

Tree Nut Allergens

Kenneth H. Roux^a Suzanne S. Teuber^b Shridhar K. Sathe^c

^aDepartment of Biological Science and Institute of Molecular Biophysics, Florida State University, Tallahassee, Fla.,

^bDepartment of Internal Medicine, School of Medicine, University of California, Davis, Calif.,

^cDepartment of Nutrition, Food and Exercise Sciences, Florida State University, Tallahassee, Fla., USA

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Abstract

Allergic reactions to tree nuts can be serious and life threatening. Considerable research has been conducted in recent years in an attempt to characterize those allergens that are most responsible for allergy sensitization and triggering. Both native and recombinant nut allergens have been identified and characterized and, for some, the IgE-reactive epitopes described. Some allergens, such as lipid transfer proteins, profilins, and members of the Bet v 1-related family, represent minor constituents in tree nuts. These allergens are frequently cross-reactive with other food and pollen homologues, and are considered panallergens. Others, such as legumins, vicilins, and 2S albumins, represent major seed storage protein constituents of the nuts. The allergenic tree nuts discussed in this review include those most commonly responsible for allergic reactions such as hazelnut, walnut, cashew, and almond as well as those less frequently associated with allergies including pecan, chestnut, Brazil nut, pine nut, macadamia nut, pistachio, coconut, Nangai nut, and acorn.

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Introduction

We have recently published a review on tree nut allergy from the clinical perspective [1]. Here we wish to focus on what is known regarding the specific allergens from a biochemical and immunological point of view. Though not the focus of this review, any discussion of food allergens must acknowledge the role that food processing conditions, food matrices, and resistance or susceptibility to enzymatic digestion and pH extremes upon consumption may play in the sensitization and effector stages in allergy. There are several recent reviews covering these topics in some detail [2–5].

Allergies to tree nut (and peanut) generally begin in childhood but unlike allergies to a number of other foods, persist throughout life [6, 7]. However, it is likely, though not yet proven, that some patients with an early onset of milder forms of allergy will become tolerant of tree nuts after a period of avoidance, analogous to what has recently been shown for a minority of peanut-allergic patients [reviewed in ref. 8]. The seriousness of tree nut allergy is indicated by the fact that all of the fatal allergic reactions to foods for individuals over the age of 6 reported to a US national registry have been caused by either peanuts or tree nuts [9].

Most tree nut allergens identified to date are seed storage proteins such as the vicilins (7S trimeric globulins composed of ~50-kD subunits), legumins (11–13S hex-

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Correspondence to: Dr. Kenneth H. Roux
Biology Unit I, Department of Biological Science
Florida State University
Tallahassee, FL 32306-4370 (USA)
Tel. +1 850 644 5037, Fax +1 850 644 0481, E-Mail roux@bio.fsu.edu

Table 1. Recognized tree nut allergens¹

Name	Allergen designation	Function/class	kD	Seq. ² source	Accession No.	Reference
<i>Castanea sativa</i>						
Chestnut	Cas s 5	chitinase Ib	9.7	P	CAA64868	Allona et al., unpubl. [79]
	Cas s 8	lipid transfer protein			N/A	
<i>Corylus avellana</i>						
Hazelnut	Cor a 1	Bet v 1 homologue	17.4	C	AF136945	[23]
	1.0401				AF323973	[23]
	1.0402				AF323974	[23]
	1.0403				AF323975	[23]
	1.0404	profilin	14	C	AF327622	Leuttkopf et al., unpubl.
	Cor a 2	lipid transfer protein	9	C	AF329829	Schocker et al., unpubl.
	Cor a 8	11S globulin-like protein	40	C	AF449424	[41]
	Cor a 9	7S vicilin-like prot.	48	C	AF441864	Lauer et al., unpubl.
<i>Bertholletia excelsa</i>						
Brazil nut	Ber e 1	2S albumin	9	C	P04403, M17146	[112]
	Ber e 2	legumin-like (11S) seed storage protein	29	C	AY221641	
<i>Juglans nigra</i>						
Black walnut	Jug n 1	2S albumin	19	C	AY102930	[92]
	Jug n 2	vicilin-like (7S) protein	56	C	AY102931	[52]
<i>Juglans regia</i>						
English walnut	Jug r 1	2S albumin	14	C	U66866	[22]
	Jug r 2	7S vicilin-like prot.	44	C	AF066055	[48]
	Jug r 3	lipid transfer protein	9	P		Pastorello, unpubl.
	Jug r 4	legumin-like (11S) seed storage protein		C		[51]
<i>Anacardium occidentale</i>						
Cashew nut	Ana o 1	vicilin-like (7S) protein	50	C	AF395894	[24]
	1.0101				AF395893	
	1.0102					
	Ana o 2	legumin-like (11S) seed storage protein		C	AF453947	[57]

¹ Extracted, in part, from the International Union of Immunological Societies, Allergen Nomenclature Sub-committee web site, <http://www.allergen.org/List.htm>.

² P = Protein, C = cDNA.

americ globulins with subunits composed of 30- to 40-kD acidic and 17- to 20-kD basic peptides), and 2S albumins ~ 15 kD with ~ 9 and ~ 5 kD subunits (generally). Vicilins appear to have defense-related properties as well. For instance, cowpea (*Vigna unguiculata*) vicilin seed storage proteins have been shown to bind to chitin which may result in inhibition of fungal growth and insect predation [10, 11]. Although not known to be an allergen, *Macadamia integrifolia* vicilin has been shown to have anti-bacterial properties [12]. The vicilins and legumin-like proteins are members of the cupin superfamily characterized structurally in plants by their β -barrel conformation and acidic and basic chain subunits [13]. The 2S

albumins (members of the prolamin superfamily [14]) and the structurally related lipid transfer proteins (LTPs, 7–9 kD) are also considered to be defense-related proteins [15]. The LTPs, as the name implies, are believed to be involved in lipid exchange between cellular membranes and are also involved in cutin biosynthesis [16, 17]. Other tree nut allergens include profilins and hevein-related proteins. Profilins, heveins, and LTPs are considered panallergens because of their contribution to the allergenicity of a wide variety of pollens, nuts, seeds, fruits, and vegetables and their propensity for exhibiting a significant degree of IgE-mediated cross-reactivity [18, 19]. One feature shared in common between many of the proteins

responsible for life-threatening reactions to tree nut (e.g., vicilins, legumin-like proteins, LTPs, and heveins) is a general resistance to proteolysis and denaturation (as assessed in vitro) [3, 16, 20].

Much of the recent work on tree nut allergens is derived from the study of recombinant proteins [21]. cDNA libraries of walnut [22], hazelnut [23], and cashew nut [24] have been described. The characterization of the allergens derived from these and other sources are described below. A current list of officially designated allergens, including tree nut allergens, may be found at <http://www.allergen.org/> [25]. The tree nut allergens from this site are listed in table 1.

Widely Consumed Tree Nuts

Hazelnut, *Corylus avellana*

Allergy to hazelnut is fairly common in Europe [26]. Discussion of the hazelnut is complicated by the fact that some patients are allergic to the nut, others to the tree pollen, and still others to both. The type of allergic reaction and the specific allergens recognized can vary considerably from one geographic area to another. Where trees of the family Betulaceae (e.g., birch, alder, hazel and hornbeam) are prevalent, the cross-reactivity between (sensitizing) pollen and nut allergens can be the leading cause of food allergies [19, 26–30]. Allergy to pollen from the common weed, mugwort (*Artemisia vulgaris*), has also been shown to be cross-reactive with hazelnut allergens and may also be a sensitizing agent [31]. Because of the ubiquitousness of this cross-reactivity [32–34] it is perhaps not surprising that the first formally named allergens fell into this category. Cor a 1, a 17-kD major allergen, is related to the major birch pathogenesis-related pollen allergen, Bet v 1, which itself is a major cross-reactive pollen allergen [19, 33, 35]. Molecules designated Cor a 1 appear to represent a complex set of gene products, some of which are more allergenic than others [23]. Four isoforms have been described, Cor a 1.01, 1.02 and 1.03, in hazel pollen and Cor a 1.04 in hazelnut.

Cor a 1.04 is a 17.4-kD protein expressed in at least four sub-isoforms, Cor a 1.0401–1.0404, which are 97–99% identical to each other but share only 63 and 71% identity with hazel pollen isoforms, Cor a 1.0101 and Cor a 1.0301, respectively [23]. Interestingly, the hazelnut allergen, Cor a 1.04, shares greater identity with the birch pollen allergen, Bet v 1 (85%), than with its own pollen homologue. Inhibition reactions comparing birch and hazel pollen extracts and hazelnut Cor a 1.04 suggest that in some populations,

the majority of patients are primarily sensitized by birch Bet v 1. Despite the high degree of identity between the four nut isoforms and the fact that between 91% and 95% of 43 hazelnut-allergic patients were reactive with three of the four versions, one form, Cor a 1.0404 reacted with only 74% of the patients [23]. An even more pronounced difference was observed in a functional histamine release assay where only minimal stimulation with a minority of sera was observed with Cor a 1.0404. This diminution of binding ability and near loss of cross-linking ability can be traced to a Cys-Pro release which may disrupt the gross conformation of the allergen [23]. These data suggest a limited number of reactive epitopes and the likelihood that they are primarily conformational.

Another example of an allergen displaying cross-reactivity between birch pollen and hazelnut is hazelnut profilin (Cor a 2) [19, 35] though the clinical relevance of this cross-reactivity has been questioned [35, 36].

Birch may not be the only source of pollen-induced cross-reactivity as mugwort pollen has been shown to substantially inhibit the binding of IgE from a pool of 28 co-reactive patients to hazelnut extract in RAST and immunoblot assays [31]. The proteins recognized and inhibited on immunoblots were at 42, 17, and <16 kD.

Schocker et al. [37] describe a patient with severe allergy to hazelnut but without cross-reactivity to birch pollen. Reactivity was observed on immunoblot with proteins at 50, 42, 38, 9, 7 kD. The data indicate that unlike those allergens cross-reactive with birch pollen, the nut-allergic patient reacted with proteins that were more heat stable. Particularly stable were those allergens in the 7- and 9-kD range. Interestingly, these allergens were not reactive with serum IgE from multiple patients with histories of birch pollinosis. Possible allergens in this size range include LTPs and 2S albumins. Subsequently, hazelnut-allergic patients with LTP-reactive IgE were described [38] and the recombinant form (Cor a 8) cloned [39]. Recently, a low-MW (17 kD) heat shock protein recognized by IgE from 10 of 14 (71%) hazelnut-allergic patients has also been described [40].

An 11S (legumin-like) globulin, Cor a 9, with homology to cashew nut Ana o 2, peanut Ara h 3, and soybean glycinin, has been cloned and sequenced, and the native form of the seed protein partially sequenced [41]. This 40 kD protein was recognized by IgE from 12 of 14 (86%) allergic patients on immunoblots. Native hazelnut legumin (35 kD) has also been identified as a major allergen [38]. Another food storage protein, a 7S vicilin-like 48-kD protein, Cor a 11, is listed in the Allergen Nomenclature registry (<http://www.allergen.org/>) but not yet described

in detail. Pastorello et al. [38] have reported patient reactivity to native 7S vicilin and a 32-kD 2S albumin.

Hansen et al. [42] recently tested 17 Danish and Swiss hazelnut-allergic patients for reactivity to Cor a 1.0401 (Bet v 1-like), Cor a 2 (profilin), and Cor a 8 (LTP) by immunoblot. They observed that 16 (94%) reacted with Cor a 1.0401, 7 (41%) with Cor a 2, and none with Cor a 8 (Cor a 9 not tested).

The susceptibility of various IgE binding hazelnut peptides to enzymatic digestion has been investigated and found to be variable depending on the enzymes and conditions used [43, 44]. Roasting of hazelnuts has been shown to significantly decrease allergenicity including reactivity to the native versions of Cor a 1.0401 and Cor a 2 [37, 42].

Walnut, *Juglans regia*, *Juglans nigra*,
Juglans californica

In the US, more patients are allergic to walnut (34%) than any other tree nut [45]. Of 54 pediatric tree nut-allergic subjects in one study, 26% were confirmed to be reactive to walnut seed protein extract [46]. Although the native North American black walnut species, *J. nigra* and *J. californica*, are fairly common in North America, they are infrequently eaten. Consequently, most walnut-allergic patients have probably been sensitized to the commercially available and widely consumed English walnut (*J. regia*).

Several English walnut allergens have been cloned and expressed. The first to be described was a 2S albumin precursor, rJug r 1, with a molecular mass of 15–16 kD [22]. When tested for binding with walnut-allergic patients' IgE by immunoblot, 12 of 16 patients (75%) were positive. Each patient had more than 50% reactivity absorbed out by soluble recombinant allergen in ImmunoCap assays emphasizing the importance of this allergen in walnut-allergic patients. The recombinant protein inhibited the binding of IgE to reduced native protein bands of 14, 10–12 and 5 kD (in reducing gel). These results are consistent with the characteristics of 2S albumin in other plant species where the larger prealbumin (14 kD) is expressed along with the large (10–12 kD) and small (5 kD) subunits of the mature protein. The deduced amino acid sequence of Jug r 1 shows similarity to 2S albumin allergens in Brazil nut (Ber e 1, 82%), castor bean (69%), cottonseed (59%), and mustard seed (45%) [22].

Jug r 1 was further analyzed by screening for linear epitopes [47]. Pools of sera from allergic patients was used to probe a solid-phase SPOTs sheet on which each member of a complete set of overlapping peptides correspond-

ing to the entire amino acid sequence of the protein was displayed. A single strongly reactive immunodominant epitope was detected by 15 of 20 (75%) Jug r 1-allergic patients' sera. This finding was somewhat surprising because similar scans on other allergens have identified multiple epitopes. Alanine mutagenesis scanning revealed a core of four critical amino acids (³⁶RGEE³⁹) for this epitope. Adsorption of patient sera with synthetic epitope revealed considerable residual reactivity toward rJug r 1, suggesting the presence of conformational epitopes as well.

Additional screening of the English walnut expression library yielded a second allergenic fusion protein, Jug r 2, which reacted with 9 of 15 (60%) allergic patients' sera [48]. Sequencing and comparison showed this to be a member to the vicilin family of seed storage proteins (a 7S globulin). Jug r 2 shows 75% similarity to Ara h 1, the peanut homologue of this allergen. The calculated size of the recombinant Jug r 2 protein was 66 kD but the mature protein was expected to be ~ 48 kD based on the probability that the N-terminal 173 amino acid hydrophobic region would be deleted in the seed, a prediction confirmed both by inhibition experiments which identified a 47–48 kD native protein and by N-terminal sequencing. The binding of patients' sera to other bands at 52 and 28 kD was also eliminated by incubation with the recombinant protein but the reactive bands were not sequenced for identification.

An English walnut LTP, Jug r 3, has also been officially recognized but not yet described [Pastorello, unreported data]. Previously, it was reported that the carrot and peach LTPs are able to inhibit IgE binding to walnut LTP in RAST and ELISA assays as might be expected for such a panallergen [49, 50]. Recently, a precursory subunit of English walnut legumin (tentatively designated Jug r 4) has been cloned and expressed and found to be reactive with sera from 15 of 23 (65%) walnut-allergic patients [51].

Preliminary reports describing the cloning and analysis of the black walnut (*J. nigra*) allergens, 2S albumin (Jug n 1) and vicilin (Jug n 2), as well as the allergens superoxide dismutase (MnSOD), and glyceraldehydes 3-phosphate dehydrogenase (GAPDH) have been published [52, 53]. The Jug n 1 and 2 were found to be 96 and 97% identical to Jug r 1 and 2, respectively, and, for Jug n 1, reactive with 8 of 12 (67%) walnut-allergic patient sera (Jug n 2 not tested). In contrast to the results of Robotham et al. [47] where only a single linear Jug r 1 epitope was IgE-reactive, 8 linear peptides were reportedly detected in Jug n 1, each of which showed complete sequence identity to the corre-

sponding region in Jug r 1 [52]. Since details of the black walnut work have not yet been published, these differences remain to be explained but could be related to variability between patients available to the two lab groups. Five of 16 (31%) and 4 of 16 (25%) walnut-allergic patients were reported to bind MnSod and GAPDH, respectively, and 4 linear reactive epitopes were identified in each [53].

Cashew nut, *Anacardium occidentale*

Cashew nuts are associated with IgE-mediated anaphylaxis as well as T-cell-mediated contact and systemic dermatitis, the latter due to constituents of the oils in the nut shell. Since in-shell cashew nuts are rarely seen outside the tropical growing region, the cell-mediated form of immunity is not a problem in Europe and North America. However, IgE-mediated cashew nut allergy is the second most commonly reported tree nut allergy in the US (20% in the FAAN registry) [45].

Garcia et al. describe three cashew-allergy patients who show reactivity to several bands in immunoblots [54]. In a recent study, we screened 15 allergic patients by immunoblot and showed that all reacted with proteins in an aqueous extract [55]. Dominant reactions were to the 31- to 35-kD peptides previously characterized as the large, acidic subunit of the 48-kD legumin-like seed storage protein (a 13S globulin) [56]. Also reactive were bands consistent with the small subunit of legumin (20–27 kD range). Both of these assumptions were borne out upon partial amino acid sequencing [55]. The 13S globulin is not only an important allergen, it is also the major protein in the cashew nut. About 50% of the aqueous-extractable proteins are represented by this fraction, also termed anacardein. The 7S globulin (vicilin) fraction represents about 5% of the extractable protein in cashew nuts (unreported data). Five of 15 (33%) patients reacted with a band at ~53 kD which was shown to be a 7S vicilin-like protein by N-terminal sequencing [55]. Patient sera also reacted with three low-MW bands in the 6- to 12-kD range (reducing gel). N-terminal sequencing demonstrated that these bands represent 2S albumin [55].

In parallel with the above studies, we generated a cashew nut cDNA expression library and screened it with human allergic sera. Thus far we have identified, sequenced, and expressed both vicilin (Ana o 1) and legumin (Ana o 2) clones [24, 57]. For the vicilin, Ana o 1, 10 of 20 (50%) of the cashew nut-allergic patients expressed IgE reactivity in immunoblot indicating that this is a major allergen. Two isoforms/alleles which differed by a single nucleotide and amino acid were identified. The

deduced 540 amino acid Ana o 1 peptide displayed 52–62% similarity to proteins found in African oil palm, macadamia nut, garden pea, soybean and English walnut, variously described as being vicilin or vicilin-like protein precursors, sucrose binding proteins and precursors, and 7S globulins. The 12-amino-acid tryptic peptide sequence of the vicilin-like protein, described above [55], fully matched the cloned sequence. A ~53-kD band was confirmed as a vicilin-like protein by immunoblot inhibition upon preincubation (adsorption) of allergic sera with the recombinant protein [24].

Further studies were conducted to identify and map the linear epitopes of Ana o 1 [24]. Eleven reactive epitopes were identified of which three were judged to be immunodominant. Curiously, one of the immunodominant epitopes mapped to the presumptive leader sequence. This map is of particular interest since it allowed us to make a direct comparison to a similar map of the major peanut allergen, Ara h 1, also a vicilin-like protein [24, 58, 59]. Significantly, none of the 11 cashew nut epitopes showed significant sequence homology with any of the 22 peanut epitopes. Perhaps even more remarkable was the fact that only 4 of the epitopes even mapped to the corresponding positions along the aligned sequences in the two allergens. These data do, however, help explain the general perception that IgE to tree nuts and peanuts are not cross-reactive in allergic patients.

Sequence analysis of the second major cashew nut allergen, Ana o 2, showed it to be a member of the legumin seed storage family (an 11S globulin) [57]. By IgE-immunoblotting, 13 of 21 (62%) serum samples from cashew nut-allergic patients were reactive with rAna o 2. Immunoblot inhibition data showed that native Ana o 2 constitutes a major band at ~33 kD and a minor band at ~53 kD. Comparison to the amino acid sequence data derived from IgE-reactive bands on an immunoblot of cashew nut protein extract shows that one N-terminal sequence and three tryptic peptide sequences shared identity with Ana o 2, but differed from the sequence of the recombinant protein by one or more amino acids suggesting that the 11S globulins represent a family of gene products [55, 57]. Probing of overlapping synthetic peptides with pooled human cashew nut-allergic sera identified 22 reactive peptides, 7 of which gave strong signals. Several Ana o 2 epitopes were shown to overlap those of the peanut legumin group allergen, Ara h 3, in position but shared little sequence similarity. Curiously, considerably greater positional overlap and identity was observed between Ana o 2 epitopes and those of an 11S protein (glycinin) from another legume, the soybean [57].

Finally, we have obtained preliminary data regarding the cloning and characterization of a third cashew nut allergen, 2S albumin (unreported data). This too was a predictable find based on immunoblotting and N-terminal sequencing of bands from IgE blots of cashew nut protein extract [55].

Almond, *Prunus dulcis*

Almonds rank first in per capita consumption of tree nuts in the USA. As a tree nut allergen, it ranks third (15% reactive) behind walnut and cashew nut in the FAAN self-reporting survey of tree nut allergies [45] and can be found in many foods. Despite these statistics and despite the fact that the proteins in almonds have been biochemically characterized [60–67], relatively little has been reported regarding the identity of the allergens involved in almond sensitivity.

Early reports by Bargman et al. [63] described the IgE from 4 of 8 patients binding to unidentified proteins at approximately 70 and 50 kD in IgE immunoblotting. Sathe et al. [60], Osborne and Campbell [64] and Acosta et al. [65] have reported the purification and biochemical characterization of the major soluble seed storage protein in almond, a 14S globulin (legumin) known as almond major protein (AMP) or, from early (1897) descriptions, as amandin. Amandin is a complex nonglycosylated storage protein with a molecular weight estimated to be in the 375- to 475-kD range and constitutes 65–70% of the extractable proteins in almond [66]. SDS-PAGE under reducing conditions separated two major pairs of polypeptides in the 42- to 64-kD range and 20- to 27-kD range that appeared to be the respective disulfide-linked acidic and basic polypeptides of amandin, consistent with the classical structural model of legumin proteins. Nineteen other polypeptides were also resolved, however, these were of lesser quantitative importance as judged by staining by Coomassie Brilliant Blue. Probing of blotted amandin with human almond-allergic sera shows that the 42- to 44-kD bands are strongly reactive [60, 67]. Preliminary sequence data indicate that these peptides are from a protein in the legumin family [unpubl. results] and are homologous to the previously reported prunin 1 and 2 cDNA sequences [68]. Additionally, amandin contains two polypeptides of approximately 66 and 50 kD, which likely represent some of the IgE-reactive bands identified by Bargman et al. [63]. We also observed that IgE from 50% or more of sera obtained from patients with serious reactions to almonds upon ingestion bind amandin, demonstrating that these polypeptides represent major almond food allergens [unpubl. data]. The data also show

that the 66-kD protein appears to be an unprocessed precursor to the 44-kD alpha chain of the legumin-like (11–13S) seed storage protein family. Recently, Poltronieri et al. [69] have identified two major bands (45 and 30 kD) by patient IgE immunoblot and N-terminal sequencing as being homologous to a 7S conglutin- γ . This group has also identified an IgE-reactive 12 kD band corresponding in N-terminal sequence to 2S albumin [69].

The various peptides recognized by human IgE appear to be inherently immunogenic in that rabbit and goat polyclonal and mouse monoclonal IgG Abs recognize most of the same bands as does human IgE on immunoblots [60, 66, 67, and unreported data]. The amandin polypeptides are highly resistant to the wet and dry heat used during food processing and have been shown to be excellent marker proteins for immunoassays to detect almond contamination in foods [67, 69].

Less Widely Consumed Tree Nuts

Pecan, *Carya illinoensis*

Pecans are widely grown and consumed in the southern US but less so elsewhere. They rank third behind almond and English walnut in total US production. Nine percent of self-reporting tree nut allergic patients list pecan as an allergen [45]. Though not well documented, the available evidence from skin prick and in vitro testing suggest considerable cross-reactivity between the allergens of pecan and the more widely consumed walnut, both are members of the Juglandaceae family [71]. In a preliminary report, complete cross-reactivity by immunoblot inhibition was demonstrated using sera from patients primarily allergic to walnut who later reported reactions to pecan [72].

Chestnut, *Castanea sativa*

Two nut allergens from chestnut have been described and cloned. One is a class I chitinase, designated Cas s 5. Native chitinase I is recognized by patients with latex-fruit allergy syndrome and is inhibitable by the avocado class I chitinase allergen, Pers a 1, indicating significant cross-reactivity [18]. Cas s 5 contains an N-terminal domain with homology to the hevein-like domain of rubber latex hevein. The hevein-like domains are a major panallergen associated with latex-fruit syndrome and include cross-reactivity between rubber latex hevein and the chestnut homologue [73–77]. By analyzing recombinant Cas s 5 with and without the N-terminal hevein-like domain, it was shown that the majority of the Cas s

5-reactive IgE from patients suffering from latex-fruit allergy syndrome was directed to this domain though some evidence for reactivity to the C-terminal catalytic domain was also found [78, 79].

Cas s 8, a second nut allergen of chestnut, is a member of another panallergenic family, the LTPs. Cas s 8 is a 9.7-kD protein with 53% identity to apple (Mal d 3) and 50% identity to peach (Pru p 3) LTP fruit allergens and 50% to mugwort weed pollen LTP by N-terminal and MALDI sequencing [79]. Skin prick testing indicates cross-reactivity between peach, apple, mugwort pollen, and chestnut LTPs and suggests that chestnut LTP-allergic patients may be primarily sensitized by mugwort pollen LTP [80].

Brazil Nut, *Bertholletia excelsa*

Allergic responses to Brazil nut are rather uncommon, possibly because of the infrequency of consumption. Nevertheless, both major and minor nut allergens have been described. The major allergen is a 2S albumin rich in methionine and is designated Ber e 1 [80]. Ber e 1 has taken on particular notoriety as a prime example of the unintended consequences of the cloning of a protein gene (in this case encoding an allergen) to create a genetically modified (GM) crop. The intent was to boost methionine content in the otherwise methionine-poor soybean. Nordlee et al. [81] determined that 8 of 9 Brazil nut-allergic patients were reactive with the 9-kD Ber e 1 band in the GM soybean extract and none reacted with unmodified soybean extract. Each of three selected allergic patients showed positive skin prick reactions to transgenic soybeans as well. Thus, not only is Brazil nut 2S albumin allergenic, it also appears to be a major allergen.

Recently, Brazil nut 2S albumin has been subjected to T cell epitope mapping using a pool of CD4+ T cells derived from 92 unscreened (for allergy) blood bank donors [83]. One major epitope was found on the small subunit and three on the large subunit. Because the T cells were not derived from patients with known allergy to Brazil nut, the significance of these findings remains to be determined.

Structural studies have shown that rBer e 1, synthesized in yeast (*Pichia pastoris*) was properly folded and cleaved and was similar to the native version in reactivity to rabbit and human antibodies [81]. These studies also revealed that rBer e 1 was rather resistant to denaturation by heat, low pH, and guanidinium chloride treatments and readily refolded upon return to physiological conditions.

In addition to 2S albumin, allergic patient sera has subsequently been reported to react with extracted proteins at 21- to 22-kD and 33.5-kD ranges (in reducing gels) which have been identified as the α - and β -subunits of 12S legumin-like globulin, respectively [84, 85]. By comparing sera from patients demonstrably allergic to Brazil nut to those with in vitro (immunoblot) reactivity to Brazil nut extract but not showing symptoms upon consumption, Pastorello et al. [86] confirmed that it is the 2S albumin (9 kD) that is the predominant allergen. The previously observed reactive proteins at 22 and 33 kD (the presumed 12S globulin subunits) were, however, recognized by both sets of sera, leading the authors to conclude that these and other reactive proteins were not involved in patient reactivity [86]. This pronouncement may have been premature as a Brazil nut-allergic patient has recently been reported whose serum reacts with 18-, 25-, 33- and 45-kD proteins (presumably including the 12S globulins) but does not react with 2S albumin [87]. Additional allergen cloning and characterization and testing of serum samples from a greater number of patients are needed to fully sort through this story.

Pine Nut, *Pinus pinea*

Pine nuts (pignolis, piñons) are commonly consumed in southern Europe and less frequently elsewhere. There have been several reports of allergic and anaphylactic responses to pine nut but relatively little published regarding allergenic peptides. Senna et al. [88] describes IgE reactivity with peptides in extracts from both pine nuts and pollen in patients who were symptomatic to the nut but not pine pollen. IgE-reactive peptides of 50 and 66–68 kD have been reported [89, 90] as has reaction to a 17-kD protein [91]. The latter had the interesting property of being rendered nonreactive upon either the transfer to nitrocellulose using conventional electrophoretic methods or reduction.

Macadamia nut, *Macadamia integrifolia*

Though not as commonly eaten as many other tree nuts, macadamia can occasionally cause serious allergic reactions [92, 93]. IgE reactivity to proteins in macadamia oil has also been reported using sera from patients with macadamia nut allergy [94]. Reactivity on immunoblot of a single patient's serum IgE with a 17 kD peptide and a second higher molecular mass peptide (kD not given) and cross-reactivity with cashew nut proteins has been described [93].

Pistachio, *Pistacia vera*

Several studies have described pistachio-allergic patients but there is relatively little information regarding the identity of the relevant allergens [45, 46, 95]. As anticipated, cross-reactivity between pistachio, cashew nut, and mango, all members of the Anacardiaceae family, has been observed in patients [95, 96–98]. Parra et al. [99] observed patient IgE binding to peptides at 34, 41, 52, and 60 kD. Fernandez et al. described significant patient IgE binding to three pistachio peptides in the 30 to 41 kD range with the strongest at 34 kD [98]. Other bands over a broad molecular mass range were also detected. Both cashew nut and mango seed (but not fruit) were effective inhibitors of IgE anti-pistachio reactivity in RAST assays. Asero et al. [50] provide some circumstantial evidence that pistachio-allergic patients are likely to be recognizing the panallergen, LTP. A connection between mild to moderate pistachio allergy and the pollinosis to the Mediterranean weed, *Parietaria* sp., has been suggested [100].

Coconut, *Cocos nucifera*

The literature suggests that very few patients are allergic to coconut [45, 101, 102] and, consequently, investigations into the allergens recognized by patients with primary coconut allergens have not been performed. However, Teuber et al. [103] did analyze the sera from two patients with primary walnut allergy and secondary coconut allergy. Interestingly, even though coconut is a monocot and walnut a dicot, and thus quite distantly related, evidence of substantial IgE cross-reactivity between the two nuts was detected. IgE-reactive coconut extract bands were detected at 35, 36.5 and 55 kD on a reducing immunoblot. Adsorption of the patients' sera with walnut as well as almond and peanut extracts inhibited IgE binding to these bands. Conversely, coconut only partially inhibited IgE reactivity to 35 and 36 kD bands on a walnut immunoblot, suggesting that IgE resulting from primary walnut exposure was responsible for a secondary cross-reaction to coconut. Indirect evidence suggests that the coconut 35- and 36.5-kD bands represent 11S legumin-like subunits [48, 104, 105].

Exotic Tree Nuts

Nuts which are not widely consumed in western markets may pose an allergy risk to some individuals. Nangai nut (*Canarium indicum*) from Micronesia is imported into Europe and has recently been shown to induce histamine release in vitro and give a positive skin prick test in

patients with multiple pollen allergies (but no history of pollen/food syndrome) [106]. The results have been interpreted as suggesting the presence of carbohydrate-containing epitopes that cross-react with these common pollen allergens [106].

Acorns from oak trees (*Quercus* sp.) were once an important food of the Native Americans and are still occasionally eaten in the US and Europe. A report of a single Spanish patient reacting with an 18-kD acorn protein on immunoblot has been published [107]. IgE reactivity to this band was partially inhibited by Bet v 1, suggesting homology to this panallergen.

Other infrequently, regionally, or locally consumed tree nuts are likely to affect some individuals but have not been studied. These include apricot kernel, candlenut, breadnut seed, beechnut, ginkgo nut, hickory nut, butter-nut, kluwak nut, kola nut, mape, paradise nut, pilinut, and water caltrop. A web site listing a wide variety of tree nuts and their edibility and uses in cooking is: <http://www.switcheroo.com/Nuts.html>.

Future Directions

Much of the descriptive and experimental work will continue to be conducted on recombinant proteins and many of the questions which remain to be adequately addressed apply to all allergenic foods and to some degree even aeroallergens [21, 108]. However, the native homologues should not be ignored since posttranslational modifications and multigene complexity are common features of several tree nut allergens and can be predicted to affect stability, allergenicity, and IgE and T cell reactivity.

The ability to manipulate the expressed genes facilitates the analysis of tree nut protein epitopes and their comparison to IgE-reactive epitopes on homologous allergens in other food types (vegetables, fruits, and animal products). Also, comparison to homologous proteins that are nonallergenic may give clues to the features that render a protein allergenic. However, as cautioned by Astwood et al. [59], simple comparisons of the sequence of potential allergens to those proteins with documented allergenicity may be of limited value since, for example, pea vicilin, which is closely related to peanut vicilin, shows 43% identity but is only rarely allergenic [109], yet cashew nut vicilin, which shares only 27% identity to peanut vicilin, is allergenic, albeit by way of a different set of epitopes [59]. Although several of the key tree nut allergens have had their linear epitopes mapped, there is a distinct paucity of information on conformational epitopes.

Such epitopes are very likely to play a major role in triggering allergic reactions [110]. T cell epitopes, which have largely been ignored, need to be identified as well.

Though some patients seem to be hypersensitive to a single nut type, others report allergy to multiple nuts and many to peanuts and other foods or pollens (though unambiguous clinical histories of 'nut' allergic patients are sometimes difficult to obtain). Whether such clusters of reactivity are the result of multiple independent sensitization events (e.g., peanut and a tree nut) in generally hypersensitive (atopic) patients or whether true cross-reactivity is the root cause needs further investigation. The further development of appropriate animal models for the study of these and other questions regarding tree nut allergies is warranted.

Cultivars producing hypoallergenic tree nuts could theoretically be derived by selection but, for a variety of reasons, not the least of which is the slow maturation of nut

trees, new varieties are more likely to be developed based on genetic engineering. Laboratory-generated hypoallergenic forms could serve as desensitizing (vaccine) agents and unmutated recombinant molecules as well-defined and characterized diagnostic reagents to replace the currently used crude extracts.

As with other allergens, the ever-present problem in data and test result interpretation brought on by the frequent discordance between various tests and challenges (e.g., ImmunoCap, ELISA, immunoblot, skin prick test, oral challenges) and clinical experiences needs to be resolved. Finally, the recent application of humanized anti-IgE to dramatically lessen the reactivity of peanut-allergic patients to peanut challenges opens the exciting possibility of attempting desensitization to the potent (recombinant) allergens of peanut and tree nuts in such treated patients [111].

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