

Changes in nasal metachromatic cells during allergen immunotherapy

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Summary

We have investigated changes of nasal metachromatic cell number, nasal symptoms and nasal provocation at the third and sixth month during allergen immunotherapy. Twenty-five subjects with perennial allergic rhinitis (house dust (23), *Alternaria* (2)) were divided into two groups: an immunotherapy-treated group ($n=14$) and a control group ($n=11$). At the first visit nasal symptom scores, nasal provocation reactions and the number of metachromatic cells in nasal mucosal epithelial scrapings were not significantly different between groups. At the third and sixth month after immunotherapy nasal symptom scores, nasal provocation and the metachromatic cells in epithelial scrapings were significantly reduced ($P<0.05$) compared with the pre-treatment values in the immunotherapy group, but unchanged in the control group. These results suggest that the reduction in metachromatic cell number at the nasal mucosal surface may be one of the mechanisms which could explain the improvement of nasal allergic symptoms by immunotherapy.

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Introduction

During IgE-mediated allergy of the airway, inflammatory cells including mast cells and basophils infiltrate locally and release chemical mediators which produce the allergic reaction [1–8]. In nasal allergy, mast cells are increased in the nasal mucosal epithelium and the nasal lamina propria [4–6]. The number of these cells in the epithelial layer has been shown to correlate with the severity of nasal symptoms and the nasal provocation reaction by allergen [5,6].

Several mechanisms have been postulated for the clinical efficacy of immunotherapy [9–15]. There are, however, no studies of the relationship between mast cell numbers and the clinical results of immunotherapy. We have investigated changes in the number of metachromatic cells at the nasal surface during immunotherapy in patients sensitized to a perennial antigen.

Subjects and methods

Subjects

Fourteen subjects (six male) with a mean age of 22 yr (range 15–65 yr) were treated with immunotherapy and 11 subjects (five male) with a mean age of 23 yr (range 13–40 yr) formed the control group and participated in the study from January to December 1982. All subjects had perennial allergic rhinitis with positive intradermal skin tests, Phadebas RAST and nasal provocation tests. All 25 subjects were mono-allergic to a panel of 13 allergens, with 23 positive to house dust and two positive to *Alternaria*. The subjects all had moderate or more severe scores of nasal symptoms as classified below. The subjects were assigned to active or control group alternatively.

Immunotherapy

In the treated group, subjects commenced immunotherapy at a dose of 0.5 ng for house dust (HD) (1 µg/ml total nitrogen at 1:1000, w/v) (Torii, Japan) or 0.7 ng for *Alternaria* extract (1.3 µg/ml total nitrogen 1:1000, w/v) (Torii), as indicated by the end-point concentration determined by the threshold skin test. The amount of

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Table 1. Treatment protocol

Trial (weeks)		
1 0.5 ng (0.7 ng)	8 5.0 ng (7.0 ng)	15 50 ng (70 ng)
↓ 50% increase	↓ 50% increase	↓ 50% increase
7 5.0 ng (7.0)	14 50.0 ng (70.0 ng)	21 500 ng (700 ng)
22→25 500 ng (700 ng) once a week		
26→29 500 ng (700 ng) once two weeks		
30→ 500 ng (700 ng) continue once a month		

Table indicates the absolute weight (total nitrogen) of HD and *Alternaria* in each intradermal injection. Beginning antigen weight could be changed at 10- to 100-fold according to the threshold of the skin test.

Values in parentheses are total nitrogens of *Alternaria*.

extract injected was increased by 50% bi-weekly to a maintenance dose of 500 ng of HD and 700 ng for *Alternaria* extract (Table 1). This amount of antigen was then injected after 1 week with a repeat dose after 2 weeks and then continued once every month for the maintenance. In the control group, subjects were not given any treatment for 6 months.

Nasal symptom scores

Nasal symptoms scores were assessed by diary cards in the 2 weeks before the examination. The mean number of sneezing attacks or nose blowing per day was recorded and graded by the subject (on a scale of +, ++ and +++). The degree of nasal stuffiness was also recorded and graded as shown (— no nasal stuffiness, + nasal stuffiness without mouth breathing, ++ mouth breathing some of the day, +++ mouth breathing most of the day) (Table 2). The severity of the nasal symptoms was classified as 0–3 (Table 2).

Nasal provocation

A dried paper disc with HD or *Alternaria* extract containing 250 ng PN/disc was placed on the surface of both inferior turbinates. The number of sneezes, nasal secretions and the appearance of the mucosal swelling was observed rhinoscopically for 5 min [16]. Scoring of the nasal provocation test was based on the three responses obtained (sneezing, secretion, swelling of inferior turbinate). A positive response in any of the parameters scored 1. If a subject showed a positive response to all three and also sneezed more than five times the score increased to 4.

All of the subjects recorded their symptoms and took

Table 2. Scoring of nasal symptoms

Number of sneezing attacks or nasal blowings a day		Nasal stuffiness*			
		—	+	++	+++
0	—	0	1	2	3
1-5	+	1	1	2	3
6-10	++	2	2	2	3
> 11	+++	3	3	3	3

* Nasal stuffiness: —, no nasal stuffiness; +, nasal stuffiness without mouth breathing; ++, mouth breathing some of the day; +++, mouth breathing most of the day.

The mean number in sneezing attacks and nasal blowing for 2 weeks before the examination was calculated from the subjects' symptom diary, and these symptom scores were automatically assessed from the table, and nasal stuffiness scores were assessed from which grade was most common during the 2 weeks.

part in the blood tests, nasal provocations and nasal scraping at the first visit, with repeats at the third and sixth months.

Stains and counts of metachromatic cells

Scrapings were obtained from the anterior surface of both inferior turbinates using a small curette cup as described previously [5,6]. By this procedure about 3 mg of each epithelial layer (mean $\pm$ s.e.) 3.06–0.09 mg, range: 2.22–4.99 mg, $n=52$) could be taken. The scrapings from the nasal cavity were smeared thinly on to a glass slide and fixed in 100% of methylalcohol for 5–10 min. Each specimen was then stained with Hansel's solution for 40 sec. The number of metachromatic cells on the slide was counted for the whole nasal scraping with a 400 $\times$ magnification. The counting was performed. From 15 subjects there was no significant difference in cell number among three specimens [6].

Blood basophil counts

Venous blood was taken from the subjects and the number of blood basophils was counted directly by Kimura's method [3].

Statistical analysis

To compare the metachromatic cell numbers between groups, and before and after treatment, a non-parametric Mann-Whitney *U*-test was used. For the comparisons of

Table 3. Scores of nasal symptoms in groups and months

Group	First month	Third month	Sixth month
Control (<i>n</i> =11)	2.2±0.1	2.3±0.1	2.4±0.1
Treated (<i>n</i> =14)	2.6±0.1	1.4±0.2**	1.3±0.2**
A (<i>n</i> =6)	2.8±0.1	0.8±0.4*	0.5±0.2**
B (<i>n</i> =8)	2.5±0.1	1.8±0.2	2.0±0.2

Values represent means ± s.e.

Statistically significant (**P*<0.05, ***P*<0.01) compared to before the treatment.

nasal symptom scores and nasal provocation tests between groups and after treatment, a chi-square test was used, although values in this paper are for mean scores.

Results

Nasal symptoms

In the control group nasal symptom scores were (mean±s.e.) 2.2±0.1 at the first visit, 2.3±0.1 at the third month and 2.4±0.1 at the sixth month, with no significant difference from the first time to the sixth month. In the treated group nasal symptom scores were 2.6±0.1 at the first visit, 1.4±0.2 at the third month and 1.3±0.2 at the sixth month with a significant reduction at the third and sixth month compared with the first visit (*P*<0.01).

The symptom scores most affected by immunotherapy at 3 months were nasal discharge and nasal obstruction, both of which decreased significantly (*P*<0.05) compared with pre-treatment values. At 6 months, sneezing, nasal discharge and nasal obstruction were all significantly decreased (*P*<0.05) compared with pre-treatment.

The subjects treated with immunotherapy were divided into two groups: group A—excellent/good improvement cases (*n*=6, HD 5, *Alternaria* 1, mean age of 24 yr), who had the improvement by a score of more than 2 points, and group B—slight or no improvement cases (*n*=8, HD 7, *Alternaria* 1, mean age of 20 yr) who improved by a score of 1 point or less at the sixth month. The mean nasal symptom scores for group A was 2.8 (before immunotherapy), 0.8 (at the third month) and 0.5 (at the sixth month), respectively. Those in group B were 2.5 (before immunotherapy) and 1.8 (at the third month) and 2.0 (at the sixth month) (Table 3).

Changes in the nasal provocation reaction

There was no significant difference in scores between control and treated groups at the first visit. In the control

Table 4. Score of nasal provocation in groups and months

Group	First month	Third month	Sixth month
Control (<i>n</i> =11)	2.9±0.2	2.6±0.3	2.7±0.3
Treated (<i>n</i> =14)	2.7±0.2	2.2±0.2	1.6±0.4*
A (<i>n</i> =6)	2.3±0.4	1.8±0.4	0.8±0.4*
B (<i>n</i> =8)	3.1±0.2	2.7±0.3	1.8±0.4

Values represent means ± s.e.

Statistically significant (**P*<0.05) compared to the first month.

group mean scores for provocation were 2.9±0.2 (at the first visit), 2.6±0.3 (at the third month) and 2.7±0.3 (at the sixth month) with no significant differences between them. In the treated group, they were 2.7±0.2 (at the first visit), 2.2±0.2 (at the third month) and 1.6±0.4 (at the sixth month) with a significant decrease (*P*<0.05) between the initial and sixth month test (Table 4).

When we examined which symptoms in the nasal provocation were most affected by immunotherapy, the results showed nasal obstruction to be significantly decreased by the third month (*P*<0.05) with nasal discharge and obstruction both significantly decreased by 6 months (*P*<0.05).

When the treated group was again divided into 2 (groups A and B) according to the improvement of nasal symptoms, for group A the mean nasal provocation scores were 2.3±0.4 (before treatment), 1.8±0.4 (at 3 months) and 0.8±0.4 (at 6 months) showing a significant reduction (*P*<0.05). In group B, the scores were 3.1±0.2 (before treatment), 2.7±0.3 (at 3 months) and 1.8±0.4 (at 6 months) with no significant differences (Table 4).

Metachromatic cells

Most of the metachromatic cells in the nasal scrapings were large, a few were small with segmented nuclei [6]. The total number of metachromatic cells in the nasal scrapings were not statistically different for the treatment and control groups at the initial visit. In the control group, mean (±s.e.) of the metachromatic cell numbers at first, third and sixth month visits were 1025±261, 1070±295, and 930±163, respectively. There were no significant differences between them. For the treated group, these values were 1295±280, 456±140, and 237±92, respectively. The mean number of metachromatic cells was significantly reduced (*P*<0.05) at both 3 and 6 months compared with pre-treatment. When subjects were again divided into two groups, there was a marked decrease in group A from 1440±599 before treatment to

Table 5. Number of metachromatic cells in groups and months

Group	First month	Third month	Sixth month
Control (<i>n</i> = 11)	1025 ± 261	1070 ± 295	930 ± 168
Treated (<i>n</i> = 14)	1295 ± 280	456 ± 140*	237 ± 92*
A (<i>n</i> = 6)	1440 ± 599	179 ± 48*	78 ± 35*
B (<i>n</i> = 8)	1186 ± 241	664 ± 220	353 ± 149*

Values represent means ± s.e.

Statistically significant (**P* < 0.05) reduction compared to before immunotherapy.

179 ± 48 at the third month after the treatment (*P* < 0.05), and a further reduction to 78 ± 35 at the sixth month (*P* < 0.05). In group B the number of these cells decreased from 1186 ± 241 before treatment to 664 ± 220 at the third month, and 353 ± 149 at the sixth month after immunotherapy. For the latter, the sixth month count showed a significant decrease from the pre-treatment value (Table 5).

Blood basophils

The number of basophils in the blood at the first, third and sixth months were 45.5 ± 9.4, 51.4 ± 8.3 and 50.5 ± 10.7, respectively in the control group, and 58.6 ± 10.7, 48.0 ± 8.0 and 56.6 ± 6.4 in the treated group. There were no significant differences demonstrated in either group.

Discussion

The role of metachromatic cells in inhalant allergic disease has previously been reported [1–8,17]. In nasal allergy the number and type of the metachromatic cells in nasal scrapings have also been described previously [4–6,17]. The sensitivity of metachromatic cells and their release of chemical mediators is reduced by immunotherapy [14]. There are, however, no previous studies which address the change in metachromatic cell numbers in the nose after immunotherapy; the purpose of this study was to investigate this change. Mast cells are abundant and basophils fewer in nasal scrapings. The numbers of these cells correlate with nasal symptoms and nasal provocation reactions to antigen [5,6] in patients with perennial allergic rhinitis.

This study was performed with attention to possible errors caused by the fluctuations in cell numbers if the weights of the scraping were not the same for each individual. We obtained the same weight (3 mg) of the scraping by curette and established the reproducibility of the method [6].

The number of these metachromatic cells in scrapings decreased greater in the group treated with immunotherapy, especially in the excellent or better effect group (group A). The number of basophils in the peripheral blood and the specific antibodies in the serum were not changed during immunotherapy in the treated group.

These findings suggest that a reduction of metachromatic cells in the epithelial layers of a local site like the nose may occur during immunotherapy and contribute to the improvement of nasal symptoms in the early stages (a few months) of treatment. Another factor which is considered to lead to improvement is the production of a blocking antibody [11,15,18], but there was no evidence of this relationship in the study. To date, it has been accepted that the immunological basis for the improvement of symptoms from immunotherapy is the production of the blocking IgG antibody, but it is agreed that this is not an entirely satisfactory explanation of the results.

Alterations in number of metachromatic cells have been reported in response to local steroid therapy and in seasonal pollen antigen exposure [19–22]. The subjects in this study were not sensitized to any seasonal allergens tested with the panel of 13 extracts (including tree and grass pollens which are clinically important in Japan), and did not take any treatment except immunotherapy. Most of the metachromatic cells which increased at the nasal surface are formalin-sensitive mast cells. These increased numbers are probably due to the growth and differentiation at the local site of mature cells from haematopoietic progenitors from the blood [23–27]. The inhibition of mechanisms in mast cell growth and differentiation might act to reduce the cells, but this is not yet proven. Recently, we have found that the epithelial cell layer of the nose stimulates growth and differentiation of metachromatic cells and eosinophils [25]. It is possible that the function of these cells may be responsible for an increase in metachromatic cells at the nasal surface with inhibition by immunotherapy.

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