

Soybean flour asthma: Detection of allergens by immunoblotting

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A 43-year-old woman developed asthma 6 years after beginning work in a food-processing plant in which soybean flour was used as a protein extender. Symptoms of sneezing, coughing, and wheezing would begin within minutes of exposure to soybean flour and resolve 2 hours after exposure ceased. Skin tests were positive to a soy extract prepared from the flour. Airway hyperreactivity was confirmed by a positive bronchial challenge to methacholine. Bronchial challenge with soybean flour produced an immediate increase in specific airway resistance from 5.0 to 22.7 L · cm of H₂O/L/sec. There was no response to challenge with lactose. The patient's allergic response to soy-flour extract was further characterized by several immunologic methods. IgE binding to soy-flour protein by direct RAST was 5.98 times that of a normal control serum. The soy-flour extract was separated by dodecyl sulfate-polyacrylamide gel electrophoresis. Twenty-four protein bands were detected in the crude soy-flour extract. After immunoblotting and subsequent autoradiography, nine proteins with molecular weights ranging from 54,500 to 14,875 were found. Cross-reactivity studies with other legumes demonstrated apparent immunologic identity between a component in green pea extract and a soybean protein with a molecular weight of 17,000. The clinical significance of this cross-reactivity is not known. We conclude that in this case of occupational asthma to soybean flour, multiple allergens were involved. Immunoblotting may be useful in identifying the allergens involved in occupational asthma. (J ALLERGY CLIN IMMUNOL 1988;82:251-5.)

Occupational asthma from exposure to soybean flour was first reported more than 50 years ago.¹ Subsequently, several other studies of asthma resulting from occupational exposure to soybean dust have appeared in the literature.²⁻⁶ Occupational asthma caused by soybean-flour exposure is mediated through an IgE mechanism. The allergens involved in the allergic response, however, have not been characterized. We have recently had the opportunity to use immunoblotting techniques to study the immunologic response of a patient who experienced asthma after occupational exposure to soybean flour.

CASE REPORT

A 43-year-old woman developed wheezing with shortness of breath 6 years after beginning work in a food-processing

Abbreviations used

BSA:	Bovine serum albumin
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SR _{aw} :	Specific airway resistance
TBS:	Tris-buffered saline

plant. The patient had no previous history of atopy, asthma, or other pulmonary diseases. She worked on an assembly line making frozen meat patties. The meat was mixed with soybean flour or wheat flour on alternate days. The flour was used as a protein extender.

After 6 years of employment, the patient noted the gradual onset of sneezing, rhinorrhea, coughing, and shortness of breath. These episodes occurred only when soybean flour was used. The symptoms began within 15 minutes of handling soy flour and usually resolved 2 hours after exposure ceased. On at least two occasions the symptoms were associated with wheezing, and they resolved after the patient used a bronchodilator. There was no fever nor sputum, and the patient did not smoke.

The chest roentgenogram revealed no abnormality. Skin tests by the prick method performed with 25 common allergens and soy extract were positive only to the soy extract. Results of pulmonary function tests were normal. FEV₁ was

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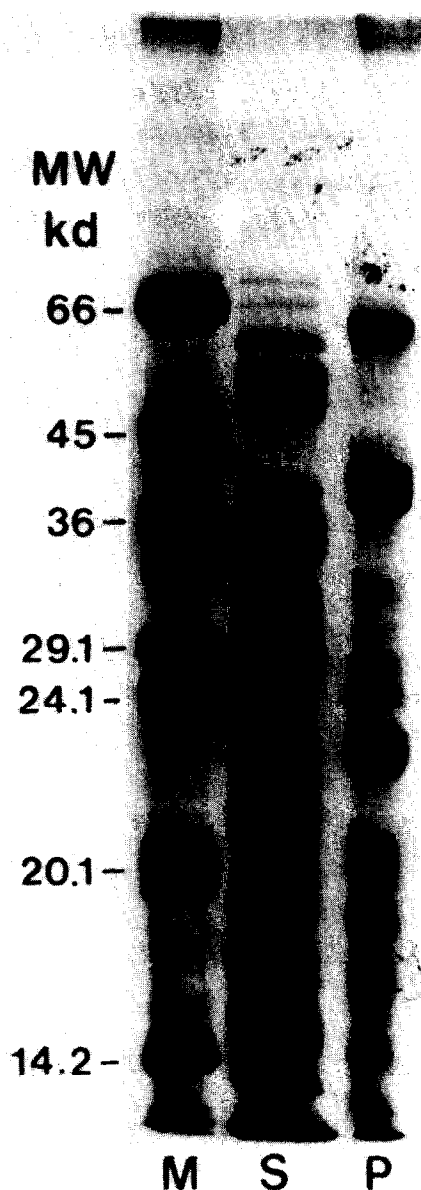


FIG. 1. SDS-PAGE of molecular weight markers (*M*), soybean protein (*S*), 25 μ g, and peanut protein (*P*), 25 μ g, stained with Coomassie blue. Molecular size (*MW*) expressed in kilodaltons (*kd*).

2.6 L (102% of predicted), FVC was 3.25 L (98% of predicted), and maximum expiratory flow rate was 366 L/min (91% of predicted).

METHODS

Bronchial challenges

The patient underwent bronchial challenge to methacholine, lactose powder, and soybean flour. Airway resistance and thoracic gas volume were measured in a constant-volume body plethysmograph (No. 09103, Warren E. Collins, Inc., Braintree, Mass.) and expressed as a product of

airway resistance and thoracic gas volume, SR_{aw} . Methacholine responsiveness was tested by measuring the subject's SR_{aw} before and after inhalation of 10 tidal breaths of phosphate-buffered saline and then after sequential doublings of methacholine (0.25, 0.5, and 1.0 mg/ml) delivered by a No. 646 nebulizer (DeVilbiss Co., Somerset, Pa.) with a dose-metering device calibrated to deliver 0.01 ml per breath.⁷ The concentration of methacholine that provoked a 100% increase in SR_{aw} from the postsaline SR_{aw} value (provocative concentration producing 100% increase in airway resistance) was calculated by log-linear interpolation. SR_{aw} was measured before and after the patient poured 100 mg of lactose powder from one 500 ml beaker to another. Inhalation challenge to soybean flour was performed with the material to which the patient was exposed at work, Procon 200 soy-protein concentrate (A. E. Staley Mfg. Co., Decatur, Ill.). The challenge methods were identical to the challenge with lactose powder.

Preparation of soybean flour and other legume extracts

Extracts of soybean flour to which the patient was exposed were prepared by soaking the flour in 0.01 mol/L of Tris HCl (pH 7.4) at a concentration of 1:10 (wt/vol) overnight with agitation of 4° C. The material was then centrifuged at 1120 g for 2 minutes, and the supernatant was stored at -20° C until it was used. Protein concentration of the extract was determined according to the method of Bradford.⁸ For comparative purposes, a crude peanut extract was provided by Dr. Steve Taylor of the Food Research Institute, University of Wisconsin. This extract was prepared by soaking defatted roasted peanuts in 10% NaCl, pH 8.0, overnight at 4° C, and followed a procedure similar to that for preparation of soybean-flour extract.

Other legume extracts (green pea, kidney bean, and lentil) were provided by Ann Herian of the Food Research Institute, University of Wisconsin. These legumes were defatted and extracted in 0.1 mol/L NH_4CO_3 , pH 8.0, overnight at 4° C, and subsequently treated as the soybean and peanut extracts.

Direct RAST measurement

Peanut and soy-flour extracts were dialyzed overnight against RAST coupling buffer (0.1 mol/L $NaHCO_3$ and 0.5 mol/L NaCl, pH 8.3). Extracts were coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia, Piscataway, N.J.) at a concentration of 3 mg of protein per milliliter of swollen gel with methods previously described.⁹ The mixture was agitated for 2 hours at room temperature and allowed to stand overnight at 4° C. The suspension was then filtered and washed twice with deionized water. The remaining active groups were blocked by letting the suspension stand in 1.0 mol/L of ethanolamine, pH 8.0, at room temperature for 2 hours with occasional stirring. The excess uncoupled protein was removed after washes with high pH buffer (0.1 mol/L $NaCO_3$ and 1.0 NaCl, pH 8.5), low pH buffer (0.1 mol/L $NaC_2H_3O_2$ and 1.0 mol/L NaCl, pH 4.0), and RAST buffer (0.05 mol/L Na_2HPO_4 , 0.2%

BSA, 0.02% NaN_3 [wt/vol], 0.5 mol/L NaCl, and 0.5% Tween [wt/vol], pH 7.5). The gel was dried to a moist cake and stored in 50% RAST buffer at 4° C.

The direct RAST was performed by incubating 0.5 ml of 3% sorbent with 0.1 ml of serum diluted 1:5 in RAST buffer with constant rotation at room temperature for 18 hours. After three washes with RAST buffer, Phadebas ^{125}I -labeled antihuman IgE (Pharmacia), 105,000 cpm, was added to each tube. The tubes were rotated overnight at room temperature. After three additional washes, the bound radioactivity was determined on a Micromedic 2/200 gamma counter (Rohm and Haas Co., Philadelphia, Pa.). All assays were performed in duplicate. The average background count was subtracted from each result before data analysis. Results of the RAST test are expressed as the ratio of radioactive binding in the serum sample to that in a negative control serum.

Immunoblotting

Immunoblotting was performed with methods similar to those previously described.¹⁰ Proteins in the soybean flour and peanut extracts were first separated by SDS-PAGE with a 3.9% polyacrylamide stacking gel and a 12.5% resolving gel. The fractions were treated at 100° C for 2 minutes in the presence of SDS and 2-mercaptoethanol. Twenty-five micrograms of protein was applied to each lane of the gel. Electrophoresis was performed at 30 mA per gel with constant current until the bromphenol blue tracking dye was about 1 cm from the bottom of the gel. A portion of the gel was sliced and stained with Coomassie blue R250, and the remainder of the gel was used for the immunoblotting experiments.

Proteins were transferred electrophoretically from the SDS gel to nitrocellulose. IgE binding to the transferred proteins was detected as follows: Unoccupied sites of the nitrocellulose were blocked by gently shaking the nitrocellulose with 3% BSA in 10 mmol/L of Tris HCl, 0.9% NaCl, pH 7.4 (TBS), plus 0.02% NaN_3 for 1 hour at 40° C. The nitrocellulose was cut into strips corresponding to the sample lanes of the original gel and transferred to incubation trays. Strips were incubated overnight in 5 ml of a solution of serum diluted 1:5 in TBS plus BSA plus NaN_3 . Control strips without antigen were blocked with BSA and incubated with TBS plus BSA plus NaN_3 only. All strips were washed with TBS for 10 minutes, then twice for 10 minutes each with TBS in 0.5% Nonidet P40 (Sigma Chemical Co., St. Louis, Mo.), and once more with TBS. They were then incubated overnight with ^{125}I -labeled antihuman IgE (Pharmacia). After a final set of washes, the strips were wrapped in plastic wrap and placed on XAR-5 x-ray film (Eastman Kodak, Rochester, N.Y.) for autoradiography at -70° C for 1 to 3 days.

Inhibition of IgE binding to immunoblots

To determine whether other legume antigens cross-react with soybean proteins to which the patient was sensitized, we conducted the following experiments: Five milliliter aliquots of a solution of the patient's serum diluted 1:5 in

TBS plus BSA plus NaN_3 were incubated for 1 hour with each of the following inhibitors: green pea, kidney bean, and lentil (50 μg of protein). BSA (50 μg) served as a control. After incubation, the serum solution was applied to nitrocellulose strips containing the separated soybean proteins, and immunoblotting was conducted as described above.

RESULTS

Bronchial challenges

In this patient, the provocative concentration producing 100% increase in airway resistance was measured to be 0.8 mg of methacholine per milliliter, confirming airway hyperreactivity. There was no significant change in SR_{aw} on challenge to lactose dust. Before the challenge, the SR_{aw} was 5.2 L · cm of $\text{H}_2\text{O}/\text{L}/\text{sec}$, and after a 10-minute exposure, it was 5.3 L · cm of $\text{H}_2\text{O}/\text{L}/\text{sec}$. One minute after exposure to soybean flour, the SR_{aw} rose from a baseline of 5.0 to 22.7 L · cm of $\text{H}_2\text{O}/\text{L}/\text{sec}$. The patient developed symptoms of sneezing, coughing, wheezing, and shortness of breath. She was treated with albuterol via inhaler, and the symptoms resolved within 1 hour.

Protein content of soybean flour and other legume extracts

The protein content of the extracts was as follows: soy flour, 3.3 mg/ml; peanut, 20 mg/ml; green pea, 41 mg/ml; kidney bean, 54 mg/ml; and lentil, 36 mg/ml.

Direct RAST

The patient's serum bound 5.65% of the total counts per minute added, whereas the control serum bound 0.94% of the total. This elicits a RAST ratio of 5.98. In a peanut solid-phase assay, the patient's serum bound 1.8% of total counts added compared to 0.83% for the control, a RAST ratio of 2.2.

SDS-PAGE

The protein separation pattern of the soybean and peanut fractions is illustrated in Fig. 1 after staining with Coomassie blue. Twenty-four protein bands were detected in the soybean-flour extract and 28 bands in the peanut extract.

Immunoblotting

The autoradiograph of the patient's serum incubated with nitrocellulose strips containing peanut and soy-flour proteins is illustrated in Fig. 2. No IgE binding could be detected toward peanut proteins. However, nine proteins in soybean-flour extract that bound IgE were recognized by the patient's serum. These had molecular weights of 54,500, 52,000, 48,000,

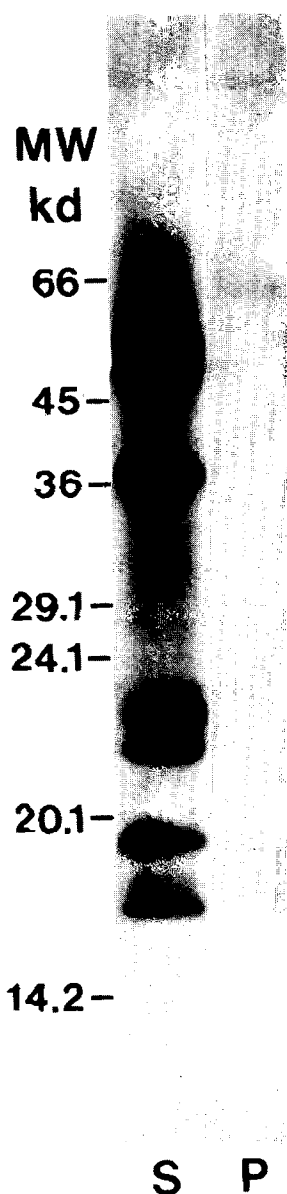


FIG. 2. Autoradiograph of soybean protein (*S*) and peanut protein (*P*) after immunoblotting with serum from a soybean-sensitive patient with asthma. Molecular size (*MW*) of IgE-binding antigens in kilodaltons (*kd*).

47,500, 38,250, 22,750, 21,250, 17,000, and 14,875, determined by their migration relative to standard molecular weight marker proteins.

Inhibition of IgE binding to immunoblots

The autoradiograph of nitrocellulose strips incubated with the patient's serum in the presence of inhibitors is illustrated in Fig. 3. The BSA control, kidney bean extract, and lentil extract did not inhibit IgE binding to soybean proteins. The green pea extract inhibited IgE binding to a soybean protein with a

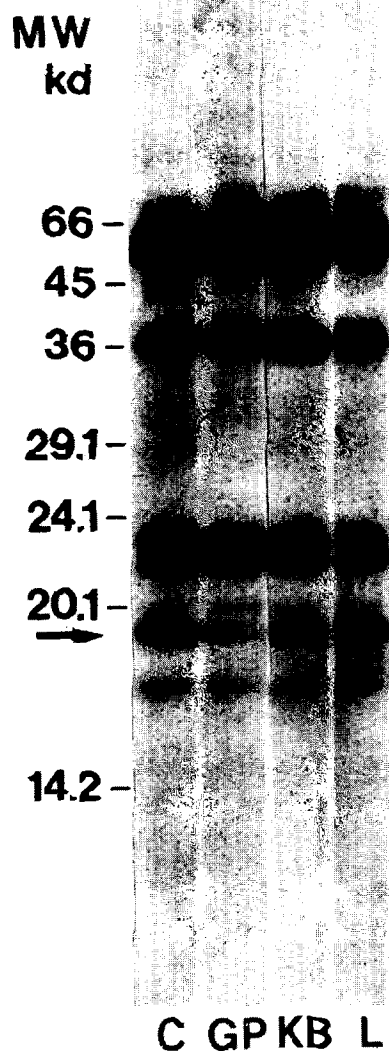


FIG. 3. Autoradiograph of soybean protein after immunoblotting with serum from a soybean-sensitive patient with asthma in the presence of various inhibitors. *Left to right:* C, BSA control; GP, green pea; KB, kidney bean; and L, lentil. Green pea extract inhibited IgE binding to a soybean protein (*arrow*) with a molecular size (*MW*) of 17,000 *kd*.

molecular weight of 17,000. This suggests that there is cross-reactivity between this particular protein in soybean flour and similar allergen in green pea.

DISCUSSION

Sensitization to soybean protein usually occurs through an oral route. Ingestion of soybean proteins may produce gastrointestinal symptoms, urticaria, angioedema, systemic anaphylaxis, and asthma in sensitive individuals. In contrast, the patient described in this study experienced occupational asthma presum-

ably caused by sensitization via an inhalation route. The patient's specific sensitivity to soybean flour was confirmed by a bronchial challenge, the positive skin test, and the demonstration of specific IgE antibodies to soy flour by the direct RAST.

We used immunoblotting to characterize further the allergens involved in the patient's IgE-mediated response to soy flour. Nine proteins were recognized from the soybean flour extract that bound IgE by the patient's serum. These proteins had molecular weights ranging from 14,875 to 54,500, determined by their migration compared to standard molecular weight marker proteins. The patient's IgE response appears to be principally directed toward soybean proteins. Peanut extract did not produce IgE binding on the immunoblots, nor did it inhibit the patient's IgE binding to soybean proteins in the RAST. Green pea extract did inhibit IgE binding to a single soybean protein with a molecular weight of 17,000, suggesting possible cross-reactivity between these two legumes. The clinical relevance of this apparent immunologic cross-reactivity is not known. No cross-reactivity was demonstrated to kidney bean and lentil, the other legumes tested.

The allergenicity of soybean proteins that cause allergic symptoms through their ingestion has been studied. Soybean protein consists of a globulin fraction (85%) and a whey fraction. The globulin fraction has been separated into four components with sedimentation coefficients of 2S, 7S, 11S, and 15S.¹¹ Specific IgE was found to bind to all fractions with considerable cross-reactivity among the 11S, 7S, and 2S globulins. The whey fraction contains a trypsin inhibitor termed "Kunitz soybean-trypsin inhibitor." This protein has been identified as an allergen in a patient experiencing anaphylactic reactions to soybean by ingestion.¹² The protein has been purified and demonstrated to have an apparent molecular weight of 20,000 to 21,000 by polyacrylamide disk gel electrophoresis. Our patient demonstrated IgE binding to a protein with a molecular weight of 21,250. Whether this represents sensitivity to the Kunitz soybean trypsin inhibitor is not known.

It is estimated that in the United States, 2% of all cases of asthma are occupationally related.¹³ The allergens involved in IgE-mediated occupational asthma have not been well characterized. The use of immunoblotting and other immunologic techniques may lead to a better understanding of the mechanisms involved in these responses. Immunoblotting studies of additional patients with occupational asthma to soybean flour could lead to the identification of major allergens and eventually to standard diagnostic reagents.

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