

Mast cells and eosinophils in the allergic mucosal response to allergen challenge: Changes in distribution and signs of activation in relation to symptoms

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An allergen challenge was performed in 10 asymptomatic patients with strictly seasonal allergic rhinitis. For comparison; seven nonallergic subjects were challenged with allergen, and seven allergic patients were challenged with diluent. Cell samples, obtained with use of a brush technique to recover cells from within the epithelium and nasal lavage to collect cells from the epithelial surface, and symptom scores were taken before challenge and at 2-hour intervals during 12 hours. The cell suspensions were cytocentrifuged onto object slides for light microscopy. Histamine was determined in the cell pellets. In brush samples from the allergic patients challenged with allergen, eosinophils, expressed as a percentage of the total granulocytes, increased from $4.3\% \pm 2.7\%$ (mean \pm SEM) to $10.3\% \pm 3.8\%$ ($p < 0.05$) 4 hours after challenge. This level was maintained for up to 12 hours. A similar increase was noted in the lavage specimens 2, 6, and 8 hours after the challenge. In the brush samples the proportion of eosinophils containing two or more cytoplasmic vacuoles, taken as a sign of activation, increased from 20% to 72% ($p < 0.05$) 8 hours after provocation. In brush samples from the allergic patients challenged with allergen, the numbers of metachromatic cells increased to a maximum of eightfold at 10 hours. In the lavage specimens, no metachromatic cells were observed before provocation, but they progressively increased in number 2 to 12 hours after provocation. Cell pellet histamine content decreased temporarily 2 to 4 hours after challenge ($p < 0.05$) in brush samples from allergen-challenged allergic patients. The local metachromatic cell density before challenge, as reflected in the brush specimens, correlated with nasal congestion, sneezing, and the degree of eosinophilia. (J ALLERGY CLIN IMMUNOL 1992;90:898-909.)

Key words: Allergic rhinitis, allergen challenge, mast cells, eosinophils, histamine, symptoms, nasal mucosal cells, nasal lavage, brush samples

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The eosinophils and mast cells have been implicated in the pathogenesis of mucosal allergy because of their content of IgE-receptors and putative mediators of allergy and because of the observation of changes in their numbers, distribution, and/or location in relation to allergy or allergen stimulation. Thus the number of eosinophils on epithelial surfaces increases rapidly as a result of allergen exposure whether in the laboratory or naturally.¹⁻⁶ Furthermore, there appears to be a correlation between the degree of eosinophilia and the degree of nasal symptoms during natural allergen exposure.¹ Although biochemical data to support the activation of the eosinophils during such conditions have been presented,^{3, 7, 8} morphologic evidence of such an activation is still lacking.

In patients with hay fever natural allergen exposure results in the redistribution of mast cells from the subepithelial stroma into the epithelium and toward the epithelial lining.^{1, 2, 6, 9-13} When the imprint technique is being used, which collects cells from the epithelial surface, mast cells could not be observed until after 4 to 5 days of natural allergen exposure.¹ Similar results were obtained in challenge experiments.² With use of a brush technique to harvest cells from the epithelium proper, an increase in metachromatic cells, interpreted as mast cells, was seen just 24 hours after the allergen challenge.^{2, 14} An intriguing observation from a study of seasonal allergic rhinitis was the finding of a positive correlation between the local mast cell density before the start of the pollen exposure and the severity of symptoms experienced during the pollen season.¹²

The aim of the present study was to investigate the kinetics of the cellular events during the first 12 hours after antigen exposure. Furthermore, we wanted to explore the theory that superficial mast cell density is an expression of the severity of the allergic disease.

PATIENTS AND METHODS

Study groups

Ten allergic patients (nine men and one woman; 20 to 31 years of age; mean age, 25.0 years) were challenged with allergen. For control, seven allergic patients (six men and one woman; 23 to 39 years of age; mean age 29.9 years) were challenged with the diluent, and seven non-allergic subjects (three men and four women; 22 to 41 years of age; mean age, 31.3 years) with negative skin test results and without nasal symptoms were challenged with allergen. All the allergic patients had strictly seasonal allergic rhinitis confirmed by history and a positive skin prick test outcome to timothy pollen and negative outcome to a standard panel of airborne allergens including molds, mites, cat, and birch pollen allergens. They had no other manifestations of their allergic disease such as bronchial or dermal symptoms and were asymptomatic at the start of the study. The study was performed during the pollen-free winter months and was approved by the Ethics Committee of the University of Göteborg.

The allergen challenge

The study was designed as an open investigation. Both nasal cavities were challenged with one spray of timothy pollen allergen, 10,000 Biological Units (BU)/ml, or the diluent (Pharmalgen, Pharmacia Diagnostics Norden AB, Uppsala, Sweden). The mechanical pump delivered 0.13 ml in each spray.

Symptom registration

Nasal symptoms were noted before the provocation and 15 minutes and 2, 4, 6, 8, 10, and 12 hours after the

provocation. The symptoms recorded were nasal congestion, rhinorrhea, and sneezing. The degree of nasal congestion and rhinorrhea was graded by each participant on a scale of 0 to 4, where none = 0, mild = 1, moderate = 2, severe = 3, and intolerable = 4. The total number of sneezes between each recording was registered. Furthermore, a composite symptom score was calculated as the sum of the scores for nasal congestion, rhinorrhea, and sneezing. The sneeze score was calculated as follows: 0 sneezes = 0, 1 to 4 sneezes = 1, 5 to 9 sneezes = 2, 10 to 19 sneezes = 3, and 20 or more sneezes = 4.

Cell sampling techniques

Samples were taken before provocation and 2, 4, 6, 8, 10, and 12 hours after provocation with use of the lavage and brush techniques.

The nasal lavage method. The nasal lavage was performed with use of a compressible plastic container equipped with a nozzle and containing 12 ml of sterile saline with 0.1% of human serum albumin.¹⁵ With the nozzle inserted into one nostril, the saline was squeezed into the nasal cavity and allowed to return into the device. This was repeated once, and the entire procedure was then repeated for the other nostril. The recovered solution was centrifuged at 1200 rpm for 10 minutes. All the supernatant was discarded, except for 1 ml in which the cell pellets were resuspended. Cell preparations were prepared by cytocentrifugation, and the remaining cell suspension was used for the analysis of histamine content (see herein).

The brush method. A commercially available 5.5 mm diameter nylon brush (Doft AB, Östhammar, Sweden) was used for cell sampling.¹⁶ The brush was used on alternate sides starting with the left nasal cavity. In brief, the brush was placed between the nasal septum and the inferior turbinate and removed gently. No anesthesia was used. After sampling, the brush was immediately placed in a 3 ml plastic tube containing 2 ml of buffered salt solution (Hanks' solution containing 0.1% of serum albumin). The brush was then shaken vigorously in the solution and carefully brushed off against the wall of the tube. Cell preparations were obtained by cytocentrifugation (see herein). The remaining suspension (1.1 ml) was then centrifuged for 10 minutes at 1200 rpm, the supernatant was discarded, and the histamine content of the pellets was determined as described in the following paragraphs.

Morphologic techniques

Aliquots (100 μ l) of the cell suspension were loaded into a cytocentrifuge (Cytocentrifuge II, Shandon Southern Products Ltd., Cheshire, England), spun for 7 minutes at 750 rpm, and air dried. One specimen from each sample was stained with the Giemsa stain, and one was fixed with ethanol-acetic acid (three parts ethanol and one part acetic acid) for 15 to 60 minutes, and this was followed by staining with toluidine blue at pH 0.5 for 30 minutes.¹⁷ Light microscopy was undertaken on coded specimens.

The total number of cells in a single high-power field

TABLE I. Selected morphologic data and symptom scores from the 10 allergic patients challenged with allergen

Patient	Metachromatic cells				Eosinophils			
	Brush		Lavage		Brush		Lavage	
	Before	Max v	Before	Max v	Before	Max v	Before	Max v
A	12	384	0	62	11.8	31.0	4.7	25.7
F	56	212	0	14	26.4	45.5	0.0	46.0
I	19	171	0	53	4.7	36.2	2.7	17.9
B	0	5	0	2	0.0	13.5	0.0	19.5
C	4	2	0	0	0.0	10.3	14.0	33.3
D	0	4	0	0	0.0	10.0	0.0	10.1
E	1	46	0	8	0.0	7.7	0.5	9.7
L	4	8	0	7	0.7	1.6	0.7	6.6
M	2	5	0	3	0.0	4.4	0.0	3.1
N	3	14	0	1	0.0	2.6	0.0	1.1

Baseline values (*before*) and the highest value (*max v*) for the total number of metachromatic cells per slide and the percentage of eosinophils of all granulocytes in cytopsin specimens from brush and lavage samples are shown. Patients' scores of nasal blockage (*BI*) and rhinorrhea (*Rh*), the total number of sneezes (*Sn*), and composite symptom score (*CSS*) for the first 15 minutes after the allergen challenge are also shown. Patients A, F, and I had substantial numbers of inflammatory cells in the samples taken before the allergen challenge, and their reaction to the challenge was in all parameters much stronger than for the other patients.

($\times 400$) was counted in the Giemsa-stained specimens. The diameter of one high-power field is one fourteenth of the diameter of the cell blanket on the cytopsin specimen.

Two hundred cells in the Giemsa-stained cytopsin preparations were differentiated into three groups. The first group comprised noneosinophilic granulocytes, which consisted in the main of neutrophils and will therefore be referred to as neutrophils. The second group was eosinophils. The third group comprised mononuclear cells, a group that consisted primarily of epithelial cells and will therefore be referred to as epithelial cells. Furthermore, eosinophils were divided into two groups; nonvacuolated and vacuolated eosinophils, the latter defined as cells with eosinophilic granules and two or more cytoplasmic vacuoles.¹⁸ In samples with few eosinophils a minimum of 20 cells was attained.

In the specimens stained with toluidine blue, the total number of cells with metachromatic granules was counted on each specimen.

Chemical assays

The histamine content of the cell pellets was determined with use of a technique previously described in detail.¹⁹ In brief, the cell pellets were resuspended in 250 μ l of 0.04 mol/L perchloric acid. After freezing and thawing, the specimens were neutralized with potassium carbonate with the precipitation of potassium perchlorate. Histamine was purified by reverse-phase, high-performance liquid chromatography with use of a C18 column with 15 mmol/L citrate buffer at pH 3 containing 5% methanol as a mobile phase and pentane sulfonic acid (5 mmol/L) as the counter ion. Histamine was detected and assayed with a two-stage, post-column, derivative-forming procedure with use of *o*-phthal-

aldehyde as the fluorescent reagent. The limit of detection for the assay is 1 ng/ml.

Statistical analysis

The statistical evaluation was performed on a microcomputer with a statistical software package (StatView II, Abacus Concepts, Berkeley, Calif.). An analysis of variance (ANOVA) was used to evaluate whether observations made at the different times differed from one another. If a statistically significant difference was found, a further analysis was made by means of the Wilcoxon signed rank sum test and Mann-Whitney U test. Spearman's correlation coefficient test was used to evaluate correlations between the different observations, *p* values of <0.05 were considered significant.

RESULTS

The primary analysis of the collected experimental data revealed that three of the patients challenged with allergen differed from the remaining seven in that their brush samples obtained before allergen challenge contained a comparatively large number of metachromatic cells and eosinophils. It was also apparent that these three patients had intense symptoms after challenge plus an intense eosinophil response after 4 to 10 hours and a pronounced mast cell response 10 to 12 hours after challenge. The relevant individual data are presented in Table I.

Symptoms

The patients reacted promptly to the allergen challenge (Fig. 1). The highest symptom score was ob-

Symptoms			
15 min after challenge			
BI	Sn	Rh	CSS
3	30	3	10
3	35	3	10
3	21	2	9
2	0	2	4
2	8	2	6
2	9	2	3
0	1	0	1
2	1	1	4
2	1	1	4
1	5	0	3

served 15 minutes after the provocation. The symptoms then decreased, and 10 hours after the provocation the patients had largely recovered their initial nasal status. Two of the patients, those with the highest numbers of metachromatic cells in the prechallenge samples, exhibited a late-phase reaction. The controls did not respond to the challenge at all. Only minimal discomfort occurred as a result of the cell sampling procedures.

Cellular findings

The cytospin preparations contained evenly distributed cells of good morphology. The total number of cells per slide was of the order of $5 \pm 1 \times 10^3$ (mean \pm SEM) in lavage samples and $20 \pm 1 \times 10^3$ in brush samples. The mean values for the different individuals varied from $2 \pm 0.5 \times 10^3$ cells (mean \pm SEM) to $25 \pm 10 \times 10^3$ cells for the lavage samples and from $8 \pm 3 \times 10^3$ cells to $40 \pm 10 \times 10^3$ cells for the brush samples. No significant differences occurred in total number of cells between the different sampling times, and no difference occurred between different study groups irrespective of the cell sampling method.

Neutrophils and epithelial cells could be identified in all the samples that were obtained. The percentage of neutrophils in the allergic patients challenged with allergen was the same as that in the control groups in overall terms. In the first two brush samples the neutrophils were significantly fewer than during the rest of the observation time (27% to 24% and 42% to 59%, respectively; $p < 0.05$). In the lavage samples the mean percentage of neutrophils varied from 64% to 82% and showed no significant changes.

In prechallenge samples, no differences occurred

in eosinophils between allergic patients challenged with allergen and allergic patients challenged with diluent, but the nonallergic controls had only very few eosinophils (Figs. 2 and 3). No increase occurred in eosinophils in samples from the two control groups. In specimens obtained from the allergic patients challenged with allergen, there was an increase in eosinophils, expressed as a percentage of the total number of granulocytes. In lavage samples, this increase was statistically significant if compared with prechallenge values as well as control groups already at 2 hours after challenge and in brush samples at 4 hours after challenge.

The evaluation of light-microscopic signs of eosinophil activation showed that the eosinophils exhibited varying numbers of granules as well as cytoplasmic vacuoles of varying size. Many of the cells contained large vacuoles as described by Tai and Spry.¹⁸ Before challenge no difference occurred in vacuolization between the groups. In brush samples from allergic patients challenged with allergen, the percentage of vacuolated eosinophils increased from $20\% \pm 12\%$ (mean \pm SEM) at baseline to a maximum of $72\% \pm 10\%$ 8 hours after the provocation ($p < 0.05$, Fig. 4). In lavage samples from allergic patients challenged with allergen, the percentage of vacuolated eosinophils varied from 28% to 57% and showed no significant changes. In samples from the controls the percentage of vacuolated eosinophils remained low during the observation period.

The specimens stained with toluidine blue were used for the specific enumeration of metachromatic cells. Staining at pH 0.5 results in a violet metachromasia of granules against a faint background, thus facilitating the identification of these cells. In the light microscope they had a variable shape and degree of granulation. Most of the cells in the prechallenge samples contained round or oval nuclei, indicating a mast-cell nature, but a few of the metachromatic cells in the 6- to 12-hour samples had lobulated nuclei, suggesting a basophil nature. Considerable interindividual variation was observed in the numbers of metachromatic cells, as can be seen in Table I.

In samples from the allergic patients challenged with allergen, the mean numbers of metachromatic cells in the brush samples decreased from 10.1 ± 5.4 (mean \pm SEM) in the prechallenge samples to 4.4 ± 2.2 in the 2-hour samples ($p < 0.05$, Fig. 5). During the late observation hours there was a significant increase in the numbers of metachromatic cells to a maximum of 79.7 ± 39 in the 10-hour samples ($p < 0.05$). In the lavage specimens from the same group, no metachromatic cells were seen before the provocation, but a few were discerned after the prov-

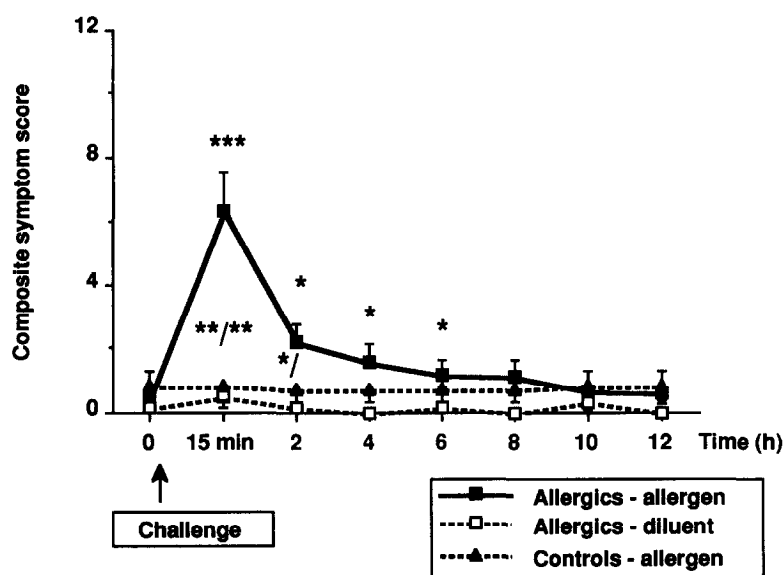


FIG. 1. The nasal symptom score (mean \pm SEM) before, 15 minutes after, and at 2-hour intervals during the following 12 hours after a nasal challenge. Highest score = 12. Statistical significance of observed differences indicated by *asterisks* (* p < 0.05; ** p < 0.01; and *** p < 0.001). *Asterisks* above the graph indicate comparison with the baseline value for the allergic patients challenged with allergen. *Asterisks* between graph lines indicate comparison between study groups: allergic patients challenged with allergen compared with allergic patients challenged with diluent before the oblique stroke and allergic patients challenged with allergen compared with controls challenged with allergen after the oblique stroke.

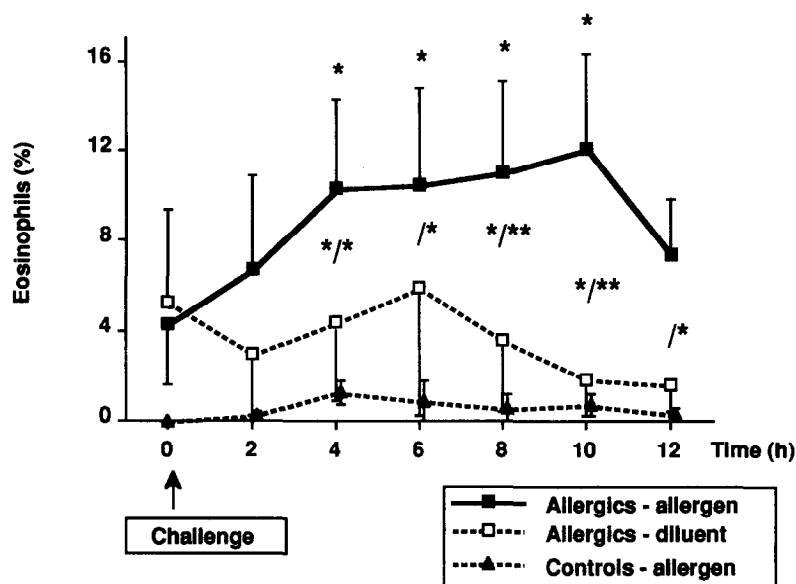


FIG. 2. The proportion of eosinophils in all the granulocytes (mean \pm SEM) in the brush specimens taken before (0 hour) and at 2-hour intervals up to 12 hours after a single nasal challenge. Statistical significance of observed differences indicated by *asterisks* (* p < 0.05 and ** p < 0.01). *Asterisks* above the graph indicate comparison with the baseline value for the allergic patients challenged with allergen. *Asterisks* between graph lines indicate comparison between study groups: allergic patients challenged with allergen compared with allergic patients challenged with diluent before the oblique stroke and allergic patients challenged with allergen compared with controls challenged with allergen after the oblique stroke.

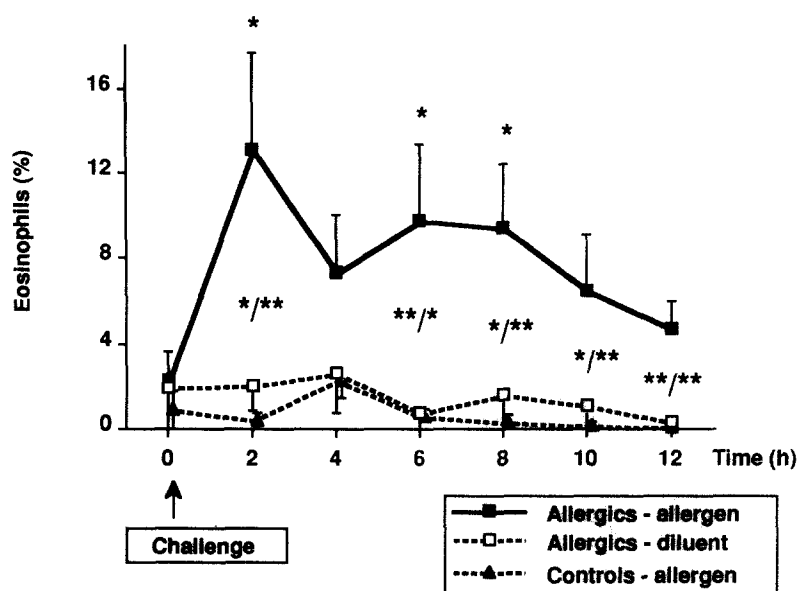


FIG. 3. The proportion of eosinophils in all the granulocytes in the returned nasal lavage fluid. Statistical significance of observed differences indicated by *asterisks* (* $p < 0.05$ and ** $p < 0.01$). *Asterisks* above the graph indicate comparison with the baseline value for the allergic patients challenged with allergen. *Asterisks* between graph lines indicate comparison between study groups: allergic patients challenged with allergen compared with allergic patients challenged with diluent before the oblique stroke and allergic patients challenged with allergen compared with controls challenged with allergen after the oblique stroke.

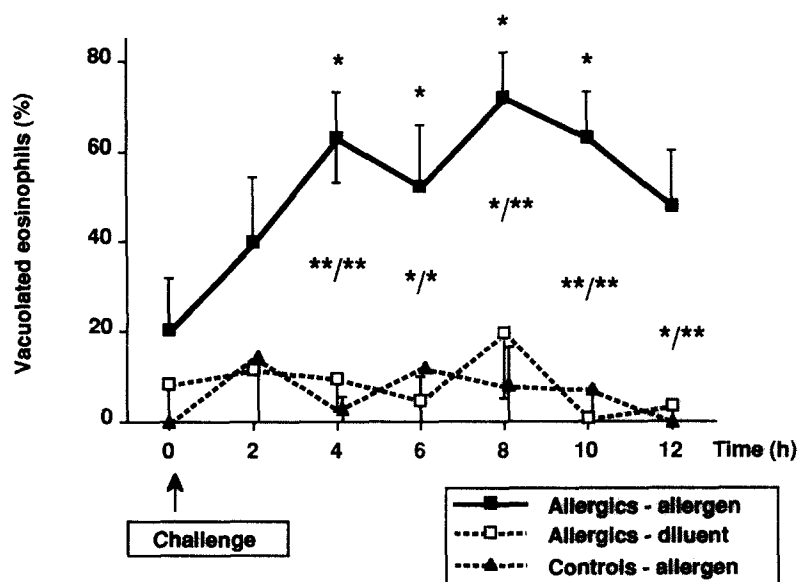


FIG. 4. The percentage of vacuolated eosinophils in all the eosinophils in brush samples. Statistical significance of observed differences indicated by *asterisks* (* $p < 0.05$ and ** $p < 0.01$). *Asterisks* above the graph indicate comparison with the baseline value for the allergic patients challenged with allergen. *Asterisks* between graph lines indicate comparison between study groups: allergic patients challenged with allergen compared with allergic patients challenged with diluent before the oblique stroke and allergic patients challenged with allergen compared with controls challenged with allergen after the oblique stroke.

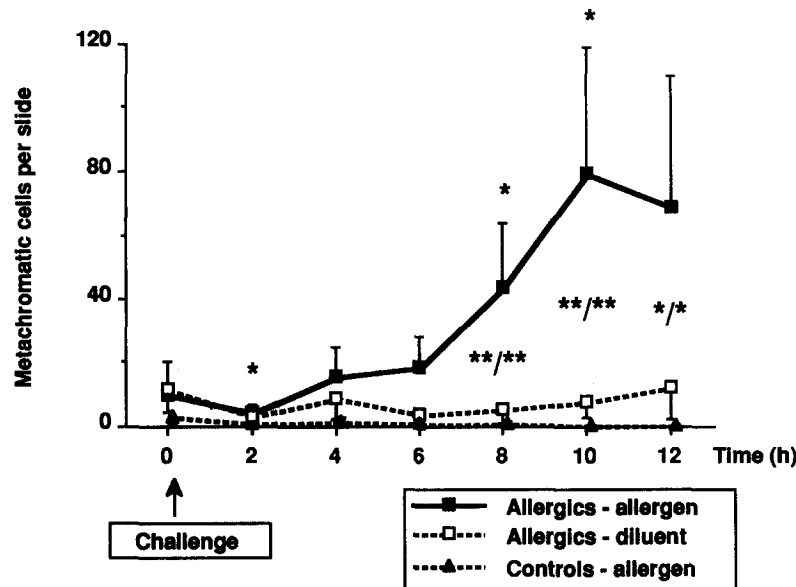


FIG. 5. The number of metachromatic cells in brush samples. Statistical significance of observed differences indicated by asterisks (* $p < 0.05$ and ** $p < 0.01$). Asterisks above the graph indicate comparison with the baseline value for the allergic patients challenged with allergen. Asterisks between graph lines indicate comparison between study groups: allergic patients challenged with allergen compared with allergic patients challenged with diluent before the oblique stroke and allergic patients challenged with allergen compared with controls challenged with allergen after the oblique stroke.

ocation, and their numbers increased to a maximum of 14.3 ± 7.3 (mean \pm SEM; $p < 0.05$) 12 hours after the provocation ($p < 0.05$, Fig. 6).

The control groups' specimens, from both the lavage and the brush samples, showed only a few metachromatic cells and eosinophils.

Histamine

Measurable quantities of histamine were in all but one of the brush samples from allergic patients challenged with allergen, taken before the provocation. A temporary decrease occurred in cell pellet histamine from 2 to 4 hours ($p < 0.05$), followed by a recovery at the later observation times (Fig. 7). The histamine content in brush samples was below the detection limit for the method in 43 of 70 samples from allergic patients challenged with allergen, in 32 of 49 samples from the allergic patients challenged with the diluent, and in 43 of 49 samples from the nonallergic controls.

The histamine content of most of the lavage specimens was below the detection limit for the method.

The histamine content per metachromatic cell before and after provocation was estimated. Because the bias in the histamine/mast-cell quotient is inversely proportionate to the number of metachromatic cells (the denominator), and in many samples these cells were very few, the means for the whole study groups could be unrealistic. Advantage was taken of the fact that the brush samples from three of the patients chal-

lenged with allergen contained significant numbers of metachromatic cells before the provocation. The mean histamine content per metachromatic cell in these three patients, estimated from the histamine/mast-cell quotients, was 15.7 ± 5.4 pg/cell (mean \pm SEM, $n = 3$) prior to challenge. After the provocation, the ratio temporarily decreased in the 2-hour and 4-hour samples to a minimum of 2 pg/cell.

Relationships

In specimens taken from the allergic patients challenged with allergen, a strong statistical correlation occurred between the eosinophil numbers with the two different cell-sampling techniques ($r_s = 0.545$; $n = 70$; $p < 0.0001$; Fig. 8). A statistically significant correlation ($r_s = 0.684$; $n = 70$; $p < 0.0001$) between the findings relating to metachromatic cell numbers when comparing the brush and lavage techniques. As expected, a statistically significant correlation occurred between metachromatic cell numbers and the histamine content of the cell pellets obtained with use of the brush procedure ($r_s = 0.639$; $n = 70$; $p < 0.0001$).

To further evaluate any relationship between the cellular findings and the symptoms in the allergic patients challenged with allergen, a correlation analysis was made. The baseline findings in mast cell and eosinophil numbers were related to the peak symptoms observed (the 15-minute observation) and the peak

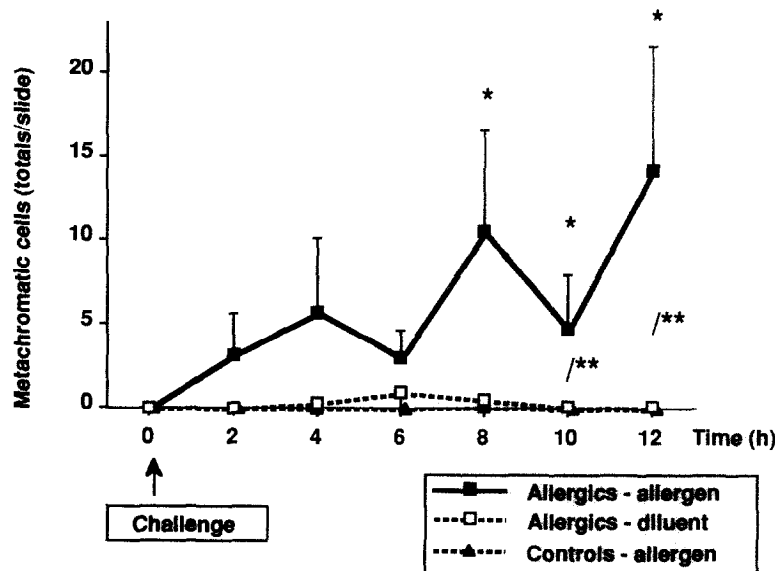


FIG. 6. Metachromatic cells in the lavage specimens. No metachromatic cells were seen before provocation. Statistical significance of observed differences indicated by *asterisks* (* $p < 0.05$ and ** $p < 0.01$). *Asterisks* above the graph indicate comparison with the baseline value for the allergic patients challenged with allergen. *Asterisks* between graph lines indicate comparison between allergic patients challenged with allergen to controls challenged with allergen.

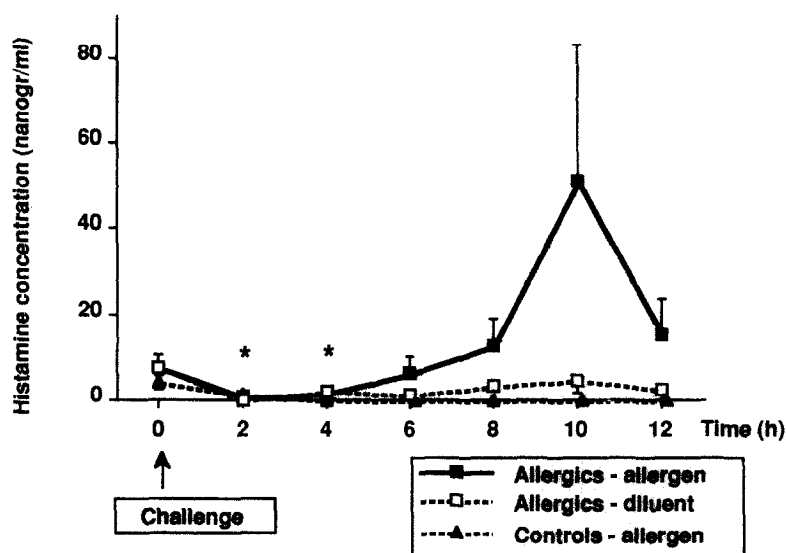


FIG. 7. Histamine concentration in resuspended cell pellets from brush samples. The pellets were resuspended in 250 μ l of 0.04 mol/L perchloric acid. Statistical significance of observed differences indicated by *asterisks* (* $p < 0.05$). *Asterisks* indicate comparison with the baseline value for the allergic patients challenged with allergen.

eosinophil count (the highest value for each individual) obtained after the provocation (Table II). A significant correlation occurred between the basal value for metachromatic cells in the brush samples and the basal value of eosinophils ($r_s = 0.867$; $n = 10$; $p < 0.01$), congestion ($r_s = 0.727$; $n = 10$; $p < 0.05$) and the number of sneezes ($r_s = 0.733$;

$n = 10$; $p < 0.05$) 15 minutes after the allergen challenge.

DISCUSSION

In this study we provide evidence that the exposure of the nasal mucosa to allergen is followed by an increase in local eosinophil and metachromatic cell

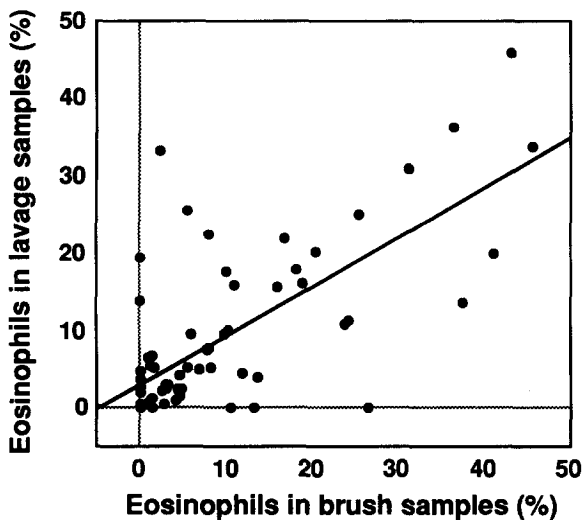


FIG. 8. Spearman correlation coefficient test of eosinophil numbers (percentage of all granulocytes) by use of the two different cell-sampling techniques shows a strong statistical correlation ($r_s = 0.545$; $n = 70$; $p < 0.0001$).

density. Furthermore, we demonstrate that the eosinophils show structural signs of activation, and in samples with representative numbers of metachromatic cells a reduction occurs in the histamine/metachromatic cell quotient, indicating the activation of these cells after allergen challenge. The crucial role of mast cells in the initiation of symptoms is also supported by the finding of a strong positive correlation between the metachromatic cell density in specimens obtained before allergen challenge and the symptoms that followed.

When studying the kinetics of putative cellular changes in the nasal mucosa, nontraumatic cell sampling techniques are preferable, since repeated samples are required. The lavage technique collects cells floating on a large part of the nasal mucosal surface. The lavage is performed without any contribution of cells outside the nasal cavity.¹⁵ The brush technique harvests a proportion of cells within the epithelium proper and provides a fairly constant cell yield.¹⁶ Both techniques are nontraumatic, do not require anesthetics, are easily tolerated, and can be used for quantitative evaluation. The difference in cell yield between the lavage and brush technique is reflected in the finding in the allergic patients challenged with allergen of only single or scant metachromatic cells in the lavage samples, whereas most brush specimens contained metachromatic cells. This can be explained by the fact that the metachromatic cells are associated with the epithelial cell layer and are seldom found floating on the surface proper.² The high degree of correlation between eosinophil numbers in the lavage

and brush specimens from allergic patients challenged with allergen shows that both techniques are useful when it comes to demonstrating changes in nonstationary inflammatory cells.

The percentage of epithelial cells and neutrophils was fairly constant throughout the study in lavage samples in which most cells were neutrophils. In the brush samples, the first brush samples from each nasal cavity (the baseline and the 2-hour samples) contained epithelial cells in abundance, but neutrophils were in the majority during the rest of the study. The finding of high epithelial cell counts in the first brush sample can be interpreted as an expression of the first crop effect, harvesting loose epithelial cells on the mucosal surface. On the other hand, the increase in neutrophils could also be interpreted as a sign of sampling trauma, as studies of experimental virus infections have shown that the sampling trauma from curette scraping as such can cause an outpouring of neutrophil granulocytes after 24 hours.²⁰

We found a rapid and significant increase in local eosinophil density in the allergic nasal mucosa as a response to the allergen exposure. This increase was noted as early as 2 hours after the provocation in the lavage specimens and after 4 hours in the brush samples. This resilient and roving nature of the eosinophils confirms the findings of several previous studies in which nasal mucosal cells were harvested with smear, brush, lavage, and imprint techniques.¹⁻⁶

Tissue and blood eosinophilia have been regarded as a hallmark of allergy, but the degree of activation of the eosinophils in vivo in humans and their putative role in allergic inflammation is still largely speculative.²¹ Although monoclonal antibodies to activated eosinophils have been used to demonstrate the activation of eosinophils in tissue sections,²² only limited attempts have been made to study the morphologic signs of eosinophil activation in vivo in humans during the allergic inflammation. The in vitro challenge of eosinophils has shown that vacuoles are a sign of activation and an ongoing secretory process.^{18, 23-25} In this study 20% of the eosinophils had such morphologic signs of activation before challenge. The allergen challenge of the allergic patients triggered not only a rapid influx of eosinophils but also a pronounced increase in the number of activated eosinophils, so that most of the cells harvested 8 hours after the challenge had cytoplasmatic vacuoles. These morphologic findings support studies that have provided biochemical evidence of eosinophil activation as part of the allergic disease process. Bascom et al.⁸ studied major basic protein and eosinophil-derived neurotoxin in nasal mucosal lavage specimens and were able to show a local increase in these proteins after allergen chal-

TABLE II. Correlation matrix between values of cellular findings and symptoms in 10 allergic patients challenged with allergen

<i>n</i> = 10	MC (brush) basal value	EOS (brush) basal value	EOS (brush) max value	EOS (lavage) basal value	EOS (lavage) max value	Congestion at 15 min	Sneezes at 15 min	Rhinorrhea at 15 min	Histamine basal value
MC (brush) basal value	1								
EOS (brush) basal value	0.867**	1							
EOS (brush) max value	0.491	0.627	1						
EOS (lavage) basal value	0.503	0.403	0.224	1					
EOS (lavage) max value	0.448	0.548	0.855**	0.394	1				
Congestion at 15 min	0.727*	0.858*	0.782*	0.370	0.673*	1			
Sneezes at 15 min	0.733*	0.752*	0.648	0.273	0.558	0.800*	1		
Rhinorrhea at 15 min	0.500	0.697*	0.852*	0.294	0.876**	0.888**	0.755*	1	
Histamine basal value	0.567	0.521	0.452	-0.070	0.167	0.385	0.527	0.261	1

The baseline findings in metachromatic cell and eosinophil density were related to the peak symptoms observed (the 15-minute observation) and the peak eosinophil count (the highest value for each individual) obtained after the provocation. A significant correlation occurred between the basal value for metachromatic cells in the brush samples and the basal value of eosinophils, congestion, and the number of sneezes 15 minutes after the allergen challenge. * $p < 0.05$, ** $p < 0.01$. MC, Mast cell numbers; EOS, eosinophil numbers; Basal value, values from samples taken before the provocation, Max value, peak value.

lenge, with the peak within a similar time frame as that found for the eosinophils in the present study.

The increase in metachromatic cells on the mucosal surface at the late points of observation in both the lavages and the brush samples supports our previous observations that a redistribution of mast cells toward the mucosal surface is part of the allergic process in mucous membranes.^{2, 9, 10} In brush samples we found a decrease in the 2-hour samples and an eightfold increase in the numbers of metachromatic cells 10 hours after a single allergen challenge. Previous results have shown as much as a 12-fold increase in the

numbers of metachromatic cells 24 hours after a single allergen challenge.¹⁴ Our earlier findings have indicated that most metachromatic cells are mast cells rather than blood basophils. This conclusion was based on light-microscopic morphology and ultrastructural observations as well as on the finding of a naphthol-AS-D chloroacetate esterase activity in the metachromatic cells. An influx of metachromatic cells was previously observed in nasal lavage specimens 8 to 12 hours after allergen challenge as part of the late-phase response. In this case the cells were interpreted as basophils on the basis of their light-microscopic

morphology.²⁶ It is of considerable biologic interest to be able to decide the role of these two cell types in mucosal allergy. Further studies are under way to further elucidate the role of mast cells and basophils in the allergic mucosal response.

When exploring the individual findings, it was evident that three patients challenged with allergen differed from the others. Some selected observations are shown in Table I. The most prominent finding was substantial numbers of metachromatic cells and eosinophils in the prechallenge samples, whereas the other patients had only a few such cells. The existence of metachromatic cells in the epithelium has been interpreted as a sign of the activation of the IgE-mediated immune system.²⁷ It is of interest to note that the three patients with substantial numbers of inflammatory cells in their prechallenge brush samples were those who showed the strongest response to the allergen challenge, two of the patients exhibited signs of late-phase reaction. Taken as a whole, this indicates that these subjects could have been "primed" in some way before challenge. Although all the patients had a history of and a skin test indicating an isolated seasonal grass pollen allergy and were asymptomatic at the start of the study, it is plausible that priming might have existed in the form of a subclinical sensitization either to some perennial allergen or to the low air pollen contents of the trees pollinating in early spring, alder (*Alnus*) and hazel (*Corylus avellana*). The three patients might also represent a subgroup of more pronounced allergic disease, without being immunologically activated before challenge.

The histamine/metachromatic cell quotient in the prechallenge brush samples was of the same magnitude as that previously reported.² The allergen challenge resulted in significantly lower histamine cell pellet contents and histamine/metachromatic cell quotient. This was taken as evidence of mast-cell activation and the loss of intracellular histamine. This cellular histamine depletion is in accordance with previous studies where this determination was made 24 hours after the allergen challenge.^{2, 12} It is interesting to note such dramatic changes in histamine and the fact that the decrease in the histamine content of the cells approximately paralleled the typical symptoms experienced by the patients after the provocation. We have interpreted the initial change in quotients as secretory activity in the existing mast cell population, although we cannot exclude the possibility that during the later observation hours an influx occurs of new metachromatic cells with lower histamine content as proposed by Bascom et al.²⁶

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Complementary DNA cloning and expression in *Escherichia coli* of *Aln g I*, the major allergen in pollen of alder (*Alnus glutinosa*)

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Previous data showed that the major pollen allergens from trees of the order Fagales, in particular alder, birch, hazel, and hornbeam, are highly interrelated. As only the complete amino acid sequence of Bet v I, the major allergen from birch, has been known, it was of interest to obtain the primary structure of other major allergens of this group, to attribute IgE-binding properties to certain features of the amino acid sequences of those allergens. cDNA was synthesized from alder pollen mRNA, sequence-specifically amplified by polymerase chain reaction and cloned into plasmid bluescript. Comparison of the deduced amino acid sequences of Aln g I and Bet v I revealed a 86.8% homology. The Aln g I encoding cDNA was subcloned into pKK223-3 and expressed in Escherichia coli as a full-length nonfusion protein. The recombinant Aln g I bound IgE from tree pollen-allergic patients and was shown to share IgE-epitopes with Bet v I by inhibition studies with recombinant Bet v I. Computer-aided calculations predicted epitopes in both Aln g I and Bet v I at the same position; the Bet v I molecule was predicted to possess two additional epitopes near the N-terminus of the molecule. (J ALLERGY CLIN IMMUNOL 1992;90:909-17).

Key words: Alder pollen, major allergen, *Aln g I*, cDNA cloning, expression, recombinant nonfusion protein, IgE-binding, epitopes

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