

Nasal mucosal immunoexpression of the mast cell chemoattractants TGF- β , eotaxin, and stem cell factor and their receptors in allergic rhinitis

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Background: Allergic rhinitis is characterized by the epithelial accumulation of cells, particularly mast cells and eosinophils.

There is little information relating to the chemotaxins

responsible for mast cell epithelial accumulation in this disease. **Objective:** Expression of the mast cell chemoattractants TGF- β , eotaxin, and stem cell factor and their receptors was investigated in tissue sections from biopsy specimens obtained from patients with naturally occurring allergic rhinitis.

Methods: Specific immunohistochemical staining was performed on thin sections of inferior turbinate biopsy specimens from patients with perennial and seasonal allergic rhinitis and, for comparison, from nonatopic and, where relevant, atopic healthy volunteers without rhinitis. Sequential staining of adjacent 2- μ m sections was undertaken to colocalize TGF- β receptors to mast cells.

Results: Evidence was found of significantly increased epithelial immunoreactivity for TGF- β 1, TGF- β 2, TGF- β 3, TGF- β receptor I, TGF- β receptor II, and TGF- β receptor III in patients with perennial and seasonal allergic rhinitis compared with that seen in healthy control subjects. TGF- β receptors I and II were found to colocalize to mast cells. Eotaxin epithelial immunoreactivity was significantly increased in the perennial group, although there were no corresponding disease-related differences found in relation to CCR-3 immunoreactivity at this site. There was no increase in stem cell factor immunoreactivity within the epithelium in naturally occurring disease. Significant correlations were found between epithelial immunoreactivity for TGF- β 1, TGF- β 2, TGF- β receptor I, TGF- β receptor II, and the number of epithelial mast cells.

Conclusion: These findings of enhanced epithelial TGF- β immunoreactivity in patients with rhinitis, the correlation with intraepithelial mast cell numbers, and the colocalization of TGF- β receptors to mast cells suggest that the epithelial expression of TGF- β might represent an important biologic process involved in either the recruitment or retention of mast

cells within the epithelium in naturally occurring allergic rhinitis. (*J Allergy Clin Immunol* 2004;114:799-806.)

Key words: Allergic rhinitis, immunohistochemistry, human, mast cells, antibodies, allergy, chemotaxis, TGF- β , eotaxin, stem cell factor

Allergic rhinitis, an inflammatory mucosal disorder of the nose, is characterized by the epithelial cell accumulation of mast cells and eosinophils. This phenomenon can be linked to the local generation and release of chemotactic factors. Although much attention has focused on chemotactic factors (in particular chemokines) of relevance to tissue eosinophil accumulation, scant attention has been given to those factors relevant to epithelial mast cell accumulation within the nose. An increase in epithelial mast cells is a characteristic finding in allergic rhinitis.^{1,2} Mast cells represent the major effector cells in rhinitis through their release of preformed and newly generated mediators, such as histamine, prostaglandin D₂, and leukotriene C₄,³ and their epithelial migration is considered to underlie the clinical occurrence of priming, whereby greater symptoms are experienced late in the pollen season in seasonal allergic rhinitis than for the same pollen count at the start of the seasonal exposure. Consistent with this, mast cells are in an activated state in symptomatic rhinitis,⁴ with ultrastructural evidence of degranulation on electron microscopy,⁵ immunostaining indicative of active cytokine secretion,^{2,6} and increased levels of the mast cell mediators histamine and tryptase, which are evident in nasal lavage fluid.³ Although it is known that CC chemokines, such as eotaxin and regulated on activation normal T-cell expressed and presumably secreted (RANTES), have mast cell chemoattractant activity, as do members of the TGF- β family, and that stem cell factor (SCF) is an important mast cell growth factor with chemotactic activity, these have not been systematically assessed in patients with naturally occurring rhinitis in relation to the epithelial accumulation of mast cells.

In an attempt to address this, we have undertaken an immunohistochemical analysis of the expression of these mast cell chemoattractants and their receptors by using inferior turbinate samples from atopic patients with allergic rhinitis (perennial and seasonal) and have compared the findings with those from subjects without rhinitis. The findings have been related both to the expression of appropriate receptors on mast cells and to the accumulation of epithelial mast cells.

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TABLE I. Summary of primary and secondary antibodies sources, species, and working dilutions

Primary antibody	Clone/lot	Source	Host species	Working dilution	Secondary antibody	Working dilution
Mast cell tryptase	AA1	Dako, Ely, UK	Mouse	1:1000	Rabbit anti-mouse	1:300
C-Kit (CD117)	Nu-ckit	Chemicon, Harrow, UK		1:50		
Eotaxin	43911.11	R&D Systems, Abingdon, UK		1:60		
TGF- β 2	IJI-MAB	GroPep Ltd, Adelaide, Australia		1:25		
TGF- β 3	20724.1	R&D Systems, Abingdon, UK		1:20		
TGF- β RI	D0803	Santa Cruz Biotechnology, Calif	Rabbit	1:150	Swine anti-rabbit	1:1200
TGF- β RII	C1303	Santa Cruz Biotechnology, Calif		1:200		
TGF- β RIII	E1503	Santa Cruz Biotechnology, Calif	Goat	1:60	Rabbit anti-goat	1:10,000
SCF	E090	Santa Cruz Biotechnology, Calif		1:60		
CCR-3	61828.111	R&D Systems, Abingdon, UK	Rat	1:80	Donkey anti-rat	1:3000
TGF- β 1	9016.2	R&D Systems, Abingdon, UK	Chicken	1:500	Donkey anti-chicken	1:1000

Abbreviations used

CIA: Computerized image analysis

SCF: Stem cell factor

TGF- β R: TGF- β receptor**METHODS****Subjects**

Ten house dust mite-sensitive subjects with perennial rhinitis (6 men and 4 women; median age [range], 32 years [19-57 years]), 10 grass pollen-sensitive subjects with seasonal allergic rhinitis (4 men and 6 women; median age [range], 33 years [23-54 years]), 3 atopic subjects without rhinitis (1 man and 2 women; median age [range], 29 years [21-31 years]), and 10 matched healthy nonatopic volunteers without rhinitis (5 men and 5 women; median age [range], 35 years [22-55 years]) participated in the study. The atopic patients were defined by a wheal response of at least 3 mm greater than that elicited by the negative control on skin prick testing to the aeroallergens *Dermatophagoides pteronyssinus* (ALK-Abello, Horsholm, Denmark) for perennial rhinitis and mixed grass pollen (Hollister-Stier, Spokane, Wash) for seasonal rhinitis. The healthy subjects were not taking any medication, and skin prick test responses to *D pteronyssinus*, grass pollen, and 8 other common aeroallergens (Allergopharma, Reinbek, Germany) were negative. The atopic control subjects all had positive skin prick test responses to *D pteronyssinus*. The patients were each asked to fill in a rhinitis questionnaire (score range, 0 [asymptomatic] to 21 [very symptomatic]) on the day of the nasal biopsy to establish the activity of the allergic condition in the rhinitis groups, as well as to exclude any symptoms in the control group.

Nasal biopsy and tissue processing

After achievement of local anesthesia (topical lignocaine and adrenaline), nasal biopsy specimens were taken under direct vision from the inferomedial border of the inferior turbinate by using Hartmann ear forceps (Medicon, Tuttlingen, Germany). The biopsy specimens were placed immediately into ice-cooled acetone containing the protease inhibitors iodoacetamide (20 mmol/L) and phenylmethylsulfonyl fluoride (2 mmol/L). After overnight fixation at

–20°C, specimens were embedded in the water-soluble resin glycol methacrylate (Park Scientific, Northampton, United Kingdom), as previously described.⁷ The study was approved by the Southampton Joint University and Hospitals Ethics Committee, and all participants provided written consent.

Immunohistochemistry

Immunohistochemistry was applied to glycol methacrylate-embedded tissue by using the streptavidin-biotin peroxidase detection method, as previously described.⁷ Antibodies were applied for 16 to 20 hours at previously titrated optimal dilutions (Table I). The following day, appropriate biotinylated secondary antibodies were applied for 2 hours (Table I), washed, and detected by using the streptavidin-biotin peroxidase system (Dako Ltd, Ely, United Kingdom). After further washing, the chromogens aminoethylcarbazole (red reaction product) or diaminobenzidine tetrahydrochloride (brown reaction product) were applied, and the sections were counterstained with Mayer haematoxylin. All experiments included negative control slides with omission of the primary antibody, as well as isotype-matched antibodies, which were performed at the titration stage. Where possible, the antibodies were preincubated with a 10-fold molar excess of immunizing peptide (TGF- β RI, TGF- β RII, and TGF- β RIII) to check for specificity.

For colocalization studies, sequential adjacent 2- μ m tissue sections were immunostained, one with AA1 to identify mast cells and the other with a TGF- β R antibody.

Assessment and quantification of immunohistochemical staining

Levels of epithelial immunoreactivity were quantified by means of computerized image analysis (CIA; Zeiss KS400; Image Associated, Bicester, United Kingdom). For each biopsy specimen, the entire intact epithelium, excluding the brush border, was systematically assessed in 2 nonserial sections on the basis of red, blue, and green color balance. By using the same system, the area of submucosa and the length of the overlying epithelium were calculated for each section. Cells exhibiting positive immunoreactivity for mast cell tryptase and c-kit were counted and expressed as the number per square millimeter of submucosa or the number per millimeter length of the epithelium. Measurements were performed by an observer who was blinded to the clinical group from which the biopsy specimen was derived. Comparison of sequential sections for the expression of the TGF- β R by mast cells was assessed by using the CIA system.

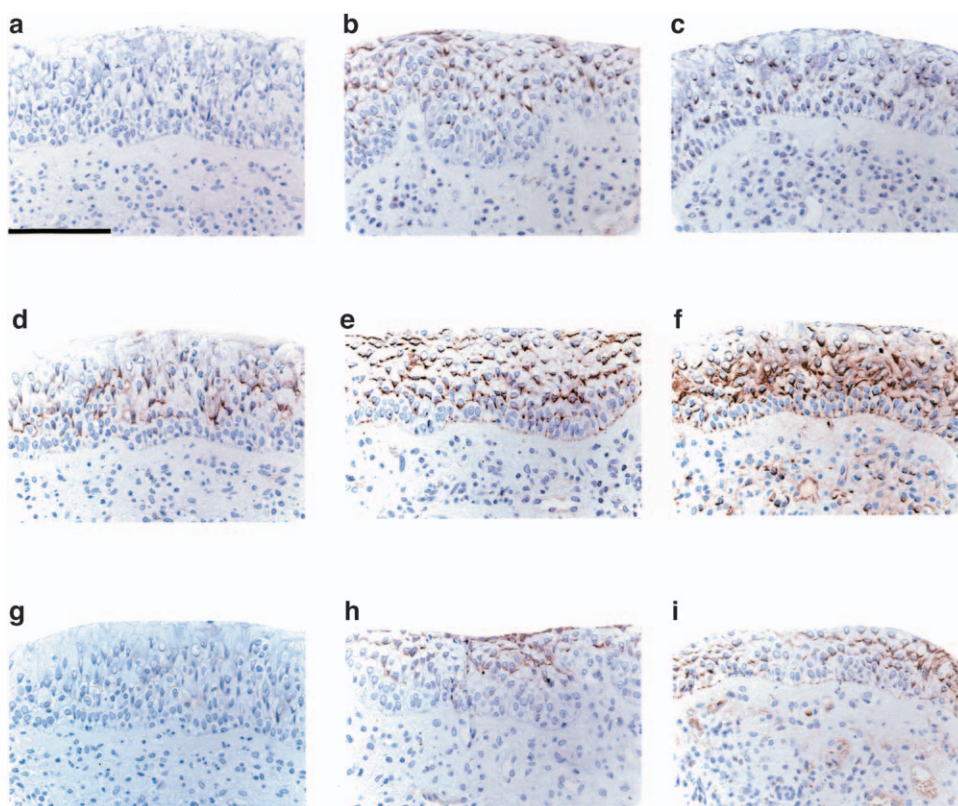


FIG 1. Immunohistochemical staining of TGF- β 1 (a-c), TGF- β 2 (d-f), and TGF- β 3 (g-i) in inferior turbinate biopsy specimens. The plate shows sections from a healthy subject (Fig 1, a, d, and g), a subject with perennial allergic rhinitis (Fig 1, b, e, and h), and a subject with seasonal allergic rhinitis (Fig 1, c, f, and i). Scale bar = 100 μ m.

Statistical analysis

Comparisons between clinical groups for immunostaining were made by using the nonparametric Mann-Whitney *U* test. Correlations were explored with the Spearman rank correlation test. The level of significance was accepted as a *P* value of less than .05.

RESULTS

The patients with rhinitis did not differ from the nonatopic or atopic control subjects in age or sex distribution. All subjects with rhinitis were symptomatic, with both the perennial (median symptom score, 12; range, 5-17) and seasonal (median symptom score, 13; range, 9-17) groups significantly more so ($P < .001$) than the atopic group without rhinitis (median symptom score, 0; range, 0-2) and the nonatopic healthy control group (median symptom score, 0.5; range, 0-3). No significant immunostaining was detected in negative control sections in which the primary antibodies were omitted, replaced with normal nonimmune serum, or preincubated with a blocking peptide.

Tryptase-positive cells

Mast cell numbers in the epithelium were significantly increased in the mucosa of patients with perennial

($P = .02$) and seasonal ($P = .01$) rhinitis when compared with those of patients without rhinitis.

TGF- β 1 to TGF- β 3

Immunoreactivity for TGF- β 1, TGF- β 2, and TGF- β 3 was observed in the epithelial layer, with predominant localization to the superficial columnar epithelial cells, although some reactivity was occasionally observed in basal cells (Fig 1). Submucosal glands were occasionally stained (mainly with TGF- β 2), whereas the submucosal connective tissue did not stain. Quantitation of epithelial immunostaining with CIA showed that there was a significant increase in TGF- β 1, TGF- β 2, and TGF- β 3 expression in patients with perennial (TGF- β 1, $P < .01$; TGF- β 2, $P < .01$; and TGF- β 3, $P = .001$) and seasonal (TGF- β 1, $P = .001$; TGF- β 2, $P = .001$; and TGF- β 3, $P = .001$) allergic rhinitis when compared with that seen in the control groups (Fig 2). There were no significant differences in TGF- β 1, TGF- β 2, or TGF- β 3 expression between the atopic and nonatopic control groups without rhinitis. Within the subjects with rhinitis, the extent of epithelial immunoreactivity was greatest for TGF- β 2, followed by TGF- β 3 and TGF- β 1, respectively. A significant correlation was observed between the number of epithelial mast cells and immunoreactivity levels for TGF- β 1 ($r_s = 0.45$, $P = .013$) and TGF- β 2 ($r_s = 0.4$, $P = .023$).

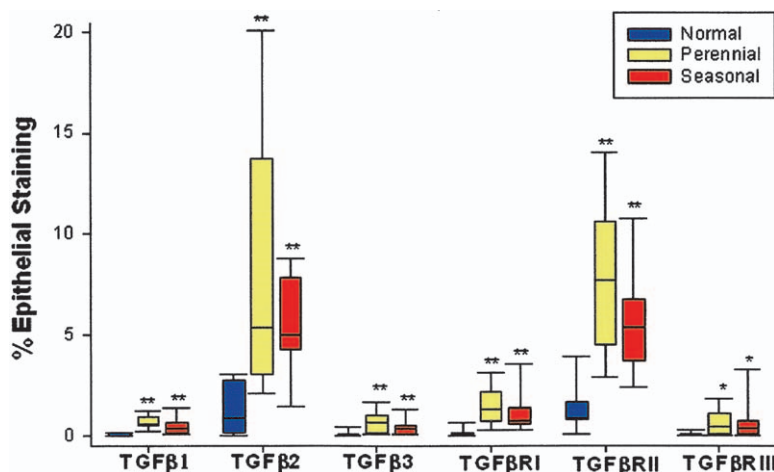


FIG 2. Quantitative analysis of TGF-β1, TGF-β2, TGF-β3, TGF-βRI, TGF-βRII, and TGF-βRIII in nasal epithelium from subjects with perennial and seasonal allergic rhinitis and nonatopic subjects for comparison. The percentage of epithelial staining for each group is shown, the *line* represents the median value, and the *error bars* denote the 5% and 95% percentile range. Data were analyzed by using the Mann-Whitney *U* test (* $P < .05$, ** $P < .01$).

There were no corresponding significant correlations observed in relation to submucosal mast cell numbers.

TGF-βRI to TGF-βRIII

Immunoreactivity for TGF-βRI, TGF-βRII, and TGF-βRIII was observed in the epithelial layer and, to a lesser degree, in the submucosal glands and vascular endothelium in the subjects with rhinitis and some of the healthy subjects. The immunostaining was predominantly localized at the level of the columnar epithelial layer (Fig 3). Quantitation of staining with CIA showed that there was a significant increase in TGF-βRI, TGF-βII, and TGF-βRIII in patients with perennial (TGF-βRI, $P = .002$; TGF-βRII, $P < .001$; and TGF-βRIII, $P = .009$) and seasonal (TGF-βRI, $P < .001$; TGF-βRII, $P = .001$; and TGF-βRIII, $P = .029$) allergic rhinitis when compared with that in the nonatopic control group (Fig 2). However, there were no significant differences in TGF-βRI, TGF-βRII, or TGF-βRIII expression in nonatopic subjects with rhinitis when compared with nonatopic control subjects without rhinitis. Of the 3 TGF-βR isotypes, TGF-βRII was most immunoexpressed within the epithelium, followed by TGF-βRI and TGF-βRIII, respectively. A significant correlation was observed between the number of epithelial mast cells and the extent of immunoreactivity for TGF-βRI ($r_s = 0.42$, $P = .02$) and TGF-βRII ($r_s = 0.45$, $P = .01$). There were no corresponding significant correlations observed in relation to submucosal mast cell numbers. Colocalization studies identified that mast cells expressed both TGF-βRI and TGF-βRII (Fig 4).

Eotaxin

Immunoreactivity for eotaxin displayed a predominantly intercellular/membrane-associated pattern within the epithelium, with the main bulk of the immunostaining localized at the level of the columnar epithelial layer (Fig 5). Quantitation of staining with CIA showed that there

was a significant increase in eotaxin expression in patients with perennial ($P = .03$) but not seasonal ($P = .09$) allergic rhinitis when compared with expression seen in the nonatopic control group (Fig 6).

CCR-3

Cellular immunoreactivity for CCR-3 was mainly nuclear membrane associated in nature and intermixed with a weaker pericellular pattern (Fig 5). Quantitation of staining with CIA showed no significant differences in CCR-3 expression in patients with perennial ($P = .57$) or seasonal ($P = .88$) allergic rhinitis when compared with that seen in the nonatopic control group (Fig 6).

SCF

Immunoreactivity for SCF showed a predominantly nuclear pattern, with occasional clearly detectable dense staining of perinuclear regions (Fig 7). Quantitation of staining with CIA showed no significant differences in SCF expression in patients with perennial ($P = .72$) or seasonal ($P = .96$) allergic rhinitis when compared with that seen in the nonatopic control group (Fig 6).

C-kit-positive cells

Immunoreactivity for c-kit demonstrated a distinct cell-surface pattern of staining of individual cells scattered in both the epithelium and submucosa (Fig 7). There was a significant increase in epithelial c-kit-positive cells in the subjects with seasonal rhinitis compared with that seen in the nonatopic control group ($P = .02$). No other significant statistical differences were apparent between the groups. Overall, there was a significant correlation between the number of mast cells in the epithelium and submucosa with the corresponding number of c-kit-positive cells ($r_s = 0.55$, $P = .002$, and $r_s = 0.63$, $P < .001$, respectively). No statistical differences were apparent between the numbers of c-kit-positive cells and AA1-

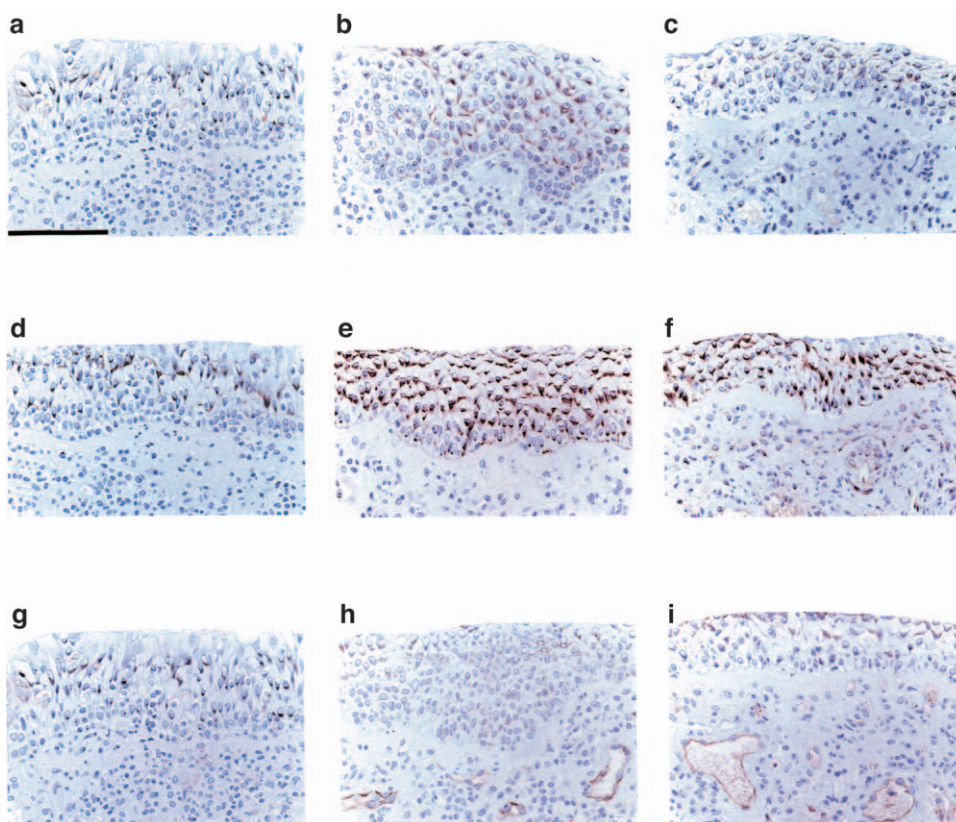


FIG 3. Immunohistochemical staining of TGF- β RI (a-c), TGF- β RII (d-f), and TGF- β RIII (g-i) in inferior turbinate biopsy specimens. The plate shows sections from a healthy subject (Fig 3, a, d, and g), a subject with perennial allergic rhinitis (Fig 3, b, e, and h), and a subject with seasonal allergic rhinitis (Fig 3, c, f, and i). Scale bar = 100 μ m.

positive cells in any of the groups in either the epithelium or the lamina propria.

DISCUSSION

An increase in epithelial mast cells was evident in patients with both seasonal and perennial allergic rhinitis in this study. This independently confirms our previous findings in patients with allergic rhinitis.^{2,6} The basis for this epithelial accumulation of mast cells is as yet unidentified. To explore this, we have quantified the epithelial expression of a range of known mast cell chemoattractants and their receptors within these biopsy specimens. Epithelial immunoreactivity was evident for the 3 TGF- β isotypes, TGF- β 1, TGF- β 2, and TGF- β 3, along with their receptors, TGF- β RI, TGF- β RII, and TGF- β RIII, as well as for eotaxin and SCF. There were differences in disease from the findings in the healthy nose in that there was significantly greater epithelial immunoreactivity for TGF- β 1, TGF- β 2, TGF- β 3, TGF- β RI, TGF- β RII, TGF- β RIII, and eotaxin in patients with perennial allergic rhinitis, and the same results were found, with the exception of eotaxin, in patients with seasonal allergic rhinitis. No disease-related difference

was evident in SCF immunoreactivity within the epithelium. With regard to TGF- β and its receptors, no such differences were evident between atopic subjects without rhinitis and nonatopic control subjects without rhinitis, suggesting that the changes demonstrated were likely to be disease rather than atopy dependent. This is the first report of the epithelial expression of TGF- β isoforms and their receptors within the nose and clearly links these known mast cell chemoattractants to clinical disease expression. We identified that TGF- β RI and TGF- β RII were also expressed on mast cells and that there were significant correlations between the number of mast cells within the epithelium and the expression at this site of TGF- β 1, TGF- β RI, TGF- β 2, and TGF- β RII. These findings would be consistent with the involvement of TGF- β in either the recruitment or retention of mast cells within the epithelium in patients with allergic rhinitis.

TGF- β is recognized as a potent mast cell chemoattractant, with maximum chemotaxis *in vitro* being evident at the femtomolar level, which is several log orders less than the picomolar concentrations of eotaxin, RANTES, and SCF that are required to induce mast cell chemotaxis under the same circumstances.^{8,9} Human gastrointestinal mast cells have previously been shown to express TGF- β receptors, and the ability of TGF- β

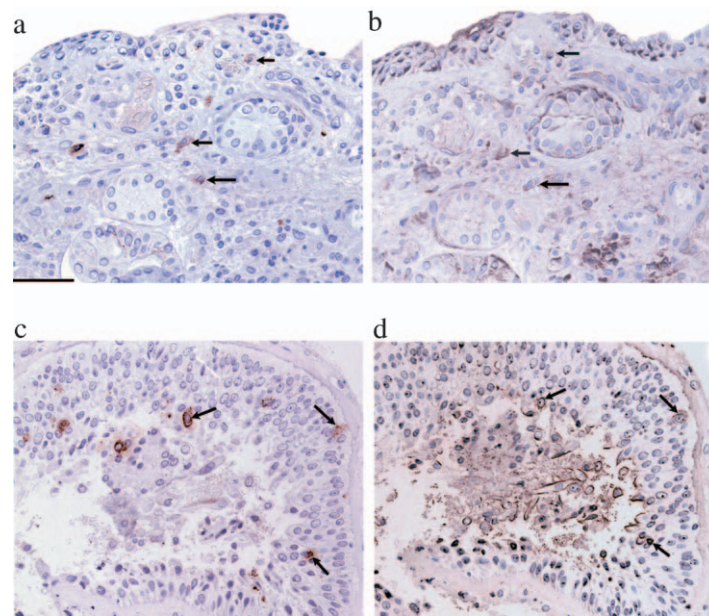


FIG 4. Sequential 2- μ m sections of nasal mucosa from 2 subjects with rhinitis stained with mouse anti-AA1 (mast cell tryptase; **a** and **c**), rabbit anti-TGF- β RI (**b**), and rabbit anti-TGF- β RII (**d**), demonstrating the expression of both receptors on some epithelial and submucosal mast cells (arrows). Scale bar = 50 μ m.

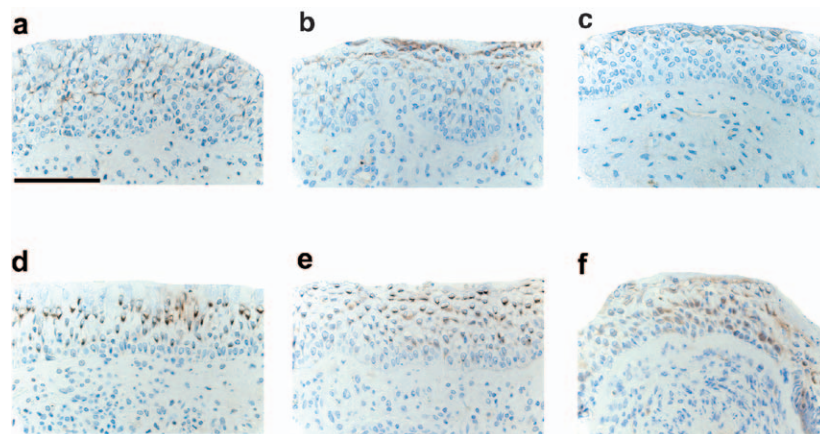


FIG 5. Immunohistochemical staining of eotaxin (**a-c**) and CCR-3 (**d-f**) in inferior turbinate biopsy specimens. The plate shows sections from a healthy subject (Fig 5, **a** and **d**), a subject with perennial allergic rhinitis (Fig 5, **b** and **e**), and a subject with seasonal allergic rhinitis (Fig 5, **c** and **f**). Scale bar = 100 μ m.

to promote mast cell chemotaxis has previously been demonstrated *in vitro* with both a human mast cell line (HMC-1) and cultured primary gut mast cells.⁹ This chemotactic response has been considered to be mediated by the TGF- β serine-threonine type I and type II receptors.⁹ In our study it was the epithelial expression of TGF- β 2, followed by TGF- β 1, that was the most prominent, and this expression correlated positively with the number of mast cells within the epithelial compartment. Although correlations cannot be taken as firm proof of cause and effect, this study provides clear evidence for TGF- β expression within the nasal epithelium that is enhanced in naturally occurring disease, and the relation-

ship to epithelial mast cell numbers supports the notion of the importance of TGF- β as a mast cell chemoattractant in the clinical setting. This is further enhanced by our identification that nasal mast cells express TGF- β RI and TGF- β RII. This is the first such identification within the airways. Our suggestion that TGF- β might be an important signal determining the attraction or retention of mast cells within the epithelium is supported by a previous publication evaluating *TGFB1* gene expression and cell recruitment within small airway samples from patients with chronic obstructive pulmonary disease.¹⁰ This study by de Boer et al reported a significant correlation between the level of *TGFB1* gene expression and epithelial mast

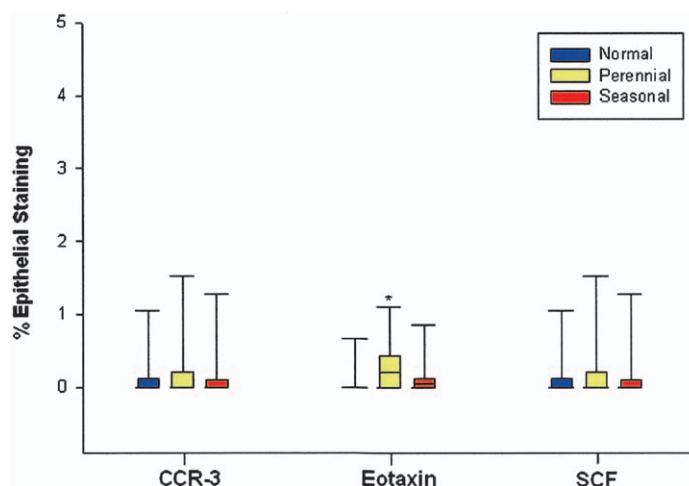


FIG 6. Quantitative analysis of CCR-3, eotaxin, and SCF in nasal epithelium from subjects with perennial and seasonal allergic rhinitis and nonatopic subjects for comparison. The figure shows the percentage of epithelial staining for each group, the line represents the median value, and error bars denote the 5% and 95% percentile range. Data were analyzed by using the Mann-Whitney *U* test (**P* < .05).

cell numbers. This would be consistent with a link between these biologic processes. It is possible that the relationship could be other than that considered, with mast cell activation within the epithelium representing an important signal for the epithelial expression of TGF- β and its receptors. This cannot be excluded by our study; however, the potency of TGF- β as a known mast cell chemoattractant would suggest otherwise.

Studies *in vitro* have identified a number of other mast cell chemoattractants, such as the CC chemokines, eotaxin, and RANTES, ligands for the CCR-3 receptor and the growth factor SCF, whose activity is mediated through the c-kit receptor and complement components, such as C3a and C5a. We thus explored the immunohistochemical expression of eotaxin and SCF, along with their respective receptors, in the same biopsy specimens as for TGF- β . There was greater epithelial expression of eotaxin in the biopsy specimens from patients with both seasonal and perennial allergic rhinitis than in the biopsy specimens from healthy control subjects, although with the limited number of biopsy specimens and the known disease variability in seasonal allergic rhinitis, this was only statistically significant in the perennial rhinitis group. There is one previous report of eotaxin immunoreactivity in nasal biopsy specimens, also identifying predominantly epithelial localization in allergic rhinitis.¹¹ This study was on seasonal allergic rhinitis and is in contrast to our findings in that the expression was significantly increased compared with that in healthy control subjects. The stimulus in seasonal allergic rhinitis is variable, depending on the pollen count. At the time our biopsy specimens were taken, all subjects were symptomatic, with a mean grass pollen count of 68 grains/m³ per 24 hours (range, 5–371 grains/m³ per 24 hours) during the period of the biopsies (May 1–July 15). Although there was a clear seasonal increase in epithelial TGF- β immunoreactivity, this was not so for eotaxin. No details of the pollen

expression are given in the article by Pullerits et al,¹¹ although the in-season biopsy specimen was taken 6 weeks into the pollen season, which was later than in our study, and it might be that a higher or more sustained level of allergen exposure is required to promote epithelial eotaxin expression, which is why it was more evident in our study in the perennial allergic rhinitis group.

Because human mast cells expressing the CCR-3 receptor migrate *ex vivo* in response to eotaxin,⁸ the epithelial expression of eotaxin might thus be thought likely to be involved in the intraepithelial accumulation of mast cells in naturally occurring disease. Animal studies have, however, questioned the importance of eotaxin in this respect because CCR-3-deficient mice have been reported to be characterized by an increased number of intratracheal epithelial mast cells, a feature that is exacerbated further by allergen challenge in sensitized animals, despite abrogation of the tissue eosinophil accumulation in these animals.¹² The role of CCR-3 and its ligands, such as eotaxin, in mast cell accumulation and differentiation is thus more complex than for eosinophils. In contrast to immunostaining for c-kit, which resulted in a ring pattern of immunoreactivity (Fig 7), as anticipated for a cell surface-expressed receptor, in our study immunostaining for CCR-3 was noted to be mainly intracellular (Fig 5). This was unexpected, and the basis for this was initially uncertain, although it appeared a consistent pattern. Price et al¹³ have, however, recently reported that the CCR-3 receptor is predominately intracellular in human mast cells and that it is only externalized after cross-linking of Fc ϵ RI by allergen. Although such externalization might have been anticipated in naturally occurring seasonal and perennial allergic rhinitis, the failure to reveal such a pattern might explain the lack of correlation between epithelial mast cell numbers and the epithelial expression of eotaxin.

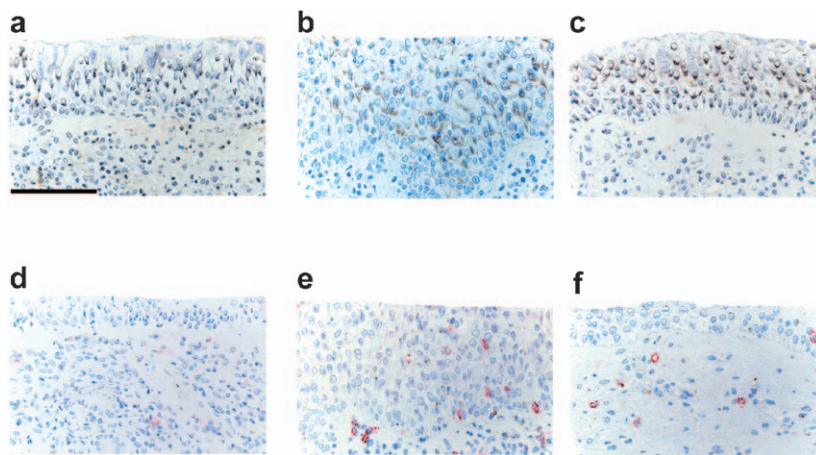


FIG 7. Immunohistochemical staining of SCF-positive (**a-c**) and c-kit-positive cells (**d-f**) in inferior turbinate biopsy specimens. The plate shows sections from a healthy subject (Fig 7, **a** and **d**), a subject with perennial rhinitis (Fig 7, **b** and **e**), and a subject with seasonal allergic rhinitis (Fig 7, **c** and **f**). Scale bar = 100 μ m.

Although we identified that SCF was expressed within the epithelium in nasal biopsy specimens, we were unable to identify any disease-related differences or a relationship between this expression and the number of intraepithelial mast cells. Fujikura and Otsuka¹⁴ have previously shown that nasal epithelial cells can generate SCF and reported a relationship between SCF mRNA within the nasal biopsy specimens and mast cell numbers and histamine content within the tissue. Their study did not, however, specifically focus on the epithelial mast cell population, and in this instance SCF might be more relevant to the larger number of submucosal mast cells.

Our study has focused on the epithelial expression of mast cell chemoattractants and their relationship to intraepithelial mast cell numbers. This study cannot draw valid conclusions about those factors relevant to the initial tissue recruitment of immature progenitor mast cell precursors or, indeed, the factors responsible for their tissue maturation and survival. Thus signals such as SCF and eotaxin might well be relevant to such processes. Our findings with the differing TGF- β isoforms, however, do suggest that further work exploring their effects on epithelial mast cell recruitment, retention, and activation in allergic airways disease is warranted.

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