

fraction = 0.0879; kinetic $k_{on} = 2.3 \text{ min}^{-1} \times 1 \times 10^{-6} \text{ mol}^{-1}$; $k_{off} = 0.005 \text{ min}^{-1}$; and $k_{off}/k_{on} = K_i = 3 \text{ nmol/L}$ (equilibrium $K_d = 3 \text{ nmol/L}$).

After a 5-mg dose, unbound (free) levocetirizine concentrations were $50 \pm 4 \text{ nmol/L}$ at 4 hours and $5 \pm 1 \text{ nmol/L}$ at 24 hours compared with 28 nmol/L at 4 hours and 4 nmol/L at 24 hours in adults. Levocetirizine H_1 -receptor occupancies at 4 and 24 hours were $94\% \pm 0.4\%$ and $60\% \pm 3\%$ compared with values of 90% and 57%, respectively, in adults.

Percentage of wheal suppression versus levocetirizine unbound (free) plasma concentrations compared with percentage of wheal suppression versus levocetirizine H_1 -receptor occupancy is shown in Fig 1. Percentage of flare suppression versus levocetirizine unbound (free) plasma concentrations compared with percentage of flare suppression versus levocetirizine H_1 -receptor occupancy is shown in Fig 2. When the percentage of suppression of wheal-and-flare responses is plotted versus the percentage of H_1 -receptor occupancy by levocetirizine, the counterclockwise loop of hysteresis is reduced, corroborating the hypothesis that the kinetics of receptor occupancy are a better representative of the time course of pharmacodynamics than are the plasma pharmacokinetics.

The H_1 -receptor occupancy data therefore provide additional pharmacodynamic support for the appropriateness of once-daily levocetirizine dosing in children age 6 to 11 years,⁴ as is recommended for adults,⁸ despite faster clearance and shorter terminal elimination half-life values in the pediatric age group.

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Disclosure of potential conflict of interest: F. E. R. Simons is part of the EPAAC Study scientific advisory board. M. Gillard is employed by UCB. The rest of the authors have declared that they have no conflict of interest.

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doi:10.1016/j.jaci.2007.02.048

Identification of 2 new sesame seed allergens: Ses i 6 and Ses i 7

To the Editor:

Sesame seed allergy is a common cause of food allergy in many countries, including Israel, Japan, the United States, and various European countries.¹ Seed storage proteins have been identified as common allergens in peanut, tree nut, and seed allergy.² Recently, several seed storage and oil body proteins in sesame seed have been identified as allergens by us and others, namely two 2S albumins (Ses i 1 and Ses i 2),^{3,4} a 7S globulin (Ses i 3),³ and 2 oleosins (Ses i 4 and Ses i 5).⁵ The purpose of this study was to determine whether 11S globulins in sesame seed, a third seed storage protein family, also have allergenic potential.

A cDNA library was constructed from green, unripe sesame seeds (*Sesamum indicum*). Total RNA was extracted and mRNA was purified on Oligotex mRNA Spin-column from Qiagen (Valencia, Calif). Synthesis and cloning of cDNA was performed by using a cDNA synthesis kit from Stratagene (La Jolla, Calif). The double-stranded cDNA was ligated into the lambda vector Uni-ZAP, packaged *in vitro* (Gigapak Gold; Stratagene), and then transfected into *Escherichia coli* XL1-Blue cells.

By using known sequence data from two 11S globulins (accession nos. AF091842 and AF240004), oligonucleotide primers were synthesized and used to screen the library. Full-length cDNA clones were isolated and sequenced at the Mount Sinai Core Facility. The sequenced clones were 100% identical to the two 11S globulins described above. These 11S globulins have only 36% identity (Fig 1).

The protein-coding regions of the 11S globulins were amplified by means of PCR with the specific primers, which allowed the introduction of unique sites suitable for ligation into the expression pET24b(+) (Novagen, Madison, Wis) vector. The ligated insert-vector constructs were introduced into the *E coli* XL1-Blue strain by means of chemical transformation, according to a protocol provided by the cell manufacturer (Stratagene). The plasmids were purified, and the inserts' orientation and nucleotide sequences were confirmed. Subsequently, each individual expression plasmid was introduced into the BL21 expression *E coli* strain. The recombinant protein was expressed on induction of the bacterial culture at 0.4 to 1.0 OD at 600 nm with 1 mM of isopropyl β -D-1-thiogalactopyranoside for 16 hours at 30°C. The recombinant protein expression



FIG 1. Shown is the alignment for the newly identified sesame seed allergens Ses i 6 and Ses i 7 with know allergens of peanut (Ara h 3), walnut (Jug r 4), and hazelnut (Cor a 9) by using Vector NTI Advance. All aligned allergens belong to the 11 S globulins.

was detected by means of probing of the Western blot with the Anti-His-Tag antibody. The recombinant proteins were purified by using a His-Bind Ni2+-chelating NTA-matrix resin (Novagen), according to the protocol provided by the manufacturer.

Finally, the recombinant proteins were screened with individual patient sera from 24 patients with sesame seed allergy. Human serum from one atopic and one nonatopic person were used as negative controls. The diagnoses of sesame seed allergy were based on clinical histories and IgE measurements. Clinical reactions involved the skin in 19 patients mostly with urticaria or angioedema, the gastrointestinal tract in 11 patients, and the respiratory system in 6 patients. One patient had a decrease in blood pressure. Twelve patients had more than one organ system

involved. Sesame seed-specific IgE levels were measured by using the Phadia FEIA-Cap System and ranged from 0.73 to greater than 100 kU/L (median, 21.2 kU/L).

For this purpose, the recombinant proteins were separated on SDS-PAGE (Nu-Page MES Bis-Tris; Invitrogen, Carlsbad, Calif), according to the protocol from the manufacturer. Two micrograms of each protein was loaded into each well. The proteins were subsequently transferred to Immobilon-P membrane (Millipore, Temecula, Calif) and then stained with 2.2% Coomassie Blue for total protein analysis. For molecular weight determination, SeeBlue Plus2 Pre-stained Standard (Invitrogen) was used.

Patient and control sera were diluted from 1:4 to 1:20 (depending on the level of sesame seed-specific IgE) in PBS-Tween plus 1% BSA and 10% normal goat serum

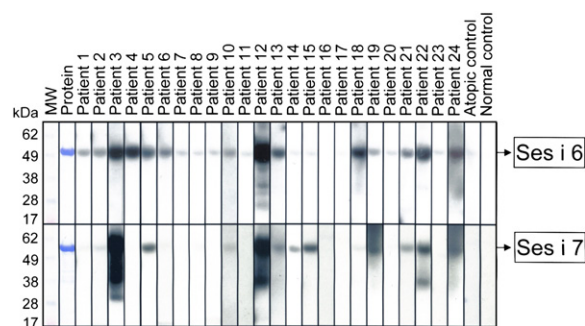


FIG 2. The recombinant proteins Ses i 6 and Ses i 7 were separated on SDS-PAGE and transferred to Immobilon-P membranes. Lane 1 shows the molecular weight (MW) markers, lane 2 shows the Coomassie Blue stain for total protein analysis, lanes 3 to 27 show immunolabeling with sera from 24 patients with sesame seed allergy, and lanes 28 and 29 show the atopic and nonatopic controls, respectively.

and incubated with immunoblots for 2 hours with gentle agitation at room temperature. After 2 hours, membranes were briefly rinsed with PBS and then iodine 125-labeled anti-human IgE (Diamed, Miami, Fla) that was diluted as per the manufacturer in PBS-Tween plus 1% BSA and 10% normal goat serum. After agitation for 1 hour at room temperature and washing with PBS, immunoblots were dried and exposed to Kodak Biomax MS imaging film (Kodak, Rochester, NY) for 6 days.

As shown in Fig 2, 13 of the 24 patients with sesame seed allergy showed a strong IgE binding to the first expressed recombinant protein, whereas 10 of the 24 patients show clear binding to the second protein. These proteins were named Ses i 6 and Ses i 7, respectively, by the allergen nomenclature subcommittee. Fig 1 shows the alignment of these new sesame seed allergens with other 11S globulin allergens of peanut, walnut, and hazelnut.

In summary, in our study 2 additional sesame seed allergens, Ses i 6 and Ses i 7, have been identified. However, future studies need to be performed to prove the

biologic activity of these newly identified allergens. These findings highlighted again the importance of seed storage proteins in peanut, tree nut, and seed allergy. The identification of new food allergens is the first step toward the use of these recombinant allergens in future diagnostic and therapeutic approaches.^{6,7}

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Disclosure of potential conflict of interest: K. Beyer has received grant support from the Food Allergy and Anaphylaxis Network, the European Union, and Institutional Funds. G. Grishina, L. Bardina, and H. A. Sampson have received grant support from the Food Allergy Research Institute.

Supported by a grant from the Food Allergy Initiative.

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doi:10.1016/j.jaci.2007.03.041