

Measurement of total IgE antibody levels in lacrimal fluid of patients suffering from atopic and non-atopic eye disorders. Evidence for local IgE production in atopic eye disorders?

V AALDERS-DEENSTRA,¹ P T M KOK,² AND P L B BRUYNZEEL²

From the ¹Royal Netherlands Eye Hospital, FC Dondersstraat 65, 3572 JE Utrecht, The Netherlands, and the ²Department of Pulmonology, University Hospital Utrecht, Catharijnesingel 101, 3583 CP Utrecht, The Netherlands

SUMMARY Total IgE levels in lacrimal fluid of patients suffering from different eye disorders were quantitatively measured by a modification of the paper radio immuno sorbent test (PRIST). The geometric mean values for patients with atopic conjunctivitis, patients with keratoconjunctivitis vernalis, and patients with asthma without conjunctivitis differed significantly from those for control persons and those for patients without atopic conjunctivitis. Besides lacrimal fluid IgE levels, serum IgE levels as well as lacrimal fluid and serum albumin levels were measured. From these values the local IgE production was calculated. Although there seemed a good correlation between the level of lacrimal fluid IgE and the amount of local IgE production, the results suggest that local IgE production in lacrimal fluid is *not* restricted to patients with atopic eye disorders only.

Since the discovery of IgE^{1,2} this immunoglobulin has been closely associated with allergic disorders. Radioimmunoassays have been developed to measure total and specific IgE antibody levels, namely, the paper radio immuno sorbent test (PRIST) and the radio allerge sorbent test (RAST). Reports concerning total IgE values in lacrimal fluid have been scarce.³⁻⁶ Since total IgE levels in lacrimal fluid seemed very low, a modification of the PRIST was used to determine quantitatively the IgE content of lacrimal fluid of controls, patients with non-atopic conjunctivitis, different atopic eye disorders, and with asthma without conjunctivitis. Measurement of the serum IgE, the albumin content of serum and lacrimal fluid, enabled calculation of the local IgE production in the same individuals.

Materials and methods

SUBJECTS

All individuals attended the outpatient department of the Clinic of Ophthalmology. The following groups were selected: (1) Normal controls (n=16:

age 20-50 years). (2) Non-atopic conjunctivitis (n=20: age 11-71 years), no atopic history, negative skin tests, symptoms of conjunctivitis. (3) Atopic conjunctivitis: seasonal conjunctivitis (n=25: age 8-79 years), seasonal symptoms of conjunctivitis, positive skin tests with or without positive atopic history; chronic conjunctivitis <3 years (n=19: age 11-65 years), chronic symptoms of conjunctivitis for less than three years, positive skin tests with or without positive atopic history; chronic conjunctivitis ≥3 years (n=17: age 15-72 years), chronic symptoms of conjunctivitis for over three years, positive skin tests with or without positive atopic history. (4) Keratoconjunctivitis vernalis non-atopic (n=9: age 8-19 years), no atopic history, negative skin tests. (5) Atopic asthma without conjunctivitis (n=9: age 7-19 years), with atopic asthma without complaints of conjunctivitis.

The diagnosis atopy was based on the results of intracutaneous skin testing. All allergen extracts came from HAL (Haarlems Allergen Laboratorium Haarlem, The Netherlands). A control prick test with histamine 1:100 000 was included. If two or more intracutaneous skin tests were found positive, the subject was considered to have an atopic constitu-

tion. On this basis the conjunctivitis group was subdivided into an atopic and a non-atopic group.

LACRIMAL FLUID

Tears were collected by means of a glass capillary (300–600 μ l). The lacrimal fluid was stored in polystyrene tubes at -70°C until analysis. In four cases the amount of lacrimal fluid collected was too small to be analysed. These cases were omitted from the study.

NASAL AND CONJUNCTIVAL SMEARS

Smears of nasal secretions and conjunctival scrapings were examined in all subjects and stained with Giemsa. Eosinophilia was graded on a nominal scale, namely, mild, moderate, and severe (1+, 2+, and 3+).

IgE DETERMINATION

The total IgE content of the different sera was measured with the commercially available Phadezym-PRIST (Pharmacia, Uppsala, Sweden). The determination and calculation of the serum IgE content (expressed in IU/ml) were performed according to the manufacturer's instructions. The total IgE content in lacrimal fluid was measured with a modification of the commercially available PRIST (Pharmacia, Uppsala, Sweden).

The modification of this assay (Johansson SGO, personal communication) deviated on several points from the normal assay procedure: (1) The incubation volume of standard of unknown sample was enlarged to 300 μ l. (2) A standard curve was constructed from 0.005 IU/ml to 20 IU/ml (1 IU/ml=2.4 ng/ml). (3) After addition of standard or unknown sample to the anti-IgE disc the incubation tubes were covered with plastic film and were incubated for ± 20 hours at room temperature under continuous shaking. Furthermore the procedure was kept similar to the unmodified assay. Since very small quantities of lacrimal fluid were collected (300–600 μ l), only single determinations were performed. Although this may have influenced the accuracy of the IgE measurements, the introduction of an IgE reference (0.110 IU/ml) giving reproducible results, convinced us of the reliability of the measured values.

ALBUMIN DETERMINATION

The albumin content was determined by radial immuno diffusion: serum albumin content was determined with NOR-partigen-albumin plates and lacrimal albumin content was determined with LC-partigen-albumin plates (Behringwerke AG, Marburg, Germany). In 12 cases the lacrimal albumin content could not be determined. These cases were excluded from the study.

CALCULATION OF THE LOCAL IgE PRODUCTION

The local IgE production in lacrimal fluid was calculated according to the following formula⁷:

$$\text{Local IgE production} = \text{IgE (lacr)} - \frac{\text{albumin (lacr)}}{\text{albumin (serum)}} \times \text{IgE (serum)}.$$

(Lacr=lacrimal).

Results

TOTAL IgE AND ALBUMIN VALUES IN SERUM AND LACRIMAL FLUID

The total IgE and albumin values in the sera and lacrimal fluid of the different investigated groups are summarised in Table 1. As can be seen from the table, there is a fair interindividual variance in serum and lacrimal IgE values within each group. The geometric mean values of the serum IgE values of the different atopic conjunctivitis groups, the keratoconjunctivitis vernalis group, and the asthma without conjunctivitis group differed significantly from those of the controls and the non-atopic conjunctivitis group. In the case of lacrimal fluid IgE values also the non-atopic conjunctivitis group differed significantly from the normal controls. When the results of the total IgE measurements in serum and lacrimal fluid are compared, it seems as if in lacrimal fluid the difference between the geometric mean values of the controls and the other groups was more striking.

The serum albumin values of the different groups were within the same range. However, the mean lacrimal fluid albumin values of most of the atopic conjunctivitis groups and the keratoconjunctivitis vernalis group were significantly higher than those of the non-conjunctivitis groups (controls, asthma without conjunctivitis).

LOCAL IgE PRODUCTION IN LACRIMAL FLUID

The calculated local IgE production (see 'Materials and methods') in lacrimal fluid of the different investigated groups is shown in Table 2. Within each group the local IgE production also showed a fair interindividual variance. The geometric mean values for the local IgE production in lacrimal fluid of all the atopic groups and the keratoconjunctivitis vernalis group differed significantly from those of the controls and the non-atopic conjunctivitis group. As can be seen from Table 2 the seasonal conjunctivitis group showed the most pronounced local IgE production. Furthermore this table illustrates that the atopic conjunctivitis group and the asthma group without conjunctivitis had a larger percentage of cases with positive local IgE production than the normal control group, the non-atopic conjunctivitis group, or the keratoconjunctivitis vernalis group. Within the atopic conjunctivitis group the percentage of patients

Table 1 Serum and lacrimal fluid IgE (in IU/ml) and albumin (in mg/ml) in normal controls and patients suffering from conjunctivitis. Geometric mean values +95% confidence intervals are presented

Clinical groups	n	IgE		
		Serum		Lacrimal fluid
		Geometric mean	Student's t test	Geometric mean
Controls	16	19.5 (1.4– 273.1)		0.058 (0.012– 0.287)
Non-atopic conjunctivitis	20	24.3 (1.2– 507.8)	NS	0.165 (0.007– 4.137)
Atopic conjunctivitis				
I seasonal	25	262.4 (14.4– 4769.5)	p<0.001	5.259 (0.053– 523.219)
II chronic <3 jr	19	131.6 (0.8–22471.4)	p<0.02	2.581 (0.006–1152.859)
III chronic >3 jr	17	208.5 (7.1– 6124.2)	p<0.001	4.464 (0.066– 304.905)
Keratoconjunctivitis vernalis (non-atopic)	9	88.0 (10.2– 765.1)	p<0.01	1.890 (0.242– 14.880)
Asthma without conjunctivitis (atopic)	9	578.3 (105.6– 3165.3)	p<0.001	6.554 (0.819– 52.457)

Statistical analysis was performed according to Student's t test.

having a positive local IgE production was greater in the chronic atopic conjunctivitis group than in the seasonal conjunctivitis group.

The correlation between the serum IgE levels and the lacrimal fluid IgE levels on the one hand and the local IgE production on the other hand was calculated. There was a poor correlation between the serum IgE levels and the local IgE production ($r=0.49$; $p<0.001$; Spearman rank correlation). However there was a fairly good correlation between the lacrimal fluid IgE levels and the local IgE production ($r=0.75$; $p<0.001$, Spearman rank correlation). This correlation was more striking when lacrimal fluid IgE levels were over 1 IU/ml.

Discussion

Since the discovery of IgE several methods have been developed to determine total IgE levels quantitatively in various body secretions. Several authors³⁻⁶ have demonstrated the presence of IgE in lacrimal fluid. Brauninger and Centifanto³ performed semi-quantitative measurements. Their total IgE values

ranged from 687.5 to 1083.3 IU/ml. Allansmith *et al.*⁴ determined lacrimal IgE by the radio immuno sorbent technique (RIST). The lowest level of IgE in lacrimal fluid they measured at that time was 10 IU/ml in a normal control person. Ballou and Mendelson⁵ measured total IgE in lacrimal fluid by the PRIST. The lowest level was measured in the lacrimal fluid of a patient with keratoconjunctivitis vernalis with positive tear RAST but negative skin tests and negative serum RAST and amounted 0.25 IU/ml. Liotet *et al.*⁶ have also performed quantitatively total IgE measurements in lacrimal fluid by means of the PRIST. They were able to measure only total IgE levels above 0.5 IU/ml. Our investigation shows that by using a modification of the PRIST much lower IgE levels (lowest level is 0.015 IU/ml) in lacrimal fluid may also be measured with reasonable accuracy. If enough lacrimal fluid can be collected (i.e., >300 μ l), the IgE level can always be determined quantitatively with this modification.

As with serum total IgE levels there is a large interindividual variation in the lacrimal fluid IgE levels in the different groups investigated. However,

Table 2 Local IgE production in lacrimal fluid of normal controls and patients suffering from non-atopic conjunctivitis, different forms of atopic conjunctivitis, keratoconjunctivitis vernalis, and asthma without conjunctivitis. Geometric mean values + 95% confidence interval are presented

Clinical groups	n	Geometric mean	Student's t test
Controls	16 (7)	0.030 (0.007– 0.134)	
Non-atopic conjunctivitis	20 (9)	0.066 (0.003– 1.377)	NS
Atopic conjunctivitis			
I seasonal	25 (17)	8.653 (0.262–287.149)	p<0.001
II chronic <3 yr	19 (15)	2.096 (0.005–908.686)	p<0.005
III chronic >3 yr	17 (15)	1.954 (0.021–181.999)	p<0.001
Keratoconjunctivitis vernalis (non-atopic)	9 (5)	0.725 (0.095– 5.540)	p<0.001
Asthma without (atopic) conjunctivitis	9 (9)	3.353 (0.185– 60.946)	p<0.001

Statistical analysis was performed according to Student's t test.

The numbers of subjects showing a positive local IgE production are in parentheses.

Measurement of total IgE antibody levels

non-atopic conjunctivitis, different forms of atopic conjunctivitis, keratoconjunctivitis vernalis, and asthma without

Student's <i>t</i> test	Albumin		Student's <i>t</i> test	Lacrimal fluid	
	Geometric mean	Student's <i>t</i> test		Geometric mean	Student's <i>t</i> test
p<0.025	41.68 (35.16–49.40) 0.04 (34.34–46.70)	NS	0.153 (0.019–1.221) 0.232 (0.030–1.822)	NS	
p<0.001	41.68 (34.32–50.62)	NS	0.333 (0.033–3.320)	p<0.05	
p<0.001	41.68 (38.32–45.33)	NS	0.326 (0.037–2.886)	p<0.05	
p<0.001	41.26 (34.12–49.90)	NS	0.419 (0.038–4.618)	p<0.01	
p<0.001	42.52 (33.65–53.73)	NS	0.589 (0.063–5.419)	p<0.01	
p<0.001	42.10 (35.30–50.20)	NS	0.170 (0.028–1.030)	NS	

comparison of the calculated geometric mean values of the lacrimal fluid IgE levels and the serum IgE levels shows that the difference between the different patients groups and the normal control group is greater in lacrimal fluid than in serum. This could be considered an indication for local IgE production, and generally the higher the lacrimal fluid IgE content the stronger the local IgE production. If the lacrimal fluid IgE content is below 1 IU/ml, it becomes difficult to predict the local IgE production. Some individuals with lacrimal fluid IgE levels below 1 IU/ml showed local IgE production where others did not. On the contrary most of the individuals with lacrimal IgE values >1 IU/ml showed local IgE production ($\pm 84\%$ of the investigated individuals). Our study suggests that the stronger the local IgE production the more severe the atopic manifestations. On the other hand a moderate local IgE production may be accompanied by severe conjunctivitis. This relation is independent of the kind of conjunctivitis (seasonal or chronic). Therefore it would be interesting to see if there is a seasonal variation in local IgE production as there is with the patients complaints and if both IgE production and complaints decrease during certain form of therapy. This problem is currently under investigation.

Although the above mentioned data seem convincing, one has to realise that the local IgE production is calculated and not measured. Diffusion from the circulation may influence the lacrimal fluid IgE content. In the formula used to calculate the local IgE production albumin is used as a kind of internal standard. Increased albumin levels in lacrimal fluid as seen in atopic conjunctivitis as well as keratoconjunctivitis vernalis may therefore be indicative of the local production of vasodilatory substances or local inflammatory processes. Both may facilitate diffusion of serum IgE and cause increased lacrimal fluid

IgE levels. The strong influx of eosinophils in both disease states may be partly responsible for that, since it has recently been established that eosinophils are capable of producing almost exclusively the strong vasoactive leukotriene C4.⁸ This finding may be important, since in those cases of atopic asthma without conjunctivitis the albumin content in lacrimal fluid is completely normal and eosinophils are rare. Therefore the eosinophil may be involved in some way in the pathogenesis of atopic conjunctivitis and keratoconjunctivitis vernalis. Nevertheless, in cases of atopic asthma without conjunctivitis a greatly increased local production of IgE has been found (see Table 1). A possible explanation for this finding may be that in atopic individuals in general IgE-producing lymphocytes may be present round most mucosal barriers, and local IgE production is a general feature. Therefore local IgE production in lacrimal fluid is not specific for atopic eye disorders only. This conclusion is in contrast with that of Liotet *et al.*⁶ However, they omitted to incorporate a control group of atopic persons without conjunctivitis.

Another explanation may be that measurement of local IgE production is too imprecise to discriminate between different atopic disorders and that specific IgE production may lead to a better discrimination. This will be investigated in the near future.

References

- Ishizaka K, Ishizaka T, Hornbrook MM. Physicochemical properties of reagin antibody. IV. Presence of a unique immunoglobulin as antibody. *J Immunol* 1966; **97**: 75–85.
- Johansson SGO, Bennich H. Immunological studies of an atypical (myeloma) immunoglobulin. *Immunology* 1967; **13**: 381–94.
- Brauninger GE, Centifanto YM. Immunoglobulin E in human tears. *Am J Ophthalmol* 1971; **72**: 558–61.
- Allansmith MR, Hahn GS, Simon MA. Tissue, tear and serum IgE concentrations in vernal conjunctivitis. *Am J Ophthalmol* 1976; **81**: 506–11.

- 5 Ballou M, Mendelson L. Specific immunoglobulin E antibodies in tear secretions of patients with vernal conjunctivitis. *J Allergy Clin Immunol* 1980; **66**: 112-8.
- 6 Liotet S, Warnet VN, Arrata M. Lacrimal immunoglobulin E and allergic conjunctivitis. *Ophthalmologica* 1983; **186**: 31-4.
- 7 Deuschl H, Johansson SGO. Immunoglobulins in tracheo-bronchial secretion with special reference to IgE. *Clin Exp Immunol* 1974; **16**: 401-12.
- 8 Verhagen J, Bruynzeel PLB, Koedam JA, *et al.* Specific leukotriene formation by purified human eosinophils and neutrophils. *FEBS Lett* 1984; **168**: 23-8.