

Specific humoral immune responses in 12 cases of food sensitization to sesame seed

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Summary

Background Hypersensitivity to sesame seeds is becoming increasingly frequent, probably owing to the larger use of this compound in international food.

Objectives This study investigated serum responses of 12 sesame sensitized patients (seven with food allergy, five with food sensitization), to a sesame protein extract, and attempted at identifying sesame major antigens.

Methods Sesame protein extracts were prepared from black, white and brown sesame seeds. Electrophoretic analysis showed similar protein patterns in the three extracts, and proper preservation of the proteins integrity. The brown sesame extract was used to set-up an ELISA assay and measure serum levels of antisesame IgG, IgA, IgM and IgE in 12 samples from sesame-sensitized individuals and six controls. It also allowed to perform western blot analyses in order to investigate the molecular weight of sesame proteins recognized by IgG, IgA and IgE.

Results Nineteen protein bands were observed upon polyacrylamide gel electrophoresis of the sesame protein extracts. Using this whole extract in ELISA, significant antisesame IgG, IgA and IgE-responses were observed in the serum of sensitized individuals, different from the lower signals obtained with control samples. Western blot analysis demonstrated highly polymorphic IgG and IgA responses and a more restricted IgE response pattern, suggesting that two proteins, respectively, 14 kDa and 25 kDa are mostly involved in sesame IgE-dependent hypersensitivity, the 25 kDa band presenting several characteristics of a major allergen.

Conclusions This study reports novel information on the possible involvement of a 25 kDa sesame protein in IgE-dependent hypersensitivity to sesame seeds.

Keywords: sesame seeds, sesame food allergy, protein profile, ELISA, Western blot

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Introduction

Sesame hypersensitivity is a disorder described with increased frequency in the literature, owing probably to the development of international alimentation and a more generalized use of sesame seeds or oil. Several series of cases [1–3] have been reported since the first description in 1950 by Rubinstein [4], and a striking increase of cases of allergy to sesame has been noted in France since 1993 [1].

Vegetarian food [2], exotic sesame preparations such as Turkish halva [3,4], fast food bread [3] and numerous bakery products (special bread, aperitive biscuits..., reviewed in [1]) are now eaten everywhere and often, and could be involved in this new phenomenon of food sensitization. Occupational allergy has also been reported in the literature in bakers preparing such special breads [5,6].

Causative mechanisms seem to be immediate hypersensitivity (type I), and the diagnosis relies on biological and clinical specific features such as specific serum IgE assessment and cutaneous or oral provocation tests with allergens.

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Table 1. Clinical and biological criteria of sesame hypersensitivity in subjects with sesame induced allergy (group 1)

Patient	Symptoms	Prick (mm)	RAST/LHRT	Food challenge
1	Baker's asthma; frequent handling of sesame	Brown S: 4 White S: 4 Comm. Extract: 2 (codeine: 4)	13.2 kU/L	DBPCFC 10 g: urticaria, asthma
2	Atopic dermatitis Asthma	Brown S: 5.5 White S: 11 Comm. Extract 2 (codeine: 2)	> 100 kU/L	DBPCFC 7 g: vomiting, abdominal pain, conjunctivitis, eczematous rash.
3	Angioedema	Comm. Extract: 2 (codeine: 2)	6.7 kU/L	Oral challenge not done Positive labial challenge
4	Angioedema	Brown S: 7 White S: 3.5 Comm. Extract: 0 (codeine: 3.5)	ND	DBPCFC 7.11 g: urticaria ++
5	Anaphylactic shock	Brown S: 7 Comm. Extract: 0 (codeine: 3.5)	RAST: negative LHRT: positive	DBPCFC 100 mg: urticaria 15 mL oil: angioedema
6	Anaphylactic shock	White S: 12 (codeine: 2)	RAST: negative LHRT: positive	Oral challenge not done Positive labial challenge
7	Laryngeal angioedema induced by cookies	Brown S: 13 (codeine: 4.5)	RAST: negative	ND

Sesame specific IgE have been reported in sensitized patients sera in many studies [1–3,5,6], but are not always present, even in proven clinical cases [1,3,7,8].

In contrast, the literature is poor in studies reporting sesame epitopes. Malish *et al.* [3] defined nine immunogenic proteins with sera from hyperimmunized rabbits. Alday *et al.* [5] reported the case of one patient occupationally exposed to sesame seed and defined a principal allergenic component in their sesame extract.

The aim of our study was, first, to detect specific IgG, IgA, IgM and IgE humoral responses in 12 clinically defined sesame-sensitized patients and secondly, to investigate sesame proteins involved in these specific humoral responses in human sera.

Materials and methods

Subjects

Twelve subjects were included in this study. They had all been investigated for food allergy, after reporting clinical symptoms following the ingestion of sesame-containing food products, as indicated in Table 1. Sensitization to sesame was defined by clinical features, skin-prick test and by the assay of specific IgE (RAST Phadebas CAP

System, Pharmacia, Uppsala, Sweden). Oral tests were however, only performed when at no risk for the patient. Positive skin-prick tests were considered upon development of a weal of at least 3 mm diameter, or of a 2 mm weal observed on two different instances. Food allergy was proven by double-blind placebo-controlled food challenges (DBPCFC), according to previously published data [1,9]. Seven patients were diagnosed to have food allergy (group 1; Table 1), and five had food sensitization to sesame (group 2; Table 2).

Six subjects (three females and three males) without sesame sensitization were recruited as controls.

Serum samples of all subjects were collected before the skin tests and oral challenges were performed, and stored at -30°C until used for the study of humoral antisesame reactivity and investigation of sesame immunoreactive proteins.

Sesame seed proteins extraction

Brown, black and white sesame seeds were purchased in a specialist shop, and ground in the laboratory. Ten gram of each preparation was used for protein extraction, with a protein extraction buffer made of Tris-HCl 0.1 M pH 7.4 and NaCl 0.14 M, supplemented with deoxycholate, Triton

Table 2. Clinical and biological criteria in subjects with latent sensitization to sesame (group 2) and negative challenges

Patient	Symptoms	Prick (mm)	RAST	Food challenge
8	Urticaria	Brown S: 5 (codeine: 4)	RAST: negative	DBPCFC 7.11 g: negative
9	Laryngeal angioedema	Brown S: 2 White S: 2 Comm. Extract: 0 (codeine: 5)	ND	ND
10	Angioedema, urticaria	Brown S: 2.5 White S: 2 Comm. Extract: 1.5 (codeine: 7)	1.6 KU/L	ND
11	Oral syndrome	Brown S: 10 (codeine: 5)	RAST: negative	Frequent intake without reaction
12	Angioedema, urticaria, asthma	Brown S: 1 White S: 1 Comm. Extract: 0 (codeine: 3.5)	3.52 KU/L	DBPCFC: negative

X-100 and Nonidet P-40 and ultrasonic deaggregation. After ultracentrifugation at 40 000 *g*, the supernatants were assayed for protein concentration and adjusted at 1.2 g/L.

SDS-PAGE and gel analysis

The protein extracts were analysed in SDS-PAGE using a 10–15 gradient PhastGel (Pharmacia), after reduction with Laemmli's buffer. The gel was then stained according to the silver stain method, dried and submitted to a computerized analysis using the ImageMaster software (Pharmacia).

Immunoenzymatic assay: ELISA

MaxiSorp Nunc ELISA plates (Nunc, Roskilde, Finland) were coated with 1 µg per well of sesame protein soluble extract in carbonate-bicarbonate buffer pH 9.6, and overcoated with phosphate buffered saline (PBS)/bovine serum albumin (BSA 5%)/Tween-20 0.1% during 30 min at 37 °C.

Aliquots of 100 µL of serum diluted 1/100 in PBS/BSA/Tween were incubated for 30 min at 37 °C in the wells. After a series of washes, each well then received 100 µL of the proper dilution of peroxidase-conjugated goat antihuman IgG (1:1000), IgA (1:1000), IgM (1:1000) or IgE (1:500) (Dako, Glostrup, Denmark). The plates were further incubated for 30 min at 37 °C in a humid atmosphere, then washed three times in PBS. One hundred microlitre of citrate buffer (pH = 5.6) containing 0.05% of orthophenylene diamin (Sigma Chemicals) and H₂O₂ were then distributed in each well. The colour was allowed to develop for

1–2 min and the enzymatic reaction was stopped with 50 µL of 2N H₂SO₄.

Absorbance in each well was measured at 492 nm (Titer-tek Multiskan II, Flow Laboratories, Helsinki, Finland). A calibrator of normal pooled human serum (different from the controls' samples) was used in each plate, which allowed to express individual results as a ratio of the sample optical density (OD)/calibrator OD.

Western blot

SDS-PAGE separations were performed as described above, using Phast gels, or a larger 12% gel. After electrophoretic transfer on nylon membranes (Millipore, Bedford, MA, USA) and overcoating in PBS/BSA/Tween-20, serum samples from 10 of the sensitized patients, diluted 1/100 in PBS, were incubated with transfer membrane strips. After 30 min incubation at room temperature and three washes each blot was further divided in three strips, being incubated, respectively, with antihuman IgG, IgA or IgE peroxidase conjugated goat antiserum (Dako). After a new series of washes, the Western blots were soaked in a diaminobenzidine-containing revelation buffer, dipped in distilled water in order to stop the reaction and air dried. They were then scanned and analysed using the ImageMaster software to determine the bands molecular weights (MW).

Statistical analysis

Comparisons between patients' groups and controls were performed using ANOVA and Student's *t*-tests, with the Myosotis software (Coultronics, Margency, France).

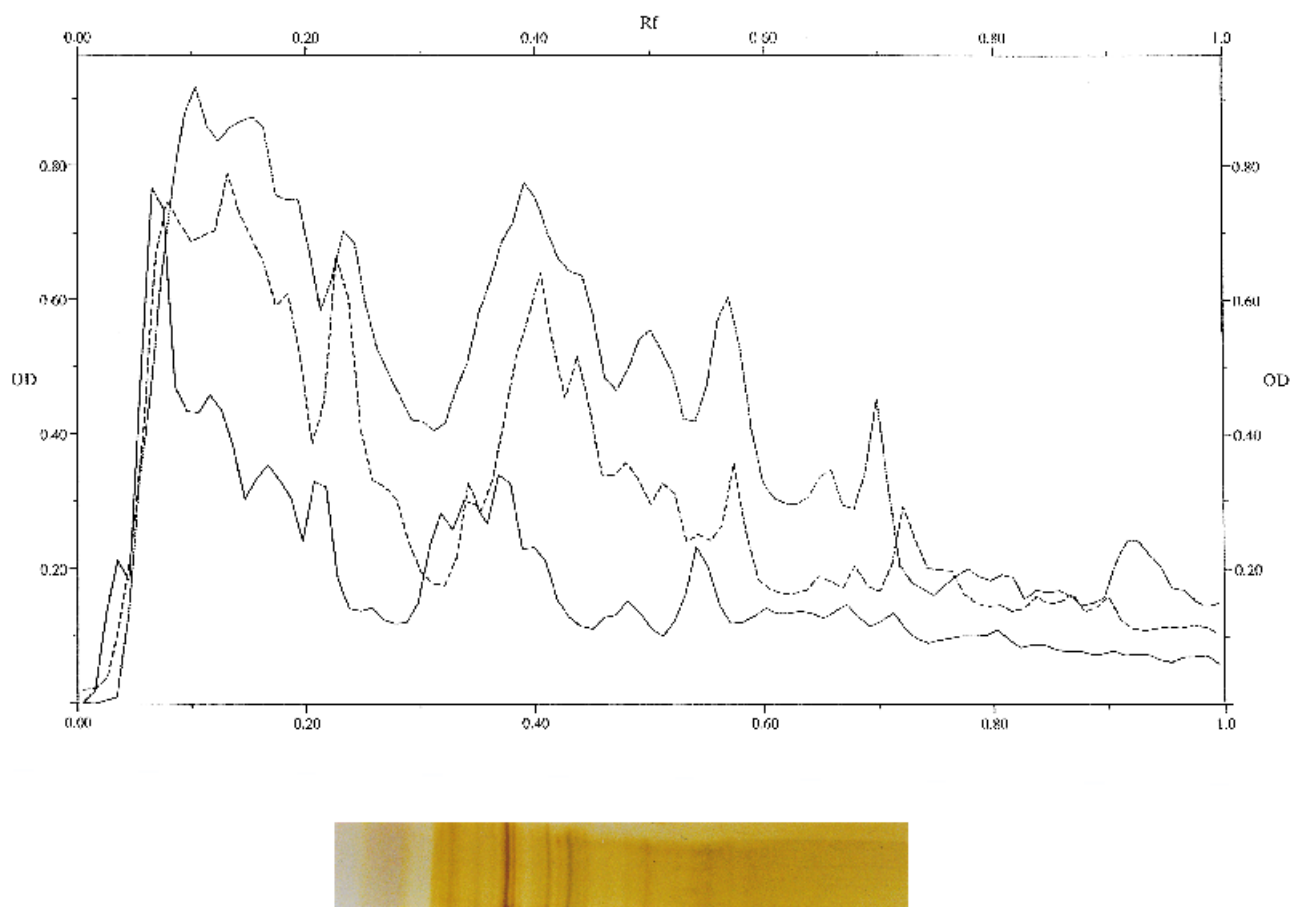


Fig. 1. SDS-PAGE and protein profiles of the three sesame extracts tested as generated by Image Master (Pharmacia) from a single SDS-PAGE 10–15 gel. (—: white sesame; - - -: black sesame;: brown sesame).

Table 3. Mean ($\pm$ SE) and range of OD values obtained in the ELISA assays of antisesame antibodies

	Group 1	Group 2	Controls
IgG	0.622 $\pm$ 0.149 (0.212–1.281)	0.549 $\pm$ 0.113 (0.243–0.849)	0.206 $\pm$ 0.22 (0.131–0.255)
IgA	0.450 $\pm$ 0.216 (0.052–1.698)	0.198 $\pm$ 0.024 (0.133–0.266)	0.096 $\pm$ 0.018 (0.035–0.166)
IgM	0.585 $\pm$ 0.090 (0.262–0.840)	0.594 $\pm$ 0.212 (0.121–1.318)	0.552 $\pm$ 0.090 (0.256–0.855)
IgE	0.069 $\pm$ 0.012 (0.012–0.115)	0.061 $\pm$ 0.005 (0.044–0.074)	0.044 $\pm$ 0.004 (0.034–0.060)

Results

SDS-PAGE and gel analysis

The three extracts yielded the same profile in SDS-PAGE analysis. Nineteen protein bands appeared in a range of

13.5–96 kDa. As the protein profiles of the three extracts tested (white, brown and black sesame) were identical, brown sesame extract was used for all immunochemical assays. This is shown on the SDS-PAGE gel and protein profiles in Fig. 1.

Gel analysis was submitted to a computerized analysis using the ImageMaster software, for protein profile and optical density analysis, which identified five major protein bands, expressed as relative percentages of each lane, at 22.6 kDa (7.4%), 25 kDa (7%), 39.4 kDa (6.7%), 51.3 kDa (5.8%) and 26.5 kDa (5.7%). The other proteins each represented less than 5% of the whole.

Immunoenzymatic assays

ELISA OD values for antisesame antibodies are reported in Table 3, and the mean levels, calculated using OD ratios, are shown in the three groups on Fig. 2. Mean levels of antisesame IgG were significantly higher in both groups of hyperreactive patients compared with controls ($P < 0.05$). Specific IgA antibodies were found at higher levels in

Fig. 2. Anti-sesame antibody levels in patients and controls sera (mean $\pm$ SE). ■, group 1, ▨, group 2, □, controls. * $P < 0.05$, ** $P < 0.005$.

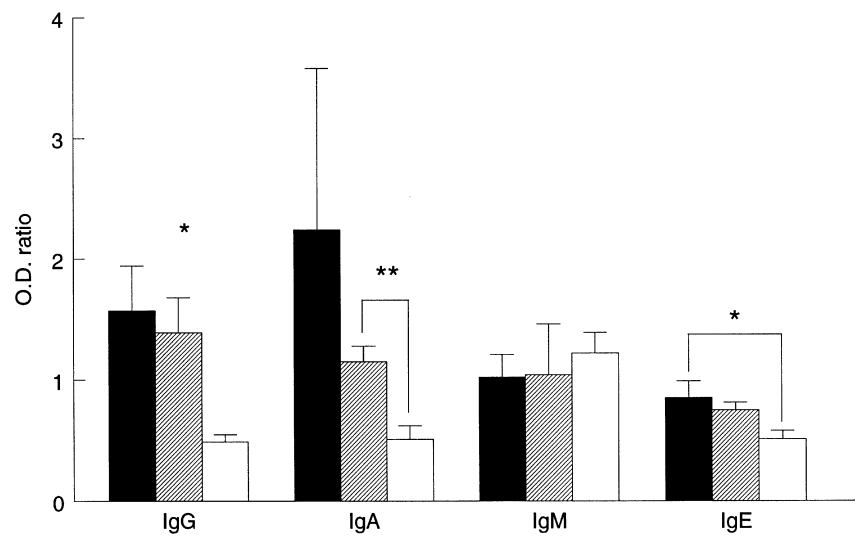
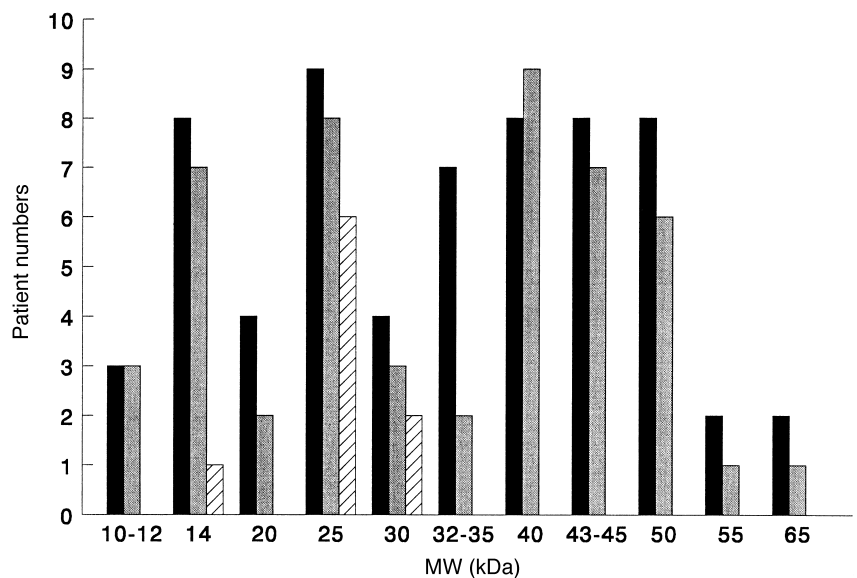


Fig. 3. Frequencies of different sesame protein bands recognized by specific IgG, IgA and IgE anti-sesame antibodies in 10 patients sera by western blot. ■, IgG; ▨, IgA; ▤, IgE.



sensitized patients than in controls, but the difference only reached statistical significance between group 2 patients and controls ($P < 0.005$) because of the large dispersion of individual IgA levels in group 1 patients. Specific IgM levels were similar in patients and controls. Using our ELISA technique for IgE detection, the signals were significantly higher in allergic patients samples compared with controls ($P < 0.05$).

There was however, no significant difference in anti-sesame antibody levels between allergic and sensitized patients.

Western-blot analysis

Figure 3 reports the results of the Western blots performed to determine sesame proteins involved in sesame-specific

humoral responses. This figure displays the number of samples that showed reactivity for each of the specific proteins recognized by IgG, IgA or IgE. Figure 4 shows a typical western blot set for these three isotypes.

More bands were recognized by specific IgG antibodies in all sera tested (6–10 proteins), with MW ranging from 12 to 65 kDa. Two bands were shared by most of the samples: 14 kDa (8/10 samples) and 25 kDa (9/10). These two protein bands yielded the strongest signals. One of the patients had no sesame-specific IgG.

IgA antibodies recognized 5–10 proteins of the extract, with MW ranging from 12 to 65 kDa. The 14 and 25 kDa proteins also recognized by IgG, were evidenced in, respectively, eight and nine samples. The patient who had no sesame-specific IgG also failed to display an IgA response.

Specific IgE antibodies recognized one or three bands in

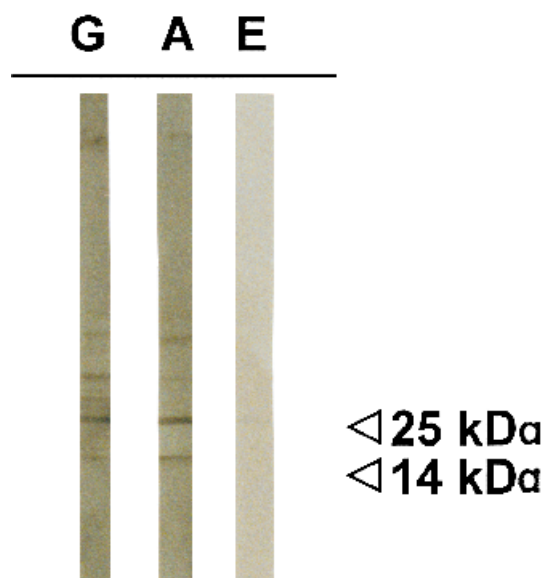


Fig. 4. Western blot proteins of 14 and 25 kDa recognized by IgG, IgA and IgE antisesame antibodies in a patient serum.

8/10 samples tested in western blot. The 14 kDa protein was recognized only by one serum, the 25 kDa protein was found for six sera, and the 30 kDa protein for two sera, with a fainter intensity. The patient who had no IgG or IgA had IgE that reacted strongly with the 25 kDa band. Another patient, who had strong IgG and IgA responses, showed no IgE reactivity. Western blot profiles were similar in the two groups of patients.

Discussion

This study confirms an increased humoral reactivity of sensitized patients to sesame proteins, with a polymorphic IgG or IgA response to 11 immunogenic sesame proteins, with molecular weights ranging between 12 and 65 kDa, and more restricted IgE responses involving two band of 14 and 25 kDa.

Sesame-specific IgE, measured by radio-immuno assays, have not always been described in sensitized subjects, even in clinically well defined cases [1,3,7,8]. Several explanations were proposed such as ancient sensitization with no re-exposure to the allergen [3] or, more frequently described, antigenic preparations inappropriate for IgE assays and provocation tests [1,8].

The preparation of antigenic extracts is indeed crucial, since immunogenic epitopes must be preserved in order to detect specific antibodies or trigger cutaneous immune reactions [8]. Before investigating for specific immunoglobulins with an ELISA assay, we checked our extract

first in electrophoresis to evidence the different proteins conserved in the solution after extraction, and in dot-blot to verify the presence of proteins involved in specific humoral responses (data not shown). Our brown sesame extract, prepared with a gentle saline-extraction method, does not seem to contain altered proteins, as evidenced by the numerous ($n = 19$) sharp protein bands visualized on SDS-PAGE analysis. Indeed, a commercial extract poorly efficient in cutaneous tests and analysed on the same gel only yielded one faint blurred band (data not shown). Alday *et al.* [5] also showed a sesame extract SDS-PAGE with a lower number of proteins (two principal components), prepared using a different technique. This stresses the importance of using well defined and protein-rich allergenic extracts for skin tests as well as epitope studies.

The ELISA-test and western blot analyses then performed with controls and patients sera confirmed the presence of immunoreactive proteins in our extract. Higher levels of sesame-specific serum IgG and IgA were found in sensitized patients compared to controls, especially in patients with proven sensitization (positive oral challenges). A 'complete' humoral response with IgG, IgA and/or IgE antibodies was found in patients and not in controls. This suggests, in patients, a phenomenon of tolerance breakdown with the development of serum reactivity, such as has been reported in food intolerance or chronic occupational exposure [10,11].

In our ELISA study, some sensitized patients had no specific IgE but IgG and/or IgA. This absence of specific IgE in hypersensitive patients confirms data reported in the literature [1,3,7,8], and perhaps indicate the participation of other hypersensitivity mechanisms involving specific IgG and immune complexes.

Western blot analysis evidenced highly polymorphic IgG and IgA responses, although the strongest reactivities were directed to 14 kDa and 25 kDa proteins. A 14 kDa protein was also evidenced by Alday *et al.* [5], involved in serological (LHRT, IgE) and cutaneous responses for the single occupationally exposed patient they have reported. Malish *et al.* [3], with a commercial sesame extract, demonstrated the antigenic activity of their sesame seed extract with hyper-immunized rabbit serum, and found nine antigens with a crossed electrophoresis assay. With an Ouchterlony double diffusion assay they evidenced antigenic activity for proteins ranging from 12 kDa to more than 150 kDa. These two studies [3,5] and that reported here confirm that numerous sesame seed proteins are recognized by specific antibodies, stress the importance of a 14 kDa protein for isotypic responses and suggest a major role for another 25 kDa protein in IgE-dependent sensitization. The latter also seems to be specifically involved in the reactivity of sesame-specific IgE.

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