

Grass pollen immunotherapy inhibits seasonal increases in basophils and eosinophils in the nasal epithelium

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Summary

Background Symptoms of allergic rhinitis are accompanied by infiltration of the nasal mucosa with inflammatory cells, predominantly eosinophils and metachromatic cells (basophils and mast cells). Specific immunotherapy (IT) reduces mucosal eosinophilia and numbers of metachromatic cells in the epithelium. A specific marker distinguishing basophils from mast cells was recently developed.

Objectives The basophil-specific monoclonal antibody 2D7 was used to determine the influence of subcutaneous IT on numbers of nasal mucosal basophils compared with the effects of IT on neutrophils, eosinophils and mast cells.

Method During a randomized, placebo-controlled trial of grass pollen IT in 44 adults with severe summer hay fever, nasal biopsies were taken at baseline, out of the pollen season, and at the peak of the pollen season following 2 years treatment. Biopsies were processed for immunohistochemistry for basophils (2D7⁺), mast cells (AA1⁺), eosinophils (MBP⁺) and neutrophils (neutrophil elastase⁺).

Results In placebo-treated (PL) patients there were significant seasonal increases in basophils ($P < 0.01$), mast cells ($P < 0.05$) and eosinophils ($P = 0.002$) in the nasal submucosa. In IT-treated patients significant increases in 2D7⁺ cells ($P < 0.01$) and eosinophils ($P = 0.01$) but not AA1⁺ cells ($P = 0.9$) were observed. These differences were significant between groups for eosinophils ($P < 0.05$). In the epithelium there were seasonal increases in AA1⁺ cells and eosinophils in both groups (PL: $P < 0.01$, IT: $P < 0.05$ for both). The between-group difference was significant for eosinophils ($P = 0.05$). Basophils were observed in the epithelium of six out of 17 in the placebo group and one out of 20 in the IT group ($P = 0.03$). Neutrophil numbers remained constant in both epithelium and submucosa.

Conclusion Successful grass pollen immunotherapy was associated with inhibition of seasonal increases in basophils and eosinophils, but not mast cells or neutrophils within the nasal epithelium. Immunotherapy may act, at least in part, by reducing seasonal recruitment of basophils and eosinophils into the epithelium.

Keywords: allergic rhinitis, immunotherapy, immunohistochemistry, basophil, mast cell, eosinophil, neutrophil

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Introduction

Infiltration of the nasal mucosa with inflammatory cells is a prominent feature of allergic rhinitis. In hayfever patients,

during the pollen season, there is an increase in the numbers of mucosal eosinophils [1] and of tryptase positive mast cells in the nasal epithelium [2]. Raised levels of mast cell mediators can be detected in nasal lavage fluid [3].

Metachromatic cells bind cationic dyes such as alcian blue at acidic pH, and are predominantly mast cells and basophils. The infiltration of metachromatic cells into the nasal mucosa is well documented in allergic rhinitis, both after allergen challenge [4,5] and during natural seasonal exposure [6]. Studies to determine the relative contribution of mast cells and basophils to this infiltration have been difficult due to the lack of a specific marker for basophils. In common with mast cells, basophils have IgE on their surface and histamine in their secretory granules. Tryptase, abundantly expressed by mast cells and in far lesser amounts by basophils, has been useful for identifying mast cells but not basophils. The recent availability of specific monoclonal antibodies, BB1 [7] and 2D7 [8], directed against basophil-specific granule proteins, has allowed basophils to be distinguished immunohistochemically.

Grass pollen immunotherapy (IT) is an effective treatment for severe summer hay fever and has long-lasting clinical effects [9]. We have previously shown that IT reduces allergen-induced tissue eosinophilia [10] and it has been demonstrated that IT inhibits seasonal increases in nasal epithelial metachromatic cells [11], and decreases the number of mast cells in the skin [12].

We report here, for the first time, using a basophil specific marker (2D7), that IT reduces seasonal basophil and eosinophil, but not mast cell, influx in the nasal mucosa in hay fever sufferers.

Materials and methods

Patients

Forty-four patients were recruited from the allergy clinic of the Royal Brompton Hospital, London, UK, and by advertisement in a local newspaper. All subjects had a history of severe summer hayfever that was not controlled by anti-allergic drugs and a positive skin test reaction (weal > 5 mm) to *Phleum pratense* (timothy grass pollen). Patients were excluded if they had multiple allergies or had received immunotherapy within the preceding 5 years. The study was performed with the approval of the ethics committee of the Royal Brompton Hospital and with the written informed consent of all participants.

Study design

This was a double-blind placebo-controlled parallel group study. After one summer of symptom monitoring and prior to randomization to injections of grass pollen immunotherapy with a depot preparation (Alutard SQ, ALK Abello, Hørsholm,

Denmark) or placebo, baseline nasal biopsies ($n = 44$) were performed outside the grass pollen season when all patients were asymptomatic. Following 2 years of treatment the 37 subjects remaining in the study had a second biopsy performed at the peak of the grass pollen season. Throughout the summer months subjects were asked to record their eye (itch, redness, swelling, streaming), nose (sneeze, blockage, running), throat (itch, dryness) and chest (breathlessness, cough, wheeze, chest tightness) symptoms, each on a scale of 0–3 and to record their intake of rescue medication: a short-acting antihistamine, acrivastine (8 mg capsules), and/or topical sodium cromoglycate for eyes and nose.

To control for possible effects of allergen exposure without atopy, additional biopsies were taken from nine non-atopic control subjects at the same time as the second set of study biopsies.

Immunohistochemistry

Nasal biopsies (2.5 mm) were taken from the inferior surface of the inferior turbinate using cup and ring forceps [13]. Biopsies were snap-frozen and processed for immunohistochemistry as previously described [14].

Immunohistochemistry for eosinophils, mast cells and neutrophils was performed on 6 µm cryostat sections, fixed in acetone/methanol (60 : 40) for 7 min, using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method as previously described [14]. Monoclonal antibodies used were BMK13 (a gift from J Barkans/R.Moqbel) for eosinophils, AA1 antitryptase (Dako Ltd, High Wycombe, UK) for mast cells and neutrophil elastase (Dako) for neutrophils.

For immunohistochemistry for basophils, sections were sent, unfixed, to the Virginia Commonwealth University, Richmond, Virginia, USA. Sections were fixed for 15 min in 10% neutral buffered formalin. Immunostaining was performed using the novel monoclonal antibody 2D7. Sections were immersed in 0.6% hydrogen peroxide and methanol for 30 min to inhibit endogenous peroxidase activity then washed twice in Tris buffered saline pH 7.6 (TBS) prior to incubation with normal horse serum for 1 hour at room temperature (22°C). Sections were incubated at 4 °C overnight with mAb 2D7 (8 µg/mL) or with the isotype matched negative control (MOPC-31C, a mouse IgG1κ; Sigma Chemical Company (Poole, UK)). Following several washes with Tween 20 TBS (Sigma) sections were incubated with biotinylated horse anti-mouse IgG (Vector ABC kit; Peterborough, UK) for 1 h at room temperature. Slides were then incubated with streptavidin-peroxidase (10 µg/mL) for 1 h at room temperature. Sections were monitored microscopically during incubation with 3-amino-9-ethyl Carbazole (AEC: 0.2 mg/mL of 0.1 M acetate buffer containing 0.01% hydrogen peroxide) for 7 min at room temperature. Finally, slides were washed and mounted in PBS containing 90% glycerol.

Adjacent sections to those stained for basophils as outlined above were simultaneously stained with G3 (Chemicon) an anti-human mast cell tryptase antibody complexed with alkaline phosphatase. Sections were incubated overnight with G3 then, after washing in Tween 20 TBS, were developed with fast blue (1 mg/mL) in 0.1 M Tris pH 8.2 + naphthol AS-Mx phosphate. The third section on each slide had 2D7 and G3 staining performed sequentially.

Quantification

Positively stained cells were counted at $\times 200$ magnification using an Olympus BH2 microscope (Olympus Optical Company Ltd, Tokyo, Japan) with eyepiece graticule. In the submucosa sections were counted to one grid's depth beneath the epithelium. All positively stained cells within the epithelium were counted and the cross-sectional area of the epithelium was measured using a computer-assisted graphics tablet (Hewlett Packard (ROM Bios) cell slides analysis; Palo Alto, CA, USA). All results were expressed as number of positive cells/mm².

Analysis

Statistical analysis was performed using a commercial software package (Minitab release 7, Mininc, State College, PA, USA). Comparisons within groups were performed using the Wilcoxon matched-pairs signed-ranks test. Comparisons between immunotherapy and placebo-treated patient groups were performed using the Mann-Whitney *U*-test. Correlations were performed using the Spearman rank correlation method. *P*-values < 0.05 were considered to be statistically significant.

Results

Clinical response to immunotherapy

The immunotherapy- and placebo-treated groups were well-matched at baseline for age, gender, hayfever severity

and cutaneous allergen sensitivity (Table 1). Detailed results of the improvements seen in both subjective and objective measures of efficacy after 2 years immunotherapy have already been presented elsewhere [15]. Briefly, between 1996 and 1998, there was a 49% improvement from baseline in diary symptom scores in the immunotherapy group that was not seen in the placebo group (15% improvement; *P* between groups = 0.01) and an 80% improvement in rescue medication requirements (placebo 18%; *P* = 0.007) (Fig. 1).

Basophils

There was an increase in the number of 2D7⁺ basophils in the submucosa during the pollen season in both the immunotherapy-treated (*P* = 0.007) and the placebo-treated (*P* = 0.001) subjects (Table 2, Fig. 2). The magnitude of the increase was greater in the placebo group but the between-group difference in numbers of basophils at peak-season was not significant (*P* = 0.2).

There were no 2D7⁺ basophils in the epithelium of baseline biopsies or in the biopsies from normal control subjects; 2D7⁺ basophils were observed in the epithelium in 35% of placebo-treated patients but only 5% of immunotherapy-treated patients (*P* = 0.03) (Fig. 2, Fig. 4)

There was no co-localization of G3 (mast cell tryptase) and 2D7 (basophil) staining (Fig. 4).

Mast cells

During the pollen season there was a 30% increase in the number of tryptase⁺ cells in the submucosa in placebo-treated patients (*P* = 0.04) that was not observed in the immunotherapy group (*P* = 0.9). This difference was not, however, significant between the groups (*P* = 0.2) and neither group differed significantly from the normal controls.

In the epithelium there was a significant increase in the number of tryptase⁺ cells in both groups (placebo:

Table 1. Patient characteristics

	Number	Gender M : F	Age (yr) Mean (range)	Skin prick test (mm ²) Median (IQ range)	Hayfever severity (visual analogue score) Median (IQ range)
Immunotherapy	22	10 : 12	32 (22–64)	49.5 (33.1, 61.8)	4.4 (3.2, 5.4)
Placebo	22	13 : 9	32 (23–59)	42.8 (27.4, 86.0)	4.5 (3.5, 5.6)

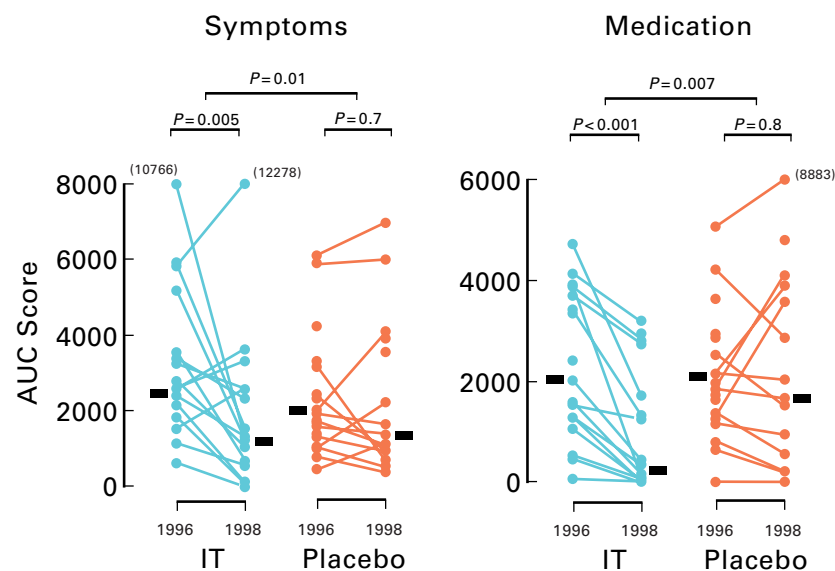


Fig. 1. Effect of 2 years of immunotherapy on symptom scores and medication requirements. Data shown are for a pre-treatment season (1996) and a post-treatment season (1998), in immunotherapy- and placebo-treated patients. Horizontal lines represent median values.

$P = 0.02$, IT: $P = 0.04$) with no difference between the two groups. (Table 2)

Eosinophils

The number of MBP⁺ eosinophils in the submucosa increased approximately eight-fold during the pollen season in the placebo-treated patients ($P = 0.002$) compared with a threefold increase in the immunotherapy group ($P = 0.01$). The number of MBP⁺ eosinophils in the submucosa at the peak of the pollen season was significantly greater in the placebo group than in the IT group ($P = 0.05$) (Table 2, Fig. 3, Fig. 4)

In the epithelium there were virtually no eosinophils observed in biopsies taken out of the pollen season or in normal control biopsies. During the pollen season there was a greater increase in epithelial eosinophils in the placebo group ($P = 0.009$) than in the IT group ($P = 0.03$). This difference between the groups was significant ($P = 0.05$).

Neutrophils

Neutrophil elastase positive cells were scattered throughout the epithelium and submucosa in both atopic and normal subjects. There were no significant differences in neutrophil

Table 2. Summary of results of immunohistochemistry (IMH). All data are cells per mm² and are expressed as median (IQ range)

	Immunotherapy		Placebo			Point estimate (95% CI)	<i>P</i> -value ⁺
	Pre-treatment	Peak season	Pre-treatment	Peak season	Controls		
<i>Submucosa</i>							
2D7	0 (0–0.4)	0.7 (0, 6.7)**	0 (0, 0)	4.2 (0.4, 10.8)**	0 (0, 0)	0.3 (– 0.1, 1.3)	0.2
Tryptase	32 (17, 45)	29 (14, 51)	28 (17, 41)	36 (24, 58)*	41 (10, 63)	1.8 (– 1, 4.5)	0.2
MBP	5.9 (2.7, 11.1)	16.4 (6.9, 75)*	11.3 (2.7, 21)	86 (41, 113)**	5 (1.1, 11.3)	44 (1.2, 81)	< 0.05
Elastase	10.6 (2, 34)	12.8 (7, 26)	12 (0, 33)	11.1 (2, 16)	4.8 (0, 25)	– 4 (– 13, 2.2)	0.2
<i>Epithelium</i>							
2D7	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 6.2)*	0 (0, 0)	0 (0, 4.3)	0.03
Tryptase	0 (0, 3.5)	2.4 (0, 17)*	0 (0, 0)	0 (0, 23)*	0 (0, 0)	0 (– 4.8, 14.5)	0.8
MBP	0 (0, 0)	0 (0, 11)*	0 (0, 0)	11.5 (0, 35)**	0 (0, 0)	15.2 (0, 28)	< 0.05
Elastase	0 (0, 37)	0 (0, 14)	0 (0, 21)	0 (0, 6)	0 (0, 0)	0 (– 9.6, 0)	0.7

* $P < 0.05$, ** $P < 0.01$ for within-group comparisons by Wilcoxon matched-pairs signed-ranks test. ⁺Between group comparisons (peak season) by Mann–Whitney *U*-test.

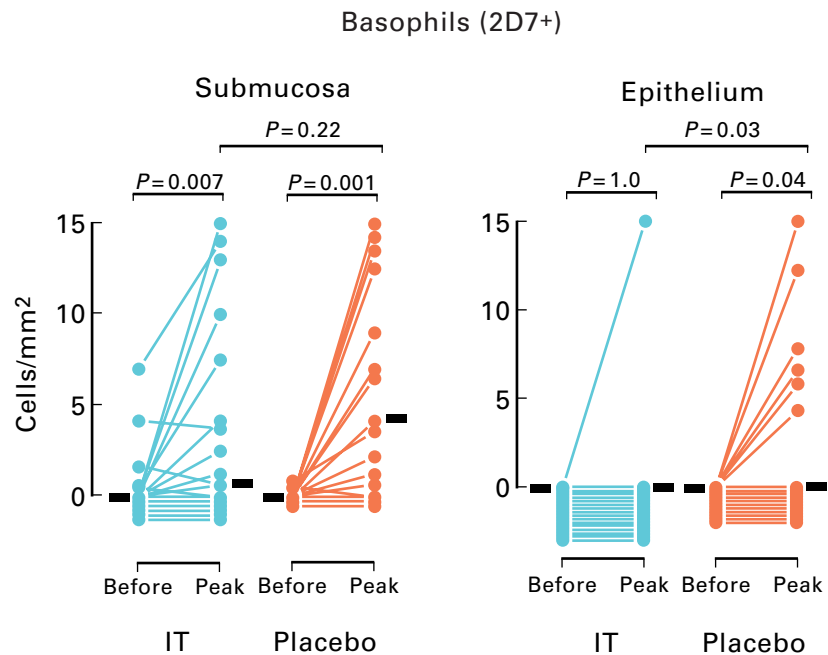


Fig. 2. Effect of 2 years of immunotherapy on numbers of 2D7-positive basophils in the nasal submucosa and epithelium. Data shown are for baseline ('before') and peak seasonal ('peak') nasal biopsies, in placebo- and immunotherapy-treated patients. Horizontal lines represent median values.

numbers either within groups or between the treatment and control groups (Table 2).

Association between mucosal cell numbers and seasonal symptoms

Significant correlations were found between seasonal symptom scores after 2 years of immunotherapy and numbers of MBP⁺ eosinophils in both the submucosa

($r = 0.6$; $P < 0.01$) and the epithelium ($r = 0.8$; $P < 0.001$). No correlations were found between symptoms and neutrophils, mast cells or basophils.

Discussion

In agreement with others [2,6,16,17], we have confirmed significant increases in the numbers of eosinophils and mast cells, but not neutrophils, in both the nasal submucosa

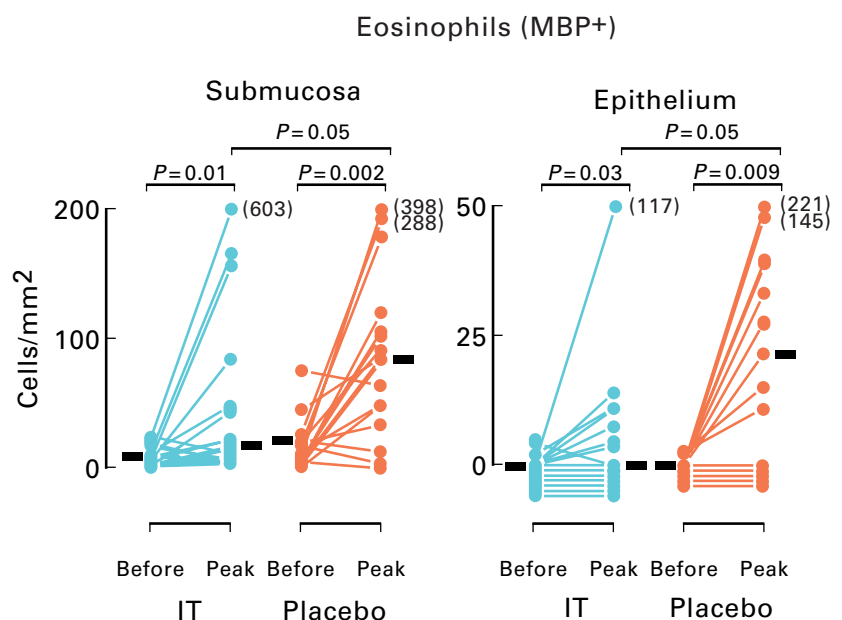
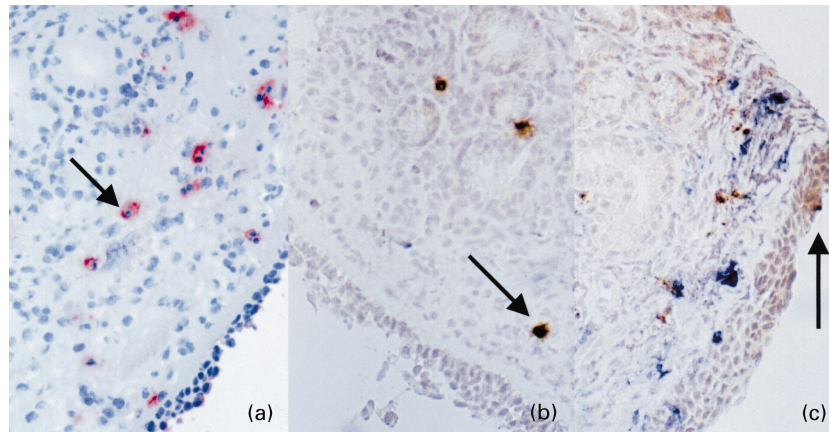


Fig. 3. Effect of 2 years of immunotherapy on numbers of MBP-positive eosinophils in the nasal submucosa and epithelium. Data shown are for baseline ('before') and peak seasonal ('peak') nasal biopsies, in placebo- and immunotherapy-treated patients. Horizontal lines represent median values.

Fig. 4. Examples of immunohistochemistry: (a) MBP⁺ eosinophils in nasal mucosa. (b) 2D7⁺ basophils in the nasal submucosa. (c) 2D7⁺ basophils in the nasal epithelium; also showing dual staining with G3 (anti-human mast cell tryptase).



and epithelium during the pollen season in subjects with seasonal allergic rhinitis. Additionally, using the novel basophil specific marker 2D7, we have shown a seasonal increase in mucosal basophils. The increases in epithelial basophils and eosinophils were inhibited by immunotherapy (IT). There was no change in neutrophil numbers and, in this study, we identified no significant reduction in mast cell numbers in the immunotherapy-treated patients. The clinical response to immunotherapy correlated closely with the reduction in both submucosal and epithelial eosinophils but appeared not to relate to any changes in other leucocytes.

These seasonal changes in mucosal cell numbers are consistent with previous studies showing increases in eosinophils after allergen challenge [14] and in both eosinophils and epithelial mast cells [2,18] during the pollen season, a phenomenon that is abolished by topical nasal corticosteroid treatment with an accompanying significant improvement in symptoms [19].

The appearance of metachromatically staining cells in the nasal epithelium during the pollen season has been well described previously [6,16,20] and has been shown to correlate with symptom scores [4] and to be inhibited by nedocromil [5]. On the basis of cell morphology and IgE and tryptase expression these cells had previously been assumed to be mast cells. Basophils may, however, produce tryptase [21] and are known to express IgE, as are eosinophils, monocytes and antigen-presenting cells that can all express the high affinity IgE receptor FcεR1.

Our data and those of KleinJan *et al.* [21] confirm increased numbers of basophils in the allergic nasal epithelium following allergen exposure. It is probable that the previously observed changes in numbers of metachromatic cells, previously attributed to mast cells, may, in fact, be due to basophils. Similarly, in atopic asthmatic patients allergen challenge results in an increase in the number of metachromatic cells in the sputum that persists for at least 7 days [22]. Subsequently, using the 2D7 antibody, these

cells have been found to be predominantly basophils, showing a 200-fold increase from relatively low baseline levels [23].

The mast cell is a pivotal effector cell in allergic responses, producing mediators of the immediate response to allergen (histamine, tryptase, prostaglandins and bradykinin) and the Th2-type cytokines IL-4 [24] and IL-5 [25] that promote IgE production and tissue eosinophilia, thereby orchestrating the late response. Mast cells are found constitutively in the nasal submucosa [20] but are found in the epithelium only in the presence of allergen [6] when greatly increased levels of tryptase are also detectable in nasal lavage [3]. Total numbers of mast cells remain unchanged, suggesting either migration from submucosa to epithelium or recruitment of mast cell progenitors that subsequently mature in the epithelium.

In common with mast cells, basophils secrete both pre-formed (histamine) and newly synthesized (leukotriene C₄ [26], cytokines, e.g. IL-4 [27], IL-13 [28]) mediators in response to both IgE-dependent (allergen) and non-IgE-dependent (chemokines) secretagogues. Basophils may, in fact, be the major source of IL-4 in allergen-induced reactions [29]. Unlike mast cells, basophils are lacking in non-inflamed tissue, as shown in our study. Presumably they are recruited to tissues, although there is the possibility that they may differentiate locally from precursor cells, as has recently been suggested for eosinophils [30]. Like eosinophils, basophils express chemokine receptors [31] and ligands for endothelial adhesion molecules [32] that may facilitate migration from the circulation into the tissues. Atopic subjects have higher levels of circulating basophils and the absolute and relative numbers of basophils increase during the pollen season [33], with an increase in basophil histamine release that correlates with seasonal symptom scores [34]. Basophils thus appear to form a mobile response to allergic stimuli, able to be rapidly directed to deliver mast cell-like properties to a variety of target organs.

In the early phase after nasal allergen challenge, increased amounts of histamine, tosyl-L-arginine methyl ester (TAME) and Prostaglandin D₂ (PGD₂) are detectable in nasal lavage. In the late phase, however, elevated levels of eosinophil major basic protein (MBP) and histamine, but not PGD₂, a mediator of mast cells but not of basophils, are detected, suggesting that eosinophils and basophils are the active cells at this timepoint [35]. Furthermore, nasal cells collected 24 h after allergen challenge have been shown to have properties comparable with simultaneously isolated peripheral blood basophils [36]. It would therefore appear that basophils play an important role in the late response and our data suggest that basophils are also likely to be implicated in the ongoing response to chronic allergen exposure, such as that experienced during the pollen season.

Using the new monoclonal antibodies 2D7 and BB1, the timecourse of basophil and eosinophil infiltration following allergen challenge in the skin [37] and the nose [38] shows both cell types appearing early: by 1 to 6 hours after challenge, and peaking at 6–24 h. Basophil numbers subsequently fall rapidly, in contrast to eosinophils, which persist in the mucosa for at least 7 days [21]. Similarly, in the lung, basophil numbers in lavage peak at 7 h after challenge and decline by at least 50% at 24 h [23]. Consistent with earlier observations, we have shown that, in placebo-treated subjects during the pollen season, basophil numbers are about 20-fold fewer than eosinophils and about 10-fold fewer than mast cells in the submucosa, while in the epithelium basophil numbers were comparable to those of mast cells but appreciably less than those for eosinophils.

Of potential interest, our observations indicate that basophils are not seen in the epithelium in all patients at the single time-point examined during the pollen season. It is possible that basophils are only present in any individual after a high allergen load or following prolonged allergen exposure. Another possibility is that their presence in the epithelium is only transient during passage into the nasal lining fluid, where they are detectable in large numbers [36]. Eosinophils, on the other hand, are more consistently detected in the nasal mucosa of all the untreated hayfever patients, suggesting that recruitment is continuous throughout the pollen season and/or eosinophils are more long-lived.

IT is extremely effective in patients with severe hayfever that persists despite optimal drug treatment. The beneficial effects persist for at least 3 years after discontinuation [9]. Otsuka [11] reported reductions in numbers of metachromatic cells in nasal brushings following IT in mite-sensitive patients, and we and others [39] have previously shown that IT reduces eosinophil infiltration into the nasal mucosa after allergen challenge [10]. More recent studies have

shown reduced numbers of mast cells in the nasal mucosa after mite-immunotherapy that correlated with the clinical response to IT [40], and a reduction in mast cells in the skin following IT [12]. In this study, we have demonstrated, for the first time, that IT inhibits the infiltration of basophils, as well as eosinophils, into the nasal epithelium during the pollen season. We have not, on this occasion, shown any change in mast cell numbers attributable to IT. There is no clear explanation for this difference from the above studies, although the allergen involved (mite vs. grass) and the organ studied (skin vs. nose) were different.

The mechanism by which IT reduces basophil and eosinophil numbers in the epithelium is not known. The local production of the Th2-type cytokines IL-4, IL-5 and IL-13 by T lymphocytes, mast cells and other cell types in the nasal mucosa of allergic subjects is widely felt to determine the nature of the response of the organ to allergen, encouraging IgE production and eosinophil recruitment to the mucosa. Previous studies by ourselves and others have provided evidence that IT induces an alteration in the local cytokine profile by decreasing IL-4 production [9], increasing IFN- γ production [10] or inducing T cell anergy [41], all of which may result in inhibition of IgE production, down-regulation of endothelial adhesion molecule expression and mast cell differentiation and a decrease in IL-5-dependent eosinophil maturation and activation. Which cytokines or chemokines are critical for basophil recruitment remains to be clarified.

In conclusion, we have shown that seasonal grass pollen exposure in allergic rhinitis is associated with infiltration of eosinophils, mast cells and basophils into the epithelium. The efficacy of injection immunotherapy may relate, at least in part, to the inhibition of basophil and eosinophil recruitment and/or migration in response to seasonal allergen exposure.

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References

- 1 Masuyama K, Till SJ, Jacobson MR et al. Nasal eosinophilia and IL-5 mRNA expression in seasonal allergic rhinitis induced by natural allergen exposure: effect of topical corticosteroids. *J Allergy Clin Immunol* 1998; 102:610–7.
- 2 Bentley AM, Jacobson MR, Cumberworth V et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis:

- increases in activated eosinophils and epithelial mast cells. *J Allergy Clin Immunol* 1992; 89:877–83.
- 3 Rasp G, Hochstrasser K. Tryptase in nasal fluid is a useful marker of allergic rhinitis. *Allergy* 1993; 48:72–4.
 - 4 Borres MP, Irander K, Bjorkstein B. Metachromatic cells in nasal mucosa after allergen challenge. *Allergy* 1990; 45:98–103.
 - 5 Lozewicz S, Gomez E, Clague J, Gatland D, Davies RJ. Allergen-induced changes in the nasal mucous membrane in seasonal allergic rhinitis: effect of nedocromil sodium. *J Allergy Clin Immunol* 1990; 85:125–31.
 - 6 Viegas M, Gomez E, Brooks J, Davies RJ. Changes in nasal mast cell numbers in and out of the pollen season. *Int Arch Allergy Appl Immunol* 1987; 82:275–6.
 - 7 McEuen AR, Buckley MG, Compton SJ, Walls AF. Development and characterization of a monoclonal antibody specific for human basophils and the identification of a unique secretory product of basophil activation. *Lab Invest* 1999; 79:27–38.
 - 8 Kepley CL, Craig SS, Schwartz LB. Identification and partial characterisation of a unique marker for human basophils. *J Immunol* 1995; 154:6548–55.
 - 9 Durham SR, Walker SM, Varga EM et al. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999; 341:468–75.
 - 10 Durham SR, Ying S, Varney VA et al. Grass pollen immunotherapy inhibits allergen-induced infiltration of CD4+ T lymphocytes and eosinophils in the nasal mucosa and increases the number of cells expressing messenger RNA for interferon-gamma. *J Allergy Clin Immunol* 1996; 97:1356–65.
 - 11 Otsuka H, Mezawa A, Ohnishi M et al. Changes in nasal metachromatic cells during allergen immunotherapy. *Clin Exp Allergy* 1991; 21:115–9.
 - 12 Durham SR, Varney VA, Gaga M et al. Grass pollen immunotherapy decreases the number of mast cells in the skin. *Clin Exp Allergy* 1999; 29:1490–6.
 - 13 Fokkens WJ, Vroom TM, Gerritsma V, Rijntjes E. A biopsy method to obtain high quality specimens of nasal mucosa. *Rhinology* 1988; 26:293–5.
 - 14 Varney VA, Jacobson MR, Sudderick RM et al. Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. *Am Rev Respir Dis* 1992; 146:170–6.
 - 15 Walker SM, Pajno GB, Torres Lima M, Wilson DR, Durham SR. Grass pollen immunotherapy for seasonal rhinitis and asthma: a randomised controlled trial. *J Allergy Clin Immunol* 2001; 107:87–93.
 - 16 Liu CM. Seasonal variation of nasal surface basophilic cells and eosinophils in Japanese cedar pollinosis. *Rhinology* 1988; 26:167–73.
 - 17 Pipkorn U, Karlsson G, Enerback L. The cellular response of the human allergic mucosa to natural allergen exposure. *J Allergy Clin Immunol* 1988; 82:1046–54.
 - 18 Fokkens WJ, Godthelp T, Holm AF et al. Dynamics of mast cells in the nasal mucosa of patients with allergic rhinitis and non-allergic controls: a biopsy study. *Clin Exp All* 1992; 22:701–10.
 - 19 Jacobson MR, Juliusson S, Lowhagen O et al. Effect of topical corticosteroids on seasonal increases in epithelial eosinophils and mast cells in allergic rhinitis: a comparison of nasal brush and biopsy methods. *Clin Exp Allergy* 1999; 29:1347–55.
 - 20 Enerback L, Pipkorn U, Granerus G. Intraepithelial migration of nasal mucosal mast cells in hay fever. *Int Arch Allergy Appl Immunol* 1986; 80:44–51.
 - 21 Kleinjan A, McEuen AR, Dijkstra MD et al. Basophil and eosinophil accumulation and mast cell degranulation in the nasal mucosa of patients with hay fever after local allergen provocation. *J Allergy Clin Immunol* 2000; 106:677–86.
 - 22 Gauvreau GM, Watson RM, O'Byrne PM. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. *Am J Respir Crit Care Med* 1999; 160:640–7.
 - 23 Gauvreau GM, Lee JM, Watson RM et al. Increased numbers of both airway basophils and mast cells in sputum after allergen inhalation challenge of atopic asthmatics. *Am J Respir Crit Care Med* 2000; 161:1473–8.
 - 24 Ying S, Durham SR, Jacobson MR et al. T lymphocytes and mast cells express messenger RNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. *Immunology* 1994; 82:200–6.
 - 25 Bradding P, Feather IH, Wilson S et al. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 1993; 151:3853–65.
 - 26 Woods JW, Evans JF, Ethier D et al. 5-lipoxygenase and 5-lipoxygenase-activating protein are localized in the nuclear envelope of activated human leukocytes. *J Exp Med* 1993; 178:1935–46.
 - 27 MacGlashan D Jr, White JM, Huang SK et al. Secretion of IL-4 from human basophils. The relationship between IL-4 mRNA and protein in resting and stimulated basophils. *J Immunol* 1994; 152:3006–16.
 - 28 Ochensberger B, Daep GC, Rihs S, Dahinden CA. Human blood basophils produce interleukin-13 in response to IgE-receptor-dependent and -independent activation. *Blood* 1996; 88:3028–37.
 - 29 Kasaian MT, Clay MJ, Happ MP et al. IL-4 production by allergen-stimulated primary cultures: identification of basophils as the major IL-4-producing cell type. *Int Immunol* 1996; 8:1287–97.
 - 30 Robinson DS, Damia R, Zeibecoglou K et al. CD34 (+)/interleukin-5Ralpha messenger RNA+ cells in the bronchial mucosa in asthma: potential airway eosinophil progenitors. *Am J Respir Cell Mol Biol* 1999; 20:9–13.
 - 31 Ugucioni M, Mackay CR, Ochensberger B et al. High expression of the chemokine receptor CCR3 in human blood basophils. Role in activation by eotaxin, MCP-4, and other chemokines. *J Clin Invest* 1997; 100:1137–43.
 - 32 Bochner BS, Sterbinsky SA, Briskin M, Saini SS, Macglashan DW Jr. Counter-receptors on human basophils for endothelial cell adhesion molecules. *J Immunol* 1996; 157:844–50.

- 33 Hirsch SR, Kalbfleisch JH. Circulating basophils in normal subjects and in subjects with hay fever. *J Allergy Clin Immunol* 1976; 58:676–82.
- 34 Winther L, Moseholm L, Reimert CM, Stahl SP, Kaergaard PL. Basophil histamine release, IgE, eosinophil counts, ECP, and EPX are related to the severity of symptoms in seasonal allergic rhinitis. *Allergy* 1999; 54:436–45.
- 35 Naclerio RM, Proud D, Togias A et al. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985; 313:65–70.
- 36 Iliopoulos O, Baroody F, Naclerio RM et al. Histamine-containing cells obtained from the nose hours after antigen challenge have functional and phenotypic characteristics of basophils. *J Immunol* 1992; 148:2223–8.
- 37 Irani AM, Huang C, Xia Z et al. Immunohistochemical detection of human basophils in late-phase skin reactions. *J Allergy Clin Immunol* 1998; 101:354–62.
- 38 Bascom R, Wachs M, Naclerio RM et al. Basophil influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. *J Allergy Clin Immunol* 1988; 81:580–9.
- 39 Furin MJ, Norman PS, Creticos PS et al. Immunotherapy decreases antigen-induced eosinophil cell migration into the nasal cavity. *J Allergy Clin Immunol* 1991; 88:27–32.
- 40 Erel F, Karaayvaz M, Caliskaner AZ et al. Effects of allergen immunotherapy on the nasal mucosa in patients with allergic rhinitis. *J Invest Allergol Clin Immunol* 2000; 10:14–9.
- 41 Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998; 102:98–106.