

# Detection of allergen-specific IgE in tears of grass pollen-allergic patients with allergic rhinoconjunctivitis

K. HOFFMANN-SOMMERGRUBER, F. D. FERREIRA\*, C. EBNER, T. BARISANI†, L. KORNINGER‡, D. KRAFT, O. SCHEINER and I. BAUMGARTNER†

*Institute of General and Experimental Pathology, University of Vienna, \*Department of Genetics and Developmental Biology, University of Salzburg, †Ophthalmological Clinic, University of Vienna, AKH, Vienna and ‡Department of Clinical Chemistry and Laboratory Diagnostics, AKH, University of Vienna, Austria*

## Summary

**Background** Allergic conjunctivitis is a common symptom amongst Type I (IgE-mediated) allergic diseases; and most frequently seen as rhinoconjunctivitis. However, the site of production and the significance of allergen specific IgE needs further elucidation.

**Objective** We investigated whether the presence of IgE in tears of grass pollen allergic patients correlated with disease and clinical symptoms, whether the IgE binding pattern to the different grass pollen antigens was different in sera and tears, and whether IgA antibodies to grass pollen allergens were present in tears. Finally, we looked whether specific IgE was produced locally or was exudated from serum.

**Methods** Sera from 44 grass pollen allergic patients suffering from either allergic rhinitis ( $n=11$ ) or allergic rhinoconjunctivitis ( $n=33$ ) and from healthy controls ( $n=7$ ) were used for the experiments. Binding of specific IgE and IgA antibodies to the different groups of grass pollen allergens (*Phleum pratense*) was evaluated by means of immunoblotting.

**Results** Specific IgE was detected in sera as well as in tears of allergic patients, whereby tear-derived allergen-specific IgE exerted similar specificities to the corresponding IgE from serum. The correlation between symptoms of ocular allergy and the presence of allergen-specific IgE in tears was highly significant ( $P<0.0001$ ). In contrast, only a poor correlation was found between specific and/or total IgE in sera and the manifestation of ocular allergy ( $P=0.73$ ).

**Conclusion** Allergen-specific IgE antibodies in tears seem to be produced locally rather than exudated from serum. IgE in tears seems to be responsible for allergic conjunctivitis. IgA in tears cannot exert a protective function since the IgA antibodies recognize different antigens in a grass pollen (*Phleum pratense*) extract than IgE antibodies. The highly significant correlation between allergic conjunctivitis and the presence of specific tear IgE emphasizes the diagnostic value of immunoblots with tear IgE, especially in cases in which serum provides inconclusive results.

**Keywords:** allergic conjunctivitis, IgE, IgA, tears, immunoblotting, grass pollen, *Phleum pratense*

*Clinical and Experimental Allergy*, Vol. 26, pp. 79–87. Submitted 15 August 1994; revised 14 December 1994; accepted 10 June 1995.

## Introduction

In industrialized countries of the temperate climate zone,

Type I allergies have increased over the last decades [1]. Up to 20% of the population suffer from allergic diseases such as rhinoconjunctivitis, rhinitis and allergic asthma bronchiale.

Major sources eliciting these IgE-mediated diseases are pollens from early flowering trees of the order *Fagales*, pollens from grasses, faecal particles from house dust mites, cat and dog dander, various fruits, vegetables, and moulds [2–8].

About 50% of pollen-allergic patients respond to the allergenic proteins from grass pollens. The majority of species from grasses of known allergenic relevance belong to the subfamily *Pooideae* (family *Festucoideae*) including species like Kentucky bluegrass (*Poa pratensis*), rye (*Secale cereale*), and timothy grass (*Phleum pratense*). A number of antigens from grass pollens have already been biochemically characterized, cloned and sequenced [9–14] and have proved to be highly crossreactive among the related species [11,12]. Major grass allergens belong to group I and V (molecular masses 28–34 kDa; [10–13]), group II and III (10–12 kDa; [14,15]), group IV (50–65 kDa; [9]) and grass profilin (14 kDa; [16]).

Patients with grass pollen-allergy suffer from seasonal rhinitis, displaying nasal irritation and blockage, sneezing, rhinorrhoea and allergic conjunctivitis with swollen conjunctivae, itching and tearing eyes up to symptoms of asthma bronchiale with attacks of bronchoconstriction on allergen exposure.

Since hay fever is an IgE-mediated disease, numerous investigations have successfully detected grass pollen-specific IgE from allergic patients' sera in RAST or immunoblots [10,11,17–19]. However, in a consistent percentage of patients with allergic rhinitis or rhinoconjunctivitis, PRIST data are either negative or inconclusive [20]. So far, IgE and IgG from tears have been measured by PRIST and radioallergosorbent test (RAST) [21–23]. Although RAST techniques offer high sensitivity, their specificity is limited and scores often lack clinical relevance. We therefore performed immunoblots, in order to discriminate the IgE-response to single allergens.

Furthermore, we investigated the following questions in the present study:

1. is allergen-specific IgE present in allergic conjunctivitis patients' tears and where does the isotype come from?
2. if there are specific IgE-antibodies present, are they directed against the same panel of allergens as the corresponding serum IgE-antibodies?
3. is specific IgA-production detectable in patients with hay fever as compared with healthy controls?

4. is transferrin present in detectable amounts in tears which can be compared with serum transferrin concentrations?

Since transferrin is known to be a good marker protein for vascular leakage of plasma proteins into tears [24,21], we considered it a helpful component to elucidate whether allergen-specific IgE in tears is produced locally in the lymphatic tissues of the eye or if it represents an exudation product from the plasma.

## Materials and methods

### Characterization of allergic patients

Forty-four grass pollen allergic patients were selected according to typical case history, positive RAST, and positive skin-prick test to SQ 200 (grass pollen mix; ALK, Horsholm, Denmark; Table 1). Out of 44 patients tested, 33 (75%) suffered from allergic rhinoconjunctivitis and 11 (25%) from allergic rhinitis alone, without a history of ocular allergic symptoms upon allergen exposure. Tear samples were collected from patients, carefully avoiding irritation, using a microcapillary pipette positioned in the inferior conjunctival sac. Informed consent was obtained from all subjects involved in the study. Sera and tears were collected during the pollen season. Six months later, during winter (no allergen exposure), tear and serum samples were collected from 11 of the patients and RAST and immunoblots were repeated. Tears and sera from seven non-allergic persons were used as a control.

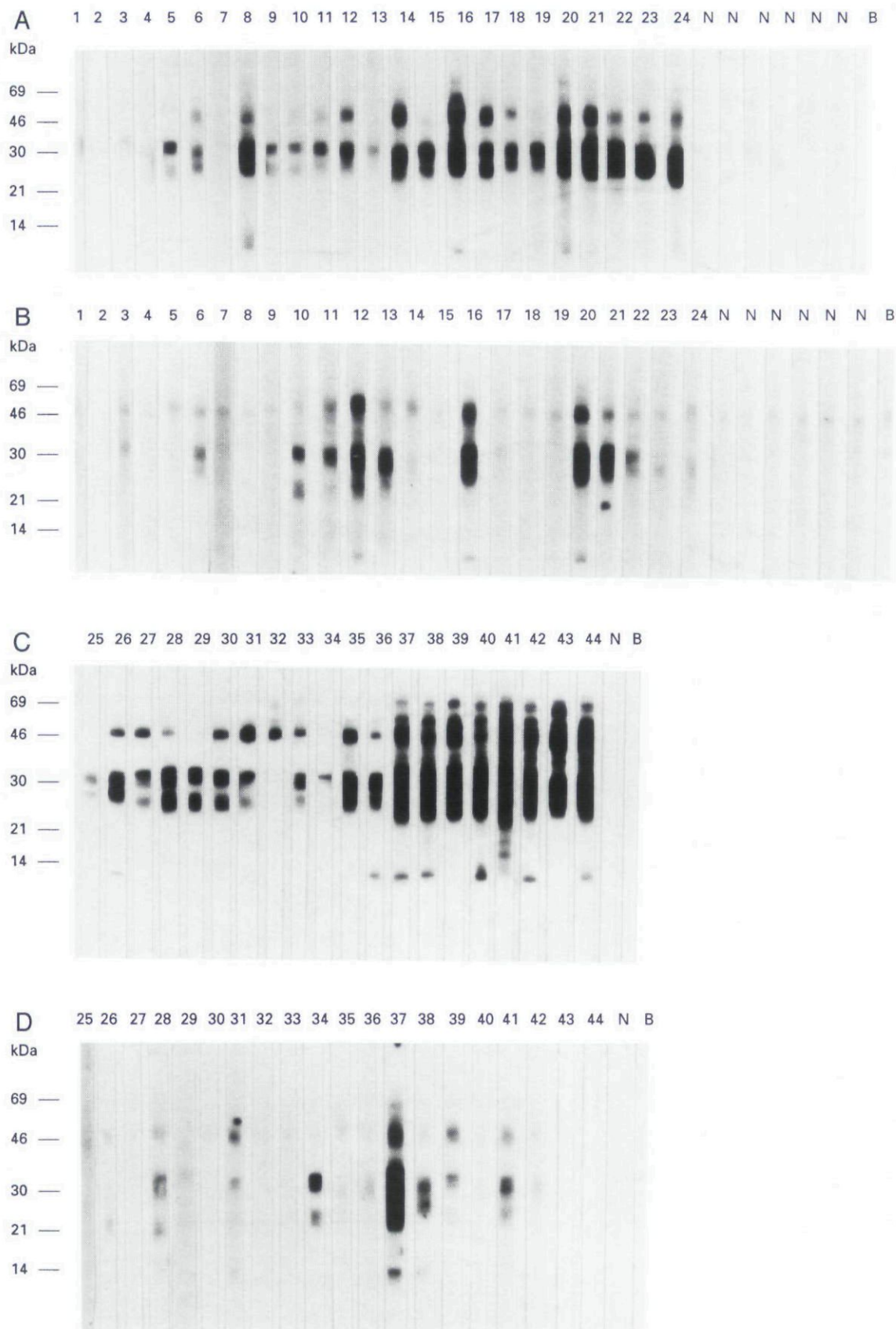
RAST to antigen g6 (Pharmacia, Uppsala, Sweden) and PRIST (Pharmacia, Uppsala, Sweden) were performed with all sera according to the manufacturer's instructions.

### Phleum pratense pollen extracts

Pollen from timothy grass (*Phleum pratense*) was obtained from Allergon, Engelhom, Sweden. Proteins were extracted (10% w/v) in potassium phosphate buffer (10 mM; pH 7.0)/polyvinylpyrrolidone (2% w/v)/ethylenediaminetetraacetic acid (2 mM)/sodiumdiethyldithiocarbamate (10 mM)/sodium azide (3 mM) at 4°C over night. After centrifugation (40 000 g; 60 min) supernatants were filtered and subsequently dialysed (Spectra/Por 1, Molecularporous Dialysis Membrane, mwco: 6–8000; Spectrum, Houston, Texas) against potassium phosphate buffer (10 mM, pH 7.0)/3 mM sodium azide for 48 h at 4°C. Subsequently, supernatants were lyophilized and stored at –20°C. Protein concentrations were measured according to Bradford (Biorad).

**Table 1.** Grass pollen-allergic patients: serum IgE (RAST PRU/mL and PRIST kU/L); Transferrin values (mg/dL) in sera and tears; conjunctivitis according to case history; and grass allergen-specific IgE derived from tears or serum detected in immunoblots (ND. Not determined). Samples from patients marked by asterisks were tested a second time out of pollen season — see Fig. 2

| Patient | RAST | PRIST<br>kU/L | Transferrin mg% Serum/tears | Conj. | Spec. IgE tears | Spec. IgE serum |
|---------|------|---------------|-----------------------------|-------|-----------------|-----------------|
| 1       | 1    | 129           | 350/0                       | +     | —               | —               |
| 2       | 2    | 247           | 322/0                       | —     | —               | —               |
| *3      | 2/2  | 16            | 310/0                       | —     | +               | +               |
| *4      | 2/2  | 51            | 322/0                       | +     | —               | —               |
| 5       | 2    | 35            | 292/0                       | —     | —               | +               |
| *6      | 3/3  | 103           | 322/0                       | +     | +               | +               |
| *7      | 3/3  | 33            | 350/0                       | +     | +               | +               |
| *8      | 3/3  | 840           | 292/0                       | +     | —               | +               |
| 9       | 3    | 111           | 350/0                       | +     | —               | +               |
| 10      | 3    | 111           | 332/0                       | +     | +               | +               |
| 11      | 3    | 23            | 332/0                       | +     | +               | +               |
| *12     | 4/4  | 86            | 292/0                       | +     | +               | +               |
| *13     | 4/3  | 1 158         | 350/23                      | +     | +               | +               |
| *14     | 4/4  | 142           | 292/0                       | +     | +               | +               |
| 15      | 4    | > 2000        | 486/0                       | —     | —               | +               |
| *16     | 5/5  | 453           | 310/0                       | +     | +               | +               |
| *17     | 4/4  | 79            | 292/0                       | +     | +               | +               |
| 18      | 4    | 72            | 292/0                       | +     | —               | +               |
| *19     | 4/4  | 51            | 310/0                       | +     | +               | +               |
| 20      | 4    | 767           | 406/0                       | +     | +               | +               |
| 21      | 6    | 191           | 350/0                       | +     | +               | +               |
| 22      | 5    | 130           | 282/0                       | +     | +               | +               |
| 23      | 4    | 56            | 332/0                       | +     | +               | +               |
| 24      | 4    | 559           | 380/0                       | +     | +               | +               |
| 25      | 3    | 44.5          | 327/0                       | +     | +               | +               |
| 26      | 3    | 82.5          | 283/0                       | +     | +               | +               |
| 27      | 3    | 455           | 283/0                       | —     | —               | +               |
| 28      | 3    | 46            | 308/0                       | +     | +               | +               |
| 29      | 3    | 46            | 296/0                       | +     | +               | +               |
| 30      | 3    | 233           | 340/0                       | —     | —               | +               |
| 31      | 3    | ND            | 283/0                       | +     | +               | +               |
| 32      | 3    | ND            | 308/0                       | —     | —               | +               |
| 33      | 3    | 44            | 308/0                       | —     | —               | +               |
| 34      | 3    | 32            | 272/0                       | +     | +               | +               |
| 35      | 4    | ND            | 400/0                       | +     | +               | +               |
| 36      | 4    | 186           | 340/0                       | +     | +               | +               |
| 37      | 4    | 126           | 308/0                       | +     | +               | +               |
| 38      | 4    | 264           | 568/0                       | +     | +               | +               |
| 39      | 4    | 348           | 283/0                       | +     | +               | +               |
| 40      | 5    | 187           | 308/0                       | —     | —               | +               |
| 41      | 5    | 221           | 500/0                       | +     | +               | +               |
| 42      | 6    | 627           | 352/0                       | +     | +               | +               |
| 43      | 6    | 1 594         | 327/0                       | —     | —               | +               |
| 44      | 6    | 404           | 283/0                       | —     | —               | +               |



## Immunoblots

### IgE-immunoblots

Aqueous pollen extracts were separated by 12% sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto nitrocellulose sheets [25]. IgE-immunoblots were performed testing allergic patients' sera and tears (1:10 diluted with sodiumphosphate buffer (50 mM; pH 7.5)/Tween 20 (0.5% v/v)/bovine serum albumine (0.5% w/v)/sodium azide (0.05% w/v) as described previously [3]. Bound IgE was detected by  $^{125}$ I-labelled rabbit anti-human IgE antibody. (Pharmacia, Uppsala, Sweden). Autoradiography was performed for 48 h at  $-80^{\circ}\text{C}$  using Kodak X-omat films and intensifying screens. In control experiments serum and tear samples from seven non-allergic individuals were tested. A buffer control omitting the first antibody was run as a comparison in all experiments.

### IgA-immunoblots

Nitrocellulose sheets with blotted *Phleum pratense* pollen proteins were prepared as described above. Human IgA present in either serum or tears (collected during grass pollen season) was detected using rabbit anti-human IgA-antibodies (Dako) and  $^{125}$ I-donkey anti-rabbit IgG. Autoradiography was performed as described above.

### Determination of transferrin in tears and sera

Sera and tears from 44 allergic patients were screened for transferrin. Five microlitres of each sample were applied into wells of a radial immunodiffusion plate (NOR Partigen-Transferrin, Behring). After a diffusion period of two days at room temperature the diameters of the precipitates were measured. Four standards (Behring) were used to plot a calibration curve. Control serum for NOR-Partigen (Behring) was used to check the accuracy of the method.

### Statistics

Statistical analysis was carried out on a personnel computer system with the use of SPSS/PC + V 5.0 (SPSS Inc. Chicago I11, USA). Analytical data for RAST classes and PRIST values were evaluated using standard linear regression analysis. Absolute frequencies were compared using the chi-square test.

## Results

### Immunoblots

#### A. IgE

*Sera (obtained during grass pollen season)* All serum samples ( $n = 44$ ) were tested for their IgE-binding capacity to *Phleum pratense* pollen allergens in immunoblots and were aligned according to their increasing RAST class scores (Fig. 1A,C; Tables 2 and 3). A typical binding pattern was observed in serum samples from grass pollen allergic patients: binding to the major allergens from group I and V, IV, II and III and *Phleum* profilin. All sera (RAST class 2.5 and higher) from patients with allergic rhinitis ( $n = 11$ ) and allergic rhinoconjunctivitis ( $n = 33$ ), showed IgE-binding activity to at least one of the *Phleum* pollen allergens (Tables 2 and 3). In three sera from allergic patients (RAST class 2.5 and less), no *Phleum* allergen-specific IgE antibodies were detected by immunoblotting technique. Sera from healthy controls ( $n = 7$ ) were negative for allergen-specific IgE (Figs. 1(A) and (C)).

*Tears (collected during grass pollen season)* The corresponding tear samples from grass pollen-allergic patients ( $n = 44$ ) were tested for the presence of specific IgE using the same technique. Twenty-nine patients (one with allergic rhinitis and 28 with allergic rhinoconjunctivitis) exhibited IgE-reactivity to at least one of the *Phleum pratense* pollen allergens (Figs 1B and D; Table 4). The tear IgE-binding reflected the pattern observed using the corresponding serum; recognizing major allergens from group I and V, II and III, IV and profilin. Non-allergic subjects showed no specific IgE in Western blots ( $n = 7$ ; Figs 1B and D).

*Sera (obtained during winter)* Sera from 11 out of the previous group of 44 allergic patients were obtained a second time during the grass pollen-free season and immunoblotting experiments were repeated. IgE-binding activity to grass pollen allergens was observed in nine out of 11 patients (10 patients with allergic rhinoconjunctivitis, one patient with rhinitis alone; Fig. 2A). All but one (patient No. 4, RAST class score 2) had shown IgE-reactivity during the pollen season.

*Tears (collected during winter)* The corresponding 11 tear samples revealed the absence of specific IgE with the

**Fig. 1.** IgE-Immunoblots: sera (A, C) and tears (B, D) from grass pollen-allergic patients taken during the grass pollen season were tested in Western blots using aqueous pollen extracts from timothy grass separated on 12% SDS-PAGE and blotted onto nitrocellulose. IgE-binding was detected using radiolabeled  $^{125}$ I anti-IgE antibodies. For controls the first antibody was omitted (B) and seven healthy persons were tested (N). For case history, RAST and PRIST see Table 1.

**Table 2.** Correlation of antigen sensitivity identified by serum-specific IgE and tear-specific IgE in 44 patients

|                    | Tear IgE positive | Tear IgE negative |
|--------------------|-------------------|-------------------|
| Serum IgE positive | 29                | 12                |
| Serum IgE negative | 0                 | 3                 |

The number of concordant and discordant test results of allergen sensitivity identified by serum-specific IgE and tear specific IgE.  $\chi^2 = 3.474$ .  $P = 0.062$ .

exception of patient No. 12, who proved by RAST to be a polyvalent allergic person (Fig. 2B).

### B. IgA

**Sera and tears** Sera and tears (collected during pollen season) from six selected patients: two patients with allergic rhinitis (serum IgE-positive/tear IgE-negative), two patients with allergic rhinoconjunctivitis (serum IgE-positive/tear IgE-negative), two patients with allergic rhinoconjunctivitis (serum IgE-positive/tear IgE-positive), and two healthy controls were tested for specific IgA-binding in immunoblots (Figs 3 A and B). The IgA-binding pattern differed individually in sera as well as in tears, and no correlation was found neither between healthy controls and allergic patients nor between the different patient groups.

### Transferrin

#### In sera and tears

A radial immunodiffusion assay was used to determine the transferrin content of sera and tear samples. Sera values ranged from 272–568 mg/dL, whereas all tear samples were negative for transferrin; except patient no. 13, who presented measurable values in tears (Table 1).

**Table 3.** Correlation of antigen sensitivity identified by serum-specific IgE and the presence of allergic conjunctivitis in 44 patients

|                    | Conjunctivitis | No conjunctivitis |
|--------------------|----------------|-------------------|
| Serum IgE positive | 31             | 10                |
| Serum IgE negative | 2              | 1                 |

The number of concordant and discordant test results of allergen sensitivity identified by serum-specific IgE and the presence of conjunctivitis.  $\chi^2 = 0.119$ .  $P = 0.730$ .

**Table 4.** Correlation of antigen sensitivity identified by tear-specific IgE and the presence of allergic conjunctivitis in 44 patients

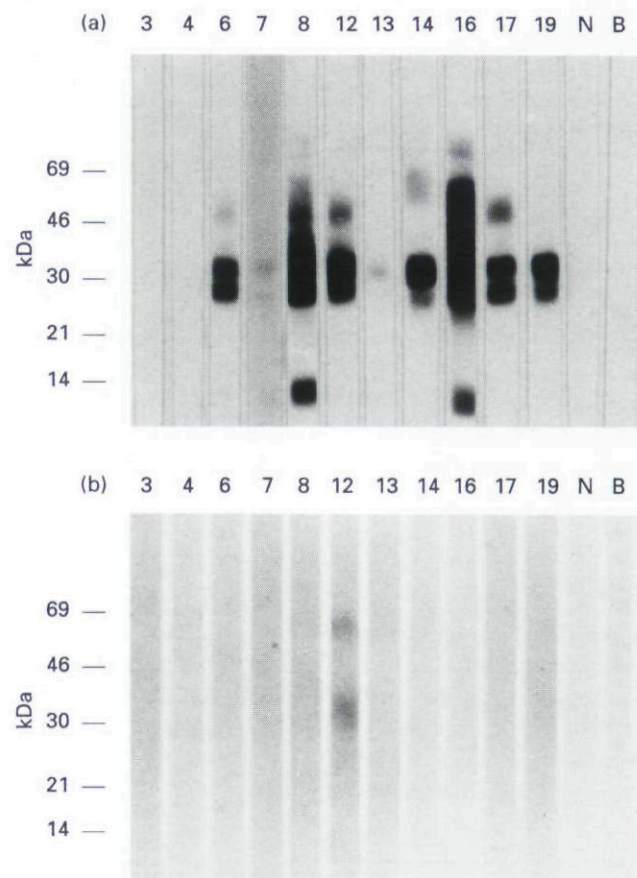
|                   | Conjunctivitis | No conjunctivitis |
|-------------------|----------------|-------------------|
| Tear IgE positive | 28             | 1                 |
| Tear IgE negative | 5              | 10                |

The number of concordant and discordant test results of allergen sensitivity identified by tear-specific IgE and the presence of conjunctivitis.  $\chi^2 = 17.836$ .  $P < 0.0001$ .

### Discussion

Grass pollens are one of the major sources of allergens eliciting IgE-mediated type I allergy leading to allergic rhinitis, conjunctivitis, and asthma bronchiale.

Allergen-specific IgE has been extensively studied in serum using RAST, PRIST, and immunoblotting

**Fig. 2.** IgE-Immunoblots: (a) sera and (b) tears from grass pollen-allergic patients were taken in winter, out of the grass pollen seasons. Immunoblots were performed as mentioned above.

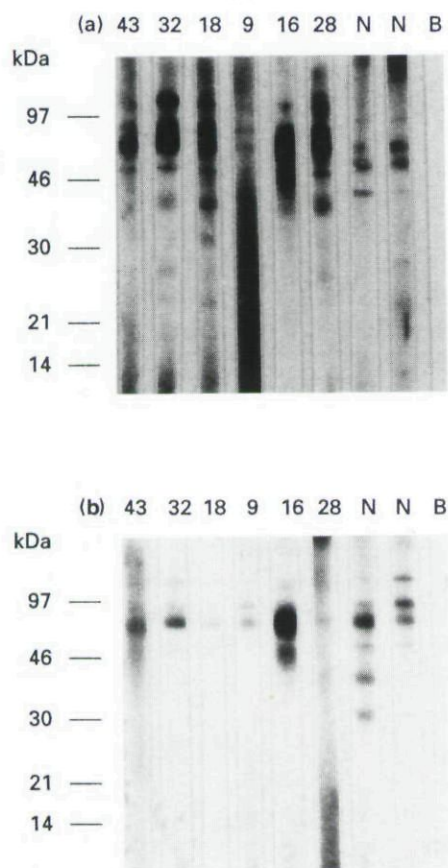


**Fig. 3.** IgA-Immunoblot: (a) sera and (b) tears from grass pollen-allergic patients and healthy persons (*n*) were tested with timothy grass pollen extract blotted onto nitrocellulose filters. Detection of bound IgA was performed with rabbit anti-human IgA followed by  $^{125}$ I-donkey anti-rabbit IgG.

techniques [3,17,26,27]. PRIST and RAST have also been used to detect IgE in mucosal secretions like saliva, tears as well as in faeces [20–23,28,29]. IgE-immunoblots were performed in order to characterize grass pollen-specific IgE in tears in more detail. Forty-four sera and tear samples from allergic patients were tested. Thirty-three patients suffered from allergic rhinoconjunctivitis, 28 of these showed IgE-reactivity in their sera as well as in their tear samples to at least one of the grass pollen-allergens tested. Three patients had IgE-reactivity in their sera only and in two cases IgE-antibodies could not be detected in either sera or tears (RAST class 2 and less).

In the group of 11 allergic patients that suffered from allergic rhinitis, nine patients had specific IgE-positive sera but negative tear samples. In one case grass pollen-specific IgE was detected in both serum and tears, while another patient displayed no IgE-reactivity in either serum or tears (RAST class score < 2).

Analysis of our data showed no correlation between RAST class scores and PRIST values ( $P = 0.02$ ). However, serum samples with high RAST class scores exerted stronger allergen-specific IgE binding capacity than serum samples with lower RAST values. Specific IgE was not always detectable by immunoblotting in patients with RAST class 2.5 or less, indicating the detection limits of this method. The same results were obtained for tear samples.

It is of interest that grass pollen-specific IgE present in tears was directed against the same panel of allergens as the IgE present in the serum, although the binding capacity of tear IgE was lower than in the corresponding serum, possibly due to dilution effects during tear collection. The highly significant correlation ( $P < 0.0001$ ) between detected IgE in tears and established allergic conjunctivitis indicates the diagnostic potential of tear IgE, especially in cases in which serum IgE is not significant. Tear samples were lacking specific IgE when collected and screened during the pollen-free season.

There are several reports about the increase in the number of mast cells in the conjunctival tissue of patients with allergic conjunctivitis as a response to allergen exposure [30–32]. Romagnani *et al.* reported cases of vernal conjunctivitis — presumably another IgE-mediated disease — where an accumulation of TH-cells producing interleukin (IL)-4 but little or no interferon gamma (IFN $\gamma$ ), exhibiting helper function for IgE-synthesis was observed [33].

Transferrin contents in tears and sera were measured to test the hypothesis that in the case of allergic rhinoconjunctivitis, allergen specific-IgE in the eye is produced locally. Since no detectable amounts of this protein were found in tears, we conclude that the grass allergen-specific IgE was not a plasma exudate but originated from local production. Final evidence of this could only be gained by the isolation of IgE-mRNA transcripts from resident plasma cells. In contrast to the findings for allergen specific IgE, IgA-immunoblots performed with sera and tears were unable to distinguish allergic patients from healthy individuals, suggesting that the IgA-antibodies in question were raised against proteins other than allergens. Our findings would also indicate that this isotype does not exert a blocking function to protect from allergic rhinoconjunctivitis. Recently, it was reported by Lozano *et al.* that allergen specific IgE levels decreased after 6, and 12 months of successful immunotherapy, but no significant change in IgA-levels were observed [34].

However, the highly significant correlation between allergic conjunctivitis and the presence of specific tear IgE emphasizes the diagnostic value of immunoblots with tear IgE especially in cases in which serum IgE

provides inconclusive results. As the conjunctival tissues surrounding the eye itself represent a lymphatic organ, approaches of therapy and/or hyposensitization treatment should be reconsidered as to whether local allergen-specific IgE production can be controlled and thus be treated by local modulation of the immune system.

### Acknowledgements

We thank Rosemary Ferris and Gabriel O'Riordain for critically reading the manuscript. This work was supported by grant SO 6707-MED of the 'Fonds zur Förderung der wissenschaftlichen Forschung'.

### References

- 1 Wüthrich B. In Switzerland pollinosis has really increased in the last decade. *Allergy Clin Immunol News* 1991; 3: 41–4.
- 2 Kaplan AP. (ed.) *Allergy*, 1985 (Cph.).
- 3 Jarolim E, Rumpold H, Endler AT et al. IgE and IgG antibodies of patients with allergy to birch pollen as tools to define the allergen profile of *Betula verrucosa*. *Allergy* 1989; 44:385–35.
- 4 Singh MB, Smith PM, Knox BR. Molecular biology of ryegrass pollen allergens. In (Baldo BA. ed): *Molecular approaches to the study of allergens*. Basel, Karger, 1990 pp. 101–120.
- 5 Tovey ER, Chapman MD, Platt-Mills TAE. Mite faeces are a major source of house dust allergens. *Nature* 1981; 289:592–3.
- 6 Spitzauer S, Rumpold H, Ebner C, Schweiger C, Valenta R, Gabl F, Anrather J, Breitenbach M, Scheiner O, Kraft D. Allergen profiles of dog hair and dander, body fluids and tissues as defined by immunoblotting. *Int Arch Allergy Appl Immunol* 1991; 94:346–48.
- 7 Calkhoven PG, Aalbers M, Koshte VL, Pos O, Eoi HD, Aalberse RC. Cross-reactivity among birch pollen, vegetables and fruits as detected by IgE antibodies is due to at least three distinct cross-reactive structures. *Allergy* 1987; 42:382–90.
- 8 Karlsson-Borga A, Jonsson P, Rolfsen W. Specific IgE-antibodies to 16 widespread mould genera in patients with suspected mould allergy. *Ann Allergy* 1989; 63:521–6.
- 9 Mathiesen F, Klysner S, Loewenstein H. Characteristics of grass pollen allergens. Epitopes of atopic allergens. pp. 9–14. The UCB Institute of Allergy. Brussels, Belgium, 1989.
- 10 van Ree R, Clemens JGJ, Aalbers M, Stapel S, Aalberse RC. Characterization with monoclonal and polyclonal antibodies of a new major allergen from grass pollen in the group I molecular weight range. *J Allergy Clin Immunol* 1989; 83:144–51.
- 11 Mathiesen F, Loewenstein H. Group V allergens in grass pollens. II. Investigation of group V allergens in pollens from 10 grasses. *Clin Exp Allergy* 1991; 21:309–20.
- 12 Laffer S, Valenta R, Vrtala S et al. cDNA cloning and expression of the major allergen Phl p 1 of timothy grass (*Phleum pratense*); recombinant Phl p 1 inhibits IgE-binding to multiple group I isoallergens from eight grass species. *J Allergy Clin Immunol* 1994; 94:689–98.
- 13 Vrtala S, Sperr W, Reimitzer I, vanRee R, Laffer S, Müller W, Valent P, Lechner K, Rumpold H, Kraft D, Scheiner O, Valenta R. cDNA cloning of a major allergen from timothy grass (*Phleum pratense*) pollen; Characterization of the recombinant Phl p 5 allergen. *J Immunol* 1993; 151: 4773–3781.
- 14 Dolecek C, Vrtala S, Laffer S et al. Molecular characterization of Phl p 2 a major timothy grass (*Phleum pratense*) pollen allergen. *FEBS Letter* 1993; 335:3:299–304.
- 15 Ansari A, Kihara TK, Marsh DG. Immunochemical studies of *Lolium perenne* (rye grass) pollen allergens, Lol p1, 2 and 3. *J Immunol* 1987; 139: 4034–41.
- 16 Valenta R, Ball T, Vrtala S et al. cDNA cloning and expression of timothy grass (*Phleum pratense*) pollen profilin in *Escherichia coli*; comparison with birch profilin. *Biochem Biophys Res Comm* 1994; 199:1:106–18.
- 17 Valenta R, Vrtala S, Ebner C, Kraft D, Scheiner O. Diagnosis of grass pollen allergy with recombinant timothy grass (*Phleum pratense*) pollen allergens. *Int Arch Allergy appl. Immunology* 1992; 97:287–94.
- 18 Westphal W, Becker WM, Schlaak M. Analysis of Rye pollen (*Secale cereale*) allergens using patients' IgE, immunoprint, Western blot and monoclonal antibodies. *Int Arch Allergy Appl. Immunol* 1988; 86:69–75.
- 19 Urisu A, Yamada K, Masuda S, Komada H, Wada E, Kondo Y, Horiba F, Tsuruta M, Yasaki T, Yamada M, Torii S, Nakamura R. 16-Kilodalton Rice protein is one of the major allergens in rice grain extract and responsible for cross-allergenicity between cereal grains in the Poaceae family. *Int. Arch Allergy Appl. Immunol* 1991; 96:244–252.
- 20 Leonardi A, Battista MC, Gismondi M, Fregona IA, Secchi AG. Antigen sensitivity evaluated by tear-specific and serum-specific IgE, skin tests, and conjunctival and nasal provocation tests in patients with ocular allergic disease. *Eye* 1993; 7:461–4.
- 21 Ballow M, Mendelson L. Specific immunoglobulin E antibodies in tear secretions of patients with vernal conjunctivitis. *J Allergy Clin Immunol* 1980; 66:112–8.
- 22 Ballow M, Mendelson L, Donshik P, Rooklin A, Rapacz P. Pollen-specific IgG antibodies in the tears of patients with allergic-like conjunctivitis. *J Allergy Clin Immunol* 1984; 73:376–80.
- 23 Kari O, Salo OP, Björkstén F, Backman A. Allergic conjunctivitis, total and specific IgE in the tear fluid. *Acta Ophthalmologica* 1985; 63:97–9.
- 24 Lundkvist U. Research and development of the RAST technology. In Evans R. editor: *Advances in diagnosis of allergy: RAST Miami, 1975, Symposia specialists*, p. 85.
- 25 Towbin H, Staehlin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets; procedure and some applications. *Proc Natl Acad Sci USA* 1979; 76:4350–54.

- 26 Birkner T, Rumpold H, Jarolim E, Ebner H, Breitenbach M, Skyvaril F, Scheiner O, Kraft D. Evaluation of immunotherapy-induced changes in specific IgE, IgG and IgG subclasses in birch pollen allergic patients by means of immunoblotting. *Allergy* 1990; 45:418–26.
- 27 Behrendt H, Krämer U, Dolgner R, Hinrichs J, Willer H, Hagenbeck H, Schlipköter HW. Elevated levels of total serum IgE in East German children: atopy, parasites or pollutants? *Allergo J* 1993; 2:31–40.
- 28 Negretti F, Casetta P. Remarkable increases of salivary IgE levels in allergic syndromes. *Int Arch Appl Immunol* 1990; 92:103–4.
- 29 Sasai K, Furukawa S, Sugawara T, Kaneko K, Baba M, Yabuta K. IgE levels in faecal extracts of patients with food allergy. *Allergy* 1992; 47:594–8.
- 30 Morgan S, Williams JH, Walls AF, Church MK, Holgate ST, McGill JI. Mast cell numbers and staining characteristics in the normal and allergic human conjunctiva. *J Allergy Clin Immunol* 1991; 87:111–6.
- 31 Holgate ST. The mast cell and its function in allergic disease. *Clin and Exp Allergy* 1991; 21:suppl.:11–6.
- 32 Pipkorn U. Mast cell behaviour in symptomatic patients with eye and nose allergies. *Clin Exp Allergy* 1991; 21:suppl.2:9–13.
- 33 Maggi E, Biswas P, DelPrete G, Parronchi P, Macchia D, Simonelli C, Emmi L, DeCarli M, Tiri A, Ricci M, Romagnani S. Accumulation of Th-2-like helper T cells in the conjunctiva of patients with vernal conjunctivitis. *J Immunol* 1991; 146:1169–74.
- 34 Lozano MR, Pasadas GF, Vacas AA, Munoz DJC, Murillo T, Guijo S. Monitoring of various types of immunotherapy with gramineal pollens. Variation of humoral immunochemical parameters. *Allergol Immunopathol Madr* 1992; 20:235–9.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.