

Presence of eotaxin in tears of patients with atopic keratoconjunctivitis with severe corneal damage

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In atopic keratoconjunctivitis (AKC), a severe form of allergic conjunctivitis, corneal ulcer or erosion is one of the main vision-threatening complications.¹ Although eosinophils are assumed to play a pathogenic role in corneal damage through the release of toxic mediators, such as major basic protein and eosinophil cationic protein,¹ the precise mechanism of eosinophil recruitment to the ocular surface remains unclear.

Recently, eotaxin has been reported to be a specific and potent chemoattractant for eosinophils.² To investigate the participation of eotaxin in allergic corneal damage, we analyzed the concentration of eotaxin and the number of eosinophils in the tears of patients with AKC.

METHODS

After informed consent was obtained, 20- μ L tear samples were collected from 38 eyes of 24 patients with AKC, including 15 eyes with corneal erosion or ulcer (erosion group), 9 eyes with superficial corneal damage (superficial punctate keratopathy [SPK] group), and 13 eyes with clear corneas (clear group). To obtain unstimulated basal tears, the tear samples were collected with microcapillary tubes at the lateral canthus of the eye lid, while patients lay on their backs inclining their heads; anesthetic was not used.³ Fourteen tear samples from 8 nonallergic volunteers were also investigated. The tear samples were immediately centrifuged. The eotaxin concentrations were measured by an ELISA with 2 mouse monoclonal anti-human eotaxin antibodies, as reported previously.⁴ The detection limit of this assay is approximately 3 pg/mL. The cells in the tear samples were centrifuged into cytospin preparations, placed on slide glasses, and stained with Diff-Quick. The number of eosinophils was counted by microscopic observation ($\times 200$) of 3 to 10 fields.

The evaluation of statistical significance was carried out by using a repeated-measures ANOVA on values of eotaxin concentration and the numbers of eosinophils, which was followed by Fisher's protected least significant difference analysis.

Abbreviations used

AKC: Atopic keratoconjunctivitis

SPK: Superficial punctate keratopathy

RESULTS

The eotaxin concentration in tears from the erosion group (1475.4 ± 870.5 pg/mL [mean $\pm$ SD]) was significantly greater than that from the SPK group (163.4 ± 81.7 pg/mL), the clear group (24.3 ± 12.7 pg/mL), and the nonallergic control subjects (21.7 ± 9.7 pg/mL) (Fig 1, A). Greater numbers of eosinophils were found in tears from the erosion group (42.2 ± 52.2 cells/field, $n = 9$) than in tears from the SPK group (0.3 ± 0.4 cells/field, $n = 5$), the clear group (0 ± 0 cells/field, $n = 8$) and the nonallergic control subjects (0 ± 0 cells/field, $n = 13$) (Fig 1, B). Moreover, the number of eosinophils correlated with the eotaxin concentration in the tear samples ($r = 0.603$, $P < .0004$ as determined by Bartlett's test; data not shown).

DISCUSSION

In this study we successfully detected extremely high concentrations of eotaxin and eosinophilic infiltration in tear samples from patients with AKC and severe corneal damage. The concentration of eotaxin in the tears from the erosion group was high enough to attract eosinophils *in vitro* (ie, approximately 1 ng/mL eotaxin is required for chemotaxis of eosinophils *in vitro*⁵). The cellular source of the eotaxin in the tears is unclear; however, we have observed that human corneal keratocytes, but not epithelial cells, produce large amounts of eotaxin in response to T_H2-derived cytokine IL-4 (unpublished observation). Further studies are necessary to gain an understanding of the production of eotaxin at the allergic ocular surface. Taken together, eotaxin in tears may play an important role in eosinophil recruitment, as well as in corneal damage occurring in allergic ocular diseases.

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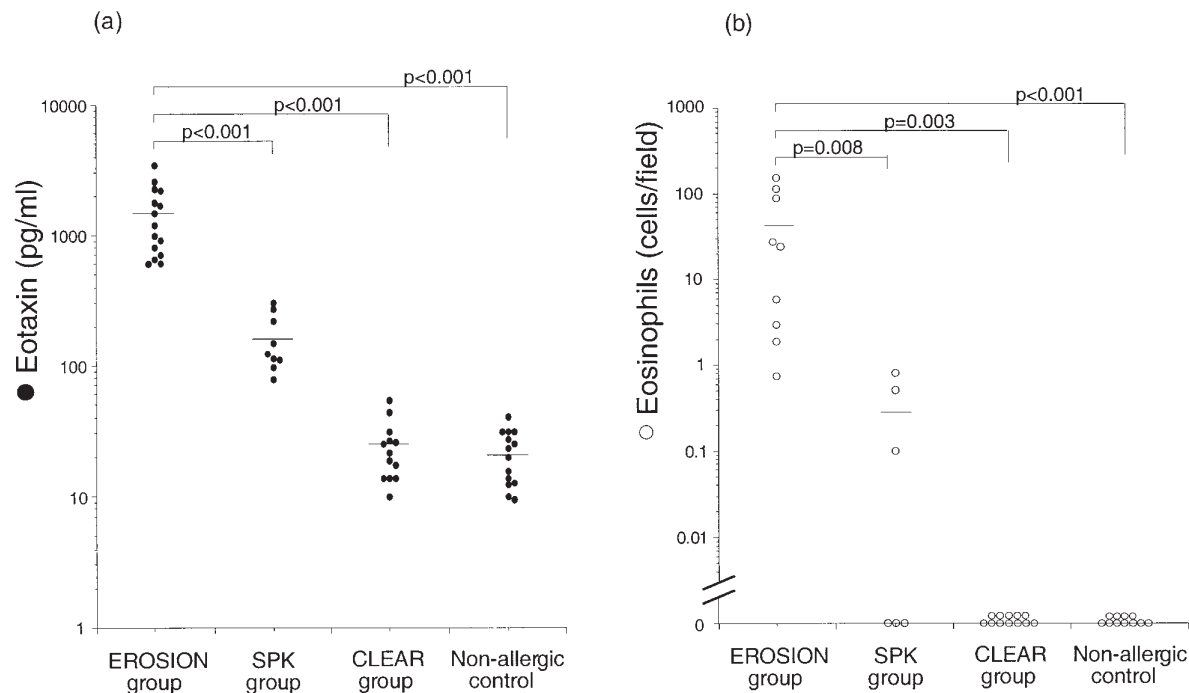


FIG 1. The concentrations of eotaxin **(a)** and eosinophil numbers **(b)** in tear samples from the erosion group (n = 15; age, 16.1 ± 3.9 years), the SPK group (n = 9; age, 17.3 ± 6.7 years), the clear group (n = 13; age, 17.0 ± 6.4 years), and the nonallergic control subjects (n = 14; age, 23.5 ± 4.4 years). Eosinophil numbers were not able to be counted in some samples (6 samples in the erosion group, 4 samples in the SPK group, 5 sample in the clear group, and 1 sample in the nonallergic control group) because of cell destruction or failures in the cytopspin method.

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