., Bedford, Mass.), and stored at -20° C until use.

For mast cell differentiation assays, NpFb conditioned medium (NpFbCM) was concentrated before sterilization by an Amicon membrane concentration system (Amicon Division, Beverly, Mass.), which retained macromolecules ranging in molecular weight from 50 to 100,000 kd.

Northern blot analysis

To determine whether human NpEps and NpFbs express SCF mRNA, total RNA was isolated from cultured NpEps and NpFbs by the guanidine isothiocyanate-phenol-chloroform method previously described³⁶ and separated on a 1.2% agarose gel. Ten micrograms of RNA was separated according to size in a 1.1% agarose gel, which was stained with ethidium bromide to ensure that equal amounts of RNA had been loaded and no degradation had occurred. RNA in 1.1% agarose gel was transferred to Genescreen filters (Dupont, Boston, Mass.). RNA was fixed to the nylon membrane by UV cross-linking and was prehybridized and hybridized in 50% formamide, 1 mol/L sodium chloride, 10% dextran sulfate, 1% sodium dodecylsulfate and 100 μ g/ml denatured salmon sperm DNA at 42° C. The filters were washed to a final stringency with 2 $\times$ standard sodium citrate and 1% sodium dodecylsulfate at 65° C for 30 minutes and 0.1 mol/L standard sodium citrate at room temperature for 30 minutes and autoradiographed with Kodak XAR film by using intensifying screens at -70° C for 2 to 7 days. As positive controls, RNA of cultured HeLa cells and RNA of

cultured human small cell lung carcinoma cells were also extracted and hybridized by the same method.

Filters were probed with α -actin to provide an indication of the amount of RNA loaded. The SCF probe is a 431 bp SmaI and SphI complementary DNA fragment (kindly provided by Dr. Krisztina Zsebo, Amgen Biologicals, Thousand Oaks, Calif.). DNA was labeled with phosphorous 32 deoxyadenosine triphosphate to a specific activity of greater than 1×10^9 cpm/ μ g.

SCF ELISA

Because mRNA expression is not always associated with production of mature protein, the secretion of SCF was assessed by ELISA in supernatants of cultured NpEps and NpFbs. Levels of extracellular immunoreactive SCF were quantified by using a modification of the double-ligand method as previously described³⁷ with commercially available sandwich-type ELISA (R&D Systems Inc., Minneapolis, Minn.). ELISA plates were coated with goat polyclonal antibodies against human (h)SCF. Dilutions of cell-free supernatant were added in duplicate, followed by the addition of a second horseradish peroxidase-conjugated specific murine monoclonal antibody against hSCF. After washing to remove any unbound antibody-enzyme reagent, a substrate solution (a 1:1 solution of hydrogen peroxide and tetramethylbenzidine) was added to the wells. The color development was stopped with 2N sulfuric acid, and the intensity of the color was measured at 540 nm on a spectrophotometer. A standard curve was generated by plotting the optical density versus the concentration of SCF in the standard wells with a curve fitting program. This ELISA is sensitive to 5.0 pg/ml SCF, and it has an intraassay coefficient of variation of less than 0.5% and an interassay coefficient of variation of less than 8%.

Mast cell differentiation assays

To determine whether SCF in NpFbCM is biologically active as a mast cell growth factor, mast cell differentiation assays from bone marrow nonadherent mononuclear cells (BMMNCs) were performed. BMMNCs were cultured in the presence of concentrated ($\times 30$) NpFbCM with or without anti-hSCF antibody (1 μ g/ml, 10 μ g/ml) for 6 weeks. BMMNCs were obtained by differential centrifugation of heparinized human bone marrow cells on 67% Percoll (Pharmacia, Uppsala, Sweden); layered cells were suspended in culture flasks with McCoy 3+ media (Life Technologies, Burlington, Ontario, Canada) and incubated at 37° C in a humidified atmosphere of 6% CO₂ and 94% air for 2 hours. After 2 hours' incubation, nonadherent cells were resuspended in RPMI-1640 media supplemented with 10% FBS, 50 μ mol/L 2-mercaptoethanol, 2 mmol/L L-glutamine, 100 IU/ml penicillin, 50 μ g/ml streptomycin, and 25 μ g/ml gentamycin. All experiments were carried out with either 30% vol/vol concentrated ($\times 30$) NpFbCM or recombinant (r)hSCF (25 ng/ml). Negative control cultures were kept in RPMI-1640 media supplemented with 10% FBS, 25 mmol/L HEPES only. To block the effect of SCF, two concentrations of anti-hSCF antibodies (1 μ g/ml and 10 μ g/ml) were preincubated with 30% vol/vol concentrated ($\times 30$) NpFbCM or rhSCF (25 ng/ml) at 37° C for 2 hours before addition to BMMNCs. The cells (1×10^6 cells/well) were cultured in suspension at 37° C in a humidified atmosphere of 6% CO₂ and 94% air in each condition. Media were changed every 3 days for 6 weeks, after which cultured total cells were counted with a hemocytometer and prepared as cytopins for staining.

Tryptase immunostaining

Cells undergoing periodate-lysine-paraformaldehyde (PLP) fixation were kept for 10 minutes in tubes on ice, then pelleted by centrifugation (10,000 rpm at room temperature) for 15 seconds, and resuspended at a final density of 5×10^5 cells/ml in 15% sucrose phosphate buffer (SPB) (0.1 mol/L, pH 7.4) for at least 10 minutes on ice. Slides were prepared by cytocentrifugation (300 rpm for 5 minutes) of 100 μ l of cell suspension. After air drying, slides were stored at -70° C. The cytopins were stained with anti-human tryptase antibodies to detect mast cells.

Immunohistochemical staining of human tryptase was done as previously described.³⁸ Briefly, after preincubation in 0.6% hydrogen peroxide in methanol to block endogenous peroxidase activity, sections were incubated in heat-inactivated normal rabbit sera for 3 hours. A mouse monoclonal anti-human tryptase antibody (G3; kindly provided by Dr. Anne-Marie Irani, Department of Medicine and Pediatrics, Medical College of Virginia, Richmond) was diluted in phosphate buffer at a concentration of 4.5 μ g/ml. After a brief rinse of normal rabbit sera with phosphate buffer, sections were incubated with anti-human tryptase antibody overnight at 4° C. Monoclonal antibodies were then detected by a peroxidase-antiperoxidase method with a Histostatin-AP kit (Zymed Laboratories Inc, San Francisco, Calif.). Counterstaining was performed with Mayer's hematoxylin. Isolated gut mucosal mast cells (kindly provided by Dr. Jean Marshall, Department of Pathology, McMaster University) were used as a positive control; mouse IgG₁ myeloma protein was used as a negative control. The number of tryptase-positive cells per 500 cells was evaluated and expressed as a percentage.

SCF immunostaining

Immunohistochemical localization of SCF in cultured NpFbs and NpEps. To detect immunoreactive SCF protein, cultured NpEps and NpFbs were trypsinized by adding trypsin-ethylenediaminetetraacetic acid (EDTA) (0.1% trypsin, 1 mmol/L EDTA) after confluence was reached. Cells undergoing PLP fixation for 10 minutes were kept for a further 10 minutes in tubes on ice, then pelleted by centrifugation (10,000 rpm at room temperature) for 15 seconds, and resuspended at a final density of 5×10^5 cells/ml in a 15% SPB (0.1 mol/L, pH 7.4) for at least 10 minutes on ice. Slides were prepared by cytocentrifugation (300 rpm for 5 minutes) of 100 μ l of cell suspension. After air drying, slides were stored at -70° C until staining. Before staining, slides were rinsed in Dulbecco's phosphate-buffered saline (DPBS) supplemented with 0.01 mol/L HEPES buffer containing 0.01% saponin (DPBS [H+S]) for 10 minutes at room temperature to enhance permeability. To block non-specific binding, sections were preincubated in DPBS (H+S) containing 75% heat-inactivated human AB sera for 1 hour and then in the same buffer with 25% heat-inactivated normal rabbit sera for 30 minutes at room temperature.

Mouse monoclonal anti-hSCF antibody was diluted in DPBS (H+S) containing 1.0% bovine serum albumin at a concentration of 10 μ g/ml. After brief washing with DPBS (H+S), sections were incubated with the monoclonal antibody overnight at 4° C and then washed with the same buffer. Monoclonal antibodies were detected by the alkaline phosphatase-anti-alkaline phosphatase method (APAAP Kit, DAKO) according to the manufacturer's instructions. Counterstaining was performed with Mayer's hematoxylin, and specific controls were included in each staining run. Mouse IgG₁ myeloma protein was used as a negative control. For verification of the antibody

SCF mRNA IN CELL LINES



FIG. 1. Northern blot analysis of SCF mRNA expression. Total RNA (10 μ g/ml) was hybridized with ³²P-radiolabeled SCF probe. RNA of NpFbs stimulated with lipopolysaccharide (*lane 2*), NpFbs stimulated with IL-1 β (*lane 3*), and unstimulated NpFbs (*lane 4*) shows 7.5 Kb and 5.5 Kb SCF-specific transcripts. *Lanes 1 and 5* (HepG₂ and HeLa cell RNA, respectively) show SCF transcripts. Ribosomal RNA (*lane 6*) was used as negative control, and RNA from HeLa cells and HepG₂ cells were used as positive controls. LPS, Lipopolysaccharide.

used, preincubated mouse monoclonal anti-hSCF antibody with rhSCF (10 μ g/ml) was used to stain Np tissues. As a positive control, cytopins of cultured HeLa cells and NCI-H69 cells were made and fixed with PLP for 10 minutes. These slides were placed in 15% SPB for 10 minutes and then stored at -70° C until immunostaining was done.

Immunohistochemical localization of SCF in nasal brushing cells. Nasal brushings were obtained after careful removal of secretions or crusts from the nose. The brush was introduced, without anesthesia, into the nose under direct visual guidance and placed between the nasal septum and the inferior turbinate. The brush was introduced in its full length, removed with a slight rotating movement, and immediately placed in a small plastic tube containing 2 ml of PLP fixative; it was then shaken vigorously and carefully rubbed off against the wall of the tube to release cells. Two brushings were done in each nasal cavity. Cells undergoing PLP fixation were kept for 10 minutes in the tube on ice, then pelleted by centrifugation (10,000 rpm at room temperature) for 15 seconds, and resuspended at a final density of 5×10^5 cells/ml in 15% SPB (0.1 mol/L, pH 7.4) for at least 10 minutes on ice. Slides were prepared by cytocentrifugation (300 rpm for 5 minutes) of 100 μ l of cell suspension. After air drying, slides were stored at -70° C. Immunohistochemical staining was performed by the same method as that used for cultured NpEps and NpFbs. After staining, a total of 500 ciliated columnar epithelial cells per slide were counted in blinded fashion for immunoreactive SCF with a conventional light microscope, and results were expressed as the percentage of stained epithelial cells.

Immunohistochemical localization of SCF in Nps. To detect the cellular sources of SCF within the Nps and changes in SCF expression after corticosteroid treatment, Np tissue sections of 5 mm in thickness were fixed in PLP for 4 hours at 4° C and washed in phosphate buffer (0.1 mol/L, pH 7.4), 10% SPB, 20% SPB, and 30% SPB each for 3 to 6 hours, respectively. The tissues were then frozen and stored in Tissue-Tek OCT (Miles, Elkhart, Ind.) in -70° C isopentane. Serial cryostat sections (6 μ m) were cut into slides coated with 3-aminopropyltriethoxysilane. Immunohistochemical staining was done with the same method as that for cultured NpEps and NpFbs. All tissue samples were coded, and sections were counted in blinded fashion by conventional light microscopy. In Np tissues the length of total stained epithelia was expressed as a percentage of the length of total basement membrane. The total number of fibroblasts and endothelia and the number of immunoreactive SCF protein-positive fibroblasts and endothelial cells were

determined in an area of 0.25 mm² of stroma. The percentage of stained fibroblasts and endothelial cells was calculated from a total of 10 fields.

The intensity of staining was divided into three grades. Grade 0 was regarded as no staining. Grade 2 was regarded as the same staining intensity as immunoreactive SCF protein-positive cultured NpEps. Grade 1 represented intensity between grades 0 and 2. The intensities of immunoreactive SCF-positive NpEps, NpFbs, and endothelial cells were measured in six high-power ($\times 400$) fields. The intensity index of each Np was calculated as follows:

$$\text{Intensity index (\%)} = \frac{(\text{Total intensity grade of counted cells})}{(\text{Expected total maximal intensity grade of counted cells})} \times 100$$

In vitro treatment of SCF-producing cells with budesonide

To determine whether in vitro treatment of cell lines with budesonide had an effect on SCF production, NpFbs, HeLa cells and NCI-H69 cells were pretreated with or without budesonide, followed by incubation of the cells with or without IL-1 β . NpFbs and HeLa cells were cultured in 24-well plates until confluent, and the NCI-H69 cells were plated at 1×10^6 per well. The cells were then pretreated with media, diluent, or doses of budesonide for 4 hours, followed by two washes with phosphate-buffered saline. Two milliliters of RPMI medium containing 10% fetal calf serum was added to each well, and IL-1 β was then added to appropriate wells at a concentration of 1 ng/ml. Cells were incubated for 48 hours in 5% CO₂, and the supernatant was harvested. SCF levels were measured by SCF ELISA.

Statistics

For statistical analysis of immunohistochemical staining of nasal brushings, Student's *t* test for nonpaired comparisons was used; for statistical analysis of immunohistochemical staining of Nps, a mixed model analysis of variance³⁹ was used to assess the differences in immunoreactive SCF-positive total cells between steroid-treated and steroid-untreated groups. Post hoc comparison was done with the Newman-Keuls' test to assess the differences in immunoreactive SCF-positive cells among NpEps, NpFbs, and endothelial cells. The Mann-Whitney rank sum test was used to assess the differences of immunoreactive SCF-positive cells in each cell type, comparing steroid-treated and steroid-untreated groups. The strength of the association

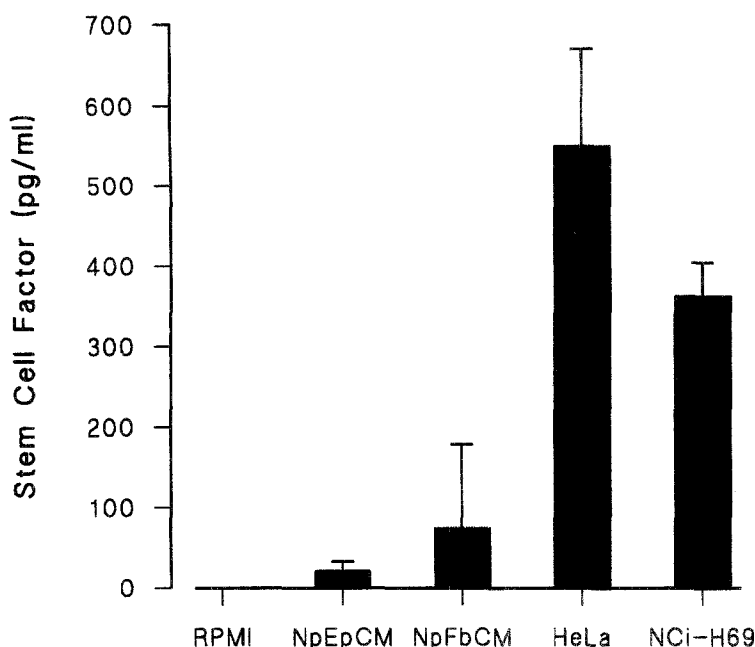


FIG. 2. SCF production by cultured NpEps and NpFbs, measured by ELISA. The level of SCF in NpEpCM is the mean of duplicate determinations in each of two experiments. The level of SCF in NpFbCM represents the mean of duplicate determinations in each of three experiments.

between the numbers of immunoreactive SCF-positive cells and the intensities of staining (intensity index) was determined by linear regression.

RESULTS

Northern blot analysis

Northern blot analysis of mRNA from the cultured NpEps and NpFbs showed hSCF-specific mRNA bands of approximately 7.5 and 5.5 Kb size (Fig. 1). SCF mRNA was also extracted from cultured HeLa cells and cultured HepG₂ cells, which are included as positive controls for SCF mRNA expression (Fig. 1). Ribosomal RNA served as a negative control.

SCF ELISA

The levels of SCF protein in the supernatants of cultured NpEps and NpFbs were 19.5 ± 12.0 pg/ml and 77.3 ± 103.4 pg/ml, respectively (Fig. 2). Lipopolysaccharide stimulation of cultured NpFbs for 6 hours did not enhance SCF production. Supernatants of cultured HeLa cells and cultured NCI-H69 cells contained much higher mean levels of SCF protein (556 ± 117 pg/ml and 359 ± 43 pg/ml, respectively) than those from Nps.

Mast cell differentiation assays

After 6 weeks, NpFbCM induced tryptase-positive cells from BMMNCs, compared with RPMI-1640. The mean percentage of tryptase-positive cells induced by NpFbCM was 3.1% of total cells (Fig. 3); absolute numbers of cells are indicated in Table I. This mast cell differentiation effect by NpFbCM was completely

inhibited by preincubation with anti-hSCF antibody at a concentration of 1 μ g/ml (Fig. 3, Table I). Although no mast cell differentiation was observed when mononuclear cells were incubated with RPMI-1640 medium alone, tryptase-positive cells were markedly increased (30.5% of total cells) when BMMNCs were incubated with 25 ng/ml rhSCF. This induction of differentiation by rhSCF was suppressed by preincubation of rhSCF with 1 μ g/ml or 10 μ g/ml anti-hSCF antibody (52.6% and 10.2% stimulation of mast cell differentiation compared with rhSCF alone, respectively), but still less than the suppression of differentiation by preincubation of anti-hSCF antibody with NpFbCM, presumably because of the much lower concentration of SCF in NpFbCM (see above). The corresponding absolute numbers of tryptase-positive cells were: rhSCF, 30.40×10^3 ; rhSCF plus 1 μ g/ml anti-hSCF, 10.56×10^3 ; NpFbCM, 4.60×10^3 ; NpFbCM plus 1 μ g/ml anti-hSCF, no cells (this despite excellent cellular proliferation, Table I).

Immunohistochemical localization of SCF in cultured NpEps and NpFbs

Cultured NpEps and NpFbs showed immunoreactive SCF protein, diffusely expressed over the cytoplasm (Fig. 4, A and B). All the positive epithelial cells showed a similar staining intensity; however, some fibroblasts revealed a stronger staining intensity than others. Cytospin preparations from cultured HeLa cells and cultured NCI-H69 cells used as positive controls showed stronger immunoreactive SCF protein staining than NpEps and NpFbs; in cultured

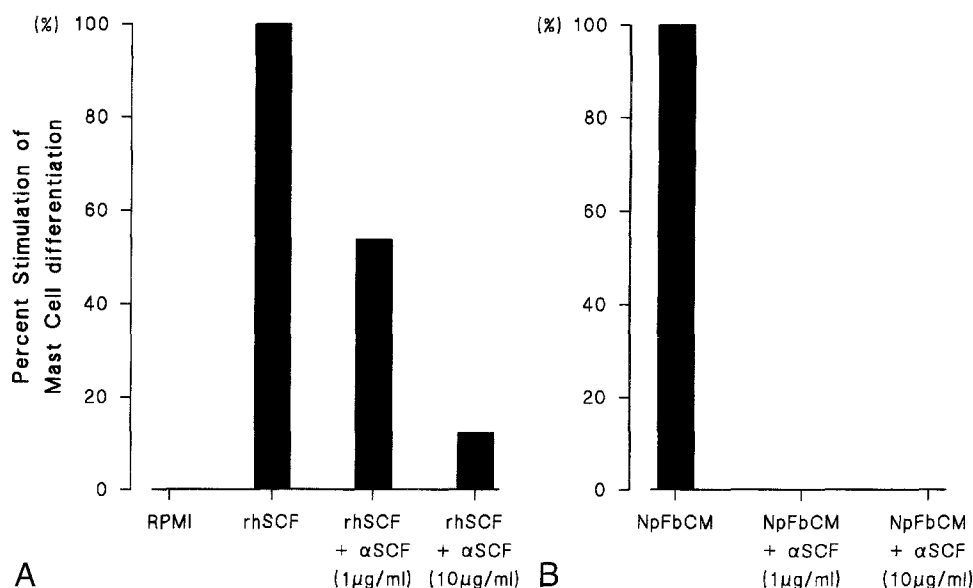


FIG. 3. Polyp fibroblast SCF induces mast cell differentiation. Percent stimulation of mast cell differentiation induced by rhSCF (25 ng/ml) (A) and NpFbCM (B). Abrogation by anti-hSCF antibody (αSCF).

TABLE I. Absolute numbers of tryptase-positive cells in SCF and CM-stimulated cultures

	Total viable cells/well ($\times 10^{-3}$)	Tryptase-positive cells/well ($\times 10^{-3}$)
RPMI	9.50	0
rhSCF	99.75	30.40
rhSCF + αSCF (1 μg/ml)	64.55	10.56
rhSCF + αSCF (10 μg/ml)	47.10	1.71
NpFbCM	146.4	4.60
NpFbCM + αSCF (1 μg/ml)	130.7	0
NpFbCM + αSCF (10 μg/ml)	109.3	0

Data represent mean of two experiments.

HeLa cells, the staining intensity of immunoreactive SCF protein was distributed evenly among cells, whereas cultured NCI-H69 cells exhibited more variable staining. Extracellular immunoreactive SCF protein was also found around HeLa cells. Slides stained with mouse IgG₁ myeloma protein (Fig. 4, C) or anti-hSCF preincubated with rhSCF showed no positive cells.

Immunohistochemical localization of SCF in nasal brushings

In all nasal brushings we detected immunoreactive SCF-positive ciliated epithelial cells. The number of SCF-positive ciliated epithelial cells among nasal brushing epithelial cells in patients with seasonal AR was higher ($97.2\% \pm 2.8\%$) than in normal control subjects

($45.6\% \pm 22.0\%$) ($p < 0.0001$). In addition, the intensity of staining was higher in nasal brushings from patients with AR in season than in control subjects (Fig. 4, D and E). Ciliated epithelial cells, basal cells, and goblet cells also showed staining for SCF protein (Fig. 4, D). Immunoreactive SCF was expressed diffusely over the entire cytoplasm, as observed in cultured NpEps and NpFbs (above). Generally, basal cells showed stronger staining than ciliated columnar epithelial cells and goblet cells. Neutrophils, eosinophils, and squamous epithelial cells were negative for immunoreactive SCF protein. The total numbers of all cells obtained by nasal brushing were very similar in patients with seasonal AR and control subjects.

Immunohistochemical localization of SCF in Nps

Substantial immunolocalization of SCF was observed in NpEps, NpFbs, and endothelial cells (Fig. 4, G and H). The percentage of SCF immunoreactivity for all cell types combined (epithelial cells, fibroblasts, and endothelial cells) was significantly higher ($p = 0.03$) in steroid-untreated than in steroid-treated subjects; for any given cell type, percentages of immunoreactive SCF-positive cells tended to be lower in the steroid-treated group, but this failed to reach statistical significance at the $p = 0.05$ level (Table II). Interestingly, Nps showed immunoreactive SCF expression in the deep stromal venular endothelium but not in arterioles; abundant immunostaining was seen in the epithelium of steroid-untreated polyps. The intensity of SCF staining was compared and found to be similar among epithelial cells, fibroblasts, and endothelial cells; a diffuse, cytoplasmic pattern was the rule. In the epithelial layer, basal cells showed stronger intensity than ciliated columnar cells. A

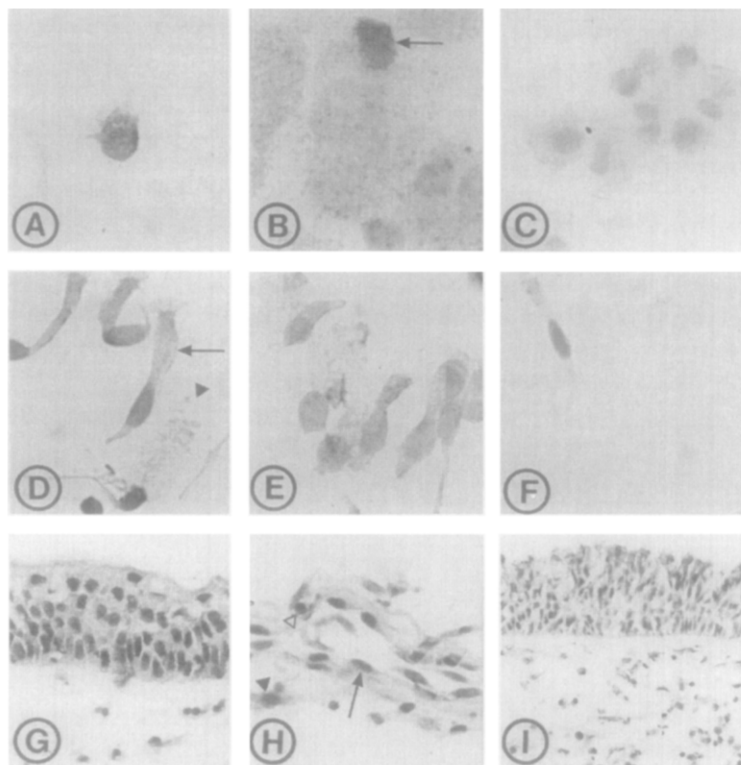


FIG. 4. Immunohistochemical localization of SCF in nasal tissues. **A**, Photomicrograph ($\times 640$) demonstrating immunoreactive SCF protein diffusely in cytoplasm of cultured NpEps with a mouse monoclonal antibody and the DAKO alkaline phosphatase–antialkaline phosphatase method. **B**, Photomicrograph ($\times 640$) demonstrating immunoreactive SCF protein in cultured NpFbs. Some fibroblasts showed stronger staining intensity than others (*arrow*). **C**, Photomicrograph ($\times 640$) demonstrating lack of staining with control protein (mouse IgG₁ myeloma protein). **D**, Photomicrograph ($\times 640$) demonstrating immunoreactive SCF protein in ciliated columnar epithelial cells (*arrow*) and goblet cells (*arrowhead*) in a nasal brushing from a subject with AR. **E**, Photomicrograph ($\times 640$) demonstrating decrease of immunoreactive SCF protein in most of ciliated columnar cells and goblet cells in a nasal brushing from a control subject. **F**, Photomicrograph ($\times 640$) demonstrating lack of immunoreactive SCF protein in a nasal brushing preincubated with control antibody (mouse IgG₁ myeloma protein). **G**, Photomicrograph ($\times 400$) demonstrating immunoreactive SCF protein in most NpEps. **H**, Photomicrograph ($\times 400$) demonstrating immunoreactive SCF protein in stromal endothelial cells (*arrow*), a fibroblast (*filled arrowhead*), and a mononuclear cell (*open arrowhead*). **I**, Photomicrograph ($\times 200$) demonstrating no staining with control antibody (mouse IgG₁ myeloma protein).

strong positive correlation was found between the numbers of immunoreactive SCF protein-positive cells and the intensity indices ($r = 0.81$, $p = 0.0007$, Fig. 5). The intensity index for SCF immunostaining was 10.90 ± 5.93 in steroid-untreated and 7.80 ± 2.98 steroid-treated subjects with Nps ($p = 0.05$) (Table III).

Effects of in vitro budesonide on SCF production

As shown in Fig. 6, budesonide inhibited SCF production by up to 53% in two primary NpFb cell lines. In one of these (line no. 2) a maximal effect on SCF production was observed at 10^{-6} mol/L budesonide, with a dose response observed to 10^{-8} mol/L. SCF production by HeLa or NCI-H69 cells could not be inhibited by budesonide at any dose, nor was any effect of budesonide observed after IL-1 stimulation of the NpFb lines (Fig. 6).

DISCUSSION

This study demonstrates that structural cells derived from or in nasal tissues produce SCF, that SCF expression is higher in nasal brushing epithelial cells from subjects with seasonal AR than in normal control subjects, and that corticosteroid treatment suppresses SCF production by primary fibroblasts in vitro. Specifically, 7.5 Kb and 5.5 Kb SCF mRNA transcripts were found in cultured NpEps and NpFbs by Northern blot analysis. Previous studies have shown that SCF mRNA is expressed in rodent regenerating liver tissue,⁴⁰ various human embryonic tissues,⁴¹ human endothelial cells,^{19, 33, 42} and a variety of human cancer cells such as small cell lung cancer⁴³ and squamous cell carcinoma of the cervix.⁴⁴ Recently, it was also reported that SCF mRNA is expressed in Sendai virus-infected rodent bronchial epithelial cells.¹⁶ These data support our findings of SCF mRNA expression by various nasal structural cells.

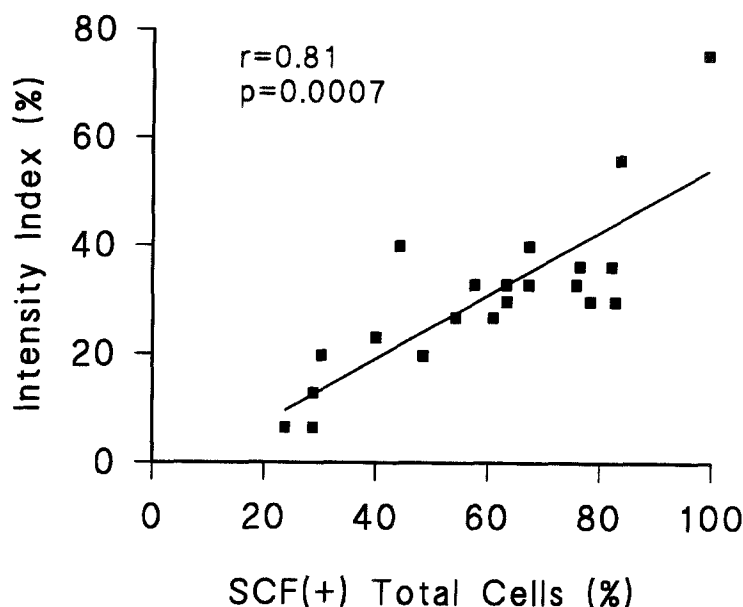


FIG. 5. Regression analysis of relationship between numbers of immunoreactive SCF protein-positive cells and intensity indices of SCF staining in Nps. The relationship is statistically significant ($r = 0.81$, $p = 0.0007$).

TABLE II. Characteristics of patients with nasal polyposis and effects of topical budesonide

	Steroid (–)	Steroid (+)
Subjects	11	10
Age (yr)	49.4 ± 12.1	48.3 ± 16.4
SCF (+) total cells (%)	67.9 ± 30.1	46.4 ± 30.3*
SCF (+) epithelial cells (%)	79.8 ± 29.2	57.3 ± 34.3†
SCF (+) fibroblasts (%)	44.1 ± 21.7	27.0 ± 17.7†
SCF (+) endothelium (%)	79.7 ± 25.3	55.6 ± 28.7‡

Average values are mean ± SD.

Steroid (–), Steroid-untreated Nps; Steroid (+), steroid-treated Nps.

* $p = 0.03$ versus steroid-untreated Nps.

† $p = 0.06$ versus steroid-untreated Nps.

‡ $p = 0.07$ versus steroid-untreated Nps.

The SCF protein we detected in NpEpCM and NpFbCM is most likely the soluble form of SCF, because it was detected in supernatants from cultured structural cells. The levels of SCF protein in these media were 19.5 ± 12.0 pg/ml (NpEpCM) and 77.3 ± 103.4 pg/ml (NpFbCM), significantly lower than the level of SCF reported in normal serum (3.3 ± 1.1 ng/ml).³⁷ There are two potential reasons for this discrepancy. First, optimal conditions for SCF production may not have been present in our culture systems, which detected mainly constitutive release of SCF. Second, most of the SCF in a cell is produced as a membrane-bound form, only small portions of which are released after proteolytic cleavage and thus detected by ELISA. The level of production notwithstanding, the expression of SCF mRNA and the release of SCF protein by Np structural cells suggest that these cells could represent an important source of SCF in vivo.

TABLE III. Intensity index of Np SCF immunohistochemistry

	Steroid (–)	Steroid (+)
Subjects	11	10
Intensity index (SD)	10.90 ± 5.93	7.80 ± 2.98*

Steroid (–), Steroid-untreated group; Steroid (+), steroid-treated group.

* $p = 0.05$ versus steroid-untreated group.

SCF production by unstimulated but by not IL-1-stimulated primary fibroblast cell lines was inhibited by budesonide in vitro. There was also no effect of budesonide on SCF production by transformed structural cells. These results give in vitro support to our in vivo findings. They also suggest that once structural cells are activated or transformed, their production of SCF may become steroid-unresponsive. This may explain the rather modest in vivo effect of budesonide, because we could not distinguish between “activated” and “nonactivated” structural cells in tissue sections from patients with Nps or AR.

The SCF present in NpFbCM was sufficient to induce a modest but significant differentiation of BMMNCs to tryptase-positive mast cells; this differentiation was specifically prevented by preincubation of NpFbCM with anti-hSCF antibody. The degree of mast cell differentiation induced by NpFbCM was less than that achieved with rhSCF. Taking into account the concentration of SCF in our NpFbCM, based on our ELISA results (77.3 ± 103.4 pg/ml), the induction observed was predictable by comparison with the results observed with rhSCF (25 ng/ml). Immunoreactive SCF was localized to cultured NpEps and NpFbs;

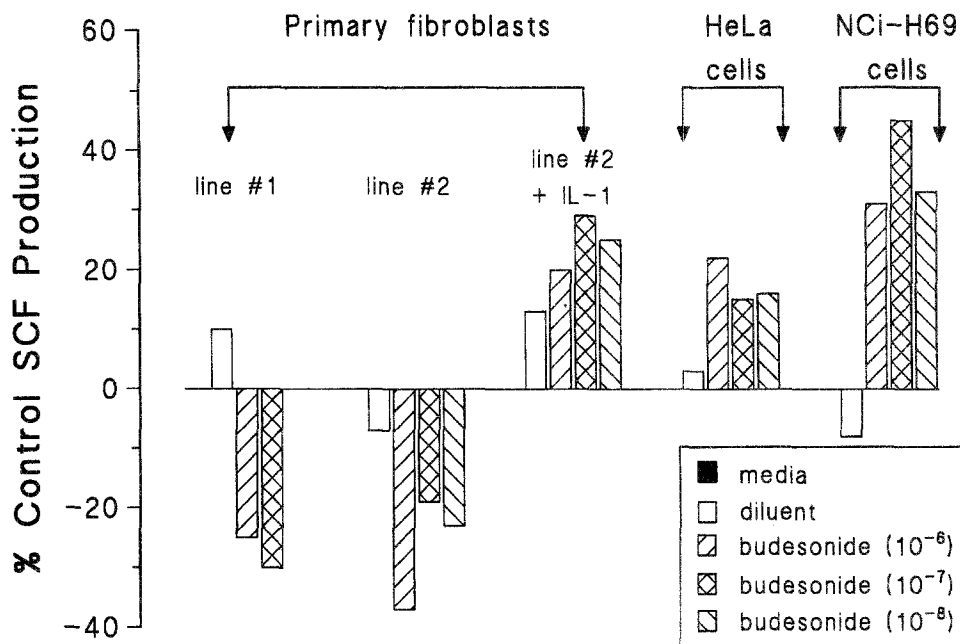


FIG. 6. Effect of budesonide on SCF production by unstimulated and IL-1-stimulated primary fibroblast cell lines. Budesonide inhibited, in a dose-response fashion, SCF production only by unstimulated primary fibroblast cell lines (nos. 1 and 2) but had no inhibitory effect on either IL-1-stimulated (cell line no. 2) or transformed HeLa or NCI-H69 cells (results of two experiments, mean of replicate samples for each experiment, $p < .05$).

this SCF probably represented the membrane-bound and/or intracellular forms. Thus these results suggest that SCF can be produced in either membrane-bound or soluble forms by NpEps and NpFbs.

In nasal brushings we detected many more immunoreactive SCF-positive epithelial cells from patients with AR than from control subjects. In a previous biopsy study, mast cells were found to be increased in the nasal epithelium in patients with AR.⁴⁵ We have also reported that NpEpCM made with epithelial scrapings can induce an increase in differentiation of metachromatic cell progenitors from the peripheral blood of atopic subjects⁴⁶; it remains possible that some of these were mast cell, and not only basophil, progenitors. It is hypothesized that the increased SCF production by AR epithelium may be responsible for mast cell accumulation in the mucosa. Although inflammatory stimuli such as lipopolysaccharide, IL-1 α , or tumor necrosis factor- β can activate tissue structural cells such as epithelial cells, it is not known what kind(s) of stimuli activate epithelial cells in AR to produce SCF or other cytokines.

The basal level of immunoreactive SCF protein expression in nasal brushings from control subjects (45.6% $\pm$ 22.0%) could have been due to exposure of the nasal mucosa to a number of stimuli, including subclinical rhinitis. Indeed, four control subjects had more than 10% neutrophils in the nasal brushings, which could have reflected subclinical infection. A separate study⁴⁷ of cytokines in nasal brushings supports this notion of microscopic and cellular evidence of inflammation in nonallergic subjects.

Immunoreactive SCF protein was also frequently localized to NpEps, stromal NpFbs, and stromal endothelial cells in Nps, with variable intensity of staining. This implies that Np structural cells may be generally activated to produce SCF, which could assist in the attachment of mast cells to structural cells and induce differentiation, maturation, and activation of mast cell progenitors or mast cells through *c-kit*.⁴⁸⁻⁵¹

Because of their strategic location, endothelial cells are intimately involved in the inflammatory response. In nasal tissues inflammatory cell trafficking occurs at the site of postcapillary venules in the stroma.⁵² Although arteriolar endothelia were usually negative for immunoreactive SCF, many stromal endothelial cells localized SCF, suggesting an important role for SCF in the nasal inflammatory response. The postcapillary venules in nasal tissues could act as a gate of entry to tissues for mast cell progenitors and participate in mast cell trafficking.

Our study revealed that when compared with untreated patients, overall SCF expression is lower in patients treated with topical steroids, which are effective in the treatment of both AR and Nps, and have been shown to abolish the immediate allergic reaction⁵³ and inflammation in Nps. Part of this effect might be due to a decrease in the density of mast cells in the tissues through direct or indirect inhibition of growth, differentiation, activation, or chemotaxis of these cells. There is, however, controversy regarding the effect of corticosteroids on mast cells in the nasal mucosa. Although some studies have demonstrated a decrease in total mast cell numbers in the nasal mucosa,⁵⁴ others have reported that only tryptase⁵⁵ or tumor necrosis

factor- α^{56} secretion (presumably derived from mast cells) decreases after topical steroid treatment. In vitro, mast cell differentiation from fetal liver cells induced by rhSCF can be prevented by dexamethasone,⁵⁷ suggesting that corticosteroids suppress SCF effects on mast cell differentiation rather than on survival. Though the mechanism of action of topical corticosteroids is complex, it does not seem unreasonable to suggest, on the basis of our findings, that topical steroid treatment decreases mast cell accumulation or activation by suppression of SCF production from structural cells. The differences in SCF production observed between the epithelium and the stromal fibroblasts in Nps may be partly responsible for phenotypic and functional differences between mast cells in these compartments.⁵⁸

The concept of an interaction between cells that are resident in tissues (structural cells) and inflammatory cell progenitors is supported by evidence in experimental¹⁸ and clinical models.²⁴ In both rodents and human beings, progenitors of basophils, eosinophils, or mast cells circulate in the blood or lymph and can be shown in vivo to traffic to tissues where they appear to undergo terminal differentiation into mature effector cells under the influence of structural cell cytokines.⁵⁹⁻⁶¹ NpEps, NpFbs, and endothelial cells could thus participate in allergic inflammation by driving the accumulation and activation of mast cells through production of SCF.

Since the submission of this manuscript, we have been saddened by the untimely death of our colleague and friend, Dr. Jerry Dolovich. This paper is dedicated to his memory.

REFERENCES

- Galli SJ, Zsebo KM, Geissler EN. The *kit* ligand, stem cell factor. *Adv Immunol* 1994;55:1-96.
- Chabot B, Stephenson DA, Chapma VM, Besmer P, Bernstein A. The proto-oncogene *c-kit* encoding a transmembrane tyrosine kinase receptor maps to the mouse *W* locus. *Nature* 1988;335:88-9.
- Geissler EN, Ryan MA, Housman DE. The dominant-white spotting (*W*) locus of the mouse encodes the *c-kit* proto-oncogene. *Cell* 1988;55:185-92.
- Muta K, Krantz SB. Apoptosis of human erythroid colony-forming cells is decreased by stem cell factor and insulin-like growth factor I as well as erythropoietin. *J Cell Physiol* 1993;156:264-71.
- Pesce M, Farrace MG, Piacentini M, Dolci S, De Felici M. Stem cell factor and leukemia inhibitory factor promote primordial germ cell survival by suppressing programmed cell death (apoptosis). *Development* 1993;118:1089-94.
- Irani AM, Nilsson G, Miettinen U, Craig SS, Ashman LK, Ishizaka T, et al. Recombinant human stem cell factor stimulates differentiation of mast cells from dispersed human fetal liver cells. *Blood* 1992;80:3009-21.
- Mitsui H, Furitsu T, Dvorak AM, Irani AM, Schwartz LB, Inagaki N, et al. Development of human mast cells from umbilical cord blood cells by recombinant human and murine *c-kit* ligand. *Proc Natl Acad Sci USA* 1993;90:735-9.
- Kirshenbaum AS, Goff JP, Kessler SW, Mican JM, Zsebo KM, Metcalfe DD. Effect of IL-3 and stem cell factor on the appearance of human basophils and mast cells from CD34+ pluripotent progenitor cells. *J Immunol* 1992;148:772-7.
- Valent P, Spanblochl E, Sperr WR, Sillaber C, Zsebo KM, Agis H, et al. Induction of differentiation of human mast cells from bone marrow and peripheral blood mononuclear cells by recombinant human stem cell factor/kit-ligand in long-term culture. *Blood* 1992;80:2237-45.
- Jozaki K, Kuriu A, Hirota S, Onoue H, Ebi Y, Adachi S, et al. Bone marrow-derived cultured mast cells and peritoneal mast cells as targets of a growth activity secreted by BALB/3T3 fibroblasts. *Exp Hematol* 1991;19:185-90.
- Iemura A, Tsai M, Ando A, Wershil BK, Galli SJ. The *c-kit* ligand, stem cell factor, promotes mast cell survival by suppressing apoptosis. *Am J Pathol* 1994;144:321-8.
- Nilsson G, Butterfield JH, Nilsson K, Siegbahn A. Stem cell factor is a chemotactic factor for human mast cells. *J Immunol* 1994;153:3717-23.
- Dasty J, Metcalfe DD. Stem cell factor induces mast cell adhesion to fibronectin. *J Immunol* 1994;152:213-9.
- Tsai M, Takeishi T, Thompson H, Langley KE, Zsebo KM, Metcalfe DD, et al. Induction of mast cell proliferation, maturation, and heparin synthesis by the rat *c-kit* ligand, stem cell factor. *Proc Natl Acad Sci USA* 1991;88:6382-6.
- Mayrhofer G, Gadd SJ, Spargo LD, Ashman LK. Specificity of mouse monoclonal antibody raised against acute myeloid leukaemia cells for mast cells in human mucosal and connective tissues. *Immunol Cell Biol* 1987;65:241-50.
- Castleman WL, Uhl EW, Busse WW, Dukes KR. Bronchiolar epithelial cells express stem cell factor (SCF) mRNA and protein [abstract]. Presented at 1995 ATS International Conference, Seattle, May 21-24. A368.
- Flanagan JG, Leder P. The kit ligand: a cell surface molecule altered in steel mutant fibroblasts. *Cell* 1990;63:185-94.
- Dvorak AM, Mitsui H, Ishizaka T. Stimulation of partial development of human mast cells by supernatant fluid from mouse fibroblast cultures. *Clin Exp Allergy* 1994;24:649-59.
- Aye MT, Hashemi S, Leclair B, Zeibdawi A, Trudel E, Halpenny M, et al. Expression of stem cell factor and *c-kit* mRNA in cultured endothelial cells, monocytes and cloned human bone marrow stromal cells (CFU-RF). *Exp Hematol* 1992;20:523-7.
- Flanagan JG, Chan DC, Leder P. Transmembrane form of the kit ligand growth factor is determined by alternative splicing and is missing in the *Sl^d* mutant. *Cell* 1991;64:1025-35.
- Anderson DM, Lyman SD, Baird A, Wignall JM, Eisenman J, Rauch C, et al. Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. *Cell* 1990;63:235-43.
- Rennard SL, Beckmann JD, Robbins RA. Biology of airway epithelial cells. In: Crystal RG, West JB, editors. *The lung: scientific foundation*. vol. 1. New York: Raven Press; 1991. p. 157-67.
- Mattoli S, Colotta F, Fincato G, Mezzetti M, Mantouani A, Patalano F, et al. Time course of IL1 and IL6 synthesis and release in human bronchial epithelial cell cultures exposed to toluene diisocyanate. *J Cell Physiol* 1991;149:260-8.
- Denburg JA, Gaudie J, Dolovich J, Ohtoshi T, Cox G, Jordana M. Structural cell-derived cytokines in allergic inflammation. *Int Arch Allergy Appl Immunol* 1991;94:127-32.
- Nakamura H, Yoshimura K, Jaffe HA, Crystal RG. Interleukin-8 gene expression in human bronchial epithelial cells. *J Biol Chem* 1991;266:19611-7.
- Black PN, Ghatei MA, Takahashi K, Bretherton-Watt D, Krausz T, Dollery CT, et al. Formation of endothelin by cultured airway epithelial cells. *FEBS Lett* 1989;255:129-32.
- Smith SM, Lee DK, Lacy J, Coleman DL. Rat tracheal epithelial cells produce granulocyte/macrophage colony-stimulating factor. *Am J Respir Cell Mol Biol* 1990;2:59-68.
- Segal GM, McCall E, Stueve T, Bagby GC. Interleukin 1 stimulates endothelial cells to release multilineage human colony-stimulating activity. *J Immunol* 1987;138:1772-8.
- Sacco O, Romberger D, Rizzino A, Beckmann JD, Rennard SI, Spurzem JR. Spontaneous production of transforming growth factor- β 2 by primary culture of bronchial epithelial cells: effect of cell behavior in vitro. *J Clin Invest* 1992;90:1379-85.
- Rochester CL, Ackerman SJ, Zheng T, Rankin JA, Elias JA. Major basic protein regulation of lung fibroblast cytokine production. Role of cytokine synergy and change. *Chest* 1995; 107:117S-8S.
- Shoji S, Rickard KA, Ertl RF, Robbins RA, Linder J, Rennard SI. Bronchial epithelial cells produce lung fibroblast chemotactic factor: fibronectin. *Am J Respir Cell Mol Biol* 1989;1:13-20.
- Holtzman MJ, Hansbrough JR, Rosen GD, Turk J. Uptake, release and novel species-dependent oxygenation of arachidonic acid in

- human and animal airway epithelial cells. *Biochim Biophys Acta* 1988;963:401-13.
33. Koenig A, Yakisan E, Reuter M, Huang M, Sykora KW, Corbacioglu S, et al. Differential regulation of stem cell factor mRNA expression in human endothelial cells by bacterial pathogens: an in vitro model of inflammation. *Blood* 1994;83:2836-43.
34. Schleimer RP. Effects of glucocorticosteroids on inflammatory cells relevant to their therapeutic applications in asthma. *Am Rev Respir Dis* 1990;141:S59-69.
35. Yankaskas JR, Cotton CU, Knowles MR, Gatz JT, Boucher RC. Culture of human nasal epithelial cells on collagen matrix supports. A comparison of bioelectric properties of normal and cystic fibrosis epithelia. *Am Rev Respir Dis* 1985;132:1281-7.
36. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156-9.
37. Langley KE, Bennet LG, Wypych J, Yancik SA, Liu XD, Westcott KR, et al. Soluble stem cell factor in human serum. *Blood* 1993;81:656-60.
38. Irani AM, Bradford TR, Kepley CL, Schechter NM, Schwartz LB. Detection of MCT and MCTC types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and antichymase antibodies. *J Histochem Cytochem* 1989;37:1509-15.
39. Keppel G. Design and analysis. 2nd ed. London: Prentice Hall; 1982.
40. Fujio K, Evarts RP, Hu Z, Marsden ER, Thorgeirsson SS. Expression of stem cell factor and its receptor, *c-kit*, during liver regeneration from putative stem cells in adult rats. *Lab Invest* 1994;70:511-6.
41. Matsui Y, Zsebo KM, Hogan BLM. Embryonic expression of a haematopoietic growth factor encoded by the *Sl* locus and the ligand for *c-kit*. *Nature* 1990;347:667-9.
42. Meininger CJ, Brightman SE, Kelly KA, Zetter BR. Increased stem cell factor release by hemangioma-derived endothelial cells. *Lab Invest* 1995;72:166-73.
43. Hibi K, Takahashi T, Sekido Y, Ueda R, Hida T, Ariyoshi Y, et al. Coexpression of the stem cell factor and the *c-kit* genes in small-cell lung cancer. *Oncogene* 1991;6:2291-6.
44. Inoue M, Kyo S, Fujita M, Enomoto T, Kondoh G. Coexpression of the *c-kit* receptor and the stem cell factor in gynecological tumors. *Cancer Res* 1994;54:3049-53.
45. Fokkens WJ, Godthelp T, Holm AF, Blom H, Mulder PG, Vroom TM, et al. Dynamics of mast cells in the nasal mucosa of patients with allergic rhinitis and non-allergic control: a biopsy study. *Clin Exp Allergy* 1992;22:701-10.
46. Ohnishi M, Ruhno J, Bienenstock J, Milner R, Dolovich J, Denburg JA. Human nasal polyp epithelial basophil/mast cell and eosinophil colony-stimulating activity: the effect is T-cell-dependent. *Am J Respir Dis* 1988;138:560-4.
47. Nonaka M, Nonaka R, Jordana M, Dolovich J. GM-CSF, IL-8, IL-1R, TNF- α R, and HLA-DR in nasal epithelial cells in allergic rhinitis. *Am J Respir Crit Care Med* 1996;153:1675-81.
48. Heard BE, Dewar A, Corrin B. Apposition of fibroblasts to mast cells and lymphocytes in normal human lung and in cryptogenic fibrosing alveolitis. Ultrastructure and cell perimeter measurements. *J Pathol* 1992;166:303-10.
49. Adachi S, Ebi Y, Nishikawa S, Hayashi S, Yamazaki M, Kasugai T, et al. Necessity of extracellular domain of *W* (*c-kit*) receptors for attachment of murine cultured mast cells to fibroblasts. *Blood* 1992;79:650-6.
50. Go S, Kitamura Y, Nishimura M. Effect of *W* and *W^v* alleles on production of tissue mast cells in mice. *J Hered* 1980;71:41-4.
51. Sperr WR, Czerwenka K, Mundigler G, Muller MR, Semper H, Klappacher G, et al. Specific activation of human mast cells by the ligand for *c-kit*: comparison between lung, uterus and heart mast cells. *Int Arch Allergy Immunol* 1993;102:170-5.
52. Widdicombe JG. Microvascular anatomy of the airways. In: Busse WW, Holgate ST, editors. *Asthma and rhinitis*. Cambridge: Blackwell Science Publication; 1995. p. 722-32.
53. Pipkorn U, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. *N Engl J Med* 1987;316:1506-10.
54. Otsuka H, Denburg JA, Befus AD, Hitch D, Lapp P, Rajan RS, et al. Effects of beclomethasone dipropionate on nasal metachromatic cell sub-populations. *Clin Allergy* 1986;16:589-95.
55. Juliusson S, Holmberg K, Karlsson G, Enerback L, Pipkorn U. Mast cells and mediators in the nasal mucosa after allergen challenge. Effects of four weeks' treatment with topical glucocorticoid. *Clin Exp Allergy* 1993;23:591-9.
56. Wershil BK, Furuta GT, Lavigne JA, Choudhury AR, Wang ZS, Galli SJ. Dexamethasone and cyclosporin A suppress mast cell-leukocyte cytokine cascades by multiple mechanisms. *Int Arch Allergy Immunol* 1995;107:323-4.
57. Irani AA, Nilsson G, Ashman LK, Schwartz LB. Dexamethasone inhibits the development of mast cells from dispersed human fetal liver cells cultured in the presence of recombinant human stem cell factor. *Immunology* 1995;84:72-8.
58. Finotto S, Dolovich J, Denburg JA, Jordana M, Marshall JS. Functional heterogeneity of mast cells isolated from different microenvironments within nasal polyp tissue. *Clin Exp Immunol* 1994;95:343-50.
59. Guy-Grand D, Dy M, Luffau G, Vassalli P. Gut mucosal mast cells: origin, traffic and differentiation. *J Exp Med* 1984;160:12-28.
60. Mullol J, Xaubet A, Gaya A, Roca-Ferrer J, Lopez E, Fernandez JC, et al. Cytokine gene expression and release from epithelial cells. A comparison study between healthy nasal mucosa and Np. *Clin Exp Allergy* 1995;25:607-15.
61. Cromwell O, Hamid Q, Corrigan CJ, Barkans J, Meng Q, Collins PD, et al. Expression and generation of interleukin-8, IL-6 and granulocyte-macrophage colony-stimulating factor by bronchial epithelial cells and enhancement by IL-1 β and tumour necrosis factor- α . *Immunology* 1992;77:330-7.

Bound volumes available to subscribers

Bound volumes of The Journal of Allergy and Clinical Immunology are available to subscribers (only) for the 1997 issues from the Publisher, at a cost of \$88.50 for domestic, \$116.10 for Canadian, and \$108.50 for international subscribers for Vol. 99 (January-June) and Vol. 100 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. Copies are shipped within 30 days after publication of the last issue in the volume. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Dr., St. Louis, MO 63146-3318; phone 1 (800) 453-4351 or (314) 453-4351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.