

Stem cell factor mRNA expression and production in human nasal epithelial cells: Contribution to the accumulation of mast cells in the nasal epithelium of allergy

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Background: In allergic rhinitis, mast cells are increased in number in the epithelium of the nasal mucosa and play an important role in the immediate response. However, the mechanism of the accumulation is not known.

Objective: The purpose of this study was to determine whether the nasal epithelial cells produce stem cell factor (SCF), the mast cell growth and chemoattractant factor, and contribute mast cell hyperplasia in the epithelium of allergic rhinitis.

Methods: We have characterized the cellular localization of SCF using immunohistochemistry, reverse transcribed-PCR, and ELISA; compared SCF production of cultured epithelial cells between patients with allergic rhinitis and nonallergic subjects; and compared the SCF production with the number of mast cells and the histamine content in the nasal epithelial scrapings. **Results:** Immunohistochemically, SCF was identified in the nasal epithelium of the biopsy specimens and in cultured nasal epithelial cells. SCF mRNA was expressed by cultured nasal epithelial cells not only in patients with allergy but also in subjects with no allergy. However, the SCF/ β -actin mRNA ratio and SCF production in day 7 cultured epithelial cells was significantly higher in allergic than in nonallergic subjects ($P = .0424$, $P = .0085$, respectively). SCF production from nasal scrapings in culture was strongly correlated with the number of mast cells ($r = 0.506$, $P = .0023$) and the histamine content ($r = 0.480$, $P = .0040$).

Conclusions: These findings demonstrate that nasal epithelial cells produce SCF and may be important in the attraction, proliferation, and activation of mast cells in allergic inflammation in the nose. (*J Allergy Clin Immunol* 1998;102:757-64.)

Key words: Stem cell factor, allergic rhinitis, epithelial cell, mast cell, cyclosporin A, steroid

In allergic rhinitis and bronchial asthma, mast cells are triggered to release several important mediators that induce inflammatory reaction.^{1,2} In humans, mast cells can increase in numbers in the epithelium of the nasal mucosa

Abbreviation used

DMSO: Dimethyl sulfoxide
MC_T: Tryptase-positive mast cells
MC_{TC}: Tryptase-chymase-positive mast cell
SCF: Stem cell factor

or bronchus and play an important role, especially in the immediate response.³⁻⁷ Two subpopulations of human mast cells have been recognized with respect to formalin sensitivity and insensitivity^{4,8,9} and neutral protease content, namely tryptase-positive mast cells (MC_T) and tryptase-chymase-positive mast cells (MC_{TC}).^{10,11} Previously, we and others reported that most mast cells in the nasal epithelium are formalin sensitive or MC_T.^{3-5,12} Recently stem cell factor (SCF) has been identified as a unique growth, differentiation,¹³⁻¹⁶ chemoattractant,¹⁷ and activation factor for mast cells in humans.¹⁸⁻²⁰ To date, the distribution and abundance of cell types producing SCF is less well known. On the basis of their immunoreactivity, keratinocytes and endothelial cells seem to be prominent sources of SCF, with fibroblasts, mast cells and Langerhans cells also exhibiting reactivity.^{21,22} To determine whether the nasal epithelial cells produce SCF and contribute to mast cell hyperplasia, we studied the localization of SCF in nasal epithelial cells. In this article we describe SCF expression and production in cultured nasal epithelial cells of not only allergic but also nonallergic subjects. However, SCF expression and production was more abundant in the former than in the latter. Moreover, SCF production in the culture of nasal scrapings was significantly correlated with the number of mast cells and the histamine content in the nasal scrapings. SCF production was significantly inhibited by cyclosporin A and prednisolone treatment of cultured epithelial cells.

METHODS

Reagents and supplies

Collagen (Vitrogen 100) was obtained from Collagen Corp (Palo Alto, Calif). McCoy's 5A medium, penicillin-streptomycin, gentamycin, trypan blue, FBS, trypsin-EDTA, superscript reverse transcriptase, and oligo primer were obtained from GIBCO BRL (Grand Island, NY), and dNTPs and Ex Tag polymerase were obtained from Takara Shuzo (Kyoto, Japan). Acid guanidinium thiocyanate-phenol-chloroform (ISOGEN) was obtained from Nippon Gene Co (Tokyo, Japan). Immunohistochemical stain kit (Histofine kit) including peroxidase, biotin-conjugated rabbit anti-mouse IgG, and diaminobenzidine (DAB) was obtained from Nichirei Co (Tokyo, Japan). 2-Mer-

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captoethanol was obtained from Wako Pure Chemical Industry Ltd (Osaka, Japan), and protease E (type XIV, 4.4 units/mg), DMSO, histopaque, and agarose were from Sigma Chemical Co (St Louis, Mo). Prednisolone was obtained from Shionogi Pharmaceutical Co (Osaka, Japan). Mouse anti-human SCF (IgG1) was obtained from Genzyme Cytokine Research Products (Cambridge, Mass), and anti-CD3 and anti-CD37 were obtained from PharMingen Co (San Diego, Calif). Monoclonal mouse anti-human cytokeratin (AEA/AE3) and antibodies 5B5, AA1, NP57, and Ber-MAC3 were obtained from Dako Corporation (Carpinteria, Calif), and nonspecific mouse IgG1 k(MOPC- 21) was obtained from Sigma Chemical Co. The ELISA kit for SCF (R and D system; Quantikine) was obtained from Quantikine Co (Minneapolis, Minn). Cyclosporin A was provided by Novartis Pharma Co (Basel, Switzerland).

Subjects

Nasal inferior turbinate specimens were obtained from subjects undergoing turbinectomy for nasal obstruction, from 31 patients with allergic rhinitis (21 men and 10 women, aged from 13 to 30 years; 22.1 ± 0.7 years [mean $\pm$ SEM]), and from 21 patients with nonallergic hypertrophic rhinitis (15 men and 6 women, aged from 15 to 55 years; 29.1 ± 3.4 years). Nasal epithelial scrapings were obtained from 14 patients with allergic rhinitis (6 men and 8 women; aged from 11 to 60 years; 23.7 ± 3.2 years) and 7 nonallergic volunteers (1 man and 6 women; aged 20 to 37 years; 25.5 ± 2.2 years). All patients with allergic rhinitis had house dust/mite positive skin test responses, mild to severe perennial nasal symptoms, CAP RAST scores of 1 to 6 for *Dermatophagoides pteronyssinus* and positive nasal provocation responses to house dust. All subjects had not been treated with any medicine including nasal corticosteroid at least for 1 month and provided informed consent for this research. These studies were approved by the Nippon Medical School's Ethics Committee.

Preparation of epithelial cell monolayers

Nasal turbinate resected from allergic or nonallergic patients were rinsed 3 times with McCoy's 5A medium supplemented with 1% penicillin/streptomycin, 1% gentamycin, and 5×10^{-5} mol/L 2-mercaptoethanol (final concentration). They were then incubated in 0.1% protease E solution in McCoy's 5A at 4°C for 16 hours. After incubation, heat-inactivated (56°C, 30 minutes) FBS was added to a final concentration of 10% (volume/volume) to neutralize protease activity. Epithelial cells were detached from the tissue by gentle agitation, filtered through a metal mesh (250 μ m), and centrifuged at 800g for 10 minutes. The cells were resuspended in McCoy's 5A medium without FBS, and 5×10^4 cells in 2 mL of medium were plated onto wells of collagen-coated culture plates, which were prepared with the method described by Yankaskas et al.²³ Cells were cultured in a humidified atmosphere at 37°C and 5% carbon dioxide. After being cultured for 24 hours, each well was washed 3 times with McCoy's 5A medium to remove nonattached cells on collagen. McCoy's 5A medium only or the medium supplemented with 10^{-7} to 10^{-4} mol/L of prednisolone or 10^{-9} to 10^{-6} mol/L of cyclosporin A (final concentration) was changed (day 0). Cyclosporin A was dissolved in dimethyl sulfoxide (DMSO) to give stock of 10^{-2} mol/L, and the final concentration of DMSO ($\leq 0.1\%$) was tested and shown to have no effect on cell growth and SCF product from cultured nasal epithelial cells. Cells derived from confluent dishes were treated with 0.05% trypsin 0.53 mmol/L EDTA4Na and counted with a hemocytometer at days 3, 7, and 14. Twenty-four or 48 wells of each test sample were prepared for reverse transcribed (RT)-PCR studies. SCF production was measured from 2 wells by ELISA. Cell viability as assessed by 0.4% trypan blue exclusion was 95% or more in all experiments.

Immunohistochemical procedures

Nasal inferior turbinates obtained from 3 patients with hypertrophic rhinitis and three patients with perennial nasal allergic were fixed in Carnoy's fluid (60% methanol/30% chloroform/10% glacial acetic acid) for 24 hours. Each specimen was embedded in paraffin and cut at 5 μ m. After the deparaffin procedure, the specimen was stained immunohistochemically for SCF with anti-human SCF(IgG1) or nonspecific mouse IgG1 as a negative control by the procedure outlined later for cell smears.

To confirm studies with isolated epithelial cells, cultured epithelial cells were studied after cytocentrifugation onto glass slides. The cell spot was stained by May-Grünwald Giemsa solution and prepared for immunohistochemistry. The specimens were fixed in Carnoy's fluid for 10 minutes. After being washed with distilled water, endogenous peroxidase in the specimen was blocked by addition of 3% peroxidase in PBS at pH 7.4 for 10 minutes. After being washed with PBS, the specimen was incubated with 10% heat-inactivated normal rabbit serum for 1 hour at room temperature. Then the specimen was washed with PBS for 2 minutes at room temperature. Monoclonal mouse anti-human cytokeratin (IgG1, 1 μ g/mL) and mouse antiSCF (IgG, 10 μ g/mL) in PBS, including 10 mmol/L Tris HCl at pH 7.8, and nonspecific mouse IgG1 (1 μ g/mL) as a negative control were incubated separately on slides overnight at 4°C. Contaminating cell types were analyzed by staining for T and B lymphocytes with mAbs CD3 and CD37, respectively; and fibroblasts, mast cells, neutrophils, and macrophages were analyzed by staining with mAbs 5B5, AA1, NP57, and Ber-MAC3, respectively.²⁴ Samples were washed with PBS for 2 minutes and incubated with a biotin-conjugated rabbit anti-mouse IgG (Histofine kit) for 2 hours at room temperature. Samples were then washed with PBS for 5 minutes and stained with diaminobenzidine substrate kit.

Mononuclear cells and red cells preparation

PBMCs were isolated from heparinized blood of patients with perennial nasal allergy and separated by density gradient centrifugation on Histopaque (specific density, 1.077g per mL) spun at 500g for 30 minutes. The PBMC viability was 95% or more by 0.4% trypan blue dye exclusion. Red cells were isolated by the removal of leukocytes and platelets from heparinized blood of allergic patients.²⁵

Semiquantitative, reverse transcribed PCR for detection of SCF mRNA

To determine whether SCF mRNA was expressed by epithelial cells, PBMCs, and red cells, the following RT-PCR procedure was used. Quantitative analysis of gene expression was performed by the methods of Kageyama et al.²⁶ and Lee et al.²⁷ After total RNA was prepared from cultured epithelial cells, PBMCs and red cells with acid guanidinium thiocyanate-phenol-chloroform by a method described previously,²⁸ 1 μ g of total RNA was used for the synthesis of first-stand cDNA with Superscript reverse transcriptase and oligo primer. Aliquots of each cDNA were amplified to the appropriate cycle with 20 pmol of a set of oligonucleotide primers, 200 μ mol/L of dNTPs, and 0.5 units of Ex Taq polymerase in 20 μ L of reaction mixture. A set of oligonucleotide primer for human SCF was synthesized as follows: sense primer, 5' CAC TAA ATT GGT GGC AAA TCT TCC 3'; anti-sense primer, 5' TGT GAC ACT GAC TCT GGA ATC TIT 3', and human β -actin was synthesized as follows: sense primer, 5' CTT CTA CAA TGA GCT GCG TG 3'; anti-sense primer, 5' TCA TGA GGT AGT CAG TCA GG 3'. The reaction was aliquoted from a master mix to minimize the tube-to-tube variation and run with a Gene Amp PCR system 2400 (Perkin-Elmer, Foster City, Calif). The conditions were initial denaturation at 94°C for 30 seconds, 63°C for 1 minute, 72°C for 2 minutes. Final extension was at 72°C for 7 minutes. RT-PCR for SCF and β -actin were performed with 27 cycles and 17 cycles, respectively,

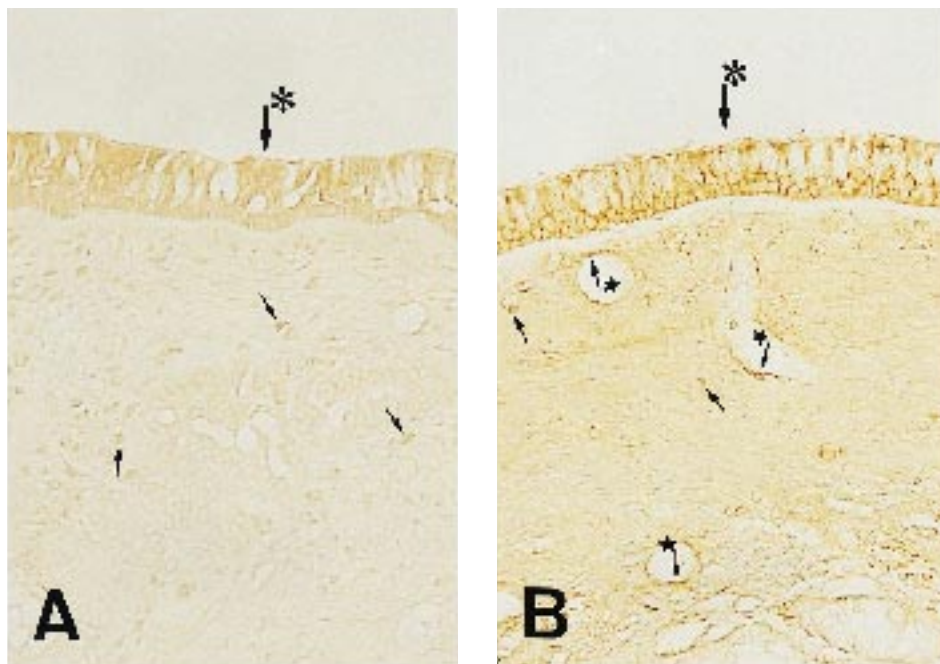


FIG 1. Immunohistochemical staining for SCF in turbinates of the nonallergic hypertrophic rhinitis (A) and allergic rhinitis (B). Staining for SCF was seen in the nasal epithelium ($\downarrow^*$) and scattered infiltrating cells ($\downarrow$) in the mucosa. In allergic rhinitis, staining was stronger in the epithelial layer, the stroma, infiltrating cells, and the vessel wall ($\downarrow^*$).

because it was determined experimentally that the DNA is exponentially amplified when these parameters were used.

The RT-PCR products were electrophoresed in 2% agarose gel at 100 V for 20 minutes. The level of SCF and actin transcripts were quantified with the Densitograph software for Macintosh (ATTO Co, Tokyo, Japan). Various amounts of cDNA were amplified with SCF and actin primer sets for each cycle to make the standard curves.

Mast cell number and histamine content in nasal scrapings

Epithelial specimens of the inferior turbinate were obtained by gentle scraping (without induced bleeding) with a small curette (Nagashima Co, Tokyo, Japan) as described previously.⁵ Five nasal scrapings were taken from 1 site in the nose. After the weight of each specimen was measured, 2 of them were dispersed as described previously.⁵ The total count in the suspension of dispersed cells and the proportion of mast cells that had mononucleus and metachromatic granules in a cytospin smear were determined. The smear was stained by May-Grünwald Giemsa solution, and the number of mast cells per 1000 cells was counted.

One scraping was incubated in cold HEPES-Tyrod buffer without Ca^{2+} and Mg^{2+} in an Eppendorf tube for 60 minutes. The specimen was then placed in 1 mL of cold HEPES-Tyrod solution without Ca^{2+} and Mg^{2+} and stored at -50°C . When the histamine content was measured, the specimens were first boiled at 90°C for 10 minutes to deactivate histaminase and centrifuged at $12,400g$ for 5 minutes. The histamine contents were measured by high performance liquid chromatography by the post-column method.²⁹

Tissue culture and SCF assays of nasal scrapings

Each of 2 epithelial scrapings was rinsed in cold RPMI 1640 medium (Nikken Seibutsu Igaku, Kyoto, Japan) supplemented with

1% streptomycin-penicillin, 0.13% gentamycin, $50\text{ }\mu\text{mol/L}$ 2-mercaptoethanol, and 5% heat-inactivated fetal calf serum for 30 minutes. The specimen was then cultured in 24-well plates containing 0.5 mL of the same medium per 1 mg of specimen (typically 2.5 mL medium for 5 mg specimen) at 37°C in an atmosphere containing 5% carbon dioxide. Two hours after the culture was started, the tissues were dispersed into single and small clusters of cells by trituration with a plastic pipette. After the cells were cultured for 7 days, the supernatants were collected and centrifuged at $800g$ for 10 minutes. SCF in the supernatant was determined by ELISA. The SCF content was calculated as an average of the SCF contents in each supernatant from 2 epithelial scrapings.

Statistical analysis

For comparisons of the SCF/actin mRNA ratio and SCF production of cultured nasal epithelial cells between allergy and nonallergy groups, *t* tests were used when variances were equal for *F* analysis. If this were not true, the nonparametric Mann-Whitney *U* statistic was used. Correlation coefficients between the mast cell number or the histamine content and SCF production of nasal scrapings were obtained by using Spearman's rank analysis.

RESULTS

Immunohistochemical study

In microscopic findings of turbinates in the patients with nonallergic hypertrophic rhinitis, immunohistochemical staining for SCF was strongly seen in the nasal epithelial layer and scattered infiltrating cells in the mucosa (Fig 1, A). However, in turbinates of allergic rhinitis, the stain for SCF was more strongly seen in the epithelial layer, the stroma, the infiltrating cells, and the vessel wall (Fig 1, B).

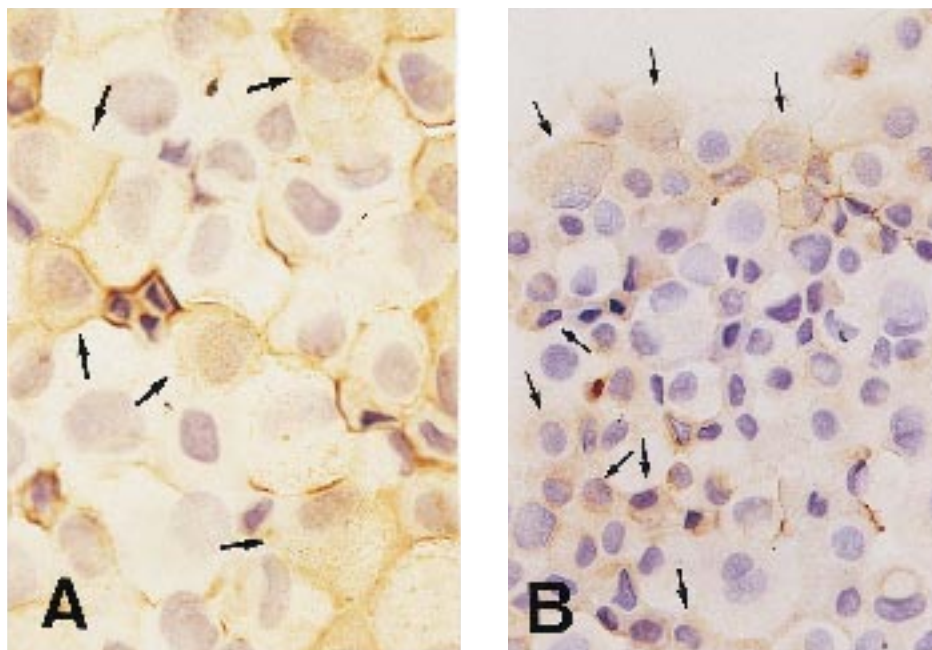


FIG 2. Immunohistochemical staining for cytokeratin (A) and SCF (B) in day-7 cultured epithelial cells of a patient with allergy. A, Most of cultured epithelial cells stained positive (↓) for cytokeratin in allergic turbinate. B, Positive stain (↓) for an anti-human SCF is shown in culture cell from a patient with allergy.

As a negative control with monoclonal mouse IgG1, no positive stain was recognized in the epithelium and stoma, neither allergic nor nonallergic turbinate.

Analysis of cultures by light microscopy and immunocytochemistry demonstrated that all these cells stained for cytokeratin in both allergic or nonallergic subjects (Fig 2, A), with no indication of contaminating cells including T and B lymphocytes, fibroblasts, neutrophils, macrophages, and eosinophils, as determined by immunocytochemical staining with mAbs specific to these cell types. Very few mast cells ($\leq 0.01\%$) were found in smears of cultured cell from some patients with allergy, but no mast cells were found in cultures from nonallergic subjects. The viability of the nasal epithelial cells was determined by trypan blue exclusion and was 95% or more in all cultures. Positive stain with anti-human SCF is shown in epithelial cells (Fig 2, B).

SCF expression in nasal epithelial cells

To confirm that nasal epithelial cells produce SCF, RT-PCR was performed, with total cellular RNA obtained from day-7 culture confluent epithelial cells. SCF transcripts were detectable in the cultured epithelial cells in both allergic patients ($n = 4$) and nonallergic subjects ($n = 3$; Fig 3). However, β -actin mRNA, but not SCF mRNA, was expressed in PBMCs; neither SCF mRNA nor β -actin mRNA was expressed in red cells of patients with nasal allergy (Fig 3). In addition for semiquantitative RT-PCR for SCF mRNA, a ratio of SCF mRNA to β -

actin mRNA was used. SCF/ β -actin mRNA ratios were 1.29 ± 0.24 (mean $\pm$ SE) in allergic patients ($n = 7$) and 0.62 ± 0.07 in nonallergic subjects ($n = 5$). There was a significant difference ($P = .0424$) between them (Fig 4).

SCF production from cultured epithelial cells

The time course and the effects of supplemental FBS on SCF production in nasal epithelial cell cultures were studied. Means $\pm$ SE of SCF contents in the supernatant of cultured nasal epithelial cells ($n = 4$ [2 with allergy and 2 without allergy]) were 50.5 ± 6.0 pg for 3-day culture, 133.6 ± 18.1 pg for 7-day culture, and 181.1 ± 18.4 pg for 14-day culture per 5×10^5 cells. The production of SCF from cultured epithelial cells was not affected by FBS (Fig 5). Means $\pm$ SE of SCF production per 5×10^5 viable cultured epithelial cells by 0%, 5% and 15% of FBS supplementation were 41.5 ± 11.1 for day 3, 128.2 ± 31.3 for day 7; 197.5 ± 35.8 for day 14; 53.2 ± 7.1 for day 3, 125.7 ± 24.6 for day 7, and 198.5 ± 33.5 for day 14; and 57.5 ± 11.1 for day 3, 119.0 ± 22.7 for day 7 and 197.5 ± 42.4 for day 14, respectively. However, when subjects were divided into 2 groups, allergic and nonallergic (Fig 6), means $\pm$ SE of SCF production per 5×10^5 viable cells in patients with allergy ($n = 8$) was 73.5 ± 8.2 pg for a 3-day culture and 125.2 ± 14.4 pg for a 7-day culture versus in nonallergic subjects ($n = 8$), 42.0 ± 4.5 pg for a 3-day culture and 74.5 ± 8.2 pg for a 7-day culture. There were significant differences in SCF production for culture day 3 and 7 ($P = .0049$ and $P = .0085$, respectively) between allergic and nonallergic subjects.

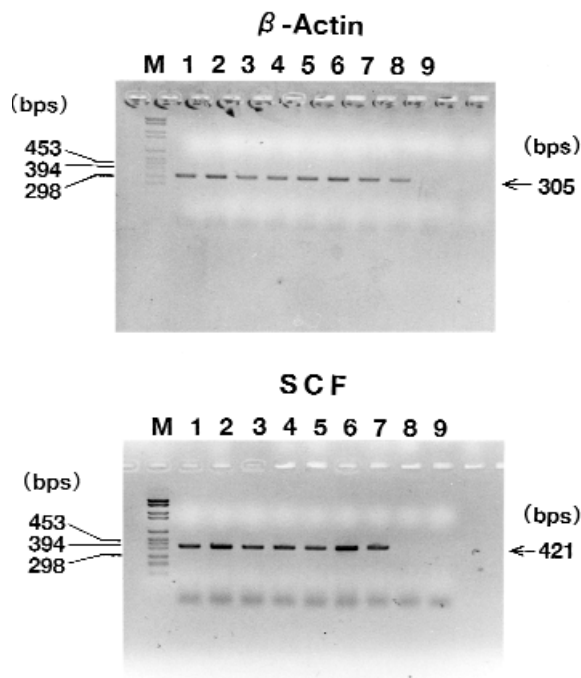


FIG 3. SCF expression in nasal epithelial cells, PBMCs, and red cells. RT-PCR was performed from total cellular RNA obtained from day-7 culture confluent epithelial cells, PBMCs, and red cells. SCF transcripts were detectable in the cultured epithelial cells in both allergic patients (lanes 1-4) and nonallergic subjects (lanes 5-7). However, SCF mRNA was not expressed; only β -actin mRNA was expressed in PBMCs (lane 8), and neither SCF mRNA nor β -actin mRNA was expressed in red cells (lane 9) of nasal allergic patient.

Inhibition of SCF production from epithelial cells by cyclosporin A and prednisolone

SCF production from viable nasal epithelial cell culture for 7 days was inhibited by cyclosporin A ($n = 4$) and prednisolone ($n = 3$; Table I). When 10^{-9} , 10^{-8} , 10^{-7} , and 10^{-6} mol/L of cyclosporin A (in which the culture cells grew with no disturbance) was added to the epithelial cell culture, SCF content was inhibited by 9%, 29%, 30%, and 45%, respectively. When 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} mol/L of prednisolone was added to the epithelial cell culture, SCF content was inhibited by 29%, 41%, 44%, and 48%, respectively.

Correlation between number of mast cells, histamine content and SCF production of nasal scrapings

The number of mast cells dispersed from nasal scrapings in 21 subjects ranged from 0 to 73 per 1000 cells. The histamine contents ranged from less than 1 ng/mg to 79.2 ng/mg. In patients with allergic rhinitis, the number of mast cells was 15.82 ± 5.18 cells (mean $\pm$ standard error), and the histamine content was 15.27 ± 5.78

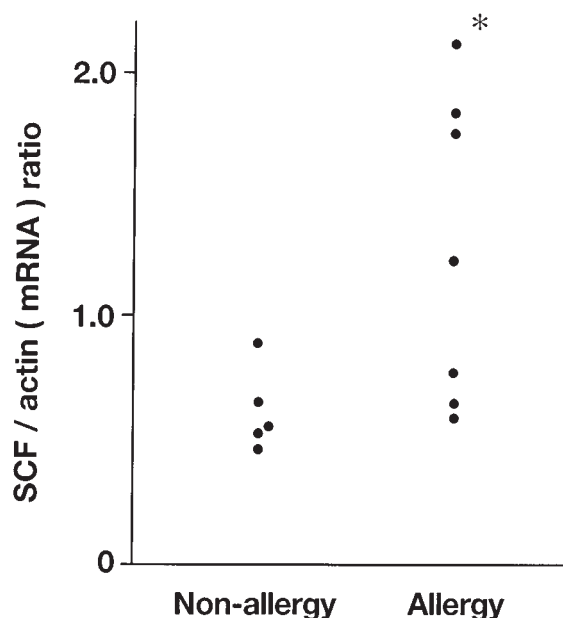


FIG 4. Semiquantitative RT-PCR for SCF was used as ratio to beta-actin mRNA. SCF/actin mRNA ratio in allergic patients ($n = 7$) was significantly higher ($*P < .05$) than in nonallergic subjects ($n = 5$). Data was shown in text.

ng/mg. Mast cells were not found and histamine contents were less than 1 ng/mg in all of the nonallergic subjects. The histamine content in a tissue in all subjects correlated well ($r = 0.965$, $P < .0001$) with the number of mast cells. On the other hand, means $\pm$ SE of the SCF amount (picograms per milligram of tissue) in the culture supernatant of nasal cells obtained from allergic and nonallergic subjects after 7-day cultures were 5.80 ± 1.74 pg/mg of tissue and 0.96 ± 0.65 pg/mg of tissue, respectively.

Both the number of mast cells and the histamine content in the nasal epithelial scrapings were strongly correlated with the SCF contents in the culture supernatants from nasal scrapings ($r = 0.506$, $P = .0023$, and $r = 0.480$, $P = .0040$, respectively; Fig 7).

DISCUSSION

In nasal allergy, mast cells increase in number and can be correlated with the degree of nasal symptoms and nasal antigen provocation.³⁻⁵ In bronchial asthma, intraepithelial mast cells in the bronchial wall increase, and their number can be correlated with the degree of the clinical severity.^{6,7} We reported that more than 80% of nasal surface metachromatic cells were MC_T, which was not seen in nonallergic normal subjects.⁵

The mechanism of mast cell hyperplasia in the nasal epithelium of patients with allergic rhinitis is unknown. In 1990 a novel hematopoietic growth factor, SCF was identified.³⁰⁻³³ It is encoded by the S1/S1^d gene. Recombinant SCF induced growth and differentiation of mast

TABLE I. Effect of cyclosporin A and prednisolone on SCF production from epithelial cells

	Control	10 ⁻⁹	10 ⁻⁸	10 ⁻⁷	10 ⁻⁶
Cyclosporin A (mol/L; n=4)					
SCF* (pg)	153.3 ± 11.9	129.6 ± 13.9	107.1 ± 11.0	104.8 ± 9.0	85.3 ± 14.0
%Inhibition		15.8 ± 5.0	29.5 ± 6.8	30.8 ± 6.3	45.3 ± 6.0
	Control	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴
Prednisolone (mol/L; n=3)					
SCF* (pg)	137.3 ± 8.6	95.0 ± 5.0	78.6 ± 9.8	75.3 ± 4.6	69.0 ± 6.1
%Inhibition		29.7 ± 8.6	41.3 ± 11.0	44.2 ± 7.0	48.9 ± 6.4

Values indicate means ± SE.

Monolayers of nasal epithelial cells from allergic patients were cultured for 7 days with prednisolone or cyclosporin A. The concentrations 10⁻⁷ to 10⁻⁴ of prednisolone and 10⁻⁹ to 10⁻⁶ of cyclosporin A did not affect cell growth and cell viability; more than 10⁻⁵ mol/L of cyclosporin A disturbed the growth of epithelial cells in culture.

*SCF production pg/5 × 10⁵ viable epithelial cells.

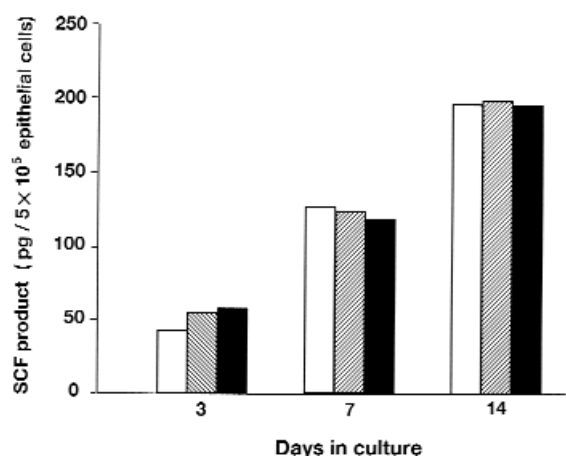


FIG 5. SCF production from cultured epithelial cells shows the relationship of the averages of SCF contents in the supernatant of cultured nasal epithelial cells (n = 4 [2 from patients with allergy and 2 from patients with no allergy]) and culture time. The SCF content means picograms per 5 × 10⁵ culture epithelial cells for 3, 7, and 14 days. The production of SCF from cultured epithelial cells was not affected by 0% (open bar), 5% (hatched bar), and 15% (black bar) of FBS supplementation.

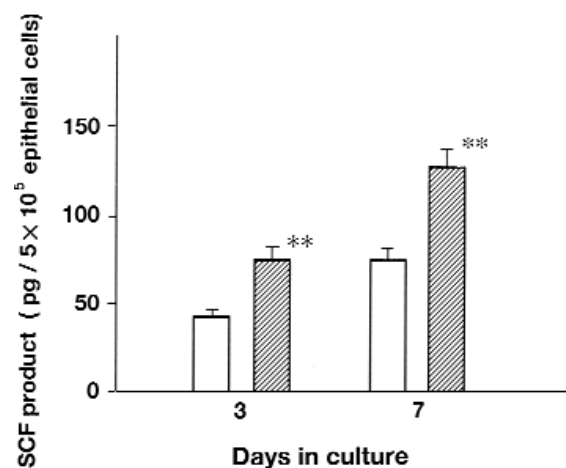


FIG 6. SCF production from cultured epithelial cells from allergic and nonallergic subjects. Average SCF production per 5 × 10⁵ viable cultured epithelial cells in allergic patients (n = 8, hatched bar) was significantly higher (**P < .01) than in nonallergic subjects (n = 8, open bar) for 3 and 7 days.

cells in mice in vitro³⁴ and in rats in vitro.³⁵ Moreover, recombinant human SCF induced the differentiation of mast cells from bone marrow and peripheral blood mononuclear cells and dispersed human fetal liver cells in long-term culture.¹³⁻¹⁶ Recently, in vitro studies of SCF have focused on its chemotactic activity¹⁷ and on its ability to activate mast cells.¹⁸⁻²⁰

To date, nasal and bronchial epithelial cells have been reported to produce granulocyte-macrophage colony-stimulating factor, IL-6, IL-8, TNF-α, RANTES, IL-1.³⁶⁻³⁹ However, these cytokines can not independently induce mast cell growth. We postulated that the accumulation of

mast cells in the nasal epithelium could be caused by a local microenvironmental factor, and we identified SCF mRNA expression in the nasal epithelial cells and SCF production from cultured epithelial cells of both allergic patients and nonallergic subjects. Interestingly, the density of immunohistochemical SCF stain in the epithelium of nasal mucosa and SCF protein in tissue culture of nasal scraping were significantly greater in patients with nasal allergy than in nonallergic subjects. Moreover, the number of mast cells and the histamine content in the nasal epithelium correlated strongly with the amount of SCF in the supernatant from cultured nasal epithelium (Fig 7). These findings suggested that the accumulation of mast cells in the nasal epithelium was contributed by

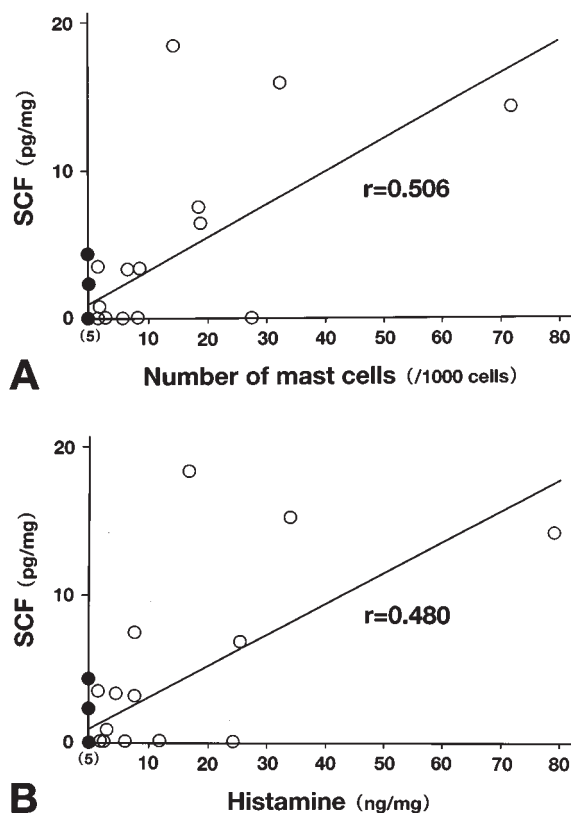


FIG 7. Correlation between SCF production by nasal epithelial cells in culture and the number of mast cells per total cells (**A**) and the histamine content (**B**) in the epithelial tissue. *Open circles* denote subjects with allergy; *filled circles*, nonallergic subjects. The *lines* are based on linear regression.

the cytokine SCF produced from the nasal epithelial cells. To date, regarding the production of SCF from respiratory epithelial cells, we,⁴⁰ Wen et al,⁴¹ and Kim et al⁴² reported the SCF production from epithelial cells of nose, normal bronchus, and nasal polyp, respectively. In this study, the data demonstrated strong immunologic stain and strong mRNA expression of SCF in epithelial cells. However, the amount of SCF that was produced by the culture epithelial cells over 3 to 14 days was low. The SCF measured in nasal epithelial cell cultures was spontaneously released protein without stimuli. It is unknown what kind of stimuli activate epithelial cells to produce SCF. We hypothesize that the SCF stored in the epithelial cells ongoing reaction is released by undefined stimuli and contributes to the accumulation of mast cells in the nasal epithelial layer. We used the corresponding primer of the soluble form domain in RT-PCR of SCF mRNA. We did not determine whether the stored SCF in cells was a soluble or nonsoluble form.

Nilsson et al¹⁷ reported that in vitro SCF was a chemotactic factor for human mast cells. Although we demonstrated that the nasal epithelial cell expressed the SCF

mRNA and produced SCF, we could not determine whether the mast cell accumulation in the nasal epithelium of allergic patients resulted from growth of mast cells in the epithelium or chemotaxis of mast cells into the epithelium from the nasal mucosa, or both. Human mast cell growth and differentiation is enhanced strongly by the addition of IL-6 in vitro.¹⁶ Indeed, nasal epithelial cells can produce IL-6 and other cytokines.³⁸

Recently, it was mentioned that the SCF not only induce mast cell differentiation or chemotactic activity but also enhance and release from mast cells.¹⁸⁻²⁰ Costa et al⁴³ and Bischoff et al⁴⁴ showed a direct activation of human mast cell by recombinant human SCF. In addition, inhalation of SCF directly induced bronchial contraction and was inhibited by antagonists of leukotriene and histamine H1 receptor, which suggested that SCF directly activated bronchial mast cells.⁴⁵ It is possible that SCF from nasal epithelial cells contributes to activation of nasal mast cells and enhances the release of mediators from nasal mast cells.⁵ In fact, we compared mast cell functions using nasal epithelial scrapings with abundant MC_T and chopped deep mucosa with abundant MC_{TC}. Functionally, antigen-induced histamine and leukotriene release from chopped nasal deep mucosa were much less than from nasal scrapings, and antigen-induced histamine release from the deep mucosa was enhanced by SCF (unpublished data). The accumulation of MC_T in the nasal epithelium can be inhibited by the topical steroid^{46,47}; we demonstrated the inhibition of SCF production from epithelial cells by steroid. The reduction of mast cells in the nasal epithelium might be induced by either the inhibition of SCF production from nasal epithelial cells by steroid and/or by the inhibition of the SCF-induced development of mast cell by steroid.⁴⁸

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