

Original article

Allergy to jackfruit: a novel example of Bet v 1-related food allergy

Background: Jackfruit allergy has been reported just once. It is unknown whether this food allergy is caused by direct sensitization or cross-sensitization to pollen allergens.

Objective: Establish whether jackfruit allergy is linked to birchpollen allergy.

Methods: Two jackfruit allergic patients and five patients with birchpollen-related apple allergy were recruited. Sensitization to pollen and plant foods was assessed by skin prick test (SPT), radio-allergosorbent test (RAST) and immunoblot. RAST analysis was performed for Bet v 1 and Mal d 1. Cross-reactivity was evaluated by RAST and immunoblot-inhibition. Biological activity of immunoglobulin E (IgE) was measured by basophil histamine release. Allergy to jackfruit was evaluated by double-blind placebo-controlled food challenge (DBPCFC) or open challenge (OC).

Results: In both patients DBPCFC confirmed the reported jackfruit allergy. SPT was 41 and 27 mm² and specific IgE to jackfruit was 5.9 and 0.8 IU/ml, respectively. Immunoblot analysis revealed IgE reactivity at Mr of approximately 17 kDa. The Bet v 1-related nature of this allergen in jackfruit was demonstrated by RAST and immunoblot inhibition. To assess whether jackfruit allergy might be common in patients with combined birchpollen-fruit allergy, five such patients underwent an OC with jackfruit. All five had OA-like symptoms.

Conclusions: Jackfruit allergy can be added to the list of birchpollen-related food allergies. Increased consumption of this fruit will result in a rise in allergic reactions.

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Key words: allergy; Bet v 1; cross-reactivity; double-blind placebo-controlled food challenge; food; jackfruit.

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Accepted for publication 9 February 2004

Immunoglobulin (Ig)E-mediated food allergy can be caused by a primary sensitization to pollen inhalant allergens (1, 2) or latex (3) resulting in cross-reactive IgE antibodies to a variety of foods. Alternatively, primary sensitization can occur by consumption of food. It is well-established that up to 70% of tree-pollen allergic patients from Northern and Central Europe (mainly birch, alder and hazel) display allergic symptoms when eating fruit (e.g. apple, peach and pear) and tree nuts (e.g. hazelnut, walnut) (4). Some patients also report allergy to vegetables-like carrot or celery (5–7). This is mainly due to cross-reactivity between the major birchpollen allergen, Bet v 1, and its homologues in fruit, nuts and vegetables. In addition, the ubiquitous protein profilin (8, 9) and cross-reactive carbohydrate determinants (CCDs) (10–12) have

been implicated in cross-reactivity between pollen and plant foods (13, 14). Symptoms in patients with pollen-plant food cross-reactivity are generally mild and limited to the oral cavity, the so-called oral allergy (OA) symptoms (15). It is characterized by immediate itching in mouth and throat and is sometimes associated with mild-to-moderate angioedema. Some patients also develop rhinoconjunctivitis and mild asthmatic symptoms (16). More recently, birchpollen-related allergens in carrot and celery and Bet v 1 homologs in soy have been implicated in more severe allergic symptoms as well (17, 18).

In this study, we describe two birchpollen allergic patients with a relatively severe allergic reaction to fresh jackfruit. Jackfruit (Nangka, *Artocarpus integrifolia*) a tropical fruit belonging to the Moraceae family (mulberry) and to the genus *Artocarpus* (breadfruit tree) is native to India, East Asia, South America and East Africa. Allergy to jackfruit has been described once before in a single patient (19). In this study, cross-reactivity to birchpollen allergens was not detected. The aim of our study was to confirm jackfruit allergy by double-blind placebo-controlled food challenge (DBPCFC) and to investigate whether cross-reactivity

Abbreviations: CCD, cross-reactive carbohydrate determinants; DBPCFC, double-blind placebo-controlled food challenge; EDTA, ethylenediaminetetraacetic acid disodium salt; mAb, monoclonal antibody; OA, oral allergy; OC, open challenge; RAST, radioallergo sorbent test; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SPT, skin prick test; VAS, visual analog scale.

with birchpollen is at the basis of the reported allergic reactions to jackfruit.

Material and methods

Patients

Two adult patients visited the outpatient department of the University Medical Center Utrecht, reporting a severe reaction to jackfruit. Both patients claimed they had never eaten jackfruit before.

Five typical birchpollen and apple allergic patients of the University Medical Center Utrecht were recruited for open food challenges with jackfruit. None of them had ever eaten jackfruit prior to the open challenge (OC). All patients had seasonal rhinitis in the period from March to May (birchpollen season in the Netherlands) and had a positive skin test ($> 7 \text{ mm}^2$) and a positive radio allerge sorbent test (RAST; $> 0.7 \text{ kU/ml}$) for birchpollen and apple. The study was reviewed and approved by the local ethical committee. Informed consent was obtained from all subjects before enrolment in the study.

Skin prick tests

The SPT were performed on the flexor aspect of the forearm with a standardized prick needle (ALK Lancet, Hørsholm, Denmark) and documented according to Dreborg (20). Histamine dihydrochloride (10 mg/ml) was used as a positive control, and the glycerol diluent of the SPT-extracts was used as a negative control (ALK-ABELLÓ, Nieuwegein, the Netherlands). Patients underwent SPT with commercial extracts from birch, grass, olive, mugwort, parietaria and ragweed pollen and from celery, hazelnut, peanut and walnut (ALK-ABELLÓ). The SPT for fresh apple, jackfruit, and peach were performed with fresh fruit using the prick-to-prick method. The wheal reaction was measured after 15 min and transferred with transparent adhesive tape on to a record sheet. The skin wheal areas were determined by computer scanning (21). The SPT were regarded positive when the wheal area was at least 7 mm^2 .

DBPCFC/open challenges

Clinical reactivity to jackfruit was investigated by DBPCFC in the two birchpollen allergic patients that had reported an adverse reaction to this food. DBPCFC was carried out using a procedure, which has been described elsewhere (22).

The challenge meals were prepared within 5 min before administration and contained: 5 and 10 g of fresh shredded jackfruit, respectively. The meals were completed with a mixture of yoghurt, orange juice, apple juice, apple sauce, and oatmeal flakes. The placebo doses consisted of the same ingredients except fresh jackfruit. Apple juice and sauce do not contain any IgE-reactive Mal d 1 allergen (Bet v 1 homolog) because of processing steps and were added for optimal blinding of taste. In addition, the patients were nose-clipped to mask odor and taste. The patients were under continuous observation during the challenge test and all symptoms were recorded.

Open challenges were performed with two samples (5 and 10 g) of fresh jackfruit. The same method of scoring was used and the same precautions were taken as in case of the DBPCFC.

Extracts and allergens

Jackfruit and apple were obtained from a local food store. Extracts were prepared essentially as described by Björkstén et al.

(23). In brief, the fruit (without peel and core) was homogenized at 10% (w/v) in 0.1 M phosphate buffer pH 7.0, containing 2% polyvinylpyrrolidone, 7 mM diethyldithiocarbamate, 2 mM ethylenediaminetetraacetic acid (EDTA) disodium salt, and 2.6 mM NaN_3 . After stirring for 1 h, particulate matter was removed by centrifugation at 18 000 g for 30 min. The supernatant was dialyzed against distilled water and lyophilized. Birchpollen extract (BPE) was made according to the protocol described by van Ree et al. (24). The rBet v 1 and rBet v 2 were purchased from Biomay (Vienna, Austria), nBet v 1 and nMal d 1 were purified by affinity chromatography using monoclonal antibody (mAb) 5H8 (directed to nBet v 1 and cross-reactive to several plant food homologs) (25). For application in RAST, 4 mg jackfruit or apple protein or 100 µg purified protein were coupled to 100 mg of CNBr-activated Sepharose 4B (Amersham-Pharmacia-Biotech, Uppsala, Sweden).

RAST and RAST-inhibition

The RAST was performed as described previously (26).

For RAST inhibition, serum was preincubated with (serial) dilutions [in 50 µl phosphate-buffered saline (PBS)-0.3% albumin, 0.1% Tween 20 (AT)] of inhibitor (BPE or jackfruit extract or nBet v 1), prior to addition of Sepharose-coupled jackfruit or birch extract. For the uninhibited value, serum was preincubated with 50 µl PBS-AT. Subsequent steps were identical to those described for the RAST. Results were either expressed as percentage inhibition, or as IU/ml.

SDS-PAGE/immunoblotting

Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; NuPAGE[®] 4–12% Bis-Tris gel, protein: 10 µg/cm) according to the protocol of the manufacturer (Invitrogen, Carlsbad, CA, USA) and silver stained using a protein silver staining kit (Bexel Biotechnology, Union City, CA, USA).

Western blotting was performed by transferring the proteins semi-dry to nitrocellulose on a Novablot electrophoretic transfer apparatus, according to the protocol of the manufacturer (Invitrogen). After blocking with PBS/ 10 mM EDTA/0.3% BSA for a minimum of 10 min, the blots were cut into strips prior to immunoprobe overnight with 150 µl human serum in 3 ml of PBS-AT. After washing away unbound serum with PBS/0.1% Tween-20, radiolabeled sheep antibodies against human IgE were used for detection of bound IgE. Blots were exposed to X-ray film (Eastman Kodak Company, Rochester, NY, USA). For blot inhibition studies, 150 µl of the inhibitor was added in several concentrations together with the patient serum. Incubation of the blotstrips and detection were performed as above.

Basophil histamine release assay

White blood cells were isolated from blood of a nonallergic donor by Percoll centrifugation (Amersham-Pharmacia Biotech) and stripped from IgE by lactic acid treatment as described elsewhere (27, 28). Subsequently cells were re-sensitized with the patients' serum. Basophil histamine release (BHR) was performed with apple, birch, and jackfruit extract (1 ng/ml to 100 µg/ml) and with purified nBet v 1 (100 pg/ml to 10 µg/ml). Liberated histamine was measured by the fluoretic method essentially as described by Siraganian (29). The protocol was approved by the medical ethical committee (MEC) of the Amsterdam Medical Center under project number: MEC97/030.

Results

Case report 1

A 31-year-old man with a history of hay fever in the birchpollen season increasingly reported episodes of OA symptoms after eating apple, hazelnut or peanut. On a holiday in Thailand, he developed OA symptoms within 5 min after eating a very small piece of fresh jackfruit. Subsequently, he developed hoarseness, swelling of the throat and dyspnea 10 min later. He had never eaten this fruit before.

Case report 2

A 27-year-old female with hay fever in the birch and grass pollen season reported increased incidence of OA-like symptoms after eating apple, hazelnut and peanut since early youth. Recently she experienced OA symptoms and abdominal cramps within 5 min after eating a small piece of fresh jackfruit for the first time.

Confirmation of jackfruit allergy by SPT, RAST, and DBPCFC

For patient 1, prick tests were strongly positive for the jackfruit (41 mm²) and RAST values (kU/ml) were positive for birchpollen (35.9), apple (21.4) and jackfruit (3.4). An allergy to jackfruit was confirmed by DBPCFC [visual analog scales (VAS) score: 90]. He reacted with OA (itching in mouth and throat) after a relatively small dose (5 g) of jackfruit. No reaction occurred on placebo.

For patient 2, the prick tests were strongly positive for jackfruit (27 mm²) and RAST values (kU/ml) were positive for birchpollen (3.8), apple (1.6), and jackfruit (0.8). The DBPCFC result was positive for jackfruit (VAS score: 40). The patient reacted with OA (itching in mouth and throat) and abdominal cramps already after 5 g of jackfruit. No placebo reaction.

Clinical histories and skin test results of both patients with reported jackfruit allergy are summarized in Table 1. Based on their clinical history, the patients were also tested by RAST for specific IgE to additional foods (Table 2).

Jackfruit allergy is related to anti-Bet v 1 IgE

To investigate whether birchpollen-related food allergies were indeed caused by Bet v 1 homologs in fruit, serum of both patients was tested on natural Bet v 1 and the four major apple allergens Mal d 1–4 (Table 2). The analysis confirmed the recognition of Bet v 1-related allergens. Binding of IgE antibodies to jackfruit was almost completely (80–85%) inhibited by jackfruit extract, BPE and nBet v 1. The inhibition with BPE was stronger (by approximately a factor 25) than with jackfruit indicating birchpollen is most likely the sensitizing agent. Specific

Table 1. Case reports 1–2: clinical history, atopy, food allergy, skin tests results and DBPCFC

Patient number	Age (years)	Sex	Rhinoconjunctivitis	Asthma	Food allergy	Positive skin test results (wheal size in mm ²)	Symptoms upon ingestion	DBPCFC	Treatment after DBPCFC
1	31	M	Birch and grass	No	ap, pea, ha, jf	Histamine (37), bi (43), gr (47), jf (41), ap (69), pe (76), pea (113), ha (38), ce (48), phl (12), ol (20)	OA, dyspnea, hoarseness	OA (90 VAS)	Antihistamine
2	27	F	Birch and grass	No	ap, pe, ha, jf	Histamine (24), bi (40), gr (41), jf (27), ap (22), pe (61), pea (16), ha (27), ce (14), phl (20), ol (10)	OA, cramps	OA (40 VAS), cramps	Antihistamine

ap, apple; bi, birchpollen; gr, grass pollen; ha, hazelnut; jf, jackfruit; ol, olive pollen; pea, peanut; pe, peach; phl, phleum pratense; OA, oral allergy (itching and swelling of the lips, mouth, throat); DBPCFC, double-blind placebo-controlled food challenge (only positive SPT results: > 7 mm² are shown); SPT, skin prick test.

Table 2. Results of specific immunoglobulin (Ig)E determination cases 1 and 2

Case report	1 (IU/ml)	2 (IU/ml)
Phleum	5.6	0.3
Birch	35.9	3.8
Peach	5.6	0.8
Hazelnut	8.8	1.5
Apple	21.4	1.6
Peanut	9.68	1.69
Cross-reactive carbohydrate determinants (CCD)	0.8	0.4
Jackfruit	5.9	0.8
nBet v 1	95.1	4.9
nMal d 1	11.1	0.6
rMal d 2	0.1	0.1
nMal d 3	0.1	0.1
rMal d 4	0.2	0.3

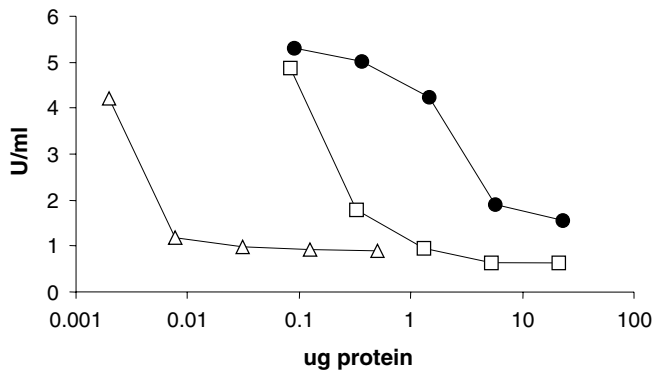


Figure 1. Radio allergeo sorbent test (RAST) inhibition studies with patient 1. Three different extracts were coupled to sepharose and jackfruit extract (circles), birchpollen extract (squares) and nBet v 1 (triangles) were used as inhibitor.

IgE to jackfruit of patient 2 was too low to perform RAST inhibition tests (Fig. 1).

Immunoblot analysis confirmed the presence of a Bet v 1 homolog in jackfruit extract. Use of the mAb 5H8 resulted in detection of a 17 kDa protein. Cross-reactivity of jackfruit allergens was further investigated by means of blot inhibition (Fig. 2). Both patients recognized a band around 17 kDa. This band was inhibited by addition of nMal d 1 and nBet v 1 and by BPE. In addition, for patient 2 the band was completely inhibited by addition of jackfruit extract (not done for patient 1).

Histamine release assay

Patients 1 and 2 were tested for their ability to induce histamine release with birchpollen, apple and jackfruit extract and nBet v 1 using the indirect histamine release assay. Patient 1 shows histamine release with all extracts/

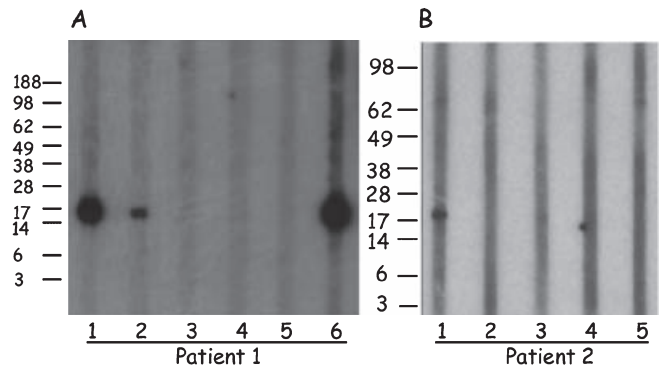


Figure 2. Immunoblot inhibition studies with patient 1 (A) and 2 (B). Lane 1, uninhibited serum; lanes 2A and 4B, 10 µg/ml Mal d 1; lane 3A, 2 µg/ml, and lanes 4A and 5B, 10 µg/ml nBet v 1; lanes 4A and 2B, 2 mg/ml birchpollen extract; lane 3B, 4.4 mg/ml jackfruit extract and lane 6A α-Bet v 1 monoclonal 5H8. Marker sizes are indicated on the left hand side.

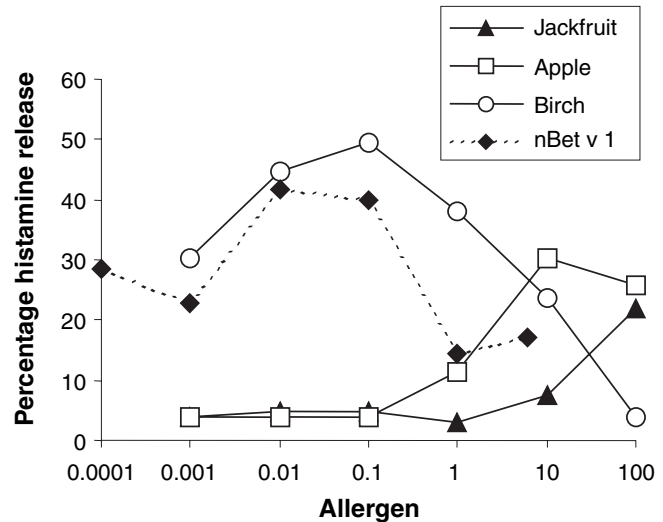


Figure 3. Basophil histamine release assay with serum of patient 1. Both apple and jackfruit extract induce a similar response, but birchpollen extract and nBet v 1 induce a higher histamine release.

allergens used and it is clear that lower concentrations of BPE and nBet v 1 are needed to induce histamine release when compared with apple and jackfruit extract (Fig. 3). For patient 2, no histamine release was detected with jackfruit and apple extract, while up to 13% release was detected with BPE and 37% release with nBet v 1 (results not shown). Stripped cells were used as a negative control and induced a histamine release lower than 3% (data not shown).

Incidence of biologically active anti-jackfruit IgE

Five adult patients (one male and four females, mean age 30.6 years), allergic to birchpollen and apple had a

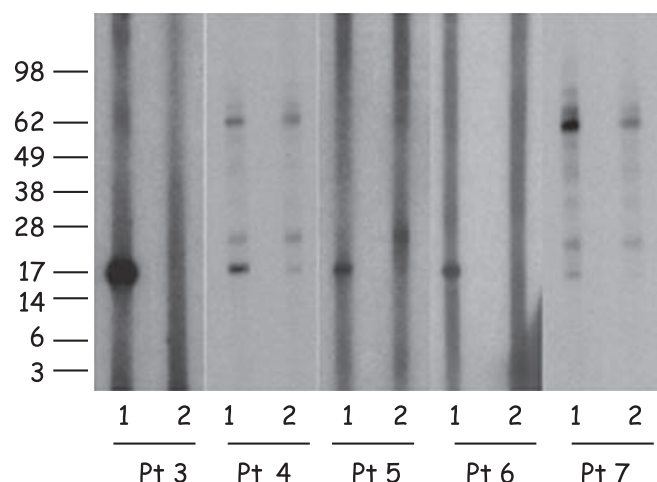


Figure 4. An immunoblot of Jackfruit extract incubated with the different sera. Lane 1: uninhibited serum, lanes 2–5: serum, preincubated with 100 µg/ml nBet v 1 (2). Blots of patients 3, 5, and 6 have been exposed for a longer time-period. Marker sizes are indicated on the left hand side.

positive skin test ($>7 \text{ mm}^2$) for birch and apple and a positive SPT and RAST to jackfruit. They were subjected to an OC with jackfruit. The mean VAS score was 70 ± 15 . Three patients reacted with OA (itching in mouth and throat) already after 5 g of jackfruit and the last two patients reacted after 10 g of jackfruit. All five patients had never eaten jackfruit before.

The immunoblot analyses revealed recognition of a 17 kDa protein band, that could be inhibited by addition of nBet v 1 (Fig. 4).

Discussion

Allergy to jackfruit was first reported for a single patient by Wüthrich et al. (19). At that time, a link to birchpollen allergy was not established. In the present study, allergy to jackfruit was for the first time confirmed by DBPCFC. In addition, it was convincingly demonstrated that allergy to jackfruit is a new member of the Bet v 1-related food allergies. Both by RAST-inhibition and by immunoblot and immunoblot-inhibition, the dominant role of cross-reactive IgE antibodies against Bet v 1 for the IgE reactivity to jackfruit was shown.

Despite the high prevalence of sensitization to birch-pollen (Bet v 1) in countries of the Northern Hemisphere

with a temperate climate, allergy to jackfruit is extremely rare compared with other Bet v 1-related food allergies such as allergy to apple and to hazelnut. The main reason for this is the insignificant role of this tropical fruit in the local diets. Open challenges with jackfruit in a group of birch-apple allergic patients that had never consumed jackfruit before, have illustrated that increased consumption of jackfruit will most likely result in a strong increase in the prevalence of allergy to this food. It can be expected that introduction of other new tropical fruit and vegetables will be accompanied by new food allergies.

Both patients in this study reported more severe symptoms in addition to their OA symptoms. One patient complained about having cramps after eating jackfruit. This was confirmed in the DBPCFC. The other patient reported hoarseness and dyspnea. It is a common generalization that Bet v 1-related food allergies only cause relatively mild symptoms limited to the oral cavity (OA symptoms). This phenomenon has been explained by the lability of Bet v 1-related food allergens in the acidic and proteolytic environment of the gastrointestinal tract. Recently, allergy to the soy homolog of Bet v 1, Gly m 4, was claimed to cause severe systemic reactions. This report and our observations suggest that some food homolog of Bet v 1 might be more stable than the extremely labile representatives like, for example, Mal d 1 from apple, enabling them to induce more severe and systemic symptoms. Whether this is the case remains to be determined. When studying stability of food allergens, both the intrinsic properties of the allergen molecules and the possible protective role of the food matrix need to be addressed. Finally, it cannot be completely excluded that reported severe symptoms are linked to allergens not (sufficiently) represented in extracts used for RAST- and immunoblot analyses. The poor IgE-binding capacity of jackfruit extracts for one of both patients (only 0.8 IU/ml), and the weak capacity to induce histamine release might indeed point in that direction.

In conclusion, allergy to jackfruit can be added to the list of birchpollen-related food allergies. Whether the jackfruit homolog of Bet v 1 is the only cross-reactive structure responsible for the observed clinical symptoms remains to be determined.

Acknowledgment

This study was supported by a grant from the EC: SAFE QLK1-CT-2000-01394.

References

1. VALENTA R, KRAFT D. Type 1 allergic reactions to plant-derived food: a consequence of primary sensitization to pollen allergens. *J Allergy Clin Immunol* 1996;**97**:893–895.
2. VAN REE R, AALBERSE R. Pollen-vegetable food crossreactivity: serological and clinical relevance of crossreactive IgE. *J Clin Immunoassay* 1993;**16**:124–130.
3. BREHLER R, THEISSEN U, MOHR C, LUGER T. 'Latex-fruit syndrome': frequency of cross-reacting IgE antibodies. *Allergy* 1997;**52**:404–410.
4. HIRSCHWEHR R, VALENTA R, EBNER C, FERREIRA F, SPERR WR, VALENT P 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;**90**:927–936.
5. WUTHRICH B, STAGER J, JOHANSSON SG. Celery allergy associated with birch and mugwort pollinosis. *Allergy* 1990;**45**:566–571.
6. HOFFMANN-SOMMERGRUBER K, DEMOLY P, CRAMER R, BREITENEDER H, EBNER C, LAIMER DA CAMARA MM et al. IgE reactivity to Api g 1, a major celery allergen, in a Central European population is based on primary sensitization by Bet v 1. *J Allergy Clin Immunol* 1999;**104**:478–484.
7. HOFFMANN-SOMMERGRUBER K, O'RIORDAIN G, AHORN H, EBNER C, LAIMER DA CAMARA MM, PUHRINGER H et al. Molecular characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross-reactivity with Bet v 1 and Api g 1. *Clin Exp Allergy* 1999;**29**:840–847.
8. VALENTA R, DUCHENE M, PETTENBURGER K, SILLABER C, VALENT P, BETTELHEIM P et al. Identification of profilin as a novel pollen allergen; IgE autoreactivity in sensitized individuals. *Science* 1991;**253**:557–560.
9. 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.
10. REINDL J, ANLIKER MD, KARAMLOO F, VIETHS S, WUTHRICH B. Allergy caused by ingestion of zucchini (*Cucurbita pepo*): characterization of allergens and cross-reactivity to pollen and other foods. *J Allergy Clin Immunol* 2000;**106**:379–385.
11. AALBERSE RC, VAN REE R. Crossreactive carbohydrate determinants. *Clin Rev Allergy Immunol* 1997;**15**:375–387.
12. FOTISCH K, ALTMANN F, HAUSTEIN D, VIETHS S. Involvement of carbohydrate epitopes in the IgE response of celery-allergic patients. *Int Arch Allergy Immunol* 1999;**120**:30–42.
13. EBNER C, HIRSCHWEHR R, BAUER L, BREITENEDER H, VALENTA R, EBNER H et al. Identification of allergens in fruits and vegetables: IgE cross-reactivities with the important birch pollen allergens Bet v 1 and Bet v 2 (birch profilin). *J Allergy Clin Immunol* 1995;**95**:962–969.
14. VAN REE R, FERNANDEZ-RIVAS M, CUEVAS M, VAN WIJNGAARDEN M, AALBERSE RC. Pollen-related allergy to peach and apple: an important role for profilin. *J Allergy Clin Immunol* 1995;**95**:726–734.
15. VAN REE R. The oral allergy syndrome. In: AMIN S, LAHTI A, MAIBACH HI, editors. *Contact Urticaria Syndrome*. New York: CRC Press, 1997: 289–299.
16. SICHERER SH. Clinical implications of cross-reactive food allergens. *J Allergy Clin Immunol* 2001;**108**:881–890.
17. BALLMER-WEBER BK, VIETHS S, LUTTKOPF D, HEUSCHMANN P, WUTHRICH B. Celery allergy confirmed by double-blind, placebo-controlled food challenge: a clinical study in 32 subjects with a history of adverse reactions to celery root. *J Allergy Clin Immunol* 2000;**106**:373–378.
18. KLEINE-TEBBE J, VOGEL L, CROWELL DN, HAUSTEIN UF, VIETHS S. Severe oral allergy syndrome and anaphylactic reactions caused by a Bet v 1-related PR-10 protein in soybean, SAM22. *J Allergy Clin Immunol* 2002;**110**:797–804.
19. WUTHRICH B, BORGA A, YMAN L. Oral allergy syndrome to a jackfruit (*Artocarpus integrifolia*). *Allergy* 1997;**52**:428–431.
20. DREBORG S, FREW A. Allergen standardization and skin tests. *EAACI position paper*. *Allergy* 1993;**14**:49–82.
21. POULSEN LK, LIISBERG C, BINDSLEV-JENSEN C, MALLING HJ. Precise area determination of skin-prick tests: validation of a scanning device and software for a personal computer. *Clin Exp Allergy* 1993;**23**:61–68.
22. WENSING M, PENNINKS AH, HEFLE SL, AKKERDAAS JH, VAN REE R, KOPPELMAN SJ et al. The range of minimum provoking doses in hazelnut-allergic patients as determined by double-blind, placebo-controlled food challenges. *Clin Exp Allergy* 2002;**32**:1757–1762.
23. BJORKSTEN F, HALMEPURO L, HANNUKSELA M, LAHTI A. Extraction and properties of apple allergens. *Allergy* 1980;**35**:671–677.
24. VAN REE R, VAN LEEUWEN WA, AKKERDAAS JH, AALBERSE RC. How far can we simplify in vitro diagnostics for Fagales tree pollen allergy? A study with three whole pollen extracts and purified natural and recombinant allergens. *Clin Exp Allergy* 1999;**29**:848–855.
25. AKKERDAAS JH, VAN REE R, AALBERS M, STAPEL SO, AALBERSE RC. Multiplicity of cross-reactive epitopes on Bet v 1 as detected with monoclonal antibodies and human IgE. *Allergy* 1995;**50**:215–220.
26. VAN DER ZEE JS, DE GROOT H, VAN SWIETEN P, JANSEN HM, AALBERSE RC. Discrepancies between the skin test and IgE antibody assays: study of histamine release, complement activation in vitro, and occurrence of allergen-specific IgG. *J Allergy Clin Immunol* 1988;**82**:270–281.
27. KNOL EF, KUIJPERS TW, MUL FP, ROOS D. Stimulation of human basophils results in homotypic aggregation. A response independent of degranulation. *J Immunol* 1993;**151**:4926–4933.
28. BUDDE IK, AALBERS M, AALBERSE RC, VAN DER ZEE JS, KNOL EF. Reactivity to IgE-dependent histamine-releasing factor is due to monomeric IgE. *Allergy* 2000;**55**:653–657.
29. SIRAGANIAN RP. Refinements in the automated fluorometric histamine analysis system. *J Immunol Methods* 1975;**7**:283–290.