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Characterization of the Soluble Allergenic Proteins of Cashew Nut (*Anacardium occidentale* L.)

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The allergens associated with cashew food allergy have not been well-characterized. We sought to identify the major allergens in cashew nut by performing IgE immunoblots to dissociated and reduced or nonreduced cashew protein extracts, followed by sequencing of the peptides of interest. Sera from 15 subjects with life-threatening reactions to cashews and 8 subjects who tolerate cashews but have life-threatening reactions to other tree nuts were compared. An aqueous cashew protein extract containing albumin/globulin was separated by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and subjected to IgE immunoblotting using patient sera. Selected IgE reactive bands were subjected to N-terminal amino acid sequencing. Each of the 15 sera from cashew-allergic subjects showed IgE binding to the cashew protein extract. The dominant IgE-binding antigens in the reduced preparations included peptides in the 31−35 kD range, consistent with the large subunits of the major storage 13S globulin (legumin-like protein). Low-molecular-weight polypeptides of the 2S albumin family, with similarity to the major walnut allergen Jug r 1, also bound IgE. The sera from eight patients who tolerate cashew but displayed allergies to other tree nuts showed only minimal or no IgE binding to cashew. Cashew food allergy is associated with the presence of IgE directed against the major seed storage proteins in cashew, including the 13S globulin (legumin group) and 2S albumins, both of which represent major allergen classes in several plant seeds. Thus, the legumin-group proteins and 2S albumins are again identified as major food allergens, which will help further research into seed protein allergenicity.

KEYWORDS: Cashew; 13S globulin; legumin-like protein; 2S albumin; vicilin; sucrose-binding protein; anacardein; food allergy; tree nut allergy

INTRODUCTION

Cashew nuts have been associated with two distinct hypersensitivity reactions. The first, contact or systemic dermatitis, has been linked to cardol and anacardic acid found in the cashew nut shell oil, both of which are related to poison ivy urushiol (*1*). The second type of hypersensitivity reported from North America and Europe is IgE-mediated food allergy; reactions can range from atopic dermatitis to fatal systemic allergic reactions (*2*−*7*). In the voluntary peanut and tree nut allergy registry maintained by the United States-based Food Allergy and Anaphylaxis Network, walnut was the most frequently reported tree nut allergy in 34% of respondents, followed by cashew (20%), almond (15%), pecan (9%), pistachio (7%), and others at less than 5% each (*8*). Cashew nuts are globally popular and are valued for their sensory qualities, especially the unique flavor and the texture. In 2001, the estimated world production of cashews was 1 470 000 metric tons. In 2000, the United States was the largest importer of shelled cashew nuts ($449,800,000), and India was the largest exporter ($418,488,000) (*9*).

Earlier Sathe (*10*) and Sathe et al. (*11*) showed that a multimeric 13S globulin, that was originally described in 1936 and termed "anacardein, dominates the total protein composition of cashew seeds and accounts for about one-half of the total soluble seed proteins (*10*, *11*). Upon ultracentrifugation, the anacardein fraction sediments primarily at 13S but also contains populations of 3S and 6S and a very small population of 16S proteins (*10*). The proteins dissociate upon sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) into multiple bands, with the major cluster of polypeptides in the 46−53 kD range. Upon reduction, two dominant polypeptide populations are seen at 30−37 and 18−24 kD, consistent with electrophoretic behavior typical of the legumin group (11−13S)

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of seed storage proteins (*10*, *11*). Herein, we identify the major allergens involved in cashew nut allergy by immunoblotting using sera from adult patients with long-standing cashew food allergies followed by sequencing of the IgE-binding polypeptides.

MATERIALS AND METHODS

Human Sera. Blood samples were drawn after informed consent from patients with life-threatening systemic reactions to cashew nut, and the sera were frozen at −70 °C until use. The study was approved by the human subjects review committee of the University of California at Davis. Skin testing and oral challenge testing were not approved for these individuals with a history of life-threatening systemic reactions because of safety concerns. Control sera were obtained from patients with a history of anaphylaxis to walnut, pistachio, or hazelnut who reported tolerance of cashews.

Cashew Protein Extracts. Raw cashews were purchased from a local grocery. The cultivar was unknown, as market samples are mixtures based on kernel size. In unpublished work (S. K. Sathe), we have not seen any differences in sodium dodecyl sulfate−polyacrylamide gel electrophoretic profiles between different market samples in Florida and Mumbai (Bombay, India). Two-gram samples were chopped with a razor blade and then added to 12 mL of Buffer D [50 mM Tris-HCl, pH 8.0, 22% v/v glycerol, 1% w/v poly(ethylene glycol) 8000, 7 mM citric acid, 6 mM L-cysteine, 6 mM L-ascorbic acid, and 2 mM ethylenediamine tetraacetic acid] and 1 g polyvinylpolypyrrolidone (*12*). A weak salt buffer such as this is expected to extract a mixture of albumins and globulins. The solution was homogenized on ice using a Polytron PT 3000 instrument (Brinkmann Instruments, Westbury, NY) and spun at 13 000 × *g* for 30 min at 4 °C. The supernatant was passed through a 5.0-μm Millex-SV syringe filter (Millipore, Bedford, MA), concentrated using a Centricon-3 concentrator (Amicon Inc., Beverly, MA), and frozen at −70 °C until use. A 13S globulin-enriched, anacardein extraction was prepared from whole raw cashew nuts ground in a blender and defatted with cold acetone (4 °C, flour-to-acetone ratio of 1:5 w/v). Defatted flour was stored at −20 °C until extracted with 0.1 M Tris-HCl, pH 8.1, and the protein extract was subjected to anion exchange (DEAE DE-53) (Whatman) and gel filtration (Sephacryl S 300) (Amersham Pharmacia Biotech AB, Uppsala, Sweden) column chromatography to yield anacardein as previously described (*11*). Protein concentrations were determined by the Bradford protein assay (BioRad Laboratories, Inc., Hercules, CA) using bovine serum albumin as the standard protein.

Polyacrylamide Gel Electrophoresis (PAGE) and Protein Transfer. Samples were boiled for 5 min in the sample buffer [60 mM Tris-HCl, pH 6.8, 2% SDS, 10% (v/v) glycerol, 0.01% (w/v) bromophenol blue, with or without 100 mM dithiothreitol (DTT)], and electrophoresis was carried out overnight at 8 mA constant current using a SE600 Vertical Slab Gel Unit (Pharmacia Biotech, Piscataway, NJ). SDS gels, 13% monomer acrylamide concentration, with 25 μg of protein/4 mm were used for immunoblotting as previously described (*13*). Parts of the gels were stained with Coomassie Brilliant Blue R (Sigma Chemical Co., St. Louis, MO). Otherwise, proteins were transferred to 0.22-μm nitrocellulose membranes (MSI, Westborough, MA) overnight at 30 V using a TE 42 Transphor Electro-Transfer Unit (Pharmacia Biotech, Piscataway, NJ). For greater resolution of low-molecular-weight proteins, cashew extract was also separated using NOVEX 16% Tricine Gels (Invitrogen,Carlsbad CA) on a Hoefer SE250 Mighty Small Mini-Vertical Electrophoresis Unit and transferred using a Hoefer TE22 Mini Tank Transphor Unit (Amersham Pharmacia Biotech).

Glycoprotein Staining. A nonreduced sample of the globulin-enriched anacardein preparation was loaded on the mini-gel apparatus as above and separated by SDS−PAGE along with a negative control, soybean 11S, and two positive controls, soybean 7S (positive control) and great Northern bean phaseolin (7S), all at 40 and 70 μg/lane. The Gelcode Glycoprotein Staining method (Pierce Chemical Co., Rockford, IL) was used according to the manufacturer's instructions.

IgE Western Immunoblotting. Nitrocellulose membranes containing blotted proteins were cut into 3−4 mm wide strips that were blocked for 1 h at room temperature in phosphate buffered saline (PBS)/3% nonfat dry milk/0.2% Triton X-100 (TX-100). Diluted sera (1:5 v/v in the blocking buffer, but 1:20 v/v was used for highly reactive sera 2, 6, 8, 9, and 14) were added to the strips and incubated overnight at room temperature. Additional strips were incubated with PBS/3% nonfat milk/0.2% TX-100 (buffer control to check for nonspecific binding of the anti-human IgE polyclonal antibodies). The strips were then washed three times for 20 min each in PBS/0.01% TX-100 and incubated overnight at room temperature with equine polyclonal ^{125}I-anti-human IgE (Hycor Biomedical Inc., Garden Grove, CA) diluted 1:5 in the nonfat milk buffer. The strips were washed three times for 20 min each and exposed to X-ray film at −70 °C overnight or up to 72 h.

Amino Acid Sequencing. SDS−PAGE was carried out as above on reduced cashew polypeptides using 10% acrylamide gels for the larger polypeptides and the 16% tricine gels for the low-molecular-weight polypeptides, and proteins were transferred to Trans-Blot PVDF membrane (0.2 μm) (BioRad). The N-terminal sequences were determined using blotted protein on an ABI 477A sequencer with an on-line 120A HPLC system (Applied Biosystems, Inc., Foster City, CA) or on an ABI Procise sequencer. Internal tryptic digests after carboxymethylation were performed on selected bands from wet acrylamide gels, separated by ABI 173 Microbore HPLC, and then sequenced as above. Sequence data were collected utilizing ABI Procise software (Applied Biosystems, Inc.) and analyzed with FASTA programming (European Bioinformatics Institute, http://www2.ebi.ac.uk/fasta3/) and BLAST programming (National Center for Biotechonology Information, National Institutes of Health, Bethesda, MD, http://www.ncbi.nlm.nih.gov/BLAST/).

RESULTS

Patient Characteristics. Patient age, age of onset of cashew allergy, other reported nut allergies, and anti-cashew ImmunoCAP or RAST results are summarized in Table 1, and Table 2 contains descriptions of the control subjects. There were 5 men and 10 women, with a mean age of 39 years. The median age of onset of cashew allergy was 3 years. Other atopic disorders were common among the group. Ten of the 15 had persistent asthma into adulthood of varying severities, and 4 had remission of asthma symptoms. Eight had a history of atopic dermatitis, persisting into adulthood in five. Seven also had a history of peanut allergy. Interestingly, all 15 had coexisting walnut allergies, which could be due in part to recruitment bias because one of the advertisements specifically asked for volunteers with walnut allergy, although other advertisements asked for individuals with any nut or seed allergy. Many patients reported that the first reaction to a tree nut was to walnut. A few recalled being told that cashews should be tolerated because they are in a different tree nut family prior to their anaphylactic reactions to cashew. Fourteen patients reported tree-nut-related emergency room visits (or a reaction that should have precipitated an emergency visit/ambulance in one of those patients) in the past 1−5 years, and the 15th had a recent reaction by trace contact with walnuts. Patient 14 and patient 9 reported that cashews were associated with more severe anaphylactic reactions than the other nuts; patient 14 was hospitalized for over 2 weeks in 1995 after a near-fatal accidental ingestion of cashew in a restaurant.

Initial Characterization of Cashew Extract by SDS−PAGE and Immunoblotting. Figure 1 shows a side-by-side comparison of the total albumin/globulin extract (T) and the globulin-enriched anacardein (A) preparation in Coomassie-stained gels in the nonreduced, dissociated form and in the reduced, dissociated form. Also shown, for comparison, is a corresponding nitrocellulose strip (right lane in each set) in which the anacardein from the same gel was transferred and probed with serum IgE from allergic patient 8 (exposed to film overnight). It can be seen that the total extract contains more

Table 1. Clinical Characteristics of Cashew-Allergic Subjects

patient	gender	age	age of onset of cashew allergy	other atopy	other food allergy	ImmunoCAP or RAST (kU/L or class)
1	M	25	3	asthma	walnut, pecan, hazel	5.66
2	F	57	53	AD,[a] asthma	adult onset to peanut, walnut	81.3
3	F	26	2	AD, AR,[b] asthma	peanut, walnut, pistachio	9.51
4	M	27	5	asthma	walnut, brazil, coconut, hazelnut	6.95
5	F	54	10	AD, AR asthma	walnuts, pecans, hazel	1.62
6	M	55	7	AD, asthma	peanut, tree nuts	class 4
7	F	30	10	AD, AR	peanut, walnut, hazelnut	4.04
8	F	43	1	AD, AR, asthma	peanut, tree nuts	41.9
9	F	35	2	AD, AR, asthma	walnuts, pecans, almond	35.1
10	F	31	2	AR, asthma	walnut, sunflower	4.42
11	M	50	1	AD, AR, asthma	tree nuts	5.19
12	F	26	3	AR, asthma	peanut, tree nuts	2.41
13	F	39	1	AD, AR, asthma	peanut, tree nuts	9.53
14	F	39	5	asthma	pistachio, tree nuts	94.7
15	M	50	2	AR, asthma	tree nuts	1.87

[a] AD = atopic dermatitis. [b] AR = allergic rhinitis.

Table 2. Clinical Characteristics of Cashew-Tolerant Subjects with Life-Threatening Food Allergy to Another Nut/Seed

patient	gender	age	age of onset of other nut allergy	other atopy	food allergy[a]	ImmunoCAP or RAST to cashew (kU/L or class)
16	M	36	4	AR,[b] AD[c]	walnuts, almonds, pecans	0.66
17	F	27	child	AR	walnuts (class 3), macadamias (only throat "irritation" to cashew)	0.43
18	M	31	18	AR, asthma	pistachio (2.29 kU/L) sunflower, mango, cumin	<0.35
19	F	13	1	AD, AR	peanut (58.4 kU/L), walnut (1.43 kU/L), pecan (1.34)	0.39
20	F	42	child	AR, asthma	walnuts (class 3), almonds	class 0
21	M	43	2	AR, AD asthma	peanuts (class 3), walnuts (2.21.kU/L), pecans	class 0
22	M	42	8	AR	hazelnut (class 2)	<0.35
23	F	30	child	AD	peanuts (class 4), walnuts (class 2)	1.26

[a] ImmunoCAP or RAST value if available [b] AR = allergic rhinitis. [c] AD = atopic dermatitis.

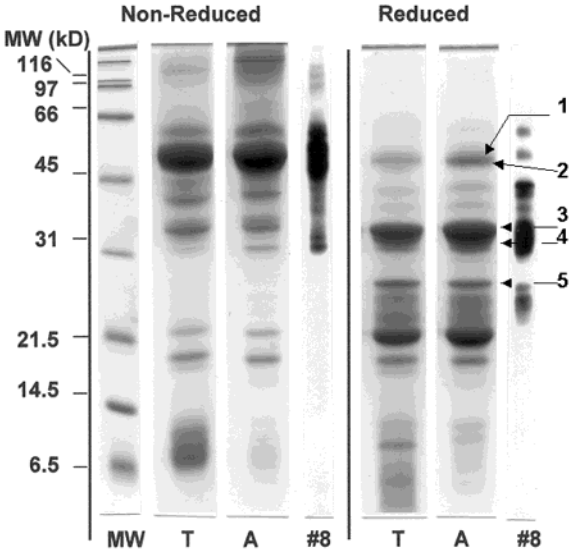

Figure 1. Comparison of nonreduced and reduced cashew proteins by protein staining and immunoblotting. Lane MW: molecular weight markers. Lane T: "total" cashew extract, stained by Coomassie Brilliant Blue R. Lane A: the globulin-enriched anacardein preparation. Lane 8: IgE immunoblot (sera from patient 8) from a lane from the same gel transferred to nitrocellulose. The numbers 1–5 on the right refer to polypeptide bands that were further separated on a 10% acrylamide gel and submitted for sequencing shown in Table 3. Polypeptide band 1 is a thin band just over band 2 that is hard to visualize with this protein load.

low-molecular-weight proteins/polypeptides than are present in the anacardein preparation, but the patterns are otherwise very similar, demonstrating that the anacardein preparation is not an

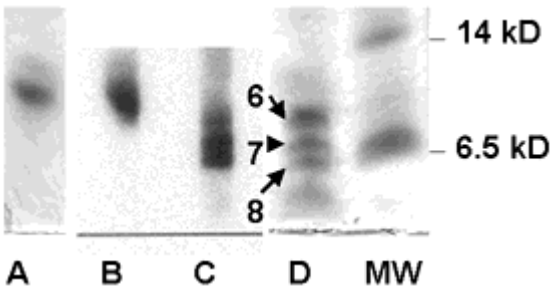

Figure 2. Comparison of nonreduced and reduced low-molecular-weight cashew proteins by protein staining and immunoblotting. Lane A: Coomassie-stained nonreduced cashew extract. Lane B: IgE immunoblot, nonreduced cashew. Lane C: IgE immunoblot, reduced cashew. Lane D: Coomassie-stained reduced cashew extract. Lane MW: molecular weight markers. The numbers 6–8 refer to polypeptide bands submitted for sequencing shown in Table 3.

"immunochemically pure" protein for the purpose of immunocharacterization and further purification steps would be needed to study the globulins separately. To assess the lower-molecular-weight proteins more carefully, Figure 2 contains a side-by-side comparison of the Coomassie-stained 16% tricine gel of total cashew extract polypeptides under 14 kD and a corresponding (proteins transferred from the same gel) immunoblot using sera from allergic patient 14 (exposed to film overnight). When the proteins are reduced and run under these conditions, four main populations of peptides are discerned, of which the upper three appear to bind serum IgE and are identified as 2S albumins below.

Reactivities of IgE toward dissociated, nonreduced total cashew extract using sera from all 15 cashew-allergic patients

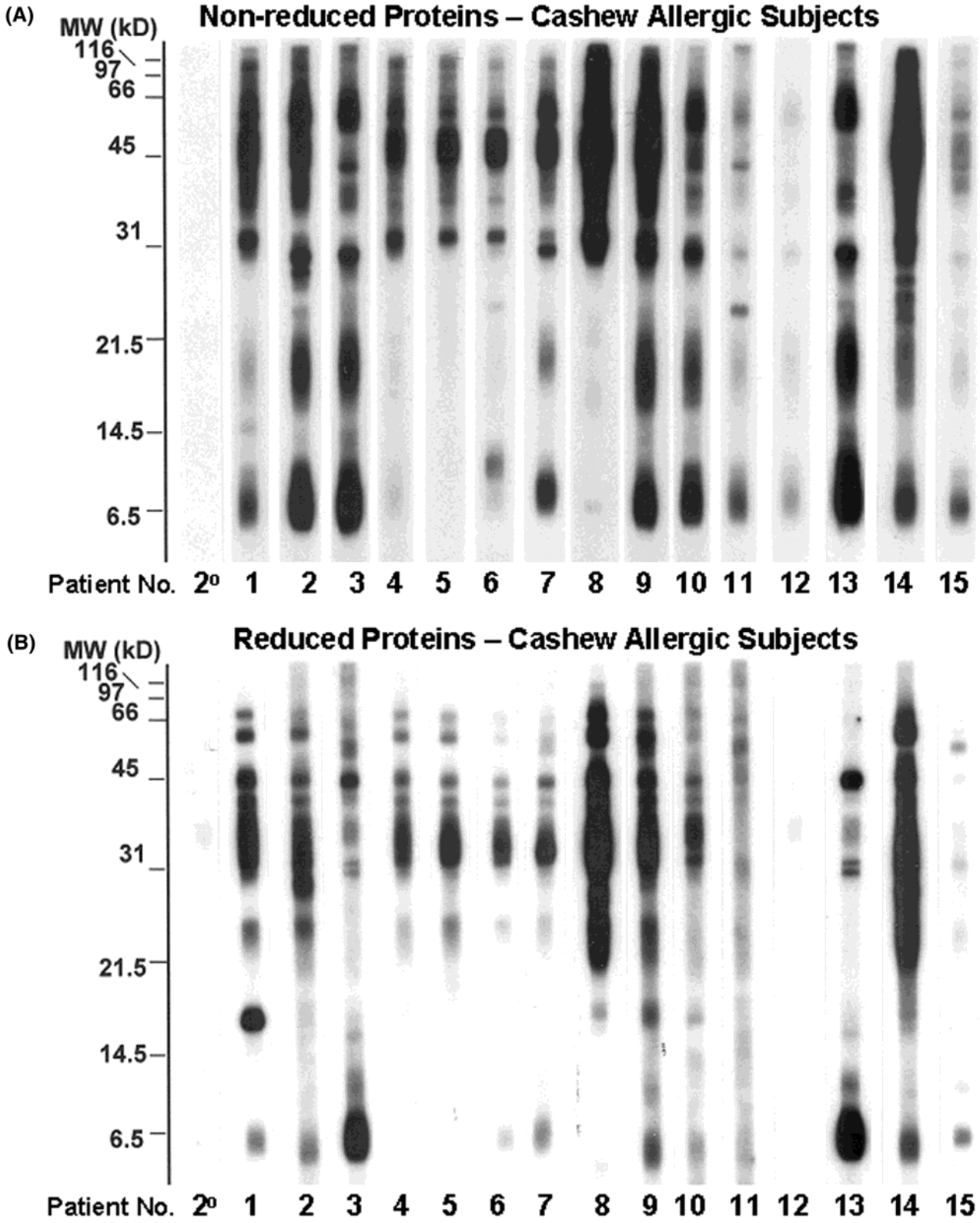

Figure 3. (A) IgE immunoblot of nonreduced cashew proteins using sera from cashew allergic subjects. 2°: secondary antibody alone. The patient numbers correspond to Table 1. (B) IgE immunoblot of reduced cashew proteins using sera from cashew allergic subjects. 2°: the secondary antibody alone. The patient numbers correspond to Table 1.

are shown in Figure 3A. Eleven patient sera (73%) (patients 1−3, 7, 9−15) show IgE binding to the lower-molecular-weight species near the 6.5 kD marker on this 13% acrylamide gel (closer to 12 kD on the 16% tricine gel); thus, the presence of a major allergen here is likely, and the allergen is probably the intact, nonreduced 2S albumin (large and small subunit). Eleven sera out of 15 (73%) show IgE binding to the large protein band-(s) just above the 45 kD marker (see Figure 1, left-hand side of the figure). Sera 3 and 11−13 have faint or absent binding in this region. This is the band region previously identified as the major 13S globulin polypeptide(s) on the basis of sedimentation behavior and stoichiometry upon reduction, and because it binds IgE from more than 50% of allergic sera, it is a major allergen (*10*, *11*). Binding of IgE to the multiple bands seen is not likely

Table 3. N-Terminal or Internal Digest Sequences from Figure 6 Showing Similarity to Databank Proteins

band	N-terminal	tryptic digest fragment	similar to	identity (%)	similarity (%)
1		GLLVPSYNNAPELVYVVQG	castor bean (*Ricinus communis*) legumin-group protein (*28*) Pfam: PF00190 11S globulin (EMBL: AF262998)	79	100
2		AFSWEILEAALK	soy (*Glycine max)* sucrose-binding protein (*29*) Pfam: PF0056 7S globulin and PF02808 7S globulin (EMBL: L06038.1)	66	91
3		LDALEPDNK	broad bean *(Vicia faba)* legumin-group protein (*30*) Pfam: PF00190 11S globulin (EMBL: ×55013)	90	100
4		GLLVPSYNNAPELVYVVQ	castor bean (*Ricinus communis*) legumin-group protein	78	100
5	GLEETICTMT M		thale cress (*Arabidopsis thaliana*) legumin-group protein, 12S (*31*) Pfam: PF00190 11S globulin (EMBL: X14313)	89	100
6	RQESFRECCQQ		English walnut (*Juglans regia*) 2S albumin [allergen Jug r 1 (*16*)] Pfam: PF00234 protease inhibitor/seed storage (EMBL: U66866)	55	91
7	RQESFRECCQELQ L Q		English walnut (*Juglans regia*) 2S albumin	46	92
8	RQESLRECCQEEQ		English walnut (*Juglans regia*) 2S albumin	46	77

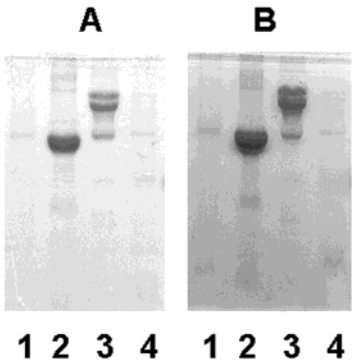

Figure 4. Glycoprotein stain. (A) 40-μg protein load. (B) 70-μg protein load. Lane 1: 13S globulin-enriched anacardein cashew preparation. Lane 2: great Northern bean phaseolin positive control. Lane 3: soybean 7S positive control. Lane 4: soybean 11S negative control.

to be influenced by glycosylation, as Figure 4 shows the lack of sugars detected by a glycoprotein stain that can react with both N- and O-linked residues. Rather, several populations of legumin-group subunits are probably present.

Western IgE immunoblotting to reduced cashew extract is shown in Figure 3B. The films were exposed for the same 72-h period as those in Figure 3A. IgE binding by patient sera 12 to the low-molecular-weight polypeptides is completely lost upon cleavage of disulfide bonds by reduction. IgE binding to this same region of proteins (2S albumins by sequence data below) also appears diminished, but not lost, in blots using sera from patient 1, 2, 7, and 9−11. At least nine sera (60%) show IgE binding to the reduced 2S albumins. Looking back at Figure 1, it can be seen that the major 13S globulin appears to dissociate into two main polypeptide populations, heavy chains at 30−35 kD and light chains at 20−27 kD, as previously described (*10*, *11*). Correspondingly, the patient sera that showed significant IgE binding to the major 13S globulin in Figure 3A now show IgE binding to the globulin heavy chains at 30−35 kD (e.g., most easily seen with sera from patients 4−7). Twelve sera (80%) appear to contain IgE directed to polypeptides here with 11, 12, and 15 basically negative, now suggesting that these subunit chains of the 13S globulin are major allergens. Ten patient sera (67%) show IgE binding of varying intensities to the globulin light chains at 20−27 kD with most binding only the uppermost peptides in this region (not including sera from patients 3, 10, and 11, which appear to have nonspecific background on the reduced immunoblot). Unidentified polypeptides that only faintly bound Coomassie stain, at approximately 43 and 40 kD, bound IgE from 12 of 15 sera (80%).

In contrast to the extensive IgE binding to the reduced, dissociated peptides by cashew-allergic patient sera, sera from patients tolerant of cashew showed very little IgE binding to the reduced total extract (Figure 5). Exceptions are sera from patients 16 and 17 that show IgE binding to a ∼16 kD protein that is also detected by sera from patients 1, and possibly 8−10 and 14. Because this particular IgE-reactive peptide was recognized by sera from two patients who were able to tolerate cashews, this peptide might not contribute to the allergic reaction in cashew-sensitive patients.

Sequence Identification of Cashew Peptides. Table 3 summarizes the results of database searches. Polypeptide band 1 appears to represent an intact subunit of the legumin-like storage protein (also known as 11S globulins, although the ones from cashew sediment at 13S). Likely, the reduction and cleavage of this subunit into heavy and light chains with the reducing agent DTT was incomplete, leaving a very faint band that became more visible at higher protein loads and separation on a 10% acrylamide gel (data not shown). These sequence data were included because it appears from Figure 1 that IgE from patient sera 8 is probably bound to this polypeptide and not the prominent band 2. Polypeptide band 2 is a member of the 7S superfamily of seed storage proteins, which includes vicilin

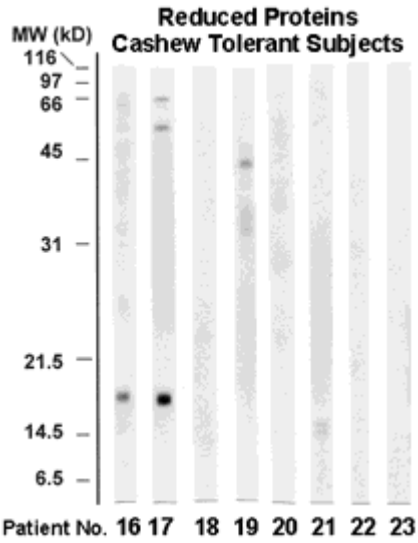

Figure 5. IgE immunoblot of reduced cashew proteins using sera from cashew tolerant subjects. The patient numbers correspond to Table 2.

proteins [e.g., Ara h 1 (*14*) and Jug r 2 (*13*)] and sucrose-binding proteins. Patient sera 3, 7, 9, 11, and 15 appear to show IgE binding to this protein in Figure 3B. Polypeptide bands 3 and 4 are confirmed to belong to the legumin group of proteins and represent heavy chains. Shown are internal digests. By HPLC, bands 3 and 4 were not identical, and hence, by inference, as for previously described legumin proteins from other seeds [pea, for instance (*15*)], there are likely to be several genes responsible for encoding the precursors. Band 5 was analyzed by N-terminal sequencing and is the only light chain of the legumin-like proteins that was subjected to sequencing. In cashew, the 2S albumin homologue of the major walnut allergen Jug r 1 (*16*) appears to have three isoforms, represented by bands 6−8, with slight sequence differences in the N-terminal sequences (which correspond to the large chain).

DISCUSSION

Virtually all of our cashew-allergic patients had clinical allergy to at least one other tree nut (walnut) or to peanut. This might represent a particularly severely affected group of patients, and it will be interesting to compare results with patients who are allergic only to cashew but tolerate walnuts. Such patients are not currently part of our database, which might reflect our geographic location in California where the majority of the U.S. walnut crop is grown, with the subsequent anecdotal clinical observation that, in this location, walnut is one of the first tree nuts introduced to the subjects (e.g., in a cookie). The report of clinical allergy correlated well with the IgE binding on immunoblots. Sera from those nut-allergic patients who tolerate cashews have essentially little evidence of IgE binding on Western blotting and low ImmunoCAP or RAST scores.

In the previous work on cashew, Parra et al. (*17*) noted extensive cross-reactivity between pistachio nut and cashew by CAP inhibition in three patients. Two of the three had never eaten cashews, and one could tolerate cashews (*17*). One of our patients with clear-cut anaphylaxis to pistachios, as well as several episodes of angioedema with nausea and vomiting to mango, could tolerate cashews—this subject (18) showed no IgE binding to cashew on immunoblot or ImmunoCAP, demonstrating that, whereas in vitro/in vivo cross-reactivity among members of the *Anacardaceae* family can occur, some allergenic epitopes within this plant family are species-specific. All of our cashew-allergic patients that had eaten pistachio reported a reaction to pistachio. This overall patient experience indicates that cross-reactivity between tree nuts can be quite complex, suggesting a need for further investigation.

To date, there have been only two publications showing IgE immunoblots to cashew, using sera from a total of five cashew allergic patients (*6*, *18*). Garcia et al. (*6*) showed IgE binding to three unreduced cashew proteins at 15, 30, and 60 kD and loss of reactivity upon reduction, whereas our patient sera showed significant IgE binding to both reduced and unreduced proteins/polypeptides, although one patient sera (12) completely lost reactivity to a low-molecular-weight species upon reduction. Fernandez et al. (*18*) describe immunoblotting using sera from two patients against cashew (although not stated, proteins appear reduced by Coomassie staining) and show several IgE reactive bands at approximately 32 kD, which correspond to the large subunits of the 13S globulin that we also found to be allergenic (*18*).

Using IgE immunoblots and partial amino acid sequencing, we have identified 2S albumins and the legumin-like 13S globulins of cashew as major allergens (being recognized by more than 50% of patient sera). We have also identified the cashew vicilin-group protein, or sucrose-binding protein homologue (7S globulin), as an allergen. Our immunoblotting data suggest that the 7S globulin is qualitatively less important to IgE binding than the 2S albumin and legumin-group proteins. This vicilin-like allergen has been named Ana o 1, and it is the first cashew allergen for which we have cloned the cDNA (*19*). We propose to name the legumin-group allergen Ana o 2 and the 2S albumin allergen Ana o 3. cDNA cloning of Ana o 2 and Ana o 3 are underway. It is not surprising that cashew 2S albumin (yet another 2S albumin) is identified as a food allergen, because several 2S albumins have been shown to be food allergens, including those from walnut [Jug r 1 (*16*)], Brazil nut [Ber e 1 (*20*)], mustard [Sin a 1 (*21*)], sunflower (*22*), cottonseed (*23*), sesame (*24*), and peanut [Ara h 2 (*25*) and Ara h 6 (*26*)]. Characterization of the cashew legumin-group allergens is anticipated to be complex because multiple genes with substantial variation in posttranslational processing of the encoded legumin-group precursors have been described in some plant species (*15*). Judging from the Coomassie-stained gels and the amino acid sequence data on hand, this is likely the case with cashew as well. Legumin-group subunits, each with a heavy and light chain connected by interchain disulfide bonds, are known to associate in a quaternary hexameric structure in the protein body of the seed (*27*). As recombinant proteins become available for study and for use in generating monoclonal and polyclonal antibodies, we will be able to further characterize and immunopurify the major and minor allergens.

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