

Identification and characterization of linear B-cell epitopes of β -globulin, a major allergen of sesame seeds

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Background: The increased consumption of foods containing sesame seeds is paralleled by an increase in reported sesame-induced allergic reactions.

Objective: This study aimed at identifying and characterizing the linear B-cell epitopes of the 14-kd β -globulin, the major allergen of sesame seed.

Methods: A peptide containing 71 amino acids (peptide B) was previously identified by us as the IgE-binding region on β -globulin. To determine the amino acid sequence of the IgE-binding sites on peptide B, we synthesized overlapping peptides 20 and 10 amino acid residues long that span the entire length of peptide B, which were offset from each other by 10 and 2 amino acid residues, respectively. Sera from 20 subjects given diagnoses of allergy to sesame β -globulin served to identify the epitopes by using the dot-blot test.

Results: At least 9 different IgE-recognition sites were identified on peptide B. Three of them, numbers 2, 3, and 13 (corresponding to amino acids 46-55, 48-57, and 76-86, respectively, in the β -globulin sequence), appeared to be immunodominant IgE-binding epitopes. Also, these peptides were best recognized in terms of intensity of response. There was no obvious sequence motif shared by the 9 different IgE-binding epitopes of β -globulin. However, approximately 60% of the amino acids represented in the epitopes are hydrophobic residues.

Conclusion: Identification of the IgE-binding epitopes might provide a better understanding of the functional role the allergens play in the disease and might have implications for immunodiagnosis and probably immunotherapy. (J Allergy Clin Immunol 2004;114:1151-8.)

Key words: Allergenicity, sesame seeds, major allergen, IgE, amino acid sequence, peptide synthesis, epitopes

It is estimated that up to 5% of children and 2% of adults have allergic reactions to foods.¹ Products derived from sesame (*Sesamum indicum*) have been recommended for young children in societies of the Mediterranean region because of their high nutritional value, with sesame proteins being rich in methionine.² During recent decades, their use has spread to North America and Europe. The increasing consumption of foods containing sesame seeds and sesame oil is paralleled by an increase in reported sesame-induced allergic reactions,³⁻⁵ often characterized by anaphylactic shock.⁶ Thus in a survey conducted in Israel on 9070 young children up to 2 years old, sesame was found to be the third most prevalent allergen after egg and cow's milk and about 3 to 4 times more frequently encountered than peanut.² This reality called for additional studies aimed at the characterization and identification of the specific sesame allergens. Recent studies have identified several allergens in sesame seeds as belonging to the family of seed storage proteins.⁶⁻⁸ IgE-mediated reactions are believed to be responsible for most food-induced allergic reactions of the immediate hypersensitivity type (type 1). IgE production involves activation of effector cells, mainly mast cells and basophils, leading to an inflammatory response and specific clinical manifestations.⁹ Currently, the only effective treatment for this is avoidance of the food in question. Total avoidance is difficult because sesame is used in many foods, and accidental consumption is almost unavoidable.^{5,6}

The identification of discrete sites in antigens that are recognized by particular antibodies or T-cell receptors is important for the characterization of allergens. Epitopes might be composed of sequential residues along the polypeptide chain (linear or continuous epitopes) or nonsequential residues from segments of the chain brought together by folded conformation of the protein (conformational or discontinuous epitopes).^{1,10} Determination of allergen-specific and IgE-binding epitopes is important for better understanding of the complex allergic reaction. Epitopes of several food allergens have already been studied.¹¹⁻¹³ However, not until recently has it been possible to systematically identify linear epitopes

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TABLE I. Clinical and biologic characteristics of sesame hypersensitivity in the 20 patients included in this study

Patient no.	Age (y)	Symptoms	SPT	UniCAP sesame IgE(kUA/L)
1*	1	Urticaria	+	2.09
2*	29	Vomiting, near syncope	+	9.13
3*	6	Angioedema, urticaria	+	3.86
4*	1.5	Diarrhea, vomiting	+	>100
5	3.5	Urticaria, AD exacerbation	+	74.60
6	20	Urticaria, angioedema	+	12.70
7	8.6	Urticaria, AD exacerbation	+	92
8	1	Urticaria, angioedema	+	10
9	2	Anaphylaxis	+	6.00
10	6	Gastrointestinal symptoms	+	32
11	9	AD exacerbation, rhinitis	+	56
12	10	Rhinitis, bronchospasm	+	19
13	32	Anaphylaxis	+	4.25
14*	4	AD exacerbation, rhinitis,	+	24.70
15	2	Asthma, anaphylaxis	+	21.10
16	1.2	Urticaria	+	3.77
17	12	AD, urticaria	+	92.80
18	18	Rhinitis, bronchospasm	+	1.45
19	7	Anaphylaxis	+	>100
20	1.2	Urticaria, AD exacerbation	+	31.30

SPT, Skin prick test; AD, atopic dermatitis.

*Skin prick tests were performed by using both a commercial preparation and a fresh extract of sesame seed proteins prepared in the laboratory. In all other cases (not marked with an asterisk), skin prick tests were performed with a commercial extract of sesame seeds.

of food allergens by using new methods for synthesizing and testing large numbers of synthetic peptides.^{10,12}

On the other hand, it is still difficult to characterize conformational epitopes, and the experimental efforts involved can be substantial.¹⁴ Sesame seeds were shown to contain 2 major storage proteins: 11S globulin (α -globulin) and 2S albumin (β -globulin), which serve as a source of amino acids during germination.¹⁵ The 2S albumin precursor (β -globulin; accession no. AF091841) was identified in our earlier study⁸ as the major sesame allergen, whereas the 11S globulin (α -globulin) was identified as a minor allergen. However, no identification and characterization of the IgE-binding sites of these allergens has been reported to date. In the present study we endeavored to map and characterize the main IgE epitopes of β -globulin.

METHODS

Subjects

The study was approved by the Committee on Ethics in Clinical Experiments on Humans of the E. Wolfson Medical Center, Holon, Israel. Twenty subjects were included, all of whom showed unequivocal clinical symptoms of immediate allergic reactions after the ingestion of sesame-containing foods within 2 hours after exposure to sesame (Table I). Clinical manifestations observed in the allergic patients included cutaneous and respiratory symptoms, as well as anaphylaxis (Table I). The subjects were tested by using the skin prick test with either a commercial sesame seed protein extract (Meridian

Biomedical, Inc. and Pharmacia or Bencard, Mississauga, Ontario, Canada) or both the commercial extract and a fresh extract of sesame seeds to verify previous sensitization to sesame.² Ten healthy subjects with no history of allergic reactions were selected as negative control subjects. In addition, a second control group consisting of 6 atopic patients with milk allergy, peanut allergy, or both but without sensitization to sesame were included. Sera from all subjects were collected and stored at -70°C until used. The serum samples of the 20 subjects recognized the β -globulin, which was previously identified as the major sesame allergen.⁸

Detection of specific IgE

Detection of IgE specific to sesame seed protein extract was performed with the Pharmacia CAP System by using a fluorimetric method (fluorescence enzyme immunoassay) described by Leimgruber et al¹⁶ with a commercial batch of sesame seed extract (Pharmacia); the units of UniCAP-specific IgE used were in kilounits of allergen per liter.

Proteolysis of β -globulin with Lys C

The β -globulin, after purification by means of fast-performance liquid chromatography and reverse-phase HPLC,⁸ was S-carboxamidomethylated with iodoacetamide and subsequently digested by the enzyme Lys C. The resulting 3 peptides were separated on a reverse-phase C18 HPLC column.⁸ One third of each of the 3 separated peptides was transferred to an electrospray-ionization mass spectrometer for peptide mapping and identification, and the remaining two thirds were subjected to a dot-blot test by using the serum samples that had previously yielded positive results with the respective β -globulin.

Peptide synthesis

A peptide containing 71 amino acid residues (peptide B) was previously identified by us as the IgE-binding region in β -globulin, the major sesame seed allergen. To determine the amino acid sequence of the IgE-binding sites on peptide B, we synthesized overlapping peptides 20 and 10 amino acid residues long that span the entire length of peptide B and that were offset from each other by 10 and 2 amino acid residues, respectively. All peptides were synthesized with an ABIMED AMS-422 multiple peptide synthesizer by using solid-phase methodology,¹⁰ according to the protocol recommended by the manufacturer (Abimed, Germany), except for one modification, as follows. After removal from the support phase, each peptide was biotinylated with 1-hydroxybenzotriazole. The esterification between the C-terminal α -COOH of the peptide and the hydroxyl group of the biotin (1-hydroxybenzotriazole) was done in the presence of dicyclohexylcarbodiimide. Product peptides were precipitated with cold tertbutyl ether (Fluka), collected by means of centrifugation (4°C at 2000 rpm), dissolved in water, and lyophilized.

Peptide analysis

Peptide separations were performed by using a Septra-Physics SP8800/8810 HPLC system with a C18 reverse-phase column. Detection at 220 nm was achieved by using an Applied Biosystems 757 variable wavelength absorbance detector. Purification was attained with a binary gradient formed from 0.1% trifluoroacetic acid in H_2O (solution A) and 0.1% trifluoroacetic acid in acetonitrile/ H_2O 75:25 (solution B). Amino acid composition was performed with a Dionex amino acid analyzer after acid hydrolysis.

IgE-binding assay

The dot-blot test of the different peptides and the serum samples was performed in Minifold II Slot Blot System (Schleicher and Schuell) that contained 72 lanes. A nitrocellulose membrane,

TABLE II. Amino acid sequence of the 6 overlapping and synthesized peptides (length, 20 amino acid residues; offset, 10 amino acids) covering the entire length of peptide B of β -globulin in sesame selected for the identification of the IgE-binding sites

Peptide no.	Amino acid sequences in the peptide	Position of each peptide within β -globulin amino acid sequence	No. of serum samples that elicited a positive response
A	TVVTTVAEEGEEENQRCGE	24-43	5
B	GEEENQRCGEWESRQCQMRH	34-53	6
C*	WESRQCQMRHCMQWMRSMRG	44-63	16
D	CMQWMRSMRGQYEESEFLRSA	54-73	0
E*	QYEESEFLRSAEANQGQFEHFREC	64-86	18
F*	EANQGQFEHFRECCNELRDVK	74-94	10

*The major IgE-binding peptides of the 6 synthesized peptides

after incubation in PBS, was installed in the system between 2 Wattmann discs immersed in PBS. Aqueous solution of streptavidin (*Streptomyces avidinii*, Sigma; 1 μ g/ μ L) was loaded in each lane (5 μ L/lane). The quantity and the concentration of streptavidin were calculated (according to information given by the manufacturer), showing that the reactivity of streptavidin was 14 U/mg protein and 1 unit of streptavidin bound to 1 μ g of biotin. Afterward, the synthesized peptides were loaded in the different lanes (10 μ L/lane). After 15 minutes' incubation, the lanes were washed with PBS, and the system was uninstalled. All other sequential steps, including blocking potential nonspecific binding sites in the nitrocellulose membrane, were described previously.⁸ Determination of the intensity of IgE binding of a specific serum sample to a specific peptide was carried out by means of densitometry (Vilber Lourmat, France) with the Bio-Profil Bio 1 d software.

Statistical analysis

Intensities of peptide-IgE binding were compared by using the Student *t* test (2-tailed independent samples). A *P* value of .05 was considered significant.

Peptide alignment

Homology analysis of the amino acid sequence of β -globulin (sesame seed 2S albumin precursor) epitopes with epitopes of known allergens was done by using alignment software available at <http://www.ncbi.nlm.nih.gov/blastp> and <http://prodes.toulouse.inra.fr/multalin/cgi-bin/multalin.pl>

RESULTS

Subjects

All 20 subjects studied exhibited positive skin prick test responses with commercial sesame seed extracts and demonstrated positive results when assayed for sesame-specific IgE with the ImmunoCAP test (Table I). The subjects participating in the negative control group have never exhibited allergic symptoms nor did their close family members. However, their sera were tested for total IgE and found to be at the normal level for nonallergic subjects to verify that they did not have allergy of any kind. In addition, this negative control group showed negative responses to sesame-specific IgE in the CAP System assay, as well as in the Western blot test.^{17,18} The second control group (with allergy to milk, peanuts, or

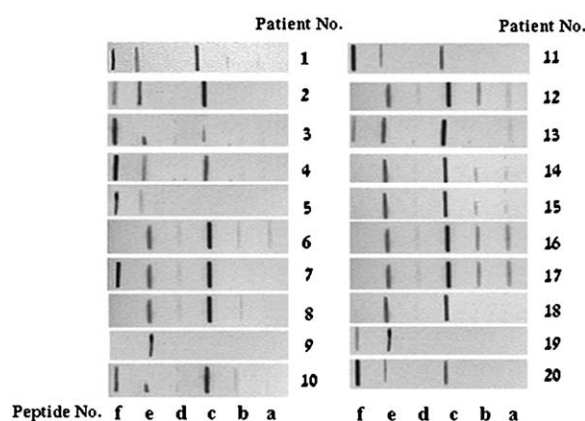


FIG 1. Dot-blot profile of IgE binding of serum samples from 20 patients with documented sesame hypersensitivity and positive reaction with β -globulin to the 6 synthesized peptides.

both) also exhibited negative responses to sesame-specific IgE but were found to possess high total IgE levels (>100 IU/mL).

Proteolysis of β -globulin with Lys C

Of the 3 peptides obtained by means of Lys C digestion of β -globulin, only peptide B, the center peptide, reacted positively in the dot-blot test with the serum samples that showed positive results in the Western blot with intact β -globulin. Peptide B, which evidently contains epitopes, corresponds to residues 24 to 94 in the whole β -globulin chain (2S albumin precursor). The sequence of this protein, the cleavage sites created by Lys C digestion, and the 3 peptides were presented in our earlier report.⁸

Peptide synthesis and their allergenic reactivity

To determine the amino acid sequence of the IgE-binding sites, we synthesized 6 overlapping peptides that span the entire peptide B, namely the IgE-binding region of β -globulin.⁸ All synthesized peptides were initially examined by means of reverse-phase HPLC and found to be $97\% \pm 1.5\%$ pure. Each peptide was 20 amino acids long (except peptides E and F, which consisted of 23 and

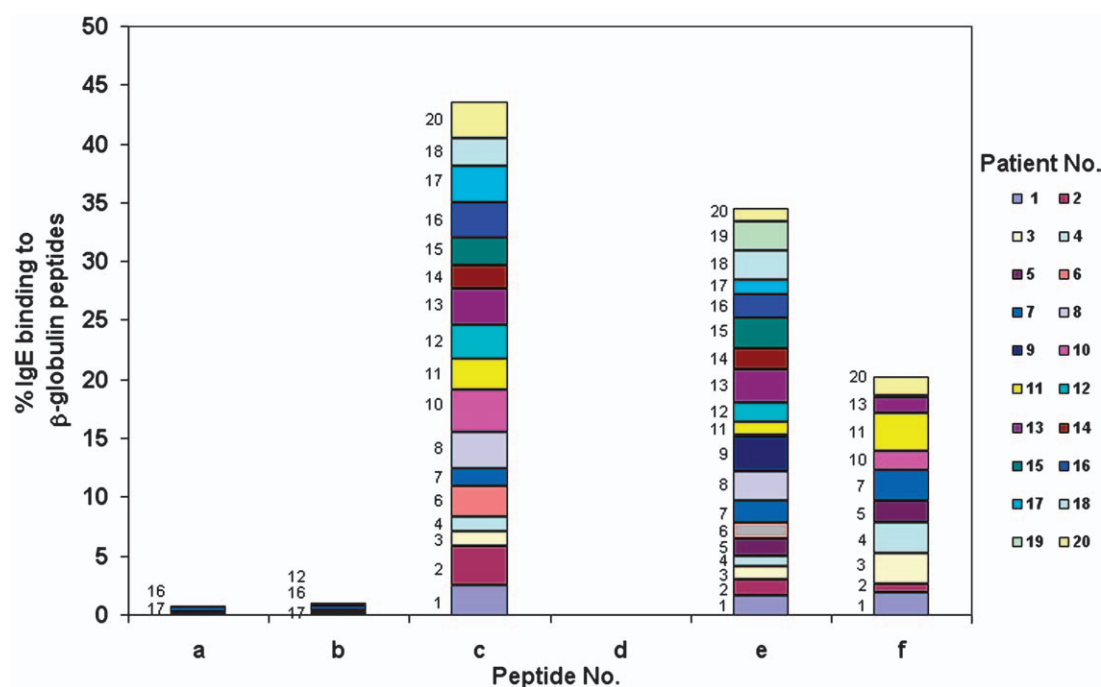


FIG 2. The relative intensity binding (percentage) of specific peptide to specific serum samples. The total IgE binding to each peptide (i) with each serum sample (j) was determined as 100% ($\sum_{i=1}^6 \sum_{j=1}^{20}$). The numbers along the bar sections denote the patients' numbers listed in the figure.

TABLE III. Amino acid sequences of the 17 overlapping and synthesized peptides (length, 10 amino acid residues; offset, 2 amino acids) covering the sequences of peptides 3, 5, and 6 of β-globulin selected for the identification of the IgE-binding regions

Peptide no.	Amino acid sequence of the peptide	Position of each peptide within β-globulin amino acid sequence	No. of sera samples of 15 in total that elicited a positive response
1	WESRQCQMRH	44-53	0
2*	SRQCQMRHCM	46-55	14
3*	QCQMRHCMQW	48-57	14
4	QMRHCMQWMR	50-59	0
5	RHCMQWMRSM	52-61	0
6*	CMQWMRSMRG	54-63	3
7	QYEEFLRSA	64-73	0
8	EESFLRSAEA	66-75	0
9	SFLRSAEANQ	68-77	0
10	LRSAEANQGQ	70-79	0
11	SAEANQGQFE	72-81	0
12*	EANQGQFEHF	74-83	3
13*	NQGQFEHFREC	76-86	14
14*	GQFEHFRECC	78-87	6
15*	FEHFRECCNE	80-89	3
16*	HFRECCNEIR	82-91	6
17*	RECCNEIRDVK	84-94	1

*The major IgE-binding peptides of the 17 synthesized peptides.

21 amino acids, respectively) and was offset from the previous peptide by 10 amino acids (Table II). In this manner, by using large overlapping fragments, the entire length of peptide B could be studied for specific IgE-binding sites.

These peptides were then probed with serum samples from the 20 patients who exhibited hypersensitivity to sesame and with serum samples from the 10 negative control subjects. Serum IgE from the negative control subjects did not recognize any of the synthesized peptides (data not shown). In contrast, Fig 1 shows that there are several IgE-binding regions along the length of peptide B that were recognized by the sera of patients with sesame hypersensitivity.

The relative intensity of IgE binding of each serum sample (j) to each peptide (i), was compared and expressed as a percentage of the total IgE binding from all serum samples to all peptides: $\sum_{i=1}^6 \sum_{j=1}^{20}$ (Fig 2).

Clearly, 3 peptides representing amino acid residues 44-63, 64-86, and 74-94 (Table II) of peptide B exhibited exceptionally high IgE-binding intensity ($P < .05$) relative to the other 3 peptides.

To focus more on the exact amino acid sequence of the IgE-binding sites, 17 small peptides consisting of 10 amino acids each, which were offset by 2 amino acids and covering the entire IgE-binding regions (peptides c, e and f), were synthesized (Table III).

All synthesized peptides were initially examined by means of reverse-phase HPLC and found to be $96\% \pm 1.8\%$ pure.

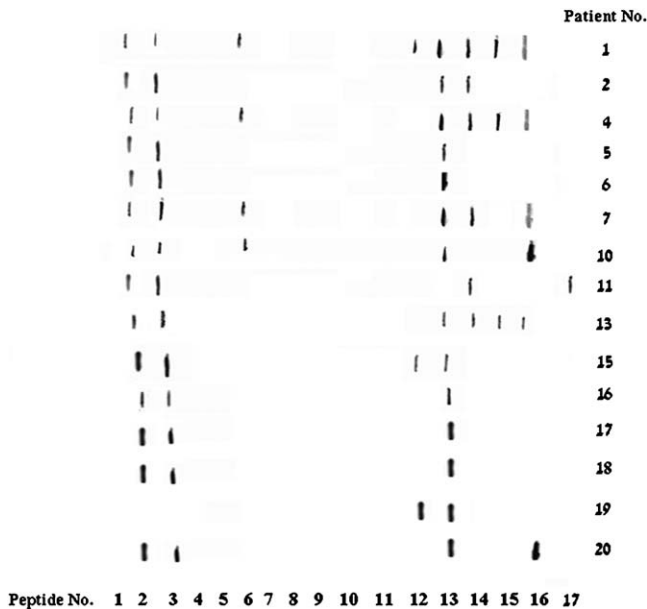


FIG 3. A dot-blot test profile of IgE binding from serum samples of 15 patients to the 17 synthesized peptides.

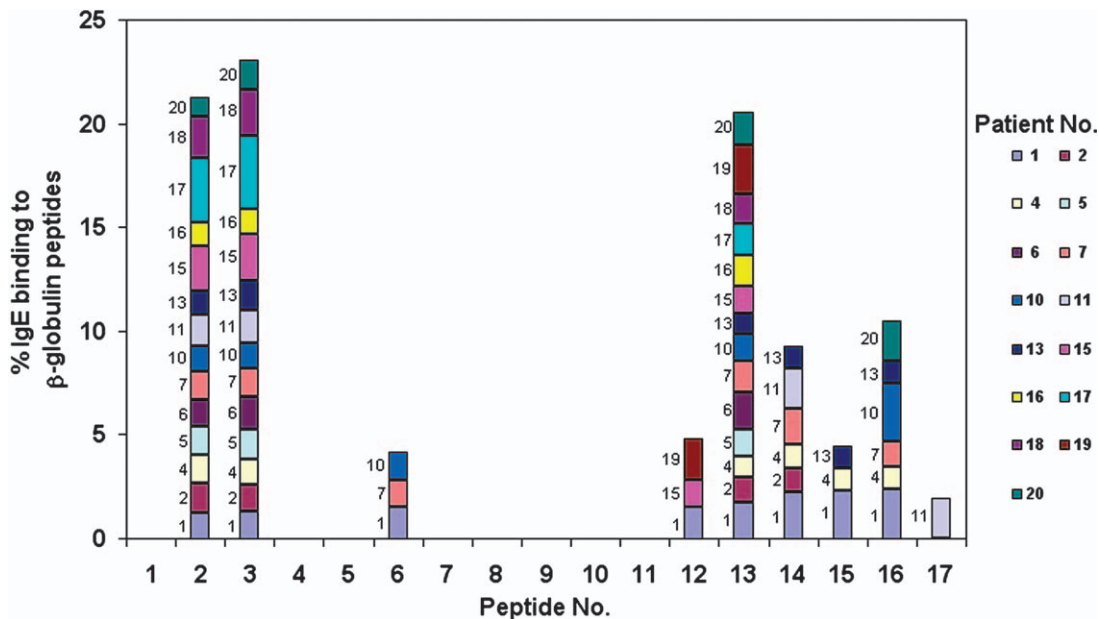


FIG 4. The relative intensity binding (percentage) of specific peptide to specific serum samples. The total IgE binding to each peptide (i) with each serum sample (j) was determined as $100\% \frac{\sum_{i=1}^{17} \sum_{j=1}^{15} I_{ij}}{\sum_{j=1}^{15} I_{ij}}$. The numbers along the bar sections denote the patients' numbers listed in the figure.

The peptide numbers were assigned arbitrarily from 1 to 17, according to their appearance along the entire length of peptides c, e, and f of peptide B. In this manner it was possible to identify individual IgE-binding epitopes within the larger IgE-binding regions of the β -globulin molecule by using 15 patients' serum samples (Fig 3). There are 9 main IgE-binding sites along the entire length of peptides c, e, and f of peptide B that were significantly ($P < .05$) recognized by the sera of the patients with sesame hypersensitivity (Table III).

The size of the epitopes seemed to be narrowed down to 6 to 10 amino acids in length. Two epitopes (amino acids 48-57 and amino acids 54-63), which partially overlapped each other, were found in the region of amino acid residues 44-63. Six epitopes (amino acids 74-83, 76-86, 78-87, 80-89, 82-91, and 84-94), which partially overlapped each other, were found in the region of amino acid residues 74-94. To determine which, if any, of the 9 epitopes was immunodominant, the relative intensity of IgE binding

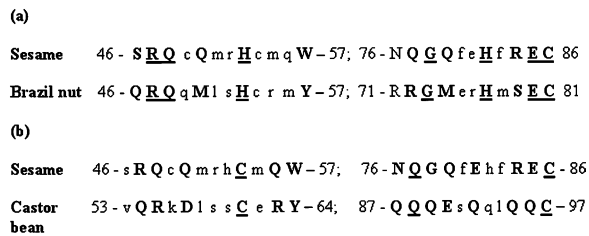


FIG 5. Alignment of deduced amino acid sequences of β -globulin (sesame seed 2S albumin precursor) immunodominant epitopes (2, 3, and 13) with epitopes of Brazil nut Ber e 1 (**A**) and of Castor bean Ric c 1 precursor (**B**). Identical amino acids are in **bold**, underlined capital letters. Similar amino acids are in **bold capital letters**. Different amino acids are in *lower-case letters*.

of each serum sample (j) to each peptide (i) was compared and expressed as the percentage of the total IgE binding from all serum samples to all peptides: $\sum_{i=1}^{17} \sum_{j=1}^{15}$ (Fig 4).

Fourteen of 15 of the patient serum samples tested recognized epitopes 2, 3, and 13. The results indicate that these 3 peptides are, or at least contain, the immunodominant epitopes of the major sesame allergen.

The serum samples of the 2 control groups exhibited no IgE binding to the 9 different 10-amino-acid-long peptides.

DISCUSSION

Sesame seeds have become one of the common food allergens for both children and adults.^{2,7} Various studies performed over the last several years elucidated the identity of several allergens in sesame seeds,^{6,7,8} but there are no reports on the characterization of their epitopes. Because antigen-specific IgE plays a critical role in the cause of allergic disease, determination of allergen-specific IgE-binding epitopes is an important first step toward better understanding of the disease process and might provide tools for immunotherapy.

Although it is difficult to characterize conformational epitopes,¹⁴ when dealing with linear epitopes, short overlapping peptides can be synthesized that can then be probed with serum samples from patients with documented food allergy. This has been demonstrated in the case of Ara h 1¹⁹ and Ara h 2 from peanuts,¹² β -lactoglobulin from cow's milk,²⁰ and Pen a 1 from shrimp.²¹ The linear epitopes are generally known to be resistant to heat and proteolysis,²² and they tend to retain their allergenic reactivity, whereas many conformational epitopes of native food proteins are modified or disrupted by heat, chemical treatments, or both, thus losing their IgE-binding potential.¹ Such treatments usually result in exposure of linear epitopes as well.^{4,23-25} Furthermore, Cooke and Sampson²⁶ observed a different pattern of specific IgE reactivity with linear and conformational epitopes of ovomucoid in children with persistent egg allergy, as opposed to children who are likely to outgrow it. They suggested that the development of IgE antibodies to linear epitopes might be related to the persistence of allergy

to egg. Also, Vila et al²⁷ reported that high levels of detectable IgE to linearized α -casein are related to the persistence of cow's milk allergy. Our study focused on identification of linear IgE-binding epitopes.

In our previous study of Lys C digestion followed by peptide separation and dot-blot testing of the individual peptides, we indicated that peptide B, which corresponds to residues 24-94 in the whole β -globulin chain, is likely to contain linear epitopes.⁸

In the present study we have determined at least 9 different IgE-recognition sites on peptide B. The identification of multiple epitopes on a single allergen is not a novel finding because there are reports on multiple IgE-binding epitopes on allergens from many foods that cause immediate hypersensitivity reactions, as in allergens from cow's milk,²⁰ hazel,²⁸ and soy.²⁹ The observation that most of these proteins have multiple IgE-binding sites probably reflects the polyclonal nature of the immune response. The 3 IgE-binding overlapping decapeptides, numbers 12, 13, and 14, of β -globulin have common amino acid residues GQFEHF (amino acids 78-83), suggesting that this 6-amino-acid sequence represents an epitope of β -globulin. Similarly, the 3 IgE-binding overlapping decapeptides, numbers 14, 15, and 16, have common amino acid residues HFRECC (amino acids 82-87), indicating that this 6-amino-acid sequence represents another epitope of β -globulin. The 2 IgE-binding overlapping decapeptides, numbers 2 and 3, have common amino acid residues QCQMRHCM (amino acids 48-55), indicating that this 8-amino-acid sequence represents yet another epitope of β -globulin. Furthermore, decapeptide 6 (amino acids 54-63), which has no common amino acid residues with another decapeptide, appears to represent an additional epitope region of β -globulin. The linear epitopes are small peptide fragments (10-18 amino acids long) and contain a minimum of 6 amino acid residues.¹ It should be pointed out that the 9 different IgE-recognition sites reacted only with sera of patients with sesame allergy and not with sera of the 2 control groups, thus excluding nonspecific IgE binding to these epitopes. Three of the β -globulin peptides (numbers 2, 3, and 13) appear to be immunodominant IgE-binding epitopes because the serum samples recognizing these epitopes amounted to more than 80% of the total sera tested. Also, these peptides are best recognized in terms of intensity of response.

Recently, Beyer et al⁷ described several allergens of sesame seeds. Amino acid sequence alignment shows that one of these allergens, a 7-kd 2S albumin precursor (accession no. AF091841), is identical to the large subunit¹⁵ of the major sesame seed allergen previously reported by us.⁸ The apparent difference in the size of the allergen between the protein reported by Beyer et al⁷ (7 kd) and by us⁸ (14 kd) is probably caused by the cleavage of the 2S albumin precursor by asparaginyl endopeptidase described by Hara-Hishimura et al.³⁰ Two of the 3 immunodominant IgE-binding epitopes observed in the present study (peptides 2 and 3) are located in the small subunit of β -globulin, which was not included in the allergen (7 kd) reported by Beyer et al.⁷

There was no obvious sequence motif shared by the 9 different IgE-binding epitopes of the sesame β -globulin allergen, yet approximately 60% of the amino acids represented in the epitopes were hydrophobic residues. Several reports in the literature indicate that there are no obvious sequence motifs shared by different IgE-binding epitopes of allergens, such as β -lactoglobulin from cow's milk³¹ and Ara h 1¹¹ and Ara h 2 from peanuts.¹² Nonetheless, comparison of the amino acid sequences of allergenic proteins and their epitopes have not yet yielded any unique or typical pattern specifically related to allergenicity.^{32,33} On the other hand, because the allergenic potential of an unknown protein can be estimated by comparing its amino acid sequence with that of known allergens, such comparisons can be of value in providing a clue regarding the putative allergenicity of a given protein. Furthermore, analysis of sequence homology to known allergens is important because homology might predict cross-reactivity and also heat stability, acid stability, or both.³³

The β -globulin 2S albumin precursor, identified as the major sesame allergen⁸ and related to the 2S albumin family, constitutes one of the 2 major storage proteins of sesame seeds.¹⁵ It is well documented that numerous seed storage proteins are associated with food allergy.³⁴ The 2S albumins of several plants, including peanut, walnut, Brazil nut, and soybean, are known food allergens. Alignment of amino acid sequence of the 9 epitopes of β -globulin identified in this study to known 2S albumin epitopes showed a mean homology of approximately 35% (eg, to the 2S albumin precursor from Brazil nut, Ber e 1 [approximately 35%], and to the 2S albumin precursor from Castor bean, Ric c 1 [approximately 45%]; Fig 5).^{35,36}

Two of the major peanut allergens (Ara h 1 and Ara h 2) are seed storage proteins that have sequence homology with proteins of other plants. This might explain the cross-reacting antibodies to other legumes found in the sera of patients who manifest clinical symptoms to only one member of the legume family.³⁷ On the other hand, many foods of plant origin belong to closely related botanical families and have structurally homologous proteins, especially in their primary amino acid sequences. Yet they are not all equally allergenic, thus making it difficult to establish the risk of cross-reactions in patients allergic to a specific food.

Knowledge of the IgE-binding epitopes might provide a better understanding of the functional role that the allergens play in the disease and might have implications in immunodiagnosis and possibly immunotherapy.

REFERENCES

1. Lehrer SB, Horner WE, Reese G. Why are some proteins allergenic? Implications for biotechnology. *Crit Rev Food Sci Nutr* 1996;36:553-64.
2. Dalal I, Binson I, Reiften R, Amitai Z, Shohat T, Rahmani S, et al. Food allergy is a matter of geography after all: sesame as a major cause of severe IgE-mediated food allergic reactions among infants and young children in Israel. *Allergy* 2002;57:362-5.

3. Pajno GB, Passalacqua G, Magazzu G, Barberio G, Vita D, Canonica W. Anaphylaxis to sesame. *Allergy* 2000;55:199-201.
4. Kanny G, De Hauteclouque C, Moneret-Vautrin DA. Sesame seed and sesame oil, contain masked allergens of growing importance. *Allergy* 1996;51:952-7.
5. Kolopp-Sarda MN, Moneret-Vautrin DA, Gobert B, Kanny G, Brodschii M, Bene MC, et al. Specific humoral immune responses in 12 cases of food sensitization to sesame seed. *Clin Exp Allergy* 1997;27:1285-91.
6. Pastorello EA, Varin E, Farioli L, Pravettoni V, Ortolani C, Trambaioli C, et al. The major allergen of sesame seeds (*Sesamum indicum*) is a 2S albumin. *J Chromatogr B* 2001;756:85-93.
7. 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;110:154-9.
8. Wolff N, Cogan U, Admon A, Dalal I, Katz Y, Hodos N, et al. Allergy to sesame in humans is associated primarily with IgE antibody to a 14 kDa 2S albumin precursor. *Food Chem Toxicol* 2003;41:1165-74.
9. Hamelmann E, Rolinck-Werninghaus C, Wahn U. From IgE to anti-IgE: where do we stand? *Allergy* 2002;57:983-94.
10. Banerjee B, Greenberger PA, Fink JN, Kurup VP. Conformational and linear B-cell epitopes of Asp f 2, a major allergen of *Aspergillus fumigatus*, bind differently to immunoglobulin E antibody in the sera of allergic bronchopulmonary aspergillosis patients. *Infect Immun* 1999;67:2284-91.
11. Burks AW, Shin D, Cockrell G, Stanley JS, Helm RM, Bannon GA. Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. *Eur J Biochem* 1997;245:334-9.
12. Stanley JS, King N, Burks AW, Huang SK, Sampson H, Cockrell G, et al. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. *Arch Biochem Biophys* 1997;342:244-53.
13. Rabjohn P, Helm EM, Stanley JS, West CM, Sampson HA, Burks AW, et al. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *Clin Invest* 1999;103:535-42.
14. Padlan EA. Structure of protein epitopes deduced from x-ray crystallography. Van Regenmortel MHV, editor. *Structure of antigens*. CRC Press, Boca Raton (FL). *Natl Inst Diabetes* 1992;1:29-42.
15. Tai SSK, Wu LSH, Chen ECF, Tzen JTC. Molecular cloning of 11S globulin and 2S albumin, the two major seed storage proteins in sesame. *J Agric Food Chem* 1999;47:4932-8.
16. Leimgruber A, Mosimann B, Claeys M, Spey M, Jaccard Y, Aubert V, et al. Clinical evaluation of a new in-vitro assay for specific IgE, the immuno CAP system. *Clin Exp Allergy* 1991;21:127-31.
17. Towbin H, Staehlin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedure and applications. *Proc Natl Acad Sci U S A* 1979;76:4350-4.
18. Burnette WN. "Western Blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein. *Anal Biochem* 1981;112:195-203.
19. Shin DS, Compadre CM, Maleki SJ, Kopper RA, Sampson H, Huang SK, et al. Biochemical and structural analysis of the IgE binding sites on Ara h 1, an abundant and highly allergenic peanut protein. *J Biol Chem* 1998;273:13753-9.
20. Ball G, Shelton MJ, Walsh BJ, Hill J, Hosking CS, Howden ME. A major continuous allergenic epitope of bovine β -lactoglobulin recognized by human IgE binding. *Clin Exp Allergy* 1994;24:758-64.
21. Reese G, Ayuso R, Leong-Kee SM, Plante MJ, Lehrer SB. Characterization and identification of allergen epitopes: recombinant peptide libraries and synthetic, overlapping peptides. *J Chromatogr B* 2001; 756:157-63.
22. Wigotzki M, Steinhart H, Paschke A. Influence of varieties, storage and heat treatment on IgE-binding proteins in hazelnuts (*Corylus avellana*). *Food Agric Immunol* 2000;12:217-29.
23. Malish D, Glovsky MM, Hoffman DR, Ghekiere L, Hawkins JM. Anaphylaxis after sesame seed ingestion. *J Allergy Clin Immunol* 1981; 67:35-8.
24. Kagi MK, Wuthrich B. Falafel burger anaphylaxis due to sesame seed allergy. *Ann Allergy* 1993;71:127-9.
25. Alday E, Curiel G, Lopez-Gil MJ, Carreno D, Moneo I. Occupational hypersensitivity in sesame seeds. *Allergy* 1996;51:69-70.

26. Cooke SK, Sampson HA. Allergenic properties of ovomucoid in man. *J Immunol* 1997;100:171-6.
27. Vila L, Beyer K, Jarviner KM, Chatchatee P, Bardina L, Sampson HA. Role of conformational and linear epitopes in the achievement of tolerance in cow's milk allergy. *Clin Exp Allergy* 2001;31:1599-606.
28. Elsayed S, Holen E, Dybendal T. Synthetic allergenic epitopes from the amino-terminal regions of the major allergens of hazel and birch pollen. *Int Arch Allergy Appl Immunol* 1989;89:410-5.
29. Herian A, Taylor SL, Bush RK. Identification of soybean allergens by immunoblotting with sera from soy-allergic adults. *Int Arch Allergy Appl Immunol* 1990;92:192-8.
30. Hara-Hishimura I, Takeuchi Y, Inoue K, Nishimura M. Vesicle transport and processing of the precursor to 2S albumin in pumpkin. *Plant J* 1993;4:793-800.
31. Selo I, Clement G, Bernard H, Chatel JM, Creminon C, Peltre G, et al. Allergy to bovine β -lactoglobulin: specificity of human IgE to tryptic peptides. *Clin Exp Allergy* 1999;29:1055-63.
32. Fuchs RL, Astwood JD. Allergenicity assessment of foods derived from genetically modified plants. *Food Technol* 1996;50:83-8.
33. Hefle SL. The chemistry and biology of food allergens. *Food Technol* 1996;50:86-92.
34. Shewry PR, Napier JA, Tatham AS. Seed storage proteins: structures and biosynthesis. *Plant Cell* 1995;7:945-56.
35. Stickler M, Mucha J, Estell D, Power S, Harding F. A human dendritic cell-based method to identify CD4+ T-cell epitopes in potential protein allergens. *Environ Health Perspect* 2003;111:251-4.
36. Bashir ME, Hubatsch I, Leinenbach HP, Zeppezauer M, Panzani RC, Hussein IH. Ric c 1 and Ric c 3, the allergenic 2S albumin storage proteins of *Ricinus communis*: complete primary structures and phylogenetic relationships. *Int Arch Allergy Appl Immunol* 1998;115:73-82.
37. Bernhisel-Broadbent J, Taylor S, Sampson HA. Cross-allergenicity in the legume botanical family in children with food hypersensitivity. *J Allergy Clin Immunol* 1989;84:701-9.