ORIGINAL ARTICLES – Laboratory science

# Eosinophil cationic protein in tears in allergic conjunctivitis

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**Abstract**

***Aims/background*—Eosinophil cationic protein (ECP) is a quantifiable product secreted by activated eosinophils. The aim of this study was to assess the degree of eosinophil activity in different clinical stages of various forms of allergic conjunctivitis.**

***Methods*—Tears were collected in glass capillary tubes from 14 subjects with seasonal allergic conjunctivitis (SAC), 23 subjects with vernal keratoconjunctivitis (VKC), 16 subjects with atopic keratoconjunctivitis (AKC), 10 subjects with giant papillary conjunctivitis (GPC), and 16 healthy control subjects. The samples were analysed in duplicate with a radioimmunoassay for ECP.**

***Results*—Statistically significant differences were evident between healthy controls and allergic subjects ($p < 0.001$). Subjects with AKC and VKC had significantly higher tear ECP values than subjects with GPC and SAC. In addition, there was a significant correlation between ECP values and disease severity in all disorders.**

***Conclusion*—The data suggest a particular pathogenic role of the eosinophil in VKC and AKC, and a less pronounced but still important eosinophil involvement in the disease processes of GPC and SAC.**

(*Br J Ophthalmol* 1996;80:556–560)

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Accepted for publication 11 March 1996

In investigations of ocular allergy, the presence of eosinophilic granulocytes has been a prominent finding in biopsies,[1 2] whereas more or less unintrusive sampling methods such as conjunctival scraping[3–5] and tear fluid collection[4] have failed to detect eosinophils in a considerable number of cases. Thus the pathological importance of the eosinophil in allergic conjunctivitis remains to be defined.

The eosinophil plays a key role in the inflammation of allergic diseases in general. It is a major cellular component in the late allergic response whether the target organ is the lung[6] or the eye.[7] In addition, the eosinophil is considered a potent proinflammatory cell[8] with truly pathogenic properties[9] in bronchial asthma. In its activated state the eosinophil liberates preformed basic proteins: eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil peroxidase (EPO), and eosinophil protein X/eosinophil derived neurotoxin (EPX/EDN). Besides the proved toxic effects of MBP and indirect evidence of damaging effects of ECP and EPO on airway epithelium in animals,[10] some data suggest an association between asthma severity and eosinophil activity, measured as released ECP.[6 9] Moreover, MBP[11] and EPO[12] may be capable of degranulating mast cells, and additional effector functions of the eosinophil can be exerted by its inflammatory mediators prostaglandin $E_2$, leukotriene $C_4$, and platelet activating factor (PAF).

To elucidate the role of eosinophil activation in allergic conjunctivitis, ECP was analysed in tears and serum of patients with various allergic eye disorders.

## Patients and methods

### PATIENTS

Sixty three patients with conjunctival signs and symptoms meeting the diagnostic criteria (Table 1) for seasonal allergic conjunctivitis (SAC) induced by pollen, vernal keratoconjunctivitis (VKC), atopic keratoconjunctivitis (AKC), or giant papillary conjunctivitis (GPC) were selected. The basis for inclusion was a history of conjunctivitis, be it seasonal for the preceding 2 years or chronically relapsing for at least 1 year. Patients with SAC were required to have had at least a 24 hour duration of symptoms before testing. Oral steroids, nonsteroidal anti-inflammatory drugs, or antihistamine treatment along with any topical medication within the previous month were reasons for exclusion, with the following exceptions: 7/23 VKC and 4/16 AKC patients were on sodium cromoglycate eyedrops, 4/23 VKC patients were on prednisolone eyedrops, and 10/16 AKC patients used steroidal lotion on the face, mostly the eyelids. Patient demographics are shown in Table 2.

### ATOPY SCREENING (DIAGNOSIS OF IgE MEDIATED HYPERSENSITIVITY)

A confirmed pollen sensitisation with radioallergosorbent testing (Pharmacia CAP Systems, RAST FEIA, Pharmacia Diagnostics, Uppsala, Sweden) was a prerequisite for being included as an SAC subject. Among patients with the chronic allergic conjunctivitis forms, diagnosis of atopy was based on the presence of

*Table 1 Diagnostic inclusion criteria for the allergic eye disorders*

| *Disease* | *Conjunctival signs* | *Age group* | *Current pollen exposure and confirmed pollen hypersensitivity* | *History/presence of contact lenses or surgical sutures* |
|---|---|---|---|---|
| SAC | Erythema, swelling, and discharge | Any | Required | No |
| VKC | Superior subtarsal papillae or limbal nodules | Children | Not required | No |
| | Superior subtarsal papillae or limbal nodules without presence or history of atopic dematitis | Adults | Not required | No |
| AKC | Conjunctival erythema with lid eczema in association with atopic dermatitis | Adults | Not required | No |
| GPC | Superior subtarsal papillae | Any | Not required | Yes |

SAC=seasonal allergic conjunctivitis; VKC=vernal keratoconjunctivitis; AKC=atopic keratoconjunctivitis; GPC=giant papillary conjunctivitis

*Table 2 Demographics of study and control subjects*

| *Diagnosis* | *No of subjects* | *No of atopics* | *No of males* | *Mean age (years)* | *(Range)* |
|---|---|---|---|---|---|
| SAC* | 14 | 14 | 11 | 29 | (11–48) |
| VKC | | | | | |
| Palpebral | 18 | 9 | 15 | 11 | (4–33) |
| Limbal | 5 | 4 | 5 | 17 | (3–44) |
| AKC | 16 | 14 | 6 | 34 | (20–47) |
| GPC† | 10 | 3 | 7 | 46 | (23–74) |
| Healthy controls | 16 | 0 | 8 | 23 | (5–50) |
| Blepharoconjunctivitis | 4 | 0 | 2 | 27 | (7–47) |

* SAC was due to birch pollen (n=9) or timothy grass pollen (n=5).
† GPC was caused by contact lens wear (n=7) or sutures (n=3).

a positive Phadiatop (Pharmacia CAP Systems), which is an in vitro test for the determination of IgE against any one constituent of a mixture of common inhalant allergens. The test has a high diagnostic precision in atopy screening.[13] Three of the children with VKC did not agree to venepuncture so atopy was diagnosed with a skin prick test using a wide panel of common allergens (Soluprick, ALK, Copenhagen, Denmark). All tests were performed according to the manufacturers' instructions. The proportion of atopic patients is indicated in Table 2.

RECORDING OF SYMPTOMS AND SIGNS

Scoring systems with a 1–3 scale (1=mild and 3=severe) adjusted to the differing manifestations of the four types of allergic conjunctivitis, were designed to assess the patients' inflammatory signs and symptoms (Table 3). The crucial occurrence of corneal lesions in AKC and VKC was defined as a score point of 3 regardless of the presence of other clinical variables.

*Table 3 Score system for signs and symptoms of the different subgroups of allergic conjunctivitis*

| *Disease form* | *Inflammatory symptoms* | *Allergic subgroup* 1 | 2 | 3 |
|---|---|---|---|---|
| SAC | Erythema and swelling of bulbar conjunctiva, tearing, itching | Mild | Moderate | Severe |
| VKC–limbal | Limbal papillae with erythema, itching | Mild | Moderate | Severe |
| VKC–palpebral | Subtarsal papillae and erythema of the upper eye lid, discharge, itching, and discomfort | Mild | Moderate | Severe or any corneal lesion |
| AKC | Erythema of bulbar or subtarsal conjunctiva, thickening of subtarsal conjunctiva, itching, and discomfort | Mild | Moderate | Severe or any corneal lesion |
| GPC | Papillae > 0.4 mm, erythema, and thickening of subtarsal conjunctiva, discharge, and discomfort | Mild | Moderate | Severe, contact lens intolerance |

CONTROL SUBJECTS

Sixteen healthy volunteer subjects, five of them children, were included in the study as a negative control group. None had a history of atopic disease, and serum screening for atopy was negative. None had experienced any ocular inflammation for the past 6 months and they were all free from medication and contact lens wear. To test the specificity of tear ECP for allergic conjunctivitis, four patients with isolated blepharitis and secondary conjunctivitis, all non-atopic and untreated, were selected as a positive control group (Table 2).

ECP ANALYSIS IN TEARS AND SERUM

Tears were collected from the lateral canthus of one eye in each subject in 50 µl glass blood caps (Kebolab, Stockholm, Sweden) and transferred to Ependorff tubes for storage at − 20°C. The samples, ranging in volume from 15 to 30 µl, were diluted 5 or 10 times to a final volume of 100 µl and analysed in duplicate with the Pharmacia ECP RIA test. The ECP values, expressed in µg/l, were adjusted to the dilution coefficient of the tear sample. Owing to the dilution factor, ECP levels <20 µg/l were undeterminable in most negative control cases. Serum samples were drawn from all healthy controls, all AKC subjects as well as from 9/14 SAC, 15/23 VKC, and 9/10 GPC subjects.The samples were handled according to the instructions of the manufacturer and kept at − 20°C until analysed. Serum ECP values > 2 µg/l were positive.

STATISTICS

Tear and serum data of all groups were analysed non-parametrically with the Kruskall-Wallis test (ANOVA). The two tailed Mann-Whitney U test was used in multiple comparisons between individual groups. p Values <0.05 corrected for ties were considered significant. To test the association between tear and serum ECP and symptoms, the Kendall rank correlation coefficient $\tau$ was used.

The project was approved by the local ethics committee.

## Results

The tear ECP values in relation to symptom scores of each allergic subject are shown in Figures 1–4. The highest median concentration was found in VKC (470 µg/l; range 19–6000), followed by AKC (215; 36–1900), SAC (70; 4–540), and GPC (53; 20–1700). In the healthy control group one subject presented 33 µg/l while all other subjects had values below 20 µg/l. The blepharitis cases exhibited a median of 74 µg/l (range 32–125). Tear ECP of negative controls was significantly lower than that of all disease groups ($p < 0.001$), as were tear ECP values of SAC subjects compared with those of AKC subjects (p=0.019) and VKC subjects (p=0.0013). In

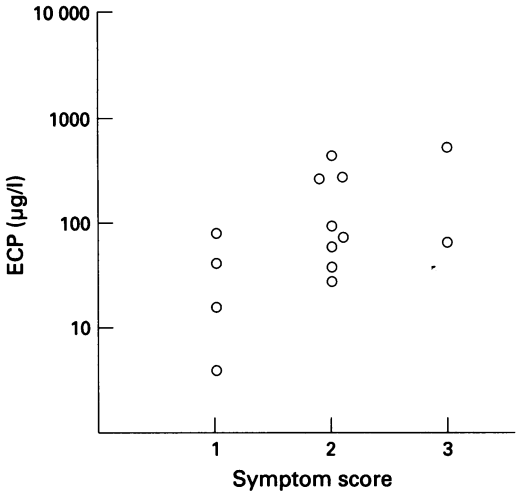


*Figure 1 Seasonal allergic conjunctivitis. Tear eosinophil cationic protein (ECP) values on a logarithmic scale are shown in relation to symptom score of each subject. Kendall correlation coefficient τ=0.451 (p=0.046). Open symbols, atopic subject; closed symbols, non-atopic subject.*

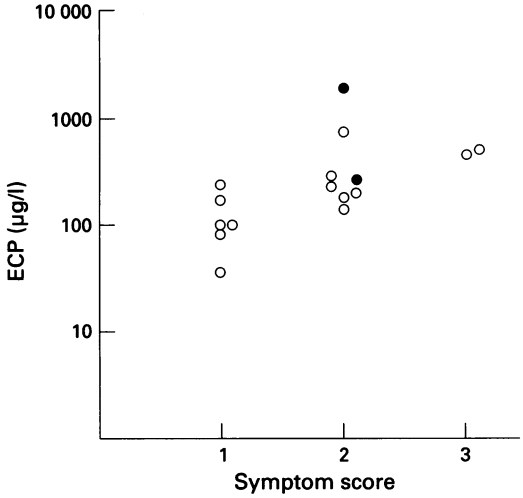


*Figure 3 Atopic keratoconjunctivitis. Tear eosinophil cationic protein (ECP) values on a logarithmic scale are shown in relation to symptom score of each subject. Kendall correlation coefficient τ=0.610 (p=0.0049). Open symbols, atopics ubject; closed symbols, non-atopic subject.*

GPC, tear ECP levels were also significantly lower than in AKC (p=0.023) and VKC (p=0.0092). The subjects with palpebral vernal disease had a greater median value than those affected by limbal vernal disease, 540 µg/l versus 215 µg/l, but the difference was not statistically significant.

Symptoms and the tear ECP amount correlated significantly in all allergic conjunctivitis forms. The Kendall correlation coefficient values are shown in Figures 1–4. Serum ECP results, given as median and range in µg/l, were for healthy controls: (8.5; 2–16), SAC: (15; 8–27), VKC: (16; 3–70), AKC: (18.5: 3–42), and GPC: (12; 4–49). Controls differed significantly from the disease groups (p=0.0048), but no significant differences were evident between the various allergic groups and no correlation was found between conjunctivitis symptoms and serum levels of ECP; neither was there any relation between tear and serum ECP in any of the disease entities.

Among subjects with chronic conjunctivitis, atopic individuals—that is, subjects with proved IgE mediated hypersensitivity, had significantly higher serum ECP concentrations than non-atopic subjects (p=0.020, Mann-Whitney U test). In contrast, no such relation was observed between atopy and levels of tear ECP.

## Discussion

The present clinical study provides evidence of local eosinophil activation and its agreement with symptom scores in the major allergic conjunctivitis disorders. Furthermore, eosinophil activity proved significantly higher in VKC and AKC, the most serious allergic conjunctivitis forms, than in the fairly benign conditions SAC and GPC.

The conspicuous association between VKC and eosinophil protein release confirms previous reports on subjects with VKC presenting

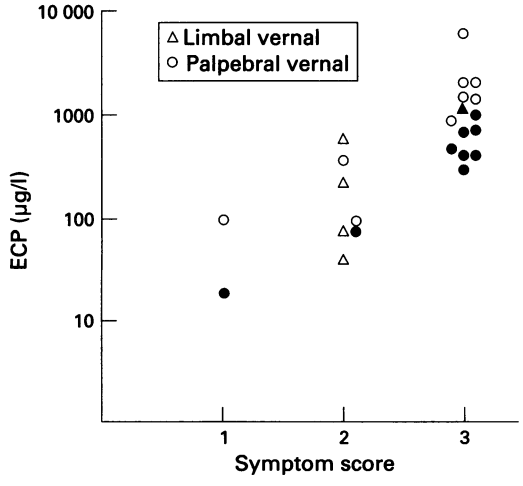


*Figure 2 Vernal keratoconjunctivitis. Tear eosinophil cationic protein (ECP) values on a logarithmic scale are shown in relation to symptom score of each subject. Kendall correlation coefficient τ=0.690 (p<0.001). Open symbols, atopic subject; closed symbols, non-atopic subject.*

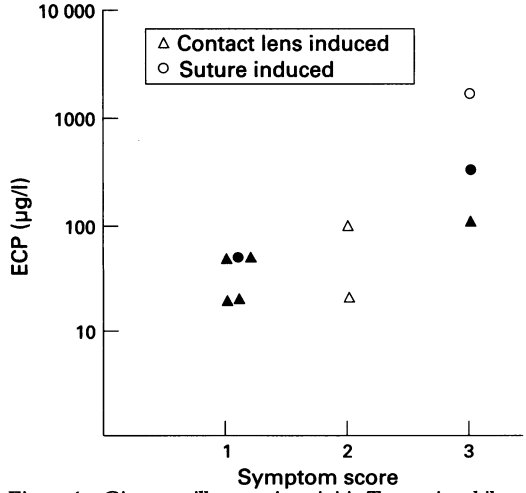


*Figure 4 Giant papillary conjunctivitis. Tear eosinophil cationic protein (ECP) values on a logarithmic scale are shown in relation to symptom score of each subject. Kendall correlation coefficient τ=0.657 (p=0.018). Open symbols, atopic subject; closed symbols, non-atopic subject.*

increased levels of tear ECP[14] and of MBP in tears[15] and tissue.[16] The subjects suffering from corneal epithelial defects—that is, 3 points in symptom score, almost consistently exhibited the highest tear ECP measurements (Fig 2). Eosinophil activity in these cases may well be as much the cause as the consequence of inflammation, thus resembling the situation in asthma where a cause and effect relation has been proposed for eosinophil secretion and tissue damage.[9] The possibility of eosinophils provoking epitheliopathy has further been suggested in an in vitro model, where MBP has proved to retard epithelial healing of the cornea.[17] In limbal vernal disease, corneal wounds rarely appear, which the somewhat lower ECP levels in comparison with those of palpebral vernal disease seem to reflect.

The AKC median tear ECP value was more than two times lower than that of VKC. Possibly, this statistically insignificant difference could be attributable to the small number of AKC subjects with corneal disease in our material (Fig 3). A recent histopathological study failed, however, to single out activated eosinophils as the cause of keratopathy in AKC.[18] Nevertheless, ECP itself could be responsible for another important tissue alteration in AKC, since it inhibits proteoglycan degradation[19] and thereby may contribute to conjunctival scarring.

Moderate, but still significant, increases of tear ECP were demonstrated in SAC. However, three out of 14 patients with SAC rendered values below the highest negative control value. The prerequisite of high allergen concentrations to elicit eosinophil recruitment has been clearly established in one human challenge model.[7] Consequently, we believe that the three SAC subjects with low tear ECP levels had experienced too low an allergen exposure with respect to their individual sensitivity to evoke cosinophil migration and activation. Still, the significant correlation between disease activity and ECP implies that eosinophils can have a pathogenic role also in severe cases of SAC.

In contact lens induced GPC, tissue eosinophilia has been previously reported as less pronounced than that of VKC,[1] which agrees with the tear ECP results of our corresponding GPC subjects. Two subjects with papillary disease secondary to exposed sutures demonstrated the highest ECP levels (Fig 4). Whether suture induced conjunctivitis is more liable to cause eosinophil activation than contact lens associated disease could not be determined, however, owing to the limited number of subjects examined.

VKC, AKC, and GPC are all deemed allergic on the basis of conjunctival mast cell infiltration,[1 2 20] the presence of mast cell derived products,[21 22] and IgE in tears.[23-25] None of the disorders is, however, invariably associated with atopy. In the present investigation the highest proportion of atopics was found in AKC and the lowest in GPC (Table 2), which confirms previously published data.[23 26-28] When atopics were compared with non-atopics of the chronic allergic groups, no significant difference was found in tear ECP. Interestingly, Bentley *et al* reported an equal increase in tissue EG2$^+$ cells—that is, 'activated' eosinophils, in non-atopic and atopic asthma.[29] It might be that the allergen specific hypersensitivity of the atopic subjects superimposes on pathogenic mechanisms that are commonly shared in atopic and non-atopic disease. Clearly, the cytokines interleukin 3 (IL-3), IL-5, and granulocyte macrophage-colony stimulating factor (GM-CSF)[30] and the chemoattractants leukotrienes,[31] PAF,[32] and histamine[33] can all be released in the wake of allergen and specific IgE interactions, but it remains unknown whether the same biological pathways are responsible for eosinophil activation and infiltration in subjects who lack evidence of specific hypersensitivity.

Isolated subjects with blepharoconjunctivitis were included as a positive control group to investigate the role of eosinophil activation in non-allergic conjunctivitis. This group also showed increased tear ECP values compared with the negative control subjects. Eosinophil participation in blepharitis has not yet been suspected and can only be established with an extension of this study group. Beyond doubt, elevated tear ECP is not specific for allergic conjunctivitis.

The serum ECP analyses revealed statistically significant increases over normal controls for all allergic disease entities, which confirms a recent report on vernal conjunctivitis[34] and numerous investigations of other allergic diseases like atopic dermatitis[35] and asthma.[36] In our study, the presence of atopy in chronically affected patients was the only disease variable which was related to high serum ECP values. It is conceivable that the serum ECP was more influenced by associated major allergic manifestations such as eczema or asthma, which were nearly ubiquitous in atopics and close to absent in non-atopics, than by the conjunctival inflammation.

In conclusion, we have demonstrated that tear ECP, in contrast with serum ECP, is a useful marker for disease severity in allergic conjunctivitis and as such could become a valuable objective variable in treatment studies. The sensitivity of eosinophil detection in conjunctival scraping[3-5] and tear fluid[4] has been low, and tear ECP testing seems more reliable since it yielded a much greater percentage of subjects with allergic conjunctivitis with concentrations exceeding the highest normal control value. Consequently, the test holds a potential as an adjunctive diagnostic tool in chronic conditions, because repeated values below 20 μg/l do not suggest allergy as being the cause. On the other hand, elevated tear ECP is not pathognomonic for allergic inflammation.

The exact pathogenic importance of the eosinophil and its releasable products is yet unknown in allergic conjunctivitis. However, the consistently high levels of tear ECP in AKC and VKC suggest an instrumental role of the eosinophil in the pathophysiological changes that characterise these severe chronic conditions.

The authors thank Gunnar Edman for his valuable help with the statistical analyses. This work was supported by grants from the Carmen and Bertil Regner Foundation, the Swedish charter for the Prevention of Blindness, the Swedish Association against Asthma and Allergy, the Swedish Medical Research Council (grant no 16X-105), the Swedish Work Environmental Fund, the Hesselman Foundation, and the Konsul Th Berg Fund, Stockholm, Sweden.

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