

Basophil and eosinophil accumulation and mast cell degranulation in the nasal mucosa of patients with hay fever after local allergen provocation

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Background: Basophils and mast cells have certain similarities and are believed to be important in upper and lower respiratory allergy.

Objective: We sought to apply immunohistology to investigate the distribution and numbers of mast cells and basophils in the nasal mucosa after allergen provocation.

Methods: Allergen challenge with grass pollen was performed in 9 patients with seasonal allergic rhinitis out of the hay fever season. Nasal biopsy specimens were taken before and approximately 1 hour, 24 hours, and 1 week after intranasal allergen provocation. We determined relative numbers and their phenotypic characteristics by using mAbs specific for tryptase, chymase, IgE, eosinophils (BMK-13), and a new mAb against basophils (BB1) by using immunohistochemistry in frozen sections.

Results: In the nasal mucosa at baseline, practically no basophils were found in the epithelium. A significant increase in numbers was found in the epithelium and lamina propria of the nasal mucosa in the early phase as early as 1 hour after allergen provocation. At 24 hours and 1 week after allergen provocation, a significant increase in basophil numbers was found in the lamina propria only. The proportion of mast cells staining for chymase in the lamina propria decreased from a median of 38% (range, 0%-82%) to 14% (range, 0%-78%) within 1 hour of allergen provocation. The proportion of mast cells staining for tryptase increased from 1% (range, 0%-86%) at baseline to 21% (range, 3%-85%) within 1 hour of allergen provocation. One week after provocation, mast cells returned to baseline numbers. A definite tissue eosinophilia was observed after allergen provocation.

Conclusion: Basophil numbers are increased in the epithelium and lamina propria of the nasal mucosa of subjects with rhinitis after allergen challenge, with influx already apparent at 1 hour. Moreover, changes in mast cell percentages and numbers

were observed within 1 hour of allergen provocation. (*J Allergy Clin Immunol* 2000;106:677-86.)

Key words: Allergen provocation, allergic rhinitis, basophils, chymase, eosinophils, hay fever, grass pollen, mast cells, nasal mucosa, tryptase

Mast cells and basophils are metachromatically staining cells, which are believed to be important in upper and lower respiratory allergy. These cells have certain similarities in structure and function. On the basis of conventional metachromatic staining procedures, it is not easy to discriminate between mast cells and basophil-like cells in tissues or brushings under a light microscope.¹⁻⁴ Nevertheless, these cells differ in their origin and in many phenotypic characteristics. Both cell types express high-affinity receptors for IgE, which can be cross-linked by allergen binding to IgE and can induce the release of secretory granule contents. By using antibodies against the mast cell proteases tryptase and chymase, mast cells may be distinguished from basophils on the basis of their granule content.^{5,6} Recent observations have indicated that Carnoy's fluid fixation, in contrast to acetone fixation, can lead to an underestimation of the number of immunohistochemically stained chymase-positive mast cells.^{7,8}

Studies measuring mediators and metachromatically staining cells in nasal lavage fluid collected from patients with rhinitis with an allergen-induced late-phase reaction have revealed high levels of histamine but relatively low levels of specific mast cell products, such as tryptase and PGD₂. These findings have implicated the basophil as an important contributor to histamine release in the late phase but not in the early phase.^{9,10}

The lack of a reliable means of detecting basophils in tissues has hindered understanding of the relative role of basophils and mast cells in allergic disease. Recently, however, the preparation of an mAb (BB1) specific for a unique granule constituent of basophils has opened the way for enumeration of this cell type in tissue.¹¹

In the present study patients allergic to grass pollen were challenged with grass pollen out of season. Biopsy specimens were taken before and 1 hour, 24 hours, and 1 week after local allergen provocation, and alterations in the numbers of basophils and mast cells were examined.

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Abbreviation used

BU: Biologic units

METHODS**Patients**

Nine patients (median age, 22 years; range, 19-51 years) participated in this study (5 women and 4 men). All patients had a history of seasonal allergic rhinitis for grass pollen of at least 2 years. Allergy to grass pollen was confirmed by a positive skin prick test response (3+) with Alutard Soluprick extract (1 SQ/mL), with no skin prick test reactions to other relevant allergens (eg, mite, cat, or dog). Patients with a positive reaction to a tree allergen were included because this study ended long before the start of the tree pollen season in the Netherlands. The patients were free of symptoms at the start of the study. None of the patients used any medication during the study or had undergone immunotherapy in the 3 years before this study. No relevant abnormalities were found on ear, nose, and throat examination. All patients gave their written informed consent, and the medical ethics committee approved the study.

Study design

This study was conducted between October and December 1995, well out of the grass pollen season. Biopsy specimens of nasal mucosa were taken from the lower edge of the inferior turbinate about 2 cm posterior to the edge by using a Gerritsma forceps with a cup diameter of 2.5 mm. Local anesthesia was obtained by placing a cotton-wool carrier with 50 to 100 mg of cocaine and 3 drops of adrenaline (1:1000) under the inferior turbinate without touching the biopsy site. The biopsy specimens were embedded in Tissue-Tek II OCT compound in a gelatin capsule and frozen immediately. No cautery was used.¹² Each patient underwent 4 nasal biopsies: at baseline, 1 hour after provocation with grass pollen, 24 hours after provocation, and 1 week after provocation. It was possible to see where the previous biopsy specimen was taken, and there was enough space for taking another biopsy specimen after 24 hours or 1 week.¹³

Provocation

The patients were allowed to acclimatize to the room for at least 15 minutes. PBS was then administered to exclude the possibility of nonspecific hyperreactivity. After 10 minutes, patients were challenged with 50 μ L of an aqueous nasal spray containing 10,000 biologic units (BU)/mL grass pollen (ALK, Groningen, Netherlands) and another 10 minutes later with 100,000 BU/mL (ALK) to initiate a strong allergic response. After both provocations, symptoms, including sneezing, rhinorrhea, itching, and nasal blockage, were recorded on a 4-point (0-3) scale. Patients were also asked to rate their symptoms during the day after provocation after 3, 6, 9, 12, and 24 hours.

Immunohistochemical staining

Staining procedures. Monoclonal antibodies directed against chymase, tryptase, IgE, eosinophil major basic protein, and basophils (Table I) were used together with the supersensitive immunoalkaline phosphatase method, as previously described.⁷

Double-staining tryptase and chymase. Frozen sections were fixed in acetone for 10 minutes and rinsed in PBS. Endogenous peroxidase was blocked with 0.1% sodium azide and 0.01% hydrogen peroxide in PBS for 30 minutes. Sections were then rinsed in PBS for 10 minutes and incubated with 10% normal goat serum (CLB, Amsterdam, The Netherlands) and 10% normal rabbit serum (CLB). After this, the slides were incubated with mouse anti-human

tryptase for 60 minutes at room temperature. The sections were then rinsed again in PBS for 5 minutes and incubated for 30 minutes with a peroxidase-conjugated rabbit anti-mouse Ig antiserum (1:200 Sigma), rinsed successively in PBS, and incubated with peroxidase-conjugated mouse anti-peroxidase (1:200 Sigma). After this, the slides were incubated with the 10% normal mouse serum (CLB) for 10 minutes and incubated with biotinylated mouse anti-human chymase (B7) for 60 minutes. They were rinsed with PBS for 5 minutes and incubated with alkaline phosphatase-conjugated goat anti-biotin (1:50 Sigma) for 30 minutes. Slides were then rinsed with PBS for 5 minutes and with TRIS buffer (0.1 mol/L, pH 8.5) for 5 minutes. This was followed by incubation for 30 minutes in Fast Blue substrate containing levamisole to block endogenous alkaline phosphatase. Finally, slides were rinsed with sodium acetate (0.1 mol/L, pH 4.6) for 5 minutes and incubated with amino-ethyl-carbazole 0.05% in sodium acetate 0.1 mol/L (pH 4.6) and 0.01% peroxide substrate for 30 minutes, and sections were rinsed with distilled water and mounted in glycerin-gelatin. Control staining was performed with an irrelevant mAb of the same subclass.

Light microscopic evaluation. Stained cells were counted in two sections of each biopsy specimen. The epithelium and lamina propria were evaluated separately. The total surface area of a section and its main parts (ie, the epithelium and the lamina propria) were estimated by using the Leica Image Analysis System. The number of cells per square millimeter was calculated for the epithelium and the lamina propria.

Statistical analysis

Statistical analysis was performed with the SPSS 7.5 software for Windows 95. Friedman two-Way ANOVA was used for statistical analysis.

The Wilcoxon signed-rank test was used for comparing data obtained at different time points to observe degranulation or redistribution (before provocation – early phase; early phase – 1 week after provocation and before provocation – 1 week after provocation). A *P* value of less than .05 was considered to indicate a significant difference between time points.

Correlation coefficients were obtained by using the Spearman rank method. Because of multiple testing, a *P* value of less than .025 was considered to indicate a significant correlation.

RESULTS**Nasal symptoms**

No symptoms were observed before allergen challenge or after challenge with saline. Nasal provocation with the allergen resulted in all patients manifesting a significant increase in immediate nasal symptoms, such as sneezing, itching, nasal blockage, and rhinorrhea. Between 6 and 12 hours after the allergen provocation in all patients, late-phase nasal symptoms were observed, especially blockage and itching. Fig 1 shows total symptoms at each measuring moment before and after allergen provocation.

Immunohistochemistry

The cryostat sections of the nasal mucosa had an average surface area of 2 mm² and mainly an intact epithelium. Staining with mAb for basophils, mast cells, eosinophils, and IgE could easily be identified by the red cytoplasm or membrane (IgE) and dark violet nucleus, as shown for basophils in Fig 2, *A*, before and Fig 2, *B*, after allergen provocation and for tryptase in Fig 2, *C*, before and Fig 2, *D*, after allergen provocation.

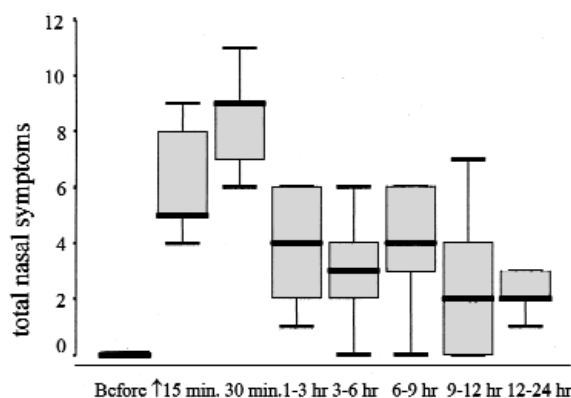


FIG 1. Total symptom score before and after allergen provocation. After both provocations (15 and 30 minutes), symptoms including sneezing, rhinorrhea, itching, and nasal blockage were recorded. Patients were also asked to rate their symptoms on a 4-point (0-3) scale during the day after provocation after 3, 6, 9, 12, and 24 hours.

TABLE I. Antibodies used

Antibodies	Clone	Concentration or dilution	Source
Anti-chymase	B7	1 µg/mL	Chemicon (Brunschwig, Netherlands)
Biotinylated anti-chymase	B7	1 µg/mL	Chemicon (Brunschwig, Netherlands)
Anti-tryptase	G3	0.7 µg/mL	Chemicon (Brunschwig, Netherlands)
Anti-basophils	BB1	6 µg/mL	A.F. Walls
Anti-IgE	HM25M	0.2 µg/mL	CLB
Anti-major basic protein	BMK-13	0.2 µg/mL	Sanbio (Zwijndrecht, Netherlands)
Biotinylated goat anti-mouse		1:50	Biogenix (Klini Path, Netherlands)
Streptavidine alkaline phosphatase/oxidase		1:50	Biogenix (Klini Path, Netherlands)
Goat anti-biotin alkaline phosphatase		1:50	Sigma (Uden, Netherlands)
Rabbit anti-mouse peroxidase		1:200	Sigma (Netherlands)
Mouse peroxidase anti-peroxidase		1:200	Sigma (Netherlands)

Basophils

At baseline, practically no (median, 0; range, 0-33 cells/mm²) basophils were found in the epithelium (Fig 3, A). Significantly increased numbers were found in the epithelium (Friedman $P = .04$) of the nasal mucosa in the early phase after 1 hour ($P = .002$); 24 hours after allergen provocation, the numbers of basophils were still raised, although this increase did not quite reach significance ($P = .06$). One week after provocation, the number of basophils was found to have returned to the baseline level. In the lamina propria, limited numbers of BB1-positive cells (median, 5; range, 2-27) were found mainly at baseline in the subepithelium layer, and numbers increased after allergen provocation (Friedman $P < .001$). Significantly higher basophil numbers relative to baseline were observed at 1 hour ($P < .001$), 24 hours ($P = .02$), and 1 week ($P = .02$) after the allergen provocation (Fig 3, B).

Mast cell tryptase

No significant changes were found in epithelial tryptase-positive cell numbers (Fig 3, C). However, in the lamina propria a trend in overall changes was observed (Friedman $P = .06$). We observed a trend of reduction in tryptase-positive cells that may indicate mast cell degran-

ulation with regeneration of mast cell tryptase 1 week later. This was mainly caused by the higher level 1 week after provocation compared with 1 and 24 hours after provocation (Wilcoxon $P = .03$ and $P = .07$, respectively; Fig 3, D).

Mast cell chymase

In the epithelium only a few chymase-positive cells were found at baseline (Fig 3, E). No overall significant changes in the numbers of chymase-positive cells were observed (Friedman). In the lamina propria the numbers of chymase-positive cells tended to be lower at 1 hour (Wilcoxon $P = .04$) and to return to baseline numbers (Wilcoxon $P = 0.03$) at 1 week after the allergen provocation (Fig 3, F).

Double staining with tryptase and chymase

Cell numbers. With a double-staining procedure, it was possible to differentiate 3 mast cell phenotypes—mast cells staining for tryptase, mast cells double staining for tryptase and chymase, and mast cells staining for chymase—as shown in Fig 4, A (before allergen provocation), and Fig 4, B (after allergen provocation). Numbers of mast cells staining for tryptase and chymase and mast cells staining for chymase in the lamina propria

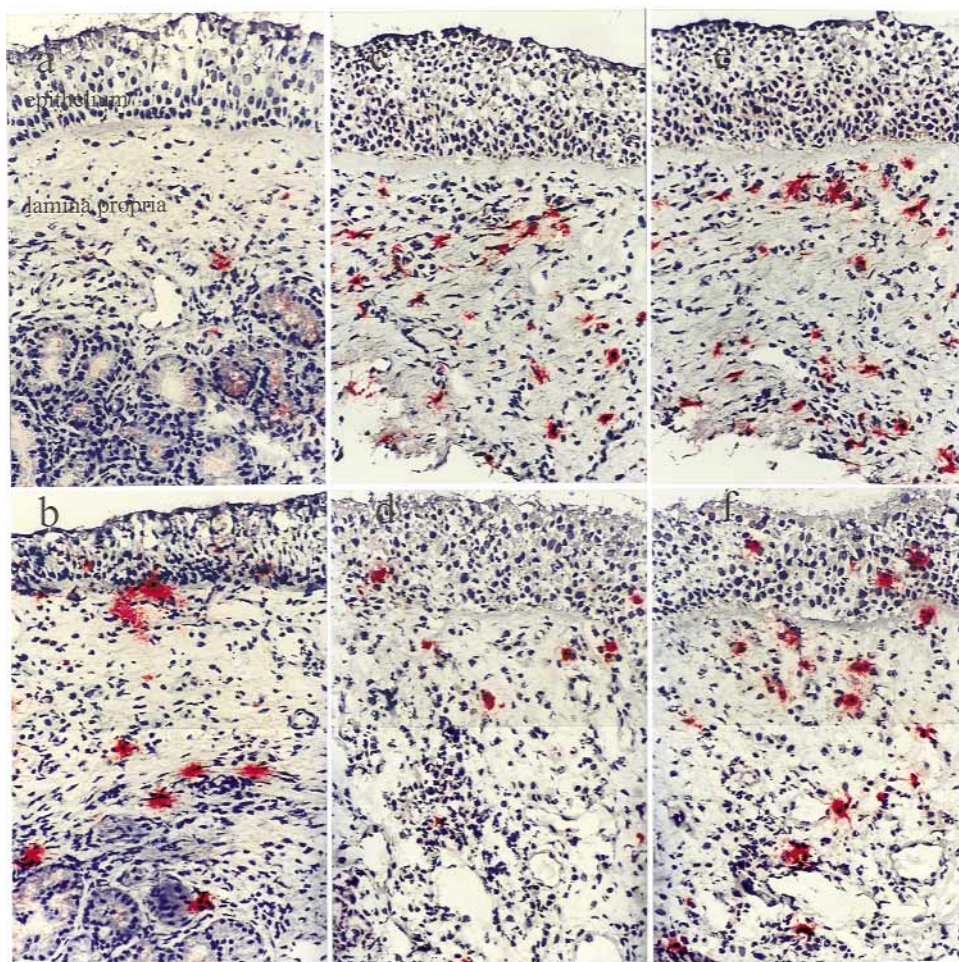


FIG 2. Photomicrograph of BB1-positive cells (red) in the epithelium and in the lamina propria of a nasal mucosal biopsy section obtained from an allergic patient before (A) and 24 hours after (B) allergen provocation. For tryptase, specimens are shown from before (C) and 24 hours after (D) allergen provocation, and for chymase, specimens are shown before (E) and 24 hours after (F) allergen provocation (counterstained with Gill's hematoxylin; magnification $\times 1000$).

showed no significant changes after allergen provocation (Fig 5, C and E). Numbers of mast cells staining for tryptase increased significantly ($P = .001$) from a median at baseline of 1 (range, 0-24 cells/mm²) to 6 cells/mm² (range, 3-94 cells/mm²) at 1 hour after allergen provocation. Numbers of mast cells staining for tryptase were still above baseline at 24 hours (median, 5 cells/mm²; range, 0-58 cells/mm²) and 1 week (median, 6 cells/mm²; range, 2-54 cells/mm²) after allergen provocation (Fig 5, A).

Percentages. Expressed as a percentage of total mast cell numbers, the proportion of mast cells staining for tryptase phenotypes was significantly raised during the provocation period (Friedman $P = .007$). There was an increase in the proportion of mast cells staining for tryptase from 1% (range, 0%-86%) at baseline to 21% (range, 3%-85%) at 1 hour ($P = .01$) after allergen provocation, and this was followed by a return to 6% (range, 0%-55%) at 24 hours and 5% (range, 2%-62%) 1 week after allergen provocation (Fig 5, B). The trend

for the proportion of mast cells staining for chymase was to decrease from 38% (range, 0%-82%) to 14% (range, 0%-78%) 1 hour after allergen provocation (Wilcoxon $P = .04$), and thereafter the proportion stayed relatively stable (Fig 5, D). The percentage of mast cells staining for tryptase and chymase reflected changes in the other phenotypes: 42% (range, 14%-83%) at baseline, 45% (range, 11%-95%) after 1 hour, 70% (range, 16%-94%) after 24 hours, and 53% (range, 23%-93%) 1 week after allergen provocation. However, these changes did not reach significance (Fig 5, F).

Eosinophils

Eosinophil numbers in the epithelium tended to be raised after allergen challenge ($P = .07$; Fig 6, A). Numbers of eosinophils in the lamina propria also increased after allergen provocation ($P < .002$). At baseline, the total numbers of eosinophils were small, and they were mainly found in the deep layers of the lamina propria

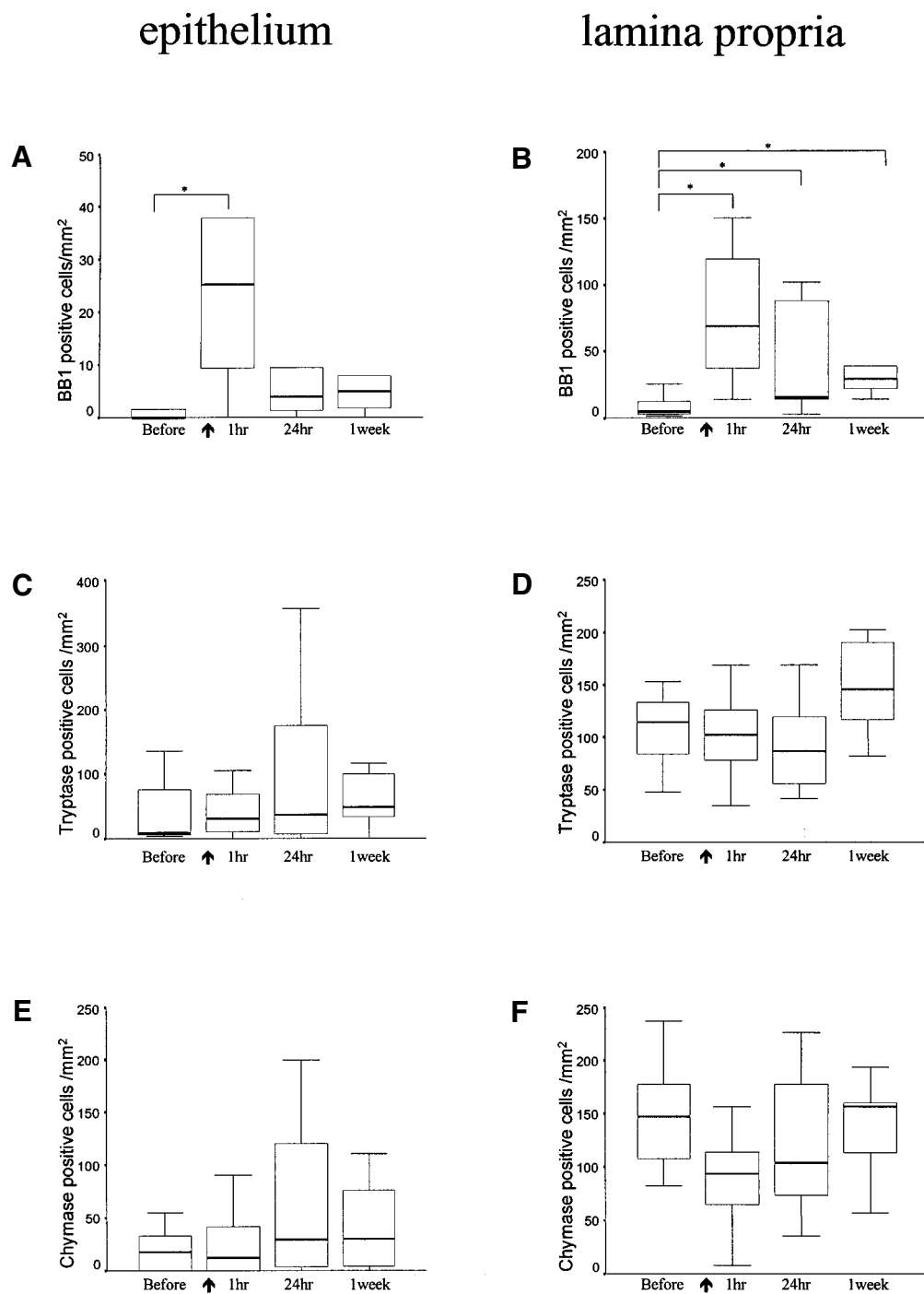


FIG 3. Numbers (in cells per square millimeter) of basophils (**A** and **B**), tryptase-positive cells (**C** and **D**), and chymase-positive cells (**E** and **F**) in the epithelium (**A**, **C**, and **E**) and lamina propria (**B**, **D**, and **F**) in nasal mucosa biopsy specimens. Nasal mucosa biopsy specimens were taken before provocation, 1 hour after provocation, 24 hours after provocation, and 1 week after provocation. Allergen challenge (arrows) was performed with 1000 and 10,000 BU grass pollen. Box plots represent the 25th-75th percentile, the range (whiskers), and the median value.

(Fig 6, *B*). At 1 hour after allergen challenge, a striking increase in numbers was observed. Eosinophil numbers were still significantly higher than at baseline, 24 hours, and 1 week after allergen provocation.

IgE-positive cells

No changes in the number of IgE-positive cells were observed in either the epithelium or lamina propria (Fig 6, *C* and *D*).

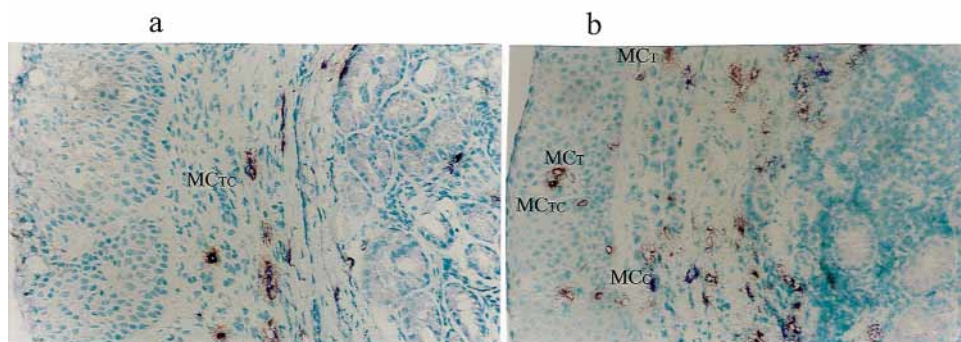


FIG 4. Mast cells. Representative sections of double staining showing tryptase (red), chymase (blue), and tryptase-chymase double-positive cells (red and blue) indicate 3 types of mast cells: tryptase-positive (MC_T), tryptase-chymase positive (MC_{TC}), and chymase-positive (MC_C). **a**, Before provocation; **b**, after provocation (counterstaining with methyl-green; magnification $\times 1000$).

Comparison of immunologically relevant cells

The numbers of all patient mast cells, eosinophils, and basophils (defined as the immunologically relevant effector cells in allergy) underwent proportional changes in the lamina propria after allergen provocation. At baseline, the level of mast cells was at a median of 88% (range, 56%-97%), with the percentages of eosinophils and basophils being low at 7% (range, 0%-37%) and 3% (range, 1%-13%), respectively. After allergen provocation in the early phase, levels of mast cells diminish sharply to a median percentage of 27% (range, 12%-42%). The eosinophils and basophils increased sharply to 51% (range, 23%-80%) and 23% (range, 6%-48%). In the late phase (24 hours after provocation) all cells are present, but numbers are lower than in the early phase: mast cells, 37% (range, 1%-97%); eosinophils, 49% (range, 1%-80%); and basophils 7% (range, 0%-25%). One week after provocation, all immunologically relevant cells were still present. Levels of mast cells were highest at 46% (range, 22%-73%), followed by the eosinophils at 38% (range, 10%-75%) and then the basophils at 9% (range, 3%-45%). Fig 7 shows the median numbers of mast cells, eosinophils, and basophils in the lamina propria at each time point.

Tryptase-positive basophils

Tryptase double staining was performed on biopsy specimens taken 1 hour and 1 week after provocation. Double staining showed tryptase (red) and BB1 (blue) double-positive cells (Fig 8). The tryptase-positive BB1 cell numbers were 6.3 cells/mm² (range, 0-15.2 cells/mm²) at 1 hour and 8.1 cells/mm² (range, 1.8-18.2 cells/mm²) 1 week after provocation. There was only one biopsy specimen that was evaluated, taken 1 hour after allergen provocation, in which no tryptase-positive BB1 cells could be observed.

DISCUSSION

This study shows that allergen provocation with grass pollen results not only in an increase in eosinophils^{13,14}

but also a significant increase in BB1-positive cells (basophils) in the epithelium and lamina propria of the nasal mucosa. This study has no placebo control group, but in a previous study we described the results of a placebo-controlled study in which repeated placebo spray was without observable effect in the nasal mucosa.¹⁵

We provide evidence that there is marked basophil infiltration in the airways as early as 1 hour after allergen challenge and a prolonged increase in numbers for up to 1 week. However, the lack of association between eosinophils and basophils after allergen provocation suggests that these cell types are attracted by different factors.

The dynamics of basophil infiltration after allergen provocation are quite different from those of mast cells. Although the influx of metachromatically staining cells (taken as mast cells) into the epithelium has been described by several authors,¹⁶⁻¹⁸ the total numbers of mast cells in the nasal mucosa are relatively constant compared with, for example, changes in eosinophil numbers.^{2,13,14,19}

Comparing the influx of eosinophils and basophils, we noted that 1 hour after allergen provocation the influx of eosinophils in the lamina propria (20 times baseline values) is twice as large as that of basophils (10 times baseline values) and that mast cells decrease (half) as a percentage of total inflammation. In the epithelium the influx of eosinophils and basophils is comparable (15 times baseline values) and also comparable with numbers found in other studies, which show that eosinophils appeared earlier in the nasal epithelium than in the lumen after allergen provocation.¹³ Extravasation of eosinophils has also been shown to occur within half an hour after allergen provocation.^{3,13} However, the lack of association between eosinophils and basophils after allergen provocation suggests that these cell types are attracted by different factors.

In earlier studies it has been suggested that basophils contribute mainly to the late-phase response. This had been concluded from the pattern of mediators released in the late phase (ie, histamine, leukotrienes, and others, but not PGD₂)^{9,10} and studies of nasal brushings and lavage fluids showing only an increase of basophils in the nasal lumen in the late phase.^{3,4,20} However, this study clearly

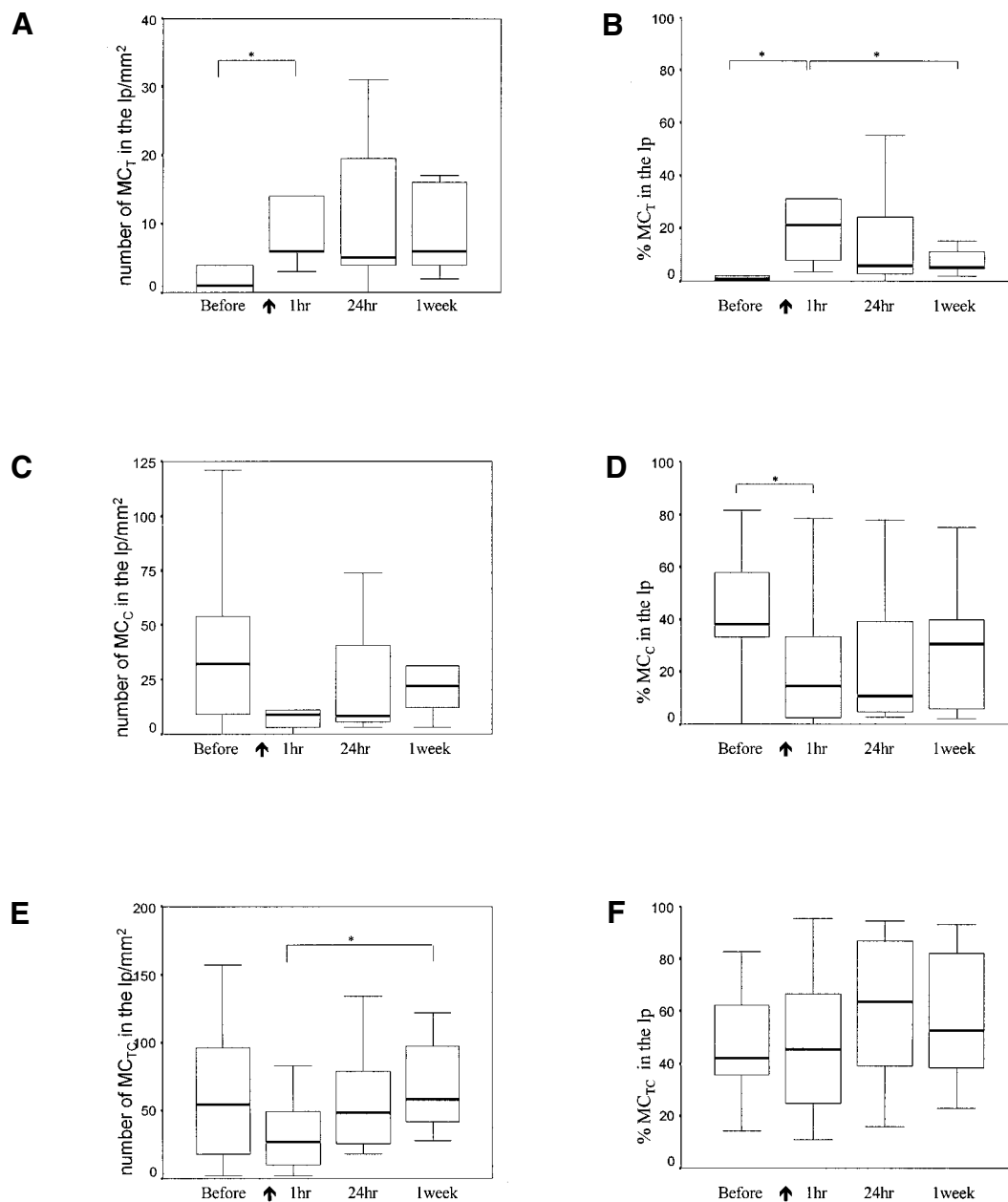


FIG 5. The numbers (**A**, **C**, and **E**) and proportion (**B**, **D**, and **F**) of tryptase-positive mast cell (MC_T) phenotype (**A** and **B**), chymase-positive mast cell (MC_C) phenotype (**C**, **D**), and tryptase-chymase double-positive mast cell (MC_{TC}) phenotype (**E** and **F**).

shows a significant increase of basophils in all layers of the nasal mucosa, even in the early phase. The pattern of mediators released cannot only be explained by differences between basophils and mast cell activation but also by a different mechanism of mast cell mediator release in the early (caused by allergen/IgE cross-linking) and the late phase. This explanation is supported by in vitro experiments showing that neuropeptide-induced mediator release results in high levels of histamine but relatively low levels of PGD_2 from mast cells, suggesting a neurogenic induction of late-phase histamine release from mast cells.²¹⁻²³ In another study carried out in col-

laboration with Braunstahl et al,²⁴ we observed that there was practically no eotaxin protein present in the nasal mucosa before allergen provocation. Moreover, in that study we compared eotaxin-stained cell numbers in healthy control subjects with those in patients with seasonal allergic rhinitis and observed no differences in cell numbers before allergen provocation. Our data does not support the idea that eotaxin release is a key mechanism for early infiltration of eosinophils and basophils. It is possible that tryptase and chymase could be stimuli for the infiltration of inflammatory cells because this has been observed in animal models.^{25,26}

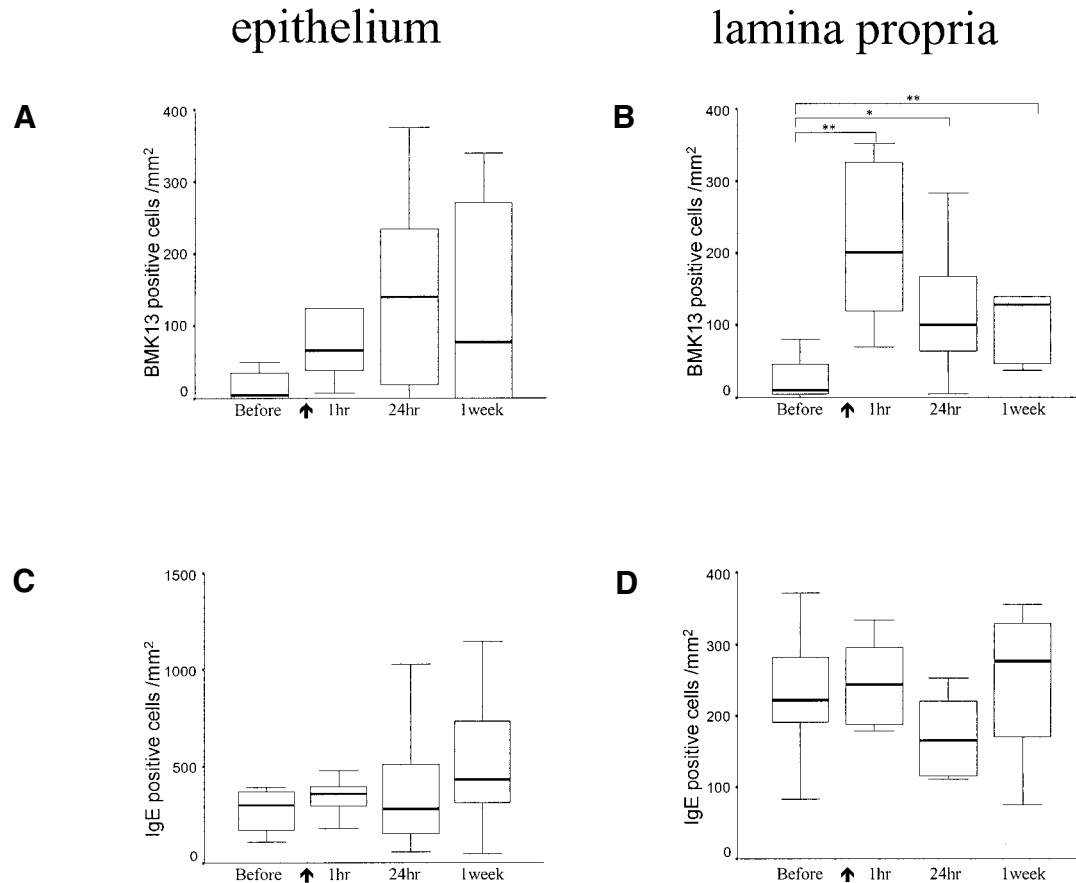


FIG 6. Numbers (in cells per square millimeter) of eosinophils (**A** and **B**) and IgE-positive cells (**C** and **D**) in the epithelium (**A** and **C**) and lamina propria (**B** and **D**) in nasal mucosa biopsy specimens. Nasal mucosa biopsy specimens were taken before provocation, 1 hour after provocation, 24 hours after provocation, and 1 week after provocation. Allergen challenge (arrows) was performed with 1000 and 10,000 BU grass pollen. Box plots represent the 25th-75th percentile, the range (whiskers), and the median value.

The trend toward a reduction in chymase-positive cell numbers at 1 hour after allergen provocation in the nasal mucosa suggests that degranulation may have occurred. However, in contrast to the reduction in chymase-positive cells, no fall in numbers of tryptase-positive cells could be observed. The correlation between epithelial basophils and tryptase-positive cells at 1 hour after allergen provocation ($r_s = 0.919$, $P = .003$) raises the possibility that these cells may, at least in part, represent the same cell. Further double staining with CD18, tryptase, and BB1 indeed showed that some of the BB1-positive cells were positive for tryptase, indicating tryptase-positive basophils (Fig 8). This concurs with the recent observation of Li et al,²⁷ who identified basophilic cells expressing the mast cell granule proteases chymase and tryptase in the peripheral blood of patients with asthma and allergies and drug-reactive patients. A possible interpretation could be that extravasated basophils are immature mast cell-committed progenitors that have left the blood circulation. The influx of tryptase-positive basophils could help to explain why numbers of tryptase-positive cells were not reduced, whereas chymase-positive cells were reduced.

Alternatively, mast cells may be able to degranulate selectively, as has been shown by Goldstein et al.²⁸

This study has again confirmed that the total numbers of IgE-positive cells do not increase in the nasal mucosa after allergen provocation.¹⁹ In a previous study we describe toluidine blue-positive/IgE-positive cells. In the epithelium most IgE-positive cells are not positive for toluidine blue. After allergen provocation, 29% of the IgE-positive cells were positive for CD1a. No IgE-positive plasma cells or B cells were observed in the epithelium.¹⁹ Unpublished data with CD117-positive cell numbers indicate that this marker detects 10 times more cells than tryptase or chymase antibodies and equal numbers of IgE-positive cells at baseline level out of season in the epithelium. This suggests that the CD117 population probably detects totally degranulated epithelial mast cells bearing IgE on the surface.

In the lamina propria it is clear that most IgE-positive cells are granulated mast cells. In earlier studies we have shown that the population of IgE-positive cells in the nasal mucosa of patients with rhinitis who are allergic to grass pollen consists of mast cells (confirmed by total of chymase-tryptase staining; median, 80%; range, 20%-

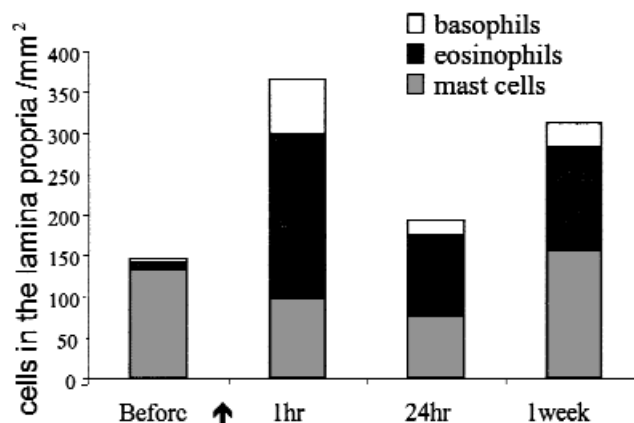


FIG 7. The inflammation of mast cells, eosinophils, and basophils related to the total inflammation of these cells in the lamina propria over time.

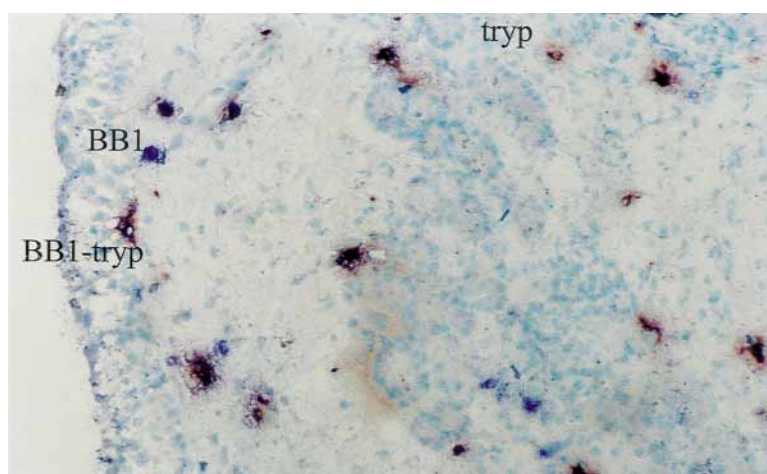


FIG 8. Tryptase-positive basophils. Double staining showed tryptase (red) and BB1 (blue) double-positive cells (counterstained methyl-green; magnification $\times 1000$). Double staining with CD18, tryptase, and BB1 indicates that BB1-positive cells are positive for tryptase.

95%; Langerhans cells: median, 10%; range, 1%-30%; B cells: median, 4%; range, 0%-14%; and plasma cells: median, 2%; range, 0%-29%), some of which may be fully degranulated mast cells containing no chymase or tryptase.²⁹

In conclusion, basophils, as identified by specific antibody BB1, increase in numbers in the epithelium and lamina propria of the nasal mucosa as early as 1 hour after provocation. This early influx is also observed in eosinophils.

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