

Systemic allergic reaction to coconut (*Cocos nucifera*) in 2 subjects with hypersensitivity to tree nut and demonstration of cross-reactivity to legumin-like seed storage proteins: New coconut and walnut food allergens

Suzanne S. Teuber, MD,^{a,b} and W. Rich Peterson, BS^b Pleasant Hill and Davis, Calif

Background: Two patients with tree nut allergy manifested by life-threatening systemic reactions reported the subsequent onset of systemic reactions after the consumption of coconut. **Objective:** Herein, the IgE-binding proteins from coconut are described, and in vitro cross-reactivity with other nuts is investigated.

Methods: The IgE-binding profile of coconut endosperm tissue extract was analyzed by SDS-PAGE followed by immunoblotting. Immunoblot inhibition studies with walnut, almond, peanut, and coconut were performed.

Results: Sera IgE from both patients recognized reduced coconut allergens with molecular weights of 35 and 36.5 kd. IgE from 1 patient also bound a 55-kd antigen. Preabsorption of sera with nut extracts suppressed IgE binding to coconut proteins. Preabsorption of sera with coconut caused the disappearance of IgE binding to protein bands at 35 and 36 kd on a reduced immunoblot of walnut protein extract in 1 patient and suppression of IgE binding to a protein at 36 kd in the other patient.

Conclusion: The reduced coconut protein at 35 kd was previously shown to be immunologically similar to soy glycinin (legumin group of seed storage proteins). The clinical reactivity in these 2 patients is likely due to cross-reacting IgE antibodies primarily directed against walnut, the original clinical allergy reported, and most likely to a walnut legumin-like protein. Coconut allergy in patients with tree nut allergy is rare; these are the first 2 patients ever reported, and therefore there is no general indication to advise patients with tree nut allergy to avoid coconut. (*J Allergy Clin Immunol* 1999;103:1180-5.)

Key words: Coconut allergy, glycinin, legumin, tree nut allergy, food allergy

We recently had the opportunity to study 2 patients who reported repeated systemic reactions after ingestion

Abbreviation used

TX-100: Triton X-100

of coconut (*Cocos nucifera*), a monocotyledonous plant of the family Arecaceae. Immediate hypersensitivity reactions to coconut have not been reported in the literature, although there is a case report of atopic dermatitis in an infant attributed to formula prepared with coconut oils.¹ Previous studies of the major albumin and globulin proteins in coconut endosperm (the edible portion of coconut) have indicated immunologic cross-reactivity with the soy (*Glycine max*) globulins conglycinin and glycinin by IgG antibodies raised against the soy proteins.² Legumes are only distantly related to the palm species.

Legume terminology has been used by some plant geneticists to group the globulin seed storage proteins of all higher plants.³ The 7S globulins from legumes are referred to as vicilins. Vicilin proteins have been given other names in the course of their study: conarachin of peanut (Ara h 1 from *Arachis hypogaea*) and β -conglycinin of soybean.^{3,4} Another globulin fraction from legumes is termed the 11S globulins, comprising legumins.^{3,5} Species-specific names for some legumin group proteins include glycinin in soybean, legumin in peas (*Pisum sativum*), and arachin in peanut (Ara h 3).⁵⁻⁷ The potential for immunologic cross-reactivity, although not necessarily clinically relevant, thus also exists with seed storage proteins of other distantly related dicots, such as the nut-bearing trees, including walnut. Herein, we describe cross-reactivity that does appear to be clinically relevant, although rare, between walnut (*Juglans regia*) and coconut.

METHODS

Human sera

Two male patients, aged 50 (patient 1) and 21 (patient 2) years, reported the onset of systemic allergic reactions to coconut beginning at least several years after the onset of severe sensitivity to tree nuts, walnuts in particular. Both of these patients had similar immediate systemic allergic reactions to walnuts, including angioedema, nausea, vomiting, and asthma, sometimes with hypotension. The reactions to coconut were also multisystemic, although described as not being quite as severe. The life-threatening nature of the respons-

From ^athe Department of Veteran Affairs Northern California Healthcare System, Pleasant Hill; and ^bthe Department of Internal Medicine, University of California, Davis, School of Medicine.

Suzanne S. Teuber was supported in part by National Institutes of Health Clinical Nutrition Research Unit grant DK 3547 and in part by a grant from the Allergy and Immunology Institute of the International Life Sciences Institute Foundation.

The opinions expressed herein are those of the authors and do not necessarily represent the views of International Life Sciences Institute Foundation. Received for publication June 8, 1998; revised Jan 13, 1999; accepted for publication Jan 14, 1999.

No reprints will be available from the authors.

1/1/97168

es precluded food challenges. Both patients had strongly positive results on Pharmacia ImmunoCAP (Pharmacia, Inc, Diagnostics, Columbus, Ohio) to English walnut (food; class 5 and 4, respectively) and coconut (class 4 in both) performed in our university hospital clinical laboratory. Both patients had also experienced reactions to almonds (class 5 and 3, respectively). One patient had reactions to peanut (class 5), although the other patient did not (class 0). Blood samples were drawn after informed consent was obtained, and the sera was frozen at -70°C . The studies were approved by the institutional review board. Skin testing was not approved in these individuals with a history of systemic reactions. Sera was used at a dilution of 1:10, except serum from patient 1, which was diluted 1:80 on the walnut immunoblots.

Protein extracts

Whole coconuts (*Cocos nucifera* L) and unsalted, unprocessed peanuts (*Arachis hypogaea*) were purchased at a local grocery store. Walnuts (*Juglans regia*, cultivar Chandler) and almonds (*Prunus dulcis*) were obtained from the University of California, Davis, Department of Pomology.

Protein extraction of coconut was done following the method of DeMason and Sekhar² (with minor modifications) to have an extracted storage protein profile similar to their results because this was the study that demonstrated immunologic cross-reactivity of some coconut proteins with soy glycinin and conglycinin. After defatting and extraction in 0.06 mol/L Tris-HCl, pH 6.8, with 2% SDS for 4 hours at 4°C , the slurry was centrifuged for 10 minutes at 4°C at 3000g, and the supernatant was passed through a 5.0- μm Millex-SV syringe filter (Millipore, Bedford, Mass) to remove particulates and then through an Extracti-Gel D AffinityPak Column (Pierce Chemical Co, Rockford, Ill) to remove residual SDS. The protein solution was concentrated by using Centricon-3 concentrators (Amicon, Inc, Beverly, Mass).

Tree nut and peanut extracts were extracted by a method developed by Dandekar et al⁸ that precipitates out the potentially interfering polyphenolic compounds and prevents degradation of seed proteins. Walnut, almond, and peanut extracts were made by finely chopping 2 g of nuts with a razor blade and then adding 12 mL of Buffer D (50 mmol/L Tris-HCl, pH 8.0, 22% vol/vol glycerol, 1% wt/vol polyethylene glycol 8000, 7 mmol/L citric acid, 6 mmol/L L-cysteine, 6 mmol/L L-ascorbic acid, and 2 mmol/L EDTA) and 1 g polyvinylpyrrolidone.⁸ The solution was homogenized on ice with a Polytron PT 3000 (Brinkmann Instruments, Westbury, NY) and spun at 13,000g for 30 minutes at 4°C . The supernatant was passed through a 5.0- μm Millex-SV syringe filter (Millipore) and concentrated by using a Centricon-3 concentrator (Amicon).

Protein concentration determinations

Protein concentration of the coconut, peanut, walnut, and almond extracts were measured with the Bradford protein assay (BioRad Laboratories, Inc, Hercules, Calif)

SDS-PAGE and protein transfer

Samples were boiled for 5 minutes in sample buffer (60 mmol/L Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 100 mmol/L dithiothreitol, and 0.01% bromophenol blue), and electrophoresis was carried out overnight at 8 mA constant current with a SE600 Vertical Slab Gel Unit (Pharmacia Biotech, Piscataway, NJ). A 12% SDS-polyacrylamide Laemmli gel was used. A nonreduced sample (without dithiothreitol) was also subjected to SDS-PAGE. Part of the gel was stained with Coomassie Brilliant Blue. Otherwise, proteins were transferred to 0.22- μm nitrocellulose membranes (MSI, Westborough, Mass) overnight at 30 V with a TE 42 Transphor Electro-Transfer Unit (Pharmacia Biotech).

Immunoblotting against coconut extract and absorption of human sera with nut extracts

The reduced protein coconut blot was cut into 4 mm-wide strips containing 25 μg of protein per strip. Strips were blocked for 1 hour at room temperature in PBS/3% nonfat dry milk/0.2% Triton X-100 (TX-100). Sera, which had incubated overnight at room temperature in PBS/3% nonfat milk/0.2% TX-100 and 400 $\mu\text{g}/\text{mL}$ nut or coconut extract, were added to the strips and incubated overnight at room temperature. Additionally, nonabsorbed sera, PBS/3% nonfat milk/0.2% TX-100 (buffer control), atopic control sera, and patient sera absorbed with an overload of 13 mg/mL ovalbumin (Sigma Chemical Co, St Louis, Mo; an irrelevant protein control) were also incubated with strips. In previous titration experiments, a coconut protein concentration of 200 $\mu\text{g}/\text{mL}$ did not consistently absorb out all IgE binding against coconut on a homologous absorption, but 400 $\mu\text{g}/\text{mL}$ was found to be sufficient. Therefore this same concentration was used for the other nut extracts and to look at relative in vitro cross-reactivity. Strips from the nonreduced coconut blot were incubated with sera from the 2 patients, atopic control sera, and a buffer control. The strips were then washed for 20 minutes 3 times in PBS/0.01% TX-100 and incubated overnight at room temperature with ^{125}I -antihuman IgE (Sanofi Diagnostics/Pasteur, Chaska, Minn) diluted 1:5 in the nonfat milk buffer. The strips were washed for 20 minutes 3 times and exposed to x-ray film at -70°C for 72 hours. Absorptions were repeated as follows: 2 times with 400 $\mu\text{g}/\text{mL}$ nut extracts (coconut, walnut, peanut, almond) and once with a high overload of 13 mg/mL (ovalbumin, walnut, peanut, almond), which did not change the outcomes.

Absorption of human sera with coconut extract and immunoblotting against walnut

The walnut blot was cut into 4 mm-wide strips containing 37.5 μg protein per strip. The strips were blocked for 1 hour at room temperature in PBS/3% nonfat dry milk/0.2% TX-100. Sera that had incubated overnight at room temperature in PBS/3% nonfat dry milk/0.2% TX-100 and coconut or nut extract were added to the strips and incubated overnight at room temperature. The same 400 $\mu\text{g}/\text{mL}$ nut extract concentration was used for this set of absorptions as for the coconut immunoblots. At this amount, coconut was able to absorb out most or all IgE binding against walnut in patient 2 but only a faint band at 36 kd seemed to be diminished in the serum IgE binding pattern of patient 1. Therefore the antigen amount was increased to 600 $\mu\text{g}/\text{mL}$ to determine whether IgE binding could be further decreased. Overall, sera was preabsorbed with coconut and walnut in replicated experiments at concentrations of 400 $\mu\text{g}/\text{mL}$ 3 times, and 600 $\mu\text{g}/\text{mL}$ twice. The coconut extract was not concentrated enough to use amounts higher than this. Sera was preabsorbed with peanut and almond at 600 $\mu\text{g}/\text{mL}$ in 2 separate experiments with duplicate results. Additionally, nonabsorbed sera, PBS/3% nonfat milk/0.2% TX-100 (buffer control), atopic control sera, and patient sera absorbed with 600 $\mu\text{g}/\text{mL}$ ovalbumin (Sigma Chemical Co; an irrelevant protein control) were also incubated with strips. The strips were washed for 20 minutes 3 times at room temperature and incubated with ^{125}I -antihuman IgE (Sanofi Diagnostics Pasteur) as earlier. The strips were washed and exposed to x-ray film at -70°C for 3 days.

RESULTS

SDS-PAGE

SDS-PAGE with the protein extraction method used, which is expected to pull out a variety of albumins and globulins, resulted in 14 bands visible on the reduced

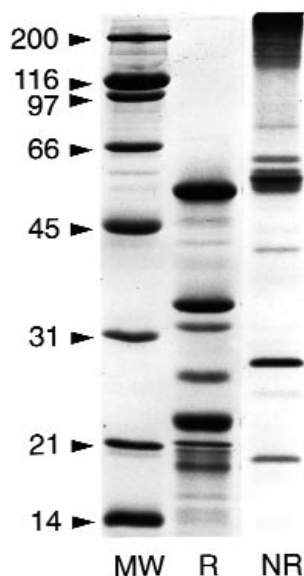


FIG 1. Coomassie-stained SDS-PAGE of coconut extract. *MW*, Molecular weight markers; *R*, reducing conditions (dithiothreitol present); *NR*, nonreducing conditions.

protein gel, with approximate molecular weights of 14, 15, 17, 18, 19, 20, 21, 23, 27, 32, 35, 43, 46, and 55 kD (Fig 1, *Lane R*). The major bands were at 55, 35, 32, 23, 21, 20, and 18 kD. The nonreduced gel had a large smear at the top of the gel and bands at about 75, 62, 58, 56, 53, 42, 29, and 19 kD (Fig 1, *Lane NR*). The results obtained are very similar to previously reported protein distributions from coconut.^{2,9-12}

Immunoblotting

Only faint binding was evident on the nonreduced coconut protein immunoblot at approximately 50 to 60 kD in patient 2 and several bands between 50 and 75 kD in patient 1 along with binding to high molecular weight proteins at the top of the gel (Fig 2). IgE from both patient sera bound with proteins at 35 and 36.5 kD on the reduced immunoblot, although the serum from patient 1 also demonstrated IgE binding to a protein band at 55 kD (lanes labeled *N* in Fig 3). The protein bands at 35 and 55 kD correlated with the visible bands on a Coomassie-stained strip from the same gel (data not shown; Fig 1 also gives this information), but there was no corresponding band on a Coomassie-stained gel for the 36.5 kD allergen. Therefore it is most likely that, quantitatively, this allergen is present in much smaller amounts in coconut endosperm and could represent an isoform or differentially glycosylated form of the 35 kD protein. Fig 3 (*lane Ov*) further shows that absorption of sera with the irrelevant protein, ovalbumin, did not remove any IgE reactivity against coconut, as expected. Control atopic sera and the buffer control (secondary antibody alone) did not show any IgE binding (data not shown). Absorption of sera with either coconut (*lane C*) or walnut extract (*lane W*) completely removed IgE binding to coconut in both patients. Absorption with almond (*lane A*) again

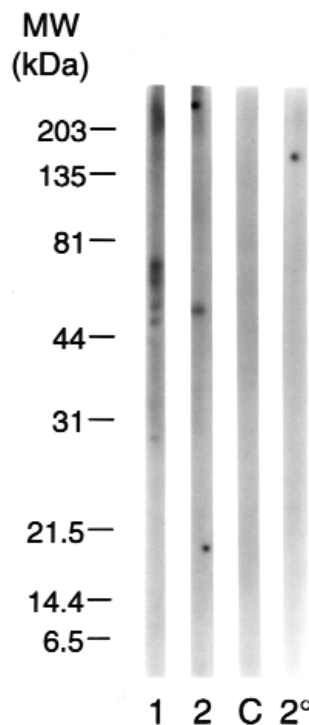


FIG 2. IgE immunoblot of coconut extract under nonreducing conditions. *Lane 1*, Patient 1; *lane 2*, patient 2; *lane C*, control atopic sera; *lane 2°*, secondary antibody only (¹²⁵I anti-human IgE). Only faint banding is present in both patients.

completely abolished binding in patient 2; in patient 1, this preabsorption removed IgE binding to the 36.5 kD protein and almost completely abolished IgE binding to the 35 kD protein and the 55 kD protein. Absorption with peanut (*lane P*) again completely removed IgE binding in patient 2 (who is not allergic to peanut); in patient 1, this preabsorption removed binding to the 35 and 36.5 kD antigens and removed nearly all IgE binding to the 55 kD antigen.

In Fig 4, the reverse absorptions are shown: patient sera was absorbed with coconut and other nuts and then incubated with a walnut blot to see which proteins were immunologically cross-reactive. On the nonabsorbed serum strips (*lanes N*), serum IgE from patient 1 showed dominant binding to bands at 35, 36 (faint), 45, 50, and 66. Absorption of the serum with ovalbumin (*lane Ov*) had no effect on binding. Absorption with 200, 400, or 600 μ g/mL walnut removed all IgE binding (*lane W*; shown is absorption with 600 μ g/mL), although absorption with up to 600 μ g/mL coconut (*lane C*; the absorption shown is with 600 μ g/mL) only removed binding to the 36 kD antigen and faint blurs between 50 and 66 kD. Absorption of the serum with almond (600 μ g/mL; *lane A*) showed results similar to that of the coconut preabsorption: the band at 36 kD was removed, although peanut preabsorption (600 μ g/mL; *lane P*) removed almost all IgE binding except at 66 and 45 kD. Control atopic sera and the secondary antibody showed no IgE binding (data not shown). Patient 2 (*lane N*) also showed

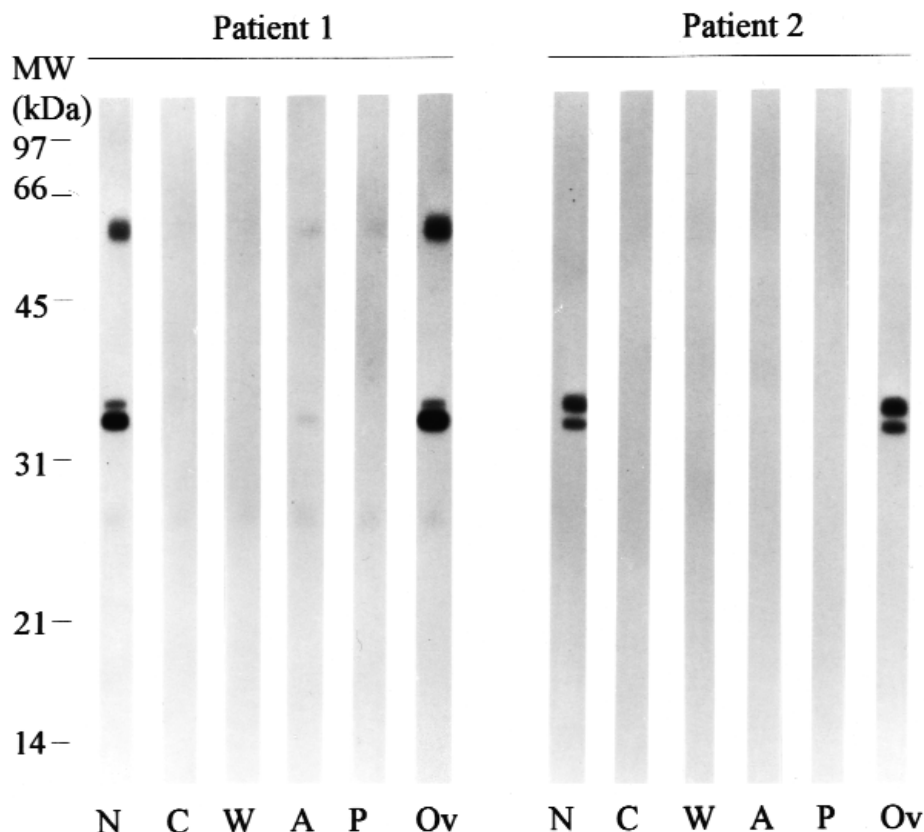


FIG 3. Coconut IgE immunoblot under reducing conditions and preabsorption of sera with extracts from walnut, almond, peanut, or ovalbumin. Results for patient 1 and patient 2 are shown separately as indicated. *N*, No other nut extract added, standard reduced immunoblot against coconut extract; *C*, sera preabsorbed with coconut extract; *W*, sera preabsorbed with walnut extract before incubation with the coconut extract strip; *A*, sera preabsorbed with almond kernel extract; *P*, sera preabsorbed with peanut kernel extract; *Ov*, sera preabsorbed with ovalbumin, an irrelevant protein control. Control atopic sera and the secondary antibody alone (buffer control) showed no reactivity and are not shown.

IgE binding to proteins at 35 and 36 kd. Absorption with walnut, coconut, almond, and peanut removed all IgE binding. However, in 2 of the replicates, one with 200 and one with 400 $\mu\text{g/mL}$ of coconut (not shown), absorption with coconut removed binding at 36 kd and greatly diminished, but did not entirely remove, IgE binding to the antigen at 35 kd. Attempts to obtain N-terminal sequence of the 35 and 36 kd bands in reduced walnut extract were unsuccessful because both were blocked.

DISCUSSION

Immediate hypersensitivity reactions to coconut are apparently rare and have never been reported in the literature. There certainly is not a high enough incidence to counsel patients with nut allergy to avoid coconut. However, the possibility that a particular patient may have IgE in a high enough titer or with particular epitope binding affinities that he or she may react to other seed storage proteins from diverse species must always be kept in mind. In this study, which is limited by the fact that we only have 2 sera because of the rarity of coconut allergy,

we have shown that the 55, 36.5, and 35 kd allergens on a reduced coconut immunoblot are immunologically cross-reactive with seed storage proteins from walnut, almond, and peanut. This is not surprising, considering that DeMason and Sekhar² previously showed that polyclonal antibodies prepared against soybean 11S globulin (glycinin, the legumin storage protein) very faintly recognize the coconut 32 kd and very strongly recognize the 35 kd proteins on a reduced blot. Our extraction technique was expected to pull out both albumins and globulins, and indeed our SDS-PAGE Coomassie-stained gels show that the albumins and globulins described by others are represented.^{2,10-13} It is likely that homologues of the various seed storage proteins exist in almost all seeds, albeit in differing amounts and with, at times, vastly different primary structures.

Also, it should be noted that the 35 kd protein discussed has been shown to be a subunit derived on dissociation and reduction of the coconut 11S globulin, which on the basis of its physical and biochemical characteristics is felt to be similar to the other described 11S globulins (legumin group).¹³ In coconut, this large globulin

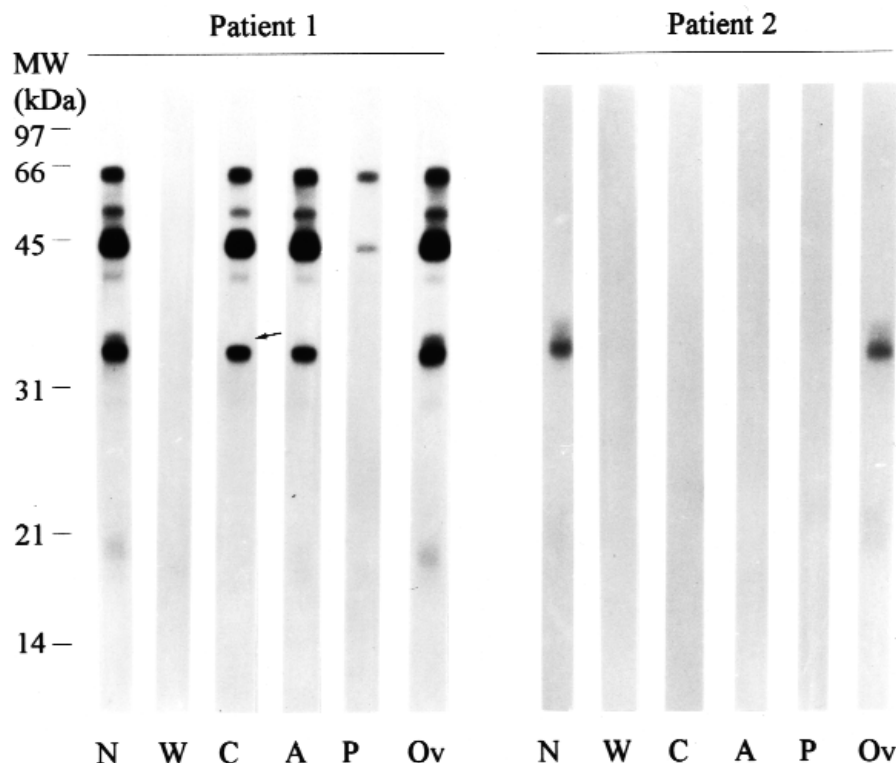


FIG 4. Walnut kernel IgE immunoblot under reducing conditions and preabsorption of sera with nuts, coconut, or ovalbumin. Results for patient 1 and patient 2 are shown separately as indicated. *N*, No preabsorption of sera with another antigen (standard IgE immunoblot against walnut kernel); *W*, sera preabsorbed with walnut extract; *C*, sera preabsorbed with coconut extract (the arrow indicates the absence of a faint band at 36 kd); *A*, sera preabsorbed with almond extract; *P*, sera preabsorbed with peanut extract; *Ov*, sera preabsorbed with ovalbumin. Control atopic sera and the secondary antibody alone (buffer control) showed no reactivity and are not shown.

was originally described in 1930 and named cocosin.¹⁴ Studies by Carr et al¹³ have shown that cocosin is a hexamer of molecular weight around 300 kd with each approximate (heterogenous) 54 kd subunit comprising an acidic and basic chain linked by a disulfide bond, which on reduction migrate as bands at about 35 and 32 kd (acidic chains) and a complex around 22 kd (basic chains). Fast protein liquid chromatography anion-exchange chromatography under dissociating conditions showed that at least 8 different subunit species were present in these bands, which could represent subunits encoded by different genes in a legumin family or post-translational processing differences.¹³ DeMason and Sekhar² could not demonstrate any cross-reactivity between polyclonal anti-soy glycinin antibodies and the 22 kd complex, implying that only the 35 and 32 kd acidic subunits were cross-reactive across the 2 species, but the 32 kd subunit was only weakly cross-reactive. Sera from neither patient 1 nor patient 2 showed IgE binding to the 22 kd complex or the 32 kd protein, supporting the previous results. The exact nature of the 36.5 kd band is not known, because this only showed up on an immunoblot and not on a Coomassie stained gel.

Interestingly, DeMason and Sekhar² also could not demonstrate immunologic cross-reactivity between the

55 kd coconut protein (reducing conditions) and soybean proteins. Serum IgE from patient 1 bound to this 55 kd protein, and we note that walnut extract completely abolished binding against this protein, although almond and peanut extracts almost completely removed IgE binding to it. At the time, DeMason and Sekhar postulated that the 55 kd protein may be unique to coconut, but from our work it appears that a homologue is present in tree nuts and probably peanuts. We would venture to say that a homologue is also present in soy but that key sequence elements are different that affect polyclonal IgG binding epitopes. The 55 kd protein on a reduced blot is definitely not a subunit of the coconut 11S globulin protein (the legumin group protein, cocosin) based on the work of Carr et al.¹³

It is interesting that peanut extract was able to absorb out the IgE activity in patient 2 against coconut and walnut. This implies that there are cross-reactive IgE epitopes on peanut legumin subunits, but the affinity to peanut may be weaker or fewer epitopes are present, because this patient has no clinical reactivity against peanut, and in fact, was negative on ImmunoCAP assay.

The genes encoding the walnut vicilin-like protein (Jug r 2; an approximate 45 kd band on a reduced immunoblot) and the 2S albumin complex (Jug r 1; 13 kd

on a nonreduced immunoblot and approximately 9 and 5 on a reduced blot) have been cloned and sequenced.^{15,16} We saw no cross-reactivity against these 2 protein species when sera was preabsorbed with coconut in patient 1, who experienced a reaction with both the vicilin and 2S albumin (note the lack of absorption of the 45 kd band for patient 1 in Fig 4, data not shown for the 2S albumin, which is not visible on this particular blot). However, both patient sera react with allergens at 35 and 36 kd on a reduced walnut blot (Fig 4, lanes N), and we infer (from immunoblot inhibition results from patient 2) that these proteins are probably both legumin-like. To confirm this finding, work is underway to sequence these proteins in walnut, which are both end-blocked on initial sequencing attempts, and to clone genes encoding members of the legumin family in walnuts.

In summary, it appears that coconut and walnut legumins are food allergens and that clinically relevant cross-reactivity can occur between tree nut species. More patients hypersensitive to coconut are needed to conclude whether the allergens described herein are major or minor. In these 2 cases, the patient histories and results of immunoblot inhibition point to walnut hypersensitivity as the primary allergy. This can be stated in particular for patient 1, because walnut extract was able to consistently absorb out all binding of his serum IgE to coconut proteins, but this was not so in the reciprocal absorption. IgE binding to almost all walnut allergens except a protein at 36 kd (to which IgE binding is barely visible) was unaffected by preabsorption of serum with coconut extract, indicating that coconut protein probably possesses only limited epitopes that can bind primarily walnut-directed IgE so that walnut could quench all IgE-binding sites on coconut, but not vice versa.

REFERENCES

- Couturier P, Basset-Stehme D, Navette N, Sainte-Laudy J. A case of coconut oil in an infant: responsibility of "maternalized" infant formulas. *Allerg Immunol* 1994;26:386-7.
- DeMason, DA, Sekhar CKN. Electrophoretic characterization and immunological localization of coconut (*Cocos nucifera* L) endosperm storage proteins. *Bot Gaz* 1990;151:302-13.
- Boroto K, Dure L. The globulin seed storage proteins of flowering plants are derived from two ancestral genes. *Plant Mol Biol* 1987;8:113-31.
- Burks AW, Cockrell G, Stanley JS, Helm RM, Bannon GA. Recombinant peanut allergen *Ara h* I expression and IgE binding in patients with peanut hypersensitivity. *J Clin Invest* 1995;96:1715-21.
- Argos P, Narayana SVL, Nielsen NC. Structural similarity between legumin and vicilin storage proteins from legumes. *EMBO J* 1985;4:1111-7.
- Lawrence MC, Izard T, Beuchat M, Blagrove RJ, Colman PM. Structure of phaseolin at 2.2 Å resolution: implications for a common vicilin/legumin structure and the genetic engineering of seed storage proteins. *J Mol Biol* 1994;238:748-76.
- Rabjohn P, West CM, Helm E, Helm R, Stanley JS, Huang SK, et al. Glycinin, a third major peanut allergen identified by soy-adsorbed serum IgE from peanut sensitive individuals [abstract]. *J Allergy Clin Immunol* 1998;101:S240.
- Dandekar AM, Vail PV, Uratsu SL, Dumanoglu H, Tebbets JS, McGranahan G, et al. Vector development for the introduction and regulation of novel transgenes in walnut plants. *Walnut Research Reports* 1993;42-58.
- Voigt J, Wrann D, Heinrichs H, Biehl B. The proteolytic formation of essential cocoa-specific aroma precursors depends on particular chemical structures of the vicilin-class globulin of the cocoa seeds lacking in the globular storage proteins of coconuts, hazelnuts and sunflower seeds. *Food Chem* 1994;51:197-205.
- Rasyid F, Manullang M, Hansen PMT. Isolation and characterization of coconut protein. *Food Hydrocolloids* 1992;6:301-14.
- Balachandran C, Armughan C. Solubility and electrophoretic characteristics of proteins in different regions of coconut endosperm. *J Food Sci Technol* 1992;29:91-6.
- Wallace RW, Dieckert JW. Isolation of coconut storage proteins by polyacrylamide-gel electrophoresis. *Anal Biochem* 1976;75:498-508.
- Carr HJ, Plumb GW, Parker ML, Lambert N. Characterization and crystallization of an 11S seed storage globulin from coconut (*Cocos nucifera*). *Food Chem* 1990;38:11-20.
- Sjogren B, Szychalski R. The molecular weight of cocosin. *J Am Chem Soc* 1930;52:4400-4.
- 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.
- Teuber SS, Dandekar AM, Uratsu SL, Haapanen LAD. Cloning and sequencing of one of the walnut (*Juglans regia*) food allergens [abstract]. *J Allergy Clin Immunol* 1995;95:332.