

Original article

Eotaxin but not MCP-3 induces eosinophil influx into nasal fluid in allergic patients

Background: Eotaxin and MCP-3 (CC chemokines), owing to their preferential action on eosinophils, seem to be of very importance in the pathophysiology of allergic rhinitis and asthma. The purpose of this study was to examine the effect of intranasally administered eotaxin and MCP-3 after specific allergen priming on the influx of inflammatory cells and their soluble mediators into the nasal mucosa.

Methods: Eotaxin and MCP-3 have been applied intranasally at the increasing doses of 1, 5 and 10 µg to allergic patients after allergen priming. The 'nasal pool' technique was used. The cell count and biochemical parameters in nasal lavage were evaluated before 30 min, and 4 and 24 h after the challenge with chemokines.

Results: Both eotaxin and MCP-3 induced the increase in clinical 'score' lasting till 24 h. Eosinophil influx into nasal mucosa after provocation with eotaxin was also observed. The challenge with MCP-3 did not induce any significant changes in nasal lavage fluid.

Conclusions: Eotaxin is likely to play an important role in the pathogenesis of allergic conditions in humans. MCP-3 did not induce inflammatory cell influx into nasal mucosa. The role of this chemokine in the pathogenesis of allergic inflammation is difficult to assess and requires further research.

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Chemokines are a group of small molecular weight (8–10 kDa) compounds which are essential for the directional migration of leukocytes during normal and inflammatory processes, especially in allergic inflammation. The chemokines family is divided into four subfamilies. Nowadays about 40 human chemokines have already been identified, but the biological function of most of them is still unknown or incompletely characterized (1–5).

Eotaxin and MCP-3 are members of the CC subfamily of chemokines. Eotaxin was originally isolated from the bronchoalveolar lavage fluid of allergen-challenged guinea pigs (6). Eotaxin is expressed constitutively in epithelial cells of normal airways. In the airways of asthmatic patients, epithelial cells are an important source of eotaxin, but inflammatory cells like T cells and macrophages are also a source of that kind of chemokine (5). Animal studies as well as *in vitro* tests have shown that eotaxin selectively attracts eosinophils and its expression is enhanced in allergic inflammation (7, 8). Atopic asthmatic patients have high concentra-

tions of eotaxin in the bronchoalveolar lavage fluid and an increased expression of eotaxin mRNA and protein in the epithelium and submucosa of their airways as compared with normal controls (9). Eotaxin levels were elevated in plasma of asthmatic patients (10). The quantities of eotaxin mRNA (accompanied by high number of eosinophils and elevated eosinophil cationic protein (ECP) concentration) were increased in the induced sputum of asthmatic subjects (11). The allergen challenge markedly increases the number of epithelial and inflammatory cells expressing eotaxin mRNA in nasal mucosa in late-phase response in atopic subjects with allergic rhinitis (12). In Hanazawa et al.'s study, intranasal administration of eotaxin with use of metered spray pumps in human subjects increased nasal eosinophils with a clinically symptomatic inflammatory response and increase in nasal nitric oxide (NO), contributing to oxidative stress (13). MCP-3 (monocyte chemoattractant protein-3) is a functional hybrid of RANTES, a powerful chemoattractant, and MCP-1, which primarily stimulates mediator release.

Thus, MCP-3 has all the properties that are needed to mobilize and activate eosinophils and basophils (14). As a result of their preferential action on eosinophils, eotaxin and MCP-3 are likely to play an important role in the pathophysiology of allergic conditions such as allergic rhinitis and asthma. However, the activity of these chemokines *in vivo* in humans is poorly recognized.

Study aim

The purpose of this study was to examine the effect of intranasally administered eotaxin and MCP-3 after specific allergen priming on the influx of inflammatory cells and their soluble mediators into the nasal mucosa.

Material and methods

Patients

Eotaxin study. The study group comprised eight allergic patients, 4 women and 4 men, aged between 23 and 62 years (average age was 39.63 ± 13.62 years). The patients were recruited from the outpatient clinic of the NIOM Department of Occupational Medicine, Lodz, Poland. Three patients were sensitized to grass or tree pollens, one to *Dermatophagoides pteronyssinus*, two to cereals and two to chloramine T (chloramine T induce IgE-mediated allergy). All subjects had a history of allergic rhinitis owing to the environmental or occupational exposure to the allergens. None was evidently symptomatic at the time of the study. Total IgE was determined in all patients and ranged from 66 to 1211 (376.25 ± 427.61) IU/ml.

MCP-3 study. Eight allergic patients, 3 women and 5 men, aged between 18 and 57 years (average age was 32.75 ± 12.68 years), participated in this part of the study. The patients were recruited from the same clinic as in the eotaxin study. One patient was allergic to *Dermatophagoides pteronyssinus*, one to chloramine T and six to cereals. All subjects had a history of allergic rhinitis as a result of environmental or occupational exposure to the allergens. None was evidently symptomatic at the time of the study. Total IgE was determined in all patients and ranged from 12 to 3329 (482.86 ± 1151.36) IU/ml.

All patients were examined for symptoms of allergen-induced rhinitis (sneezing, rhinorrhea, itching and nasal congestion). Patients refrained from taking oral antihistamines and topical nasal medication for a period of 14 days before and throughout the study. Because of the long half-life of astemizole, volunteers receiving this drug were excluded from the study. Patients with concurrent asthma, pregnancy, severe systemic illness and recurrent nasal bleeding were also excluded.

All subjects gave their written consent to participate in the study, and the study protocol was reviewed and approved by the Regional Ethics Committee.

Allergic status

Allergic status was confirmed by skin-prick testing with a panel of environmental allergens (commercial set of Allergopharma, Germany) including mites, grass and tree pollens, feathers, moulds and occupational allergens- cereals, chloramine (T). The latter agent was prepared as 1%, 0.1%, 0.01%, 0.001% solution in 0.9% NaCl in our laboratory. A negative control was made with the allergen diluent, and a positive control with histamine solution. All the sites

were examined after 20 min; the grading of the wheal (4 mm > control was considered positive) and flare (5 mm > control – positive) reaction was conducted following standard methods. Total IgE was determined with the use of FEIA (Pharmacia, Uppsala, Sweden).

Allergen extracts were purchased from Allergopharma Joachim Ganzer KG (Hamburg, Germany). Eotaxin and MCP-3 were obtained from Pepro Tech Inc., Rocky Hill, NJ, USA.

Study design

The study was performed in several steps as shown in Fig. 1. On the first day, after nasal lavage, each patient was intranasally challenged with a specific, skin test-positive allergen. This was followed by a challenge with placebo (diluent – Dulbecco's buffer (Sigma-Aldrich, Poznan, Poland) containing 0.3% (HSA) or 1 µg of chemokine in 5 ml of Dulbecco's buffer 48 h later in a random manner. One week later, another specific allergen challenge was performed and followed by a challenge with 5 µg of chemokine (or placebo) 48 h later. On the following week the procedure was repeated with 10 µg of MCP-3 or eotaxin.

Nasal challenge technique

The technique of nasal challenge with allergen has been described elsewhere (15, 16). In brief, allergen extracts in 5 ml of Dulbecco's buffer were instilled into the nasal cavity. Nasal washings with saline were performed by means of the 'nasal pool' technique described by Greiff et al. (17), 10 min before, and 30 min, 4 h and 24 h after the challenge. The 'nasal pool' device (5 ml syringe with closely fitting nostril piece), filled with 5 ml saline, was inserted in the nasal cavity for 5 min and then recovered. Patients were asked to record symptom scores according to the following protocol: rhinorrhea (none, 0 scores; mild, 1 score; moderate, 2 scores; severe, 3 scores), sneezes (none, 0 scores; 1–3/10 min, 1 score, 3–6/10 min, 2 scores; more than 6/10 min, 3 scores), nasal congestion (none, 0 scores; mild, 1 score; moderate, 2 scores; severe, 3 scores). A positive clinical challenge was defined as more than 3 scores.

The lavage fluid was centrifuged and the total and differential count of the cell pellet was analyzed. A centrifugation (10 min at $400 \times g$) of saline washings separated the cell pellet from the supernatant. The pellet was washed in a sterile Dulbecco's buffer (Sigma) with 0.1% HSA and then suspended in 1.0 ml of that buffer. Total leukocytes, eosinophils and metachromatic cells were counted following suspension in Turk solution, Dunger's solution, and toluidine blue solution, respectively. The sample was then centrifuged at $800 \times g$, transferred onto a slide and stained by the Pappenheim method. A minimum of 200 cells per smear was counted for each specimen. Cells were classified as epithelial cells, neutrophils eosinophils, basophils, lymphocytes and monocytes.

Concentrations of eosinophil cationic protein (ECP) and tryptase were measured by FEIA method (UniCAP, Pharmacia) according to the manufacturer's protocol. The nasal samples for the assays were collected before, 30 min, 4 and 24 h after provocation.

Statistical analyses

The data were analyzed by a parametric two-way ANOVA and Tukey's test for multiple comparisons. The differences were regarded as significant at $P < 0.05$.

Results

Analysis of symptom scores

Eotaxin Eotaxin induced the symptom of rhinorrhea, which started several minutes after provocation and

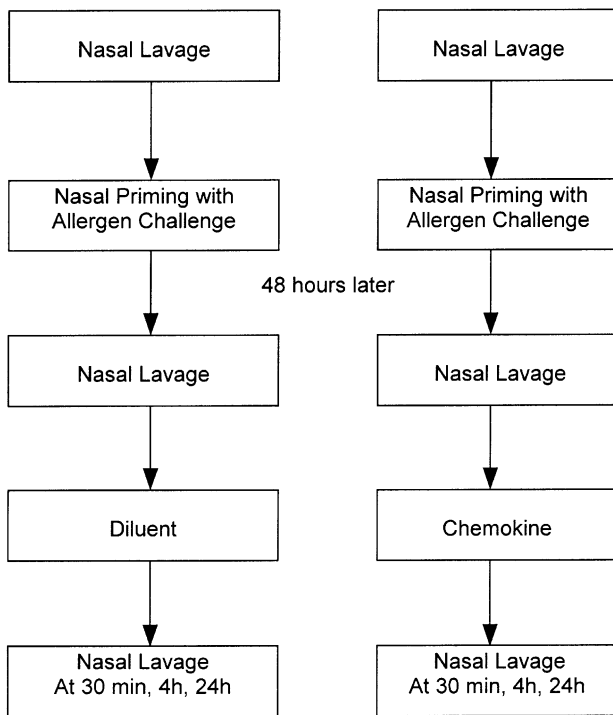


Figure 1. Study design. Eotaxin and MCP-3 challenge of allergen-primed nasal mucosa of allergic patients.

lasted for 6 h. In every subject this symptom appeared after the instillation of 1 µg of eotaxin, and intensified after the challenge with the 5 µg dose. Instillation of 10 µg eotaxin did not change the effects seen at the 5 µg dose. In all cases we observed similar effects: frontal headache (with mild hypertension) and itching (mainly facial skin and whole surface in two persons). One subject developed papules. These symptoms appeared from 10–20 min after eotaxin instillation and disappeared within 48 h. In one case we had to administer metyloprednisolon intravenously and oral steroids in two other cases. None of the patients experienced symptoms of conjunctivitis.

Statistical analysis of symptom scores revealed significant differences between the groups – immediately after the challenge ($F_{4,128}=3.42$, $P<0.05$), 4 h ($F_{4,128}=6.79$, $P<0.001$) and 24 h after the provocation ($F_{4,128}=8.85$, $P<0.001$), the score after challenge with allergen and subsequent doses of eotaxin was significantly higher than after challenge with placebo at all time points.

Also within, group comparisons revealed significant differences after allergen provocation ($F_{3,96}=10.95$, $P<0.001$), eotaxin at 1 µg ($F_{3,96}=19.89$, $P<0.001$), 5 µg ($F_{3,96}=13.27$, $P<0.001$) and 10 µg ($F_{5,175}=28.56$, $P<0.001$). All provocations except that with placebo induced statistically significant increase in symptom score at all time points after the provocation (Fig. 2).

MCP-3 induced the symptom of rhinorrhea which started several minutes after the provocation and lasted

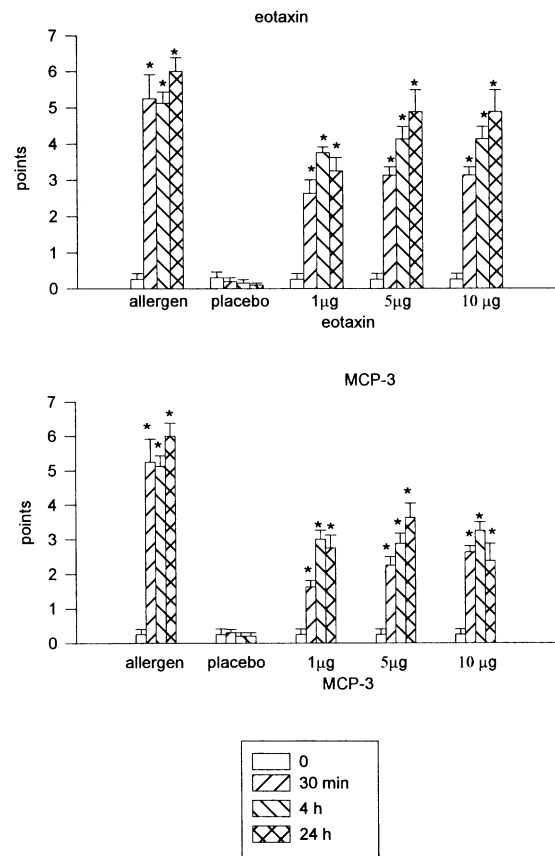


Figure 2. Composite symptom scores after allergen, placebo, eotaxin and MCP-3 challenge of the nasal mucosa. Both eotaxin and MCP-3 induced significant increase in clinical 'score' at 1, 5 and 10 µg doses at all time points after provocation.

6 h. As with eotaxin, the instillation of the highest dose (10 µg) did not cause additional effects in seven of the subjects. In one patient, 10 µg MCP-3 caused whole body itching and cloddy rash requiring methylprednisolon administration. One subject developed symptoms of conjunctivitis.

Statistical analysis of symptom scores revealed significant differences between groups immediately after the challenge ($F_{4,136}=10.91$, $P<0.001$), 4 h ($F_{4,136}=12.99$, $P<0.001$) and 24 h after the provocation ($F_{4,136}=18.60$, $P<0.001$), at all time points the score after challenge with allergen and subsequent doses of MCP-3 was significantly higher than after challenge with placebo and also the score after allergen provocation was significantly higher than after all doses of MCP-3.

Within, group comparisons revealed significant differences after allergen provocation ($F_{3,102}=38.57$, $P<0.001$), MCP-3 at 1 µg ($F_{3,102}=18.31$, $P<0.001$), 5 µg ($F_{3,102}=14.59$, $P<0.001$) and 10 µg ($F_{3,102}=15.55$, $P<0.001$). All provocations except that with placebo induced statistically significant increase in symptom score at all time points after the provocation (Fig. 2).

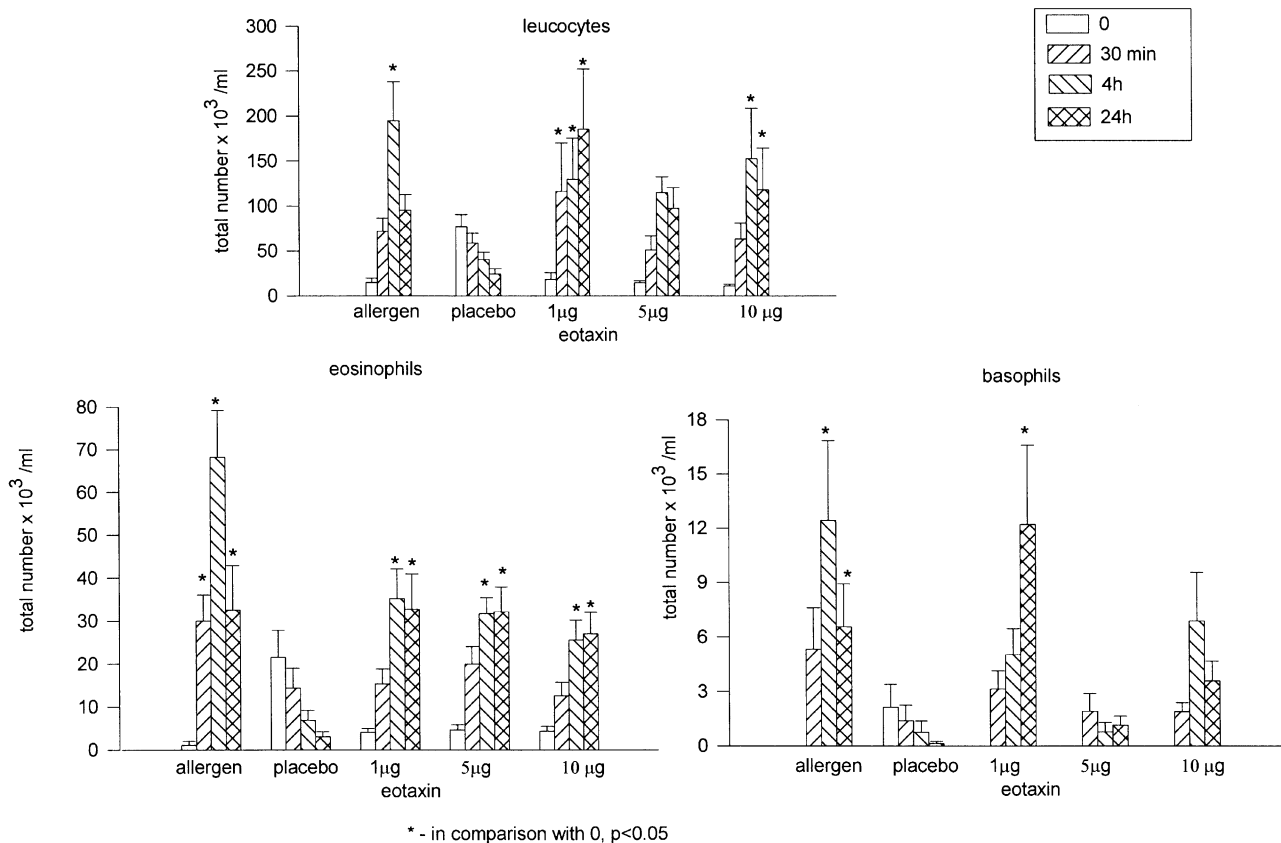


Figure 3. Time course of number of leukocytes, eosinophils and basophils in nasal fluid in the patients challenged with placebo, allergen and three doses of eotaxin. The number of cells was assessed before and 30 min, 4 h and 24 h after provocation. The influx of eosinophils into the nasal mucosa was significant after all doses of eotaxin at 4 and 24 h.

Cellular findings

Eotaxin

Leukocytes The provocation with allergen and eotaxin resulted in an increase in the total number of leukocytes at all time points. The differences between the groups were statistically significant at 4 h ($F_{4,140}=3.39$, $P<0.05$) and 24 h ($F_{4,140}=3.50$, $P<0.01$): total leukocyte count after allergen challenge at 4 h and after instillation of 1 µg eotaxin at 24 h were significantly higher than after placebo. Within-group comparisons revealed also significant differences in the total leukocyte count after allergen challenge ($F_{3,105}=9.35$, $P<0.001$), after provocation with eotaxin at 1 µg ($F_{3,105}=8.03$, $P<0.01$) and 10 µg ($F_{3,105}=6.44$, $P<0.01$). Allergen provocation induced significant increase in leukocyte count at 4 h. Also 30 min, 4 h and 24 h after the challenge with 1 µg eotaxin total leukocytes count was significantly higher than before the provocation. The instillation of 10 µg eotaxin induced a statistically significant increase in the number of leukocytes at 4 h and 24 h (Fig. 3).

Eosinophils Between-group comparisons of total eosinophil count revealed significant differences at 4 h ($F_{4,140}=14.58$, $P<0.001$) and 24 h after the challenge

($F_{4,140}=4.81$, $P<0.01$). At these time points the values were significantly higher after allergen and eotaxin provocation (doses 1 and 5 µg) as compared to placebo. Moreover, the eosinophil influx induced by 10 µg of eotaxin was significantly higher than after placebo at 24 h.

Within-group comparisons of total eosinophil count revealed statistically significant differences after allergen challenge ($F_{3,105}=34.87$, $P<0.001$), eotaxin at 1 µg ($F_{3,105}=10.06$, $P<0.001$), 5 µg ($F_{3,105}=7.75$, $P<0.01$) and 10 µg ($F_{3,105}=5.41$, $P<0.05$). In all cases the number of eosinophils at 4 h and 24 h was significantly higher than before the challenge. Moreover, after the allergen provocation, the increase in the eosinophil count was significant at 30 min (Fig. 3).

Basophils Our analysis of the total basophil count revealed significant differences between groups at 4 h ($F_{4,140}=7.21$, $P<0.001$) and 24 h after the provocation ($F_{4,140}=7.20$, $P<0.001$). At 4 h, the value was significantly higher after allergen than after placebo and eotaxin at the doses of 1 and 5 µg. At 24 h, the instillation of 1 µg eotaxin induced significantly higher increase in the basophil count than the challenge with placebo and eotaxin at the doses of 5 µg and 10 µg.

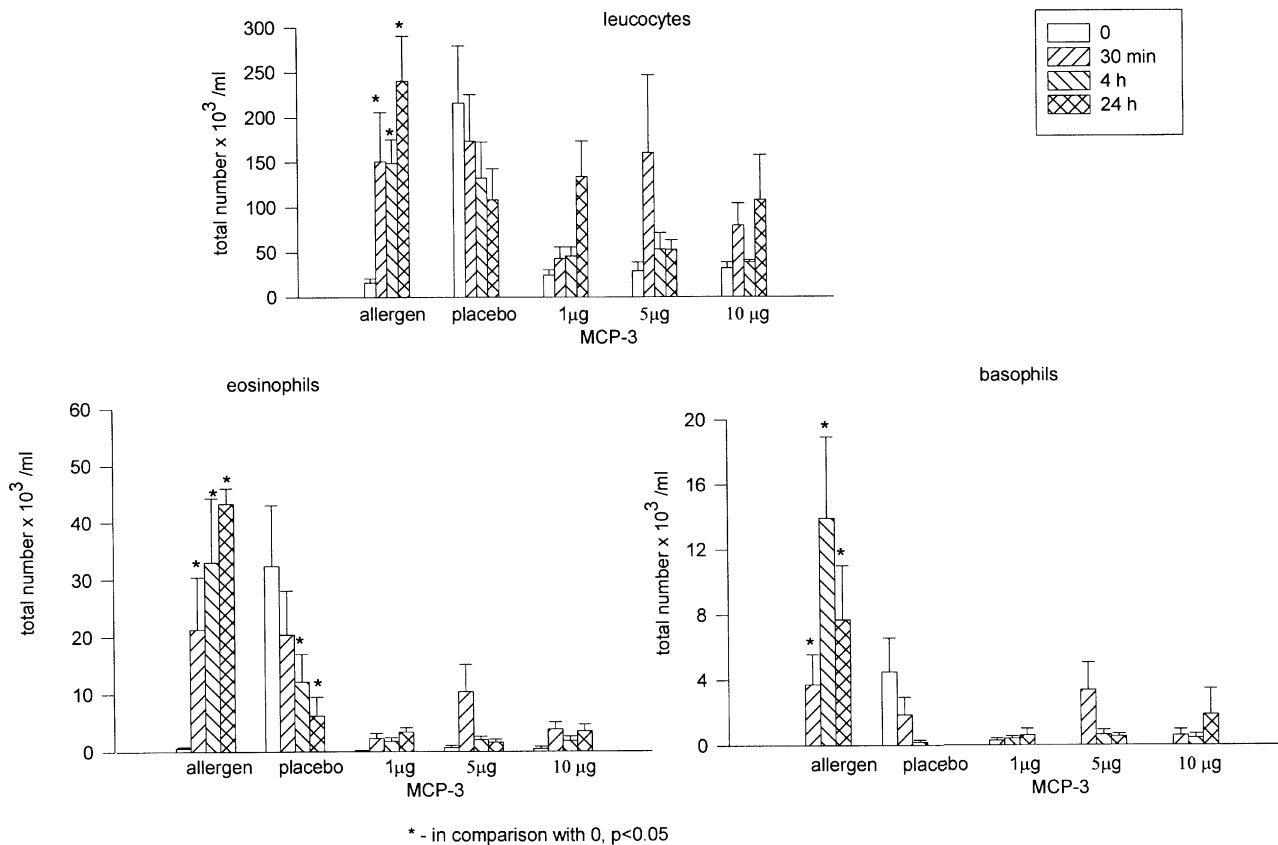


Figure 4. Time course of number of leukocytes, eosinophils and basophils in nasal fluid in the patients challenged with placebo, allergen and three doses of MCP-3. The number of cells was assessed before and 30 min, 4 h and 24 h after provocation. No significant changes were observed after MCP-3 challenge at all time points.

Within-group analysis revealed significant differences after allergen provocation ($F_{3,105} = 9.12$, $P < 0.001$) and 1 µg ($F_{3,105} = 9.37$, $P < 0.001$). Allergen challenge induced significant increase in total basophil count at 4 h and 24 h in comparison with values before the challenge. After instillation of 1 µg eotaxin, the number of basophils was significantly higher at 24 h than at all other time points (Fig. 3).

Leukocytes The provocation with allergen and MCP-3 resulted in an increase in the total number of leukocytes at all time points. The differences between the groups were statistically significant at 24 h ($F_{4,140} = 3.03$, $P < 0.05$) after the provocation; the value after allergen challenge was significantly higher than after 5 µg MCP-3.

Within-group comparisons revealed significant differences in the total leukocyte count only after allergen challenge ($F_{3,105} = 7.75$, $P < 0.01$), at all time points the values were significantly higher than before (Fig. 4).

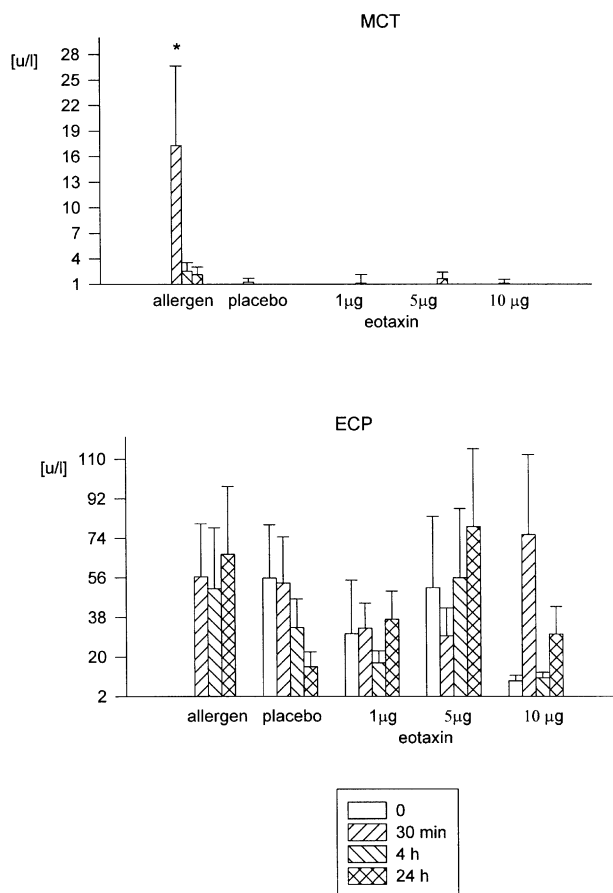
Eosinophils Between-group comparisons of total eosinophil count revealed significant differences at 4 h ($F_{4,140} = 5.84$, $P < 0.001$) and 24 h after the challenge

($F_{4,140} = 10.11$, $P < 0.001$). At these time points the values were significantly higher after allergen, MCP-3 at 1 µg, 5 µg and 10 µg provocation in comparison with placebo.

Within-group comparisons of total eosinophil count revealed statistically significant differences only after allergen challenge ($F_{3,105} = 21.25$, $P < 0.001$). The number of eosinophils at all time points after the provocation was significantly higher than before the provocation (Fig. 4).

Basophils Our analysis of the total basophil count revealed significant differences between groups at 4 h ($F_{4,140} = 14.37$, $P < 0.001$) and 24 h after the provocation ($F_{4,140} = 3.99$, $P < 0.01$). At all these time points, allergen provocation induced significant increase in basophil count as compared with the placebo provocation and MCP-3 at all doses.

Within-group analysis revealed significant differences only after allergen provocation ($F_{3,105} = 14.50$, $P < 0.001$), total basophil count at 4 h and 24 h was significantly higher in comparison with values before challenge (Fig. 4).



* - in comparison with 0, $p < 0.05$

Figure 5. Time course of mast cell tryptase (MCT) and eosinophil cationic protein (ECP) concentration in nasal fluid in the patients challenged with placebo, allergen and three doses of eotaxin. The concentrations were assessed before and 30 min, 4 h and 24 h after provocation. No significant changes were observed at all time points.

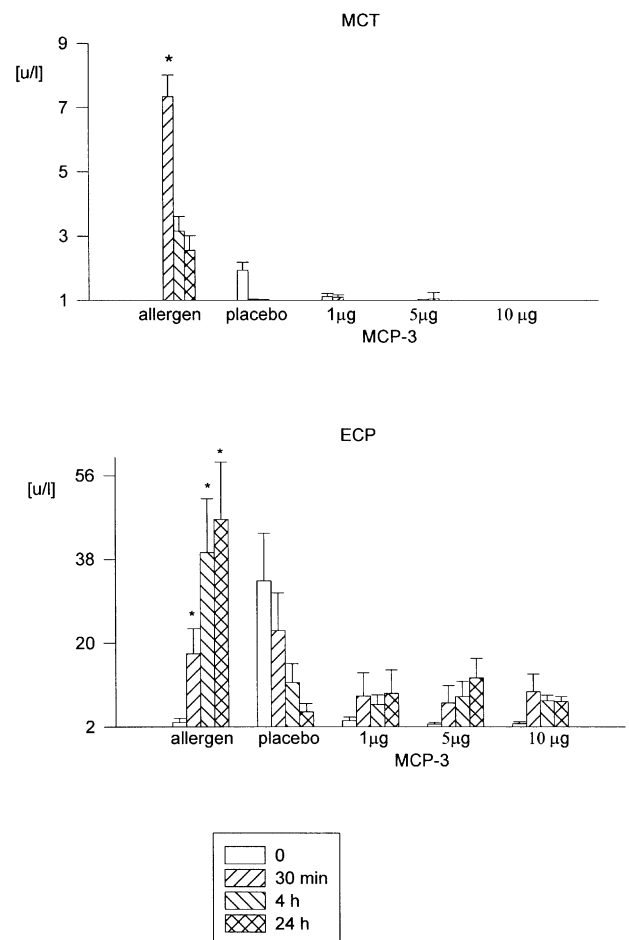
Biochemical findings: permeability index

Eotaxin Between-group comparisons revealed significant differences at 30 min ($F_{4,140} = 8.95$, $P < 0.001$), 4 h ($F_{4,140} = 3.07$, $P < 0.05$) and 24 h ($F_{4,140} = 9.81$, $P < 0.001$). At 30 min and 24 h, the increase induced by allergen and eotaxin (all doses) challenge was significantly higher after placebo. At 4 h only values after allergen provocation were significantly higher than after placebo.

Within-group comparisons revealed significant differences after challenge with allergen ($F_{3,105} = 7.19$, $P < 0.01$), the value was significantly higher at 30 min than before, at 4 h and 24 h.

Between-group comparisons revealed significant differences at 30 min ($F_{4,140} = 5.40$, $P < 0.001$), 4 h ($F_{4,140} = 12.47$, $P < 0.001$) and 24 h ($F_{4,140} = 12.39$, $P < 0.001$). At these time points the permeability index after allergen challenge was significantly higher after placebo and than after MCP-3 at all doses.

Within-group comparisons revealed significant dif-



* - in comparison with 0, $p < 0.001$

Figure 6. Time course of MCT and eosinophil cationic protein (ECP) concentration in nasal fluid in the patients challenged with placebo, allergen and three doses of MCP-3. The concentrations were assessed before and 30 min, 4 h and 24 h after provocation. No significant changes were observed at all time points.

ferences after challenge with allergen ($F_{3,105} = 14.78$, $P < 0.001$), MCP-3 at the dose of 1 µg ($F_{3,105} = 6.03$, $P < 0.05$) and 10 µg ($F_{3,105} = 5.95$, $P < 0.05$). After the allergen challenge, the index was significantly higher at all time points compared to before the challenge. MCP-3 induced a significant increase in the permeability ratio at 30 min compared with all other time points.

Mediator levels

Eotaxin Between-group comparisons of MCT levels revealed significant differences at 30 min ($F_{4,140} = 12.19$, $P < 0.001$); after the allergen provocation it was higher than after placebo and eotaxin at all doses. Similarly, within-group comparisons revealed significant differences after allergen challenge ($F_{3,105} = 14.11$, $P < 0.001$); at 30 min the values were significantly higher than at the other time points (Fig. 5).

The analysis of ECP levels did not reveal significant differences (Fig. 5).

Between-group comparisons of the MCT level revealed significant differences at 30 min ($F_{4,140} = 11.13$, $P < 0.001$); after the allergen provocation it was higher than after the placebo and all doses of MCP-3. Similarly, within-group comparisons revealed significant differences after allergen challenge ($F_{3,105} = 0.82$, $P < 0.001$); at 30 min the level of MCT was significantly higher than before the challenge (Fig. 6).

Between-group comparisons of ECP level revealed significant differences at 4 h ($F_{4,140} = 6.19$, $P < 0.001$) and 24 h ($F_{4,140} = 9.47$, $P < 0.001$). At these time points, allergen provocation induced a significant increase in the level of ECP as compared with placebo and all doses of MCP-3. Within-group comparisons revealed significant differences only after allergen challenge ($F_{3,105} = 26.24$, $P < 0.001$). The ECP level was significantly higher at all time points after the challenge than before it (Fig. 6).

Discussion

We have demonstrated that eotaxin caused a significant influx of eosinophils into nasal mucosa *in vivo* in human allergic subjects after specific allergen provocation ('priming'). MCP-3 did not induce allergic inflammatory cells influx, in spite of clinical symptoms. This is one of the first demonstrations of the effect of nasal instillation of these chemokines in human upper airways and the first demonstration with the use of allergen 'priming'.

Eosinophil infiltration of the airways in response to allergen exposure is characteristic of allergic rhinitis and allergic asthma. The role of eosinophils in allergic conditions is well documented. These cells have the potency to cause tissue damage through the release of their cytotoxic granule-derived proteins, oxygen metabolites and lipid-derived mediators, such as PAF and LTC₄. Indeed, a good correlation between the number of eosinophils in the blood and airways has been found, indicating clinical severity. Recruitment of these inflammatory cells into the airways involves a series of events including adhesion to endothelial cells, transendothelial migration and subsequent chemotactic movement (18). In recent years a number of CC chemokines have been identified that cause eosinophil chemotaxis. They include RANTES (19), eotaxin (20), MCP-3 (21), MCP-4 (22), and MIP-1 α (23). Neutralizing antibodies against RANTES, MIP-1 α , and MCP-3 (24) have been shown to block eosinophilic inflammation in a mouse model of asthma. The eosinophil influx into nasal mucosa in mice was elicited by eotaxin challenge (25). Intratracheal administration of eotaxin to mice induces a rapid and substantial accumulation of eosinophils in bronchoalveolar lavage

fluid. In guinea pigs, eotaxin causes the accumulation of eosinophils within the lung (7, 8). The knockout mice for eotaxin have a reduced capability to mount an eosinophilic inflammation in the early period (e.g., at 18 h) after allergen challenge (26). Eosinophils predominantly express the CC chemokine receptor CCR-3 which binds eotaxin, MCP-3, RANTES and MCP-4 (27). CCR-3 is also expressed on basophils. It is likely that CCR-3 plays a critical role in chemotaxis and the activation of eosinophils in allergic inflammation.

Eotaxin was considered to be one of the most important chemokines for eosinophil recruitment *in vivo* (28). This chemokine is a more effective eosinophil chemoattractant than RANTES when injected into the skin of the rhesus monkey (29). Human eosinophils express high numbers of the eotaxin receptor, CCR-3. Eotaxin binds this receptor with a 10-fold affinity than RANTES (25). This chemokine causes basophil degranulation *in vivo* but its effect on mast-cell activation is not clear. Neutrophils and monocytes do not appear to express CCR-3.

MCP-3, as a functional hybrid of RANTES, a powerful chemoattractant, and MCP-1, which primarily stimulates mediator release, seemed to have all the properties that are needed to mobilize and activate eosinophils and basophils (30, 31). In basophils, MCP-3 induces cytosolic free-calcium concentration changes and chemotaxis, histamine release and LTC₄ formation after IL-3 pretreatment. In contrast to MCP-1, MCP-3 is an eosinophil stimulant inducing Ca²⁺ changes and chemotaxis of these cells. MCP-3 has been reported to interact with several CC chemokine receptors, which can be simultaneously or selectively expressed on leukocyte subpopulations.

In our study we tried to investigate the effect of eotaxin and MCP-3 *in vivo* in human subjects after specific allergen challenge ('priming'). We chose this model because of results of previous studies. The lack of effects after nasal instillation of eotaxin without allergen 'priming' was reported in the literature (13). Nobody tried using the method with 'priming'. Also results of our previous studies with other CC chemokines (RANTES) showed that this chemokine did not induce statistically significant influx of inflammatory cells to unprimed nasal mucosa as compared with the placebo control. In the allergen-primed mucosa RANTES induced a very significant influx of eosinophils (32). For an inflammatory response of leukocytes, a chemotactic gradient must be established between epithelium and mucosal post-capillary venules. The allergen-challenged mucosa may be characterized by increased permeability to chemokines owing to the action of histamine or leukotrienes. The work by Douglas et al. who studied IL-8 (33) clearly demonstrated that the permeability of mucosa was important for chemokine action. Another explanation for the enhanced accumulation of leukocytes after allergen challenge is that the cells have already been

mobilized into the nasal mucosa as a result of allergen stimulation, and the chemokines attracted them into the airway lumen. The potentiating role of allergen priming is also connected with the production of proeosinophilic (e.g., IL-5, granulocyte macrophage colony stimulating factor GM-CSF) and proinflammatory (e.g., IL-1, tumour necrosis factor- α TNF- α) cytokines in the allergen-challenged mucosa. The importance of the sequential action of various cytokines has been well documented. For example, the injection of eotaxin into the guinea pig skin does not cause an eosinophilic inflammation. However, if the animals earlier receive IL-5 intravenously followed by eotaxin locally, there is a significant eosinophilic infiltration at the site of the injection (34). IL-1 and TNF- α upregulate adhesion molecules on endothelium. The nasal pool technique has proved to be a valuable model to investigate inflammatory mechanisms in allergic rhinitis. Provocation with specific allergen in all subjects caused allergic reactions with characteristic clinical symptoms and changes in nasal lavage fluid (significant increase of eosinophil number, permeability index, mast cells derived tryptase and ECP concentration). The 48 h time point for the provocation has been selected because by that time the amount of cells was stabilized. The 1-week wash-out period between provocations is reported in the literature as optimal (13). We instilled three doses of studied chemokines. The tissue level of eotaxin and MCP-3 in acute inflammation is still unknown. In our previous study with RANTES we assumed the concentration of 5 μ g to be reasonable for an *in vivo* organ challenge. However, in the RANTES study, we observed a clearly dose-dependent inflammatory response. In this study, it is difficult to evaluate the optimal dose for *in vivo* provocations. Hanazawa chose 8 μ g dose which induced the largest change of nasal NO after eotaxin provocation.

On the graphs there are average value from three allergen provocations. The results that were taken under concern came from subjects of which whom eosinophil number in particular time points after provocation did not differ more than three cells per one ml of lavage. This criterion was taken from previous evolved standard method (15).

Eotaxin caused clinical inflammatory response with rhinorrhea and sneezing but it did not cause nasal blockage in all patients. This response is larger than in

the Hanazawa study. The cause of this difference is probably the method of chemokine administration (instillation vs. metered spray pump). Intranasal administration of eotaxin induced a significant migration of eosinophils selectively as expected. In the study by Hanazawa et al. the maximal increase in the nasal NO was observed 8 h after eotaxin challenge. Eosinophils were increased significantly in the 8th hour and returned to the baseline level 24 h after the eotaxin challenge. In our study a significant increase of these cells was observed 4 and 24 hours after each studied doses. The lack of ECP increase after eotaxin provocation showed that this chemokine revealed chemotactic activity on eosinophils but did not cause activation of these cells. Influx of basophils was not significant (except after 1 μ g after 24 h, probably nonspecific effect). Hanazawa also did not observe statistically significant influx of basophils. The lack of basophils migration after eotaxin challenge is probably related to the low selectivity for these cells. We found no significant difference in the influx of neutrophils and macrophages at any point as expected.

MCP-3 caused inflammatory clinical response, but eosinophil influx into the nasal mucosa was not significant. The number of basophils after instillation of MCP-3 was similar to that before the challenge. As with eotaxin, MCP-3 did not cause an increase in the level of mediators in the nasal washings. Kuna et al. have reported the phenomenon of receptor class desensitization on human basophils for C-X-C and C-C chemokines, showing that low concentrations of various chemokines desensitized basophils to subsequent challenge with an optimal concentration of MCAF/MCP-1 (35). It is difficult to say whether the absence of effect of studied chemokine is related to desensitization, but this mechanism must be taken into consideration.

To sum up, eotaxin causes eosinophil recruitment into the nasal mucosa *in vivo* in human allergic subjects after specific allergen 'priming'. Therefore, this chemokine is likely to play an important role in the pathogenesis of allergic conditions. The role of MCP-3 in the pathogenesis of allergic rhinitis and asthma in humans is difficult to assess and requires further research. Knowledge about mechanisms of eosinophil recruitment shows a potential novel target for therapeutic intervention in allergic inflammation.

References

1. VANACKER AA, VOSS HP, TIMMERMAN H. Chemokines: structure, receptors and functions. A new target for inflammation and asthma therapy? *Med Inflammation* 1996;**5**:393–416.
2. ALAM R. Chemokines in allergic inflammation. *J Allergy Clin Immunol* 1997;**99**:273–277.
3. BACON KB, SCHALL TJ. Chemokines as Mediators of Allergic Inflammation. *Int Arch Allergy Immunol* 1996;**109**:97–109.
4. BAGGIOLINI M, DEWALD B, MOSER B. Human Chemokines: an Update. *Annu Rev Immunol* 1997;**15**:675–705.
5. HUMBERT M. Pro-eosinophilic cytokines in asthma. *Clin Exp Allergy* 1996;**26**:123–127.
6. ROTHENBERG ME, LUSTER AD, LILLY CM, DRAZEN JM, LEDER P. Constitutive and allergen-induced expression of eotaxin mRNA in the guinea pig lung. *J Exp Med* 1995;**181**:1211–1216.
7. JOSE PG, GRIFFITHS-JOHNSON PD, COLLINS DT, WALSH R, MOQBEL NF, TOTTY O, TRUONG O, HSUAN JJ, WILLIAMS TJ. Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airway inflammation. *J Exp Med* 1994;**179**:881–887.
8. GRIFFITHS-JOHNSON DA, COLLINS PD, JOSE ROSSI PJ, WILLIAMS TJ. The chemokine eotaxin activates guinea pigs eosinophils in vitro and causes their accumulation into the lung in vivo. *Biochem Biophys Res Commun* 1993;**197**:1167–1172.
9. LAMKHOUEB B, RENZI PM, ABI-YOUNES S, GARCIA-ZEPADA EA, ALLAKHVERDI Z, GHAFFAR O, ROTHENBERG MD, LUSTER AD, HAMID Q. Increased expression of eotaxin in bronchoalveolar lavage and airways of asthmatics contributes to the chemotaxis of eosinophils to the site of inflammation. *J Immunol* 1997;**159**:4593–4601.
10. LILLY C, WOODRUFF PG, CAMARGO CA, NAKAMURA H, DRAZEN JM, NADEL ES. Elevated plasma eotaxin levels in patients with acute asthma. *J Allergy Clin Immunol* 1999;**104**:786–790.
11. ZEIBECOGLOU K, YING S, MENG Q, KAY AB, ROBINSON DS, Papageorgiou N. Expression of eotaxin in induced sputum of atopic and nonatopic asthmatics. *Allergy* 2000;**55**:1042–1048.
12. MARSHALL EM, CAMERON L, LAVIGNE F, LEUNG DYM, HAMILOS D, GARCIA-ZEPADA EA. Eotaxin mRNA and protein expression in chronic sinusitis and allergen-induced nasal responses in seasonal allergic rhinitis. *Am J Respir Cell Mol Biol* 1997;**17**:683–690.
13. HANAZAWA T, ANTUNI JD, KHARITONOV SA, BARNES PJ. Intranasal administration of eotaxin increases nasal eosinophils and nitric oxide in patients with allergic rhinitis. *J Allergy Clin Immunol* 1999;**105**:58–68.
14. NICKEL R, BECK LA, STELLATO C, SCHLEIMER RP. Chemokines and allergic disease. *J Allergy Clin Immunol* 1999;**104**:723–742.
15. GÓRSKI P, KRAKOWIAK A, PAZDRAK K, PALCZYNSKI C, RUTA U, WALUSIAK J. Nasal challenge test in the diagnosis of allergic respiratory diseases in subjects occupationally exposed to a high molecular allergen (flour). *Occup Med* 1998;**48**:91–97.
16. KUNA P, LAZAROVICH M, KAPLAN AP. Chemokines in seasonal allergic rhinitis. *J Allergy Clin Immunol* 1996;**97**:104–112.
17. GREIFF L, PIKORN U, ALKNER U, Persson CGA. The 'nasal pool' divide applies controlled concentrations of solutes on human nasal airway mucosa and samples its surface exudations/secretions. *Clin Exp Allergy* 1990;**86**:599–605.
18. GLEICH GJ. Mechanism of eosinophil-associated inflammation. *J Allergy Clin Immunol* 2000;**105**:651–663.
19. TERAN LM, NOSO N, CAROLL M, DAVIES DE, HOLGATE S, SCHRODER JM. Eosinophil recruitment following allergen challenge is associated with the release of the chemokine RANTES into asthmatic airways. *J Immunol* 1996;**157**:1806–1812.
20. GARCIA-ZEPADA EA, ROTHENBERG RT, OWNBEY J, CELSTIN J, LEDER P, LUSTER AD. Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia. *Nat Med* 1996;**2**:449–456.
21. DAHINEN CA, GEISER T, BRUNNER T, VON TSCHARNER V, CAPUT D, FERRERA P, MINTY A, BAGGIOLINI M. Monocyte chemotactic protein 3 is a most effective basophil- and eosinophil-activating chemokine. *J Exp Med* 1998;**179**:751–756.
22. UGGUIONI MP, LOETSCHER P, FORSSMAN U, DEWALD B, LI H, LIMA SH, LI Y, KREIDER B, GARROTA G, THELEN M, BAGGIOLINI M. MCP-4 a novel structural and functional analog of MCP-3 eotaxin. *J Exp Med* 1996;**183**:2379–2384.
23. ROT A, KRIEGER M, BRUNNER T, BISCHOFF SC, SCHALL TJ, DAHINEN CA. RANTES and macrophage inflammatory protein 1 α induce the migration and activation of normal human eosinophil granulocytes. *J Exp Med* 1992;**176**:1489–1495.
24. STAFFORD S, LI H, FORSYTHE PA, RYAN M, BRAVO R, ALAM R. Monocyte chemotactic protein – 3 (MCP-3) / fibroblast induced cytokine (FIC) in eosinophilic inflammation of the airways and the inhibitory effects of an anti-MCP-3/FIC antibody. *J Immunol* 1997;**158**:4953–4960.
25. RIJN MVD, MEHIHOP PD, JUDKINS A, Rothenberg ME, LUSTER AD, OELIGEN HC. A murine model of allergic rhinitis studies on the role of IgE in pathogenesis and analysis of the eosinophil influx elicited by allergen and eotaxin. *J Allergy Clin Immunol* 1998;**102**:65–74.
26. ROTHENBERG ME, MACLEAN JA, PEARLMAN E, LUSTER AD, LEDER P. Targeted disruption of chemokine eotaxin partially reduces antigen-induced tissue eosinophilia. *J Exp Med* 1997;**185**:785–790.
27. DAUGHERTY BL, SICILIANO SJ, DEMARTINO JA, MALKOWITZ L, SIROTINA A, SPRINGER MS. Cloning, expression and characterization of the human eosinophil eotaxin receptor. *J Exp Med* 1996;**183**:2349–2354.
28. CONROY DM, HUMBLE AA, RANKIN SM, PALFRAMAN RT, COLLINS PD, GRIFFITHS-JOHNSON DA, JOSE PJ, WILLIAMS TJ. The role of the eosinophil-selective chemokine, eotaxin in allergic and non-allergic airways inflammation. *Mem Inst Oswaldo Cruz* 1997;**92**:183–191.
29. PONATH PD, QUIN DJ, RINGLER I, CLARK-LEWIS J, WANG J, KASSAM N, SMITH H, SHI X, GONZALO JA, NEWMAN W, GUTIERREZ-AMOS JC, MACKAY CR. Cloning of the human eosinophil chemoattractant eotaxin: expression, receptor binding and functional properties suggest a mechanism for the selective recruitment of eosinophils. *J Clin Invest* 1996;**97**:604–612.
30. PROOST P, WUYTS A, VAN DAMME J. The role of chemokines in inflammation. *Int J Clin Laboratory Res* 1996;**26**:211–223.
31. STOKLOSA T. Chemokines – the new family of inflammatory cytokines. *C Europ J Immunol* 1997;**22**:69–77.
32. KUNA P, ALAM R, RUTA U, GÓRSKI P. RANTES Induces Nasal Mucosal Inflammation Rich in Eosinophils, Basophils and Lymphocytes In Vivo. *Am J Respir Crit Care Med* 1998;**157**:873–879.
33. DOUGLAS JA, DHAMI D, GURR CE, BULPITT M, SHUTE JK, HOWARTH DH, LINDLEY IJ, CHURCH MK, HOLGATE ST. Influence of IL-8 challenge in the nasal mucosa in atopic and nonatopic subjects. *Am J Resp Crit Care Med* 1995;**152**:927–933.

34. COLLINS PD, MARLEAU S, GRIFFITHS-JOHNSON DA, JOSE PJ, WILLIAMS TJ. Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo. *J Exp Med* 1995;**182**:1169–1174.
35. KUNA P, REDDIGARI SR, RUCINSKI D, SCHALL TJ, KAPLAN AP. Chemokines of the α - β -subclass inhibit human basophils' responsiveness to monocyte chemotactic and activating factor/monocyte chemoattractant protein. *J Allergy Clin Immunol* 1995;**95**:574–586.
36. JOHANSSON SGO, HOURIHANE JO'B, BOUSQUET J, BRUIJNZEEL-KOOMEN C, DREBORG S, HAAHTELA T, KOWALSKI ML, MYGIND N, RING J, VAN CAUWENBERGE P, VAN HAGE-HAMSTEN M, WÜTRICH B. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;**56**:813–824.