

Monitoring nasal allergic inflammation by measuring the concentration of eosinophil cationic protein and eosinophils in nasal secretions

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Quantitative measurement of the eosinophil cationic protein (ECP) concentration and the percentage of eosinophils in nasal secretions has greatly improved our understanding of the inflammatory process after natural allergen exposure. ECP and eosinophils were measured in the nasal secretions of 40 symptomatic patients with seasonal allergic rhinitis during the pollen season. Results showed a significant relationship between a high concentration of ECP (median: 410 ng/g, range: 6–2380 ng/g) and a high percentage of eosinophils (median: 13.5%, range: 1–85%). This quantitative study again demonstrated that infiltration by eosinophils and release of ECP play a key role in allergic rhinitis. It also suggests that the combined measurement of the percentage of eosinophils together with the ECP concentration in nasal secretions seems to be a very useful model in monitoring and assessing the condition of chronic nasal inflammation in patients with allergic rhinitis.

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The study of cellular distribution and levels of inflammatory mediators has provided improved insight into the pathophysiology of allergic rhinitis. Many studies reported that in sensitized individuals a nasal challenge with an appropriate allergen results in an early- and late-phase nasal allergic reaction. Some literature data suggest that the late nasal reaction may be a more clinically relevant model of ongoing allergic disease than the early phase (5). The authors of two recent studies showed that in 17 atopic patients, after nasal allergen challenge, infiltration of eosinophils and consequent release of the various inflammatory mediators into nasal secretions were evidence of important pathophysiologic features in the late-phase nasal allergic reaction (11, 12).

The aim of this study was to quantitatively determine the eosinophil cationic protein (ECP) concentrations and eosinophil counts in patients with hay fever during the pollen season and to compare these data with the previous data found in normals and patients outside the season after nasal challenge.

Material and methods

Patients

A total of 40 patients with seasonal allergic rhinitis (17 males and 23 females) aged from 18 years to 50 years (mean age 29 years) were studied. All patients were seen at the beginning of the pollen season with complaints of itchy nose, sneezing, rhinorrhea, and nasal obstruction since the start of the pollen season. Their nasal atopy to grass pollen was confirmed by medical history and skin tests. During the previous 2 weeks they had not taken any medication. None of them had any other nasal inflammation except allergy. The subjects gave their informed consent. This study was approved by the local ethics committee.

Sample collection and preparation

The authors used Biewenga et al.'s aspiration system (2). The samples were collected by repeated

aspiration from the middle meatus and from the floor of both nasal cavities into a preweighed plastic sampling tube. This was immediately followed by aspiration of a known volume (1.0 ml) of PBS (pH 7.4) including 10% of Mesna (Mistabron, UCB Pharmaceuticals Company, Belgium). Mesna acts by disrupting the disulfide bonds of the mucus polypeptide chains, which is especially necessary in order to obtain a good-quality supernatant. The amount of secretion sampled was measured by weighing the collection tube and the tube with the aliquot of PBS before and after sampling. The samples were cooled on ice immediately after sampling, and then mixed in a Vortex mixer, and centrifuged at 1000 g at 4°C for 15 min. The supernatants were stored at -20°C or -80°C until used.

Measurement of ECP

The ECP concentration was determined by following the instructions for radioimmunoassay (RIA, RIA kits from Pharmacia, Sweden).

Cellular examination

After removing the supernatant, the cell pellets were resuspended in 1 ml PBS, pH 7.2, containing 5% bovine serum albumin (BSA). A volume of 100 µl of Mesna was added, and the tubes were incubated for 30 min at 37°C. Longer incubations were sometimes necessary to dissolve very sticky mucus present in the secretions. The cells were washed with PBS-1% BSA, and cytocentrifuge preparations were made (Shandon Scientific Limited). They were then stained using May-Gründwald-Geimsa and examined by light microscopy. Three hundred leukocytes were evaluated and a differential count was made.

Objective evaluation

Four major nasal symptoms (itching in the nose, sneezing, rhinorrhea, and nasal obstruction) were recorded for the day on which the patient visited the clinic, using scales to grade the severity of symptoms, i.e.: 0 = none: no symptom evident; 1 = mild: symptom clearly present but minimal awareness; 2 = moderate: definite awareness of symptom which is bothersome but tolerable; 3 = severe: symptom is hard to tolerate and causes interference with activities of daily living/sleeping. A total nasal symptom score (Sum) was calculated by adding these four scores together for each patient.

Nasal airway resistance (NAR) was measured by passive anterior rhinomanometry (PAR, Heyer Company, Germany) expressed in Pa/cm³/s of the left and right sides. The total (R_{tot}) and the mean (R_{mean}) nasal resistances were computed from the

equation: $R_{tot} = R_l \times R_r / (R_l + R_r)$ (4); and $R_{mean} = (R_l + R_r) / 2$. In addition, a unilateral (the higher resistance side) nasal resistance (R_{max}) was also selected as a comparative parameter. Sometimes, unilateral nasal obstruction was mentioned by the patients.

Statistical evaluation

All statistical tests were carried out two-tailed at the 1% level of significance in order to correct for multiple comparison. The calculations were performed using the SPSS/PC+ program on an IBM compatible microcomputer. The Spearman rank correlations coefficients were used to quantify the relationship between the variables of nasal symptom scores, the concentrations of ECP, the percentage of eosinophils, and nasal resistance (R_{tot}, R_{mean}, and R_{max}).

Results

The study was performed without any problem. The amount of secretion obtained from the patients varied between 140 and 410 mg (median: 260 mg). A total of 40 cytopins were obtained from all study patients. The quality of cell definition on the cytopins was excellent. Therefore, a total number of 300 inflammatory cells could be counted from each cytospin.

Table 1 shows that all the patients had at least a mild score for all four symptoms, and in most of them the severity of the nasal symptoms was at a moderate level. This was also confirmed by the total symptom score (Table 2, median of Sum: 8, range: 6–11). For all these symptoms, a significant relationship was statistically found only between sneezing and itching in the nose. Furthermore, the symptoms of nasal itching, sneezing, and rhinorrhea correlated very well with the total nasal symptoms score (Sum). Only the complaint of nasal obstruction correlated very well with nasal resistance (R_{tot}, R_{mean}, and R_{max}).

Generally, the cell population consisted of epithelial cells and leukocytes. Red blood cells were very rarely seen. The share of the leukocytes varied from

Table 1. Severity of nasal symptoms observed in patients (n=40)

		Symptom scores			
		0	1	2	3
Sneezes	(40=100%)	0	5(12.5%)	25(62.5%)	10(25%)
Itching	(40=100%)	0	14(35%)	18(45%)	8(20%)
Rhinorrhea	(40=100%)	0	10(25%)	22(55%)	8(20%)
Congestion	(40=100%)	0	0	32(80%)	

Table 2. Median and mean values of percentage of eosinophils and concentration of ECP in nasal secretions, and of all objective evaluations in all 40 patients

	Median	Mean	Minimum	Maximum
Eosinophils (%)	13.5	22.98	1	85
ECP (ng/g)	410	564	6	2380
Sum	8	8.13	6	11
NARtot	0.32	0.39	0.16	0.93
NARmean	0.71	0.92	0.4	2.7
NARmax	0.9	1.18	0.4	4.2

10 to 90% of the total cell population. Eosinophils were counted at a median percentage of 13.5 (Table 2; range: 1–85%). The percentage of neutrophils varied in inverse proportion to the percentage of eosinophils. Other cells such as lymphocytes, mast cells, basophils, and monocytes were rarely found.

The median concentration of ECP was 410 ng/g (Table 2; range: 6–2380 ng/g). There existed a significant correlation ($P = 0.003$) between the concentrations of ECP and the percentage of eosinophils. Of the 20 patients (50% of the total population) showing ECP concentration above the median value, 17 (85%) had eosinophil counts higher than 10% (Fig. 1). On the other hand, of the 25 patients (62% of the total population) with nasal eosinophilia (higher than 10% of eosinophil count), 17 (68%) had high ECP concentration (more than 410 ng/g).

No significant correlation was found between the percentage of eosinophils or ECP concentration on the one hand, and any single nasal symptom or nasal resistance (Rtot, Rmean, and Rmax) on the other.

Discussion

It seems that the use of inflammatory markers in the clinical management of allergic rhinitis has great clinical potential and opens a new interesting field of research. To reach this goal, the choice of the nasal sampling technique is of paramount importance because it will determine whether obtaining quantitative information on the inflammatory cells and/or mediators is feasible. Then, quantitative data on the normal and pathologic levels of these inflammatory components in nasal secretions need to be determined.

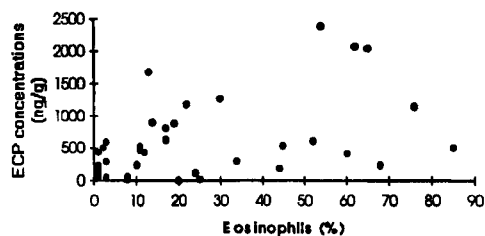


Fig. 1. Percentage of eosinophils and ECP concentrations in nasal secretions of patients ($n = 40$).

By using the microsuction technique, the quantitative determination of the ECP concentration in nasal secretions was performed. It also provided uniform and adequate nasal cytologic specimens for nasal cytology analysis. The major advantage of this technique is that it gives more precise information than the nasal lavage technique as the dilution factor can be simply calculated and easily controlled by the investigator. When using the nasal lavage technique, however, a considerable variation in the total range of ECP from 6 to 6000 $\mu\text{g/l}$ in the lavage fluids of 17 atopic patients before and after nasal allergen challenge was reported (3). Hence, several important questions have so far remained unanswered; i.e., "Which concentration of ECP in the nasal secretions should be considered pathologic?" and "At what level of percentage should the inflammatory cells in nasal secretions be considered pathologic during the allergic reaction?"

The possible activities of eosinophils as inflammatory cells in human allergic disease have been discussed for many years. An increase in total count and actively secreting eosinophils within the nasal submucosa and epithelium has been demonstrated by an immunohistologic study during seasonal grass pollen-induced rhinitis (1). It has been suggested that the diagnosis of allergic rhinitis must be considered when eosinophils appear in more than 10% of inflammatory cells (7, 8). The disadvantage of expressing the presence of eosinophils as a percentage is that it does not tell anything about a possible eventual increase in the absolute number. On the other hand, however, it is highly improbable that a mucosal inflammation occurs due to an increase in total number of inflammatory cells without a shift in the percentages of the different cell populations.

Iliopoulos et al. reported that after nasal allergen challenge, more subjects showed an increase in cells (i.e., eosinophils and neutrophils) than developed a late-phase reaction (LPR), suggesting the need for additional factors to explain the activation of the incoming cells and the development of LPR (6). Recently, it has been suggested that the determination of the ECP concentration in various body fluids made a quantitative estimate possible of the part played by the eosinophil in pathologic processes (10). Therefore, combined measurement of the percentage of eosinophils and ECP concentration in nasal secretions provides information not only on the participation of eosinophils but also on their activity. It is reported that there is a highly significant correlation between serum ECP levels and histamine sensitivity in patients with atopic asthma (9).

To this date, no detailed quantitative data on the normal and pathologic concentrations of ECP in the nasal secretions have been published. Therefore, the authors compared the present quantitative data on

ECP concentrations (11) and percentage of eosinophils (12) with those obtained from a previous study in hay fever patients after nasal allergen challenge outside the pollen season and in normal adults. In the control group of healthy volunteers, results showed that the percentage of eosinophils was almost 0 (range: 0–0.7%), and the median baseline ECP concentration was measured at 105 ng/g (range: 2–281 ng/g). In this group, challenge did not induce significant changes in either eosinophils or ECP. In the patient group out of season compared to the normal group, there was a significantly ($P < 0.01$) higher baseline percentage of eosinophils (median: 2.7%, range: 0–45%) than in the control group but not for baseline ECP concentration (median: 160 ng/g, range: 6–1475 ng/g). In the latter group, 1 h after nasal allergen challenge (NAC), a simultaneous increase was seen both in the percentage of the eosinophils and in the ECP concentration of the nasal secretions. They simultaneously continuously increased for at least 10 h. There was, however, a clear-cut difference in the time at which the maximum value was reached and the duration of this peak. The eosinophil count reached a peak 2 h after nasal allergen challenge with a duration of 8 h (median percentage of eosinophils: 8.8–13.3%), while the highest ECP level (median: 401 ng/g, range: 32–2298 ng/g) was reached only after 24 h with no clear-cut plateau.

These high levels of both percentage of eosinophils and the concentrations of ECP found during the late phase after experimental provocation correspond very well with the values found in patients with allergic rhinitis during the season. During the season, there was a significant correlation between ECP concentration and percentage of eosinophils. From the clinical point of view, this finding suggests that in the season the patient seems to be in a continuous late-phase condition (both high eosinophil count and ECP value) or in a continuous state of mucosal inflammation. This condition can explain the “priming phenomenon” and hyperreactivity of atopic patients during the season.

In this study, it has not been possible to demonstrate a good correlation between high ECP values and eosinophilia on the one hand, and nasal symptom scores on the other. This may be due to the fact that the major symptom of the late phase is nasal blockage, which fluctuates with the nasal cycle. The eosinophilia and ECP values do not show a nasal cycle. Furthermore, during the season a patient will show a combination of a continuous late-phase condition (mainly nasal obstruction) with repetitive early phases (repetitive allergen exposures accompanied by mainly sneezing and rhinorrhea). This mixture of symptoms due to different pathophysiologic mechanisms cannot be expected to correlate well with just

two late-phase parameters. On the other hand, ECP is not the only inflammatory marker. According to our data, some patients ($n = 8$) showed quite low ECP concentrations (less than 100 ng/g) during the season.

Mostly during the season, however, one sees in atopic patients ECP levels (median: 410 ng/g) that are far higher than the ECP levels in normal volunteers (median: 105 ng/g) and are similar to the late phase in atopic patients after provocation (median: 401 ng/g). So, in conclusion, one can state that the combined assessment of eosinophil count and ECP concentration (one of their granule proteins) in nasal secretions is a good indicator of mucosal inflammation in the allergic patients and that ECP levels reflect the state of the activation of the eosinophils present.

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