

Tear levels of interferon- γ , interleukin (IL) -2, IL-4 and IL-5 in patients with vernal keratoconjunctivitis, atopic keratoconjunctivitis and allergic conjunctivitis

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Summary

Background A predominance of TH2 activity in chronic allergic diseases, such as vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC), has been suggested recently. However, there is no published study on tear levels of cytokines of the two different subgroups, TH1 and TH2, in patients with ocular allergy.

Objectives We measured interferon (IFN)- γ , interleukin (IL)-2, IL-4 and IL-5 levels in tears by ELISA, to determine whether the levels of these cytokines are elevated in allergic ocular diseases when compared among patient groups and normal controls.

Methods Tear levels of IL-2, IFN γ , IL-4 and IL-5 were measured by ELISA using samples from patients with VKC, AKC (AKC-NP, without proliferative lesions; and AKC-P, with proliferative lesions), allergic conjunctivitis (AC) and normal subjects. The levels of these cytokines in tears and the clinical severity of AD were also compared.

Results Tear IL-4 level in patients with AKC was significantly higher than those in VKC, AC and controls, and tear IL-4 levels in patients with AKC-P vs VKC differed significantly. Tear IL-5 levels in patients with diseases associated with proliferative lesions, VKC and AKC-P, were higher than those in AC and normal controls. However, tear level of IL-5 in patients with AKC-P was significantly higher than that in AKC-NP. Although the dermatological severity of AD correlated significantly with tear IL-4 level, IFN γ , IL-2 and IL-5 levels did not correlate with dermatological severity of AD.

Conclusion These results indicate that the TH2-like cytokines play an important pathophysiological role in severe ocular allergic conditions such as AKC and VKC and that tear level of IL-5 may be a candidate marker to evaluate the clinical status of ocular allergy. The different patterns of tear levels of IL-4 and IL-5 among ocular allergic diseases may reflect the origin and immunological basis of these cytokines.

Keywords: allergic conjunctivitis, atopic dermatitis, atopic keratoconjunctivitis, interferon- γ , interleukin-2, interleukin-4, interleukin-5, tears, vernal keratoconjunctivitis

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Introduction

The pathogenesis of allergic conjunctival disorders is not fully understood. Recent progress in immunological research has revealed that murine CD4⁺ T cells can be

divided into two groups based on their patterns of cytokine production [1]. T helper (TH)1 cells produce interleukin (IL)-2 and interferon (IFN)- γ . TH2 cells produce IL-4, IL-5 and IL-6. Cytokines have been detected in conjunctival cells of ocular pemphigoid immunohistochemically [2], and TH2 cytokines have been demonstrated in the conjunctiva [3]. IL-4 plays a critical role in the switching of B lymphocytes to produce immunoglobulin (Ig) E [4], and may therefore be

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of critical importance in the development of allergic diseases, although other cytokines including IL-6 and IL-13 have synergistic effects [5]. IL-5 is the principal cytokine responsible for eosinophilia. In many settings, monoclonal antibodies directed against IL-5 have been shown to abolish eosinophilia. IL-5 also activates mature eosinophils, prolongs their survival and contributes to their accumulation at sites of inflammation [6]. The activating effect of IL-4 is promoted by IL-5 and IL-6, but inhibited by IFN α and γ , transforming growth factor- β and prostaglandin E₂ [7,8]. IL-4 is increased in culture supernatants of samples obtained by brush collection in allergic conjunctivitis (AC) patients [9]. A previous study showed a significantly elevated mean level of IL-4 in tears of patients with AC compared with controls, and levels in patients with vernal keratoconjunctivitis (VKC) and giant papillary conjunctivitis were also significantly higher than those in AC and normal controls [10]. Increased transcripts of IL-4 and IL-13 were detected by RT-PCR method in samples from AC patients [11]. From these studies, a predominance of TH2 activity in ocular allergy has been suggested.

Atopic keratoconjunctivitis (AKC), which also belongs to the ocular allergic diseases, is a bilateral, chronic hypersensitivity disease of the ocular surface seen in association with systemic atopic dermatitis (AD), characterized by lesions of the conjunctiva and cornea that are variable in severity. The anterior ocular findings of AKC have a wide spectrum, and a subset of patients has the same signs as those of AC. However, in some cases, corneal complications such as scarring, neovascularization, and plaque formation often result in compromised vision. Due to the presence of systemic AD in AKC, most systemic parameters, such as serum IgE, cytokines and chemical mediators, depend on the systemic clinical severity of AD or the distribution of atopic eczema. VKC is a recurrent seasonal disease of childhood, characterized by severe inflammation. It is most commonly seen in male patients and, unlike the severe group of AKC, tends to resolve spontaneously after several years. Thus, comparison of tear cytokine levels

between VKC, AKC and AC enables us to investigate whether the local immunological reaction in patients with ocular allergy, which includes diseases complicated by AD, reflects systemic immunological activation. Moreover, to our knowledge, there is no published study on tear levels of cytokines of the two different subgroups, TH1: IFN γ , IL-2, and TH2: IL-4, IL-5, in patients with VKC, AKC and AC.

In the present study, we used sensitive antigen capture enzyme-linked immunoassays (ELISA) for tear IFN γ , IL-2, IL-4 and IL-5 to determine whether these cytokines exist free in the tears in VKC, AKC and AC and whether the levels of these cytokines are elevated in these diseases when compared among patient groups and normal control subjects. We also investigated the correlation between levels of these cytokines in tears and the clinical severity of AD.

Materials and methods

Patients

We studied 49 patients with VKC, AKC and AC who presented to the Department of Ophthalmology, Yokohama City University Hospital, Yokohama, Japan, between February and July, 1997. Informed consent was obtained from all patients before sample collection. Tear samples were obtained from five male and two female VKC patients with a mean age of 13.6 years (range 11–17), from 15 male and 13 female AKC patients with a mean age of 23.6 years (range 8–64) and from six male and eight female AC patients with a mean age of 24.7 years (range 8–42). VKC, AKC and AC were diagnosed according to the guidelines of diagnosis and treatment of conjunctivitis proposed previously [12], which are summarized in Table 1. VKC was diagnosed by slit lamp examination showing typical cobblestone excrescences (giant papillary conjunctivitis of the upper palpebral conjunctiva more than 1 mm in size), perilimbal gelatinous change and corneal lesions, such as stromal lipid deposition or pannus formation.

Table 1. Diagnostic inclusion criteria for allergic ocular disorders

Disease	Conjunctival signs	Age group	Current pollen exposure and confirmed allergy	History/presence of atopic dermatitis
AC	Hyperemia, swelling and discharge without proliferative lesions	Any	Required	No
AKC	Conjunctival erythema or giant papillae with lid	Any	Not required	Yes
VKC	Superior tarsal giant papillae or limbal nodules	Children or puberty	Not required	No

AKC patients had atopic dermatitis (AD) and conjunctival and/or corneal involvement. Corneal complications may vary in severity from punctate keratitis to frank necrosis. Therefore, AKC was divided into two subgroups; AKC-NP (nonproliferative) and AKC-P (proliferative) according to the severity and pathophysiological findings of the cornea and conjunctiva. Keratoconjunctivitis in the AKC-NP group (14 cases) was non-proliferative, showing conjunctival papillae, especially on the upper tarsal conjunctiva, or superficial punctate keratopathy, in contrast with that in the AKC-P group (14 cases) which exhibited proliferative lesions such as giant papillae or corneal plaques, similar to VKC [12]. The patients with AKC met the criteria of Hanifin and Rajka [13]. None of the patients was receiving systemic or local steroids or anti-allergic drugs on sample collection. The clinical severity of the AD lesions was graded as mild, moderate, or severe, according to the criteria proposed previously [14]. Patients with AC who showed seasonal symptoms such as ocular itching and tearing without proliferative lesions had positive RAST or skin prick test to grass pollen. Tear samples from 13 normal individuals (five male, eight female; age range 17–50 years; mean 26.5) served as controls.

Sample preparation and measurement of IFN γ , IL-2, IL-4 and IL-5

A volume of tears exceeding 40 μ L was collected with a plastic tube without the use of saline solution according to a previous report [15]. In brief, microhematocrit tube (NRIS, Herlev, Denmark) was introduced into the inferior tear meniscus. Tears were expelled into the plastic tube by using a bulb attached to one end of the tube. This process was performed two or three times at 4–6 min intervals. In bilateral cases, the tear sample was collected from the clinically more severely affected eye. Tears were stored at -20°C until assay. The tears were diluted 10-fold, and the tear levels of IFN γ , IL-2, IL-4 and IL-5 were determined using a two-epitope 'sandwich' enzyme-linked immunosorbent assay (ELISA) kit (CytoscreenTM human IFN γ , IL-2, IL-4 and IL-5 ELISA kits; BioSource International, Camarillo, CA, USA). The procedures recommended by the manufacturer were followed without any modification, and the optical absorbance values were read on a micro-ELISA autoreader (model MR 580; Dynatech, Cambridge, MA, USA) at 490 nm. All samples were tested in duplicate, and serum levels of cytokines were calculated by comparing the mean absorption of duplicate samples with that of the standard curve. The detection limits for IFN γ , IL-2, IL-4 and IL-5 were 4 pg/mL, 5 pg/mL, 65 fg/mL and 4 pg/mL, respectively. Samples were assayed on two separate occasions, and interassay variation was 9.2–12.4% (mean). All samples were coded and read blind in the assay.

Statistical analysis

Non-parametric analysis was conducted in this study. Mann–Whitney test was used to identify differences between patient groups and controls. Spearman's rank correlation test was used to establish the significance of correlation between paired groups. A level of $P < 0.05$ was accepted as statistically significant.

Results

Tear level of IL-4

The tear level of IL-4 in VKC patients was 220 ± 580 fg/mL (mean $\pm$ SD); 1914 ± 2820 fg/mL in AKC patients (AKC-NP: 1444 ± 2351 fg/mL; AKC-P: 2384 ± 3244 fg/mL); 350 ± 450 fg/mL in AC patients; and 60 ± 160 fg/mL in normal controls (Fig. 1). Tear level of IL-4 in patients with AKC was significantly higher than those in VKC, AC and normal controls ($P < 0.01$). Tear levels of IL-4 in patients with AC was significantly higher than that in controls ($P < 0.05$). Tear level of IL-4 in patients with AKC-P was significantly higher than those in VKC, AC and controls ($P < 0.05$). No significant difference was observed in tear IL-4 levels between AKC-NP and AKC-P.

Tear level of IL-5

The tear level of IL-5 in patients with VKC was 276 ± 287 pg/mL; 175 ± 144 pg/mL in AKC; and 73.9 ± 57.2 pg/mL in AC; while that in normal controls was 40.2 ± 28.9 pg/mL (Fig. 2). Tear IL-5 level in AKC patients was significantly

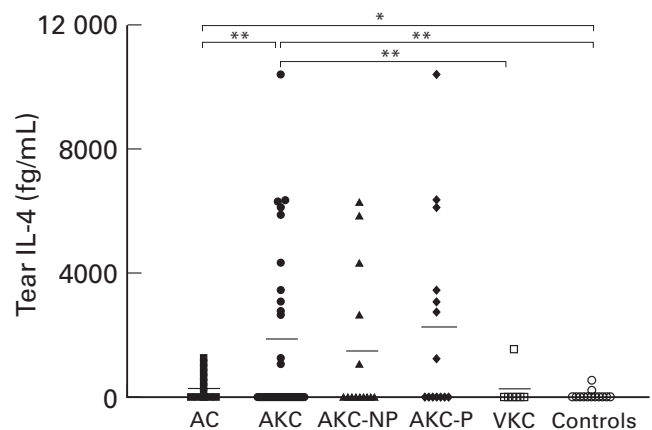


Fig. 1. Tear IL-4 levels in patients with vernal keratoconjunctivitis (VKC), atopic keratoconjunctivitis (AKC; AKC-NP [without proliferative lesions] and AKC-P [with proliferative lesions]), allergic conjunctivitis (AC) and normal controls. Each horizontal line represents the mean IL-4 level. Significance between patient groups and controls or among patient groups is indicated by symbols: ** $P < 0.01$, * $P < 0.05$.

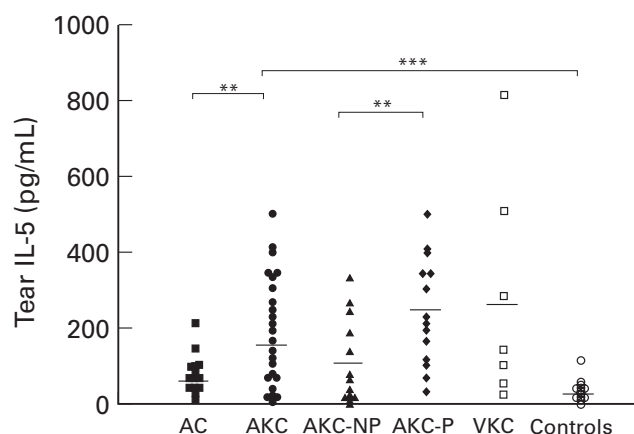


Fig. 2. Tear IL-5 levels in patients with VKC, AKC (AKC-NP and AKC-P), AC and normal controls. Each horizontal line represents the mean IL-5 level. Significance between patient groups and controls or among patient groups is indicated by symbols: *** $P < 0.001$, ** $P < 0.01$.

higher than those in AC and normal controls ($P < 0.01$ and 0.001 , respectively). Tear level of IL-5 in patients with AKC-P was significantly higher than those in AKC-NP, AC and controls ($P < 0.01$, 0.001 and 0.001 , respectively). In contrast, there was no significant difference in tear levels of IL-5 in patients with AKC-P and VKC.

Tear level of IFN γ

The tear level of IFN γ in patients with VKC was 104 ± 60.7 pg/mL; 105 ± 56.9 pg/mL in AKC patients (AKC-NP: 111 ± 72.4 ; AKC-P: 98.9 ± 37.4 fg/mL); and 101 ± 137 pg/mL in AC patients; while that in normal controls was

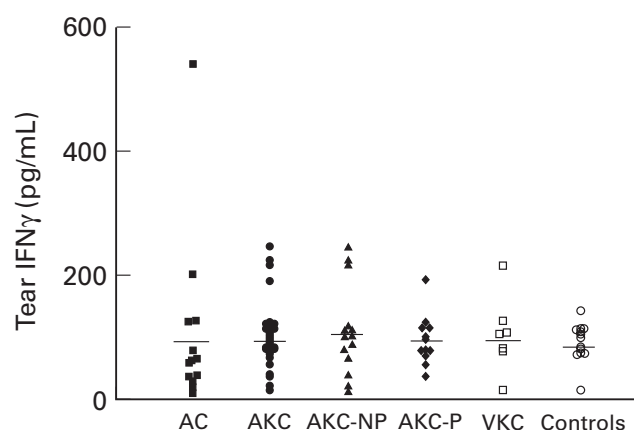


Fig. 3. Tear IFN γ levels in patients with VKC, AKC (AKC-NP and AKC-P), AC and normal controls. Each horizontal line represents the mean IFN γ level.

91.7 ± 31.2 pg/mL (Fig. 3). No significant difference was found in any patient groups or controls.

Tear level of IL-2

The tear IL-2 level in patients with VKC, AKC, AC and healthy controls was 114 ± 179 , 101 ± 227 , 75.7 ± 122 and 36.8 ± 111 pg/mL, respectively (Fig. 4). Tear IL-2 level in patients with VKC was higher than those in patients with AKC, AC and normal controls, but these differences did not reach significance.

Relationships between tear cytokine levels and dermatological severity of AD

As shown in Fig. 5, dermatological severity of AD correlated significantly with IL-4 level ($P = 0.022$). However, IFN γ , IL-2 and IL-5 levels did not correlate with dermatological severity of AD. In this analysis, the two subgroups of AKC patients (AKC-NP and AKC-P) were deemed a single group.

Discussion

It has been demonstrated in this study that healthy controls produce detectable levels of IFN γ and IL-2 in tears, similar to those in patients with AC, AKC and VKC. The sources of IFN γ and IL-2 in tears are not known. However, because there are no known endogenous conjunctival cells that secrete IFN γ or IL-2 either spontaneously or when activated, the most likely source is the small number of CD3⁺ T cells seen in conjunctival follicles [11]. Because IFN γ inhibits IL-4-induced IgE production by human B cells [16], it is speculated that the decreased level of IFN γ in the tears

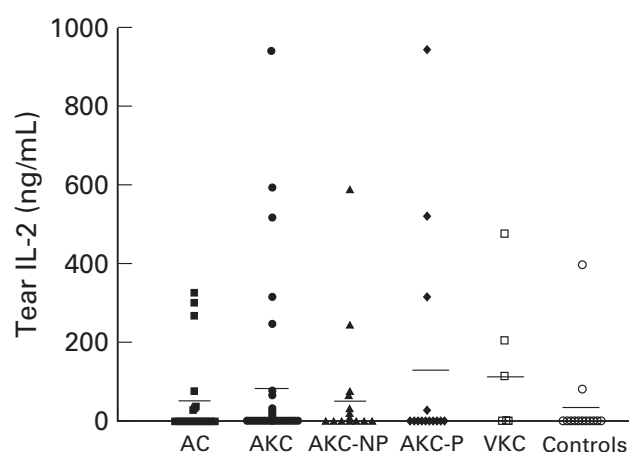


Fig. 4. Tear IL-2 levels in patients with VKC, AKC (AKC-NP and AKC-P), AC and normal controls. Each horizontal line represents the mean IL-2 level.

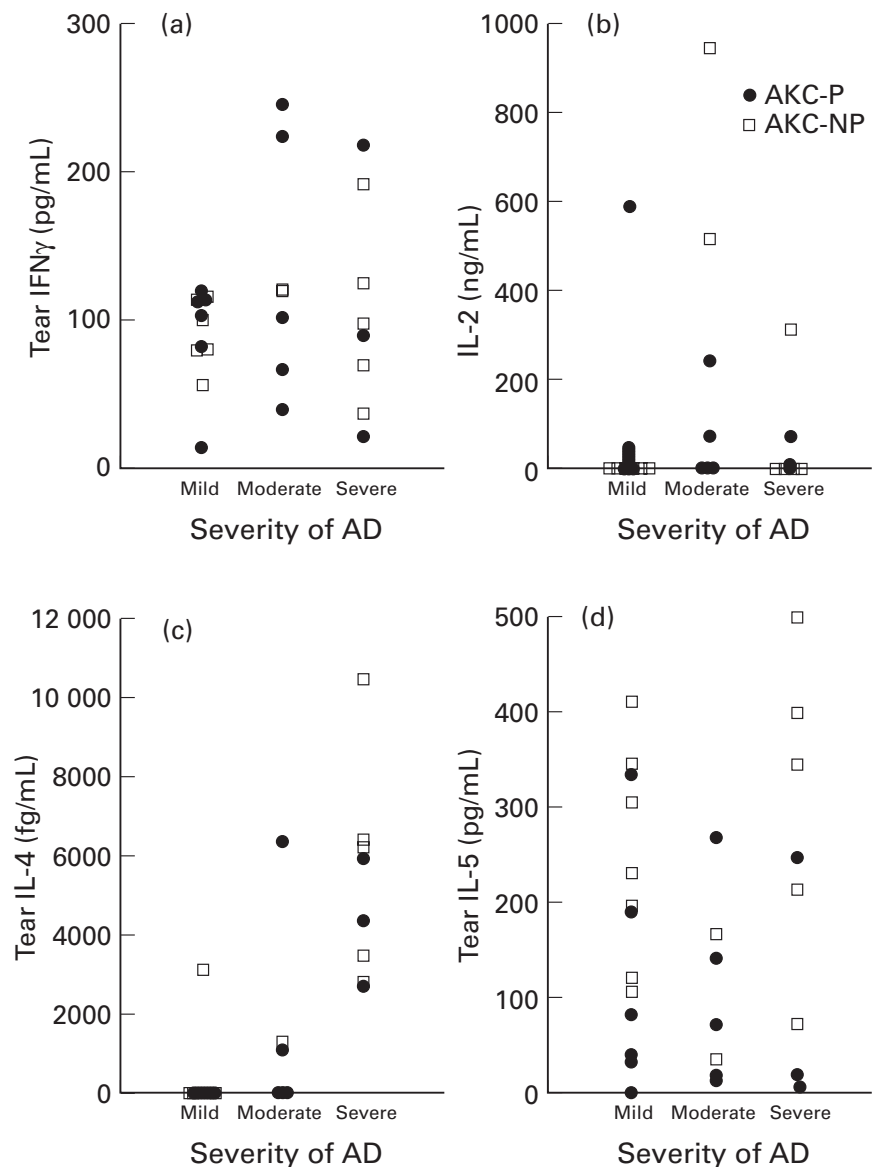


Fig. 5. Correlation between tear cytokine levels and dermatological severity of AD. (a) No correlation between IFN γ level in tears and dermatological severity of AD in patients with AKC ($P=0.745$). (b) No correlation between IL-2 level in tears and dermatological severity of AD in patients with AKC ($P=0.476$). (c) Correlation between IL-4 level and dermatological severity of AD in patients with AKC (rank correlation coefficient = 0.588, $P=0.022$). (d) No correlation between IL-5 level in tears and dermatological severity of AD in patients with AKC ($P=0.414$).

of AKC and VKC patients with AD is related to the over-production of IgE in AD [17]. A recent study revealed significantly higher IL-2 mRNA expression in subjects with AKC than those with VKC [3], but no significant difference in tear IL-2 levels between these diseases was found in our present study.

It is possible that IL-4 may be produced by mast cells in seasonal AC, in which leucocyte infiltration into the conjunctiva is absent [18]. However, significantly more T-helper cells and macrophages are found in the epithelium of conjunctiva from patients with severe chronic ocular allergic diseases such as AKC [19]. Therefore, IL-4 in tears is thought to be derived mainly from TH cells in AKC and VKC. Tear IL-4 levels in patients with AKC differed

significantly from those in VKC, AC and controls in our present study. Moreover, a significant correlation between tear IL-4 level and severity of AD was demonstrated in patients with AKC, but no correlation was found between other tear cytokine levels and severity of AD. These findings indicate that a high level of IL-4 in tears reflects the severity of the atopic state. Recent studies have supported the possible involvement of IL-4 in the pathogenesis of AD [20], and it has been suggested that IL-4-mediated immune response may be augmented in atopic disorders that manifest over-production of IgE [21]. Although the local clinical features of AKC-P and VKC are similar, a significant difference was demonstrated in tear IL-4 levels between these diseases; therefore, it can be considered that IL-4 level

in tears in these diseases does not reflect the degree of local immunological abnormality but reflects the severity of AD. A significant difference in tear IL-4 levels was also noted between AC and controls in our study. It has been demonstrated that the level of IL-4 in the tears of normal subjects is similar to that in serum, but in patients with AC, the level in tears exceeds that in serum, indicating the possibility that the allergic reaction also occurs locally at the ocular surface in AC [10]. This hypothesis may explain the significant elevation of tear IL-4 in patients with AC compared with normal controls.

In the present study, we observed elevation of tear level of IL-5 in patient groups with proliferative keratoconjunctival lesions, i.e. VKC and AKC-P. Although the elevated tear level of IL-5 in patients with VKC failed to reach significance compared with those in AC and controls, this could be explained by the limited number of patients with VKC. Interestingly, tear IL-5 level in patients with AKC-NP was significantly lower than that in AKC-P. Tear IL-5 could possibly be used as a marker to evaluate the clinical status of proliferative ocular allergic diseases or differentiate them from non-proliferative ocular allergic diseases such as AC and AKC-NP. The results of our present study revealed a dissociated pattern of elevated levels of IL-4 and IL-5 on the ocular surface in ocular allergy. Evidence for the dissociation of IL-4 and IL-5 has been observed in a number of pathological settings including AD [22]. Histopathological studies have demonstrated that the conjunctival stromal inflammatory infiltrate in VKC was mainly composed of mononuclear cells, eosinophils and mast cells. This histopathological change may cause the elevation of tear levels of IL-5 observed in AKC-P and VKC.

In conclusion, the results of this study demonstrate that the TH2-like cytokines, IL-4 and IL-5, may play an important pathophysiological role in severe ocular allergic conditions, such as AKC and VKC. Measurement of tear IL-5 level indicated that AKC-P shows similar immunological features to VKC. However, it was also observed that although the local clinical features of AKC-NP are quite different from those of AKC-P, these conditions have similar allergic characteristics as reflected by tear IL-4 levels. The finding of increased tear level of IL-5 in ocular allergic diseases with proliferative lesions and the dissociated pattern of tear levels of IL-4 and IL-5 among ocular allergic diseases suggest the clinical relevance of these cytokines.

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