

Linear IgE epitope mapping of the English walnut (*Juglans regia*) major food allergen, Jug r 1

Jason M. Robotham, BS,^a Suzanne S. Teuber, MD,^b Shridhar K. Sathe, PhD,^c and Kenneth H. Roux, PhD^a Tallahassee, Fla, and Davis, Calif

Background: Peanut and tree nut allergies can be life-threatening, and they appear to be growing in prevalence. Jug r 1, a 2S albumin seed storage protein, was previously characterized as a major English walnut food allergen.

Objective: We sought to identify the linear IgE-binding epitopes of Jug r 1 and to determine which, if any, amino acids are necessary for this binding to occur.

Methods: Pools of sera from walnut-allergic patients and overlapping peptides synthesized on an activated cellulose membrane were used to screen for IgE-binding epitopes. Mutational analysis of the immunodominant epitope was carried out through single and multisite amino acid substitutions. Inhibition assays were performed through use of affinity-purified IgE, soluble forms of the epitope peptide, and the recombinant 2S albumin, rJug r 1.

Results: One immunodominant linear epitope was identified. Amino acid mutations to the epitope demonstrated that the residues RGEE, at positions 36 through 39, were minimally required for IgE binding. Probing of this epitope with sera from each of 20 patients revealed 15 of the sera to be positive. Binding of patients' IgE to the epitope was inhibited with a soluble form of the peptide; however, soluble peptide did not completely inhibit the binding of IgE to the intact rJug r 1.

Conclusion: One major linear IgE-reactive epitope and its critical core amino acid residues have been identified. Mutation of any of these core amino acids resulted in loss of IgE binding to the epitope, and this points toward the feasibility of reducing allergenicity in genetically modified walnuts. However, strong evidence for the existence of conformational epitopes was also obtained. (*J Allergy Clin Immunol* 2002;109:143-9.)

Key words: Epitope mapping, 2S albumin, walnut, nut allergy, Jug r 1, food allergy

Abbreviations used

DMF: N,N-dimethylformamide

Fmoc: 9-fluorenylmethoxy carbonyl-derived

NC: Nitrocellulose

RT: Room temperature

TBS: Tris-buffered saline [solution]

TBS-T: Tris-buffered saline [solution] with 0.2% Tween-20

It is estimated that up to 8% of children less than 3 years of age and 2% of adults are affected by food allergies.¹ Although food-induced allergic reactions are the most common cause of outpatient anaphylaxis,² most severe reactions of this kind are caused by peanuts and tree nuts.^{3,4} Most plant food allergens can be found among pathogenesis-related proteins, seed storage albumins and globulins, and α -amylase and protease inhibitors.⁵ A previous study by Teuber et al⁶ documented the cloning and sequencing of a gene encoding Jug r 1, a major allergen in the English walnut, *Juglans regia*. Jug r 1 is a 2S albumin seed storage protein and possesses important homologies in amino acid sequence with other 2S albumin proteins from Brazil nut, cottonseed, castor bean, and mustard.⁶ Like many 2S albumins, Jug r 1 is synthesized as a precursor protein and cleaved into a large subunit and a small subunit, these being joined by disulfide bridges. It is currently believed that patients with life-threatening allergies to walnuts and other tree nuts will rarely become tolerant of these foods⁷ and consequently face a lifetime of avoidance.⁸ Avoidance, however, is often difficult because of the ubiquitous nature of these foods and the potential for cross-contamination during their processing.⁷

Many approaches to allergen immunotherapy under investigation today are based on a detailed knowledge of the amino acids found in IgE-reactive B-cell epitopes.⁹⁻¹² These reactive sites can be either linear or conformational. Typically, a linear epitope contains a stretch of contiguous amino acids spanning 5 to 10 residues in the antigen, whereas residues distant in the primary sequence but proximate in the folded protein constitute a conformational epitope.¹³ In recent years, the genes encoding several proteins with allergenicity, such as Jug r 1, have been cloned and expressed through use of molecular biology techniques.^{6,14-16} Despite the rapidly increasing number of recombinant allergens, relatively few IgE-reactive B-cell epitopes have been defined.¹⁷⁻²⁹

From ^athe Department of Biological Science and Structural Biology Program, Florida State University, Tallahassee; ^bthe Department of Internal Medicine, Division of Rheumatology, Allergy and Clinical Immunology, University of California, Davis, School of Medicine; and ^cthe Department of Nutrition, Food, and Exercise Science, Florida State University, Tallahassee.

Funded in part by the International Life Sciences Institute, the Allergy and Immunology Institute (Suzanne S. Teuber), USDA NRICGP grant (#9901530), and a training grant fellowship from Florida State University NSF-RTG (#9602233).

Received for publication July 26, 2001; revised September 20, 2001; accepted for publication September 25, 2001.

Reprint requests: Kenneth H. Roux, PhD, Department of Biological Science, Biology Unit I, Florida State University, Tallahassee, FL 32306-4370.

Copyright © 2002 by Mosby, Inc

0091-6749/2002 \$35.00 + 0 1/87/120558

doi:10.1067/mai.2002.120558

The specific aim of this research was to screen the large and small subunits of the English walnut allergen Jug r 1 for linear IgE-binding epitopes. We report the finding of a single immunodominant IgE-binding epitope residing in the large subunit, and we have identified 4 core amino acids necessary for this binding to occur.

METHODS

Human sera

The study was approved by the Human Subjects Review Committee at the University of California, Davis. Sera from 20 walnut-allergic patients with convincing histories of life-threatening systemic allergic reactions to walnuts, positive ImmunoCAP assays (Pharmacia Diagnostics, Columbus, Ohio), and evidence of IgE against rJug r 1 (see below) were used in this investigation. Control sera were obtained from atopic patients with no history of walnut sensitivity and 1 patient who had life-threatening reactions to walnuts, positive ImmunoCAP assay, and positive serum IgE immunoblot to walnut proteins but was negative for binding to the 2S albumin.

Expression and isolation of rJug r 1

Two protocols for isolation of rJug r 1 were used. In the first, used to produce rJug r 1 for screening patient sera by Western blotting, the Jug r 1 insert was subcloned into the pPROEXHT expression vector (Gibco BRL, Invitrogen Life Technologies, Carlsbad, Calif) and transformed into *Escherichia coli* BL21-CodonPlus (Stratagene Inc, La Jolla, Calif). Cells were grown overnight at 37°C in 5 mL 2X YT broth with 50 µg/mL ampicillin and chloramphenicol. This was then added to 500 mL 2X YT broth and grown to an A_{600} of 1.0. Isopropyl thiogalactose was added to 0.5 mmol/L, and the culture was grown at 37°C for 4 hours. Cells were pelleted, washed with 0.02 mol/L PBS (pH 7.3), and resuspended in BRL Lysis Buffer (Gibco BRL). Lysozyme was added to 500 µg/mL on ice for 15 minutes; this was followed by the addition of sarkosyl to 1% w/v. The cells were sonicated, and the insoluble matter was pelleted by centrifugation at 12,000g for 20 minutes at 4°C. Ni-NTA resin (Gibco BRL) was added in BRL Buffer A (Gibco BRL) to the supernatant and allowed to incubate overnight at 4°C. The resin was washed 4 times with BRL Buffer A, and the His-tagged fusion protein was then eluted with BRL Buffer C (Gibco BRL) containing 0.25% sarkosyl.

In the second protocol, rJug r 1 was expressed in fusion with glutathione-S-transferase and purified after the Frangioni and Neel³⁰ protocol with slight modifications, as previously described by Teuber et al.⁶ This glutathione-S-transferase-rJug r 1 fusion protein was used in all dot-blot assays, as described below.

Immunoblotting of rJug r 1

rJug r 1 samples were either subjected to SDS-PAGE and electrophoretically transferred to nitrocellulose (NC) or blotted directly onto the membrane. For SDS-PAGE analysis of rJug r 1, samples were boiled for 5 minutes in sample buffer (60 mmol/L Tris-HCl, pH 6.8, 2% SDS, 10% v/v glycerol, 0.01% w/v bromophenol blue) and electrophoresis was carried out with an 8-mA constant current through use of a SE600 Vertical Slab Gel Unit (Pharmacia Biotech, Piscataway, NJ). An SDS-PAGE gel (13% monomer acrylamide concentration) with 1 µg of protein per 4 mm was used for immunoblotting, as previously described.⁶ Protein was transferred to a 0.22-µm NC membrane (MSI, Westborough, Mass) overnight at 30 V through use of a TE42 Transphor Electro-Transfer Unit (Pharmacia Biotech). The blot was cut into 4-mm-wide strips and blocked for 1 hour at room temperature (RT) in PBS/3% nonfat dry

milk/0.2% Triton X-100 (TX-100). Diluted sera, 1:5 v:v in the blocking buffer, were added to the strips and incubated overnight at RT. The strips were then washed for 20 minutes 3 times in PBS/0.01% TX-100 and incubated overnight at RT with equine polyclonal ¹²⁵I-antihuman IgE (Hycor Biomedical Inc, Garden Grove, Calif) diluted 1:5 in the nonfat milk buffer. The strips were washed for 20 minutes 3 times and exposed to Kodak Biomax x-ray film (Kodak, Rochester, NY) at -70°C for 48 hours. All incubations were carried out with rocking.

For dot-blot assays, a graphite pencil was used to circumscribe 4 mm × 8 mm ellipses on a dry 0.4-µm NC membrane (Shleicher & Schuell, Keen, NH). The NC was incubated in distilled-deionized water on a rocking table for 5 minutes and allowed to air-dry before each dot was loaded with 0.11 µg of rJug r 1 in 2 µL distilled-deionized water. The antigen-loaded NC was then rinsed in distilled-deionized water as described above, placed protein-side-up on 3MM paper (Whatman Intl Ltd, Maidstone, United Kingdom), and dried under a 60-W light until all moisture was removed. Once dry, dots were rinsed for 2 minutes in TBS-T (Tris-buffered saline solution [TBS; 20 mmol/L Tris, 137 mmol/L NaCl, pH 7.6] containing 0.2% Tween-20), blocked for 1 hour at RT in TBS-T containing 2% BSA (Sigma, St Louis, Mo), and then incubated overnight at 4°C with sera diluted 1:40 in TBS-T. Membranes were then washed once for 15 minutes and 3 times for 5 minutes in TBS-T before being incubated for 1 hour at RT with horseradish peroxidase-labeled goat-antihuman IgE (Biosource International, Camarillo, Calif) diluted 1:2000 in TBS-T. Washing in TBS-T was repeated as described above, and the reactive dots were identified after (1) a 5-minute incubation in Enhanced Chemiluminescence Plus (Amersham Pharmacia, Piscataway, NJ), prepared according to the manufacturer's instructions, and (2) subsequent exposure to Kodak X-OMAT x-ray film.

Solid-phase peptide (SPOTs) synthesis

On the basis of the published amino acid sequence of Jug r 1 and our unpublished data determining the subunit cleavage sites,⁶ twenty-five overlapping 13-amino acid peptides, offset by 3 amino acids, were synthesized; these corresponded to the entire 64-amino acid length of the large subunit (Fig 1, A) and the 26-amino acid length of the small subunit (Fig 1, B). Additional peptides bearing the target epitope (QGLRGEEMEMV), mutated forms of this peptide, and a non-IgE-binding peptide (LSQRGLQSSSV) were also synthesized. Thirteen versions of the target peptide were created through a single-site alanine substitution at each position along the amino acid sequence, and 6 mutated peptides were synthesized through use of multiple alanine substitutions (Table I).

Peptides were synthesized on derivatized cellulose sheets through use of 9-fluorenylmethoxy carbonyl-derived (Fmoc) amino acids, as described by the manufacturer (Genosys Biotechnologies, Inc, The Woodlands, Tex). Briefly, cellulose membranes containing free hydroxy groups were esterified with an Fmoc-amino acid dissolved in 1-methyl-2 pyrrolidinone. The coupling reaction was followed by washing in N,N-dimethylformamide (DMF), and the amino acids were acetylated with acetic anhydride. The membranes were deprotected by washing in DMF and incubation with a solution of 20% piperidine in DMF; this was followed by another wash in DMF. Coupling, acetylation, and deprotection steps were repeated for each cycle. During the final cycle, a mixture of dichloromethane, trifluoroacetic acid, and triisobutylsilane (1:1:0.05) was used to deprotect the acid-labile amino acid side chains.

IgE binding to solid-phase synthetic peptides (SPOTs analysis)

The peptide-containing membranes were washed in TBS and incubated overnight at RT in blocking solution as directed by the

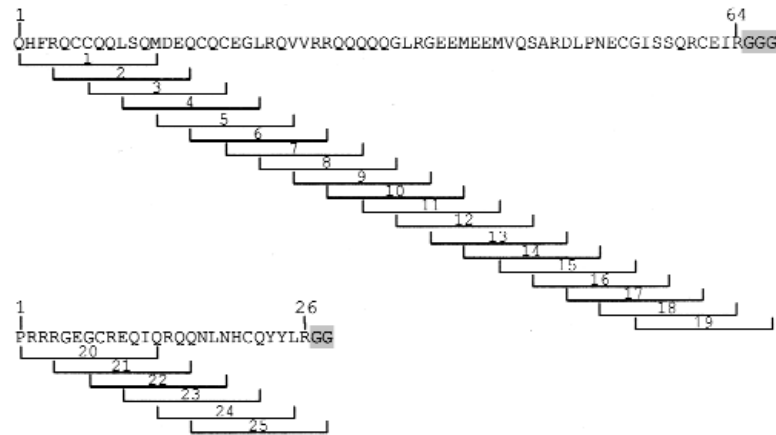


FIG 1. Overlapping peptide synthesis of Jug r 1. **A**, Nineteen 13-amino acid peptides, offset by 3 amino acids, were synthesized; they corresponded to the entire 64-amino acid large subunit. **B**, Similarly, 6 peptides were synthesized; they corresponded to the entire 26-amino acid small subunit. Glycine residues were added to the C-terminal end of each sequence (*shaded*) so that all synthesized peptides were of equal length.

manufacturer (Genosys Biotechnologies, Inc). Membranes were then washed in TBS-T for 10 minutes and incubated overnight at 4°C with an individual patient's serum or pooled patients' sera diluted 1:5 in blocking buffer (total sera:blocking buffer). This incubation was followed by three 5-minute washes in TBS-T and an overnight (4°C) incubation with ¹²⁵I-antihuman IgE (Hycor Biomedical Inc, Garden Grove, Calif) diluted 1:10 in a mixture of PBS, 5% nonfat dry milk, and 0.05% Tween-20. Three final 10-minute washes in PBS/0.05% Tween-20 were performed, and IgE-peptide reactivity was identified after a 48-hour exposure at -70°C to Kodak Biomax x-ray film.

Soluble epitope peptide synthesis

A soluble form of an identified IgE-reactive peptide (QGLRGEEMEEMV) was synthesized by Fmoc protocols on an automated peptide synthesizer (Model 433A, Applied Biosystems, Foster City, Calif).

Isolation and inhibition of epitope-specific IgE

The epitope-reactive IgE was isolated from patients' sera by affinity chromatography. A 0.5-mL pool of patients' sera, known to be reactive to the reactive synthetic solid-phase epitope peptide, was slowly added to a 5-mL disposable polypropylene column (Pierce Chemical Company, Rockford, Ill) containing cyanogen-bromide-activated beads (Sigma) to which 5 mg of the reactive peptide had been covalently coupled, as described by the manufacturer; the effluent was collected. Bound IgE was eluted with the addition of 0.2 mol/L glycine sulfate (pH 2.3) collected in a beaker containing 10 μL 1% BSA in buffered saline solution borate and subsequently neutralized with 1.0 mol/L Tris. The column was then rinsed with buffered saline solution borate and the initial effluent repassed over the column. The process was repeated for a total of 3 times, yielding 3 eluates and the column effluent.

To test the specificity and reactivity of the epitope-specific IgE, control and pooled and fractionated patients' sera were preincubated overnight at 4°C with different amounts (70, 7.0, and 0.7 μg) of the soluble epitope peptide or a soluble nonspecific peptide. The preincubated sera were used to probe either solid-phase IgE-reactive and non-IgE-reactive peptides or rJug r 1. IgE binding to the solid-phase synthetic peptides and dot-blotted rJug r 1 was detected

TABLE I. Mutational analysis of the Jug r 1 IgE binding epitope, E1

Amino acid sequence*	Substitution	IgE binding†
<u>A</u> GLRGEEMEEMV	Q1A	++
Q <u>A</u> L RGEEMEEMV	G2A	++
QGA RGEEMEEMV	L3A	+
QGL <u>A</u> GEEMEEMV	R4A	-
QGLR <u>A</u> EEEMEEMV	G5A	-
QGLRG <u>A</u> EEMEEMV	E6A	-
QGLRGE <u>A</u> EMEEMV	E7A	-
QGLRGE <u>E</u> AEMEEMV	M8A	++
QGLRGEEM <u>A</u> EEMV	E9A	++
QGLRGEEME <u>A</u> MV	E10A	+
QGLRGEEME <u>E</u> AV	M11A	++
QGLRGEEMEEM <u>A</u>	V12A	++
QAA RGEEMEEMV	G2A,L3A	+
QAAA GEEMEEMV	G2A,L3A,R4A	-
QAAAA EEMEEMV	G2A,L3A,R4A,G5A	-
QGLRGEEME <u>AA</u> V	E10A,M11A	+
QGLRGEEM <u>AAA</u> V	E9A,E10A,M11A	-
QGLRGE <u>EE</u> AAAAV	M8A,E9A,E10A,M11A	-

*The critical core amino acid residues, located at positions 36 through 39 of the large subunit, and an influential glutamic acid residue at position 42 are indicated in bold type. Mutated residues are underlined.

†Peptides were probed with pooled with pool 1, comprising sera from 6 walnut-sensitive patients.

through use of ¹²⁵I-antihuman IgE and Enhanced Chemiluminescence Plus, respectively, as described earlier.

RESULTS

IgE binding to rJug r 1

IgE from 20 walnut-sensitive patients was shown to bind the 20-kDa His-tagged rJug r 1 (Fig 2). Three sera showed only faint binding, whereas the other 17 sera gave moderate or strong signals. Recombinant Jug r 1

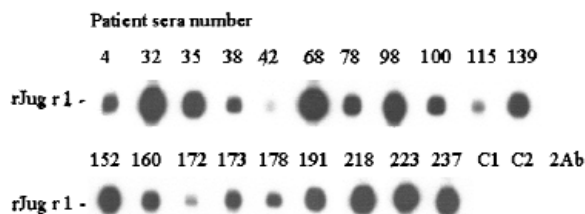
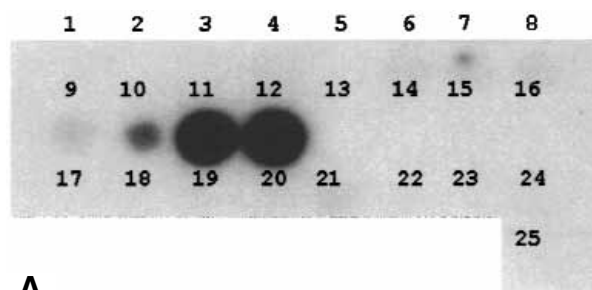


FIG 2. IgE immunoblotting of rJug r 1. C1, Serum from a walnut-allergic patient that does not bind to Jug r 1; C2, serum from an atopic control patient not allergic to walnuts; 2Ab, the secondary antibody alone.



A

SPOT	Amino Acid Sequence
10	RQQQQQLRGEEM
11	QQQLRGEEMEEM
12	GLRGEEMEEMVQS

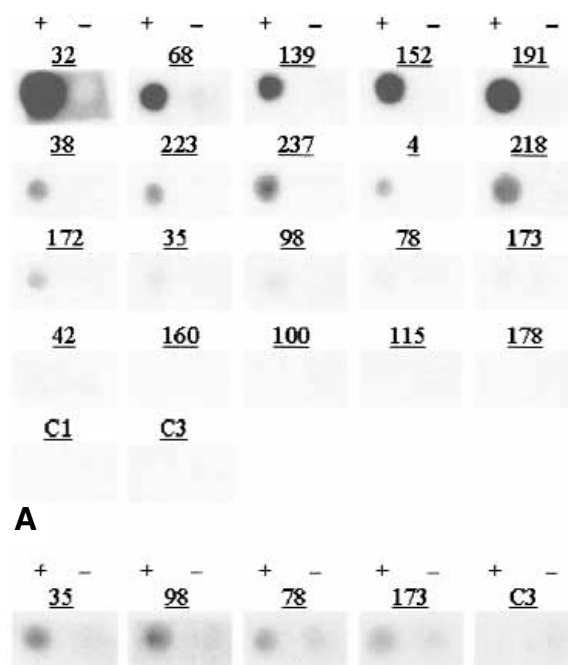
B

FIG 3. IgE binding analysis of Jug r 1. **A**, SPOTs array depicts all 25 overlapping 13-amino acid Jug r 1 large and small subunit peptides probed with pool 1, comprising sera from 6 walnut-sensitive patients. **B**, Sequence comparison of 3 adjacent overlapping peptides—10, 11, and 12—recognized by walnut-allergic sera. The amino acid sequence common to each is indicated (shading).

was previously shown to completely inhibit binding to the native 2S albumin and to substantially inhibit binding of patient sera IgE to English walnut in walnut ImmunoCAP assays (up to 84% of measurable antiwalnut IgE)⁶; the folding thus appears to preserve most of the presumed conformational epitopes present on the native mature protein.

Identification and recognition of IgE-reactive linear epitopes on rJug r 1

The entire lengths of both the large and the small subunits of Jug r 1 were studied by probing overlapping solid-phase synthetic peptides with sera from 20 patients randomly assigned to 4 pools. Each pool recognized 3 adjacent peptides from the large subunit; 2 peptides were recognized very strongly (no. 11 and no. 12) and 1 peptide less so (no. 10); no peptides were identified from the small subunit (Fig 3, A). A common sequence, GLRGEEM, was observed in all 3 large-subunit peptides (Fig 3, B). A fourth partially overlapping sequence (no. 9 in Fig 3, A) showed a slight positive reaction. Some other peptides showed slight positive signals in some assays (eg, no. 7 in Fig 3, A) but were



A

B

FIG 4. Differential binding of IgE from the sera of 20 walnut-allergic patients to the E1 epitope (+, left SPOT) and negative control (-, right SPOT) peptides. **A**, Autoradiograph shows patients' sera binding to the E1 peptide strongly (nos. 32, 68, 139, 152, and 191), moderately (nos. 38, 223, 237, 4, 218, and 172), weakly (nos. 35, 98, 78, and 173), or not at all (nos. 42, 160, 100, 115, and 178). **B**, Longer exposure of weakly reactive sera (nos. 35, 98, 78, and 173). C1, Serum from a walnut-allergic patient that does not react with Jug r 1; C3, serum from an atopic subject not allergic to walnut.

not reproducible, leading us to examine only the identified dominant linear epitope-bearing peptides. Additional peptides were tested in which alanine was substituted at each of the 12 amino residues of the IgE-reactive peptide, QQLRGEEMEEMV. In addition, peptides were tested with varying numbers of alanines substituted at the N-terminus and the C-terminus. Together, these data demonstrated that the core amino acids RGEE, at positions 36 to 39, and an additional glutamic acid residue at position 42, were necessary for maximum IgE binding to occur (Table I).

SPOTs containing a 12-amino acid peptide (QQLRGEEMEEMV) bearing the immunodominant epitope, designated E1, and SPOTs containing a negative control (-) peptide (LSQRSQQCRQ) selected from the large subunit of Jug r 1 were used to test the degree of individual reactivity and specificity of allergic and control sera. Of the 20 sera tested, 5 exhibited strong recognition of the epitope peptide, 6 exhibited moderate recognition, 4 exhibited weak recognition, and 5 showed no recognition; none reacted with the (-) peptide (Fig 4).

Isolation and inhibition of E1-specific IgE

To determine whether the identified epitope E1 is the major epitope recognized by patients' sera, we separated E1-specific IgE from the total antiserum and tested both fractions for reactivity with rJug r 1. Epitope-specific IgE

was isolated from patient serum by passage over an E1-affinity column. To assure removal of anti-E1 antibodies, the 3 sequential eluate and the serum effluent fractions were first assayed against our solid-phase IgE-binding (+) and non-IgE-binding (–) peptides. Fig 5, A shows binding of IgE to the positive (E1) peptide in unfractionated patient serum, progressively less binding in the first 2 eluate fractions, and no binding in the third fraction, indicating complete removal of the E1-reactive antibody by the column. Significantly, the E1-adsorbed serum (effluent) also showed no reactivity to the E1 peptide, confirming removal of E1-specific IgE from the serum. The eluate and effluent fractions were then used in dot-blot assays to determine whether IgE antibodies to the E1 epitope represent the bulk of the rJug r 1-reactive IgE. As expected, there was demonstration of binding of IgE to rJug r 1 from total sera and from the first 2 eluate (anti-E1) fractions but not from the third (anti-E1-depleted) fraction (Fig 5, B). However, there was considerable rJug r 1-specific IgE remaining in the effluent (anti-E1 depleted fraction), demonstrating the presence of additional IgE antibody specific for 1 or more conformational epitopes.

To further examine the specificity of both our peptide-reactive fractions and unfractionated Jug r 1-reactive IgE, a soluble form of the epitope peptide was preincubated with whole patient serum as well as the E1-specific IgE antibodies in an attempt to inhibit their reaction with both our solid-phase peptides and rJug r 1. Varying amounts (70, 7.0, 0.7 μ g) of the peptide were incubated with whole patients' sera before the E1 epitope (positive) and negative solid-phase peptides were probed. Partial to complete inhibition was achieved (Fig 5, C), demonstrating that the solid- and fluid-phase versions of the peptide are similarly recognized.

The amount of soluble peptide (70 μ g) needed to completely inhibit binding of E1-specific IgE to the solid-phase version of this epitope was similarly used for inhibition studies involving unfractionated patient serum IgE and affinity column purified IgE fractions against the rJug r 1. Inhibition was again observed in the eluate (E1 epitope-specific) IgE fractions. These data demonstrate that the epitope is similarly recognized in both the peptide and the recombinant protein. However, IgE reactivity in both whole patient serum and the E1-adsorbed effluent fraction reacted with rJug r 1 (Fig 5, D), again demonstrating the existence of additional (presumably conformational) IgE-reactive epitopes on rJug r 1.

DISCUSSION

On a global basis, walnuts rank second in tree nut production.³¹ In children, anaphylactic reactions to peanut and tree nuts are responsible for most fatal and near-fatal food-allergic reactions.^{3,32} In a recent study, 32 fatal cases of anaphylactic reactions to foods reported to a national registry were analyzed; it was determined that up to 31% were due to tree nuts.³³ The severity and prevalence of some food-induced allergic reactions have prompted investigation to identify and characterize offending allergens.

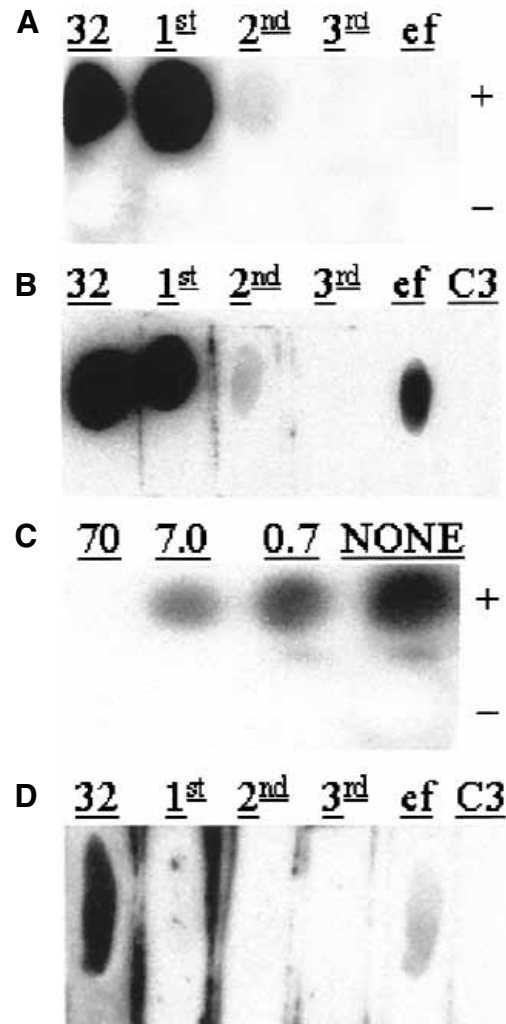


FIG 5. Isolation and inhibition of E1-specific IgE. **A**, Strips containing the E1 epitope peptide (top SPOT, +) and a negative control peptide (bottom SPOT, –) probed with whole patient serum (32), each of 3 E1 eluates (1st, 2nd, 3rd) and the effluent (ef). **B**, Strips dotted with rJug r 1 and probed as in Fig 5, A. C3, Sera from an atopic subject not allergic to walnut. **C**, Strips containing SPOTs as in Fig 5, A and probed with whole patient serum (32) containing varied amounts (in μ g) of soluble E1 peptide. **D**, Strips dotted with rJug r 1 and probed as in Fig 5, B, but with added soluble E1 inhibitor.

Jug r 1, a major allergen in the English walnut, has previously been characterized as a 2S albumin seed storage protein.⁶ 2S albumins are found in almost all edible seeds. In view of the importance of the members of this class of protein as food allergens and the possibility of cross-reactivity among class-related proteins,³⁴ a detailed knowledge of these proteins would be valuable. In particular, given the critical role that allergen-specific IgE plays in the allergic reaction, determination of allergen-specific IgE binding epitopes—be they linear or conformational—appears to be of great importance for a better understanding of the allergenic nature of foods and for possible therapeutic intervention.

We have found a single linear IgE-reactive epitope and have defined its core amino acid residues. To date, no common structural character of linear IgE epitopes has been identified,³⁵ but this could change as more epitope mapping studies are completed. A key step in the allergic reaction is the binding of at least 2 IgE antibodies to a multivalent allergen. The fact that we have found only 1 linear epitope on Jug r 1 is unique in that all previously analyzed allergens have contained multiple linear IgE-binding sites.³⁵ Although we cannot rule out the existence of weakly binding peptides that are undetected by our methods, the affinity of such peptides would be much lower, as indicated by the high intensity of the signal on the epitope E1. Our findings, in combination with the knowledge that allergens must be multivalent to elicit an allergic response, point toward the existence of conformational epitopes on Jug r 1 as well. Although the data are qualitative, it is noteworthy that all 20 patients' sera bound rJug r 1 (Fig 2), whereas only 15 sera bound the linear epitope, many of these exhibiting disproportionately faint binding. This too is evidence that conformational epitopes are relevant and key in IgE binding for some patients' sera. In fact, it has been estimated that most protein epitopes are conformational.³⁶ It is worthwhile to note that the labeling of an epitope as "linear," based on the use of synthetic epitopes, is imprecise because the identified epitope might be a fragmented part of a larger, discontinuous epitope.³⁵

Clinically, insight into the IgE-binding epitopes of allergens, linear or conformational, is extremely useful. Identifying the immunodominant linear IgE-binding epitope of Jug r 1 might lead to better designs for walnut allergy therapy. Currently, there is no generally agreed-on treatment for IgE-mediated food allergy, and complete avoidance of the food is therefore recommended.³⁷ With tree nuts such as walnuts, however, avoidance is often difficult because equipment used for food processing is often shared; the implicated allergen thus might be present in trace amounts or might be added to foods in which its presence is not expected by the consumer. Because tree nut allergies are rarely resolved, reactions can be severe, and accidental ingestion is almost inevitable, life for afflicted patients can be fraught with anxiety.³⁸

Among new approaches to allergen immunotherapy and diagnosis are use of blocking peptides to inhibit the triggering of IgE-mediated hypersensitivity reactions, immunization with specific peptides to alter the type of immune response elicited, allergen reengineering to enhance protective responses, and use of defined peptides in diagnostic assays.⁹⁻¹² A common factor for the application of these approaches is a required knowledge of the amino acids in the IgE-reactive sites of the allergen. We have mapped the major linear IgE epitope of Jug r 1 and identified the critical core amino acid residues within the epitope. Additional studies are planned to identify the conformational epitopes on this allergen.

We thank Rich Peterson for his valuable assistance.

REFERENCES

1. Sampson HA. Food allergy. Part 1: immunopathogenesis and clinical disorders [review]. *J Allergy Clin Immunol* 1999;103:717-28.
2. Yocum MW, Khan DA. Assessment of patients who have experienced anaphylaxis: a 3-year survey. *Mayo Clin Proc* 1994;69:16-23.
3. Sampson HA, Mendelson LM, Rosen JP. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1992;327:380-4.
4. Yunginger JW, Sweeney KG, Sturmer WQ, Giannanadra LA, Teigland JD, Bray M, et al. Fatal food-induced anaphylaxis. *JAMA* 1988;260:1450-2.
5. Breiteneder H, Ebner C. Molecular and biochemical classification of plant-derived food allergens. *J Allergy Clin Immunol* 2000;106:27-36.
6. Teuber SS, Dandekar AM, Peterson WR, Sellers CL. Cloning and sequencing of a gene encoding a 2S albumin seed storage protein precursor from English walnut (*Juglans regia*), a major food allergen. *J Allergy Clin Immunol* 1998;101:807-14.
7. Bock SA, Atkins FM. The natural history of peanut allergy. *J Allergy Clin Immunol* 1989;83:900-4.
8. Sicherer SH, Munoz-Furlong A, Burks WA, Sampson HA. Prevalence of peanut and tree nut allergy in the US determined by a random digit dial telephone survey. *J Allergy Clin Immunol* 1999;103:559-62.
9. Valenta R, Vrtala S, Focke-Tejkl M, Bugajska-Schretter A, Ball T, Twardosz A, et al. Genetically engineered and synthetic allergen derivatives: candidates for vaccination against Type I allergy. *Biol Chem* 1999;380:815-24.
10. van Neerven RJJ. The role of allergen-specific T cells in the allergic immune response: relevance to allergy vaccination. *Allergy* 1999;54:552-61.
11. Bindslev-Jensen C, Poulsen LK. In vitro diagnostic methods in the evaluation of food hypersensitivity. In: *Food allergy: adverse reactions to foods and food additives*. Boston: Blackwell Science Limited; 1997. p. 137-50.
12. Vrtala S, Ball T, Spitzauer S, Pandjaitan B, Suphioglu C, Knox B, et al. Immunization with purified natural and recombinant allergens induces mouse IgG1 antibodies that recognize similar epitopes as human IgE and inhibit the human IgE-allergen interaction and allergen-induced basophil degranulation. *J Immunol* 1998;160:6137-44.
13. Zhao Y, Chait BT. Protein epitope mapping by mass spectrometry. *Anal Chem* 1994;66:3723-6.
14. Dolecek C, Vrtala S, Laeffler P, Steinberger P, Kraft D, Sheiner O, et al. Molecular characterization of Phl p 2, a major timothy grass (*Phleum pratense*) pollen allergen. *FEBS Lett* 1993;335:299-304.
15. Ghosh B, Perry MP, Marsh DG. Cloning the cDNA encoding the Amb t V allergen from giant ragweed (*Ambrosia trifida*). *Gene* 1991;101:231-8.
16. Scheiner O, Kraft D. Basic and practical aspects of recombinant allergens. *Allergy* 1995;50:384-91.
17. Elsayed S, Titlestad K, Apold J, Aas K. A synthetic hexadecapeptide derived from allergen M imposing allergenic and antigenic reactivity. *Scand J Immunol* 1980;12:171-5.
18. Atassi H, Atassi MZ. Antibody recognition of ragweed allergen Ra3: localization of the full profile of the continuous antigenic sites by synthetic overlapping peptides representing the entire protein chain. *Eur J Immunol* 1986;16:229-35.
19. Mazur G, Baur X, Modrow S, Becker WM. A common epitope on major allergens from non-biting midges (Chironomidae). *Mol Immunol* 1988;25:1005-10.
20. van 't Hof W, van Milligen FJ, van den Berg M, Lombardero M, Chapman MD, Aalberse RC. Epitope mapping of the cat (*Felis domesticus*) major allergen Fel d I by overlapping synthetic peptides and monoclonal antibodies against native and denatured Fel d I. *Allergy* 1993;48:255-63.
21. Helm RM, Cockrell G, Connaughton C, West CM, Herman E, Sampson HA, et al. Mutational analysis of the IgE-binding epitopes of P34/Gly m Bd 30K. *J Allergy Clin Immunol* 2000;105:378-84.
22. Helm R, Cockrell G, Herman E, Burks A, Sampson H, Bannon G. Cellular and molecular characterization of a major soybean allergen. *Int Arch Allergy Immunol* 1998;117:29-37.
23. 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.
24. van 't Hof W, Driedijk PC, van den Berg M, Beck-Sickinger AG, Jung G, Aalberse RC. Epitope mapping of the Dermatophagoides pteronyssinus house dust mite major allergen Der p II using overlapping synthetic peptides. *Mol Immunol* 1991;28:1225-32.
25. Walsh BJ, Howden ME. A method for the detection of IgE binding

- sequences of allergens based on a modification of epitope mapping. *J Immunol Methods* 1989;121:275-80.
26. 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.
 27. 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.
 28. Stanley JS, King N, Burks AW, Huang SK, Sampson HA, Cockrell G, Helm RM, West CM, Bannon GA. 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.
 29. 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. *J Clin Invest* 1999;103:535-42.
 30. Frangioni JV, Neel BG. Solubilization and purification of enzymatically active glutathione S-transferase (pGEX) fusion proteins. *Anal Biochem* 1993;210:179-87.
 31. Sze-Tao KWC, Sathe SK. Walnuts (*Juglans regia* L): proximate composition, protein solubility, protein amino acid composition and protein in vitro digestibility. *J Sci Food Agric* 2000;80:1393-401.
 32. Foucard T, Malmheden Yman I. A study on severe food reactions in Sweden—is soy protein an underestimated cause of food anaphylaxis? *Allergy* 1999;54:261-5.
 33. Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001;107:191-3.
 34. Pastorello EA, Farioli L, Pravettoni V, Ispano M, Conti A, Ansaloni R, et al. Sensitization to the major allergen of Brazil nut is correlated with the clinical expression of allergy. *J Allergy Clin Immunol* 1998;102:1021-7.
 35. Bredehorst R, David K. What establishes a protein as an allergen? [review]. *J Chromatogr B Biomed Sci Appl* 2001;756:33-40.
 36. Berzofsky JA. Intrinsic and extrinsic factors in protein antigenic structure. *Science* 1985;229:932-40.
 37. Smith LJ, Muñoz-Furlong A. The management of food allergy. In: Metcalfe DD, Sampson HA, Simon RA, editors. *Food allergy: adverse reactions to foods and food additives*. Boston: Blackwell Science Limited; 1997. p. 431-44.
 38. Sicherer SH, Sampson HA. Peanut and tree nut allergy. *Curr Opin Pediatr* 2000;12:567-73.

Receive tables of contents by e-mail

To receive the tables of contents by e-mail, sign up through our Web site at

<http://www.mosby.com/jaci>

Choose *E-mail Notification*

Simply type your e-mail address in the box and click the *Subscribe* button

Alternatively, you may send an e-mail message to majordomo@mosby.com.

Leave the subject line blank and type the following as the body of your message:

subscribe jaci_toc

You will receive an e-mail message confirming that you have been added to the mailing list.

Note that TOC e-mails will be sent out when a new issue is posted to the Web site.