

Tumour necrosis factor alpha (TNF α) in tears of atopic patients after conjunctival allergen challenge

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Summary

Background Proinflammatory cytokines, such as TNF α are involved in the pathogenesis of allergic rhinitis and asthma.

Objective Our aim was to study the tear fluid TNF α levels in atopic patients before and after topical ocular allergen challenge.

Methods Thirteen patients were first challenged with topical allergen in the left eye and then dilution buffer in the right eye. Tear fluid samples were collected before the challenges and after 5, 30 and 60 min. Clinical symptoms were scored. Tear fluid TNF α concentrations were measured using a double-antibody radioimmunoassay. TNF α release rates were calculated using the tear fluid flow in the collection capillary as an estimate for the tear secretion rate.

Results The mean baseline TNF α concentrations (ng/L) were 1310 (allergen-challenged eye) and 967 (control eye), and release rates (pg/min) 1.81 (allergen-challenged eye) and 1.39 (control eye), respectively. In the allergen-challenged eye, TNF α concentrations and release rates were 1479 and 4.30 (5 min), 1367 and 3.20 (30 min) and 1426 and 3.80 (60 min). In the control eye, TNF α concentrations and release rates were 746 and 0.67 (5 min), 1001 and 0.92 (30 min) and 1504 and 1.05 (60 min). The release rates of the allergen-challenged eye were significantly increased at all time points after the challenge when compared with the baseline or the control eye.

Conclusion Increased TNF α release rates suggest that this cytokine is an early mediator of allergy after conjunctival challenge.

Keywords: allergen challenge, conjunctivitis, cytokine, tear fluid, TNF α

Clinical and Experimental Allergy, Vol. 29, pp. 537–542. Submitted 6 October 1997; revised 22 January 1998; accepted 23 March 1998.

Introduction

The role of cytokines, such as tumour necrosis factor-alpha (TNF α) in the pathogenesis of allergy and asthma have been the subject of intensive investigations [1,2]. Among other proinflammatory cytokines TNF α may be involved in the development of diseases of the ocular surface. Increased expression of conjunctival TNF α mRNA has been detected in Sjögren's syndrome [3] or after conjunctival microtrauma [4]. Only recently TNF α was found in human tears with a

sensitive radioimmunoassay method [5]. The aim of this work was to study the role of TNF α in ocular allergy by investigating whether conjunctival allergen challenge of atopic patients in a symptom-free period induces release of TNF α into tears.

Materials and methods

The study was performed according to The Declaration of Helsinki, and was approved by the Ethical Review Committee of Helsinki University Eye Hospital. Informed consent was obtained from each patient. Thirteen volunteering patients (12 women, one man, mean age 26.5 years, range

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16–40 years) suffering from ocular allergic symptoms when in contact with pollen or animal dander were included in the study. The patients were selected on the basis of anamnestic criteria of ocular involvement and a positive skin-prick test to the respective allergen (weal diameter 3 mm, Solu-Prick®, 10 HEP, ALK, Hørsholm, Denmark). Seven patients suffered from bronchial asthma, and all of the patients had rhinitis together with the ocular symptoms. Exclusion criteria were: any symptoms or signs of clinical activity of allergic ocular involvement, any other eye diseases, or use of topical ophthalmic solutions within 2 weeks before the study.

About 10 µL of tear fluid was collected from the left eye with a scaled flame-polished microcapillary tube (baseline), as previously described [6]. Ten microlitres of 100 000 U/mL allergen solution (ALK, Hørsholm, Denmark) was applied into the conjunctival sac of the left eye with an electronic pipettor (Biohit, Helsinki, Finland), whereafter the eye was kept closed for 30 s. The medial canthus was compressed to avoid the flow of allergen into the lacrimal channel. Depending on the verified allergic reactivity of the patient, one of the

following allergen solutions was used for provocation: cat dander, timothy or birch. The allergic symptoms (redness, tearing, chemosis, itching) were scaled from 0 to 13+ (total score) according to Abelson *et al.* [7]. Tear samples were collected 5 min, 30 min, and 1 h after the challenge, and the tear volume and collection time were recorded. After collecting the tear samples from the allergen-challenged left eye the right eye was similarly challenged with the dilution buffer solution.

Tears were immediately centrifuged 9000g for 2 min, after which the supernatants were transferred to –20 °C until assayed. The tear fluid flow in the collection capillary (µL/min) was used as an estimate of the tear secretion rate. It was calculated by dividing the volume of the tear fluid sample by the tear fluid collection time. Tear flow-corrected TNFα release (the TNFα concentration in the sample multiplied by the tear fluid flow in the collection capillary, given as pg/min) was also calculated in order to compensate for the relative aqueous hypersecretion expected to be induced by the allergen challenge.

Table 1. The tear fluid flow in the collection capillary before challenge with topical conjunctival allergen or diluent buffer (baseline) and after challenge (5 min, 30 min, 60 min).

Flow µL/min	Baseline allergen	Diluent	5 min allergen	Diluent	30 min allergen	Diluent	60 min allergen	Diluent
<i>n</i>	12	12	12	12	12	12	12	12
Mean	1.1	1.3	3.3*†	0.9†	3.4*†	0.9+	2.9*†	0.8†
Median	0.8	1.2	2.1	0.7	1.5	1.0	1.7	0.6
SE	0.3	0.2	1.0	0.2	1.3	0.1	1.1	0.1
Min.	0.3	0.5	1.0	0.4	0.6	0.4	0.9	0.3
Max.	3.6	2.9	11.1	2.5	15.0	1.8	14.3	1.8

*Statistically significant difference between allergen vs diluent challenge. †Statistically significant difference when compared with baseline. min., minimum; max., maximum.

Table 2. The tear fluid TNFα concentration before challenge with topical conjunctival allergen or diluent buffer (baseline) and after challenge (5 min, 30 min, 60 min).

Conc ng/L	Baseline allergen	Diluent	5 min allergen	Diluent	30 min allergen	Diluent	60 min allergen	Diluent
<i>n</i>	10	12	12	12	12	12	12	11
Mean	1310	967	1479*	746	1367	1001	1426	1504
Median	1050	1000	1304	600	1300	792	1243	1200
SE	212	163	236	185	163	190	222	439
Min.	586	< dl (1)	740	< dl (2)	< dl (1)	< dl (4)	617	< dl (2)
Max.	2500	1866	3625	2120	2600	1780	3600	4500

*Statistically significant difference between allergen vs diluent challenge. < DL indicates the number of the samples below the detection limit of the assay. min., minimum; max., maximum.

Table 3. The tear fluid TNF α release before challenge with topical conjunctival allergen or diluent buffer (baseline) and after challenge (5 min, 30 min, 60 min).

Release pg/min	Baseline allergen	Diluent	5 min allergen	Diluent	30 min allergen	Diluent	60 min allergen	Diluent
<i>n</i>	10	12	12	12	12	12	12	11
Mean	1.81	1.39	4.30*†	0.67	3.20*†	0.92	3.80*†	1.05
Median	1.14	0.89	2.47	0.48	1.75	0.91	2.57	0.99
SE	0.66	0.41	1.32	0.19	1.31	0.19	1.38	0.26
Min.	0.476	< DL (1)	1.414	< DL (2)	< DL (1)	< DL (4)	0.821	< DL (2)
Max.	7.44	4.74	15.28	2.23	16.11	1.89	18.57	2.88

*Statistically significant difference between allergen vs diluent challenge. †Statistically significant difference when compared with baseline. < DL indicates the number of the samples below the detection limit of the assay. min., minimum; max., maximum.

TNF α was measured using a double-antibody radioimmunoassay, as previously described [5]. Non-parametric Wilcoxon matched pairs signed-ranks test was used to obtain the probability (*P*) values to find out the significance of changes in tear fluid flow, TNF α concentration or release in tears. The levels of allergen-challenged eyes were compared with those of the control eyes challenged with the dilution buffer. In addition, the parameters measured at different times were compared with the baseline level. Because of the high variability in baseline TNF α values we also calculated for each individual the ratios of concentration or release values at different time points to the baseline values. All results are given in means, medians, standard error of means (SE) and ranges. Probability (*p*) values less than 0.05 were considered significant.

Results

The tear fluid flows in the collection capillary (μ L/min),

TNF α concentrations (ng/L), TNF α release rates (pg/min) are shown in Tables 1–3, and symptom scores in Table 4.

The allergen challenge induced a slight increase in tear TNF α concentration in 5 min. The tear secretion rate estimated with the fluid flow in the collection capillary raised at the same time. When the relative dilution effect was taken into account by calculating the TNF α release (fg/min), the results showed that TNF α release increased significantly in 5 min after the allergen challenge. The magnitude of this change was significantly different from that induced by the challenge with the dilution buffer. There was also a significant difference in TNF α release at all time points after the allergen challenge (5 min, 30 min, 60 min) when compared with the contralateral eye.

The ratios of concentration or release values at different time points to the baseline are given in Tables 5 and 6. The results showed that 5 min after the allergen challenge the mean concentration was 1.3-fold, where as in the control eye it was 1.2-fold (NS). On the other hand, the release ratios 5 min after allergen- vs diluent challenged eyes showed a

Table 4. Clinical score before challenge with topical conjunctival allergen or diluent buffer (baseline) and after challenge (5 min, 30 min, 60 min)

Score	baseline allergen	Diluent	5 min allergen	Diluent	30 min allergen	Diluent	60 min allergen	Diluent
<i>n</i>	12	12	12	12	12	12	12	12
mean	0	0	4.6*†	0.3	5*†	0.1	2.1*†	0.1
median	0	0	5	0	4	0	1.5	0
SE	0	0	0.5	0.1	0.7	0.1	0.6	0.1
Min.	0	0	1	0	2	0	0	0
Max.	0	0	7	1	9.0	1	6	1

*Statistically significant difference between allergen vs diluent challenge. †Statistically significant difference when compared with baseline. min., minimum; max., maximum.

	Allergen challenge					
	5 min to baseline		30 min to baseline		60 min to baseline	
	Conc	Release	Conc	Release	Conc	Release
Mean	1.3	4.7*	1.3	5.0	1.6	2.8
Median	1.4	2.1	1.2	1.8	1.1	1.7
SE	0.2	2.1	0.4	3.2	0.6	0.8
Min.	0.5	1.0	0.2	0.7	0.6	1.3
Max.	2.3	21.1	4.1	27.6	6.1	7.0

*Statistically significant difference between allergen vs diluent challenge. min., minimum; max., maximum.

clearly significant elevation (4.7-fold vs 1.3-fold, respectively; $P < 0.05$).

Discussion

In the present study we evaluated the effect of conjunctival allergen challenge on the tear fluid TNF α levels in atopic patients. The patients were challenged during a symptom-free period with the allergen in the left eye and the dilution buffer solution in the right eye, which served as an intraindividual control. The results showed that the baseline concentrations of atopic patients tended to be higher than those of non-atopic individuals (mean 358 ng/L). This may be due to the fact that the tear fluid flows also appeared lower in atopic patients than in patients without ocular surface diseases [5]. The TNF α concentrations given as reference from our earlier study may not present a normal population because this study included relatively young and healthy patients scheduled for PRK (the mean age was 31.6 years, and range 21–49 years. Clinical investigation prior to PRK

showed no signs of ocular inflammation or allergy. On the other hand, these former levels can be compared with the present levels because the patients challenged with topical allergen also presented the same age group.

The fact that the allergen-challenged eyes (the first challenged eyes) had higher baseline concentration and release values was interesting. The irritation of the allergen solution in the first eye might also have induced a hypersecretion in the fellow eye, which was sometimes clinically observed. Unfortunately we did not measure the tear secretion rate of the other eye at this point. The lacrimal gland might have released its TNF α 'storages' in the other unchallenged eye, too. While the tear collection for the diluent challenge took place about one hour later, the TNF α concentrations were lower despite the fact that the hypersecretion was over by that time.

Our present results showing increased tear TNF α release rates after topical allergen challenge are in accordance with the finding that the TNF α concentrations in nasal lavage fluid are higher in acute induced allergic rhinitis than after

Table 5. The ratios showing how many fold the TNF α concentration or release is at different time points when compared with baseline. The ratios have been calculated for each individual, and the data is presented as means, medians, SE and ranges.

Ratio	Challenge with the diluent					
	5 min to baseline		30 min to baseline		60 min to baseline	
	Conc	Release	Conc	Release	Conc	Release
Mean	1.2	0.9	1.4	1.1	1.9	1.8
Median	0.9	0.5	1.0	0.7	1.1	0.6
SE	0.5	0.3	0.4	0.4	0.8	1.2
Min.	0.1	0.04	0.6	0.5	0.2	0.1
Max.	5.3	3.3	3.6	3.3	8.3	11.1

min., minimum; max., maximum.

Table 6. The ratios showing how many fold the TNF α concentration or release is at different time points when compared with baseline. The ratios have been calculated for each individual, and the data is presented as means, medians, SE and ranges.

endonasal administration of saline [8]. Increased TNF α concentrations have also been reported in bronchoalveolar lavage (BAL) fluid in atopic asthma patients after segmental allergen challenge [9]. Furthermore, the TNF α concentrations in BAL fluid of symptomatic asthmatics are higher than those of asymptomatic patients [10].

TNF α is produced by many different cells, such as activated monocytes and macrophages, stimulated lymphocytes, neutrophils, endothelial cells, smooth muscle cells [11], mast cells [12] and eosinophils in nasal polyposis [13]. Production of TNF α after allergen challenge in lungs of bronchial asthma patients has been localized to alveolar macrophages [14] and mast cells in the airways [15]. In the allergic nasal mucosa mast cells seem to be the main source of this cytokine [8]. TNF α has been found in the epithelium and subepithelial substantia propria as well as in inflammatory cells of normal conjunctiva [16]. Healthy tears also contain measurable amounts of TNF α , but its origin is not known [5].

What is the potential function of tear fluid TNF α in the ocular allergic reaction? Tear fluid levels may merely reflect the rate of release from the cells of the ocular surface (epithelial or inflammatory cells) or they may be due to enhanced production of TNF α in the lacrimal glands. TNF α is an important inducer of many endothelial cell adhesion molecules, such as E-selectin, ICAM-1 and VCAM-1 [17], which are needed for recruitment of inflammatory cells. TNF α itself also has the capacity to attract neutrophils and monocytes [18]. TNF- α has also been shown to degranulate mast cells, which leads to liberation of histamine and tryptase [19]. Allergen exposure induces ICAM-1 expression on conjunctival epithelial cells of allergic subjects [20], which suggests that conjunctival epithelium has a special role in the recruitment of inflammatory cells during the allergic reaction. Accumulation of neutrophils in tear fluid occurs at 20 min after ocular allergen challenge, whereas eosinophils and lymphocytes increase during a clinical late-phase reaction at 6 h [21]. The tear TNF α release was increased immediately after the allergen challenge, simultaneously with the first clinical symptoms. The elevated release was, however, sustained for at least 60 min, even though the clinical symptoms had nearly disappeared by this time. The patients were not followed long enough to reveal a possible second elevation of tear TNF- α release during the late phase reaction.

Tear fluid studies have proved useful in the diagnosis of ocular allergy. Tear IgE and tryptase levels are very high in allergic conjunctivitis, vernal keratoconjunctivitis or giant papillary conjunctivitis [22–25]. Interleukins IL-4 [26] and IL-6 [27] have been shown in increased amounts in allergic tears. Fujishima *et al.* (1995) could show that the IL-4 concentrations correlated with the severity of the ocular allergy [26]. The present study contributes to the knowledge on the

role of cytokines in ocular allergy, even though it did not allow conclusions between the severity of the clinical symptoms and the TNF α concentrations or release rates.

Acknowledgements

We are grateful to Ms Leena Petman for help in recruiting patients to the study. The study was financially supported by Mary and Georg C. Ehrnrooth Foundation, The Eye and Tissue Bank Foundation, Finland, The Allergy Research Foundation, Finland, The Eye Foundation, Finland, The Finnish Medical Foundation, The Finnish Medical Council and Helsinki University Eye Hospital.

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