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Immunogenicity and allergenicity of 2S, 7S and 11S soy protein fractions

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It is known that in a part of the population, mainly among children, some are hypersensitive to soybean protein, although it is not yet completely elucidated which protein fraction is more immunogenic/ allergenic. The objective of the study was to compare the immunogenicity and allergenicity of the soy protein fractions. The 2S (conglycinin), 7S (β conglycinin) and 11S (glycinin) fractions were isolated from soy protein by affinity chromatography. These purified soy protein fractions were used as antigens for immunizing BALB/c mice to evaluate their immunogenicity by following the appearance of specific IgM and IgG antibodies in blood serum by ELISA. The allergenicity of these soy protein fractions was evaluated by the following approaches: i) the production of IgE antibodies against 2S, 7S and 11S soy protein fractions by BALBc mice in the anaphylactic cutaneous passive test (PCA), and ii) the production of IgG1 specific antibodies against the 7S fraction in BALB/c mice. The 7S and 11S fractions induced the formation of IgM and IgG antibodies. The PCA test showed that only the 7S fraction was allergenic leading to the production of IgE antibodies. Another evidence that reinforces the allergenicity of the 7S soy protein fraction is the presence of IgG1 specific antibodies reactive to this protein fraction in immunized mice. Our study shows that the 7S soy protein fraction is important to elicit allergic reactions in mice and may contribute to elucidate the allergenicity of soy-derived products.

Uniterms
- Food allergy
- Soybean/allergenicity
- Soybean/immunogenicity
- ELISA – PCA

INTRODUCTION

Allergic reactions to foods result from heightened immunologic responses to glycoprotein components present in the foods and constitute a frequent health complaint (Metcalfe, 1991; Shah, Walker, 2002). Children and adults who develop such reactions are said to have a food hypersensitivity or food allergy that are terms proposed to define a reaction to food exposure causing objectively reproducible symptoms or signs at a dose

tolerated by normal subjects (Crespo *et al.*, 2004). The prevalence of food allergy in children appears to be increasing for reasons that are still poorly understood. It has been estimated that approximately 6% of young children and about 2% of the general population have food allergy, with the majority experiencing gastrointestinal symptoms (Sampson, 2003).

A number of factors can affect the development of food allergy. One is the amount of a particular food that is ingested, which is influenced by diet and culture of a country. This can have a significant effect on the prevalence of a specific food allergy in that region. Examples of this are codfish allergy in Norway, rice and soy allergy in Japan, and peanut allergy in the U.S. Other factors involved in food allergies are the gut permeability, that has been considered to be a major factor in food allergy sensitization, and the food processing that can substantially modify food allergen content. Most food allergens are stable molecules that resist the effect of food processing and cooking and the digestive processes (Taylor *et al.*, 1987). There are, however, exceptions such as the labile allergens present in apple and other fresh fruits and vegetables (Dreborg, Foucart, 1983). Conversely, it has been suggested that digestion can modify antigens. Finally, intrinsic factors are present. Most known food allergens have molecular weights between approximately 10 and 70 kDa. However, proteins larger than 70 kDa can also be allergens. by releasing or uncovering new epitopes, thus increasing allergenicity.

Although several immunologic mechanisms may operate in allergic reactions to foods, the IgE-mediated immediate hypersensitivity reaction is, by far, the most well established mechanism. The IgE-mediated allergic immune response can be divided into three phases: i) the sensitization phase in which B cells switch to the production of specific IgE; ii) the effector phase consisting of an acute reaction and a facultative late-phase reaction; iii) a chronic phase that may be the result of repetitive late-phase reactions (Bischoff *et al.*, 2000). The acute reaction causes activation of mast cells and basophils releasing histamine, leukotrienes, and other mediators known to be responsible for the wheal and flare reaction occurring in the skin and at various mucosal sites including the eye, nose, lung, and gastrointestinal tract. This reaction, that occurs within seconds to minutes, may be followed by a late-phase reaction starting within 2-24 hours after an allergen challenge and is characterized by a cellular infiltration of the tissue with granulocytes (basophils, eosinophils) and lymphocytes (mainly Th2 cells).

Allergy to proteins of cow milk, eggs, wheat, and soy is more common in infants and young children, whereas seafood, peanuts, and tree nuts are the most common cau-

ses of food allergy in adult life (Bischoff *et al.*, 2000).

Once the diagnosis of food hypersensitivity has been established, the only proven therapy is elimination of the offending allergen (Sampson, 1983). The first description of the use of a soy product as a cow milk substitute was made in 1909 (Ruhrah, 1909). Subsequently, soy protein formulae have been widely used to feed infants to ensure not only survival but also a normal life for many children who were affected by the wide spectrum of clinical manifestations of cow milk protein allergy (Kay *et al.*, 1960; Juto *et al.*, 1978; Naidoo *et al.*, 1981; Steichen, Tsang, 1987; Businco, Cantani, 1990). Surprisingly, a variety of adverse reactions have been reported involving soy proteins (Cook, 1960; Sampson, 1991), including allergenicity of soybean protein fractions (Ratner, 1955; Shibasaki *et al.*, 1980; Burks *et al.*, 1988; Codina *et al.*, 2003). The prevalence of a concomitant intolerance to soy in the allergy to cow milk is between 0% and 63% with high levels in the enterocolitis/enteropathy syndromes not associated with IgE (Van Sickle *et al.*, 1985; Bock, Atkins, 1990; Ragno *et al.*, 1993; Burks *et al.*, 1994; Harikul *et al.*, 1995). However, the true prevalence of allergy to soy proteins in documented allergies to proteins in cow milk is not yet well established (Kuitunen *et al.*, 1975; Jakobsson, Lindberg, 1979; Zeiger *et al.*, 1999; Ahn *et al.*, 2003).

The immunogenicity and allergenicity of soy proteins is a matter of discussion by several investigators and it is not completely clear which protein fractions of soy are the most allergenic. It has been suggested that the 2S and 7S soy protein fractions are allergenic (Shibasaki *et al.*, 1980; Ogawa *et al.*, 1991). With the purpose to contribute to the elucidation of soy allergenicity this study evaluated: i) the capacity of the 2S, 7S and 11S soy protein fractions to induce the production of IgM and IgG antibodies in mice; ii) the induction of the IgE type antibodies by 2S, 7S and 11S soy protein fractions in mice using the PCA test; iii) the production of IgG1 specific antibody against 7S protein fraction in mice.

MATERIAL AND METHODS

Animals

Male BALB/c mice and male Wistar rats, weighing 30-40 g and 150-200 g, respectively, from the "Instituto de Ciências Biomédicas - Universidade de São Paulo" were used. All animals had free access to water and food (commercial chow from Purina®, São Paulo, Brasil). This experimental study was approved by the Ethics Committee of Animal Experimentation of the Faculty of Pharmaceutical Sciences of the University of São Paulo.

Isolation of soy protein fractions

The 7S fraction was purified from the soy protein isolate and the 11S fraction from the defatted soy flour (Bünge Alimentos, São Paulo, Brazil), according to the procedures described by Burks *et al*. (1988) and Thanh, Shibasaki (1976), respectively, with minor modifications (Bittencourt *et al*., 2005). The 7S fraction was extracted with 20 parts (m/v) of a solution containing 0.03M Tris-HCl buffer (pH 6.2), 0.001 M dithioerytritol (Sigma Chem. Co., St Louis, USA), kept under stirring, at 9 °C for 1 h; then, the preparation was centrifuged at 35,000 x g at 4 °C for 20 min. The pellet was discarded and pH of the supernatant adjusted to 4.8 with 1N HCl (Merck Co., Rio de Janeiro, Brazil) and centrifuged at 35,000/g for 20 min, at 4 °C. After the elimination of the supernatant, the pellet was diluted in 10 parts 0.03M Tris-HCl buffer (pH 6.2) and pH adjusted to 7.6 with 1N NaOH (Merck Co., Rio de Janeiro, Brazil). This suspension was kept under agitation at 9 °C until the pellet dissolved. The pH was adjusted again to 6.2 and the solution was centrifuged at 35,000 x/g for 20 min, at 4°C, and supernantant was used as the crude extract. Then, 0.4 M NaCl (Merck Co., Rio de Janeiro, Brazil) was added to this extract and the solution was chromatographed on an affinity column with Concana-valin-Sepharose 4B (Sigma-Aldrich Ltd., St. Louis, USA). The second protein peak eluted from this column was dialyzed against distilled water for 12 h and filtered on 100 kDa membranes. The retained sample in the membrane was dissolved, concentrated under vacuum and fractionated by electrophoresis on a 5-20% gradient SDS-PAGE and followed by Coomassie blue staining. The 2S and 11S fractions were extracted and purified from the defatted soy flour that was initially extracted with 20 parts 0.03M Tris-HCl, pH 8.0, containing 0.01M 2-mercaptoethanol (Sigma-Aldrich Ltd., St Louis, USA). This sample was allowed to rest for 1 hour at room temperature and afterwards was centrifuged at 10,000 rpm for 20 min, at 20 °C. The first pellet was rejected and the supernatant considered as the crude extract. The pH was then adjusted to 6.4 with 2 N HCl and the sample centrifuged at 10,000 rpm for 20 min at 4 °C. The pH of the supernatant was adjusted to pH 4.8 and centrifuged again. To eliminate the possible presence of the soy 2S fraction, whose MW is identical to the basic subunit of the 11S fraction, the supernatant was filtered on a 100 kDa membrane (Filtron Technol. Corp., Northborough, USA) and the retained sample on the membrane was dissolved and concentrated under vacuum. The supernatant was analyzed by 5-20% gradient SDS-PAGE and Coomassie blue staining. The filtrate was separated on 5-20% gradient SDS-PAGE and the bands were stained with Coomassie blue for identification of the 2S soy protein fraction.

Protein Concentration

The total protein concentration in the serum of mice immunized with 2S, 7S and 11S protein fractions was evaluated according to Lowry *et al*. (1951), using bovine serum albumin as standard.

Immunization

The immunization of BALB/c mice was made in three steps with different purposes: i) to evaluate the production of IgM and IgG, groups of 10 BALB/c mice were immunized with 2S, 7S and 11S protein fractions, separately, in two steps. Initially these animals received 50 µg of antigen emulsified in complete Freund's adjuvant (1:2) administering 200 µL/animal intraperitoneally (i.p). After 15 days, these animals received 100 µg of the same antigen, emulsified in incomplete Freund's adjuvant (1:2) by injecting the same amount of antigen/animal (i.p.); ii) to evaluate the presence of IgE, groups of 8 BALB/c mice were immunized with 50 µg of the 2S, 7S and 11S protein fractions, separately, using 7.5 mg aluminum hydroxide in 0,5 mL saline (i.p.) as adjuvant, followed by a booster of 5 µg of the same antigen 28 days after the first immunization; iii) to evaluate the presence of specific IgG1 antibodies, groups of 8 BALB/c mice were immunized with 50 µg 7S protein fraction utilizing 7.5 mg of aluminum hydroxide in 0,5 mL saline (i.p.) as adjuvant, and after 28 days the animals received a booster of 5 µg of the same antigen. After immunization, blood was collected periodically in the ophthalmic plexus and serum (pool) was obtained after centrifugation at 800 g for 10 minutes. Following the collection of blood each animal was sacrificed with ethyl ether in a closed chamber. These sera were used to evaluate the production of IgE directed against the 2S, 7S and 11S protein fractions by using the PCA test in Wistar rats, as well as the production of IgM, IgG and IgG1 by ELISA tests.

IgM, and IgG antibodies directed against 2S, 7S and 11S soy protein fractions in mice

Polystyrene plates with 96 wells (EIA/RAI, Costar, Cambridge, MA, USA) were coated with 50 µL 2S, 7S or 11S fractions (1 µg/well) and incubated for 12 h at 37 °C. Excess protein fractions not adsorbed onto plates was removed by washing with PBS + Tween 20 (0.05% v/v) followed by blocking with defatted milk at 5% in PBS, pH 7.2 and incubation for 2 h at 37 °C. After blockade, plates

were washed, as described above, and then incubated for 2 h at 37 °C with 50 μL/well of the test serum. The following steps included washing the plates, addition of 50 μL anti-IgG (alkaline phosphatase conjugated affinity purified sheep anti-mouse IgG, Rockland) or anti-IgM (alkaline phosphatase conjugated affinity purified goat anti-mouse IgM heavy chain, Rockland) conjugate labeled with alkaline phosphatase, diluted to 1:1000 in defatted milk at 1% in PBS, pH 7.2, followed by incubation at 37 °C for 2 h. After washing the plates, a solution containing the alkaline phosphatase substrate (*p*-nitrophenylphosphate in 0.1 M glycine, 1 mM $MgCl_2$ and 1 mM $ZnCl_2$, pH 10.4) was added and left for 30 minutes at room temperature, protected from light. The reaction was blocked with 50 μL 3 M NaOH and the absorbance measured at 405 nm. The immunogenicity was evaluated by measuring the immune humoral response against the soy protein fractions considering the absorbance obtained for the different sample dilutions without defining a threshold for high and low responsiveness

Test of Passive Cutaneous Anaphylaxis

The titers of IgE anaphylactic antibodies in serum from BALB/c mice were determined using the PCA test. After immunization of mice with 2S, 7S or 11S protein fractions, separately, blood was collected weekly through the orbital plexus and serum was obtained after centrifugation at 800 g for 10 minutes. The serum obtained from each mouse was kept at -20 °C until analysis. The serum pool was prepared from equal volumes of serum obtained from each mouse at 7, 14, 21, 28 and 35 days after immunization. Wistar rats, shaved on the back, were sensitized intradermically with 100 μL of a pooled serum (undiluted, 1/5, 1/10, 1/20, 1/40 and 1/80 serial serum dilutions) obtained from mice immunized with 2S, 7S or 11S of soy protein fractions, separately. After 18 and 24 h, the rats were challenged intravenously (caudal vein) with 1 mL 0.25% Evans Blue containing 0.5 mg of the same antigen used in the immunization. At 2 h after challenging, the rats were sacrificed with ethyl ether in a closed chamber and the reaction was observed on the inverted skin by measuring the diameter of the reaction in the skin. The IgE titer was expressed as the reciprocal of the largest serum dilution which resulted in a positive reaction in the skin with a diameter ≥ 5 mm. The tests were made in triplicate considering at least two animals with positive reactions.

### IgG1 antibodies directed against 7S soy protein fraction in mice

Polystyrene plates with 96 wells (EIA/RAI, Costar, Cambridge, MA, USA) were coated with 7S soy protein

fraction (10 μg/mL) and incubated overnight. After incubation the plates were washed with PBS + Tween 20 (0.05% v/v) followed by blocking with defatted milk at 5% in PBS, pH 7.2 and incubation for 2 h at 37 °C. The following steps included washing the plates, addition of 50 μL of test serum and incubation for 2 h at 37 °C. Then, the plates were washed and 50 μL LOMG1-PO (peroxidase conjugated affinity purified rat anti-mouse IgG1) was added, followed by incubation at 37 °C for 1h. After washing the plates, a solution containing 10 μL H_2O_2 and 10 mg OPD in 10 mL citrate buffer 0.2 M, pH 5.0 was added and left for 30 minutes at room temperature, protected from light. The reaction was blocked with 30% sulfuric acid and the absorbance measured at 490 nm.

RESULTS

Purification of 2S, 7S and 11S protein fractions from soybean

The analysis by SDS-PAGE in denatured conditions (50V in the loading phase (7%) and 150V in the separation phase (20%) confirmed the presence of purified 2S fraction (MW 20 kDa), α (MW 63.17 kDa), α' (MW 58.06 kDa) and β (MW 42.09 kDa) subunits of the 7S fraction and the acidic (MW 38.8 KDa) and basic (MW 21.04 KDa) subunits of the 11S fraction of soy (Figure 1).
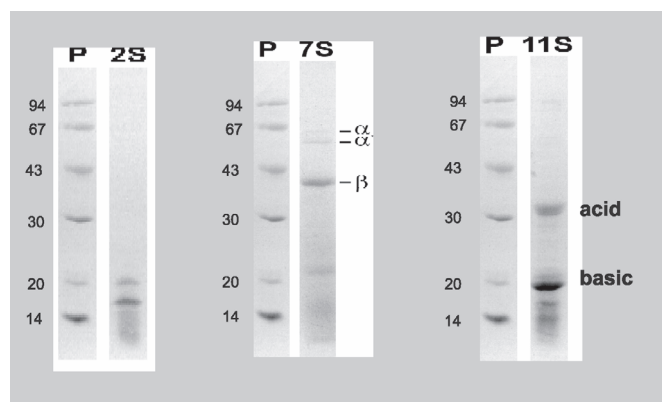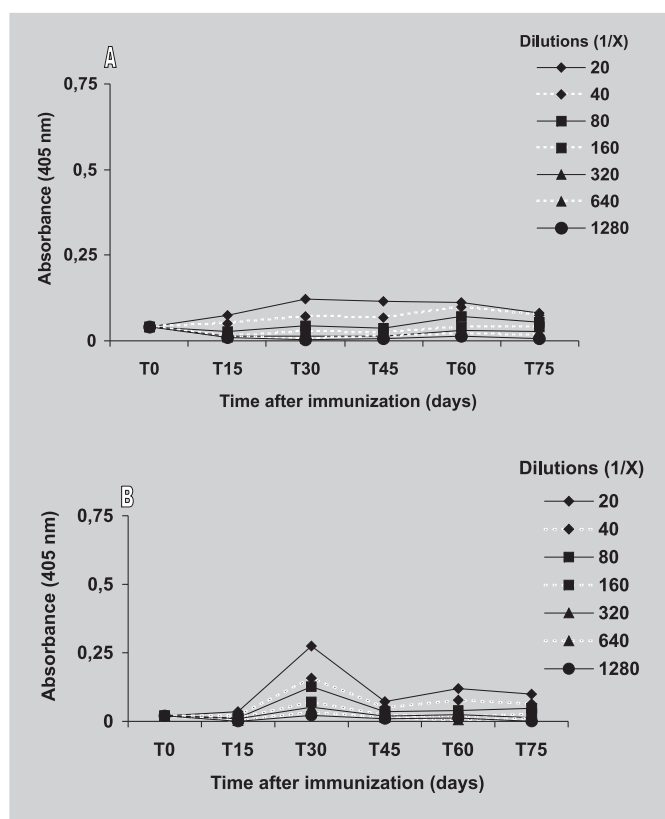

FIGURE 1 – SDS-PAGE of 2S soy protein fraction, α, α' and β subunits of the 7S protein fraction and acidic and basic subunits of the 11S protein fraction of the soy, stained with Coomassie blue. Numbers on the left indicate the migration of molecular weight markers.

IgM and IgG antibodies reactive to 2S, 7S and 11S soy protein fractions produced in mice

The immunogenicity of the 2S, 7S and 11S soy protein fractions was investigated by evaluating the
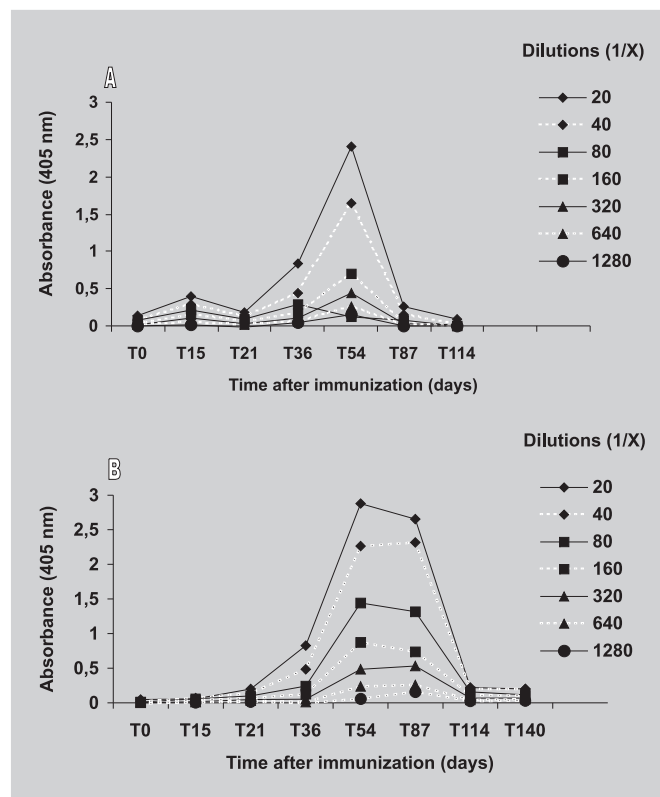
humoral immune response in mice through the production of IgM and IgG antibodies. The 2S protein was not immunogenic in mice as no production of IgM and IgG antibodies was detected post-immunization of mice with this soy protein fraction (Figure 2). In contrast, the 7S fraction induced a strong immune response with a high production of IgM and IgG, respectively, between the 21st – 87th and the 21st – 114th days after immunization (Figure 3). The 11S fraction induced an initial and low-intensity IgM response between the 18th and 43rd days, as well as an intense IgG production from the 18th to the 90th day after immunization with two maximal peaks on the 27th and 57th days (Figure 4).

FIGURE 3 - Time course of formation of IgM (A) and IgG (B) antibodies reactive to the 7S soy protein fraction after immunization of BALB/c mice. The data are expressed as mean of duplicates.

FIGURE 2 - Time course of production of IgM (A) and IgG (B) antibodies reactive to the 2S soy protein fraction after immunization of BALB/c mice. The data are expressed as mean of duplicates.

Evaluation of the 2S, 7S and 11S soy protein fractions allergenicity by the Passive Cutaneous Anaphylaxis test (PCA)

The capacity of the 2S, 7S and 11S soy protein fractions to induce the formation of IgE antibodies in the immunized BALB/c mice due to the binding capacity of IgE to mast cells in the skin of rats was investigated by PCA. The results obtained with the PCA test showed that the 2S and 11S soy protein fractions did not induce IgE formation in mice immunized with these proteins. Nevertheless, the PCA test for the 7S soy protein fraction was positive in the secondary response (35th day), when an intense production of IgE due to the 7S booster on the 28th day after the initial immunization was observed. This positive reaction was evidenced by a blue stain with a diameter larger than 5 mm in the rat skin. The diameters of these positive reactions for undiluted and diluted serum were 16 mm (undiluted) 14 mm (1/5), 11 mm (1/10), 9 mm (1/20), 8 mm (1/40), 5 mm (1/80) and 3 mm (1/160), considering 80 as the serum titer, which corresponded to the inverse of the largest dilution which resulted in a positive reaction ≥ 5 mm (Figure 5).

Production of specific IgG1 antibodies directed to 7S soy protein fraction in mice

The presence of specific IgG1 antibodies against the 7S soy protein fraction in BALB/c mice was investigated, considering that in mice this immunoglobulin isotype, like
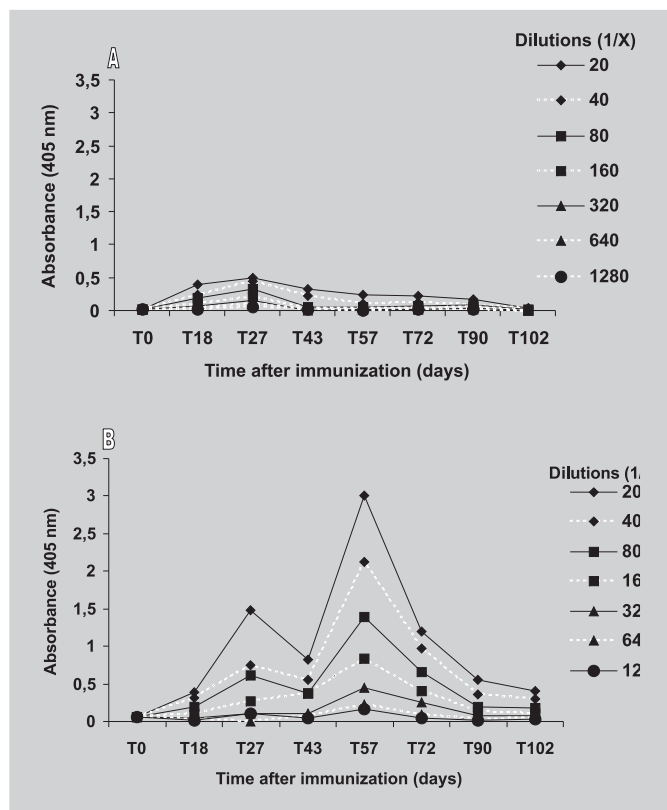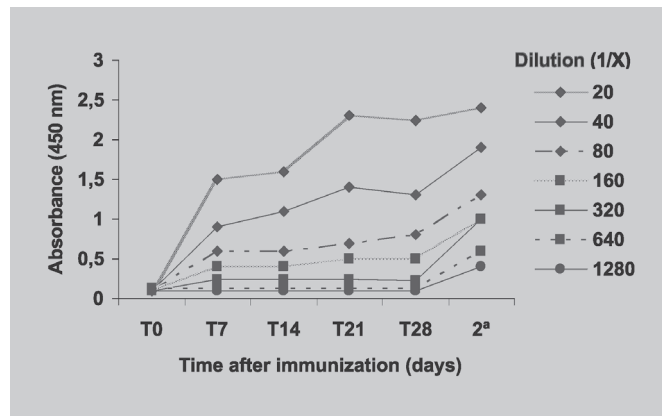
FIGURE 4 - Time course of formation of IgM (A) and IgG (B) antibodies reactive to the 11S soy protein fraction after immunization of BALB/c mice. The data are expressed as mean of duplicates.
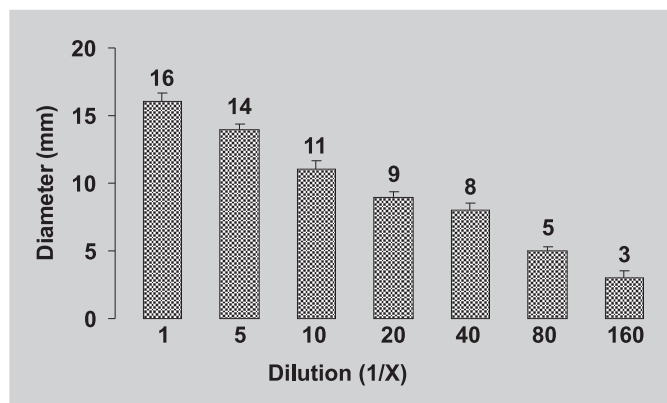
FIGURE 5 - Production of IgE antibodies reactive to the 7S soy protein fraction after immunization of BALB/c mice. Data are presented as mean ± S.D. and were obtained by the PCA test at the secondary response (35th day) after a booster on the 28th day from the initial immunization of BALB/c mice.

IgE, has the ability to elicit anaphylactic reactions (Ogawa *et al.*, 1991). The 7S fraction stimulated the immunological system of mice to produce IgG1 specific antibodies on days

7, 14, 21 and 28 after immunization with a titer equal to 1/160. After the 7S soy protein fraction booster received on day 28 after the initial immunization, the animals presented a strong immune response with the highest titer equal to 1/320 on the 35th day after the initial immunization (secondary response) (Figure 6).

FIGURE 6 - Time course of the production of IgG1 antibodies reactive to the 7S soy protein fraction after immunization of BALB/c mice. The results were obtained by ELISA at the secondary response (35th day) after a booster on the 28th day from the initial immunization of BALB/c mice. Data are expressed as mean of duplicates. 2ª – Secondary response on the 35th day of initial immunization, after booster on the 28th day.

DISCUSSION

The elective treatment for allergy to cow milk protein is the elimination of this protein from the diet. Prolonged breast-feeding and the avoidance of cow milk during the first three months of lactation of high-risk babies decreases the prevalence and severity of atopic diseases (Businco *et al.*, 1993). A variety of hypoallergenic formulas have appeared on the market over the last years for feeding lactating infants prone to a high atopic risk of allergy to cow milk. The soy formulas containing sucrose are particularly indicated in children with protein allergy to cow milk. On the other hand, the soy proteins may also be allergenic (Botey *et al.*, 1993; Galli *et al.*, 1996) and there is a need to define which of the soybean protein fractions is involved in this allergic process.

To investigate the allergenicity of the soy protein fractions, animals models are often used considering that allergic responses upon reexposure to the offending food reflecting the human situation, has been demonstrated to be a useful tool to expand the knowledge of mechanisms

underlying the development of food allergy and to assess the potential allergenicity of novel food products. Several animal models have been developed for research on food allergy. In the past decade the studies were initiated in small animal models including guinea pig (Piacentini *et al.*, 1994), rat (Knippels *et al.*, 1998), and mouse (Li *et al.*, 2000). Recently, larger animals as dogs (Buchanan and Frick, 2002) and pigs (Helm *et al.*, 2002), that more closely mimic human physiology, anatomy, and allergic disease have been used for food allergy investigation. In relation to mice, several strains have been used for food allergy investigation (i.e.: BALB/c, DO11.10, C3H/He, C57BL/6, CBA, SJL). All these strains show sensitization to allergens followed by increased formation of IgE and IgG1 antibodies (Wang *et al*, 1999; Watanabe *et al.*, 2003; Hsieh *et al.*, 2003; Lifrani *et al.*, 2005), demonstrating that different mouse strains display similar patterns of allergen-specific antibody responses.

The presence of IgM and IgG antibodies directed to 7S and 11S soy protein fractions in mice shown in this study, reinforces the hypothesis that these fractions are immunogenic (Figures 3 and 4). These data are in accordance with the study by Christensen *et al.* (2003) who analyzed blood from mice fed a soy-containing diet, by ELISA and immunoblotting, and showed antibody reactivity towards various soy protein fractions. They also found antigenic specificity of the serum antibodies of soy-consuming mice towards both the basic and the acidic chains of glycinin and the β conglycinin subunits by immunoblotting.

Other important findings in our study are those obtained with the PCA test. This test represents an animal model for inflammatory reactions in type I allergy and has been a gold standard method to measure allergen-specific IgE antibody levels in allergy mouse models. The normal mechanism of the production of IgE antibody starts with the antigen presentation by antigen presenting cells that can lead to activation of T cells. These T-helper subsets include Th1, Th2, Th3 and Tr cell types and through the pattern of cytokines released, T cells have a different effect on the differentiation and secretion of antibodies by B cells. Allergic diseases have been studied in models for Th2 cells polarization and it is well known that the synthesis of the human IgE results from interaction between Th2 and B cells through cytokines, such as, IL-4 and IL-13 and surface molecules (CD40-CD40L) (Ramos *et al.*, 2003). The binding of allergens to IgE on mast cells triggers the release of several mediators, of which histamine is the most prevalent, regulating several essential events in the immune response (Mazzoni *et al.*, 2001).

In our experiments with the PCA test, the allergenic potential of the 7S soy protein fraction was clearly demonstrated (Figure 5). The positive PCA test for 7S fraction means that it stimulated the production of IgE antibodies in mice that sensitized mast cells from the skin of Wistar rats. The challenge with 7S fraction and Evans Blue stain in the caudal vein of the rat caused the degranulation of mast cells that increased the vascular permeability and caused the overflow of the Evans Blue stain evidenced by a blue color in the rat skin. Considering these data it can be suggested that the 7S soy protein fraction is highly immunogenic and allergenic in mice as it is capable of triggering degranulation of mast cells through the production of specific IgE. In contrast, the 2S and 11S protein fractions did not induce histamine release from mast cells in this same test. Another important evidence of the 7S fraction's allergenicity is the finding of specific IgG1 antibodies in the serum of BALB/c mice which was highly significant especially after the antigenic booster on the 28th day of immunization (Figure 6). This finding is extremely relevant because it has been demonstrated that the anaphylactic IgG1 enhances the pulmonary eosinophilic inflammation and airway hyperreactivity (Macedo-Soares *et al.*, 2004) which reinforce the implication of the 7S fraction in the allergenicity of soybean.

Considering that the allergenic protein fraction must be immunogenic, the findings observed here are relevant and polarize the discussion of the soy protein allergenicity to the 7S fraction, while our experiments with animals indicate that the 2S fraction seems not to be implicated in the allergy to soy proteins. This is in contrast with data reported by Shibasaki *et al.* (1980) showing that 2S-globulin inhibitor could inhibit 90% of the RAST against 2S, 7S and 11S globulins, suggesting that 2S-globulin is the most potent allergen of the soybean globulins. On the other hand, Ogawa *et al.* (1991) suggested that the 7S fraction is a main allergenic soy protein fraction. In our study with the 2S, 7S and 11S soy protein fractions we used 50 µg of protein for the initial immunization of mice followed by 5 mg booster of each soy protein fraction for the PCA test. These differences of experimental protocols could lead to the different results found in our experiments when compared with those of Shibasaki *et al.* (1980).

In conclusion, our study indicated that the 7S soy protein fraction is allergenic and immunogenic in mice which can be helpful for those interested in the field of allergic reactions to soy-derived foodstuffs.

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RESUMO

Imunogenicidade e alergenicidade das frações protéicas 2S, 7S and 11S da soja

Sabe-se que uma parte da população, principalmente crianças, são hipersensíveis à proteína de soja, embora não esteja completamente esclarecido qual a fração protéica mais alergênica. O objetivo do estudo foi comparar a imunogenicidade e alergenicidade das frações protéicas 2S (conglicinina), 7S (β conglicinina) e 11S (glicinina) da soja, isoladas por cromatografia de afinidade. Essas frações protéicas foram usadas como antígenos para imunizar camundongos BALB/c e avaliar sua imunogenicidade pela produção de anticorpos IgM e IgG por ELISA. A alergenicidade dessas frações foi avaliada de acordo com os seguintes procedimentos: i) produção de anticorpos IgE contra as três frações protéicas por camundongos BALBc pelo teste de anafilaxia cutânea passiva (PCA) e ii) produção de anticorpos IgG1 contra a fração 7S em camundongos BALB/c por ELISA. As frações 7S e 11S induziram a formação de anticorpos IgM e IgG. O teste de PCA mostrou que somente a fração 7S foi alergênica pela produção de anticorpos IgE. A fração protéica 7S induziu a formação de anticorpos IgG1 em camundongos imunizados com essa fração. Nosso estudo mostra que a fração protéica 7S da soja é importante para desencadear reações alérgicas em camundongos e pode contribuir para esclarecer a alergenicidade dos produtos derivados da soja.

UNITERMOS: Alergia alimentar. Soja/ alergenicidade. Soja/imunogenicidade. ELISA – PCA.

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