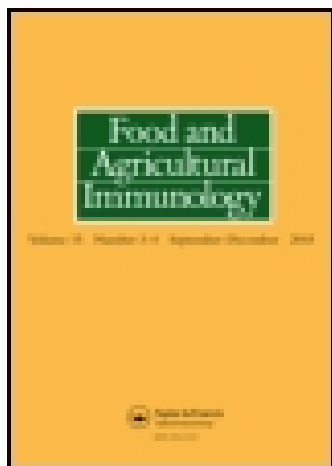




Food and Agricultural Immunology

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/cfai20>

Allergenicity and tolerance to proteins from brazil nut (*Bertholletia excelsa* H. B. K.)

Vânia Maria Maciel Melo ^a, José Xavier-Filho ^a, Maria Silva Lima ^a & Annie Prouvost-Danon ^{b c}

^a Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, Caixa Postal, 1065, Fortaleza, Ceará, 60001-970, Brazil

^b Departamento de Fisiologia e Farmacodinâmica, FIOCRUZ, Av. Brasil, 4365, Rio de Janeiro, 21045-900, Brazil

^c Unité d'Immuo-Allergie, Institut Pasteur, Paris, France

Published online: 16 Sep 2008.

To cite this article: Vânia Maria Maciel Melo, José Xavier-Filho, Maria Silva Lima & Annie Prouvost-Danon (1994) Allergenicity and tolerance to proteins from brazil nut (*Bertholletia excelsa* H. B. K.), Food and Agricultural Immunology, 6:2, 185-195, DOI: [10.1080/09540109409354829](https://doi.org/10.1080/09540109409354829)

To link to this article: <http://dx.doi.org/10.1080/09540109409354829>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

Allergenicity and Tolerance to Proteins from Brazil Nut (*Bertholletia excelsa* H. B. K.)

VÂNIA MARIA MACIEL MELO,¹ JOSÉ XAVIER-FILHO,¹ MARIA SILVA LIMA¹ AND ANNIE PROUVOST-DANON²

¹*Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, Caixa Postal, 1065, 60001-970 Fortaleza, Ceará, Brazil;* ²*Departamento de Fisiologia e Farmacodinâmica, FIOCRUZ, Av. Brasil, 4365, 21045-900 Rio de Janeiro, Brazil*

(Received for publication 4 August 1993)

Allergenicity to Brazil nut protein fractions and to a purified methionine-rich 2S protein, considered to be similar to the allergens of other oilseeds, has been followed through IgG₁ and IgE synthesis after mouse subcutaneous immunization. The allergenic capacities of all protein fractions and of the 2S protein are not as prominent. Oral intake of Brazil nuts in several doses was also tested in mice and rats as a possible route of immunization, but instead of immunization, a systemic tolerance was induced. This state of immunological tolerance was more efficiently induced by a scheme of multiple feeding than by a single feeding.

Keywords: Allergenicity, Brazil nut protein, immunological tolerance

INTRODUCTION

Brazil nut is known to have high levels of the sulphur-containing amino acids methionine and cysteine (Antunes, 1975) probably being the richest source of these essential amino acids (Sun, 1987). In an analysis of low molecular weight proteins in several oilseeds, Youle and Huang (1981) reported that a 2S albumin fraction from Brazil nut contained high levels of methionine and cysteine. After this finding there was an increasing interest in the nutritional value of Brazil nuts, as many vegetable proteins (mainly those from legume seeds) are deficient in these essential amino acids (De Clercq, 1990). This interest is easily understood when it is considered that approximately 700 million people include legume seeds in their diet (Abbot, 1966) and legumes, besides comprising up to 9% of the total plant production in developing countries, are still a major component of the diet of people in several countries (Lumen, 1990). Molecular approaches have been used for improving

Correspondence to M. S. Lima.

A. Prouvost—Danon is on sabbatical leave from Unité d'Immuno-Allergie, Institut Pasteur, Paris, France.

the nutritional quality of seed proteins. Among such studies, changes in the amino acid composition of seeds involving modifications of the specific seed storage protein genes were envisaged (Altenbach *et al.*, 1989). However, the use of 2S sulphur-rich protein-containing seeds to improve food may be hindered by the fact that the 2S albumin from the castor bean (*Ricinus communis*) and cottonseed (Youle & Huang, 1978, 1979), and the protein *Sin a 1* from yellow mustard, *Sinapis alba* (Menendez Arias *et al.*, 1988) have been recognized as atopic allergens for hypersensitive individuals (Sharief & Li, 1982; Menendez-Arias, 1988). More recently, Monsalve and Rodriguez (1990) showed that 11 purified proteins from the 2S protein fraction of *Brassica rapa*, *Brassica oleracea* and *Brassica napus* seeds cross-reacted with antibodies against *Sin a 1*, the protein from yellow mustard previously reported as an atopic allergen.

The aim of the work reported in this paper was to study the allergenicity or the tolerance to Brazil nut proteins, especially to its isolated 2S fraction, as assessed by the IgE and IgG₁ responses in mice, either after parenteral immunization or after food ingestion.

MATERIALS AND METHODS

Seeds and Seed Meal

Seeds of Brazil nut (*Bertholletia excelsa* H. B. K.) were obtained from commercial suppliers from Belém, State of Pará, Brazil. After decortication the seeds were coarsely ground and delipidated with *n*-hexane. After oil extraction the fragments were further ground in a coffee grinder to obtain a finely granulated meal. The seed meal was kept in hermetically sealed flasks at 4°C until use.

Protein Extraction

The finely divided meal was extracted (meal to buffer ratio 1:10) with 0.035 M-phosphate buffer, 1.0 M-NaCl, pH 7.5, for 2 h at 24°C. A crude extract was obtained by filtration and centrifugation (20 000 × *g* for 30 min at 4°C). This crude extract was exhaustively dialyzed against water, and proteins were recovered by freeze drying. This material is described as protein crude extract.

Protein Purification

Low molecular weight proteins were separated by gel filtration in a Sephadex G-150 column (1.75 × 50 cm) equilibrated with phosphate buffer (0.035 M-phosphate buffer, 1.0 M-NaCl, pH 7.5). A total of 10 ml of the crude extract (250 mg protein) were applied to the column and 3.0 ml fractions were collected. Protein was estimated by measuring the absorbance at 280 nm. Fractions (SI through SIV) were pooled and recovered by dialysis and freeze drying. Fraction SIII was submitted to ion-exchange chromatography in DEAE-Sephadex equilibrated with 0.005 M-phosphate buffer, pH 7.4, and eluted with a NaCl gradient (0–1.0 M in 100 ml) in the same buffer. Fractions (3.0 ml) were collected and protein was estimated by measuring the absorbance at 280 nm. Protein fractions (DI and DII) were pooled and recovered by dialysis and freeze drying. Protein fraction DI (200 µl of a 1.0 mg ml⁻¹ solution) was submitted to further fractionation in reverse-phase high-pressure liquid chromatography (HPLC) in a C₁₈-µ-Bondapak (Waters, Milford, MA, USA) column (3.9 mm × 30 cm) equilibrated with 0.1% trifluoroacetic acid (TFA) and eluted with an acetonitrile gradient (0–60%) in 0.1% TFA. Elution was performed with a flow rate of 1.0 ml min⁻¹ and protein was monitored by measuring the absorbance at 280 nm. Fractions (HPLC-I and HPLC-II) were manually collected and freeze-dried.

Reduction and Carboxymethylation of Protein

Protein fraction DI was reduced by beta-mercaptoethanol in the presence of 6.0 M-guanidine

hydrochloride and carboxymethylated by using iodoacetic acid according to the procedure of Crestfield *et al.* (1963).

Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in the presence and absence of beta-mercaptoethanol according to the method of Laemmli (1971). When proteins were reduced by beta-mercaptoethanol 8.0 M-urea was included. Proteins were stained by Coomassie brilliant blue.

Amino Acid Analysis

Protein fraction DI (10 μ g) was hydrolyzed (6.0 M-HCl + 0.5% phenol at 112°C for 24 h) and derivatized by phenylisothiocyanate. Amino acid analysis was performed in a Waters Pico-Tag HPLC system according to the recommendations of the manufacturer (Waters, 1986).

Antigens

The crude extract and fractions SI, SII, SIII and DI, were prepared as described above.

Subcutaneous Immunization

Groups of 10 2-month-old Swiss mice of both sexes were subcutaneously injected with 100 μ g CE, SI, SII, SIII and DI protein fractions admixed with 0.5 ml of a gel containing 1.0 mg of aluminium hydroxide, as adjuvant (Prouvost-Danon *et al.*, 1972). Booster injections were given on the 21st and 35th day after the primary injections. Mice were bled from the retro-orbital plexus at 7, 14, 21, 28, 35 and 42 days after the primary injection.

Oral Administration

Groups of 10 2-month-old mice of both sexes were fed with 0.1, 0.4, 0.8, 1.2 or 5.4 g of decorticated Brazil nuts, either in a single dose or in multiple (10) doses after fasting for 24 hours. The animals were bled from the retro-orbital plexus at 3, 7, 14, 21, 28, 35 and 42 days after ending the oral regimen with Brazil nuts.

Oral Administration and Subcutaneous Immunization

Groups of 10 animals previously fed with single or multiple amounts of Brazil nuts were subcutaneously immunized with 100 μ g of crude extract admixed with 0.5 ml of a gel containing 1.0 mg of aluminium hydroxide 3 days after the oral regimen with Brazil nuts. Booster injections were given on the 21st and 35th days as for the primary injections. The animals were bled at 7, 14, 21, 28, 35 and 42 days after immunization. Control groups of mice without feeding and/or without subcutaneous immunization were included in the study.

Antibody Titrations: Passive Cutaneous Anaphylaxis (PCA)

The IgG₁ antibodies were measured in pools of sera from the immunized mice by PCA in mice using a 2 h latent period for skin sensitization (Ovary, 1958). The PCA reactions were elicited by intravenous (retro-orbital plexus) injections of the antigens (250 μ g of crude extract or 125 μ g SI, SII, SIII and DI) in 0.250 ml of saline containing 0.5% Evans blue. Each test was carried out in four mice.

Controls without antigen gave negative results. The titers of IgE antibodies were determined in pooled sera from mice by PCA in rats (Mota, 1969) using an 18 h latent period for skin sensitization. The PCA reactions were elicited by intravenous injections of

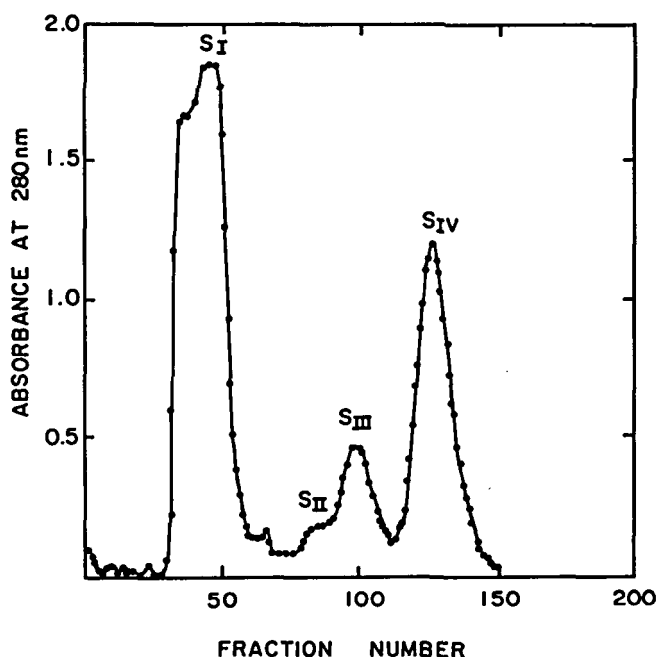


FIG. 1. Gel filtration chromatography of proteins (250 mg) of a crude extract of *B. excelsa* (Brazil nut) seeds. A column (1.75 × 50 cm) of Sephadex G-150 was equilibrated and run with 0.035 M-phosphate buffer containing 1.0 M-NaCl, pH 7.5. Fractions of 3.0 ml were collected. Absorbance was measured at 280 nm.

the antigens (2.0 mg of crude extract or 1.0 mg SI, SII, SIII and DI) in 1.0 ml of saline containing 0.5% Evans blue. Each test was carried out in two rats. Controls without antiserum or without antigen gave negative results. In both types of PCA the titers were recorded as the reciprocal of the highest dilution giving a positive reaction.

Statistical Analysis

Statistical analysis was performed using two-way analysis of variance (ANOVA) (Montgomery, 1991). Differences between group means were evaluated by the Tukey range test procedure (Lison, 1958) with values of $P \leq 0.05$ being considered significant.

RESULTS

Isolation of a Methionine-rich Protein from *B. excelsa* Seeds

The crude extract obtained from the seed meal was fractionated by gel filtration in Sephadex G-150 (SI, SII, SIII and SIV in Figure 1). Ion-exchange chromatography of the low molecular weight fraction SIII in DEAE-Sepharose showed that it was contained mainly in one fraction (DI in Figure 2). SDS-PAGE of this fraction in the absence of beta-mercaptoethanol showed only a band with a relative molecular weight of 13 000 (not shown). Reduction and carboxymethylation suggested that this protein was formed by two chains linked by disulphide bridges. Reverse-phase HPLC of fraction DI protein and SDS-PAGE of the eluted peak confirmed the molecular weight of 13 000 (not shown). Amino acid analysis of fraction DI showed that this fraction contained a high proportion of

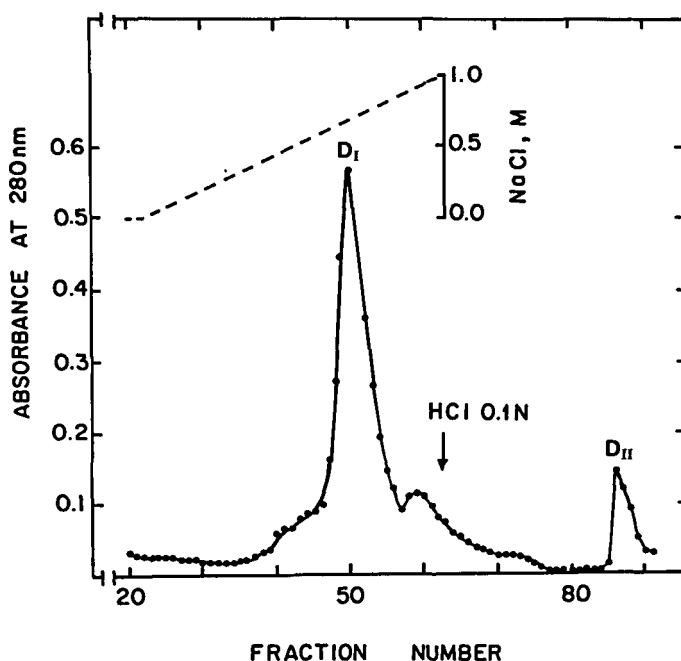


FIG. 2. Ion-exchange chromatography of the SIII fraction (20 mg) obtained in the gel-filtration step. A column of DEAE-Sephacrose was equilibrated with 0.005 M-phosphate buffer, pH 7.4, and eluted with the same buffer followed by a gradient of NaCl (0–1.0 M). Fractions of 3.0 ml were collected. Absorbance was measured at 280 nm.

methionine (13.0%) suggesting that it was possibly one of the molecular forms of the 2S seed albumin (Castro *et al.*, 1987).

Allergenicity of a Crude Extract of Protein Fractions (SI, SII and SIII) and of a Sulphur-rich Protein (DI) from Brazil Nuts

The allergenic response to Brazil nut proteins in mice was detected by measuring the production of IgG₁ and IgE. They were estimated by PCA titers in mice and rats respectively (Figure 3).

As shown in Figure 3(a) the titers of the IgG₁ response to crude extract increased with time until 7 days after the first booster injection, after which there was a slight drop.

Fractions SI, SII and SIII from the gel filtration step induced a lower IgG₁ synthesis than that obtained using the crude extract. Only the SI fraction gave a primary response and all three promoted increasing IgG₁ synthesis after the second booster injection.

Statistical analysis of the data showed that SI-, SII- and SIII-induced IgG₁ responses are similar and only the SII response differs significantly from the crude extract-induced IgG₁ response ($P \leq 0.05$). On the other hand, IgG₁ synthesis induced by DI, a sulphur-rich protein (Figure 3(a)), is slightly higher than that induced by the crude extract ($P \leq 0.05$).

IgE synthesis induced by crude extract SI, SII, SIII and DI is shown in Figure 3(b). The crude extract promoted a transient primary response at the seventh day. IgE synthesis started again after the 14th day but mainly after the first booster injection at the 21st day. The second booster injection induced an increase in IgE synthesis that again reached the same level as after the first booster. The IgE synthesis induced by SI, SII, SIII and by DI

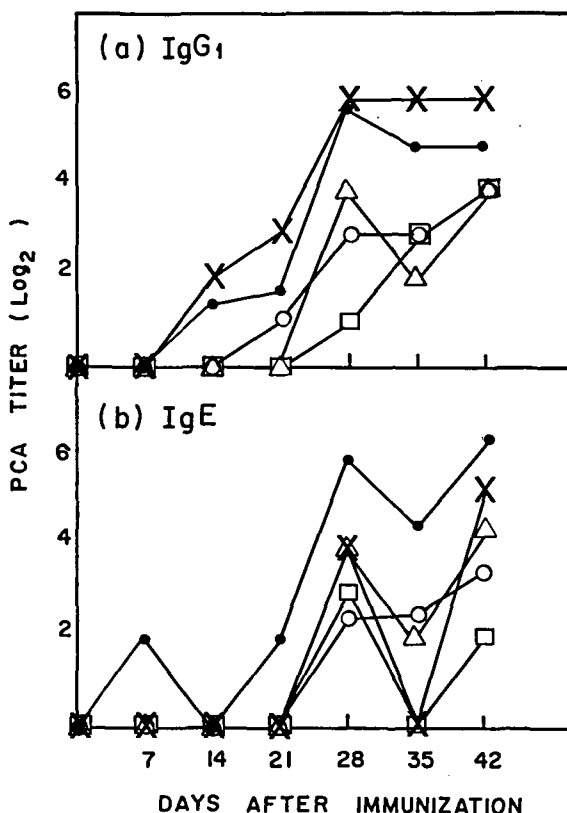


FIG. 3. IgG₁ and IgE immune responses to protein fractions of Brazil nuts. Swiss mice were immunized with 100 μ g of crude extract (●); SI (○); SII (□); SIII (△); and DI (X) mixed with 1.0 mg of aluminium hydroxide in saline by injection. Boosters with each protein fraction are indicated by an arrow. PCA reactions were challenged in mice for IgG₁ (a) with 250 μ g of crude extract or 125 μ g of the corresponding fraction used for immunizations, and in rats for IgE (b) with 2.0 mg of crude extract or 1.0 mg of the corresponding fraction used for immunizations. All values refer to pooled sera of 10 mice and are means of three experiments. Differences between control and SI, SII and SIII are significant with values of $P \leq 0.05$, and between control and DI $P \leq 0.01$.

did not exhibit the transient primary response seen with crude extract (Figure 3(b)), the immune response starting only after the first booster. In all cases (SI, SII, SIII and DI) IgE synthesis was lower than that induced by crude extract (Figure 3(b)) and increased after the second booster injection. These lower syntheses of IgE by SI, SII and SIII differ significantly with values of $P \leq 0.05$ and a value of $P \leq 0.01$ for DI, when compared with the control.

Induction of Tolerance by a Single Feeding with Increasing Amounts of Brazil Nuts
IgG₁ and IgE synthesis could not be detected after single feedings with 0.1, 0.4, 0.8, 1.2 and 5.4 g of Brazil nuts at days 3, 7, 10, 14, 21, 28, 35 and 42.

Three days after a single feeding dose of 0.1, 0.4, 0.8, 1.2 or 5.4 g of Brazil nuts, groups of 10 mice were immunized subcutaneously with 100 μ g of crude extract with aluminium hydroxide as adjuvant. Synthesis of IgG₁ and IgE was compared in all cases with that induced by subcutaneous immunizations with crude extract in mice without previous feeding. Figure 4(a) shows that a previous single feeding dose of 0.1, 0.4 or 0.8 g induces

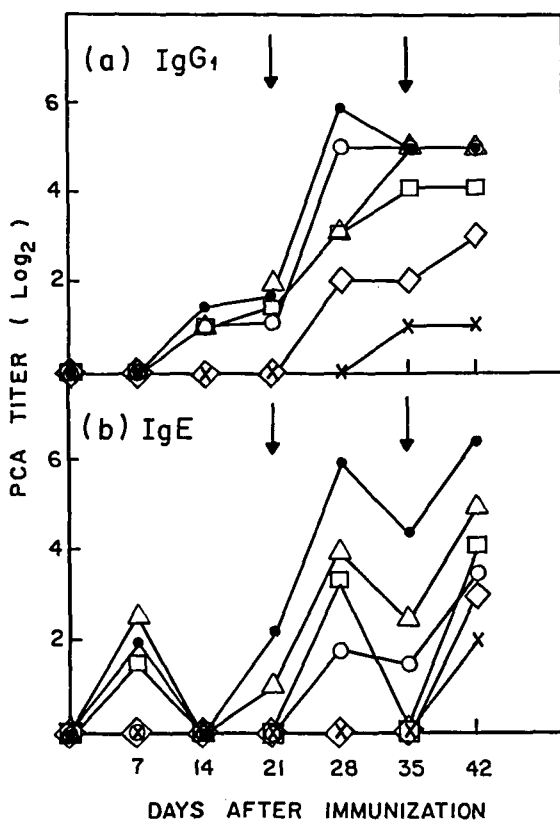


FIG. 4. Effect of previous feeding of Brazil nuts in a single dose on the IgG₁ and IgE responses. Swiss mice were fed with 0.1 g (□); 0.4 g (Δ); 0.8 g (○); 1.2 g (◇); and 5.4 g (X) of Brazil nuts. Control immunization was without previous feeding (●). Boosters with 100 μ g of crude extract are indicated by an arrow. PCA reactions were challenged in mice for IgG₁ (a) with 250 μ g, and in rats for IgE (b) with 1.0 mg of crude extracts. All values refer to pooled sera of five mice and are means of six experiments. Differences in (a) between control (non-fed) and animals fed with 1.2 and 5.4 g of Brazil nuts are significant, with $P \leq 0.05$. Differences in (b) between control (non-fed) and animals fed with 0.1, 0.8, 1.2 and 5.4 g of Brazil nuts are significant, with $P \leq 0.05$.

IgG₁ synthesis similar to that induced by crude extract alone. However, single feeding doses of 1.2 or 5.4 g induced progressive tolerance according to the dose, the IgG₁ titers being very low with the highest amount (5.4 g) of Brazil nut pre-treatment. These results are statistically significant with values of $P \leq 0.05$.

The IgE specific synthesis shown in Figure 4(b). All IgE titers were lower than the control. All treatments, apart from that with the 0.4 g dose, induced increasing levels of tolerance compared with a control group subcutaneously immunized with 100 μ g of crude extract without previous feeding (statistical significance with values of $P \leq 0.05$).

Induction of Tolerance by Multiple Feedings with Increasing Amounts of Brazil Nuts

Neither IgG₁ nor IgE synthesis could be detected after multiple feedings with any dose of Brazil nuts and at any time as observed until day 42.

Three days after an oral regimen of 10 feedings totalling 0.1, 0.4, 0.8 and 1.2 g of Brazil nuts, groups of 10 mice were subcutaneously immunized with 100 μ g of crude extract with

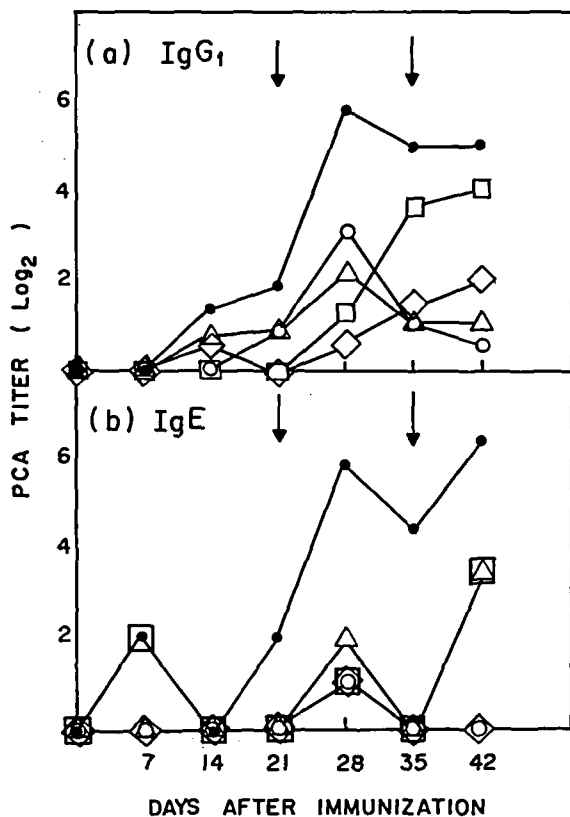


FIG. 5. Effect of previous feeding of Brazil nuts in multiple amounts on IgG₁ and IgE responses. Swiss mice were fed with 0.1 g (□); 0.4 g (△); 0.8 g (◇); and 1.2 g (●) of Brazil nuts. Control immunization was without previous feeding (●). Boosters with crude extract are indicated by an arrow. PCA reactions were challenged in mice for IgG₁ (a) with 250 µg, and in rats for IgE (b) with 1 mg of crude extract. All values refer to pooled sera of five mice and are means of six experiments. Differences in (a) and (b) between control (non-fed) and animals fed with 0.1, 0.4, 0.8 and 1.2 g of Brazil nuts are significant, with $P \leq 0.05$.

aluminium hydroxide as adjuvant. The synthesis of IgG₁ and IgE was compared to that induced by subcutaneous immunization with crude extract in mice without feeding. Figure 5(a) shows that this regimen induced a declining IgG₁ synthesis and tolerance starting with the dose of 0.1 g, the tolerance being progressively intensified up to the highest quantity of Brazil nuts ingested (differences statistically significant with values of $P \leq 0.05$).

IgE-specific synthesis for this regimen is shown in Figure 5(b). The IgE titres were progressively lower with increasing doses of Brazil nuts and there was no difference between the IgE titres with previous feeding either with 0.8 or 1.2 g, which were practically nil. All patterns of IgE synthesis were compared to that induced by subcutaneous immunization with crude extract of mice not submitted to previous feeding with Brazil nuts (differences statistically significant with values of $P \leq 0.05$).

DISCUSSION

A low molecular weight fraction from Brazil nut crude extract (SIII in Figure 1), when submitted to ion-exchange chromatography, was found mainly in one fraction (DI in Figure 2) and had a relative molecular weight of 13 000 as shown by SDS-PAGE. This protein

was formed of two chains linked by disulphide bridges (not shown) and its amino acid composition showed a high methionine content (not shown). These properties are similar to those earlier determined by Sun *et al.* (1987) and Castro *et al.* (1987) for the 2S albumin of Brazil nut. We refer to this methionine-rich protein as the 2S protein fraction.

The allergenicity of several protein preparations from Brazil nuts was demonstrated by the PCA assay which showed specific IgG₁ and IgE synthesis after subcutaneous immunization of Swiss mice (Figure 3).

The patterns of IgG₁ synthesis induced by SI, SII and SIII (Figure 3(a)) fractions were significantly lower than that induced by the crude extract (Figure 4(a)), while that for the 2S protein fraction (DI in Figure 2) was higher (Figure 3(a)).

The IgE titers induced by SI, SII and SIII (Figure 3(b)) and by the 2S protein fraction DI (Figure 5(b)), were lower than that promoted by the crude extract. As judged from the production of IgE, the allergenic capacity of all protein fractions including the 2S sulphur-rich protein of Brazil nut is not as prominent. According to recently published data on Brazil nut-associated allergy in humans (Breuil & Dore, 1993), only 12 such cases have been described in the literature. However, it has been known that cottonseed, nuts and other oilseeds contain potent allergens (Spies, 1974) that can be isolated according to the classical procedure of Spies *et al.* (1951). More recently, Youle and Huang (1978, 1979) have separated 2S albumins from castor beans and cottonseeds that were shown to be similar to the allergens previously isolated by Spies *et al.* (1951). Furthermore, the protein *Sin a 1* from yellow mustard (Menendez-Arias *et al.*, 1988) has been reported to be an atopic allergen to hypersensitive individuals, being related to other low molecular weight albumins such as those from rapeseed, sunflower and Brazil nut (Kortt & Caldwell, 1990; Monsalve & Rodriguez, 1990; Youle & Huang, 1981). Indeed, previous data from allergens of oilseeds based only on chemical analysis or immunodiffusion are poor proof to the establishment of their allergenicity. More sensitive techniques are required for a complete characterization of such allergens (Ory & Sekul, 1983).

In the present work, feeding with Brazil nuts was also tested as a possible route of immunization. Mice were fed decorticated Brazil nuts, either in single or multiple doses, with the quantities varying between 0.1 and 5.4 g. In neither of the two schemes (single or multiple doses), was any specific IgG₁ or IgE against the crude extract of Brazil nuts detected. Indeed, both schemes of oral feeding suppressed specific IgG₁ and IgE synthesis in mice according to the amounts of ingested nuts when it was followed by subsequent subcutaneous immunization with the crude extract. Instead of immunization, a systemic tolerance has developed.

As it has already been mentioned, orally administered antigens may induce effective tolerance suppressing subsequent immune response to otherwise immunogenic contacts with the antigen (Thomas & Parrot, 1974; Vaz *et al.*, 1977; Tomasi, 1980; Mowat *et al.*, 1986; Aizpurua & Russel-Jones, 1988).

The present experiments show that previous ingestion of Brazil nuts induced tolerance more efficiently if a scheme of multiple feeding was used (Figure 5) rather than a single feeding scheme (Figure 4), and by increasing the amounts of Brazil nuts in both schemes (Figures 4 and 5). Furthermore, the IgE isotype was more sensitive to the tolerance effects of Brazil nut extract than the IgG₁ isotypes. This result agrees with those of other researchers which have shown that T-cell mediated immunological tolerance is preferentially expressed against IgE antibody isotype (Snapper *et al.*, 1988; Menamin *et al.*, 1991).

These results establish the allergenic abilities of proteins of Brazil nuts by direct measurements of specific IgG₁ and IgE antibodies. Apparently, these proteins and the 2S protein among them, are not such potent allergens when administered subcutaneously. Furthermore, they induce tolerance when administered orally. The allergenic properties inferred to the allergens of other oilseeds by the identification of the 2S protein fraction (Youle & Huang, 1981; Ampe *et al.*, 1986; Sun *et al.*, 1987; Guerche *et al.*, 1990) were not confirmed in relation to IgE synthesis.

It has been shown (Elson & Ealding, 1984; Aizpurua & Russel-Jones, 1988; Restum-Miguel & Prouvost-Danon, 1991; Lima *et al.*, 1991) that the proteins that are active in oral immunization are known to possess 'lectin or lectin-like' binding activities, and that they act at low doses (in the order of μg). This is in contrast with Brazil nut proteins and other non-lectin antigens (Thomas & Parrot, 1974; Vaz *et al.*, 1977; Tomasi, 1980; Mowat, 1986) which induce oral tolerance and act at higher doses (in the order of mg).

It has been suggested that the balance between mucosal and systemic response may be related to the route by which antigen is initially encountered (Owen, 1983). Brazil nut protein could be used in this way as a tool in designing experiments for understanding the mechanisms that regulate systemic immunity to antigens orally administered and thus allowing a wide nutritional use of proteins acting as antigens (Altenbach *et al.*, 1989; De Clercq, 1990). Considering these characteristics, Brazil nuts should be considered a high-quality food.

Furthermore, the possibility of using the gene coding for the 2S protein through genetic manipulation should be explored in order to improve the nutritional quality of food plants (Ampe *et al.*, 1986; Guerche *et al.*, 1990).

ACKNOWLEDGEMENTS

This work was supported by the Brazilian agencies CAPES (J. X.-F., V. M. M. M.) and CNPq (J. X.-F., M. S. L. and A. P.-D.) and the French CNRS (A. P.-D.).

REFERENCES

- ABBOT, J. C. (1966) Protein supplies and properties. The problem, in *World Protein Resources* (Gould, R. F., Ed.), American Chemical Society, Washington DC, USA pp. 1–15.
- AIZPURUA, H. J., DE & RUSSEL-JONES, G. J. (1988) Oral vaccination. Identification of classes of proteins that provide an immune response upon oral feeding, *Journal of Experimental Medicine*, **167**, 440–451.
- ALTENBACH, S. B., PEARSON, K. W., MECKER, G., STARACI, L. C. & SUN, S. S. M. (1989) Enhancement of the methionine content of seed proteins by the expression of a chimeric gene encoding a methionine-rich protein in transgenic plants, *Plant Molecular Biology*, **13**, 513–522.
- AMPE, C., VAN DAMME, J., DE CASTRO, L. A. B., SAMPAIO, M. J. A. M., VAN MONTAGU, M., & VANDEKERCKHOVE, J. (1986) The amino-acid sequence of the 2S sulfur-rich proteins from seeds of Brazil nut (*Bertholletia excelsa* H. B. K.), *European Journal of Biochemistry*, **159**, 597–604.
- ANTUNES, A. J. (1975) Protein supplement of navy bean with Brazil nuts. PhD Thesis, Department of Food Science and Human Nutrition, State University of Michigan, East Lansing, MI, USA.
- BREUIL, K. & DORE, D. (1993) Allergie à la noix du Brésil, *Journées Parisiennes d'Allergie* (Société Française d'Allergologie et d'Immunologie Clinique) Paris, 8 January.
- CASTRO, L. A. B. DE, LACERDA, Z., ARAMAYO, R. A., SAMPAIO, J. A. M. & GANDER, E. S. (1987) Evidence for a precursor molecule of Brazil nut 2S seed proteins from biosynthesis and c-DNA analysis, *Molecular General Genetics*, **206**, 338–343.
- CRESTFIELD, A. M., MOORE, S. & STEIN, W. H. (1963) The preparation and enzymatic hydrolysis of reduced and S-carboxymethylated proteins, *Journal of Biological Chemistry*, **238**, 622–631.
- DE CLERCQ, A., VANDEWIELE, M., VAN DAMME, J., GUERCHE, P., VAN MONTAGU, M., VANDEKERCKHOVO, J. & KREBBUS, E. (1990) Stable accumulation of modified 2S albumin and storage proteins with higher methionine contents in transgenic plants, *Plant Physiology*, **94**, 970–979.
- ELSON, C. O. & EALDING, W. (1984) Cholera toxin feeding did not induce oral tolerance in mice and abrogated oral tolerance to one unrelated protein antigen, *Journal of Immunology*, **133**, 2892–2897.
- GUERCHE, P., DE ALMEIDA, E. R. P., SCHWARZTEIN, M. A., GANDER, E., KREBBUS, E. & PELLETIER, G. (1990) Expression of the 2S albumin from *Bertholletia excelsa* in *Brassica napus*, *Molecular General Genetics*, **221**, 306–314.
- KORTT, A. A. & CALDWELL, J. B. (1990) Low molecular weight albumins from sunflower seed: identification of a methionine-rich albumin, *Phytochemistry*, **29**, 2805–2810.
- LAEMMLI, U. K. (1971) Cleavage of structural proteins during the assembly of the head of bacteriophage TH, *Nature*, **227**, 680–685.
- LIMA, M. S., ALBUQUERQUE, D. A., NUNES, D. C. S. & PUSZTAI, A. (1991) Modulation of IgE and IgG₁ responses in mice by jacalin, *Joint Meeting BSI-SFI*, London, Abstracts, p. 105, OP 37.
- LISON, L. (1958) In *Statistique Appliquée à la Biologie Expérimentale. La Planification de l'Expérience et l'Analyse des Résultats* VILLARS, G., Ed., Paris pp. 110–132.

- LUMEN, BENITO, O. (1987) Molecular approaches to improving the nutritional and functional properties of plant seeds as food sources. Developments and comments, *Journal of Agricultural and Food Chemistry*, **38**, 1779–1897.
- MENAMIN, C., SCHON-HEGRAD, O. J., GIRN, B. & HOLT, P. G. (1991) Regulation of IgE responses to inhaled antigens: cellular mechanisms underlying allergic sensitization versus tolerance induction, *International Archives of Allergy and Applied Immunology*, **94**, 78–82.
- MENENDEZ-ARIAS, L., MONEO, I., DOMINGUEZ, J. & RODRIGUEZ, R. (1988) Primary structure of the major allergen of yellow mustard (*Sinapis alba* L.) seed *Sin a I*, *European Journal of Biochemistry*, **177**, 159–166.
- MONSALVE, I. & RODRIGUEZ, R. (1990) Purification and characterization of proteins from the 2S fraction from seeds of the *Brassicaceae* family, *Journal of Experimental Botany*, **41**, 89–94.
- MONTGOMERY, D. C. (1991) In *Design and Analysis of Experiments* (Wiley, Ed.), New York, 418 pp.
- MOTA, I. & WONG, D. (1969) Homologous and heterologous passive cutaneous anaphylactic activity of mouse anti-sera during the course of immunization, *Life Science*, **8**, 813–820.
- MOWAT, A. MCL., THOMAS, M. J., MAC KENZIE, S. & PARROT, D. M. V. (1986) Divergent effects of bacterial esposaccharide on immunity to orally administered protein and particulate antigens in mice, *Immunology*, **58**, 677–683.
- ORY, R. L. & SEKUL, A. A. (1983) Allergens in oilseeds, in *Seed Proteins* (DAUSSANT, J., MOSSÉ, J. & VAUGHAN, J., Eds), Academic Press, London, pp. 83–89.
- OVARY, Z. (1958) Passive cutaneous anaphylaxis in the mouse, *Journal of Immunology*, **81**, 355–358.
- OWEN, R. L. (1983) Ultrastructure of antigen trapping epithelia of mucosal lymphoid organs, in *Regulation of the Immune Response* OGRA, P. L. & JACOBS, D. M., Eds, Karger, Basel, pp. 88–98.
- PROUVOST-DANON, A., BINAGHI, R., ROCHAS, S. & BOUSSAC-ARON, Y. (1972) Immunochemical identification of mouse IgE, *Immunology*, **23**, 481–491.
- RESTUM-MIGUEL, N. & PROUVOST-DANON, A. (1991) Effects of oral feeding with jacalin on IgE and IgG₁ responses in mice, *Joint Meeting BSI-SFI*, London, Abstracts, p. 105, OP 36.
- SHARIEF, T. S. & LI, S. S. (1982) Amino acid sequence of small and large subunits of seed storage protein from *Ricinus communis*, *Journal of Biological Chemistry*, **257**, 14753–14759.
- SNAPPER, C. M., FINKELMAN, F. D. & PAUL, W. E. (1988) Differential regulation of IgG₁ and IgE synthesis by interleukin-4, *Journal of Experimental Medicine*, **167**, 183–196.
- SPIES, J. R. (1974) Allergens, *Journal of Agricultural and Food Chemistry*, **22**, 30–35.
- SPIES, J. R., COULSON, E. J., DORRIS, C., CHAMBERS, B., HARRY, S., STEVENS, H. & SHIMP, J. H. (1951) The chemistry of allergens. XI. Properties and composition of natural protease isolated from oilseeds and nuts by the CS-IA procedure, *Journal of the American Chemical Society*, **73**, 3995–4001.
- SUN, S. S. M., LEUNG, F. W. & TOMIC, J. C. (1987) Brazil nut (*Bertholletia excelsa* H. B. K.) proteins: fractionation, composition and identification of a sulfur rich protein, *Journal of Agricultural and Food Chemistry*, **35**, 232–235.
- THOMAS, H. C. & PARROT, M. V. (1974) The induction of tolerance to a soluble protein antigen by oral administration, *Immunology*, **27**, 631–640.
- TOMASI, T. B. (1980) Oral tolerance, *Transplantation*, **29**, 353–356.
- VAZ, N. M., MAIA, L. C. S., HANSON, D. G. & LYNCH, J. M. (1977) Inhibition of homocytotropic responses in adult inbred mice by previous feeding of the specific antigen, *Immunology*, **60**, 115.
- WATERS (1986) Pico-tag amino-acid analysis system, *Operator's Manual*, no. 88140, revision 3.
- YOULE, R. J. & HUANG, A. H. C. (1978) Albumin storage proteins in the protein bodies of castor bean, *Plant Physiology*, **61**, 13–16.
- YOULE, R. J. & HUANG, A. H. C. (1979) Albumin storage proteins and allergens in cottonseeds, *Journal of Agricultural and Food Chemistry*, **27**, 500–503.
- YOULE, R. J. & HUANG, A. H. C. (1981) Occurrence of low molecular weight and high cysteine containing albumin storage proteins in oilseeds of diverse species, *American Journal of Botany*, **68**, 44–48.