Immunological Characterization of Dutch Sesame Seed-Allergic Patients

Malgorzata Teodorowicz^a Rozine J. Terlouw^a Ad Jansen^b
Huub F.J. Savelkoul^a Janneke Ruinemans-Koerts^c

^aDepartment of Cell Biology and Immunology, Wageningen University, Wageningen, ^bDepartment of Otorhinolaryngology, Radboud University Medical Centre, Nijmegen, and ^cDepartment of Clinical Chemistry and Haematology, Rijnstate Hospital, Arnhem, The Netherlands

Key Words

Sesame allergy · Oleosin · Basophil activation test · IgE inhibition test · Allergen extracts

Abstract

Background: Sesame seed is an allergen of growing importance worldwide. However, knowledge of the clinically relevant sesame allergen and its cross-reactivity with homologous allergens is limited. The aim of this study was the immunological characterization of Dutch sesame seed-allergic patients and evaluation of cross-reactivity between sesame seed, tree nut and pollen allergens using different sources of allergen extracts. **Methods:** Six patients with a medical history of sesame seed allergy were included, i.e. 5 with an anaphylactic reaction and 1 with an oral allergy syndrome (OAS). The immunological background of the sesame seed and tree nut IgE sensitization was characterized with Western blotting and a basophil activation test (BAT). The major sesame allergen was identified by nanoLC-MS/MS. Cross-reactivity was measured using an immuno-inhibition assay with the Phadia ImmunoCAP system. **Results:** Oleosin was identified as the major allergen for the 5 patients with an anaphylactic reaction to sesame seed, but no cross-reactivity between sesame and tree nut proteins was observed. For the patient

with OAS, IgE specific to oleosin was not detected but cross-reactivity between sesame seed and tree nut proteins was observed. The BAT and ImmunoCAP inhibition test added value to the clinical and immunological characterization of sesame seed-sensitized patients, distinguishing relevant and non-relevant sensitizations. **Conclusions:** Our immunological approach enabled us to fully characterize the sensitization pattern of 6 sesame seed-allergic patients. The different protein composition of commercially available allergen extracts influences the outcomes of the immunological assays and thus also the diagnosis to a large extent.

© 2016 S. Karger AG, Basel

Introduction

Sesame seed (*Sesamum indicum* L.) is a highly immunogenic food which may trigger severe allergic reactions including life-threatening ones [1–3]. The growing importance of sesame seed allergens is reflected in the directives of food safety authorities imposing the inclusion of sesame seed on the list of allergen labelling [4, 5].

M.T. and R.J.T. contributed equally to the work.

Most cases of sesame seed allergy are diagnosed in early childhood and adolescence. It has been estimated that only 20–30% of these patients develop tolerance to sesame seed [6]. Accurate diagnosis is thus important, as most sesame seed-allergic patients need lifelong dietary restrictions and emergency medication. On the other hand, misdiagnosis of food allergy, e.g. treating clinically non-relevant cross-sensitization as true food allergy, may lead to malnutrition, eating disorders, psychosocial problems and severe family disruption [7].

Diagnosis of food allergy is generally based on a clear medical history, skin-prick tests (SPT), specific IgE (sIgE) blood tests and a double-blind placebo-controlled food challenge (DBPCFC). The DBPCFC is considered the gold standard for food allergy diagnosis [8–10], but it does have some limitations like high costs, greater time investment and a risk of life-threatening reactions. As sensitization does not necessarily signify a clinically relevant food allergy, additional *in vitro* methods with high diagnostic power are required [11]. The basophil activation test (BAT) has been shown to be a sensitive and specific tool for *in vitro* diagnosis of an immediate-type allergy [11–13]. An important advantage of the BAT is the ability to measure cross-linking capacity as a functional activity of sIgE antibodies and the absence of risk for the patient [14]. Particularly in the case of discrepancies between medical history, SPT and/or sIgE testing, the BAT can be a useful additional tool in the diagnosis of food allergy.

Diagnosis of sesame seed allergy requires knowledge of the clinically relevant allergens and cross-reactivity with homologous allergens of other foods or pollens. So far, 7 sesame seed allergens have been identified; these show cross-reactivity with other seeds, tree nuts and peanut on the basis of protein homology [15–19]. However, there is a lack of knowledge of the major sesame seed allergens responsible for sensitization in European countries, including the Netherlands. Identifying the sIgE profiles of sesame seed allergens and clinically relevant cross-reactivity with other foods might provide indications for the clinical relevancy and severity of sesame seed-allergic reactions.

This study shows the clinical relevance of sIgE patterns in 6 Dutch sesame seed-sensitized patients, using different sources of allergen extracts and combining 2 standard immunological assays (testing of sIgE levels and Western blotting) with 2 additional ones (BAT and immuno-inhibition with the Phadia ImmunoCAP system) providing added value to the immunological characterization.

Materials and Methods

Allergen Extracts

The following allergen extracts were obtained from Bühlmann (Basel, Switzerland): almond, cashew nut, hazelnut, peanut and sesame seed. Allergen extracts of hazelnut, peanut, sesame seed and walnut were obtained from Allergopharma B.V. (Zeist, The Netherlands). Almond extract was obtained from ALK (Round Rock, Tex., USA).

Patients

The study was approved by the Medical Ethics Committee CMO Region Arnhem-Nijmegen, The Netherlands (No. NL42526.091.12). Seven controls were included, i.e. 4 healthy, non-atopic controls and 3 atopic controls with clinical symptoms of an inhalation and/or food allergy other than sesame seed. Patients (aged >18 years) with sesame seed allergy were selected from the patient database of the Allergy Clinic Arnhem according to the following inclusion criteria: (1) a clear and well-documented medical history of sesame seed-allergic reactions (5 patients with anaphylactic reactions and 1 with oral allergy syndrome, OAS) and (2) positive sIgE (via blood testing) and/or positive SPT to sesame seed. All these patients refused a DBPCFC because of the anticipated risks. Medical history and sensitization (SPT/sIgE) results for other foods like legumes and tree nuts were also obtained. The presence of a significant amount of sIgE (>2 kU/l) for an allergen for at least 2 patients was the determining factor for Western blot and ImmunoCAP inhibition analysis in order to detect differences between the protein sources and to observe different sensitization patterns for an allergen between patients. All controls and patients donated EDTA and serum blood samples.

Skin-Prick Test

SPT were performed and graded as described elsewhere [20, 21]. Reactions were considered positive if the mean wheal diameter was 3 mm larger than that of the histamine control.

sIgE Blood Test

For all patients and controls, serum sIgE levels were determined for sesame seed, almond, cashew nut, peanut, walnut, hazelnut, grass pollen and tree pollen. For the atopic controls, additional sIgE levels were also determined for house-dust mite and/or cat dander, according to self-reported clinical relevancy. Measurements were done according to the manufacturer's instructions on an ImmunoCAP 250 (Phadia, Uppsala, Sweden, now part of ThermoFisher Scientific). Allergen-specific IgE values <0.35 kU/l were considered negative.

Basophil Activation Test

The Flow2 CAST BAT (Bühlmann) was performed according to the instructions of the manufacturer. EDTA blood samples were freshly tested or stored for a maximum of 24 h at 2–8°C. Two positive controls, formyl-methionyl-leucyl-phenylalanine (fMLP) and an anti-IgE receptor mAb (FcεRI), and 1 negative control (PBS) were used. The test was considered positive when CD63 expression on basophils was >15% and the response was dose-dependent (corrected for negative control) [22]. Two BATs were performed on each patient on 2 subsequent days. On the first day, EDTA blood samples were collected and tested on basophil activation in response to all included allergen extracts (sesame seed, almond, cashew nut,

Fig. 1. Principle and results of the IgE-based inhibition of sesame seed sIgE with hazelnut CAP for patient 6. Patient serum was loaded to 5 hazelnut (Haz) sponges and incubated for 30 min. Eluates were collected, pooled and transferred to new Haz sponges. After 3 cycles of incubation, the eluates were pooled and transferred onto 2 sesame seed (Ses) sponges. After incubation, eluates of the Ses sponges were pooled and transferred to 1 Ses sponge. All capsules were placed back in the right CAP pens and analyzed for sIgE binding with physiological salt with ImmunoCAP 250.

hazelnut, peanut and walnut) at an optimized concentration of 100 ng/ml. The next day, depending on the individual patient results of the first day, concentration series (i.e. higher and lower allergen concentrations) were performed for the extracts that had triggered a basophil activation of >15% on the first day. In the case of 2 allergen extracts of different companies being used and 1 of them resulting in a negative BAT, the BAT with this extract was repeated with higher allergen concentrations (maximum: 400 ng/ml).

IgE-Based Inhibition with Allergen-Coated CAP Sponges Preparation of Allergen-Coated CAP Sponges

Allergen-coated cellulose CAP sponges (Phadia) were carefully removed from the capsule. The preserving liquid was first removed by drying the sponge between filter paper twice. Subsequently, the sponge was washed 3 times with 0.5 ml of wash solution and the remaining liquid squeezed out with a sterile wire, followed by drying between filter paper. The sponge was then placed back in a clean and dry capsule and the capsule was placed in an Eppendorf cup.

Inhibition Part

Patient serum (40 µl) was loaded onto 5 sponges coated with the first potentially cross-reactive allergen, e.g. hazelnut (fig. 1) and

incubated for 30 min at 37°C. The sponges were centrifuged at 12,000 g for 3 min. Eluates of serum were pooled, gently mixed and 40 µl was transferred to new sponges coated with the same allergen, incubated and centrifuged according to the same procedure as in the first incubation step. After 3 cycles of incubation, the sponges were placed back in the right CAP pens and analyzed for sIgE binding with physiological salt with the ImmunoCAP 250.

Read-Out Part

Eluate of serum from the last inhibition step was transferred onto 2 sponges coated with the second cross-reactive allergen, e.g. sesame seed (fig. 1) and incubated for 30 min at 37°C. The sponges were centrifuged at 12,000 g for 3 min and eluates of serum were pooled and gently mixed, and then 40 µl was transferred to new sponges coated with the same allergen. After incubation, the sponges were placed back in the right CAP pens and analyzed for sIgE binding with physiological salt with the ImmunoCAP 250.

The percentage of inhibition was calculated as follows: $(1 - \text{sIgE}_x / \text{sIgE}_i) \times 100$, where 'x' is the amount of sIgE for the tested allergen after inhibition with another allergen and 'i' is the initial sIgE amount of the tested allergen.

The detection limit of the ImmunoCAP method is 0.1 kU/l with an imprecision of 10%.

The method was validated with the use of a control allergen (house-dust mite) and related to the classic inhibition ELISA method before its application in this study (unpubl. data).

SDS-PAGE

Allergen extracts were separated by SDS-PAGE under reducing conditions using BioRad equipment. Proteins were loaded onto a 12.5% polyacrylamide gel in an amount estimated at 20 µg/well (according to the manufacturer). After protein separation, the gel was stained using GelCode blue stain reagent (Thermofisher Scientific). A molecular weight marker (Precision Plus Protein dual color standards, BioRad) was included.

Western Blotting

The separated proteins (SDS-PAGE) were transferred to a Whatman membrane using the Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (BioRad) at 15 V for 35 min. The membrane was blocked for 1 h at room temperature (RT) with 3% BSA in Tris-buffered saline with 0.5% of Tween-20 (TBST). After washing, the membrane was incubated overnight at 4°C with patient serum diluted (3–5 times) in TBST. After incubation, the membrane was washed and incubated for 1 h at RT with mouse monoclonal anti-human IgE (BD) diluted at 1:800 in 0.5% non-fat dried milk in TBST. Thereafter, the membrane was washed and incubated with goat anti-mouse-HRP antibodies (Dako PO447) diluted at 1:1,000 in 0.5% non-fat dried milk in TBST for 30 min at RT. After washing the membrane, a chemiluminescent detection was performed. The blot was incubated with ECL Western blotting detection reagent mix for 2 min. It was then scanned using a Molecular Imager Gel Doc XR System (BioRad), and each membrane was analyzed with ImageLab 4 software. The intensity of each band was calculated and subsequently graded as intense, medium or weak (table 1).

Identification of the Most Immunoreactive Fraction by nanoLC-MS/MS Peptide Sequencing and Database Search

The protein fractions with a molecular weight estimated to be around 14 kDa were cut off of the SDS-PAGE gel and analyzed by Alphalyse (Odense, Denmark). Samples were reduced and alkylated with iodoacetamide, i.e. carbamidomethylated, and subsequently digested with trypsin that cleaves after lysine and arginine residues. The resulting peptides were concentrated by Speed Vac lyophilization and redissolved for injection on a Dionex nano-LC system and MS/MS analysis on a Bruker Maxis Impact QTOF instrument. The MS/MS spectra were used for Mascot database searching. The data are searched against in-house protein databases downloaded from UniProt and NCBI containing >49 million known non-redundant protein sequences.

Results

Clinical Characteristics of the Patients

All sesame seed-allergic patients were females and had experienced their first allergic reactions during childhood or adolescence. At the time of this study, their age ranged

from 18 to 54 years. Table 2 shows the SPT/sIgE profile and clinical relevancy of sensitizations to the tested allergens. Patient 1 had an isolated sesame seed allergy. Patients 2 and 3 also had hay fever (grass pollen allergy) and patients 4 and 5 had a concomitant allergy to both grass and tree pollens. Patient 5 also had a clinically relevant OAS for walnut and hazelnut. Patient 6 had hay fever (grass pollen allergy) and relatively mild allergic symptoms (OAS) to sesame seed as well as to some tree nuts (hazelnut, almond and cashew nut).

The 4 non-atopic female controls (aged 24–54 years) were not IgE-sensitized to all tested allergens (sIgE <0.35 kU/l). The 3 atopic controls (aged 24–58 years; 2 men and 2 women) displayed sIgE against at least 1 allergen. Control 5 displayed sIgE against house-dust mite and cat dander. Control 6 displayed sIgE against hazelnut, grass pollen and tree pollen. Control 7 displayed sIgE against hazelnut, peanut, sesame seed, grass pollen, tree pollen and house-dust mite (data not shown).

Immunological Characteristics of the Patients

Basophil Activation Test

Figure 2 shows the BAT results for all patients, except for the peanut and walnut extract, which was due to the absence of basophilic activation for all patients. Except for patient 6, positive BAT results for the sesame seed extract from Bühlmann were obtained for all patients, and were dose-dependent. Patients 2–5 showed no/less stimulation with the sesame seed extract from Allergopharma than with the Bühlmann extract at a concentration of 100 ng/ml. Increasing the dose to 400 ng/ml only resulted in a higher basophilic stimulation for patients 2 and 4, i.e. from 15 and 30% to 44 and 56%, respectively. Only patient 6 showed a positive BAT for almond and cashew nut extract. With the Bühlmann hazelnut extract, basophilic stimulation was detected for patients 5 and 6 only. All sesame seed, almond, cashew nut, hazelnut, peanut and walnut extracts were tested in the control group. For the patients in the control group, no basophilic activation was observed (data not shown).

IgE-Based Inhibition with Allergen-Coated CAP Sponges

Inhibition experiments were performed for patients 5 and 6. Patient 5 represented the group that also included patients 2–4 with respect to the grass pollen and tree pollen sensitization in addition to sesame seed. Patient 6 was unique, being the only patient with multiple tree nut sensitizations in addition to sesame seed. In total, 5 inhibitions were performed for patient 5: tree pollen/hazelnut

Table 1. Immunoreactive fractions detected by Western blot for each patient

Extract	Protein fraction, kDa	Protein family	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Sesame seed (Bühlmann)	79, 62, 43, 36, 22	11S/7S subunits	XXX	XX	n.r.	X	n.r.	X (62 kDa)
	14	oleosin	XXX	XXX	XXX	XXX	XXX	n.r.
Sesame seed (Allergopharma)	80–20 (smear)	11S/7S subunits	XXX	XXX	XX	XX	XX	n.r.
	12–17	oleosin	XXX	XXX	XXX	XXX	XXX	n.r.
Almond (Bühlmann)	79, 60, 45, 37	11S/7S subunits	n.t.	n.t.	n.r.	n.t.	n.r.	XXX
Almond (ALK)	63–50, 37	11S/7S subunits	n.t.	n.t.	n.r.	n.t.	n.r.	XXX
Hazelnut (Bühlmann)	55	11S/7S subunits	n.r.	n.t.	n.r.	n.r.	X	XX
	18	Bet v 1	n.r.	n.t.	n.r.	n.r.	X	n.r.
Hazelnut (Allergopharma)	55	11S/7S subunits	n.r.	n.t.	n.r.	X	n.r.	n.r.
	25	11S/7S subunits	n.r.	n.t.	n.r.	n.r.	n.r.	X

Only clinically relevant food allergens for each patient were tested. The band intensity was specified as intense (XXX), medium (XX) or weak (X) based on calculations made with ImageLab 4 software (BioRad) and approximate molecular weights were estimated also with ImageLab 4 software. Based on these molecular weights, the protein family was specified using the InformAll Allergenic Food Database [23]. n.r. = Non-reactive; n.t. = not tested.

Table 2. Diagnostic test results for the enrolled patients

Patient No.	Test	Sesame seed	Inhalation allergens		Other nuts				
			grass pollen	tree pollen	almond nut	cashew nut	hazelnut	peanut	walnut
1	SPT	+	–	–	+	–	–	–	–
	sIgE, kU/l	>100	<0.35	<0.35	0.37	0.7	4.5	<0.35	<0.35
	Clin relevant	yes	no	no	no	no	no	no	no
2	SPT	+	+	–	–	–	–	–	–
	sIgE, kU/l	26.9	1.7	<0.35	<0.35	<0.35	<0.35	<0.35	<0.35
	Clin relevant	yes	yes	no	no	no	no	no	no
3	SPT	–	+	+	–	–	+	–	+
	sIgE, kU/l	9.8	15.5	7.8	0.4	<0.35	2.2	<0.35	<0.35
	Clin relevant	yes	yes	no	no	no	no	no	no
4	SPT	+	+	+	+	–	+	–	–
	sIgE, kU/l	55.9	3.6	1.6	1.1	0.7	4.1	<0.35	<0.35
	Clin relevant	yes	yes	yes	no	no	no	no	no
5	SPT	+	+	+	+	+	+	+	+
	sIgE, kU/l	13.2	34.8	19.1	2.2	<0.35	21.9	3.6	2.5
	Clin relevant	yes	yes	yes	no	no	OAS	no	OAS
6	SPT	+	+	–	+	+	+	–	+
	sIgE, kU/l	7.1	0.95	<0.35	5.7	49.5	10.4	0.47	1.9
	Clin relevant	OAS	yes	no	OAS	OAS	OAS	no	no

Clin = Clinically; – = negative; + = positive.

Fig. 2. Results of the BAT for all patients (P1–P6) at protein extract concentrations of 100 ng/ml. For P2 and P4, results with sesame seed extract from Allergopharma are presented at a dose of 400 ng/ml. Alm B = Almond extract (Bühlmann); Cas B = cashew nut extract (Bühlmann); FcεRI = anti-FcεRI; fMLP = formyl-methionyl-

leucyl-phenylalanine; Haz AP = hazelnut extract (Allergopharma); Haz B = hazelnut extract (Bühlmann); Neg Co = negative control; PC = positive control; Ses AP = sesame seed extract (Allergopharma); Ses B = sesame seed extract (Bühlmann).

Fig. 3. SDS-PAGE of analyzed extracts: (1) sesame seed extract (Bühlmann), (2) sesame seed extract (Allergopharma), (3) almond extract (Bühlmann), (4) almond extract (ALK), (5) hazelnut extract (Bühlmann), (6) hazelnut extract (Allergopharma) and (M) marker. The molecular weights of the major sesame seed allergens (left) are indicated according to Tai et al. [24].

(93% inhibition), grass pollen/sesame seed (21%), grass pollen/hazelnut (32%) and hazelnut/sesame seed (0%). Four inhibitions were performed for patient 6: cashew nut/almond (94%), cashew nut/sesame seed (91%), hazelnut/sesame seed (88%) and hazelnut/almond (72%).

Characterization of Allergen Extracts by SDS-PAGE

The SDS-PAGE gel showed the separation of the tested protein extracts by molecular weight (fig. 3). Significant qualitative differences were observed between the 2 sesame seed extracts: the Bühlmann extract consisted of 12 protein fractions, but that from Allergopharma appeared as a smear and only 5 bands could be identified. For the almond extracts, large differences were also seen: the Bühlmann extract consisted of 24 protein fractions, but only 12 bands were identified for the ALK extract. Both hazelnut extracts showed a similar separation pattern, although the first extract contained 2 extra-low molecular fractions with molecular weights estimated at 18 and 15 kDa.

Identification of Immunoreactive Fractions with Western Blotting and the Most Potent Fraction by nanoLC-MS/MS

For patients 1–5, the most immunogenic fraction of sesame seed extract from both Bühlmann and Allergo-

pharma was the 12- to 17-kDa protein (table 1; fig. 4). This fraction was identified by nanoLC-MS/MS as oleosin. Immunogenic fractions with higher molecular weights (79, 62, 43, 36 and 22 kDa) were seen with the Allergopharma extract for patients 1–5 and with the Bühlmann extract for patients 1, 2 and 4. These fractions could be identified as subunits of 7S and 11S globulins based on the InformAll Allergenic Food Database [23]. The qualitative differences in band intensity between 2 different extracts may be explained by qualitative differences between the extracts and/or the different biological activity of the tested allergen extracts due to differences in protein extraction and purification (as in results for BAT above). Almond extracts were tested for patients 3, 5 and 6. No visible bands were observed for patients 3 and 5, but a similar pattern was observed with the Bühlmann and ALK extracts for patient 6. The molecular weight of the most intense protein fraction, estimated to be around 60 kDa, corresponds with the major polypeptides of amandin, which is a storage protein and regarded as a major allergen (11S globulin) [25]. Hazelnut extracts were tested for patient 1 and patients 3–6. Visible bands were observed with the Bühlmann extract for patients 5 and 6 and with the Allergopharma extract for patients 4 and 6. Fractions with molecular weights estimated at around 55 and 25 kDa, respectively, are subunits of storage proteins (7S/11S globulin, based on the InformAll Allergenic Food Database) which, according to Nitride et al. [26], may be the subunits of one of the Cor a 9 isoforms. The 18-kDa fraction observed in patient 5 was most probably Cor a 1, which belongs to the Bet v 1 family and may be responsible for the observed cross-reactivity between tree pollen and hazelnut.

Discussion

In this Dutch study population of sesame seed-allergic patients, the major allergen was a 14-kDa protein identified as oleosin (Ses i 4 and/or Ses i 5). This finding is in line with data previously published for a sesame seed-allergic population in France [27]. The other studies on Italian and US populations of sesame seed-allergic patients showed high reactivity to seed storage proteins including 7S/11S globulins and 2S albumin fractions (Ses i 2, Ses i 3, Ses i 6 and Ses i 7) [15–17]. In our study, 5/6 patients from a Dutch population also displayed immunoreactivity towards other seed storage proteins (7S/11S), but a much stronger reaction against oleosin was observed. This kind of difference according to geographical

Fig. 4. Western blot membranes of all 6 patients (P1–P6). (1) Sesame seed extract (Bühlmann), (2) sesame seed extract (Allergopharma), (3) almond extract (Bühlmann), (4) almond extract (ALK), (5) hazelnut extract (Bühlmann) and (6) hazelnut extract (Allergopharma). The red circle shows the most immunogenic sesame fraction, with a molecular weight estimated at 14 kDa (Bühlmann) and 12 to 17 kDa (Allergopharma) and identified by nanoLC-MS/MS as oleosin (color refers to the online version only).

location, often observed even within Europe, might be explained by botanical variability, cultural and dietary habits and genetic factors [18, 19]. Differences in the sensitization patterns observed between different geographic regions may also result in differences in clinically relevant cross-reactivity with other foods or pollen.

From the clinical view, it is important to know the patterns of cross-reactivity; this provides knowledge about an allergic sensitization. Classically, cross-reactivity is tested by inhibition assays in which an allergen extract is added to a patient's serum, and the remaining unbound IgE is subsequently detected by ELISA. This procedure can be limited by the availability of allergen extracts (different sources of allergens) and the methodological differences in the ELISA protocols. In this study, we used a novel assay applying the widely used ImmunoCAP detection method. As IgE sensitization profiles are often measured with this technique, the same allergen extracts may be used for the inhibition studies. So far, clinical cross-reactivity with sesame seed has been reported with hazelnut, rye, kiwi, poppy seed, black walnut, cashew, macadamia, pistachio and peanut [28]. In our patient group, 5 patients were sensitized to hazelnut, but only patients 5 and 6 had a clinically relevant hazelnut allergy and so the

cross-reactivity for these 2 was analyzed. In patient 5, the CAP immuno-inhibition experiments showed no cross-reactivity between hazelnut and sesame seed (supported by the Western blot results). In this patient, a well-known cross-reactivity was shown between tree pollen and hazelnut (para-birch syndrome) and a partial inhibition between grass pollen and hazelnut and between grass pollen and sesame seed. These partial cross-reactive patterns might be due to profilins, which have not been reported as clinically relevant allergens. For patient 6, the immuno-inhibition experiments showed that the sesame seed sensitization might be due to cross-reactivity between sesame seed and hazelnut and cashew nut. Moreover, the Western blot analysis suggested possible cross-reactivity between sesame seed and almond proteins. Therefore, the inhibition experiments together with the results of Western blotting enabled us to describe 2 different sensitization patterns observed in patients 5 and 6.

In this study, the BAT assay gave an added value to the ImmunoCAP inhibition experiments and Western blot analysis in determining the clinical relevance and immunological cross-reactivity profile of IgE-sensitized sesame seed-allergic patients. The great advantage of the BAT over the sIgE measurements is the possibility of dis-

criminating between clinically relevant and non-relevant sensitizations. The BAT analysis confirmed the clinically relevant sesame seed sensitization in patients 1–5. The negative BAT for patient 6 agreed with the Western blot analysis. In contrast to the other sesame seed-allergic patients (patients 1–5), for patient 6, only 1 protein band with a molecular weight estimated at around 62 kDa was observed, which was most probably the subunit of storage protein 7S or 11S. sIgE against this protein is probably due to cross-reactivity with almond, hazelnut or cashew nut. The medically proven OAS of this patient might have been caused by a tree nut instead of sesame seed. Another explanation could be that the patient had outgrown her sesame seed allergy (she was a child at diagnosis but 18 years of age at the time of this study). The BAT also confirmed the clinically relevant sensitization to cashew nut for patient 6 and to hazelnut for patients 5 and 6. However, the BAT for hazelnut was positive only with the Bühlmann extract and not with that from Allergopharma. These results were confirmed by Western blot analysis where a 55-kDa protein band was seen with the Bühlmann extract but not with the Allergopharma extract. The walnut allergy for patient 5 could not be confirmed by the BAT, maybe due to the quality of extract used.

The qualitative differences in allergen content of the various commercial extracts used for diagnostic testing that we observed in this study are a point of great concern. The differences in the degree of basophil activation with the sesame seed extracts from Bühlmann and Allergo-

pharma could be explained by the different protein composition of these extracts seen on SDS-PAGE. Knowledge of the protein content and the influence of the manufacturing process on composition and allergenicity are important as such factors may influence the test outcome and thus diagnosis.

The results of this study show the clinical relevance and immunological background of sIgE patterns in 6 Dutch, sesame seed-sensitized patients, by combining additional (BAT and ImmunoCAP immuno-inhibition) diagnostic approaches with conventional ones (testing sIgE levels and Western blot). With our immunological approach, we characterized the major allergen, oleosin, which did not show cross-reactivity with tree nuts. Patient 6 showed a different profile characterized by 1 clinically less-relevant immunoreactive fraction of sesame seed, which showed cross-reactivity with hazelnut and cashew nut. Based on our findings, we can conclude that Dutch sesame seed-sensitized patients can be better clinically characterized with in vitro laboratory tests. Such tests support the decision of the medical doctor, who has then to decide if further analysis like DBPCFC is necessary.

Acknowledgements

We thank Yvonne Schmidt-Hieltjes for her excellent work on the inhibition experiments with the CAP system and all patients for their willingness to donate blood samples.

References

- 1 Kanny G, De Hauteclouque C, Moneret-Vautrin DA: Sesame seed and sesame seed oil contain masked allergens of growing importance. *Allergy* 1996;51:952–957.
- 2 Derby CJ, Gowland MH, Hourihane JO: Sesame allergy in Britain: a questionnaire survey of members of the Anaphylaxis Campaign. *Pediatr Allergy Immunol* 2005;16:171–175.
- 3 Ben-Shoshan M, Harrington DW, Soller L, Fragapane J, Joseph L, St Pierre Y, Godefroy SB, Elliott SJ, Clarke AE: A population-based study on peanut, tree nut, fish, shellfish, and sesame allergy prevalence in Canada. *J Allergy Clin Immunol* 2010;125:1327–1335.
- 4 Canada H: Sesame. One of the ten priority food allergens (2012-10-26). http://www.hc-sc.gc.ca/fn-an/pubs/securit/2012-allergen_sesame/index-eng.php.
- 5 Becker W, Brasseur D, Bresson JL, Flynn A, Jackson AA, Lagiou P, Mingrone G, Moseley B, Palou A, Przyrembel H, Salminen S, Strobel S, van Loveren H: Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission Relating to the Evaluation of Allergenic Foods for Labelling Purposes. *EFSA J* 2004;32:1–197.
- 6 Cohen A, Goldberg M, Levy B, Leshno M, Katz Y: Sesame food allergy and sensitization in children: the natural history and long-term follow-up. *Pediatr Allergy Immunol* 2007;18:217–223.
- 7 Dalal I: Allergy to seeds. *Pediatr Allergy Immunol* 2011;22(1 Pt 2):e150–e154.
- 8 May CD: Objective clinical and laboratory studies of immediate hypersensitivity reactions to foods in asthmatic children. *J Allergy Clin Immunol* 1976;58:500–515.
- 9 Maloney JM, Rudengren M, Ahlstedt S, Bock SA, Sampson HA: The use of serum-specific IgE measurements for the diagnosis of peanut, tree nut, and seed allergy. *J Allergy Clin Immunol* 2008;122:145–151.
- 10 Hourihane JO, Dean TP, Warner JO: Peanut allergy in relation to heredity, maternal diet, and other atopic diseases: results of a questionnaire survey, skin prick testing, and food challenges. *BMJ* 1996;313:518–521.
- 11 Ebo DG, Bridts CH, Hagendorens MM, Aerts NE, De Clerck LS, Stevens WJ: Basophil activation test by flow cytometry: present and future applications in allergology. *Cytometry B Clin Cytom* 2008;74:201–210.
- 12 Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ: Flow cytometric analysis of in vitro activated basophils, specific IgE and skin tests in the diagnosis of pollen-associated food allergy. *Cytometry B Clin Cytom* 2005;64B:28–33.
- 13 Tokuda R, Nagao M, Hiraguchi Y, Hosoki K, Matsuda T, Kouno K, Morita E, Fujisawa T: Antigen-induced expression of CD203c on basophils predicts IgE-mediated wheat allergy. *Allergol Int* 2009;58:193–199.

- 14 Chirumbolo S: Basophil activation test in allergy: time for an update? *Int Arch Allergy Immunol* 2012;158:99–114.
- 15 Asero R, Cecchi L, Cervone M, Crivellaro M, Lodi Rizzini F, Pravettoni V, Quercia O, Amato S, Mistrello G: Detection of 20 and 32 kDa IgE-binding proteins as the major allergens in Italian sesame seed allergic patients. *Eur Ann Allergy Clin Immunol* 2014;46:22–25.
- 16 Magni C, Ballabio C, Restani P, Fuggetta D, Alessandri C, Mari A, Bernardini R, Iacono ID, Arlorio M, Duranti M: Molecular insight into IgE-mediated reactions to sesame (*Sesamum indicum* L.) seed proteins. *Ann Allergy Asthma Immunol* 2010;105:458–464.
- 17 Beyer K, Bardina L, Grishina G, Sampson HA: Identification of sesame seed allergens by 2-dimensional proteomics and Edman sequencing: seed storage proteins as common food allergens. *J Allergy Clin Immunol* 2002;110:154–159.
- 18 Vereda A, van Hage M, Ahlstedt S, Ibañez MD, Cuesta-Herranz J, van Odijk J, Wickman M, Sampson HA: Peanut allergy: clinical and immunologic differences among patients from 3 different geographic regions. *J Allergy Clin Immunol* 2011;127:603–607.
- 19 Mills EN, Mackie AR, Burney P, Beyer K, Frewer L, Madsen C, Botjes E, Crevel RW, van Ree R: The prevalence, cost and basis of food allergy across Europe. *Allergy* 2007;62:717–722.
- 20 Jansen A, de Lijster de Raadt J, van Toorenenbergen AW, van Wijk RG: Allergy to pistachio nuts. *Allergy Proc* 1992;13:255–258.
- 21 Norman P: Skin testing; in Rose NF, Friedman H, Fahey JL (eds): *Manual of Clinical Laboratory Immunology*. Washington, American Society for Microbiology, 1986, pp 660–663.
- 22 Den Hartog G: An alternative competitive inhibition method for diagnosis of allergy: two patients with a ficus (*Ficus benjamina*) and fig (*Ficus carica*) sensitization; in *Relevance of IgA in Allergy: Regulation by Mucosal Factors* (chapt 3); thesis, Wageningen University, 2013.
- 23 Manchester University: InformAll Allergenic Food Database. <http://www.inflammation-repair.manchester.ac.uk/informall/allergenic-foods/>.
- 24 Tai SS, Wu LS, Chen EC, Tzen JT: Molecular cloning of 11S globulin and 2S albumin, the major seed storage proteins in sesame. *J Agric Food Chem* 1999;47:4932–4938.
- 25 Sathe SK, Wolf WJ, Roux KH, Teuber SS, Venkatachalam M, Sze-Tao KW: Biochemical characterization of amandin, the major storage protein in almond (*Prunus dulcis* L.). *J Agric Food Chem* 2002;50:4333–4341.
- 26 Nitride C, Mamone G, Picariello G, Mills C, Nocerino R, Berni Canani R, Ferranti P: Proteomic and immunological characterization of a new food allergen from hazelnut (*Corylus avellana*). *J Proteomics* 2013;86:16–26.
- 27 Leduc V, Moneret-Vautrin DA, Tzen JT, Morisset M, Guerin L, Kanny G: Identification of oleosins as major allergens in sesame seed allergic patients. *Allergy* 2006;61:349–356.
- 28 Gangur V, Kelly C, Navuluri L: Sesame allergy: a growing food allergy of global proportions? *Ann Allergy Asthma Immunol* 2005;95:4–11.