

Mast cell and eosinophil activation during early phase of grass pollen–induced ocular allergic reaction

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ABSTRACT

Both mast cells and eosinophils were implicated in the pathophysiology of allergic conjunctivitis; however, the potential role of eosinophils in an early phase of allergic reaction has not been elucidated. The aim of this study was to assess the relation between clinical symptoms and sequence of mast cells and eosinophils specific mediators release into tear fluid during conjunctival allergen provocation. Patients with grass pollen rhinoconjunctivitis ($n = 38$) and healthy volunteers ($n = 10$) were challenged with increasing doses of allergen applied on the conjunctiva. The clinical symptoms were assessed by clinical score. Tear fluid was collected from 12 patients before provocation, at 20 and 40 minutes after positive response. Tryptase and eosinophil cationic protein (ECP) were measured using UniCap and 15-hydroxyeicosanoid acid (15-HETE) with a specific immunoassay. All allergic patients (but no control subjects) had a positive clinical response to the challenge. In 1 patient symptoms appeared after 50 BU/mL of grass allergen administration, in 3 patients symptoms appeared after 500 BU/mL (7.9% of patients), in 14 patients symptoms appeared after 1600 BU/mL (36.8%), and in 20 patients symptoms appeared after 5000 BU/mL (52.6%). The allergen dose was not correlated with the skin-prick test diameter. The mean tryptase concentration increased at 20 minutes from “nondetectable” to 5.89 ± 1.97 micrograms/L and then decreased to 1.77 ± 1.07 micrograms/L ($n = 12$; $p < 0.05$) at 40 minutes. ECP concentration was not changed at 20 minutes but increased at 40 minutes from 1.38 ± 0.98 micrograms/L before provocation to 10.61 ± 7.78 micrograms/L ($n = 7$; $p < 0.05$). There was no change in 15-HETE release. Both mast cells and eosinophils are activated during allergic reaction in conjunctiva and activation of eosinophils is preceded by activation of mast cells.

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Seasonal allergic conjunctivitis (SAC), often associated with rhinitis, is a common disease that may affect up to 40–60% of patients with respiratory allergy. Clinical symptoms of SAC include itching of eyes, redness of the conjunctiva, lacrimation, and eyelid swelling and are usually accompanied by sneezing and nasal obstruction.^{1–6} Symptoms of SAC appear in the specific pollen season depending on the sensitizing allergen and the geographical area.^{7–9}

Seasonal allergic rhinoconjunctivitis is a type I hypersensitivity reaction involving IgE-mediated mast cell activation with subsequent release of inflammatory mediators responsible for triggering acute ocular symptoms.^{7,9} The normal conjunctiva is a thin layer with goblet cells producing mucin. Mast cells are located mostly in the lamina propria of the conjunctiva and are concentrated around blood vessels and glands but during the ongoing allergic inflammation they migrate into the epithelium.^{10,11} Histamine released from

mast cell leads to vasodilatation observed as eye redness, increased vascular permeability, and sensory nerves activation resulting in itching.¹¹ Other mast cell–derived mediators such as prostaglandins, leukotrienes, kinins, and mast cell tryptase can be also detected in the tear fluid and seems to contribute to development of acute symptoms.¹² Furthermore, mast cell–derived mediators including chemokines lead to recruitment of other inflammatory cells: eosinophils, basophils, B cells, and T cells resulting in development of conjunctival inflammation and persistence of symptoms even in the absence of exposure to allergens. Eosinophils and basophils are not seen in healthy conjunctiva.¹⁰ Graziano *et al.* showed, using the animal models, that mast cells activation leads to the cytokines release and further eosinophil activation—moving to the ocular surface, their adhesion, and mediators release.¹³ The presence of the late-phase ocular reaction may influence the severity of the disease, changing ocular reactivity, and predict the disease progression from localization in the eye to a systemic atopic disease.¹⁴

Eosinophils, which are absent in normal conjunctiva, infiltrate intensively the tissue during the inflammation process and are considered to be the key cells involved in the pathophysiology of the chronic phase of allergic conjunctivitis.^{14,15} Producing toxic cationic

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proteins (major basic protein and eosinophilic cationic protein [ECP]), enzymes, oxygen metabolites, and arachidonic acid metabolites eosinophils may be responsible for chronic symptoms and damage of conjunctival epithelium.^{16,17} ECP is a specific marker of eosinophil activation and increased levels were found in tear fluid in SAC and perennial allergic conjunctivitis.¹⁸ Moreover, ECP levels were found to be increased 6 hours after challenge with specific allergen, suggesting involvement of eosinophil activation in the developing of the late phase of allergic reaction in the conjunctiva.¹⁹ Bonini *et al.* detected eosinophils (in addition to neutrophils and lymphocytes) in the tears at 12–24 hours after the challenge.²⁰ However, the involvement of eosinophils into the early phase of allergic reaction in the conjunctiva has not been established.

The allergen conjunctival provocation test (CPT) is a well-established model of ocular allergy and it may be useful tool for the diagnosis of allergic conjunctivitis and for the study of the pathophysiology of allergic reaction.^{21–23} We used this model to assess the relationship between development of allergen-induced clinical symptoms and activation of mast cells and eosinophils assessed with specific mediator release into tear fluid.

PATIENTS AND METHODS

Study Subjects

Thirty-eight patients with seasonal allergic rhinoconjunctivitis (14 men and 24 women; 19–42 years old) were recruited into the study. All patients had a history of ocular and nasal symptoms during grass pollen season and pollen sensitization was confirmed by a positive skin-prick test (SPT) to grass pollen allergens. Patients with history of other eye diseases (glaucoma, cataract, and/or uveitis), treated with β -blockers, having history of bronchial asthma, grass pollen immunotherapy, clinically important allergy to perennial allergens, nasal septum deviation, or polyps were excluded from the study. Patients were tested before the grass pollen season and on the day of provocation, patients were asked not to wear contact lenses. Oral antihistamines were withheld for at least 1 week before the study and topical medications that could interfere with the CPT results were withheld for at least 12 hours (cromones) or 24 hours (prednisone and prednisolone), *i.e.*, at least the time equivalent to five to seven times of the drug half-time according to the French recommendations.²⁴

The control group used as negative control for CPT specificity consisted of 10 subjects (3 men and 7 women; aged 27–49 years old). Eight subjects were healthy, nonatopic volunteers and two subjects were sensitive to others allergens (one to *Dermatophagoides* sp. and one to trees and weeds). This study was approved by local

Ethics Committee. All subjects signed written informed consent.

Conjunctival Provocation

Grass pollen lyophilizate of allergen in a concentration of 5000 BU/mL (Allergopharma J. Ganzer KG, Reinbek, Germany) was diluted in *aqua ad* injections (containing 0.4% phenol, human albumins, sodium chloride, and carbonate) to contain six final concentrations: 5000, 1600, 500, 160, 50, and 5 BU/mL. Starting with negative control solution (diluent), one drop of increasing concentrations of allergen solution was applied every 10 minutes alternatively to the right and then to the left eye. The higher dose was added only if there was no reaction after the previous one.

Subjective symptoms were evaluated by patients after each dose of allergen using the following scale: 0, no itching; 1, slight itching; 2, moderate itching; and 3, intensive itching. The physician assessed the reaction by estimating the redness according to the following scale: 0, no redness; 1, palpebra conjunctival redness; 2, palpebra conjunctival redness + eyeball conjunctiva redness (no more than one-half of it); and 3, palpebra conjunctival redness + eyeball conjunctiva redness (more than one-half). If either the subjective or the objective symptoms score reached at least 2 points, the CPT was interpreted as positive and the challenge was stopped at this point. The clinical reaction was finally assessed by summing up both objective (graded from 0 to 3) and subjective symptom scores (graded from 0 to 3).

Collection of Tear Fluid

Tear fluid was collected from 12 patients before provocation and then 20 and 40 minutes after positive response.^{25,26} Three filter paper strips (Schirmer's test strips) were placed in the lower eye fornix without anesthesia and remained until they became completely wet. During this time the patient was asked to avoid frequent blinking. The paper was removed and inserted into 500 μ L of phosphate-buffered saline for 15 minutes to dissolve absorbed mediators. The collected fluid was stored at 20°C until assayed. To diminish any local discomfort, patients' eyes were finally washed with saline and if the reaction was strong, antihistamine drops were administered.

Measurement of Mediators in Tear Fluid

Tryptase and ECP were measured using UniCap (Pharmacia, Upsal, Sweden). Sensitivity for tryptase was 1.00 μ g/L and sensitivity for ECP was 2.00 μ g/L. A 15-hydroxyeicosanoid acid (15-HETE) concentration was evaluated by competitive enzyme-linked immunoassays (Assay Designs, Inc., Ann Arbor, MI) according to the original manuals. Minimal 15-HETE sensi-

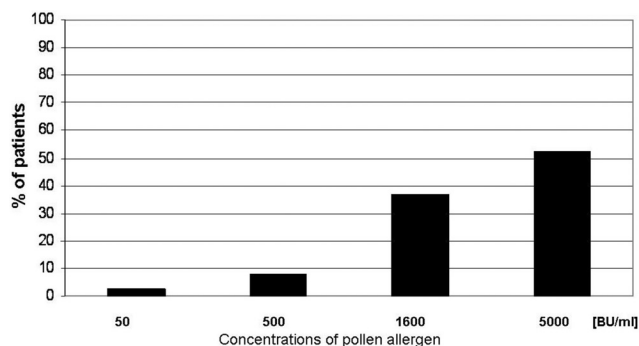


Figure 1. The percentage of patients with rhinoconjunctivitis showing a positive response to increasing concentrations of pollen allergen ($n = 38$).

tivity was 69.2 pg/mL. The following cross-reactivity occurred: 15-HETE, 100%; 12-HETE, 7.2%; 5-HETE, 0.6%; 5, 15-diHETE, 0.3%; 8,15-diHETE, 0.3%; arachidonic acid, 0.1%; prostaglandin D₂ (PGD₂), 0.1%; PGE₂, 0.3%; and PGF_{2 α} , 0.3%.

Statistical Analysis

Mann-Whitney U test was used to compare data between two groups of patients. Analysis of differences within the group were performed using Wilcoxon matched pairs test. Results of mediator measurements were expressed as median $\pm$ SEM, and $p < 0.05$ was considered statistically significant.

RESULTS

Clinical Results of CPT Regarding Allergen Concentration

All patients reported itching as the first symptom immediately after allergen application. Conjunctival redness appeared within 3–5 minutes in the eye to which the last allergen dose was administered and within 5–10 minutes it spread to the other eye in all patients with positive response. Some patients reported nasal symptoms such as watery nasal discharge ($n = 4$), nasal blockage ($n = 2$), and sneezing ($n = 1$). None of the subjects from the control group had any ocular or nasal symptom after conjunctival provocation.

All allergic patients had positive response to CPT; only 1 of 38 patients reacted to the lowest concentration of 50 BU/mL, 3 subjects (7.9%) reacted to 500 BU/mL, 14 subjects (36.8%) reacted to 1600 BU/mL, and 20 subjects (52.6%) responded to the highest concentration of grass pollen allergen of 5000 BU/mL (Fig. 1). The median value of clinical symptoms score after diluent (negative control) administration was 0.013 because of one nonspecific eye reaction. Administration of allergen at the 5 BU/mL concentration did not change statistically the median value clinical score but starting from 500 BU/mL with increasing allergen con-

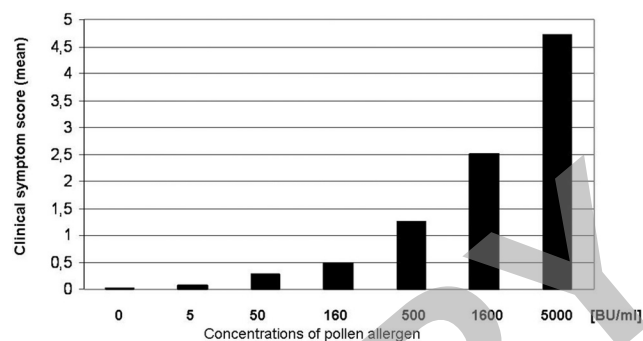


Figure 2. Clinical symptom scores according to the amount of allergen necessary to induce symptoms during the challenge ($n = 38$).

centrations there were statistically significant increases in median symptoms score from 1 to 2.5 and to 5.0 for 500, 1600, and 5000 BU/mL, respectively ($p < 0.05$; Fig. 2). There was no correlation between the diameter of positive SPT wheal to grass pollen allergen mixture and the provocation dose of allergen in CPT (mean wheal diameter was 10.65 ± 3.45 mm, 11.71 ± 4.02 mm, and 11.33 ± 1.52 mm in patients responding to 5000, 1600, and 500 BU/mL, respectively; NS).

Tryptase and ECP Release into the Tear Fluid

None of the 12 patients tested had detectable tryptase concentration in tear fluid before the provocation. Tryptase was detectable at 20 minutes after positive clinical response in 10 patients and in 7 of them tryptase concentration returned to a “nondetectable” level at 40 minutes. In three subjects tryptase concentration decreased at 40 minutes when compared with the 20-minute time point, but it was still higher than at the baseline. In one patient tryptase increased at 40 minutes and in another no tryptase was detectable at any time point. The median concentration of tryptase increased from “nondetectable” to 5.89 ± 1.97 μ g/L ($p < 0.05$) at 20 minutes after provocation and then decreased to 1.77 ± 1.07 μ g/L ($p < 0.05$) at 40 minutes (Fig. 3a). There was no detectable tryptase in tear fluid in the control group ($n = 6$) in any of the time points measured.

Before the challenge ECP was detectable in the tear fluid only in one of seven allergic patients tested and at 20 minutes in none of them. However, at 40 minutes in the positive reaction in five of seven patients a significant concentration of ECP could be measured. The median concentration of ECP in tear fluid increased from 1.38 ± 0.98 μ g/L before provocation to 10.61 ± 7.78 μ g/L at 40 minutes ($p < 0.05$; Fig. 3 b). There was no detectable ECP level in tear-fluid in the control group at any time point.

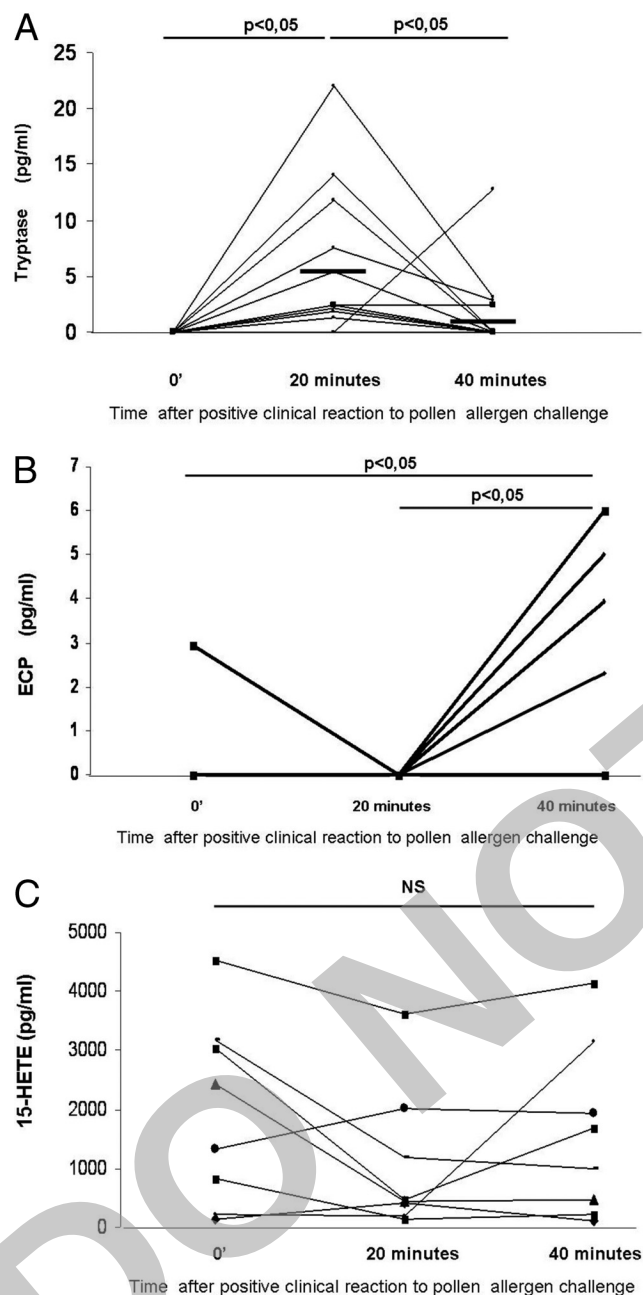


Figure 3. Concentrations of (a) tryptase, (b) eosinophilic cationic protein (ECP), and (c) 15-hydroxyeicosanoid acid (15-HETE) in tear fluid from individual patients at baseline and 20 and 40 minutes after the challenge with pollen allergens.

15-HETE Generation

15-HETE was detectable at baseline and at 20 and 40 minutes after provocation in all patients with allergic conjunctivitis ($n = 8$). For individual patients there were fluctuations up and down in 15-HETE concentration after the challenge, but they were not consistent. The median concentration of 15-HETE in tear fluid before the challenge was 1878.5 ± 559.6 pg/mL, at 20 minutes was 456 ± 427.2 pg/mL, and at 40 minutes was 1344.5 ± 512.8 pg/mL; however, the changes were

not statistically significant (NS; Fig. 3 c). In control patients 15-HETE concentrations were not assessed.

DISCUSSION

This study confirmed that CPT is a highly sensitive tool for diagnosis of SAC because all 38 patients with a history of seasonal disease reacted to the challenge. The specificity of the test was confirmed by negative response to pollen allergen challenge in eight healthy volunteers and in two patients with allergic conjunctivitis and sensitive to other than grass pollen allergens. CPT may be used to confirm ocular allergy in patients with positive SPT results but it is particularly useful when a positive clinical history is confronted with negative SPT results and lack of allergen-specific serum IgE.^{1,24,26} It can be also helpful to determine the most clinically relevant allergens in polysensitized patients before allergen-specific immunotherapy and then to assess the effectiveness of the immunotherapy.^{27–32} In patients with allergic rhinitis, CPT can be used as a diagnostic method alternative to nasal provocation test.³³

Conjunctival allergen provocation seems to be a good model to study pathophysiology of ocular allergy because both early and late-phase response were observed after conjunctival allergen challenge. When combined with tear fluid sampling, CPT allows for measurement of mediator release and, indirectly, for assessment of inflammatory cell activation. In our study, measurement of mast cell tryptase turned out to be a sensitive and specific marker correlating with clinical reaction. Significant increase in tryptase concentration in tear fluid was observed at 20 minutes after allergen challenge but at 40 minutes concentration of tryptase in tear fluid tended to decrease, indicating that mast cell activation was no longer present. Such early increase in tryptase is consistent with other studies showing early increase in the concentration of other mast cell-specific mediators: histamine, PGD_2 , and neutrophil chemotactic activity in tear fluid after allergen challenge.^{34–39} In contrast to histamine, which was usually detectable in tear fluid at baseline conditions,³⁴ tryptase was not measured before the challenge, which facilitated subsequent assessment of mast cell activation. Our study suggests that measurement of mast cell tryptase may be practical if assessment of mediator release in the tear fluid is used to increase reliability of the CPT.

This study shows a relatively early increase in ECP level, observed already at 40 minutes after the challenge. Those results could indicate that eosinophils may be activated during the early phase of the allergen-induced immediate reaction. This study is in agreement with Wang and colleagues who showed a simultaneous increase in a percentage of eosinophils as

well as ECP concentration at 1 hour after the nasal allergen challenge. The eosinophil count reached the peak at 2 hours after the nasal provocation and lasted for 8 hours. The highest ECP level was observed at 24 hours after the challenge but with no plateau phase.⁴⁰ Interestingly, a similar early ECP increase in nasal washes was previously reported during non-IgE-mediated hypersensitivity reaction induced by local aspirin challenge in patients with aspirin exacerbated respiratory disease.⁴¹ The clinical symptom appearance was associated with the eosinophil influx into the nasal secretions and ECP increase. They were also correlated with a higher tryptase level after the nasal challenge, indicating that both eosinophils and mast cells play a role in the aspirin-induced nasal mucosa reaction.

Early studies indicated the ECP role only in the late-phase response. Montan *et al.* analyzed ECP in tears during a 72-hour time period after a single dose of allergen challenge.⁴² Tear fluid ECP was significantly elevated in challenged eyes compared with contralateral eyes only at 6, 8, and 24 hours while at earlier time points of 10 minutes and 2 and 4 hours no change in ECP level was observed.⁴³ The clinical relevance of the ECP level has not been explained by showing no correlation with the chronic disease. Muromoto *et al.* measured the ECP level in patients with SAC and found that it was useful regarding the pathology; however, the ECP dose does not facilitate the diagnosis because of many false negative subjects.³⁸ Because our immunoassay has similar sensitivity to detect ECP as in a study by Muromoto, the lack of false negative results in our study could be explained either by different doses of allergen or different technique of the tear fluid sampling.³⁸

This study reports for the first time the presence of an arachidonic acid metabolite 15-HETE in tear fluid from patients with allergic rhinoconjunctivitis. 15(S)-HETE may be produced by epithelial cells, endothelial cell eosinophils, and macrophages, although the source of this mediator detected in the tear fluid is not known.^{44–46} 15-HETE has mostly proinflammatory activity but its contribution to IgE-mediated reactions is not known. High levels of 15-HETE were observed in bronchoalveolar lavage in children with bronchial obstruction.⁴⁷ We have already found that aspirin triggers specific generation of 15-HETE from nasal polyp epithelial cells and peripheral blood leukocytes from aspirin-sensitive but not aspirin-tolerant patients with asthma/rhinosinusitis.^{48,49} In this study, although 15-HETE was detectable in the tear fluid at baseline, no increase in 15-HETE generation could be consistently found after the challenge. However, high levels of 15-HETE detected in the tear fluid suggest that it may be involved in the pathophysiology of chronic ocular inflammation and further studies are warranted to clarify its potential role.

In conclusion, we showed that the conjunctival response to provocation with allergen is associated with early activation of both mast cells and eosinophils, suggesting a contribution of both cell types to the pathophysiology of acute ocular reaction.

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