

IDENTIFICATION OF SOY PROTEIN ALLERGENS IN PATIENTS WITH ATOPIC DERMATITIS AND POSITIVE SOY CHALLENGES; DETERMINATION OF CHANGE IN ALLERGENICITY AFTER HEATING OR ENZYME DIGESTION

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INTRODUCTION

A common problem encountered by primary care physicians is the possible association between ingestion of a particular food and symptoms experienced by a child (Bock, 1980). The Federal Register in 1983 reported 15%, or approximately 34 million people, in the United States thought symptoms to be associated with ingestion of certain foods (USDA, 1983). A variety of adverse reactions have been reported to soy proteins (Cook, 1960; Duke, 1934; Sampson, 1983; Burks, 1988). Soy protein has been consistently one of the five major foods implicated in causing food hypersensitivity in over 90% of children with documented adverse food reactions (Sampson, 1988; Burks, 1988). However, there are very few studies on the allergenicity of the soybean protein fractions (Ratner, 1955; Shibasaki, 1980; Burks, 1988). Soy proteins are being used increasingly in a number of foods and food products (Anderson, 1984). Thus, the potential for possible sensitization is also increasing.

In some children with atopic dermatitis (AD), food hypersensitivity has been demonstrated to have a pathogenic role in the development of eczematous lesions (Sampson, 1984; 1985). Approximately 35% of children with AD undergoing double-blind placebo-controlled food challenges (DBPCFC) have food hypersensitivity reactions (Burks, 1988). Patients undergoing a DBPCFC were found to develop an eczematous, pruritic, morbiliform

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rash within 2 hours of ingesting the offending food antigen. Soy, peanuts, milk, egg, wheat, and fish accounts for 90% of the foods involved in these reactions (Sampson, 1983; Burks, 1988).

An enzyme-linked immunosorbent assay (ELISA) was used to investigate allergen-specific IgE and IgG antibody to soy protein and its fractions in patients with AD and a positive DBPCFC to soy. Serum from these same individuals was used to examine the antigens in soy proteins by immunoblotting techniques. We then used pooled serum from similar patients to investigate the change in antibody response after heating or enzyme digestion of the crude soy protein and its fractions.

EXPERIMENTAL SECTION

Approval for this study was obtained from the Human Research Advisory Committee at the University of Arkansas for Medical Sciences and Johns Hopkins University Medical School. Eight patients (mean age 6.18 ± 5.12 years, range - 18 months to 16 years) with AD and allergic rhinitis and a positive immediate prick skin test to soy (wheal ≥ 3 mm) had a positive DBPCFC to soy. (Sampson, 1983) Control sera were obtained from normal children being followed at Arkansas Children's Hospital. These children were not atopic and reported previous use of soybean products without symptoms.

Soybean proteins and fractions

For the ELISA and immunoblotting investigations protein was fractionated into 7S, 11S and whey fractions in addition to a crude extract. The two major soy protein fractions, 7S and 11S were isolated and purified according to a modification of the procedure Thanh and Shibasaki. (Thanh, 1976) Commercial defatted soy flakes were extracted with 20 parts (w/v) of 0.03M Tris-HCl buffer (pH 8.0) containing 0.001M dithioerythritol (DTE) at room temperature (20-22°C) for 1 hour. Following centrifugation at 35,000 x g for 20 minutes (20°C), the supernatant was recovered and adjusted to pH 6.0 with 1N HCl. A crude 11S protein precipitate was obtained by centrifuging this mixture at 4°C as above. Purified 11S protein was obtained by resolubilizing the precipitate in 50 parts (w/v) 0.03M phosphate buffer (pH 7.6) containing 0.4M NaCl, 0.001M DTE, and 0.05% sodium azide and passing through a concanavalin-A Sepharose 4B column to remove contaminating glycoproteins. To obtain the 7S protein fraction, commercial defatted soy flakes were extracted with 20 parts (w/v) of 0.03M Tris-HCl buffer (pH 6.2) containing 0.001M DTE at room temperature for 1 hour and centrifuged at 35,000 x g for 20 minutes (4°C). The supernatant was recovered, adjusted to pH 4.8 with 1N HCl and centrifuged as above. The precipitate, thus obtained, was dispersed in 10 parts Tris-HCl buffer, adjusted to pH 7.6 with 1N NaOH and stirred at 9°C until the precipitate dissolved. The solution was readjusted to pH 6.2 and centrifuged. Following the addition of 0.4M NaCl, the crude 7S supernatant was warmed to room temperature (20-22°C) and bound to a concanavalin-A Sepharose 4B column pre-equilibrated against 0.03M Tris-HCl buffer (pH 6.2) containing 0.4M NaCl and 0.001M DTE. After removing the unbound proteins, purified 7S protein was recovered by eluting the column with the buffer containing 0.1M α -methyl-D-mannoside. The purified 7S solution was equilibrated against 0.035M phosphate buffer (pH 7.0) containing 0.4M NaCl, 0.001M DTE, 0.05% sodium azide by repeated dilutions (times) concentration in an Amicon stirred cell containing a YM-1 membrane followed by redilution (1:10 v/v).

To obtain the crude extract, soy protein (w/v) adjusted to pH 7.0 by centrifugation as above then the supernatant and adjusting the pH. The whey protein fraction will precipitate at pH 4.0. The supernatant is the whey fraction. The pH is adjusted to 7.0 with alkali.

ELISA for IgE and IgG

A biotin-avidin ELISA was used to detect soy protein antibodies with microtiter plates (Burks, 1986) Titer was determined (Gibco, Santa Clara, CA) using anti-human IgE (Tago, Inc., Elkhart, IN) as the index of the plate was determined (7S, 11S, whey) at room temperature (0.1 sodium carbonate buffer (phosphate-buffered saline, pH 7.2; Sigma, St. Louis, MO) was used. The upper two wells were used to generate a curve for IgE,

The patient's serum samples were diluted (1:10) and dispensed in duplicate into the microtiter plates and incubated for one hour at room temperature. The plates were then incubated again with peroxidase-avidin complex (1:1000) for one hour. After washing, the plates were incubated with O-phenyl diamine (1:100) for one hour. The absorbance was read at 492 nm using a microtiter reader (Bio-Rad, Richmond, VA). The standard curve was a simple linear regression line. The results were read from the standard curve (ng per milliliter).

IgG antisoy protein was determined by using a modified biotin-avidin ELISA. The plates were incubated with 11 microtiter plate (1:100) of human IgG (Miles, Elkhart, IN) as the index of the plate was determined (7S, 11S, whey) at room temperature. The plate was washed five times with phosphate-buffered saline (pH 7.2) and incubated. To generate a curve for IgG, the patient's serum samples were diluted in duplicate into the microtiter plates and incubated for one hour at room temperature. The plates were then incubated with peroxidase-avidin complex (1:1000) for one hour. The absorbance was read at 405 nm (Titer

To obtain the crude soy fraction commercial defatted flakes were extracted at room temperature for 45 minutes with 20 parts deionized water (w/v) adjusted to pH 8.0 with 1N NaOH. Following centrifugation as above the supernatant recovered is the crude soy preparation. The whey preparation is obtained by taking the crude supernatant and adjusting the pH to 4.0 with 1N HCl. The principle globulin proteins will precipitate when centrifuged as above. This supernatant is the whey fraction and is adjusted to pH 7.0 with dilute alkali.

ELISA for IgE and IgG

A biotin-avidin ELISA was developed to quantify IgE antiso protein antibodies with modifications from an assay previously published. (Burks, 1986) The upper two rows of a 96-well microtiter plate (Gibco, Santa Clara, CA) were coated with affinity purified goat anti-human IgE (Tago Diagnostics, Burlingame, CA). The remainder of the plate was coated with one of the soy products (crude, 7S, 11S, whey) at a concentration of 5 μ g/ml in coating buffer (0.1 sodium carbonate-bicarbonate buffer, pH 9.5). The plate was incubated at 37°C for one hour and then washed five times with rinse buffer (phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20; Sigma, St. Louis, MO) immediately and in between subsequent incubations. The upper two rows used a standard reference to generate a curve for IgE, ranging from 0.25 to 10 ng/ml.

The patient's serum samples were diluted (1:2, 1:10, 1:50, 1:100) and dispensed in duplicate in the lower portion of the plate. After incubation for one hour at 37°C and washing, biotinylated, affinity-purified goat anti-human IgE (Tago) was added to all wells. Plates were incubated again for one hour at 37°C and washed before horseradish peroxidase-avidin conjugate (Vector, Burlingame, CA) was added. After washing, the plates were developed by the addition of a buffer containing O-phenylenediamine (Sigma). The reaction was stopped by the addition of 2N-hydrochloric acid to each well, and absorbance was read at 492 nm (Titertek Multiscan; Flow Laboratories, McLean, VA). The standard curve was plotted on log-logit paper by means of simple linear regression, and values for each patient's sample were read from the curve as "nanogram-equivalent units" per milliliter (ng per milliliter).

IgG antiso protein antibodies to the different fractions were determined by using a modification of a previously described enzyme-linked immunoassay. (Burks, 1986) The upper two rows of a 96-well microtiter plate (Gibco) were coated with a monoclonal antibody to human IgG (Miles Scientific; Naperville, IL). The remainder of the plate was coated with one of the soy products (crude, 7S, 11S, whey) at a standard concentration of 5 μ g/ml in coating buffer. The plate was incubated at 37°C for one hour and then washed five times with rinse buffer immediately and in between subsequent incubations. The upper two rows used a standard reference to generate a curve for IgG, ranging from 5 to 250 μ g/ml. The patient's serum samples were diluted (1:10, 1:50, 1:100, 1:500) and dispensed in duplicate in the lower portion of the plate. After incubation for one hour at 37°C and washing, alkaline phosphatase conjugated goat antihuman IgG (Tago) was added to all wells. After incubation for one hour at 37°C and washing, nitrophenyl phosphate in carbonate buffer was added and the plate was developed. Plates were read at 405 nm (Titertek Multiscan). The standard curve was

plotted on log-logit paper, and patient values were read from the curve in "nanogram equivalent units" per milliliter (ng per milliliter).

Immunoblotting: IgE and IgG

The electrophoresis procedure is a modification of Sutton et al (Sutton, 1982) SDS-PAGE electrophoresis was carried-out with a 5-20% polyacrylamide separating gel and a stacking gel of 3%. The protein

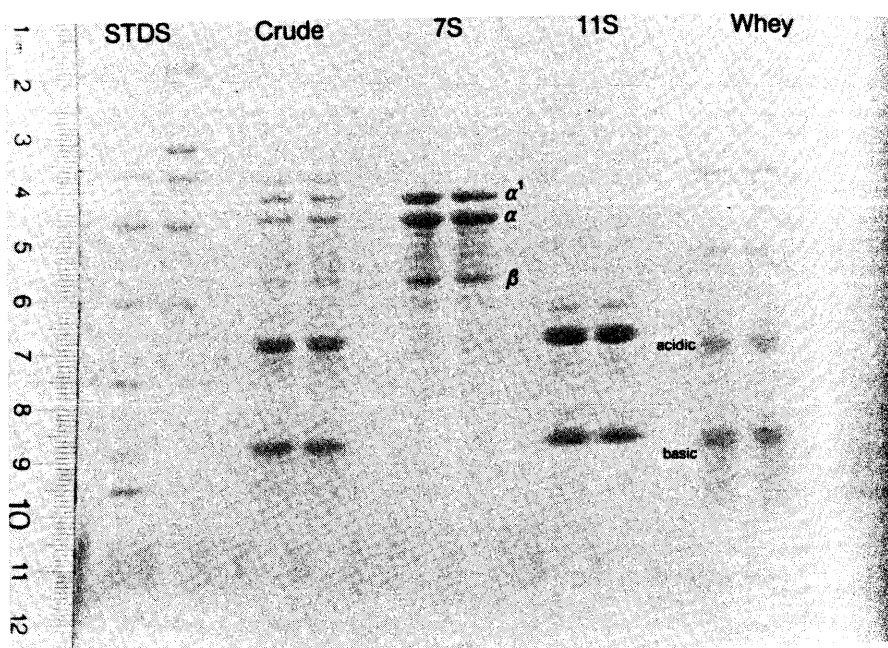


Fig. 1. Coomassie blue stain of 5% to 20% SDS-PAGE. Lanes are marked with low and high molecular weight standards, crude soy preparation and 7S, 11S, and whey fractions of soy protein. The major subunits of 7S are α' , α , and β . The 11S subunits are the acidic and basic fractions.

concentration of soy product added to each well in the gel was 1 mg/ml. Electrophoresis was performed overnight for 16 hours at 30 V (E-C Apparatus Corp., St. Petersburg, FL). To assure proper protein separation Coomassie Blue (Sigma) stains were done on preliminary gels.

Proteins were transferred from the separating gel to a nitrocellulose membrane in a transfer buffer with 10% SDS and methanol. The procedure was done in a transblot apparatus (Bio-Rad) for 5 hours (0.150 A). To assure transfer of the protein an Amido Black stain (Bio-Rad) was done on one portion of the nitrocellulose

After removal from the transblot apparatus, the nitrocellulose was placed in blotto (25 gm non-fat dry milk, 0.5 gm sodium azide in 500 ml of phosphate buffered saline with EGTA added) and rocked for 1 hour. The nitrocellulose was then washed 3 times with TBS (2.42 g Tris base, 29.42 gms NaCl in 1 liter of water and adjusted to pH 7.5). Next the nitrocellulose was incubated with the patient's se

1:10 dilution) overnight with TBS 3 times, goat antiserum incubated at room temperature, anti-goat alkaline phosphatase then added after washing with rocking. After a 10 min start by adding 250 μ l of tetrazolium in 70% dimethylformamide; Bio-Rad temperature with rocking the NBT/BCIP solution 10 minutes with distilled

The procedure for IgE except in staining IgG (1:500, Bio-Rad) and alkaline phosphatase c

EATING AND ENZYME DIG

The crude soy product was subjected to heat treatment at 100°C or 120°C for 60 minutes. Digestion by the method of Langer is an immobilized system is an immobilized system consists of 2 bioreactors containing trypsin, ch

LISA INHIBITION

A competitive ELISA was performed on the extract and the digest (system). One hundred challenge patients was either the crude soy product or the digest. The inhibited described above. The food specific IgE value divided by the food-specific IgE to get the percent

DATA ANALYSIS

Statistical comparison of the Wilcoxon sign

RESULTS AND DISCUSSION

Preliminary gels to evaluate protein separation pattern for the crude, the 7S fraction are action the acidic and basic. The ELISA results are shown in Table 1. The normal controls the atopy to the children with atopy difference in the IgE

(1:10 dilution) overnight at 4°C with rocking. After washing again with TBS 3 times, goat anti-human IgE (1:100, Bio-Rad) was added and incubated at room temperature with rocking for 2 hours. Rabbit anti-goat alkaline phosphatase conjugated IgG (1:100, Bio-Rad) was then added after washing with TBS, at room temperature for 1.5 hours with rocking. After again washing with TBS 3 times, development was started by adding 250 µl of the NBT solution (30 mg nitro blue tetrazolium in 70% dimethylformamide; Bio-Rad) and 250 µl of the BCIP solution (15 mg 5-Bromo-4-Chloro-3-Indolyl phosphate in 70% dimethylformamide; Bio-Rad) into 25 ml of carbonate buffer at room temperature with rocking. The reaction is then stopped by decanting the NBT/BCIP solution and incubating the nitrocellulose for 10 minutes with distilled water. The paper is then air dried.

The procedure for specific IgG to the crude product is identical to IgE except in staining the first antibody added is goat anti-human IgG (1:500, Bio-Rad) and the second antibody is rabbit anti-goat alkaline phosphatase conjugated IgG (1:100, Bio-Rad).

HEATING AND ENZYME DIGESTION

The crude soy protein and the 7S, 11S and whey fractions were subjected to heat treatment. Each protein was subjected to either 80°C or 120°C for 60 minutes. Crude soy was subjected to enzyme digestion by the method of Thresher et al. (Thresher, 1989). The system is an immobilized digestive enzyme assay (IDEA). The assay consists of 2 bioreactors, one containing pepsin, and the other containing trypsin, chymotrypsin and intestinal mucosal peptidases.

ELISA INHIBITION

A competitive ELISA inhibition was done using the crude soy extract and the digested soy product (after passing over the IDEA system). One hundred µl of pooled serum (1:20) from the positive challenge patients was incubated with various concentrations of either the crude soy or digested soy (0.00005 to 50 ng/ml) for 18 hours. The inhibited pooled serum was then used in the ELISA described above. The percent inhibition was calculated by taking the food specific IgE value minus the incubated food-specific IgE value divided by the food-specific IgE value. This number is multiplied by 100 to get the percentage of inhibition.

DATA ANALYSIS

Statistical comparisons were done by the Mann-Whitney U test and the Wilcoxon signed-rank test.

RESULTS AND DISCUSSION

Preliminary gels were stained with Coomassie Blue to ensure adequate protein separation. One can see in Figure 1 the banding pattern for the crude, 7S, 11S and whey fractions. The major bands in the 7S fraction are the α' , α and β subunits. In the 11S fraction the acidic and basic subunits are also easily identified.

The ELISA results for specific IgE to crude soy, 7S, 11S and whey are shown in Table 1. Comparing the IgE in the atopic patients to the normal controls there was a statistically significant increase in the atopics to the crude extract ($p < 0.005$) and 7S ($p < 0.05$). The children with atopic dermatitis did not show a significant difference in the IgE response to whey ($p > 0.2$) or 11S ($p > 0.2$).

Table 2 gives the results of the IgG ELISA to crude soy, 7S, 11S and whey. The atopic patients did have a significant increase in IgG to the crude extract ($p < 0.02$) and 11S ($p < 0.005$). Their IgG specific for whey ($p > 0.2$) and 7S ($p > 0.05$) was not significantly different from the normal controls.

Comparisons were then made among the eight patients found to have soy protein hypersensitivity to determine if there was significantly more IgE specific for any of the three fractions. There was an increase in IgE specific for 7S versus whey ($p < 0.05$) but not when comparing 7S versus 11S ($p > 0.10$) or 11S versus whey ($p > 0.05$). A similar comparison was done for specific IgG. There was no significant difference in 7S versus 11S ($p > 0.10$), 7S versus whey ($p > 0.20$) and 11S versus whey ($p > 0.10$).

TABLE 1.

IgE ELISA (ng/ml) to crude soy protein, 7S, 11S, and whey fractions

Patients	Crude+	7S*	11S	Whey
1	30	70	25	30
2	10	15	3	0.25
3	60	220	120	40
4	2	4	4	2
5	60	70	25	60
6	15	40	40	15
7	3	0.72	3.1	7.5
8	3.3	2.5	1.3	0.6
Normals (+/-S.D.)	7.69 10.62	11.42 19.45	9.87 9.94	22.83 19.75

+p<0.005

*p<0.05

IgG ELISA (ng/ml) to

Patients

1

2

3

4

5

6

7

8

Normals 2
(+/-S.D.) 2

+p<0.02
*p<0.005

1 2

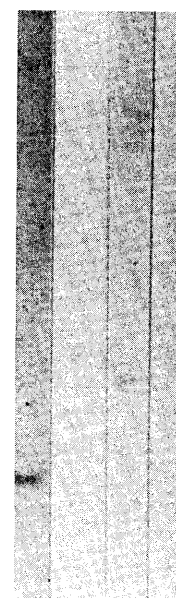


Fig. 2 IgE immunoblotting for four patients (5 to 8)

Immunoblotting for specific IgE is shown in Figure 2. Numbers 5-8 are samples from the eight patients with positive DBPCFC's to soy (patients 1, 3, 5 and 6 from Table 1). These four patients had the highest specific IgE values and also had the strongest blotting pattern. All eight patients had immunoblots done. The intensity of their binding pattern correlated with their specific IgE values. Patient #5 had bands against the α' , α and β subunits of 7S. Patient #6 had similar results against 7S but in addition had bands against the acidic and basic subunits of 11S. Patients #7 and #8 had bands representing 7S and 11S with the major band against the acidic subunit of 11S. Numbers 1-4 are representative samples from the sera of the control patients. Minimal bands can be seen in each strip with the strongest band in number 4 to the acidic subunit of 11S.

TABLE 2

IgG ELISA (ng/ml) to crude soy protein, 7S, 11S, and whey fractions

Patients	Crude+	7S	11S*	Whey
1	28,214	9,050	15,600	21,607
2	30,714	12,000	6,400	9,464
3	8,203	12,364	54,167	5,664
4	7,644	12,813	14,375	1,180
5	9,375	44,160	47,040	23,214
6	23,961	19,500	45,506	6,027
7	1,402	1,623	1,583	633
8	1,548	629	3,366	2,376
Normals	2,766.11	4,024.44	2,016.44	4,150.67
(+/-S.D.)	2,948.76	4,948.76	1,874.86	4,281.81

+p<0.02

*p<0.005

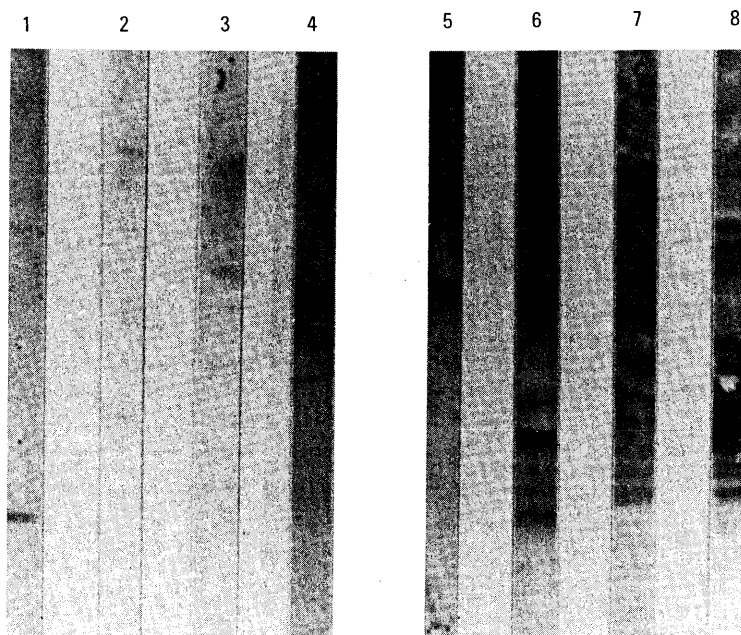


Fig. 2 IgE immunoblot of four normal control subjects (1 to 4) and four patients (5 to 8) with positive DBPCFC to soy.

The immunoblot for specific IgG to the crude product is shown in Figure 3. These are the same eight patients serum used in the IgE immunoblot. Numbers 1-4 are the control patients and numbers 5-8 are the patients with positive DBPCFC's to soy. Bands can be seen in each of the atopic's immunoblot to both the 7S and 11S fractions. As in the IgE immunoblots no one fraction is stained consistently darker.

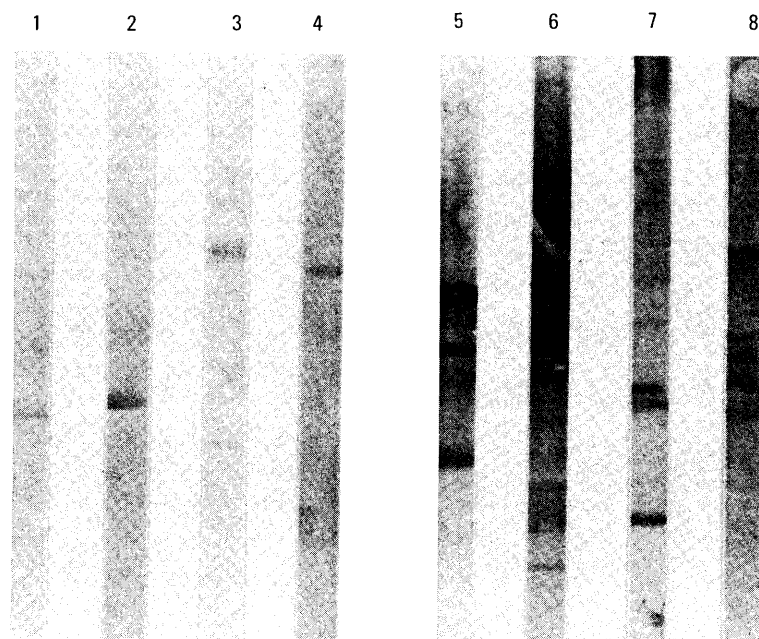


Fig. 3. IgG immunoblot of four normal control subjects (1 to 4) and four patients (5 to 8) with positive DBPCFC to soy.

To address the question of whether heating would change the allergenicity of the crude soy protein, and the 7S, 11S, and whey fractions. Heating of these proteins was done at 80°C and 120°C for 60 minutes. In the IgE specific ELISA there was a significant decrease in specific antibody for each protein except whey when heated to 80°C (Table 3) When the proteins were heated to 120°C there was a significant reduction in IgE specific for each one.

In the IgG specific ELISA heating to 80°C did not consistently decrease the immunoglobulin-specific binding as in the IgE ELISA. (Table 4) In fact, the IgG specific for the 11S component was higher when heated at 80°C. Heating to 120°C did significantly decrease the IgG binding to each protein fraction although not to the degree that IgE binding was affected.

IgE

Protein

Crude Soy

Whey

7S

11S

IgG

Protein

Crude soy

Whey

7S

11S

TABLE 3

IgE (ng/ml) to heated soy proteins

<u>Protein</u>	<u>Untreated</u>	<u>80°C</u>	<u>120°C</u>
Crude Soy	8	0.4	0.005
Whey	4	4	0.2
7S	12	4	0.05
11S	24	10	0.05

TABLE 4

IgG (ng/ml) to heated soy proteins

<u>Protein</u>	<u>Untreated</u>	<u>80°C</u>	<u>120°C</u>
Crude soy	60	60	10
Whey	50	50	10
7S	200	100	10
11S	10	20	3

Crude soy was passed over the IDEA system to compare the specific IgE to the crude soy product versus the digested soy product. An ELISA inhibition assay was used for the comparison. We measured the IgE specific to crude soy with either crude soy or digested soy incubated overnight to inhibit IgE binding (data not shown). There was at least a 10,000 fold difference in the concentration required to inhibit the ELISA by 75%, suggesting a significant change in IgE binding of the digested soy product compared to the crude soy product.

Discussion

Allergic reactions to legumes including soybean and peanut are not uncommon. Soybean protein is used in many commercial foods. Approximately 90% of the seed proteins are salt-soluble globulins with the bulk of the remainder being water-soluble albumins. (Naismith, 1955; Wolf, 1972) They comprise a broad range of molecular sizes and display four major sedimentation components in the ultracentrifuge, designated 2, 7, 11 and 15S. (Wolf, 1956) Each fraction is a complex mixture of proteins. The 7S and 11S each make up approximately one-third of the extractable proteins followed by the 2S and 15S which comprise 20% and 10%, respectively. (Nielson, 1985)

Glycinin has a molecular weight of 320,000 to 360,000 and is made up of six subunits. Each subunit is comprised of one acidic and one basic polypeptide linked by a disulfide bond. Both the acidic and basic polypeptides are heterogeneous and possess molecular weights ranging from 34,000 to 45,000 and 19,000 to 22,000, respectively. (Brooks, 1985) One exception is an acidic polypeptide designated A₅, which has a molecular weight of approximately 10,000. (Morreira, 1981) β -conglycinin, a glycoprotein, has a molecular weight of approximately 180,000. (Koshiyama, 1976) It is a trimer composed of three major subunits, α' , α and β , which have molecular weights of 76,000, 72,000 and 53,000, respectively. (Shattuck, 1985)

The allergenicity of the globulin fractions 2, 7 and 11S have previously been examined by RAST and RAST-inhibition experiments in patients with asthma and atopic dermatitis felt to be soybean-sensitive. (Shibasaki, 1980) There was considerable cross-reactivity among the 3 fractions with specific IgE antibody found against each one. But the 2S-globulin had the highest allergic potency to inhibit the RAST against 11S-, 7S- and 2S-globulin. Heat treatment of 80, 100 and 120°C for 30 minutes enhanced the RAST activity of the 2S-globulin (when heated at 80° for 30 minutes) while the activity of the others was reduced to 40 to 75% of the native globulin.

The results of the IgE and IgG ELISA's in these atopic patients with positive DBPCFC's to soy show a significant difference in both IgE and IgG specific for the crude protein extract compared to the normal controls. There was also an increase in IgE specific for 7S in the atopic population as demonstrated by IgE ELISA. The atopic group had more specific IgG for 11S, as shown by IgG ELISA.

The immunoblotting experiments reveal a great variation in the protein subunit polypeptides recognized by human antibodies. Variable patterns suggested that no one component of the soy fraction bands is more antigenic than others. There appeared to be IgE

antibodies to both 7S and 11S in the patients. The majority of immunoblots also were detected. It is that most of the antigen binding that IgE and IgG is to different soy fractions and not to another is similar to wheat. (Theobald) Increased amounts of IgE in the patients compared to their normal controls. Reaction of the food proteins.

Very little is known about the effect of heating on soy foods by heating. Simple heating of alpha-lactalbumin denatured and beta-lactoglobulin are more stable. Studies with soy protein isolate show that heat causes aggregation of subunits of 11S (Utsumi, 1981) and that heat treatment at 100°C for 30 minutes inactivation and its component. Binding of the 2S fraction to the IgE binding to the other crude product.

Similarly, very few studies have examined the allergenicity of foods after heating. To examine the difference in the binding of beta-lactoglobulin, the patient antibodies to the digested soy protein.

CONCLUSION

This study demonstrates that DBPCFCs to soy have significant differences in the binding of antibodies to a crude soy extract. An IgE and IgG response was observed to the crude soy and to the heated soy but significantly reduced by enzyme treatment provided to reduce IgE binding.

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antibodies to both 7S and 11S fractions in varying amounts in most of the patients. The majority of protein bands identified in the IgG immunoblots also were detected in the IgE blots. It appears from this that most of the antigens are also allergens as well. The finding that IgE and IgG is present in varying amounts to the different soy fractions and that no one fraction is more antigenic than another is similar to findings in earlier studies with patients sensitive to wheat. (Theobald, 1986) These publications showed increased amounts of IgE in their population sensitive to these foods compared to their normal controls. They did not however, find any fraction of the food protein to be more consistently allergenic.

Very little is known about the alteration of the allergenicity of foods by heating. Simple heating of bovine gammaglobulin and alpha-lactoalbumin denatures these proteins while alpha-casein and beta-lactoglobulin are more heat stable (Anderson, 1984). From other studies with soy protein it is known that heating of the crude soy bean causes aggregation of the β subunits of 7S with the basic subunits of 11S (Utsumi, 1984). Shibasaki et al (Shibasaki, 1980) examined heat treatment at 80°C, 100°C and 120°C of the crude soy fraction and its components and its effects on IgE binding. The IgE binding of the 2S fraction was enhanced with heating at 80°C, whereas the IgE binding to the other fractions were reduced 40% to 75% of the crude product.

Similarly, very few studies have determined the change in allergenicity of foods after enzyme digestion. In one study done to examine the difference in IgE activity to pepsin digests of β lactoglobulin, the patients had significantly lower levels of IgE antibodies to the digested proteins (Anderson, 1984)

CONCLUSION

This study demonstrates that children with AD and positive DBPCFCs to soy have significantly elevated levels of IgE and IgG antibodies to a crude soy protein extract. It also provided evidence of an IgE and IgG response to multiple fractions of the soy protein. Heating the crude soy and its fractions appears not to enhance IgE activity but significantly reduces its binding. The significant enzyme treatment provided by the IDEA system also appears to greatly reduce IgE binding.

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