Allergic rhinitis to grass pollen: Measurement of inflammatory mediators of mast cell and eosinophils in native nasal fluid lavage and in serum out of and during pollen season

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Background: In allergic rhinitis, mast cells, activated by cross-linking of allergen to mast cell–bound specific IgE, release both vasoactive mediators related to the early nasal symptoms and chemotactic mediators that attract inflammatory cells, such as eosinophils, related to the late-phase response.
Objective: We have analyzed, during and out of pollen season, in blood and nasal fluid from patients allergic to grass pollen, histamine and tryptase to monitor the early phase markers and eosinophil and eosinophil cationic protein (ECP) to monitor the late phase.
Methods: Twenty patients were enrolled in the study. As a control, we studied 10 nonatopic subjects. Mediators and eosinophils were assessed in blood and nasal fluid. Histamine was tested only in nasal fluid.
Results: During pollen season, tryptase but not histamine increased in nasal fluids from patients (2.96 vs 0.22 U/ml, $p = 0.001$) and correlated with symptom scores ($r_s = 0.63$, $p = 0.003$). Tryptase was not detected in serum. Eosinophils increased in nasal cytology (17.0% vs 2.0%, $p = 0.001$) and in the blood (26.5 vs 12.7 × 10^6 L, $p = 0.001$) from patients, but they did not correlate with symptom scores. ECP increased only in the nasal lavage (16.33 vs 1.30 ng/ml, $p = 0.001$) and correlated with symptom scores ($r_s = 0.53$, $p = 0.016$).
Conclusions: Both ECP and tryptase increase in nasal secretion in natural disease. Therefore the measurement of tryptase and ECP levels in nasal fluid might be a useful clinical test for monitoring disease activity and the effects of therapeutic agents. (J Allergy Clin Immunol 1997;100:832-7.)

Key words: Eosinophils, eosinophil cationic protein, histamine, tryptase, nasal lavage fluid, grass pollen

Abbreviations used
ECP: Eosinophil cationic protein
SPT: Skin prick test

The pathophysiology of allergic rhinitis is characterized by ongoing tissue inflammation that follows the classic type I immediate hypersensitivity reaction.[1, 2] The activation and recruitment of effector cells causes morphologic changes, hyperreactivity of target tissue, and, finally, clinical symptoms. The role of mast cells and eosinophils has been strongly inferred both through the increased numbers of these cells locally during periods of disease exacerbation and through chemical evidence of participation documented by the presence of their specific products in nasal fluids of patients during active disease.[3-8]

After surface IgE cross-linking with allergen, there are several morphologic changes within the mast cells that release several mediators responsible of vascular permeability, vasodilatation, and mucous secretion. In many sensitized subjects, this early phase is then followed by the recrudescence or development of symptoms some hours later. This late phase is associated with recruitment of eosinophils that (at least in asthma) may damage epithelial cells and other structures through the release of toxic constituents of their granules.[9, 10] However, both in rhinitis[11] and in asthma,[12] treatment with topical corticosteroids may lead to a depletion of mucosal eosinophils of the nose and, respectively, of the bronchi, which is associated with clinical improvement.

For some years, commercial assays have been available to quantify specific products of these inflammatory cells in blood as well as in local fluids close to the inflammatory process, for example, in nasal lavage fluid.

In this study, to gain insight into the natural history of allergic rhinitis, we have analyzed, in blood and nasal fluid from patients allergic to grass pollen, histamine and tryptase as mast cell markers and eosinophil cationic protein (ECP) as a marker for activated eosinophils. Patients were studied during and out of pollen season, and results were compared with those obtained in non-

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TABLE I. Mediators in nasal fluid lavage

	Healthy subjects		Subjects with allergic rhinitis	
	Out of pollen season[a]	During pollen season[b]	Out of pollen season[c]	During pollen season[d]
Histamine (ng/ml)	22.7 (18.6-26.9)	22.6 (20.0-26.8)	24.4 (19.3-27.1)	26.0 (18.4-31.8)
Tryptase (U/ml)	0.21 (0.12-0.23)	0.21 (0.12-0.23)	0.22 (0.14-0.26)	2.96 (2.57-4.12)
ECP (ng/ml)	1.10 (1.08-1.23)	1.14 (1.07-1.30)	1.30 (1.14-1.52)	16.33 (10.33-25.08)

Significant differences: Histamine: **a** vs **b**, p = NS; **a** vs **c**, p = NS; **c** vs **d**, p = NS; **b** vs **d**, p = NS. Tryptase: **a** vs **b**, p = NS; **a** vs **c**, p = NS; **c** vs **d**, p = 0.001; **b** vs **d**, p = 0.001. ECP: **a** vs **b**, p = NS; **a** vs **c**, p = NS; **c** vs **d**, p = 0.001; **b** vs **d**, p = 0.001.

TABLE II. Eosinophils in nasal scraping and in blood

	Healthy subjects		Subjects with allergic rhinitis	
	Out of pollen season [a]	During pollen season[b]	Out of pollen season[c]	During pollen season[d]
In nasal scraping (%)	1.5 (1.0-2.0)	2.0 (1.0-2.0)	2.0 (1.0-2.0)	17.0 (11.5-26.0)
In blood (cells $\times$ 10^6 L)	13.5 (10.0-19.0)	14.0 (11.0-18.0)	12.7 (11.5-15.5)	26.5 (19.0-30.0)

Significant differences: Eosinophils in nasal scraping: **a** vs **b**, p = NS; **a** vs **c**, p = NS; **c** vs **d**, p = 0.001; **b** vs **d**, p = 0.001. Eosinophils in blood: **a** vs **b**, p = NS; **a** vs **c**, p = NS; **c** vs **d**, p = 0.001; **b** vs **d**, p = 0.001.

allergic subjects. Furthermore, data were related to the severity of symptoms.

METHODS

Subjects

Twenty patients (10 men and 10 women, 23 to 38 years of age, mean age 28.8 ± 3.8) with strictly seasonal allergic rhinitis to grass pollen entered the study. The study was performed out of and during the pollen season when the patients were symptomatic. Their allergy had been verified by their medical history, a skin prick test (SPT), and RAST. The patients had no symptoms of asthma, urticaria, or eczema, and their symptoms were present only during the pollen season for at least 4 years before the study. All patients during the previous pollen season had taken topical steroids for controlling their symptoms. At the time of the study, both out of and during pollen season, none of the patients received nasal steroid or antihistamine therapy but they used topical decongestant drugs, such as naphazoline, when needed. However, no patient received any medication for at least 5 days before the study. As a control group, we studied 10 nonatopic subjects from the laboratory staff (three men and seven women, 22 to 35 years of age, mean age 28.4 ± 3.4). They had no history of seasonal or perennial rhinitis, asthma, or urticaria and had negative prick test responses to a panel of allergens. Blood samples were drawn in Vacutainer SST-tubes, and after allowing clotting for 60 minutes at room temperature (20° to 24° C), the blood was centrifuged for 10 minutes at 1300g at room temperature. The sera were frozen at −70° C and analyzed at the end of the study period.

Grass pollen count per cubic meter of air was continuously taken with a volumetric pollen trap (Burkard Manufacturing; Rickmansworth, U.K.) placed on the roof of the hospital building at a height of approximately 20 meters. Microscopic analysis was performed at magnification of 400×, and results were calculated as mean counts per day. The patients were studied in January, when the grass pollen was absent, and during the pollen season, after 2 weeks of peak values that were reached at the end of May. Accordingly, patients experienced their symptoms in the months of May and June.

Informed consent was obtained from each subject, and the study was approved by the Institute of Internal Medicine and Geriatrics Committee.

SPTs

SPTs were performed and evaluated in accordance with the European Academy of Allergy and Clinical Immunology Subcommittee on Allergen Standardization and skin tests, with the use of a standard allergen panel (Bayer; Milan, Italy).[13] The panel included the following extracts: five pollens (grass, parietaria, mugwort, birch, and olea), two molds *(Alternaria* and *Cladosporium)*, two animal danders (cat and dog), two house dust mites *(Dermatophagoides pteronyssinus* and *Dermatophagoides farinae)*, a negative control (glycerinated saline), and a positive control (histamine, 10 mg/ml). A skin test response was regarded as positive if the wheal diameter was 3 mm greater than that of the glycerinated saline control.

Clinical score

Before and during the pollen season, the patients gave an overall assessment of their rhinitis symptoms. The symptoms of nasal blockage, nasal itching, sneezing, and rhinorrhea were rated on a four-point scale in which 0 = no symptoms, 1 = mild; 2 = moderate; and 3 = severe. Total symptom scores ranged from 0 to 12 and represented the sum of scores of nasal blockage, nasal itching, sneezing, and rhinorrhea.

Nasal fluid

Nasal lavage was performed with the use of a disposable, metered-dose nasal inhaler (Markos; Monza, Italy) filled with sterile, room-temperature, normal saline solution. The device consisted of a plastic cup with two compartments. The central compartment was filled with sterile saline solution with a syringe; the external compartment collected the liquid after

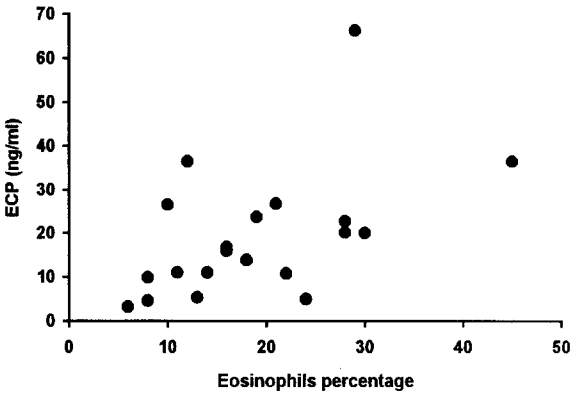

FIG. 1. Correlation between eosinophil percentage and levels of ECP in nasal lavage fluid (r_s = 0.53, p = 0.016).

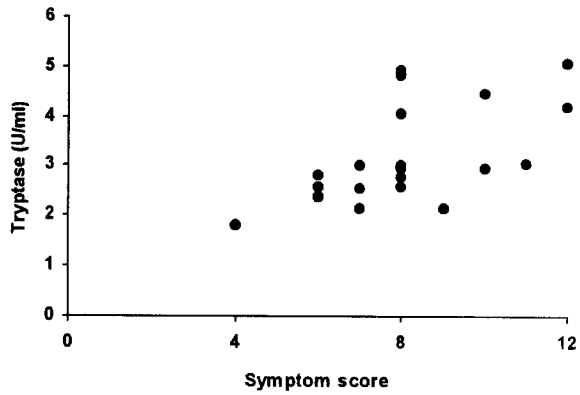

FIG. 2. Correlation between symptom score and levels of tryptase in nasal lavage fluid (r_s = 0.63, p = 0.003).

washing. Total input of saline solution was approximately 8 ml (4 ml in each nostril for 5 minutes). To collect the nasal washings, the subjects were instructed to actively breathe during a Valsalva maneuver to harvest nasal fluid in the cup. Obtained samples were stored on ice and centrifuged at 400*g* for 10 minutes at 4° C; supernatant aliquots were stored at −70° C. The individual variation in the recovered versus introduced volume was 86% ± 8%.

Laboratory tests

Tryptase levels were determined with a modified Pharmacia Tryptase RIACT assay with two monoclonal antibodies for tryptase. In the RIACT, 50 μl of nasal lavage fluid or serum, 50 μl of sample diluent, and 50 μl of ^{125}I-labeled antitryptase antibody were simultaneously added to plastic tubes with antitryptase antibody. After overnight incubation, the tubes were washed three times and then counted in a gamma counter.[14] The detection limit was less than 0.35 U/L and the interassay variation was less than 6%. Histamine assay was performed by radioimmunoenzyme assay on nasal washing by use of a modification of the radioimmunoenzymatic method of Pharmacia, Histamine RIA 50.[15] The detection limit was less than 2 ng/ml. The assay of serum and nasal fluid ECP was performed with a Fluorescent Enzyme Immunoassay (FEIA) with the Pharmacia CAP™ system (Pharmacia AB; Uppsala, Sweden). The detection limit was less than 0.5 ng/ml, and the coefficients of variation were within assay less than 5.9% and total less than 7%.[16]

Nasal cytology

Nasal eosinophil counts were performed on nasal scraping. After the patient cleared the nose of excess secretions, nasal mucosal samples were collected under direct vision. The cupped tip of a disposable probe was gently passed on the mucosal surface of the medial aspect of the inferior turbinate. The specimen was spread onto a plain slide and immediately fixed in 95% ethyl alcohol and dipped in Wright-Giemsa stain. The slide was examined under oil immersion by light microscopy at 400× magnification. Eosinophil counts were expressed as a percentage of 300 cells counted.[11] All specimens were examined by the same microscopist.

Blood eosinophil counts

Blood eosinophil counts were obtained with a Technicon model H2 analyzer and expressed as cells × 10^6 L.

Statistics

All assays were performed by operators without knowledge of the protocol. All data were expressed as median together with percentile P25 and P75 values and were analyzed with the Kruskal-Wallis nonparametric test. Correlations between histamine, tryptase, ECP values in the nasal fluid, and both mucosal eosinophil percentage and sum of score symptoms were analyzed with Spearman's correlation coefficient analysis. Probability values of less than 0.05 were considered significant.

RESULTS

Mediator levels in nasal fluids

The results of nasal fluid assays from atopic grass-sensitive subjects compared with those from control subjects are shown in Table I. Concerning histamine, no significant differences were detected between all groups under study, that is, patients with rhinitis and control subjects out of and during pollen season. In regard to tryptase and ECP out of pollen season, no significant differences were detected between patients with rhinitis and control subjects. During pollen season, their levels significantly increased in atopic patients, whereas no significant changes were observed in nonatopic subjects. Accordingly, significant differences were observed between patients with rhinitis and control subjects.

Mediator levels in serum

Tryptase was not detectable in serum. Concerning serum ECP, no significant differences were detected between all groups under study: patients with rhinitis, 3.0 (3.0 to 3.0) versus 3.0 (2.0 to 3.0), and control subjects, 3.0 (2.0 to 3.0) versus 3.0 (3.0 to 3.0), respectively, out of and during pollen season.

Eosinophil counts

Out of pollen season, no significant differences in nasal samples were observed between the patients with rhinitis and control subjects. During pollen season, the eosinophil percentages increased only in the patients with rhinitis. Accordingly, a significant difference was observed between patients with rhinitis and control subjects during pollen season (Table II).

Out of pollen season, no significant differences in blood were observed between the patients with rhinitis and nonatopic subjects (Table II). As expected, during pollen season we observed an increase of blood eosinophils in patients with rhinitis, but these cells were in normal range. Accordingly, a significant difference was observed between patients with rhinitis and nonatopic subjects during pollen season.

Correlations

We also analyzed data of the patients with rhinitis during pollen season for correlations between the levels of histamine, tryptase, ECP in nasal lavage fluids, and of eosinophil percentages in nasal mucosal samples. As shown in Fig. 1, there was a correlation between ECP and eosinophil percentages in nasal mucosal samples. No other correlations were observed (data not shown). By analyzing data for correlations between the levels of histamine, tryptase, ECP, and eosinophil percentages with symptom scores, we found significant correlations between both tryptase and ECP levels and symptom scores (Figs. 2 and 3). On the other hand, no correlations were observed between both histamine levels and eosinophil percentages and symptom scores (data not shown).

DISCUSSION

The pathophysiology of allergic rhinitis is associated with the presence of inflammatory cells such as mast cells and eosinophils in the nasal mucosa. The role of nasal mast cells in the immediate hypersensitivity reaction of patients with allergic rhinitis has been well documented. After exposure to allergen, mast cells are activated by cross-linking of allergen to mast cell–bound specific IgE. Mast cell degranulation leads to a biphasic mediator release. The activated mast cells release both vasoactive mediators related to the early nasal symptoms and chemotactic mediators that attract inflammatory cells such as eosinophils related to the late-phase response.[17-21]

In this study we have analyzed, in blood and nasal fluid from patients allergic to grass pollen, histamine and tryptase to monitor the early phase markers and eosinophil and ECP to monitor the late phase.[17-21] Furthermore, to gain insight into the pathophysiology of allergic rhinitis, data were related to the severity of symptoms.

The role of nasal mast cells in the immediate hypersensitivity reaction of patients with allergic rhinitis has been well documented. Immediate allergic nasal symptoms of itching, sneezing, and watery rhinorrhea occur as a consequence of mediator release after IgE-dependent activation of mast cells in the nasal mucosa. The biologic properties of these mediators include vasodilatation and an increase in vascular permeability, which may cause nasal blockage. In our study, however, during pollen season, tryptase but not histamine levels appeared to increase in nasal fluids from patients with allergic rhinitis and to correlate with symptom scores. In fact, nasal lavage fluids

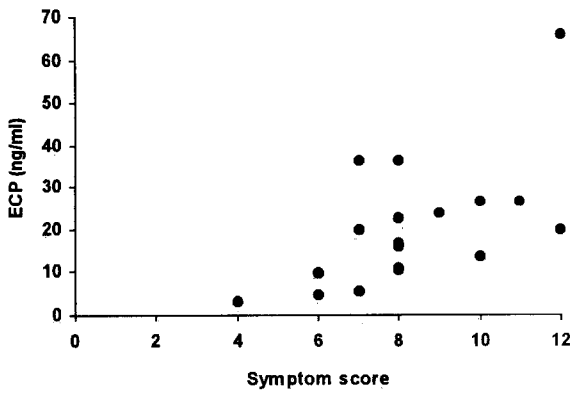

FIG. 3. Correlation between symptom score and levels of ECP in nasal lavage fluid (r_s = 0.65, p = 0.002).

in patients with rhinitis and nonatopic subjects contained high levels of histamine out of and during pollen season. This is in agreement with previous studies demonstrating that measurement of histamine in nasal lavage fluid as a marker of an allergic response appears not to be ideal. The origin of the histamine has not been defined. A possible explanation for the discrepancy in tryptase and histamine release might be that histamine is metabolized or removed more rapidly than tryptase. Alternatively, it can be produced by mucosal bacteria. However, the tryptase increase is not observed in blood.[19-24] On the other hand, the immunoassay used in our study measures primarily β-tryptase, whereas the α form of human tryptase is the predominant type in blood at baseline in normal subjects and is elevated in those with systemic mastocytosis.[24] Tryptase has been demonstrated to be elevated in nasal fluid after allergen challenge. In the present study, we investigated serum and nasal fluid of atopic and nonatopic subjects under natural allergen exposure to show natural mast cell activity in disease. The results show that active nasal allergy is associated with elevated tryptase in nasal fluid. On the other hand, patients with allergies tested out of the pollen season showed no increase or only slightly increased tryptase levels. In other studies it has been shown that patients with severe inflammatory nasal diseases without an allergic component show no elevated nasal tryptase levels. Elevated nasal tryptase values can be provoked not only by allergen challenge as described but also by natural allergen exposure and are a marker of active rhinitis only.[18, 25, 26]

During the late-phase response, activated eosinophils are involved.[17, 19, 27] Accordingly, the eosinophil number increased in nasal cytology and in the blood from patients with allergic rhinitis during pollen season. On the other hand, ECP levels increased only in the nasal lavage fluid. However, the measurement of ECP in nasal fluid is a more feasible marker to monitor nasal inflammation. In fact, ECP levels but not eosinophil counts correlate with symptom score. This observation suggests

that enumeration of eosinophils alone may not accurately reflect their involvement in inflammation. Eosinophil blood levels but not ECP levels are increased in the blood. It is well known that serum ECP levels are dependent not only on the number of blood eosinophils but mostly on their ECP content and on their propensity to secrete ECP, that is, on their activation state.[27-29] These results suggest that assessments of eosinophils and their mediators in peripheral blood may underestimate the contribution of these cells to the inflammatory process at the mucosal surface in the respiratory tract. In the past decades, a great number of studies have led to the understanding that eosinophils are closely involved in the pathogenesis of allergic diseases such as allergic asthma and allergic rhinitis.[30, 31] The propensity of eosinophils to release ECP in vitro may be related to the extent to which the cells have been exposed to various priming agents in circulation, such as different cytokines, for example, IL-5, IL-8, RANTS, or mediators such as platelet-activating factor and leukotrienes.[26, 32] Moreover, it is important to note that the major difference between upper and lower airways is in the surface area, which is approximately 150 cm^2 in the nose and several square meters in the lower airways.[33]

In conclusion, the measurement of tryptase and ECP levels in nasal fluid might be a useful clinical test for monitoring disease activity and the effects of therapeutic agents. Mast cell activation also can be demonstrated without artificial allergen challenge: in other words, simply by means of natural allergen exposure in untreated patients. However, on the whole, these results strengthen our previous suggestion that in Sicily, grass rhinitis is not a risk factor for the development of asthma.[34] This study demonstrates, then, that isolated pollen allergy leads to an at least partly reversible nasal disease and that mast cell activity is not detectable out of the pollen season. Therefore we can conclude that if a patient with allergies has nasal symptoms out of the pollen season, these problems should not be related to enhanced mast cell activity but must be explained by different mechanism(s), for example, nonspecific nasal hyperreactivity.[22]

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