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SHORT COMMUNICATION

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Eotaxin-1 (CCL11) up-regulation in tears during seasonal allergic conjunctivitis

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Abstract *Purpose:* To compare in-season eotaxin-1 levels in tears of patients suffering from seasonal allergic conjunctivitis (SAC) with (1) tears of normal subjects and (2) tears of SAC patients out of season. *Methods:* Tears of 11 SAC patients and six control volunteers were collected during the pollen season. Tears of five SAC patients showing a strong sensitivity to grass pollen (skin-prick tests and specific serum IgE) were collected both in season and out of season. ELISA measured eotaxin-1 level. *Results:* Eotaxin-1 concentration in tears of SAC patients [2,100±503 (SEM) pg/ml] and normal subjects (1,193±176 pg/ml) were significantly different (P=0.0049). Regarding allergic patients, the clinical score (sum of five allergic criteria) was significantly different in season and out of season (P=0.0043) as was also the case with eotaxin-1 concentration (P=0.024). *Conclusions:* The eotaxin-1 concentration in tears of patients showing hay fever could confirm a diagnosis of seasonal ocular allergy.

Introduction

Human eotaxin-1, now referred to as CC chemokine ligand 11 (CCL11) [10], has been cloned, and a specific eosinophil eotaxin receptor, CC chemokine receptor 3 (CCR3), characterized [4, 6, 11]. Eotaxin plays a critical role in allergic diseases [11, 12].

Eotaxin-1 has been found in tears of patients suffering from atopic keratoconjunctivitis (AKC) with severe corneal damage [3] and in the mucus of patients with vernal keratoconjunctivitis (VKC) [9]. An increase in eotaxin staining has been histologically demonstrated in the conjunctiva of patients with VKC as compared to control subjects [1]. In vitro, eotaxin-1 expression is inducible in human corneal keratocytes and conjunctival fibroblasts [4, 7, 9]. Eotaxin-1 has been reported in tears of seasonal allergic conjunctivitis (SAC) patients [5] but, surprisingly, never in comparison with nonallergic subjects.

The aims of our study were to compare the tear eotaxin-1 level of SAC patients (1) with that of normal subjects and (2) both during pollen season (in season) and out of season.

Materials and methods

Study groups

Seventeen consecutive subjects were included in this prospective study performed in accordance with the ethical standards (Declaration of Helsinki, Lausanne ethical committee, informed consent form). Medical history, anamnesis, and skin-prick tests served to determine which patients suffered from SAC (n=11) and which were nonallergic (n=6). Allergic patients abstained from taking anti-inflammatory drugs for 10 days before the start of the experiment.

All 11 SAC subjects [three males, eight females, mean age 26.8±2.5 (Standard error on the mean, SEM) years] presented ocular itching, tearing, and a burning sensation during the grass-pollen season (May–July), and some of them multiple papillae. Tears were collected during May/June, corresponding to the highest charge in grass pollen per meter^3 of air for the given study year. Seven patients underwent skin-prick tests (ALK, Hørsholm, Denmark, Table 1) with negative (solvent), positive control (histamine), and 14 allergens (grass, rye, alder, ash tree, beech, birch, hazel tree, oak, mugwort, plantain, cat, dog, *Dermatophagoides pteronyssinus*, *D. farinae*). Prick tests for the remaining four patients were not available. Six normal subjects (three males, three females, 39.2±10.7 years) had no history of allergy, ocular, or systemic symptoms.

In a second step, of the seven patients whose skin-prick tests were available, patients 2 and 4 were excluded (doubtful prick for grass pollen), and five patients, 7–11, remained (positive prick for grass pollen, one male, four females, 24.4±1.4 years). Serum-specific IgE levels (kU/l) against a mixture of five Swiss grass pollens (Pharmacia CAP System) were: negative (<0.35), limit positive in class 1 (0.35–0.7), and positive in class 2 (0.71–3.5), class 3 (3.51–17.5), class 4 (17.51–50), and class 5 (50.1–100 kU/l). Tears of these subjects were first collected between 30 March and 23 April, when no patient presented with itching. The date of collection corresponded to both grass pollen out of season according to the daily pollen graphs in Lausanne and a nil-to-low charge in tree pollens for patients allergic to trees (patients 8, 10, 11). Tears from the same five subjects were again collected between 11 May and 15 June, this corresponding to the grass-pollen period. At both visits, patients were scored from 0–3 according to itching, conjunctival hyperemia, chemosis, eyelid swelling, and tearing (Table 2). The clinical score was the sum of the scores for each criteria, where 0 represented no symptoms/signs, 2 was considered as the minimal symptomatic score, and 15 was the maximum.

Tears and ELISA

Unstimulated basal tears (5–10 μl) were collected with flame-worked glass microcapillaries from the lateral canthus of the eyelid of patients over 5 min in absence of topical anesthesia. Eotaxin-1 levels were measured by an Enzyme-linked immunosorbent assay (ELISA). A plate was coated with a mouse antihuman eotaxin-1 monoclonal antibody (Becton-Dickinson, San Diego, CA, USA)

Table 1 Grading scale for skin-prick tests

Observation	Score	Interpretation
No reaction or erythema	0	Negative
Wheal, diameter <2 mm	1	Negative
Wheal, diameter 2–3 mm	2	Doubtful
Wheal, diameter ≥4 mm	3	Positive
Wheal, diameter >4 mm, with pseudopodia	4	Positive

Table 2 Seasonal allergic patients 7–11

Patient	Specific IgE class[1]	Prick tests[2]							Itching[3]		Hyperemia[3]		Chemosis[3]		Eyelid swelling[3]		Tearing[3]	
		Grass	Plantain	Alder	Beech	Birch	Hazel tree	Oak	Out	In	Out	In	Out	In	Out	In	Out	In
7	5	4	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0
8	3	4	0	2	0	0	1	0	0	2	1	1	0	0	0	0	0	1
9	5	4	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	1
10	2	4	0	0	0	1	0	0	0	2	0	1	0	1	0	0	0	1
11	2	3	3	3	4	4	3	3	0	1	0	2	0	1	0	0	0	1

[1] Serum-specific IgE class for a mixture of five Swiss grasses. The result is positive up from class 2
[2] Skin-prick tests for seven allergens relevant for at least one patient. For grading scale, see Table 1
[3] Five clinical symptoms/signs scored during the grass pollen "out of season" and the grass pollen "in season"

and washed. A serial dilution of recombinant human eotaxin-1 (16–500 pg/ml) and dilutions of samples were prepared in 0.05% Tween and 1% BSA in PBS. Two replicates of r-eotaxin-1 dilutions and samples, a biotinylated mouse antihuman eotaxin-1 monoclonal antibody (Becton-Dickinson), a streptavidin-alkaline phosphatase conjugate (Becton-Dickinson), and alkaline phosphatase substrate (Sigma, St Louis, MO, USA) were successively applied with in-between washes. The OD was read at 405 nm. Test sensitivity was 60 pg/ml. The nonparametric Wilcoxon/Kruskal-Wallis test and the matched pairs *t* test were used.

Results

Eleven SAC patients symptomatic in season were compared to six nonallergic patients. On skin-prick tests, patients 2, 4, 10, and 11 were also allergic to cats and patients 10 and 11 to dogs, but none of them was exposed daily to these animals. All patients were negative for mites (*D. pteronyssinus* and *D. farinae*). During the pollen season, eotaxin-1 concentration in tears of allergic patients ranged from 1,147 to 3,150 pg/ml [mean 2,099.9± 503.2 (SEM) pg/ml] compared to 894–1602 pg/ml (mean 1,192.7±1,76.2 pg/ml) in control subjects (Fig. 1, P= 0.0049). In season, allergic patients showed 76% more eotaxin in mean than control subjects.

For the second part of the study, five SAC patients were selected as showing the strongest reaction to grass allergens on skin-prick tests (Table 2). All were positive for rye (not reported here) and had positive serum-specific IgE to grass pollen. Plantain, alder, beech, birch, hazel tree, and oak were also taken in consideration, since each was positive for at least one patient. Four patients presented itching on the date of in-season collection, and patient 7 one day before collection. The clinical score was 0.4±0.2 out of season and 4±0.5 in season (Fig. 2. P=0.0043).

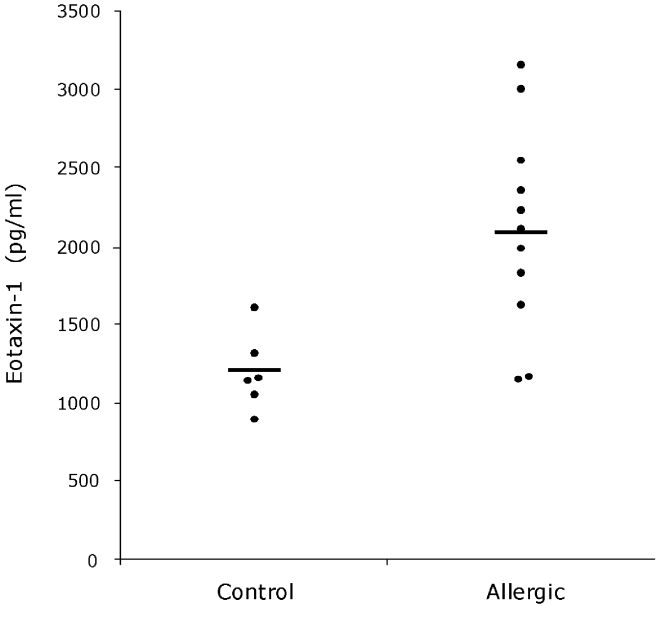

Fig. 1 Eotaxin-1 level in tears of seasonal allergic patients versus control nonallergic subjects during the grass-pollen season. *Horizontal lines* represent the means

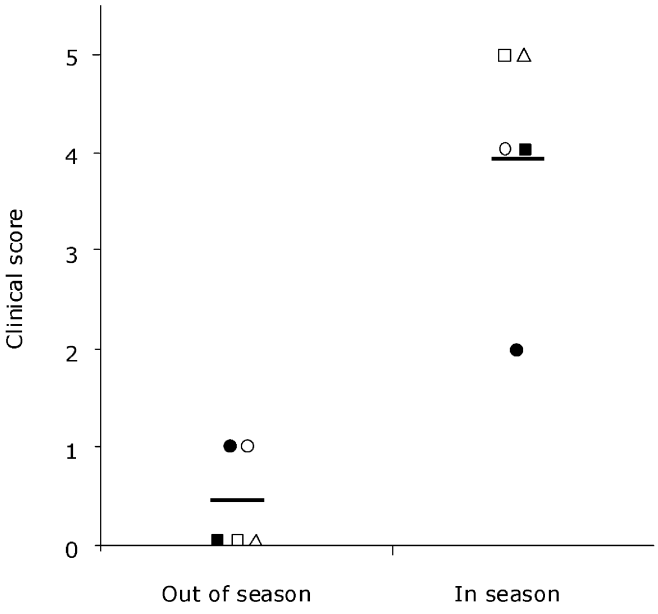

Fig. 2 Clinical score in SAC patients: Grass pollen in season versus out of season. One symbol refers to one patient: ● *filled circle* (patient 7), ○ *empty circle* (patient 8), ■ *filled square* (patient 9), ❑ *empty square* (patient 10), Δ *empty triangle* (patient 11). The horizontal lines represent the means

Out of season, the five SAC patients showed between 1,033 and 2,622 pg/ml of eotaxin-1 in their tears (mean 1,742.8±663 pg/ml) (Fig. 3). In season, patients showed between 1,147 and 3,150 pg/ml of eotaxin-1 (mean 2,135.4±784.3 pg/ml) (P=0.024). Thus, eotaxin-1 concentration in tears of SAC patients significantly increases by 23% in pollen season. Interestingly, in both seasons, allergic patients conserved their position relative to one another regarding eotaxin-1 tear concentration.

Discussion

This study demonstrates that eotaxin-1 is significantly upregulated in tears of SAC patients compared to nonallergic subjects. Moreover, SAC patients showed a higher level of eotaxin-1 in their tears in season than out of season. Allergic patients did not show more serious ocular disorders than SAC. Eotaxin-1 seems to be constitutively expressed in tears of nonallergic patients. This result confirms a positive cytoplasmic eotaxin staining previously demonstrated in superficial epithelial cells of conjunctiva from normal subjects [1].

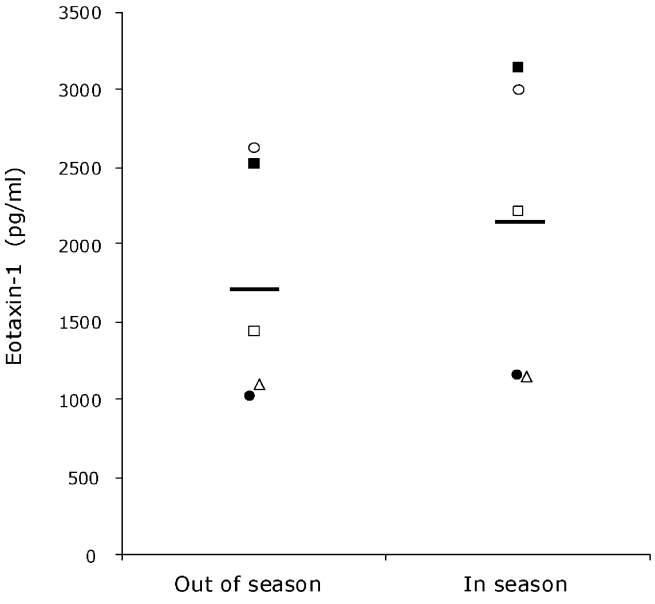

Fig. 3 Eotaxin-1 level in tears of SAC patients: Grass pollen in season versus out of season. One symbol refers to one patient: ● *filled circle* (patient 7), ○ *empty circle* (patient 8), ■ *filled square* (patient 9), ❑ *empty square* (patient 10), Δ *empty triangle* (patient 11). *Horizontal lines* represent the means

Why do seasonal allergic patients show a relatively high eotaxin-1 baseline, and why is this baseline variable between patients?

1 It could be that an underlying subclinical inflammatory process continues out of season
2 Patients could be both sensitive, and exposed, to inhalant allergens not tested
3 Patients could be exposed to other pollen allergens throughout the year in a professional or private context, although the questionnaire and anamnesis were negative in this regard
4 Tear production differs from one person to another [14] and will differentially dilute the chemokine. This is confirmed by the observation that allergic patients maintain their relative low or high eotaxin-1 concentration with regard to one another throughout the year.

The allergy of some patients to tree pollens could not influence our results regarding the out-of-season tests for grass pollen. On the day of collection, no ocular symptoms or signs were present for either patient 10, allergic to birch, or patient 11, polyallergic to tree pollen. The corresponding tear eotaxin-1 concentration out of season was moderate for patient 10 and weak for patient 11. Alder and hazel tree pollens being over by the time of collection, these could not bias the eotaxin-1 baseline concentration of patient 8, allergic to both trees.

Eotaxin-1 has been reported in tears of patients suffering from AKC with severe corneal damage or ulceration. However, when AKC patients had clear corneas, eotaxin-1 concentration was very low [3]. Eotaxin-1 was demonstrated in the mucus of VKC patients [9]. In a Turkish study, eotaxin-1 was present in tears of SAC patients during the pollen season [5], but the levels reported were much lower than those in our study. This is most likely due to regional differences in pollen charge for Turkey and Switzerland (M. Irkec, personal communication).

Eotaxin-1 participates in allergic disorders via both the recruitment of eosinophils to the site of inflammation and their activation [12]. Indeed, this chemokine is a specific ligand for CCR3, highly expressed on eosinophils [6, 11]. In eye pathologies, eosinophils were revealed in VKC and AKC [2, 3]. Eotaxin-1 has other target cells, namely, Th2 lymphocytes and mast cells. Th2 cell recruitment by eotaxin could represent a key mechanism in allergy because it promotes the allergen-driven production of IL-4 and IL-5 [10, 13]. Conjunctival mast cells play a central role in ocular allergy [8] as they lead to the release of histamine, leukotriene C4, and various cytokines responsible for serious forms of ocular allergy. Eotaxin-1 is one mediator of allergy in tears of SAC patients and could help specify the clinical score.

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