

Anaphylactic reaction to lychee fruit: evidence for sensitization to profilin

J. FÄH, B. WÜTHRICH and S. VIETHS*

Allergy Unit, Department of Dermatology University Hospital, Zürich, Switzerland and Institute of Food Chemistry, Technische Universität, Berlin, Germany

Summary

Background and Objective Due to the increasing popularity of exotic fruits in the Western diet, allergologists are confronted with allergic reactions to substances in these plants. The present report describes an anaphylactic reaction after the consumption of lychee fruit (*Litchi sinensis*). The atopic patient also suffers from rhinoconjunctivitis due to a sensitization against pollen of the *Compositae* family, as well as from dyspnoea after eating sunflower seeds. Our goals were to determine crossreactivity between antibodies against lychee fruit and other plants and to characterize the allergen.

Methods and results Specific IgE against lychee fruits were detected by an EAST assay. The allergen was characterized by immunoblot, immunoblot inhibition and EAST inhibition assays. Broad crossreactivity between lychee fruit and other plants was found and profilin identified as the protein responsible for the patient's complex allergy syndrome.

Conclusion Lychee fruit contains a significant amount of profilin. Consumption of this exotic fruit can cause severe anaphylactic reactions in patients being sensitized against the plant pan-allergen profilin.

Keywords: lychee fruit, profilin, anaphylaxis, allergy, skin-prick tests, EAST, cross-reactivity

Clinical and Experimental Allergy, Vol. 25, pp. 1018–1023. Submitted 15 December 1994; revised 7 April 1995; accepted 28 April 1995.

Introduction

Allergologists are often confronted with complex food allergy syndromes comprising inhalant allergens as well as food allergens, sometimes extending over a range of botanically unrelated vegetables or fruits. Well-known examples are the association between birch pollen allergy and allergic reactions to fruits, nuts and some uncooked vegetables [1–3] and the connection between 'mugwort' (*Artemisia*) allergy and allergic reactions to cooked celery and spices [4,5]. The proteins responsible for these syndromes were recently identified: the plant pan-allergens profilins [6–11], cytochrome c [12] and the *Bet v*

I related allergens [13–20]. Because these proteins are ubiquitous in plants, an allergic reaction to any one of these substances can produce a clinically complex allergy syndrome.

Due to the increasing popularity of exotic fruits or vegetables in the Western diet, we must expect to be confronted with increasing numbers of patients exhibiting allergic reactions to these delicacies. Anaphylactic reactions against mango, avocado and kiwi fruit have been recently described [4,21–23].

In this paper we investigated the allergenic potential of lychee fruit (*Litchi sinensis*) in a patient with a severe fruit and seed anaphylaxis. Lychee belongs to the family of *Sapindaceae*, which also contains the two related species rambutan (*Nephelium lappaceum*) and longan (*Euphoria longana*). The three fruits, which in appearance and taste are quite similar, are native to south-east Asia. Strictly

Correspondence: Dr J. Fähr, Allergy Unit, Department of Dermatology, University Hospital Zürich, Gloriastrasse 31, 8091 Zürich, Switzerland.

speaking, the lychee, rambutan and longan are not fruits but nuts. Their closest non-tropical 'cousins' are the walnut, cashew nut and pistachio nut of the family Anacardiaceae.

Materials and methods

Skin tests

Skin-prick tests (SPT) of routine inhalants were carried out using conventional glycerol skin-prick solutions and standardized needles (Stallerpoint, France) according to the manufacturer's protocol (Alyostal extracts, Stallergènes S.A. Fresnes Cedex, France). Further prick to prick testing was performed with native allergens as described by Dreborg *et al.* [24]; briefly, a standardized needle was pricked into fresh fruits and immediately thereafter into the skin. Intracutaneous testings (ICT) with routine allergens were performed according to the manufacturers protocol (Stallerpoint, France) using unitized, disposable syringes.

The results of the skin tests were evaluated after 20 min; results were graded according to the guidelines of the European Academy of Allergology and Clinical Immunology [25]. Histamine (1 mg/mL) in 50% glycerol was used as positive control.

Sera and antisera

Serum from the patient (W.B.) was stored at -20 °C until use. A serum pool from seven non-atopic donors (code: Wn 13.26), as well as serum from one non-atopic individual with no history of food allergy (W.Z.) were used as negative controls.

Two polyclonal rabbit antisera raised against celery profilin and ragweed profilin were kindly supplied by Dr P. Deviller, Laboratoire de Biochimie, Hôpital Louis Pradel, Lyon, France [9].

Food extracts for in vitro analyses

All extracts from fruits and vegetables were prepared from uncooked material by a low temperature method as previously described [20,26]. Extracts were stored freeze-dried at -20 °C until used. Commercially available birch and mugwort pollen extracts were obtained from Allergopharma (Reinbek, Germany).

Chemicals

If not otherwise mentioned, all chemicals were of analytical grade.

Measurement of specific IgE levels

EAST assay: Specific IgE in the serum of patients and control persons were measured with a commercial enzyme allergosorbent test (EAST) (Allergopharma, Reinbek, Germany [27]). Food allergen disks were prepared at the Institute of Food Chemistry, Berlin, using cyanogen bromide-activated filter paper disks as previously described [26]. The concentration of total protein in the coupling solution was 12 µg/mL. After incubating the disks with serum, a mouse monoclonal anti-IgE antibody coupled to alkaline phosphatase was utilized. P-nitrophenylphosphate served as the chromogenic substrate. Absorbance was measured at 405 nm using the reference system of the manufacturer. The results were expressed in EAST classes and in units/mL (U/mL), corresponding to Phadebas RAST units.

Crossreactivity of lychee IgEs

Competitive EAST assay Lychee fruit allergen disks were simultaneously incubated with 50 µL of twofold diluted serum and 50 µL of various food and pollen extracts at a protein concentration of 0.25 mg/mL overnight at room temperature. The same concentration of ovalbumin was used to control for non-specific inhibition. A positive control (100% value) was obtained with 50 µL of incubation buffer instead of inhibitor solution. After the first incubation step, the disks were washed three times and the EAST continued as described above. The results were compared with the 100% value and expressed as % inhibition. All EAST determinations were run in duplicate.

Identification of IgE binding lychee proteins

SDS-PAGE Lychee proteins were separated by discontinuous sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as previously described [11,20]. Briefly, 14 µg/cm of protein from vegetable food extracts was loaded on the gel (stacking gel 5% (w/v), separation gel 13% (w/v) acrylamide). The semi-dry blotting procedure was based on a modification of the system described by Khyse-Andersen [28].

Protein transfer The separated proteins were transferred to nitrocellulose (NC) membranes (0.2 µm, Schleicher and Schüll, Dassel, Germany) using a Novablot semi-dry electroblotting apparatus (Pharmacia, Uppsala, Sweden) at 0.8 mA/cm² for 45 min as described [11]. The membranes were cut into 3–4 mm wide strips. The separated proteins were visualized with india ink [29].

IgE binding and visualization The lychee protein strips were incubated overnight at room temperature with 150 µL of serum diluted to 1 mL with incubation buffer. Excess IgE were removed by washing and bound IgE visualized with an amplified peroxidase immunostaining procedure using 3,3',5,5'-tetramethylbenzidine/diethylsodiumsulphosuccinate (TMB/DONS) as substrate [11,30]. Non-specific binding was checked by incubating one strip with incubation buffer instead of human serum, and with serum of the non atopic control patient W.Z.

Identification of profilins The polyclonal rabbit anti-profilin antisera were diluted 1:30 000 in incubation buffer. Incubation with the NC strips and detection was carried out as previously described [20].

Competitive assay Patient serum (75 µL) was pre-incubated with 100 µg of protein from the plant food extracts or 100 µg of ovalbumin (control) in a total volume of 1.0 mL incubation buffer for 60 min at room temperature. Afterwards, the immuno-detection was carried out by the standard procedure as described above.

Case report

A 23-year-old female (W. B.), in general good health and with no family history of atopy, suffered from rhinitis and conjunctivitis during her work as a florist. She noticed a worsening of her symptoms, with sneezing, rhinorrhoea, obstruction of the nasal passages and conjunctival itching, when working with plants belonging to the Compositae family. Six months after onset of her rhinoconjunctivitis she experienced an acute episode of severe dyspnoea after eating a fruit yoghurt containing sunflower seeds. Two months later another episode occurred after eating two to three fresh lychee fruits. A few minutes after taking the fruits she felt an itching in her mouth and throat. Five minutes later her lips and throat swelled, followed by severe dyspnoea. She was immediately admitted to the hospital, where she received an inhalation therapy with epinephrine as well as intravenous treatment with antihistamines and corticosteroids. After these two episodes the patient was referred to our unit for allergological examinations.

Results

Skin-prick (SPT) and intradermal (ID) tests

The patient reacted strongly in skin-prick tests with

Table 1: Skin tests with pollen extracts and food allergens

Allergen	SPT	ICT
Grass rye	-	+
Mugwort	++	
Other composites (dandelion/sunflower/ daisy/golden rod)	+ to +++	
Northern trees (birch/alder/hazel)		+ to ++
Sunflower seeds	+++*	
Lychee (fresh and canned)	+++	

Skin-prick test (SPT): +, weal > 3 mm; ++, weal > 5 mm; +++, weal > 10 mm. Intracutaneous test (ICT): +, weal > 5 mm; ++, weal > 10 mm. * Prick to prick.

pollen of the Compositae family (mugwort, dandelion, sunflower, marguerite, golden rod), with sunflower seeds, and with fresh and tinned (pasteurized) lychee fruits. Prick tests with house dust mites, dog-, cat- and horsehair, tree pollen (birch, hazel, alder) and grass pollen were negative. Intracutaneous testings were positive with birch, hazel and alder-pollen as well as with grass and rye. The results of the skin tests are summarized in Table 1.

EAST and EAST inhibition

Specific IgE against lychee fruit and sunflower seeds were quantified in the patient's serum (W. VB.) by EAST. Lychee fruit resulted in an EAST class 2 (1.3 U/mL), whereas no specific IgE were found against sunflower seeds (EAST class 0). No IgE binding to the disks occurred with the negative control serum pool Wn 13/26.

Crossreactivity measurements were done by EAST inhibition assays. Lychee fruit allergen disks were simultaneously incubated with diluted patient's serum and 0.25 mg/mL of protein from 11 plant food extracts as the inhibitors including ovalbumin as the negative control. The specific inhibitions of the IgE binding were 94% (lychee fruit), 77% (sunflower seed) 85% (celery), 67% (anise seed), 82% (potato), 89% (tomato), 78% (Golden delicious apple), 81% (strawberry), 83% (birch pollen), 92% (timothy grass), 70% (mugwort pollen) and 0% (ovalbumin).

Immunoblotting

The immunoblot results with lychee fruit extracts are presented in Fig. 1. The IgE antibodies of the allergic patient, W. B., bound to two bands at approximately

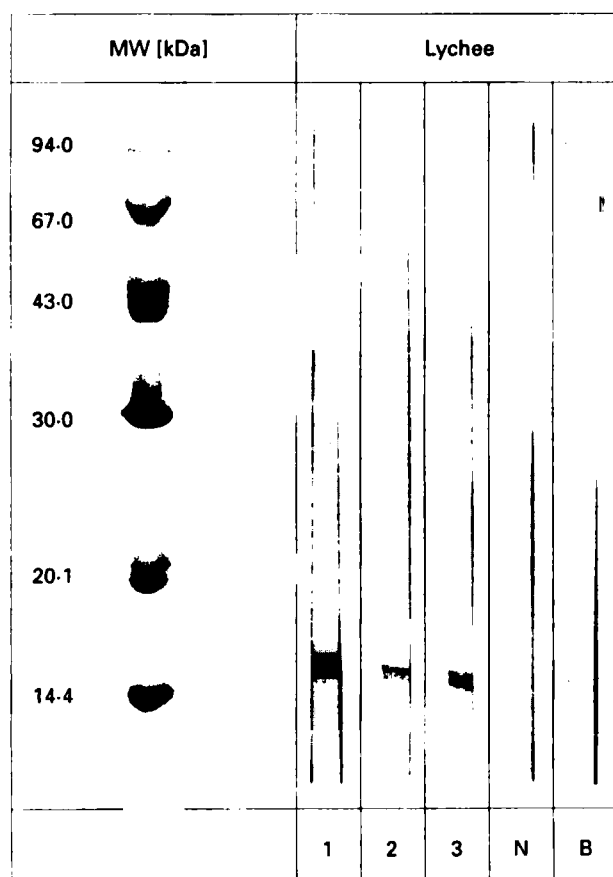


Fig. 1. Binding of IgE from human sera and IgG from rabbit antisera to lychee fruit allergens separated by SDS-PAGE and transferred to NC. Lane 1: Patient Wu; lane 2: rabbit anti-celery profilin; lane 3: rabbit anti-ragweed profilin; N: non-allergic control serum (patient W.Z.); B: buffer control.

16 kDa and to one additional band at 43 kDa (Fig. 1, lane 1). The non-allergic control serum revealed very weak non-specific antibody binding to the 43 kDa protein, but no reactivity in the region of 16 kDa (Fig. 1, lane N). Both rabbit antisera against celery profilin and ragweed profilin clearly recognized the 16 kDa lychee proteins, even at the very high dilution factor of 1:30 000 (Fig. 1, lanes 2 and 3).

Immunoblot inhibition experiments were performed using extracts from birch pollen, celery and lychee fruit as the inhibitors (Fig. 2). A total of 100 µg of protein from each of the three extracts totally inhibited the IgE reactivity with the 16 kDa lychee proteins (Fig. 2, lanes 2–4). In contrast, binding to the 43 kDa band could not be inhibited, even by the lychee fruit extract itself (Fig. 2, lane 2). We therefore conclude that this band represents non-specific binding or a latent sensitization against ovalbumin; 100 µg of ovalbumin (Fig. 2, lane 1) had no

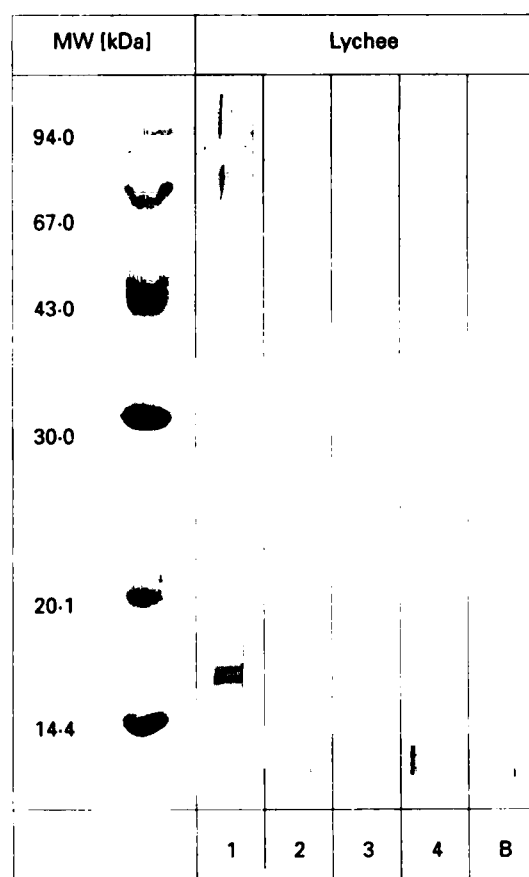


Fig. 2. Measurement of cross-reactivity by immunoblot inhibition experiments. Binding of IgE from Patient W.B. to lychee fruit allergens, inhibited by 100 µg of protein from various allergen extracts or ovalbumin. Inhibitors: Lane 1: ovalbumin; lane 2: lychee fruit; lane 3: celery; lane 4: birch pollen; B: buffer control.

influence on the IgE antibody reactivity with the 16 kDa proteins.

Discussion

In this paper we presented a patient suffering from inhalant allergic reactions to pollen of the Compositae family and anaphylaxis after eating lychee fruit and sunflower seeds. To our knowledge this is the first report describing an allergic reaction against lychee fruit. *In vivo* skin-prick tests (SPT) confirmed immediate-type sensitization to pollen of the Compositae family, sunflower seeds and fresh as well as tinned lychee fruits. These findings were in perfect concordance with the patient's symptoms. Interestingly, although specific IgE against lychee fruit were detected in the patient's serum, specific IgE against sunflower seeds were not detected.

Because there is no close botanical relationship between the *Compositae* (e.g. sunflower and mugwort) and *Sapindaceae* (lychee fruit), a plant pan allergen was suspected as being responsible for the complex allergy syndrome. In order to characterize this allergen, immunoblot experiments were performed with extracts from lychee fruit and the patient's serum. IgE antibodies from the patient's serum bound to two bands at approximately 16 kDa. In a parallel experiment, two different rabbit anti-profilin antisera bound to the 16 kDa proteins (Fig. 1), giving strong evidence that the patient's reaction against lychee fruit is due to a profilin sensitization, and that the two proteins represent iso-allergens of profilin. Profilins are highly conserved proteins, present in almost all eucaryotic organisms. As a family of plant allergens, they are highly crossreactive [6–8,10]. To confirm the crossreactive nature of the patient's IgE antibodies, EAST inhibition assays were performed with 11 different plant extracts (birch pollen, grass pollen, mugwort, celery, sunflower seed, anise seed, potato, tomato, Golden delicious apple and strawberry), most of which were known to contain high amounts of profilin [10]. As expected, binding of the patient's IgE to the lychee fruit allergen disks was strongly inhibited with all extracts in a competitive manner. Interestingly, sunflower seeds also acted as a potent inhibitor, although the EAST-assay of these seeds with the patient's serum was negative. This apparent discrepancy might be due to a relatively low profilin concentration in sunflower seeds: dilution of the profilins by other sunflower proteins on the EAST-disk might have compromised the sensitivity in this assay. The immunoblot inhibition results indicate that the 16 kDa allergen bands represent the crossreactive proteins. The appearance of profilin as a double band and the molecular weight of approximately 16 kDa is in accordance with previous reports on celery allergens [9,19].

Lychee fruit profilin appears to be a quite thermostable protein, since skin-prick tests were positive not only with fresh lychee fruits but also with the tinned products. Inquiries into the canning procedure revealed that the fruits were pasteurized at a temperature of 90 °C 10–15 min.

In summary, the patient's sensitizations to the plant pan-allergen profilin seems to be the factor responsible for the anaphylactic reactions against lychee fruit and sunflower seeds, as well as for the rhinitis and conjunctivitis she experienced during her work as a florist.

Acknowledgement

We are grateful to Mrs Roswitha Tschirnich for excellent

technical assistance and to Dr P. Deviller for his collaboration.

References

- 1 Lahti A, Björkstén F, Hannuksela M. Allergy to birch pollen and apple, and cross-reactivity of the allergens studied with the RAST. *Allergy* 1980; 35:297–300.
- 2 Eriksson NE, Formgren H, Svenonius E. Food hypersensitivity in patients with pollen allergy. *Allergy* 1982; 37:437–43.
- 3 Halmepuro L, Vuontela K, Kalimo K, Björkstén F. Cross-reactivity of IgE antibodies with allergens in birch pollen, fruits and vegetables. *Int Arch Allergy Appl Immunol* 1984; 74:235–40.
- 4 Wüthrich B, Hofer I. Nahrungsmittelallergie: Das Sellerie-Beifuss-Gewürz-Syndrom: Assoziation mit einer Mangofrucht Allergie? *Dtsch Med Wochenschr* 1984; 109:981–6.
- 5 Wüthrich B, Stäger J, Johansson SG. Celery allergy associated with birch and mugwort pollinosis. *Allergy* 1990; 45:566–71.
- 6 Valenta R, Duchene M, Pettenburger K et al. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 1991; 253:557–60.
- 7 Valenta R, Duchene M, Vrtala S et al. Profilin, a novel plant pan-allergen. *Int Arch Allergy Immunol* 1992; 99:271–3.
- 8 Valenta R, Duchene M, Ebner C et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med* 1992; 175:377–85.
- 9 Vallier P, DeChamp C, Valenta R, Vial O, Deviller P. Purification and characterization of an allergen from celery immunochemically related to an allergen present in several other plant species. Identification as a profilin. *Clin Exp Allergy* 1992; 22:774–82.
- 10 van Ree R, Voitenko V, van Leeuwen WA, Aalberse RC. Profilin is a cross-reactive allergen in pollen and vegetable foods. *Int Arch Allergy Immunol* 1992; 98:97–104.
- 11 Vieths S, Schöning B, Baltes W. Allergy to fruits and vegetables in pollen-sensitive patients: allergen characterization by IgE immunoblotting and peroxidase staining. *Food Agric Immunol* 1992; 4:181–97.
- 12 Matthews PA, Baldo BA, Howden ME. Cytochrome c allergens isolated from the pollens of the dicotyledons English plantain (*Plantago lanceolata*) and Paterson's curse (*Echium plantagineum*). *Mol Immunol* 1988; 25:63–8.
- 13 Breiteneder H, Pettenburger K, Bito A et al. The gene coding for the major birch pollen allergen Bet v I, is highly homologous to a pea disease resistance response gene. *Embo J* 1989; 8:1935–8.
- 14 Ipsen H, Hansen OC. The NH₂-terminal amino acid sequence of the immunochemically partial identical major allergens of Alder (*Alnus glutinosa*) Aln g I, birch (*Betula verrucosa*) Bet v I, hornbeam (*Carpinus betulus*) Car b I and oak (*Quercus alba*) Que a I pollens. *Mol Immunol* 1991; 28:1279–88.

- 15 Ebner C, Birkner T, Valenta R et al. Common epitopes of birch pollen and apples studies by western and northern blot. *J Allergy Clin Immunol* 1991; 88:588-94.
- 16 Vieths S, Schöning B, Aulepp H, Baltes W. Identifizierung kreuzreagierender Allergene in Pollen und pflanzlichen Lebensmitteln. *Lebensmittelchemie* 1993; 47:49-53.
- 17 Hirschwehr R, Valenta R, Ebner C et al. Identification of common allergenic structures in hazel pollen and hazelnuts: a possible explanation for sensitivity to hazelnuts in patients allergic to tree pollen. *J Allergy Clin Immunol* 1992;.
- 18 Schöning B, Vieths S, Petersen A, Baltes W. Identification and characterization of allergens related to Bet v I, the major birch pollen allergen, in apple, cherry, celery and carrot by two-dimensional immunoblotting and microsequencing. *J Sci Food Agric* 1995; in press.
- 19 Vieths S, Jankiewicz A, Wüthrich B, Baltes W. Immunoblot study of allergens in celery using sera of patients sensitized to birch pollen, mugwort pollen and celery root. *Ann Allergy* 1994; submitted.
- 20 Vieths S, Schöning B, Petersen A. Characterization of the 18-kDa apple allergen by two-dimensional immunoblotting and microsequencing. *Int Arch Allergy Immunol* 1994; 104:390-404.
- 21 Blanco C, Carrillo T, Castillo R, Quiralte J, Cuevas M. Avocado hypersensitivity. *Allergy* 1994; 49:454-9.
- 22 Falliers CJ. Anaphylaxis to Kiwi fruit and related "exotic" items. *J Asthma* 1983; 20:193-6.
- 23 Fine AJ. Hypersensitivity reaction to kiwi fruit (Chinese gooseberry, *Actinidia chinensis*). *J Allergy Clin Immunol* 1981; 68:235-7.
- 24 Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. *Allergy* 1983;38:167-72.
- 25 Skin tests used in type I allergy testing Position paper. Subcommittee on Skin Tests of the European Academy of Allergology and Clinical Immunology. *Allergy* 1989; 10:1-59.
- 26 Vieths S, Schöning B, Brockmann S, Aulepp H. Untersuchungen zur Allergie gegen Lebensmittel pflanzlicher Herkunft: Herstellung und Charakterisierung von Obst- und Gemüseextrakten für serologische Untersuchungen. *Dtsch Lebensm Rdsch* 1992; 88:239-43, 273-9.
- 27 Wahl R. Vergleichsuntersuchungen von Sammelallergenscheiben und Vergleich des Allergopharma spezifischen IgE: ELISA mit RIA. *Allergologie* 1992; 15:384-8.
- 28 Khyse-Andersen J. Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose. *J Biochem Biophys Meth* 1984; 10:203-9.
- 29 Hancock K, Tsang VCW. India ink staining of proteins on nitrocellulose paper. *Anal Biochem* 1993; 133:157-62.
- 30 Vieths S, Jankiewicz A, Schöning B, Aulepp H. Apple allergy: the IgE binding potency of apple strains is related to the occurrence of the 18-kDa allergen. *Allergy* 1994; 49:262-71.

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.