

Original article

Eosinophil cationic protein and specific IgE in serum and nasal mucosa of patients with grass-pollen-allergic rhinitis and asthma

Background: After allergen exposure, IgE-bearing mast cells surface in respiratory mucosa. Eosinophils are also recruited locally by chemotactic mediators; they are the main cell directly involved in the late phase of allergic inflammation. IgE antibody and eosinophil cationic protein (ECP) are routinely determined mainly in serum although they exert their pathogenetic role more directly on mucosal surfaces.

Methods: We performed a comparative study of IgE antibody to grass and ECP on nasal mucosa and blood samples in order to evaluate the relevance of monitoring allergic inflammation in the target organ. Thirty-one patients and 10 nonatopic controls were enrolled in the protocol. Twenty-six subjects allergic to grass, 11 with rhinitis (group 1) and 15 with asthma and rhinitis (group 2), completed the study. Five patients dropped out. Specific IgE to grass and ECP was determined in nasal mucosa by our method based on *in situ* incubation.

Results: Serum IgE to grass did not increase during the pollen peak, as did nasal IgE, in group 1 from before the pollen peak, from 2.3 to 3.2 kU/l ($P=0.02$), and in group 2 at the pollen peak, from 4.8 to 12.2 kU/l ($P=0.01$). Serum ECP did not show any significant variation in group 1, but it increased at pollen peak from 6 to 11.2 $\mu\text{g/l}$ ($P=0.01$) in group 2. Nasal ECP increased significantly in both groups even before the pollen peak. In group 1, ECP values rose from 15 to 39.9 $\mu\text{g/l}$ ($P=0.01$). In group 2, ECP increase was much higher than in group 1, from 9 to 213 $\mu\text{g/l}$ ($P=0.001$). Serum eosinophils, like nasal ECP, showed a significant increase of values from before the pollen peak in both groups, without correlation with serum ECP in rhinitic patients.

Conclusions: Both specific IgE and ECP in the nasal mucosa showed a better correlation with allergen exposure than serum evaluations. With an appropriate method, allergic inflammation may be best monitored in the nasal mucosa.

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In patients with allergic respiratory diseases, eosinophils, by release of cationic protein, play a major role in allergic inflammation (1–5).

Eosinophil cationic protein (ECP) levels are elevated in biopsies and bronchoalveolar lavage (BAL) obtained from patients with allergic asthma and correlate with the severity of symptoms and with the results of respiratory function tests (6, 7). Furthermore, several studies have observed a similar pattern in serum ECP concentrations (8, 9). Therefore, serum ECP measurements have been proposed to monitor the effects of anti-inflammatory asthma therapy, but the results have been discordant (10–13).

Significantly increased ECP and IL-8 concentrations in the nasal lavage fluid (NAL) of asthmatics without symptoms (14) suggest that the level of ECP may be high because of minimal exposure to the allergen and may therefore be a predictor of possible subsequent

exacerbation (15). On the other hand, serum ECP levels in asthmatic patients allergic to birch pollen and *Parietaria* were high only after a period of high pollen concentrations (16–18).

In NAL from symptomatic patients with allergic rhinitis, ECP was elevated and levels rose significantly after a specific nasal challenge (19, 20). ECP levels in NAL from patients allergic to birch pollen were significantly high during the pollen peak of a season with low pollen concentrations (14).

As sensitivity in serum determinations of ECP is poor (21, 22), and NAL is difficult to measure and results vary greatly (19), we adopted a method of direct mucosa incubation (26, 27) to observe the kinetics of specific IgE and ECP in the nasal mucosa of patients allergic to grass pollen who were affected by either rhinitis alone or associated with asthma.

The aim of the study was to compare nasal and serum

specific IgE, nasal and serum ECP, and blood eosinophils, in relation to seasonal allergen exposure, focusing on the priority of response between serum and nasal mucosa.

Material and methods

Patients and control subjects

Thirty-one patients (17 males, 14 females, mean age 26 years, range 12–42) were divided into two groups according to clinical symptoms shown during exposure: group 1, with rhinitis alone ($n=14$), and group 2, with rhinitis associated with asthma ($n=17$). Ten age-matched normal nonallergic subjects were also recruited to the study as controls.

The inclusion criteria for patients were as follows:

- 1) clinical history of mild to moderate seasonal rhinitis alone or associated with asthma
- 2) sensitization only to grass pollen, as shown by skin prick tests with glycerinated extracts (Bayropharm DMS, Milan, Italy) and serum RAST (RAST-FEIA CAP-System, Pharmacia, Uppsala, Sweden)
- 3) acceptance of a regimen based only on oral antihistamines and β_2 -agonists when needed.

All patients and controls gave their written informed consent to the study, which was approved by the Regional Ethics Committee of Tuscany, Italy.

Study design

The study was carried out from January to July. During this period, all patients underwent four determinations of serum and nasal specific IgE to timothy grass, serum and nasal ECP, and blood eosinophils. The first determination was performed outside the season (end of January), the second before the pollen peak (last week of April), and the third at the pollen peak (first week of June). A fourth determination was also made after the pollen peak (first week of July).

Symptoms of rhinitis and asthma were recorded in a diary. An overall evaluation of severity of symptoms during the period preceding serum and mucosal assessments was performed on the basis of diary records and medical examination, on a four-point scale from 0 to 3, where 0 represented no symptoms and 3, severe symptoms.

Nasal and serum specific IgE, ECP, and blood eosinophils were evaluated also in 10 normal nonatopic controls outside the season and at the pollen peak.

Methods

Serum specific IgE to timothy grass was determined by fluoroimmunoassay (RAST-FEIA CAP-System, Pharmacia, Uppsala, Sweden).

Serum ECP was measured by fluoroimmunoassay (ECP-FEIA Pharmacia, Uppsala, Sweden).

Nasal levels of specific IgE and ECP were determined by direct incubation on nasal mucosa by a new method previously described (26, 27). Briefly, the same type of substrates that had been used for the serum assay, i.e., sponges covalently coupled with timothy grass or with anti-ECP antibody, were utilized. The substrates, in the nasal applicator covered by a permeable membrane (Fig. 1), were allowed to incubate in the nasal mucosa at the level of the anterior part of the lower turbinate for 5 min. After nasal incubation, the substrates were washed in Tween 20 NaCl 0.9% (w/v) solution at a concentration of 16 ml/l, put in test tubes with 1 ml 0.9% (w/v) NaCl solution containing sodium azide 0.02% (w/v), and stored at -20°C until

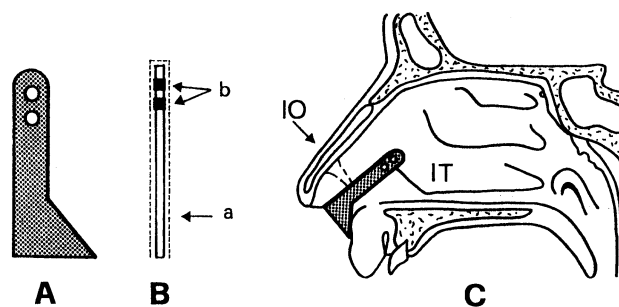


Figure 1. Nasal applicator and its positioning on nasal mucosa. A) Applicator; B) cross-section of applicator with sponges (a) and membrane (b); C) position of applicator on nasal mucosa. IO: internal ostium; IT: inferior turbinate.

used. From this point, specific IgE was determined with the same fluoroimmunoassay used for the serum. The substrates for nasal ECP determination were incubated for 30 min with enzyme-labeled anti-ECP antibody, instead of 150 min as for serum determinations; washed twice; and then subjected to the same procedure used for serum. All four determinations of nasal and serum samples were run together.

Blood eosinophils were assayed by a counter-cytometer (Max M, Hialeah-Fi).

Pollen count

A Hirst pollen trap (model VPPS 2000, Lanzoni, Milan, Italy) was used to take daily pollen counts. Pollen counts were expressed as the mean daily number of pollen grains per cubic meter (m^3) of filtered air.

Statistical analysis

Statistical analysis was done by nonparametric tests for nongaussian distribution of values, which were expressed as median and range. The Mann–Whitney test for unpaired data was used to compare serum and nasal specific IgE and ECP, and serum eosinophils in the two groups. Wilcoxon's rank sum test for paired data was performed to evaluate baseline against April and June determinations. In view of the multiple comparisons, the significance level was reduced to 0.026.

Data referring to the evaluations after the pollen peak were given only as descriptive analysis.

Results

Dropouts

Three patients of group 1 were excluded because of lack of compliance with checkup times and two patients of group 2 because they took oral or topical corticosteroids. Therefore, the results refer to 26 patients, 11 subjects of group 1, with rhinitis alone, and 15 subjects of group 2, with rhinitis and asthma (Table 1).

Pollen data

The mean daily pollen count was $2/\text{m}^3$ of air during March. The mean daily pollen count was very low in April (11 pollen grains/ m^3 of air), with the highest value (24 pollen grains/ m^3) in the last week of the month. The

Table 1. Characteristics of subjects studied

Clinical group	Number of subjects	Age (years) Mean value (and range)	Sex	Total serum IgE (baseline) Median (and range) kU/l
Rhinitis (group 1)	11	29 (14–42)	6 M, 5 F	76 (23–606)
Asthma and rhinitis (group 2)	15	24 (12–42)	9 M, 6 F	136.5 (35–280)
Controls	10	24 (12–40)	4 M, 6 F	14 (10–50)

pollen peak reached a mean daily value of 55 pollen grains/m³ in the first week of June. The pollen count dropped below 10 in the first week of July (Fig. 2).

Baseline (January)

Patients vs controls. No significant difference was found in any parameter assessed except for serum and nasal specific IgE, which were negative in all controls ($P < 0.35$ kU/l) and positive in all patients but three who had negative nasal IgE (Table 2).

Group 1 vs group 2. No significant difference emerged in serum specific IgE, serum ECP, blood eosinophils, or nasal specific IgE and nasal ECP (Table 2).

Before pollen peak (April)

Group 1 vs group 2. Symptoms of rhinitis developed in 8/11 subjects of group 1 (mean score 0.9) and in 14/15 subjects of group 2 (mean score 1.3). Symptoms of asthma were present in only 2/15 patients of group 2 (Fig. 2). No significant difference emerged in any of the parameters examined (Table 2).

April vs January. No variation was found in serum IgE or in serum ECP in either group. A slight increase in nasal IgE was found in both groups, and this was significant in group 1 ($P = 0.02$). A significant increase of nasal ECP was evident in both groups (group 1, $P = 0.006$; group 2, $P = 0.00065$), accompanied by an increase in eosinophil number (group 1, $P = 0.02$; group 2, $P = 0.005$) (Fig. 2).

Pollen peak (June)

Group 1 vs group 2. Moderate symptoms of rhinitis were present in all patients of both groups (mean scores of 1.8 for group 1 and 1.9 for group 2) and mild to moderate symptoms of asthma (mean score 1.3) in 14/15 subjects of group 2. No significant difference was found between the two groups in any parameter examined (Fig. 2).

June vs January. Serum specific IgE was not significantly increased in either group. Serum ECP was increased only in group 2 ($P = 0.01$), although 7/15

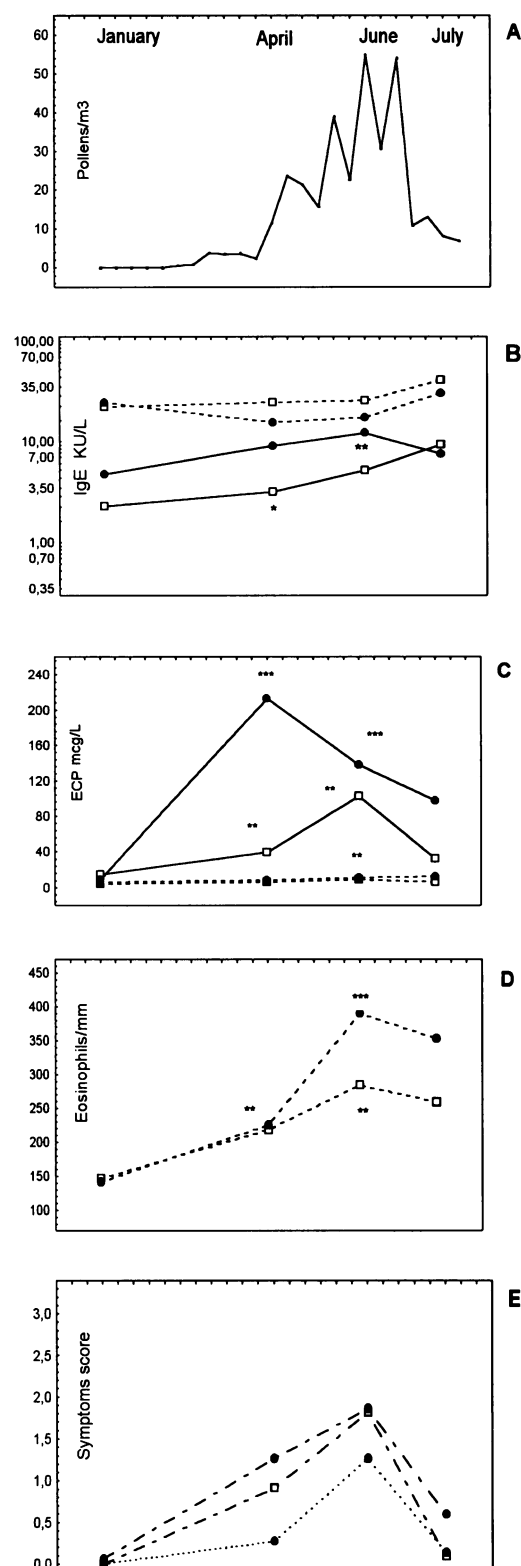


Figure 2. (□) group 1; (●) group 2; (---) serum; (—) nasal mucosa; A) Grass pollen mean daily concentration (pollen grains/m³) during period of study; B) serum and nasal IgE; C) serum and nasal ECP; D) blood eosinophils; E) (—) nasal symptoms in group 1 and group 2; (....) asthma symptoms in group 2. * $P < 0.026$; ** $P < 0.01$; *** $P < 0.001$. Significance of variation compared to baseline.

Table 2. Values (median and range) of serum and nasal IgE, ECP, and blood eosinophils in patients (groups 1 and 2) and controls

	Serum (kU/l)	Nasal IgE (kU/l)	Serum ECP (μ g/l)	Nasal ECP (μ g/l)	Eosinophils (cells/mm ³)
Group 1 (n=11)					
Baseline	22.1 (2.6–128.6)	2.3 (0.8–24.4)	4.2 (2.3–47.8)	15.0 (3.4–33.4)	147 (72–283)
Before peak	24.3 (3–151.3)	3.2 (0–72)	6.3 (3.6–16.6)	39.9 (19.2–272)	218 (70–1144)
Pollen peak	25.4 (3–152.3)	5.2 (0–60)	8.8 (3.7–13.4)	103.0 (11.2–278)	285 (129–594)
After peak	41.1 (4–181.9)	9.3 (2.1–117.8)	6.6 (3.1–12.5)	32.8 (9–124)	259 (138–970)
Group 2 (n=15)					
Baseline	23.4 (3–96.7)	4.8 (0–161.2)	6.0 (0–12.9)	9.2 (3.3–93.6)	142 (37–442)
Before peak	15.4 (2.9–130.6)	9.1 (0.17–69)	8.6 (3.6–64.5)	213.0 (33.5–266)	226 (70–730)
Pollen peak	17.3 (1.8–144.1)	12.2 (0.7–84.4)	11.2 (2.1–46.5)	138.0 (13.8–275)	390 (62–1462)
After peak	30.5 (1.9–150.6)	7.5 (0.9–453)	12.9 (2.5–22.5)	97.7 (9–247)	353 (84–830)
Controls (n=10)					
Baseline	<0.35 (<0.35–<0.35)	<0.35 (<0.35–0.38)	4.5 (2.5–10.9)	17.5 (2.5–29)	117 (47–156)
Pollen peak	<0.35 (<0.35–<0.35)	<0.35 (<0.35–0.38)	5.0 (3.5–12)	15.0 (5–29)	103 (56–148)

subjects showed values below 16 μ g/l. Blood eosinophils were significantly increased in both groups (group 1, $P=0.0044$; group 2, $P=0.00065$). Nasal specific IgE was increased in both groups, significantly so in group 2 ($P=0.0076$).

Nasal ECP was increased significantly in group 1 ($P=0.004$); nasal ECP of group 2 showed lower values than in April, with a median value still above 130 μ g/l, and was significantly different from January ($P=0.00065$) (Fig. 2).

After the pollen peak, symptoms of rhinitis were absent in all subjects except for one of group 1 and were still present in 7/15 subjects of group 2 (mean score 0.6). Symptoms of asthma subsided in all but two subjects.

According to symptoms, nasal ECP and serum eosinophils showed a decrease that was not evident in the case of serum ECP, and serum and nasal IgE.

Discussion

Some authors have recently demonstrated that IL-4 mRNA expression and ϵ germ-line gene transcripts increase after allergen challenge in the nasal mucosa of hay fever patients (23). Furthermore, IgE antibodies against the allergens responsible for symptoms have been detected on nasal mast cells by a highly specific immunohistochemical method performed on mucosal biopsies (24). Supported by the increasing evidence that IgE is locally produced and is responsible for local activation of cells expressing high-affinity receptors after allergen exposure (25), we studied specific IgE and ECP in nasal fluid in comparison with specific IgE, ECP, and eosinophils in blood samples before and during pollen exposure. With our easy method of *in situ* incubation, which offers higher sensitivity and reproducibility than nasal washings (26–27), we could show that the primary response to an allergic stimulus is in the target organ and is followed only later by a systemic response. In fact, unlike that from serum, specific IgE in the nasal mucosa of both groups of patients rose significantly during exposure. This increase, which was observed in patients

with allergic rhinitis as early as April, i.e., well before the pollen peak, was present in serum determinations only in patients with asthma and at the end of pollen exposure. In accordance with previous observations made on patients sensitive to mites (28), mucosal IgE in these grass-pollen-allergic patients also showed a closer relation to pollen exposure than serum. This suggests that the immune response to allergen can be best studied in the target organ.

ECP concentrations in the nasal mucosa showed a more significant increase before the pollen peak, when pollen concentrations were below 25 granules per m³ of air, at a time when patients presented only slight symptoms of rhinitis. Furthermore, in the patients with rhinitis and asthma, nasal ECP showed significantly higher values than in patients with rhinitis alone, even if at that time symptoms of rhinitis were quite similar in the two groups and symptoms of asthma were present in only two patients. A significant increase of ECP has been found in nasal washings during pollen exposure by other authors, underlining that mucosal inflammation may precede the development of symptoms (14, 15). As symptoms of rhinitis were not evidently different in the two groups, the higher values of nasal ECP in group 2 at the beginning of pollen exposure might be correlated with the following development of asthmatic symptoms. This higher releasability of eosinophils shows that the extent of symptoms to lower airways may be dependent on the level of allergic inflammation in the respiratory mucosa, where the nose and bronchi may be considered as two parts of a single organ. Blood eosinophil concentrations were significantly increased in both groups of patients from before the pollen peak, paralleled by ECP in the nasal mucosa. Therefore, despite a significant increase of blood eosinophils, an activation of ECP was not observed in the serum of patients with rhinitis, according to other authors (20), but was observed locally in the nasal mucosa. This seems to confirm that eosinophils are mainly activated in the tissue of the target organ rather than in peripheral blood.

In asthmatic patients, a slight but significant rise in

serum ECP was observed at the pollen peak. Previous observations on asthmatic patients allergic to pollen have reported an increase in serum ECP during seasonal exposure, but this occurred after the pollen count had been high for several weeks or at the end of pollen peak (17, 19). The results of this study show that prolonged exposure to a low pollen count (<25 granules per m³ of air, i.e., minimal allergen exposure but lasting at least 4 weeks) increases ECP in the nasal mucosa of patients much more than prolonged exposure to high pollen counts increases serum ECP. These data show that the kinetics of IgE and ECP in the nasal mucosa is different from serum IgE and ECP kinetics, and can be correlated with the behavior pattern of cells involved in the allergic inflammatory response, from which they are derived. In fact, during exposure to allergens, mast cells surface in the respiratory epithelium, and large numbers of basophils and eosinophils are recruited on the epithelial surface of

target organs (29, 30). The significant increase in nasal ECP observed after a low but sufficiently long pollen exposure may induce a relevant inflammatory reaction and suggests that anti-inflammatory prevention might be more effective if administered well before the pollen peak.

Because this method is easy to perform and more reproducible and sensitive than washing procedures, ECP measurements on nasal mucosa may be used to monitor acute allergic inflammation, yielding relevant data for a more appropriate anti-inflammatory strategy in seasonal rhinitis and asthma prevention.

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