

Dynamics of mast cells in the nasal mucosa of patients with allergic rhinitis and non-allergic controls: a biopsy study

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Summary

Mast cell degranulation is thought to be an important component of the pathogenesis of allergic rhinitis. Quantitative studies on mast cells in nasal mucosa after allergen exposure have given widely divergent results, ranging from an overall decrease via redistribution to an overall increase. We investigated this problem by employing a combination of anti-IgE and toluidine blue staining of biopsy specimens. In allergic patients anti-IgE was found to identify all mast cells and toluidine blue to detect mast cells that were not (totally) degranulated.

The study was composed of two parts done in different patient groups. In the first part of the study biopsies were performed in 23 patients with isolated grass-pollen allergy, once during natural provocation in the summer and once in the winter. Biopsies were also performed in 12 controls. Non-allergic controls were found to have the same number of mast cells in the lamina propria as asymptomatic allergic patients. The controls seldom have mast cells in the epithelium. The patients with isolated grass-pollen allergy showed an increase in the numbers of mast cells in the lamina propria during natural provocation and the same seemed to occur in the epithelium as well. During natural provocation almost all of the mast cells in the epithelium and half of those in the lamina propria were degranulated.

In the second part of the study 17 patients with isolated grass-pollen allergy and four controls were challenged daily with allergen extract during a 2-week period in the winter. During this period biopsies were performed at eight different occasions, i.e. once before, six occasions during and once after the provocation period. The results of this part of the study showed that during provocation mast cells migrate to the surface of the nasal mucosa, where they become degranulated, and that the pool of mast cells in the lamina propria was apparently replenished by migration of mast cells from the vessels in the lamina propria. The total number of mast cells in the lamina propria remained approximately the same while the mast cells residing in an increasingly thick layer measured from the basal membrane into the lamina propria became degranulated. After 2 weeks, 82% of the mast cells in the lamina propria was degranulated and it was only in the deepest layers that some toluidine blue positive cells were found.

This study can explain the seemingly conflicting reports in the literature on mast cell dynamics and degranulation and shows that the reported differences are due to differences in the techniques used and the time of evaluation.

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Introduction

Mast cells appear to play an important role in allergic

rhinitis. The allergic reaction of the mucosa is induced by an interaction between allergen and IgE antibodies on the surface of the mast cell. Most of the studies performed on the role of metachromatically staining cells (mast cells and basophils) in the nasal mucosa have been performed in nasal smears or scraping material [1,2]. Few quantita-

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tive studies have been done in biopsies of nasal mucosa. We are aware of only two quantitative studies in which biopsies of normal human mucosa were used [3,4]. Otsuka *et al.* described the existence of considerable numbers of mast cells in the epithelium and lamina propria of two allergic patients and eight non-allergic controls [3]. In a comparative study on allergic patients and non-allergic controls, Okuda found an increased number of mast cells in the epithelium of the allergic patients but not in the lamina propria [4].

More studies have been done on mast cells in allergic nasal mucosa and on the numerical changes shown by mast cells in epithelium and lamina propria during (natural) allergen provocation [5–10]. The changes in mast cell numbers during allergen provocation have not been conclusive. Wihl and Mygind did not find any change after allergen challenge at all [5]. Enerbäck *et al.* saw no change in the total number of mast cells but did find a redistribution of mast cells into the epithelium during natural provocation [6]. The latter, however, was denied by Pipkorn and Enerbäck in 1988 [7]; instead, they reported a decrease in the number of mast cells in epithelium and lamina propria. A temporary redistribution of metachromatic cells toward the mucosal surface, occurring 5–24 hr after allergen challenge, was found by Borres [8]. Finally, the group of Davies found increased numbers of nasal mucosal mast cell numbers during natural provocation [9,10].

Studies on mast cells have employed a variety of different tissue fixatives and staining methods, which makes it difficult to compare the results reported by different authors. The method mentioned most often in the recent literature is toluidine blue staining at pH 0.5 after fixation with ice-cold, iso-osmotic solution of formaldehyde and acetic acid (IFAA) or ethanol. There is evidence suggesting that not all of the mast cells in the nasal mucosa are detected in this way [11,12]. In a recent study we used an immuno-alkaline phosphatase method with anti-IgE to study mast cells [13]. Contrary to the toluidine blue staining, in this way all mast cells are discovered in biopsy specimens of allergic patients [14]. A possible disadvantage may be that other cells which are known to express Fc ϵ II receptors could be positive for IgE [15–19]. The anti-IgE staining showed more positive cells in the epithelium and lamina propria of allergic patients during natural provocation than in non-allergic controls [13]. These results are in agreement with those of a study reported by Bachert [20].

To study changes in the number and degranulation of mast cells, we investigated these cells in the epithelium and lamina propria of the nasal mucosa of the lower inferior turbinate in patients with isolated grass-pollen allergy and normal controls. In this study we used

Toluidine Blue (TB) and anti-IgE immuno-alkaline phosphatase methods. First, a group of patients was studied during natural provocation. Biopsy specimens of nasal mucosa were taken during the grass-pollen season and also out of the season, i.e. between October and January. These biopsies were compared with those of controls. A second group of patients with isolated grass-pollen allergy and controls were studied in a provocation study done during two weeks in January. In this period biopsies of nasal mucosa were taken 3–4 times in each patient. Other cells that could be IgE⁺ were determined quantitatively.

Materials and methods

Patients and controls during natural provocation

The first part of the study on natural provocation was done in 23 patients with isolated grass-pollen allergy and 12 controls. The patients (mean age 34 years [range 12–57], 14 male, 9 female) had an isolated grass-pollen allergy confirmed by a positive skin prick-test reaction to ALK Soluprick (Diephuis, Groningen, The Netherlands) extract of 1 HEP/ml and no other positive skin prick-test reaction to 13 common allergens, a mean (range) radio-allergosorbent test (RAST) score of 5+ (3+–5+) and a mean (range) total IgE value of 394 (19–1900) IU/ml. The controls comprised 12 volunteers and patients (mean age 28 years [range 17–42], 3 male, 9 female) visiting the Ear, Nose and Throat (ENT) department with an ear or throat disease not related to the nose and nasal sinuses, without nasal complaints, no abnormalities in the nose at ENT examination, a negative RAST score, and a mean (range) total IgE value of 39 (4–90) IU/ml. None of the controls in this part of the study used any medication that could have influenced the results of this study. Some of the patients used the nasal corticosteroid spray budesonide and/or the antihistamine drug terfenadine. No significant differences in age or serum IgE level were found between the groups with and without medication use. Despite the treatment all patients had serious complaints. Terfenadine tablets were always discontinued at least 48 hr before a biopsy was performed. Budesonide was not discontinued during the study. The effect of budesonide, either on the migration or the degranulation, could not be evaluated because patients were not randomly assigned to the use of budesonide which meant that it was the patients with more severe complaints who used the budesonide spray.

Patients and controls in the provocation study

Seventeen patients and four controls participated in the provocation part of the study. The patients (mean age 29

Table 1. Timing of biopsy for different patients. Patients were randomly divided in two groups (I and II). The first biopsy of the patients in group II was either taken at 30 min after provocation or at 8 hr after provocation

Patient group	Timing of biopsy after first provocation			
I	Before provocation <i>n</i> = 7	24–48 hr <i>n</i> = 5	8–10 days <i>n</i> = 6	1–2 weeks after provocation <i>n</i> = 9
II	30 min <i>n</i> = 5	4–6 days <i>n</i> = 9	14 days <i>n</i> = 9	
	8 hr <i>n</i> = 5			

years [14–48], 7 male, 10 female) had an isolated grass-pollen allergy for at least 1 year, confirmed by a positive skin prick-test reaction to ALK Soluprick extract of 1 HEP/ml and no other positive skin prick-test reaction to 13 common allergens, a median (range) RAST score of 4+ (3+–5+) and a median (range) total IgE value of 290 (22–1900) IU/ml determined during the grass-pollen season. The four controls (volunteers, mean age 29 years [24–44], 2 male, 2 female) without nasal complaints, had no abnormalities in the nose at ENT examination and a negative skin prick-test reaction to ALK Soluprick extract of 13 common inhalant allergens. None of the patients or controls in this part of the study used any medication. None of the patients or controls had an infection of the respiratory tract during the week preceding the challenge.

Design of the provocation study

Patients and controls were daily challenged with allergen during a 2-week period. Symptoms and signs were recorded each day. In the patients biopsies were performed three or four times, in the controls two times. To allow investigation of a time-related effect, the patients were randomly divided into two groups for statistical reasons, which meant that a sufficient number of different biopsy times could be analysed. Thus biopsies were taken from the allergic patients at seven different times as shown in Table 1. One patient in group I and one in group II were biopsied only once because they withdrew from the study before the time of the second biopsy. One patient in group I was biopsied only twice. All patients and controls gave their informed consent and the study was approved by the

medical-ethical committees of the Leyenburg and the Dijkzigt Hospitals.

Nasal biopsy

Biopsies of nasal mucosa were taken from the lower edge of the inferior turbinate, about 2 cm posterior to the anterior edge with use of a Gerritsma forceps with a cup diameter of 2.5 mm. Local anaesthesia was obtained by placing a cotton-wool carrier with 50–100 mg cocaine and three drops of adrenaline (1:1000) under the inferior turbinate without touching the place where the biopsy would be taken [21]. The biopsy specimens were embedded in Tissue-Tek II OCT compound in a gelatin capsule and frozen immediately [22].

The biopsies of the patients with isolated grass-pollen allergy collected during natural provocation were taken in the first week of July at the height of the grass-pollen season and between October and January, well out of the season. The controls were biopsied between July and November.

Biopsies taken from the patients with isolated grass-pollen allergy during the provocation study were collected before the provocation, 1/2 hr, 8 hr and 24 hr after the first provocation, and 2, 4, 6, 8, 10 and 14 days after the first provocation and 1 and 2 weeks after the last provocation (Table 1). Biopsies were always taken before another provocation took place. The biopsy specimens of the controls were collected before the first provocation, and 24 hr and 12 days after the first provocation.

Symptom scores

Patients were asked daily prior to the provocation what symptoms they had had during the preceding day. The severity of these symptoms, e.g. nasal blockage, nasal discharge, sneezing, nasal itching, and eye watering/irritation were recorded on a four point scale (0–3). A total symptom score was calculated as the sum of the individual symptom scores recorded. The same symptom score was used to grade symptoms occurring 30 min after the provocation test.

Nasal allergen challenge

Patients and controls were challenged daily with grass-pollen extract (1000, 3000, 10 000, 30 000, 100 000 SQ/ml; ALK obtained from Diephuis, Groningen, The Netherlands) with a pump spray delivering a fixed dose of 50 ± 2 μ l. Before provocation, the subjects waited 15 min to allow the nasal mucosa to become acclimatized to room

temperature. On the first day of the provocation study the patient was challenged with 1000 SQ/ml followed by increasing doses of allergen to establish a threshold dose. The duration of the interval between two doses was 15 min. Within 15 min of each challenge the number of sneezes was recorded and the amount of secretion, collected in preweighed paper tissues, was determined. The lowest concentration which gave either more than 0.5 g of secretion or more than five sneezes within 15 min was called the threshold dose. In the following days the same concentration was used until the patient experienced serious complaints, i.e. a score of 3 (severe symptoms) for two or more items of the symptom score of the previous day, at which point a new threshold dose was established.

Staining procedure

Each specimen of nasal mucosa was cut into serial 6 μm -thick sections on a Reichert-Jung 2800 Frigocut cryostat and transferred to gelatin-coated microscope slides. Serial sections were stained with toluidine blue at pH 0.5 for 30 min, anti-IgE monoclonal antibody using the immunoalkaline phosphatase method, and both anti-IgE and toluidine blue in a double staining technique. The immunoalkaline phosphatase method used has been described elsewhere [23]. Briefly, after being transferred to gelatin-coated microscope slides, the sections were dried and fixed in acetone for 10 min at 20°C, rinsed in phosphate-buffered saline (PBS, pH 7.2), and incubated with monoclonal mouse anti-IgE (CLB, Amsterdam, The Netherlands) for 30 min at 20°C. The sections were then rinsed again in PBS for 5 min, and incubated for 15 min with a rabbit anti-mouse immunoglobulin antiserum conjugated to alkaline phosphatase (DAKO D314, Dakopatts, Copenhagen), rinsed successively in PBS and Tris buffer pH 8.0, and incubated for 30 min with a New Fuchsin solution. Finally, the sections were rinsed with distilled water, counterstained with Mayer's haematoxylin, and mounted in glycerin gelatin (Chroma, Schmid GmbH, Köngen, Germany). For double staining, after incubation with the New Fuchsin solution, the sections were rinsed with distilled water, stained with toluidine blue at pH 0.5 for 30 min and counts were performed immediately. In the provocation study, immunofluorescence double-staining was performed with monoclonal CD1a (Netherlands Cancer Institute, Amsterdam, The Netherlands) or CD38 (Ortho, Raritan, New Jersey, U.S.A.), which stain Langerhans cells and B cells/plasma cells, respectively, followed by incubation with goat anti-mouse immunoglobulin labelled with TRITC (GaMIg-TRITC, CLB, Amsterdam, The Netherlands) and reincubation with anti-IgE labelled with FITC (RaHuIgE-FITC, CLB, Amsterdam, The Netherlands). Control

staining was performed by substitution with PBS and incubation with non-relevant monoclonal antibodies of the same subclass.

Light-microscopical evaluation

In two sections of each specimen, all mast cells with nuclei in the plane of the section were counted. The number of mast cells in the epithelium and lamina propria were counted separately. The total surface area of two sections and of the epithelium and lamina propria was estimated by Kontron Image Analysis System Videoplan. The number of mast cells/mm² section area of epithelium and lamina propria was calculated. The percentage of degranulation of the mast cells in the provocation study was calculated by dividing $100 \times$ the number of TB⁻IgE⁺ cells by the number of IgE⁺ cells

% degranulation =

$$\frac{100 \times \text{cells positive for IgE but negative for TB}}{\text{Total IgE positive cells}}$$

Statistical analysis

For the first part of the study concerning natural provocation, use was made of Student's (paired) *t*-tests.

In the provocation part of the study three repeated measurements were made for each of the two groups before and during the provocation, together constituting six points on a time axis (see Table 1). Because the evaluated variables were symmetrically distributed (i.e. with skewness shown to be non-significant), a repeated measurement analysis of variance was performed. In this analysis the six time effects on the dependant variable are obtained as the estimates of six coefficients in a model with three within-subject repetitions (two coefficients), two between-subject repetitions (one coefficient), the interaction between repetition and groups (two coefficients) and the constant term (one coefficient). The differences between the end of the provocation period and the period 1–2 weeks after provocation were analysed with the sign test. Rank correlation coefficients between symptoms and number of IgE⁺ cells and TB⁺ cells in epithelium and lamina propria were also determined.

Results

General description

The sections of nasal mucosa had an average surface area of 3 mm² and usually showed a lining of ciliated columnar epithelium with or without goblet cells and/or partially stratified cuboidal epithelium. All but one section (excluded from the study) in the study on natural

Table 2. Number of Toluidine Blue positive (TB⁺) and IgE positive (IgE⁺) cells/mm² during natural allergen provocation. Mean (s.d.) in epithelium and lamina propria of nasal mucosa of controls without nasal complaints, patients with isolated grass-pollen allergy during the grass-pollen season (symptomatic) and patients with isolated grass-pollen allergy in winter (asymptomatic)

	Epithelium		Lamina propria	
	TB ⁺	IgE ⁺	TB ⁺	IgE ⁺
Controls	3 (4)	5 (14)	83 (45)	17 (24)
Symptomatic patients with isolated grass-pollen allergy during season	6 (18)	126 (206)	68 (50)	134 (138)
Asymptomatic patients with isolated grass-pollen allergy in winter	1 (4)	67 (93)	64 (42)	80 (58)
<i>P</i> -value I	*	0.01	n.s.	0.001
<i>P</i> -value II	*	0.005	n.s.	0.0001
<i>P</i> -value III	*	0.20	n.s.	0.03

P-value I = *P*-value from Student's *t*-test comparing controls with symptomatic patients. *P*-value II = *P*-value from Student's *t*-test comparing controls with asymptomatic patients. *P*-value III = *P*-value from paired Student's *t*-test comparing symptomatic and asymptomatic patients. *, numbers too small for reliable statistics.

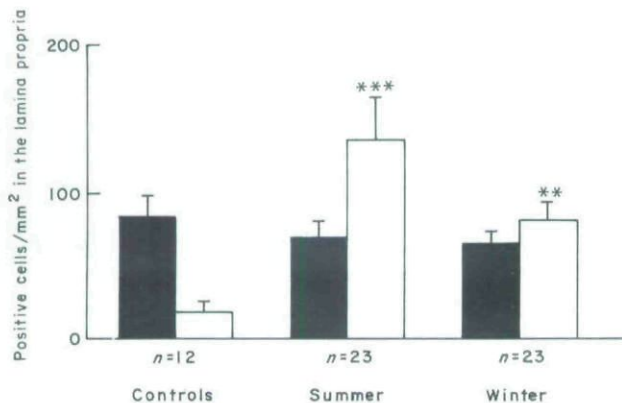


Fig. 1. Mean and standard error of the number of IgE⁺ cells (□) and Toluidine Blue positive cells (■) in the lamina propria of the nasal mucosa of controls, symptomatic patients with isolated grass-pollen allergy, and asymptomatic patients with isolated grass-pollen allergy (biopsied in the winter). The allergic group showed approximately the same number of TB⁺ cells as the non-allergic group did. However, the number of IgE⁺ cells in the lamina propria was significantly higher in the allergic patients than in the controls. Furthermore a significant difference was found between numbers in the summer and winter in the allergic group.

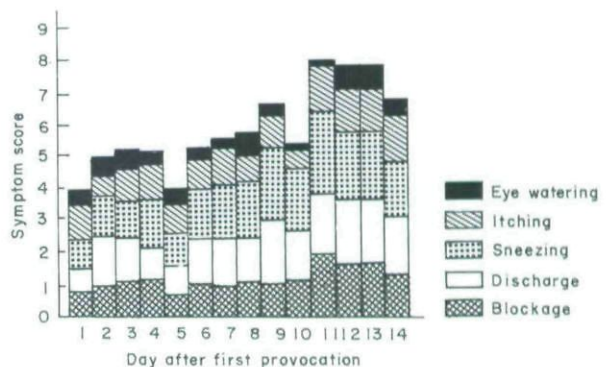


Fig. 2. Mean of the symptoms nasal blockage, nasal discharge, sneezing, nasal itching and eye watering/irritation experienced in the day before challenge recorded on a four point scale (0–3) during the 2 week daily provocation period. The symptom score of nasal blockage, nasal discharge, sneezing, nasal itching and the total symptom score increased significantly.

provocation showed intact or virtually intact epithelium. In the provocation study the epithelium could not be evaluated in three sections (before, 6 and 8 days after provocation). The lamina propria usually consisted of a looser subepithelial cell-rich layer with most of the mucous glands and a deeper collagenous cell-poor layer

Table 3. Number of Toluidine Blue positive (TB⁺) and IgE positive (IgE⁺) cells/mm² during allergen provocation study. Median (range) in epithelium and lamina propria of nasal mucosa of patients with isolated grass-pollen allergy during a 2 week provocation study in winter

Timing of biopsy	Epithelium		Lamina propria		% degranulation in the lamina propria
	TB	IgE	TB	IgE	
Before provocation	5 (0-73)	144 (0-327)	34 (1-188)	61 (15-160)	22 (0-80)
30 min after first provocation	0 (0-0)	82 (4-383)	25 (17-39)	52 (16-85)	46 (27-72)
8 hr after first provocation	0 (0-39)	357 (61-472)	26 (6-98)	55 (39-117)	62 (13-86)
24-48 hr after first provocation	0 (0-17)	91 (8-239)	60 (29-63)	65 (16-145)	34 (0-80)
4-6 days after first provocation	0 (0-9)	111 (0-364)	31 (6-96)	48 (0-120)	47 (11-86)
8-10 days after first provocation	5 (0-34)	6 (0-166)	34 (17-51)	51 (0-151)	34 (0-67)
14 days after first provocation	0 (0-0)	68 (0-279)	11 (0-51)	49 (2-197)	82 (0-100)
1-2 weeks after last provocation	0 (0-150)	189 (45-700)	23 (0-76)	83 (36-123)	67 (12-100)

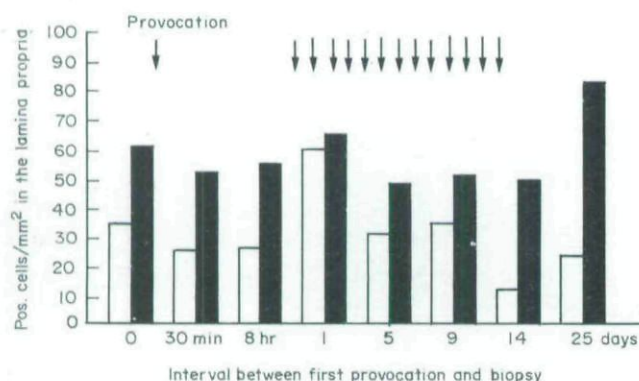


Fig. 3. Median number of IgE⁺ cells (□) and Toluidine Blue positive cells (■) in the lamina propria of the nasal mucosa of patients with isolated grass-pollen allergy before, during, and after a 14-day provocation study. A significant decrease in Toluidine Blue positive cells occurred during the provocation period. The number of IgE⁺ cells did not decrease significantly.

onto the bone. The sections were all deep enough to allow assessment of both layers.

After Toluidine Blue staining, mast cells could easily be identified by their dark-violet, metachromatic granules against a background of faintly stained tissue. The anti-IgE staining showed red cells against a blue counterstained background. The double staining with anti-IgE and TB showed violet cells, in which usually a blue centre and a red surface could be distinguished and also cells staining only red as were found in the sections stained with IgE only. Most of the IgE⁺TB⁺ cells in the epithelium and all of these cells in the lamina propria morphologically resembled mast cells and not basophils.

The numbers of mast cells in controls and allergic patients during natural provocation

The numbers of TB⁺ cells and IgE⁺ cells found per square millimetre tissue section in the natural provocation part of the study are shown in Table 2. The epithelium of the controls showed virtually no positive cells; in the allergic patients the number of TB⁺ cells in the epithelium was low in the summer as well as in the winter. The number of IgE⁺ cells in the epithelium was significantly higher in the allergic patients (126/mm² in summer, 67/mm² in winter) than in the controls (5/mm²). In the allergic group the difference between summer and winter did not reach significance. In the lamina propria the allergic group showed approximately the same number of TB⁺ cells as the non-allergic group did. However, the number of IgE⁺ cells in the lamina propria was significantly higher in the allergic patients (134/mm² in summer, 80/mm² in winter) than in the controls (17/mm²). Furthermore, a significant difference between summer and winter was found in the allergic group (Fig. 1).

Symptom score during the provocation study

During the 2-week long daily provocation period the symptom score for nasal blockage, nasal discharge, sneezing, nasal itching, and the total symptom score, recorded the preceding day, increased significantly (Fig. 2). The symptom score for eye watering/itching did not increase significantly. No significant increase in the symptom score was found within 30 min immediately after the daily provocation. No significant correlation could be found between symptom score and mast cell numbers or extent of degranulation.

Number of mast cells in the epithelium during the provocation study

Before provocation, IgE⁺ cells were found in all sections analysed and TB⁺ cells in four out of eight sections. The number of IgE⁺ cells seemed to decrease 30 min after provocation (from 144 cells/mm² before to 82 cells/mm² after provocation) and to increase again during the next 8 hr after provocation (from 82 cells/mm² to 357 cells/mm²). The number of biopsy specimens evaluated in each group, however, was too small to allow detection of significance. During the rest of the 2-week provocation period the number of IgE⁺ cells decreased significantly ($P=0.002$) (Table 3). One to 2 weeks after the provocation period, a second increase in the number of IgE⁺ cells seemed to occur.

The number of TB⁺ cells in the epithelium was too small for statistical analysis. During provocation, 28 out of 37 sections showed no TB⁺ cells in the epithelium. In the controls virtually no IgE⁺ or TB⁺ cells were found in the epithelium.

Number of mast cells in the lamina propria during the provocation study

The numbers of TB⁺ and IgE⁺ cells found per square millimetre of lamina propria in the provocation study are shown in Table 3. Throughout the provocation period the number of IgE⁺ cells in the lamina propria remained about the same. The number of TB⁺ cells seemed to decrease slightly 30 min and 8 hr after provocation and to increase by a factor of two, 24 hr after provocation. During the remainder of the provocation period the number of TB⁺ cells decreased significantly ($P=0.03$).

Double-staining with anti-IgE and TB showed that early in the provocation period IgE⁺TB⁻ cells occurred only in the subepithelial layer and that most of the cells in the deeper layers were IgE⁺TB⁺ cells. During the provocation period the depth of the area with mainly IgE⁺TB⁻ cells increased steadily until at the end of the provocation period most of the cells were IgE⁺TB⁻. The median percentage of IgE⁺TB⁺ cells decreased from 78 before provocation to 18 at the end of the provocation period. One to 2 weeks after the last provocation this percentage of IgE⁺TB⁺ cells had increased to 33 (Fig. 3). In the controls the numbers of IgE⁺ and TB⁺ cells did not change during the provocation period (results not shown).

IgE⁺ cells not being mast cells

Occasionally, IgE⁺ cells with a lymphoid morphology were seen, mainly in the lamina propria. Morphologically, such cells could easily be distinguished from mast

cells and were therefore not counted; eosinophils positive for IgE were not found. Other possibly IgE⁺ cells that could be erroneously taken for mast cells are Langerhans cells and plasma cells.

Immunofluorescence double-staining for the presence of both IgE and CD1a (for Langerhans cells) or both IgE and CD38 (for plasma cells) showed that in the epithelium of the allergic patients 29% (0–100%) of the IgE⁺ cells were positive for CD1a. Of the IgE⁺ cells in the epithelium, none were found to be simultaneously positive for CD38. In the lamina propria IgE⁺ cells were occasionally found to be positive for CD1a or CD38 as well; these numbers always represented <3% of the IgE⁺ cells. No IgE⁺ cells in the biopsy specimens of the controls were found to be simultaneously positive for either CD1a or CD38.

Discussion

Mast cell degranulation is an important component of the pathogenesis of allergic rhinitis. The results of the available quantitative studies on mast cells in the nasal mucosa differ widely, ranging from an overall decrease via redistribution to an overall increase after allergen exposure [5–10]. These differences could be due to the use of different staining techniques which do not always guarantee that all mast cells, including the degranulated ones, are counted. Another possible explanation of these differences is that the interpretation of the results is impeded by the lack of knowledge about the dynamics of the mast cell response in relation to allergen exposure. It is conceivable that some of the literature cited refers to different stages of an ongoing process.

In the present study we attempted to overcome both of these problems: on one hand, by using a method that always detects the mast cells in allergic patients and, on the other hand, by collecting data of the entire nasal mucosa on a large number of occasions after allergen challenge. We chose anti-IgE staining combined with Toluidine Blue staining of sections. After staining with anti-IgE it is certain that all mast cells in the epithelium and lamina propria of allergic patients will be detected [14]. However, in the controls mast cells are not positive for IgE or do not have enough IgE on their surface to be detected with anti-IgE in immunohistochemistry on sections. Double staining with IgE and TB in the allergic patients showed that TB stains the mast cells that are not (totally) degranulated. IgE⁺ cells which were not mast cells were found in relatively small numbers that did not influence the results of this study, except for the Langerhans cells in the epithelium of asymptomatic allergic patients. The use of biopsy in a provocation period of 2 weeks enabled us to study the dynamics of the mast cell response.

The results of this study show that mast cells are present in the lamina propria but seldom in the epithelium of non-allergic controls. This finding is in agreement with results obtained by Okuda *et al.* [4]. Allergic patients without symptoms (isolated grass-pollen allergy in the winter) have approximately the same number of mast cells in the lamina propria as non-allergic controls do. In the winter, when degranulation does not occur, the number of IgE⁺ cells is greater than the number of TB⁺ cells; this is in all likelihood due to other IgE⁺ cells that are not mast cells, such as IgE⁺ CD1a⁺ Langerhans cells [19].

During natural provocation of allergic patients the number of mast cells in both the epithelium and the lamina propria increases. This is in agreement with the findings of Lozewicz *et al.* who, although they did not indicate results concerning epithelium and lamina propria separately, found an increase during natural provocation [9]. Although these authors used a different method (α -Naphthol chloroacetate esterase reaction), their mast cell numbers are in the same range as ours [9]. These findings differ from those made in studies done by Pipkorn and Enerbäck [6,7], who found a mean mast cell number of only 1.55 (range 0–4.7) cells/mm² in the subepithelial layer in one study, a range of 0–30 in another study, and an increase and a redistribution to the epithelium of mast cells in these two studies, respectively. This difference might be explained by the use of the TB staining in the studies done by Pipkorn and Enerbäck [6,7], since this method detects only the mast cells that are not degranulated.

During the natural provocation study, the allergic patients used a nasal corticosteroid spray when they had severe complaints despite the use of antihistamines. Okuda *et al.* [24] and Gomez *et al.* [25] demonstrated a lower increase of nasal mucosal mast cells during provocation after use of nasal corticosteroid spray. This suggests that the use of nasal corticosteroids has led to an underestimation of the increase of mast cells found in the natural provocation study.

In the provocation study three periods could be distinguished. The first is the period from before provocation up to 8 hr after provocation. In this period, biopsy specimens were taken at three different times (see Table 1). Thirty minutes after provocation the number of epithelial mast cells seemed to decrease, probably due to migration to the surface. Eight hours after provocation an increase in epithelial mast cells was found, probably due to migration from the subepithelial layer to the epithelium. Simultaneously a considerable number of mast cells were seen perivascularly in the lamina propria. Mast cells in the lamina propria can either arise from monocytic cells [29] or can migrate into the lamina propria from the blood. As a result, the total number of

mast cells in the lamina propria remained approximately the same. The percentage of degranulated mast cells, however, increased from 22 before provocation to 62, 8 hr after provocation. This degranulation is seen mainly in the subepithelial layer. These results correlate well with the findings of Borres *et al.* [8], who demonstrated a temporary redistribution of mast cells toward the mucosal surface 5–24 hr after provocation. Twenty-four hours after the first provocation, the number of mast cells in the epithelium had returned to a level somewhat lower than that seen before provocation. In the lamina propria the level had equalled that seen before provocation.

The second period covers the 14 days of daily provocation, each of which can be seen as a recurrence of the preceding day. Biopsies were always taken shortly before the new provocation, and thus 24 hr after the last provocation. In this period the number of mast cells in the epithelium decreased slowly. The number of mast cells in the lamina propria remained approximately the same, but they became degranulated in an increasingly thick layer measured from the basement membrane. After 2 weeks 82% of the mast cells in the lamina propria had become degranulated, and only in the deepest layers were some TB⁺ cells found.

In the 2 weeks after the last provocation, i.e. the third period, the total number of mast cells seemed to increase in both the epithelium and the lamina propria. Surprisingly, 2 weeks after provocation, 62% of the mast cells in the lamina propria were still degranulated. This suggests either a prolonged degranulation of the mast cells in the lamina propria or a slow regranulation of degranulated mast cells.

An interesting difference between the natural provocation and the provocation studies is that in the latter a running out of mast cells in the epithelium occurred that did not occur after natural provocation and that the number of mast cells in the lamina propria, contrary to the situation after natural provocation, did not increase. During the provocation studies an increase in mast cells occurred in the epithelium and lamina propria after the daily provocation was terminated. During natural provocation, too, the percentage of degranulation of the mast cells in the lamina propria was only 49 (unpublished results) as compared with the 82% during the provocation study. These findings suggest that the stimulus during the daily provocation study was stronger than during natural provocation. Apparently, restoration of mast cell numbers is possible during natural provocation but not during provocation studies.

The provocation study showed an increase in the symptom score of nasal blockage, nasal discharge, sneezing, nasal itching, and the total symptom score experienced the previous day. This indicates that the priming

effect, described by others [26–28], was seen in our study, too. A possible explanation for the priming effect could be the increased release of mediators from mast cells [8]. However, the absence of correlation between symptom score and number of mast cells or amount of degranulation does not support the hypothesis that the priming effect is due to increased numbers of degranulating mast cells at the mucosal surface. It seems more likely that the priming effect is due to a combination of different processes taking place after allergen provocation.

The findings in the present study have shed light on the seemingly conflicting recent results reported on mast cell dynamics and degranulation, and show that the reported differences must be due to differences in the techniques applied and the times at which the response was evaluated.

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References

- Hastie R, Chir B, Heroy III JH, Levy DA. Basophil leukocytes and mast cells in human nasal secretions and scrapings studied by light microscopy. *Lab Invest* 1979; 40:554–61.
- Okuda M, Otsuka H. Basophilic cells in allergic nasal secretions. *Arch Otorhinolaryngol* 1977; 214:283–9.
- Otsuka H, Denburg JA, Dolovich J *et al.* Heterogeneity of metachromatic cells in human nose: significance of mucosal mast cells. *J Allergy Clin Immunol* 1985; 76:695–702.
- Okuda M, Otsuka H, Kawabori S. Basophil leukocytes and mast cells in the nose. *Eur J Respir Dis* 1983; 64 suppl 128: 7–14.
- Wihl JA, Mygind N. Studies on the allergen-challenged human nasal mucosa. *Acta Otolaryngol* 1977; 84:281–6.
- Enerbäck L., Pipkorn U, Granerus G. Intraepithelial migration of nasal mucosal mast cells in hay fever. *Int Arch Allergy Appl Immunol* 1986; 80:44–51.
- Pipkorn U, Karlsson G, Enerbäck L. Secretory activity of nasal mucosal mast cells and histamine release in hay fever. *Int Arch Allergy Appl Immunol* 1988; 87:349–60.
- Borres MP, Irander K, Björkstén B. Metachromatic cells in nasal mucosa after allergen challenge. *Allergy* 1990; 45:98–103.
- Lozewicz S, Gomez E, Claque 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.
- Viegas M, Gomez E, Brooks J, Davies RJ. Changes in mast cell numbers in and out of the pollen season. *Int Arch Allergy Appl Immunol* 1987; 82:275–6.
- Drake-Lee AB, Price JM, Milford CM, Bickerton RC. Nasal mast cells: a preliminary report on their ultrastructure. *J Laryngol Otol* 1987; suppl 13:1–17.
- Claman HN, Giorno R, Choi KL. Degranulated ('phantom') mast cells in GVHD revealed by double immunofluorescence. *J Allergy Clin Immunol* 1987; 180:77.
- Fokkens WJ, Holm AF, Lijntjes E, Mulder PGH, Vroom ThM. Characterization and quantification of cellular infiltrates in nasal mucosa of patients with grass-pollen allergy, non-allergic patients with nasal polyps and controls. *Int Arch Allergy Appl Immunol* 1990; 93:66–72.
- Feltkamp-Vroom ThM, Stallman OJ, Aalberse RC, Reerink-Brongers EE. Immunofluorescence studies on renal tissue, tonsils, adenoids, nasal polyps and skin of atopic and nonatopic patients with special reference to IgE. *Clin Immunol Immunopathol* 1975; 4:392–404.
- Capron M, Capron A, Dessaint J, Torpier G, Johansson S, Prin L. Fc-receptors for IgE on human and rat eosinophils. *J Immunol* 1981; 126:2087–92.
- Yodoi J, Ishizaka K. Lymphocytes bearing Fc-receptors for IgE. I. Presence of human and rat T-lymphocytes with Fc_γ-receptors. *J Immunol* 1979a; 122:2577–83.
- Joseph M, Tonnel AB, Capron A, Voisin C. Enzyme release and super oxide anion production by human alveolar macrophages stimulated with immunoglobulin E. *Clin Exp Immunol* 1980; 40:416–22.
- Bruijnzeel-Koomen CAFM, Wichen DF v, Toonstra J, Berrens L, Bruijnzeel PLB. The presence of IgE molecules on epidermal Langerhans cells from patients with atopic dermatitis. *Arch Dermatol Res* 1986; 278:199–205.
- Fokkens WJ, Rijntjes E, Vroom ThM, Mulder PGH. Fluctuation of the number of CD-1 (T6)-positive dendritic cells, presumably Langerhans cells, in the nasal mucosa of patients with an isolated grass-pollen allergy before, during and after the grass-pollen season. *J Allergy Clin Immunol* 1989; 84:39–43.
- Bachert C. Die immunhistochemische Darstellung von IgE an der Nasenschleimhaut als diagnostisches Kriterium bei der Soforttyp-Allergie. *Allergologie* 1989; 12:372–6.
- Fokkens WJ, Vroom ThM, Gerritsma V, Rijntjes E. A mild biopsy method to obtain high-quality specimens of nasal mucosa. *Rhinology* 1988; 26:293–5.
- Feltkamp-Vroom ThM, Boode JHM. An embedding and sectioning technique for immunohistochemical studies of minute specimens of tissue. *J Clin Pathol* 1970; 23:188–9.
- Fokkens WJ, Vroom ThM, Rijntjes E, Mulder PGH. CD-1 (T6) HLA-DR-expressing cells, presumably Langerhans cells, in nasal mucosa. *Allergy* 1989; 44:167–72.
- Okuda M, Senba O. Effect of beclomethasone dipropionate nasal spray on subjective and objective findings in perennial rhinitis. *Clin Otolaryngol* 1989; 53:15–21.
- Gomez E, Claque JE, Gatland D, Davies RJ. Effect of topical corticosteroids on seasonally induced increases in nasal mast cells. *Br Med J* 1988; 296:1572–3.

- 26 Connell JT. Quantitative intranasal pollen challenge. II. Effect of daily pollen challenge, environmental pollen exposure, and placebo challenge on the nasal membrane. *J Allergy* 1968; 41:123-9.
- 27 Wachs M, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Observations on the pathogenesis of nasal priming. *J Allergy Clin Immunol* 1989; 84:492-501.
- 28 Gerth van Wijk R, Dieges PH, Toorenenbergen AW van. Seasonal variability in nasal sensitivity to house dust mite extract. *Rhinology* 1987; 25:41-8.
- 29 Czarnetski BM, Figdor CG, Kolde G, Vroom ThM, Aalberse R, Vries JE de. Development of human connective tissue mast cells from purified blood monocytes. *Immunol* 1984; 51:549-54.

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