

Sensitization to the major allergen of Brazil nut is correlated with the clinical expression of allergy

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Background: Only a few studies have investigated the clinical role of food allergens, especially the relationship between sensitization to a given allergen and occurrence of adverse reactions when eating the relevant food item.

Objective: This study evaluated the clinical role of the allergens of Brazil nut by comparing the patterns of IgE binding in sera from 11 patients with anaphylaxis after eating Brazil nuts with those from 10 subjects with no symptoms to this food item. Both groups had specific IgE to Brazil nut.

Methods: Allergens in the in-house extract of Brazil nut were identified by SDS-PAGE/immunoblotting, the major allergen was purified by HPLC, and its N-terminal sequence was determined by a protein sequencer.

Results: SDS-PAGE/immunoblotting detected a number of allergenic components with molecular weights ranging from 4 to 58 kd. All sera from symptomatic patients recognized a 9-kd allergen corresponding (as established by amino acid sequencing) to a 2S albumin already described as a major allergen of Brazil nut, whereas the other allergens each bound IgE from less than 50% of sera. No sera from asymptomatic subjects showed IgE binding to the 9-kd allergen, but they did recognize components from 25 to 58 kd, which are minor allergens. **Conclusions:** These findings indicate that the allergen underlying clinical reactions to Brazil nut is a 2S albumin of 9 kd and that in vitro reactivity to this allergen identifies subjects who react in vivo to ingestion of this food. (*J Allergy Clin Immunol* 1998;102:1021-7.)

Key words: Food allergy, Brazil nut, immunoblotting, major allergen

The clinical role of major allergens from foods has been only scantily investigated. The first report on their importance was by Aas and Lundkvist¹ who used the purified allergen M (Gad c 1 according to current allergen nomenclature) from codfish for in vitro diagnosis, attending 100% sensitivity and specificity. To determine the role of food allergens, sera from patients who are clinically allergic (not simply sensitized) to a given food item must be used. With adequate characterization of patients, the role of major allergens, such as Ara h 1 from peanut² and Pen a 1 from shrimp,³ has been clearly established. Sound clinical data may even lead to changes in allergen nomenclature as with ovomucoid, which Bernhisel-Broadbent et al⁴ demonstrated was the major allergen from egg and as such deserved the name of Gal d 1 instead of ovalbumin.⁴

Allergy to Brazil nut (*Bertholletia excelsa*), first described in 1983,⁵ is considered quite uncommon, probably because the nut is eaten only rarely as a fairly exotic item. One study reported 12 cases of hypersensitivity reactions observed during 8 years in the Isle of Wight,⁶ but in a case list of patients allergic to nuts, Brazil nut was preceded only by peanut in causing allergic reactions.⁷ This nut has aroused interest lately because of the possibility of transferring hypersensitivity to its allergens by introducing transgenic soybean grafted with a methionine-rich 2S albumin derived from Brazil nut.⁸ That study underlined the importance of assessing this protein's ability to elicit allergic reactions because it is used to increase the nutritional quality of soybean; however, the relative risk of anaphylaxis has not been taken into consideration.

This study investigated whether allergic symptoms to the Brazil nut might be related to sensitization to its major allergen, already described in the literature. We studied the pattern of IgE reactivity to Brazil nut components in a group of patients with severe clinical reactions to this nut compared with a group with specific IgE but no clinical symptoms. Particular attention was paid to the bioimmunochemical characterization of the allergenic proteins through chromatography purification, amino acid N-terminal sequence, and blotting inhibition.

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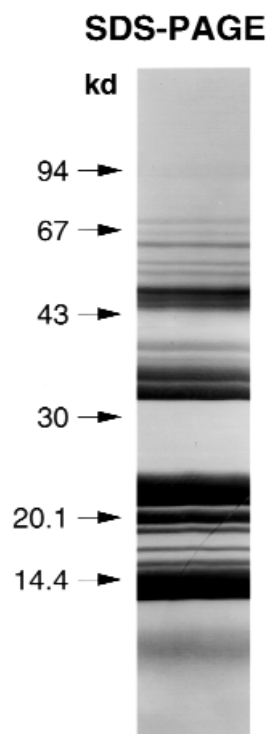


FIG 1. SDS-PAGE of raw Brazil nut extract stained with Coomassie Brilliant Blue. Protein content was 0.084 mg $\times$ 1 cm of gel; because this lane was 2.5 cm, the protein content of the gel in the figure was 0.21 mg.

METHODS

Patients

Among the outpatients referred to the Third Department of General Medicine, University of Milan, and to the Bizzozzero Division of Niguarda Ca' Granda Hospital, Milan, 11 patients (6 men and 5 women) who reported severe anaphylactic reactions after the ingestion of Brazil nut were selected for this study. The reaction to Brazil nut was established only by medical reports detailing the kind of symptoms, and the clinical history focused on the relationship between ingestion of Brazil nut and occurrence of symptoms and any allergic reactions to other food items. The IgE-mediated mechanism of the reaction was demonstrated by detection of specific IgE to Brazil nut by the CAP system (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden).

To evaluate the clinical significance of the single allergens, the *in vitro* investigation included the pattern of IgE reactivity to Brazil nut allergens in a group of patients with a positive CAP system result for Brazil nut but no clinical reactions, as definitely assessed by an open challenge with 2 Brazil nuts. The CAP system test was done with a panel of food allergens, including Brazil nut, in all patients referred to our clinics for food allergy.

Brazil nut extract

The extract was prepared in our laboratory according to the following method. The pulp of the nut was ground in a mixer and then defatted with several passages in acetone followed by paper filtration. The powdered, defatted nut pulp was dried overnight and suspended 1:10 wt/vol in 0.1 mol/L PBS, pH 7.4, for 2 hours at 4° to 8°C. The extract was centrifuged, filtered, and stored at -20°C. The protein

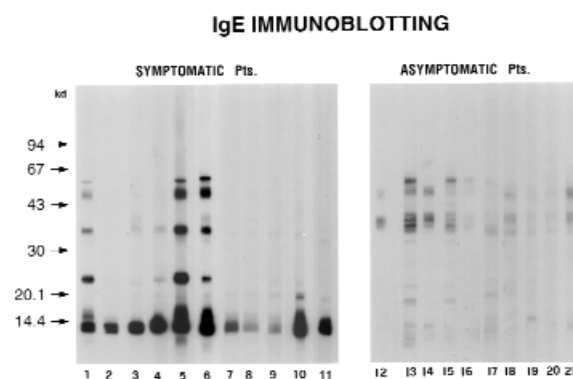


FIG 2. IgE immunoblotting of raw Brazil nut extract, with 25 μ g of Brazil nut extract loaded for every strip, in sera from 11 symptomatic patients and 10 asymptomatic patients.

concentration of the extract was determined with Folin reagent, according to the method of Lowry et al,⁹ giving a result of 27 mg/mL.

SDS-PAGE/immunoblotting

The Brazil nut extract, diluted 1:16 in distilled water, was mixed 1:2 with tris(hydroxymethyl)-aminomethane, pH 6.1, containing 4% SDS and 6% β -mercaptoethanol, glycerol (50% vol/vol), and 2% bromophenol blue (1%). The sample was then denatured by heating at 100°C for 5 minutes. Optimal conditions for the run into the gel was 0.084 mg of protein $\times$ 1 centimeter of gel; this concentration gave good separation of the bands without excessive protein diffusion.

Low molecular weight markers were as follows: rabbit muscle phosphorylase b, 94 kD; BSA, 67 kD; ovalbumin, 43 kD; bovine erythrocyte carbonic anhydrase, 30 kD; soybean trypsin inhibitor, 20.1 kD; and bovine milk α -lactalbumin, 14.4 kD. The extract and the markers were separated in a discontinuous buffer system in an SDS-polyacrylamide 7.5% to 20% separation gradient gel with a 6% stacking gel at 6 mAmp for 16 hours in a BIO-RAD protein IIx vertical electrophoresis slab cell, essentially according to the method of Neville,¹⁰ which has already been described in detail.¹¹ After separation, part of the gel was stained with Coomassie Brilliant Blue R-250 and destained until the background was clear.

SDS-PAGE gels were electroblotted to nitrocellulose paper (Amersham, Buckinghamshire, UK) by using a Trans-Blot cell from BIO-RAD (Richmond, Calif). The blot-nitrocellulose paper was then cut into strips and incubated overnight with single sera from the patients under investigation diluted 1:4 in blocking solution. To determine the IgE-binding components, the nitrocellulose strips were incubated for 6 hours with ¹²⁵I-labeled anti-human IgE antiserum (Pharmacia Diagnostics AB, Uppsala, Sweden) diluted 1:5. The unoccupied nitrocellulose membrane-binding sites were then blocked by incubation in PBS, pH 7.4 $\pm$ 0.2, with 0.1% Tween 20 for 30 minutes at 37°C.

The molecular weight of a protein under investigation was determined by comparing its electrophoretic mobility with the migration of proteins with known molecular weight (low molecular weight markers, Pharmacia Biotech AB). The reference calibration curve was prepared by measuring the migration distance of the protein bands on a photograph of the gel and calculating their values (Rf):

$$Rf = \frac{\text{distance protein migrated from origin}}{\text{distance from origin to reference point}}$$

The curve was then drawn by plotting the Rf values of the marker against the logarithms of their corresponding molecular weights.

The molecular weight of the IgE-binding components was determined by determining their R_f , locating this value on the calibration curve, and then reading the corresponding weight.

The immunoblotting results indicated that the protein appearing out of range of our reference curve, with a molecular weight less than 10 kd, was the major allergen of Brazil nut.

Purification of the 9-kd protein

To determine the amino acid sequence of the major allergen and its ability to inhibit immunoblotting of the raw extract, we did a 1-step protein purification by using the 27 mg/mL preparation. The raw Brazil nut extract was separated on a Superdex 75 HPLC column (Pharmacia) by size-exclusion chromatography. The column was equilibrated and eluted with 1 mol/L NaCl in 0.035 mol/L sodium phosphate buffer, pH 7.5, according to the method of Sun et al.¹² Fractions corresponding to chromatogram peaks were collected, and proteins in the different fractions were analyzed by gel electrophoresis. The fractions containing the 2S protein were pooled and concentrated by centrifugation in Centricon 3 concentrators (Amicon Inc, Beverly, Mass) with a 3000 d molecular weight cut-off at 3000 rpm for 6 hours at 4°C. The protein concentration was measured by using Lowry's method, and it gave a result of 1.8 mg/mL. This pooled protein was used for immunoblotting inhibition experiments.

N-terminal sequencing

The 9-kd protein purified by HPLC was adsorbed by centrifugation on polyvinylidene difluoride membranes with the ProSpin (Applied Biosystems) device, and the N-terminal sequence was determined on a gas-phase Applied Biosystems Model 470A Protein Sequencer with an on-line Model 120A PTH Analyzer.

Isoelectrofocusing

The 9-kd purified protein was focused by an LKB 2117 MULTIPHOR II system (LKB Bromma, Sweden) on a polyacrylamide gel plate (30% acrylamide, 0.8% bisacrylamide), pH gradient 3.5 to 10. Four microliters of undiluted, 1:10, 1:50, and 1:100 samples were applied at a constant current of 1000 V for 3 hours by using a 2197 LKB Power Supply (LKB). The protein content for each line was as follows: 5.76 μ g, 0.57 μ g, 0.11 μ g, and 0.057 μ g, respectively. The gel was fixed and stained with Coomassie Brilliant Blue R-250 (Pharmacia) according to the manufacturer's directions.

Periodic acid Schiff stain

Periodic Acid Schiff staining was done to detect glycosylation of proteins. Ten microliters of 9-kd purified protein, corresponding to the protein content of 1.8 mg/mL, was run into mini gel blotted onto Problot membrane (Applied Biosystems) as described by Towbin and Gordon.¹³ This was done at a constant current of 16 V for 60 minutes, omitting the methanol and SDS in the transfer buffer. Two identical membranes were blotted at the same time, 1 stained with Coomassie R-250 and the other used for PAS staining for glycoproteins. For periodic acid Schiff staining, the membrane was fixed in 12% trichloroacetic acid for 1 hour, tested with a 7% acetic acid solution containing 2% KIO_4 , and then held at 4°C for 1 hour. The membrane was immersed in Schiff's reagent and kept in the dark at 4°C overnight. The background was destained in methanol; glycoproteins appeared as purple bands. Milk whey proteins were used as control.

Immunoblotting inhibition experiments with 9-kd purified protein

For SDS-PAGE and immunoblotting of the raw Brazil nut extract and of the 9-kd purified protein, we used pooled sera from

allergic patients. In the immunoblot inhibition experiment Brazil nut extract was electrophoresed and immunoblotted as described before. The serum pool, at a final dilution of 1:4, was inhibited with 500 μ L of the 9-kd purified protein diluted 1:10 (corresponding to 0.09 mg) and 1:100 (corresponding to 0.009 mg) for 1 hour and incubated overnight with the strips of total Brazil nut extract. The strips were washed and incubated with ^{125}I -labeled anti-human IgE for 6 hours and then exposed to x-ray film at -70°C .

Statistical analysis

The levels of specific IgE to Brazil nut in patients with and without symptoms were compared by means of the Mann-Whitney U test. The frequency of reactivity to any single allergen of Brazil nut identified by SDS-PAGE/immunoblotting was assessed separately in patients with and without symptoms to the nut.

RESULTS

Study population

Eleven patients (6 men and 5 women) with clinical reactions to ingestion of Brazil nut and a positive CAP system result were selected for the study. Six patients had a documented history of anaphylactic shock, and 5 had laryngeal edema associated with diffuse urticaria/angioedema syndrome. As shown in Table I, some patients had other sensitizations, with only 2 being symptomatic, 1 with an oral allergy syndrome to foods, and 1 with grass pollinosis. Ten patients (5 men and 5 women) with positive IgE test results for Brazil nut but no clinical symptoms to this food were included in the in vitro study. These patients were referred to the Allergy Center because they had oral allergy syndrome for fresh fruits and vegetables. All of them had a positive CAP system result for a number of other foods (as reported in Table I), and most had pollinosis to grass and, to a lesser extent, to birch pollen.

The mean value for Brazil nut-specific IgE according to the CAP system in patients with anaphylactic reactions to the nut was 36.41 kU/L compared with 1.79 kU/L in asymptomatic subjects. The difference between the values from symptomatic and asymptomatic patients, analyzed by means of the Mann-Whitney U test, was highly significant ($P < .0001$).

Extract

SDS-PAGE. The raw extract of Brazil nut had a protein concentration of 27 mg/mL. SDS-PAGE of the extract, after Coomassie Brilliant Blue staining, showed several polypeptidic components, with molecular weights ranging from 9 to about 70 kd. The most important bands appeared near 9, 20, and 35 kd (Fig 1).

Immunoblotting. IgE immunoblotting of the 11 patients with anaphylaxis to Brazil nut is shown on the left side of Fig 2. All the patients had specific IgE against the allergenic component at 9 kd, which must thus be considered the major allergen of Brazil nut because 100% of the patients showed IgE reactivity to this protein. Other allergenic polypeptides were detected at less than 5 kd (2 of 11 patients, 18.2%), 14.4 kd (4 of 11 patients, 36.6%), 22 kd (4 of 11 patients, 36.6%), 35 kd (4 of 11 patients, 36.6%), and 48 and 55 kd (3 of 11 patients, 27.3%).

TABLE I. Clinical characteristics of the 11 symptomatic and 10 asymptomatic patients under investigation

	Sex	Age	CAP system	Symptoms	Specific IgE Abs to other food items*
Symptomatic patients					
1	M	10	50.3	Anaphylactic shock	Peanut, 1.02; hazelnut, 1.8
2	M	16	2.87	Urticaria, glottis edema	
3	M	8	3.21	Urticaria, glottis edema	Peanut, 9.80; hazelnut, 15; soybean, 6.24; carrot, 9.28; celery, 8.22; apple, 26.4; pear, 16.8.
4	F	5	48.7	Urticaria, glottis edema	
5	M	35	>100	Anaphylactic shock	Peanut, 1; hazelnut, 1.5
6	F	26	67.2	Urticaria, glottis edema	Peanut, 2; hazelnut, 2.8
7	F	27	6.3	Anaphylactic shock	
8	F	19	14.7	Urticaria, glottis edema	
9	M	25	16.3	Anaphylactic shock	Peach, 1.2; sesame seed, 11.7
10	F	5	83.1	Anaphylactic shock	
11	M	16	7.86	Anaphylactic shock	Hazelnut, 5.1; carrot, 2.1; apple, 13.7
Asymptomatic subjects					
12	F	18	0.8	—	Peanut, 3.44; rye, 10.1; barley, 4.43; oat, 5.24; tomato, 2.82
13	M	40	3.12	—	Peanut, 23.1; hazelnut, 3.69; wheat, 17.9; rye, 20.2; barley, 17.7; oat, 15.1; rice, 13.1; carrot, 12.1; celery, 14.3
14	M	21	2.12	—	Peanut, 32.1; almond, 3.55; nut, 5.24; wheat, 14.2; barley, 14.4; maize, 15.7; tomato, 19.7; carrot, 14; apple, 13.7; apricot, 20
15	M	25	1.43	—	Peanut, 25.8; nut, 6.79; wheat, 8.11; rice, 7.28; tomato, 7.07; celery, 8.04; apple, 34.4
16	M	27	1.19	—	Peanut, 10.1; wheat, 11; rye, 9.47; barley, 10; oat, 6.27; white bean, 6.58; tomato, 5.79; celery, 6.92
17	F	32	2.87	—	Peanut, 26.7; almond, 6.47; wheat, 16; rye, 17.6; barley, 16.9; oat, 12.9; maize, 15.9; rice, 14.4; tomato, 17.8; carrot, 17.2; celery, 15.3; apple, 9.58; kiwi, 6.08; banana, 11.7
18	F	43	2.10	—	Peanut, 3.59; hazelnut, 3.99; maize, 4.01; tomato, 5.22; carrot, 8.54; apple, 11.9; pear, 5.33; peach, 4.28; apricot, 4.30; plum, 3.75
19	M	23	1.54	—	Peanut, 78.8; hazelnut, 32.6; nut, 44.5; wheat, 22.5; barley, 32; maize, 82.1; soybean, 34.5; tomato, 35.1; carrot, 19.9; apple, 59.8
20	F	25	1.81	—	Peanut, 10.9; hazelnut, 9.93; nut, 20; wheat, 9.53; barley, 9.54; oat, 9.95; maize, 14.5; rice, 10.6; soybean, 9.68; tomato, 15.1; fennel, 13.4; apple, 24.6; peach, 27.3; cherry, 8.20; apricot, 19.4; plum, 14.1
21	F	54	0.99	—	Peanut, 7.42; hazelnut, 4.11; wheat, 12.2; barley, 11.5; rice, 8.7; soybean, 9.07; carrot, 7.23; peach, 6.21; cherry, 5.72; apple, 5.12; tomato, 6.10

Abs, Antibodies.

*As determined by the CAP system.

IgE immunoblotting of the 10 asymptomatic subjects is shown on the right side of Fig 2. Four patients presented weak radio staining with the allergic components ranging from 25 to 58 kd, which in symptomatic patients represent minor allergens.

We performed IgE immunoblottings with a pooled serum from healthy subjects to detect any aspecific binding to the raw extract and with a pooled serum from the asymptomatic patients with a positive CAP system result for Brazil nut (Fig 3).

Purification in HPLC

The chromatogram in Fig 4 shows the elution profile of separation of the raw Brazil nut extract with a protein concentration of 27 mg/mL. Besides the exclusion peak there were 2 other peaks whose good resolution suggested satisfactory protein purification. The right lane in Fig 4 shows electrophoresis of peak number 2, which contains the protein fraction corresponding to only the 2S polypeptides.

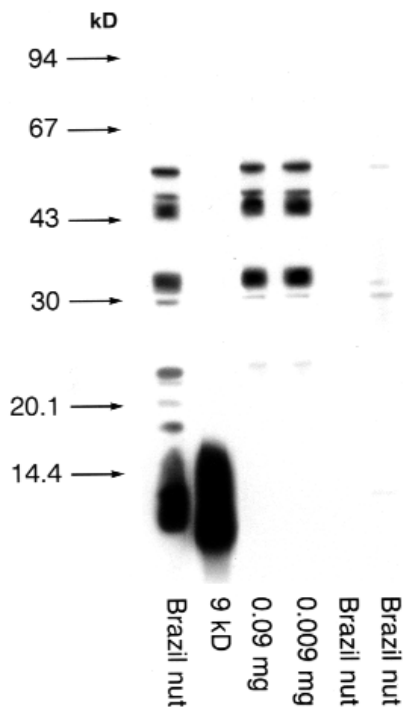


FIG 3. IgE immunoblot of the Brazil nut extract with pooled sera from symptomatic patients (*lane 1*). The same pooled serum was used to detect 9-kd purified protein (*lane 2*) and inhibition of raw Brazil nut extract by 9-kd purified protein at different protein concentrations (*lanes 3 and 4*). Negative serum was used for the IgE blot of the raw extract to detect aspecific binding (*lane 5*). *Lane 6* shows IgE blot of raw extract with pooled sera from asymptomatic patients under investigation.

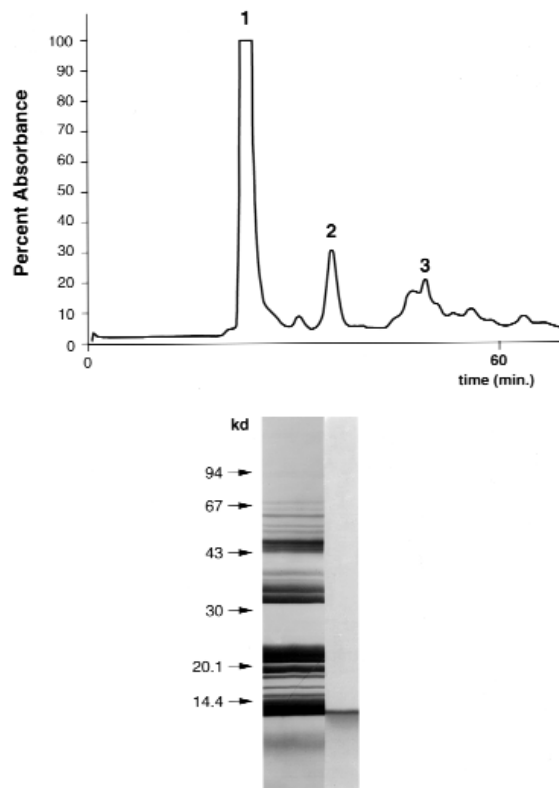


FIG 4. Chromatographic profile of raw Brazil nut extract separated on Superdex 75 HPLC column by size-exclusion chromatography (*top*). SDS-PAGE of raw Brazil nut extract (*left lane*) and of purified 2S albumin (*right lane*) stained with Coomassie Brilliant Blue (*bottom*).

Amino acid sequencing

Analysis of the first amino acids of the allergenic component at low molecular weight gave the following result: Pro-Arg-Arg-Gly-Met-Glu-Pro-His-Met-Ser-Glu-X-X-Glu-Gln. A search in the Swiss Protein Bank showed that this sequence corresponds to the initial 15 amino acids of the sequence 70-142, representing the large-chain subunit of a 2S sulphur-rich seed storage protein precursor of Brazil nut, already described by Ampe et al.¹⁴ This enabled us to establish a molecular weight of 8966 daltons, which we refer to as 9 kd.

Isoelectrofocusing

SDS-PAGE and immunoblotting of the gel filtration fraction showed that protein migrating at 9 kd contained the major allergen of Brazil nut. Isoelectrofocusing was done on this fraction, which gave a mean isoelectric point of 9 to 10 with Coomassie Brilliant Blue R-250 staining.

Periodic acid Schiff stain

The result of periodic acid Schiff staining for glycosylation on the 9-kd protein and on a positive control

excluded any glycosylation of this protein from Brazil nut extract.

Immunoblotting inhibition

The major allergen in the raw extract of Brazil nut IgE immunoblotting was completely inhibited by the 9-kd purified allergen at different dilutions (Fig 3).

DISCUSSION

This study demonstrates that the only clinically important major allergen of Brazil nut is a protein of about 9 kd. As shown by analysis of the IgE reactivity to the nut extract, all subjects with IgE and clinical symptoms recognized the 9-kd component, whereas those with IgE but no symptoms did not. This strongly suggests that IgE reactivity to this allergen governs the development of symptoms to the ingestion of Brazil nut.

We characterized this allergen by a series of methods addressing the aspects of immunochemistry and biologic function. First, SDS-PAGE and immunoblotting allowed its identification and definition of its allergenic importance. The molecular weight was difficult to establish, being lower than the standard of 14.4 kd, and thus pre-

venting precise measurement by interpolation with the reference curve using the standard proteins with known molecular weights. By calculating the molecular weight as a sum of the amino acids composing the proteins under investigation, a weight of 8966 daltons (about 9 kd) was achieved. The protein was purified by HPLC with a gel-filtration technique, which yielded a single fraction by a new SDS-PAGE analysis found to be composed of 2 chains, both with low molecular weights.

Determination of the amino acid sequence identified the 9-kd component as the "large chain" of a 2S sulphur-rich seed storage protein already detected as the main proteic component of Brazil nut.¹⁴ The whole protein seems to consist of a small chain of about 4 kd plus the large chain of about 9 kd, thus giving a theoretical molecular weight of about 13 kd. The fact that with SDS-PAGE we did not detect a band at this molecular weight that corresponds to the whole protein is probably due to the splitting of the 2 chains caused by the reduction of disulphuric bridges during the sample preparation with β -mercaptoethanol.

Our findings about the allergenic importance of this protein confirm the observations by Nordlee et al⁸ who found IgE reactivity to the 2S protein from Brazil nut after transferring this protein to soybean by transgenic engineering. The transgenic soybean was grafted with the gene of the 2S protein from Brazil nut, which is particularly rich in cysteine and methionine, to improve its nutritional value. The authors of that study assumed the 2S protein was an important allergen of Brazil nut, which is confirmed by our findings that the occurrence and severity of anaphylaxis to Brazil nut are related to sensitization only to this allergen. The complete inhibition of binding of the ¹²⁵I-labeled anti-serum anti-IgE to the nitrocellulose strip after incubation with the purified protein definitely confirmed the origin of the 3 bands detected with immunoblotting (about 13, 9, and 4 kd), making it reasonable to suppose they belong to a single protein, which is the main allergen of Brazil nut.

In 3 symptomatic patients we observed IgE reactivity against a 35 kd allergen and a 22-kd allergen. Bartolome et al¹⁵ described a similar reactivity with the serum of a patient allergic to Brazil nut with specific IgE not only to the 2S albumin but even to allergens of 33.5 and 21 kd. These proteins were identified as the α - and β -subunits of a 12S Brazil nut globulin. However, in our study reactivity to these proteins was also observed in asymptomatic patients' immunoblotting, and therefore it is reasonable to assume they are not involved in causing the clinical symptoms.

Interesting data are provided by the positive RAST result in subjects with no symptoms who did not show specific IgE to the 2S albumin. The low specificity of the commercial diagnostic test indicates the inadequacy of the solid phase-conjugated allergen. The close correlation we noted between clinical symptoms and reactivity to the 9-kd allergen would suggest assaying specific IgE only to this allergen so as to avoid false-positive results. The question of sensitivity and specificity of RAST to food allergens has been evaluated in a number of clinical studies, which have

confirmed its reliability only for a few foods.¹⁶ For some foods, not including Brazil nut, cutoff levels of specific IgE distinguishing symptomatic from asymptomatic subjects among those with a positive IgE test were reported.¹⁷ In our study, even considering patients with comparable levels of specific IgE, such as asymptomatic patients 13 and 17 and symptomatic patients 2 and 3, only sera from the latter bound the 9-kd protein, indicating that the clinical expression of allergy is linked to IgE reactivity to this allergen more than to the overall specific IgE level. The positive CAP/RAST result for Brazil nut in asymptomatic subjects is likely to be caused by allergens cross-reacting with other foods to which they were sensitized. The high molecular weights define these components as molecules not yet identified and not comprised in the already known cross-reacting allergens, all of which have a low molecular weight.

Brazil nut is an uncommon example of an allergen source with a single important allergen governing clinical reactivity, similar to food items such as codfish¹ and shrimp¹⁸; most allergenic foods (eg, egg, cow's milk, and peanut) have several allergens responsible for clinical symptoms.¹⁶ The Brazil nut major allergen is biochemically a 2S albumin particularly rich in methionine and cysteine. A number of other 2S albumins acting as allergens, including yellow mustard (*Sinapis alba*),¹⁹ rapeseed (*Brassica napus*),²⁰ and castor bean (*Ricinus communis*),²¹ are already known. These proteins belong to the group of so-called seed storage proteins, sharing characteristics such as high biosynthesis in specific tissues at given stages of plant development, the presence in mature seeds of separate storage called "protein bodies," and polymorphism in single genotypes, as well as in genotypes of the same species.²²

The 2S albumins differ from other seed storage proteins on the basis of their extraction and solubility in water and the sedimentation coefficient of about 2. They are widespread in seeds from Dicotyledons and have been thoroughly investigated in Cruciferae, especially *Brassica napus* and *Arabidopsis thaliana*, in which 2 interchain bonds linking the 9- and 4-kd subunits were directly demonstrated.²³ All the 2S albumins are compact globular proteins conserving cysteine residues, but they differ in subunit structure and synthesis.²² Because of their storage function, the 2S albumins have a content in sulphur and nitrogen high enough to maintain protein synthesis, and Brassicaceae plants also have antifungal activity, probably connected with preservation of seeds.²⁴

Storage proteins have a clinical role in some human diseases, being associated with gluten enteropathy, baker's asthma, and other allergic manifestations.

Considering that these proteins are particularly widespread in nature, it would be important to investigate their allergenicity and the cross-reactivity within different 2S albumins, which is as yet unexplored, to establish whether they act as panallergens similar to pathogenesis-related proteins or profilins.²⁵

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