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Source of the aeroallergen of soybean dust: A low molecular mass glycopeptide from the soybean tela

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Airborne soybean allergens in the dust generated during the unloading of soybeans in the harbor caused asthma epidemics in Barcelona, Spain. The major allergen causing the epidemics was a glycopeptide <14 kd molecular mass abundant in soybean dust. This allergen occurs in all parts of the soybean plant at all stages of growth, but the telae (hulls) and pods are by far the richest source. Small amounts of a similar cross-reacting allergen are found in some other grain dusts. The botanical function and significance of this soybean plant component is not known nor is the potential for airborne dispersion of this allergen at other grain-handling sites. (J ALLERGY CLIN IMMUNOL 1991;87:783-8.)

Dust discharged into the air in the harbor during soybean unloading has been identified as the cause of the epidemics of asthma in Barcelona, Spain.[1-3] We previously reported that soybean telae (hulls) collected in Barcelona from beans shipped both from the United States and Brazil were rich in an allergen that is a glycopeptide with a low MM of about 14 kd.[3] Only trace amounts of the allergen were found in cleaned soybeans or soybean flakes. Furthermore, asthma from soybean dust has been reported in only a few isolated cases in farmers or workers in soybean-processing plants in the United States. Therefore, we considered the possibility that the allergen developed during the sea voyage from Toledo, Ohio, or Sàn Paulo, Brazil, where the beans were loaded, per-

Abbreviations used
SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
PBS: Phosphate-buffered saline
MM: Molecular mass

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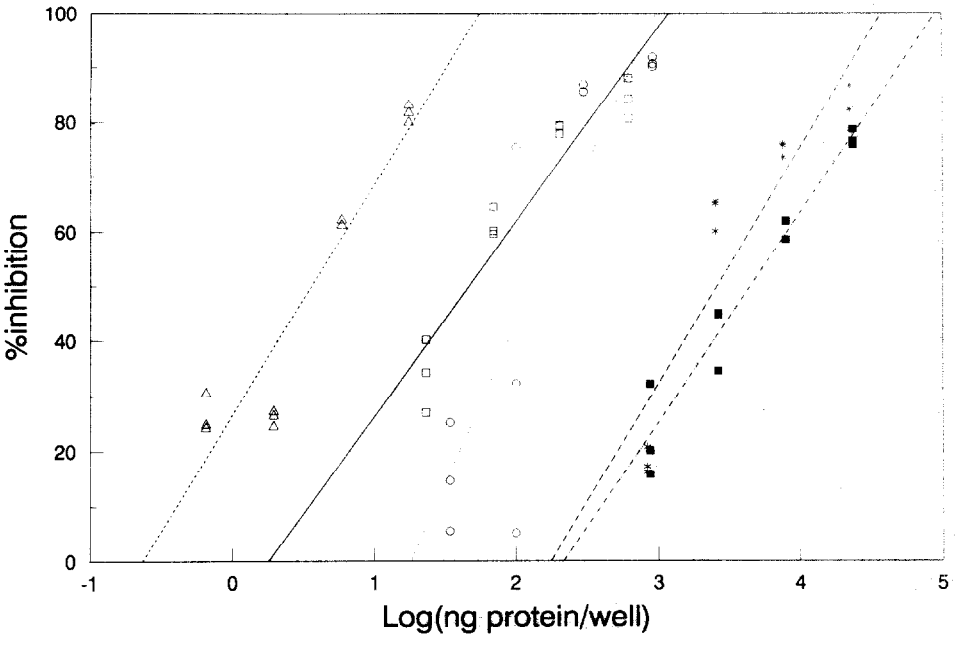

FIG. 1. RAST-inhibition curves demonstrating the cross-reactivity and relative potency of corn and oat dusts and soybean-plant pods to the soybean-hull preparations as they compete for solid-phase binding sites of the low MM allergen component; dust (△), low MM allergens (□), immature pods (○), corn dust (*), and oat dust (■).

TABLE I. Low MM allergen in soybean-plant parts: Developmental stage

Plant part	June (seedlings)	July (flowering)	Aug (immature pods)	Sept (mature beans)
Stems	2892*	2247	68,175	3,693
Leaves	3843	3684	11,409	
Flowers	—	8748		
Pods	—	—	72,491,940	22,086,006
Telae	—	—	742,380	41,049,129
Beans	—	—	15,540	497,148

*Units per gram of dry weight.

haps from a microorganism growing on the beans. In addition, preliminary studies of extract dusts from corn, oats, wheat, and barley suggested that a cross-reacting allergen was present, which lent some support to the hypothesis of a common microbial source of the allergen or that there was a similar antigen present in the plant dusts themselves.

Therefore, we collected soybean plants at various stages of growth from a field near Rochester, Minn., and various grain dusts from Rochester and Edmonton, Alberta, Canada, stored under conditions in which contact with soybeans had not occurred. Small amounts of allergen were present in the leaves, stems, and flowers of immature soybean plants, and the immature soybean telae contained large amounts of the allergen. Telae from fresh ripe beans picked before the pod opened and therefore with very little opportunity for microbial growth contained large amounts. Although this is clear evidence that the allergen arises from the soybean plant, we also found a small amount of cross-reacting material in dust from stored wheat, corn, oat, and barley, but we did not find any from rape seed.

MATERIAL AND METHODS

Sera

Sera from three Barcelona, Spain, residents who experienced asthma after exposure to soybean dust were used to identify and quantify soybean allergens in soybean-plant parts from growing and mature plants, dusts from grain-storage areas, a soybean-hull extract, and a low-molecular weight fraction of the hull extract. These sera contained IgE antibodies to several soybean components.

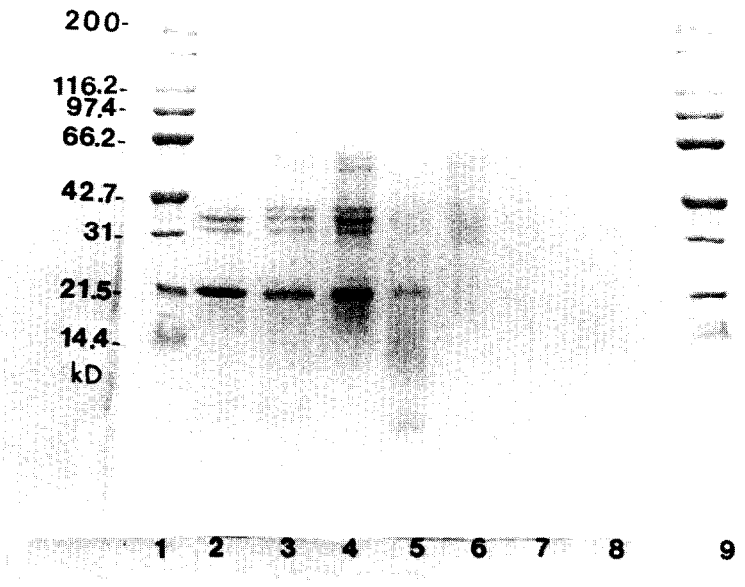

FIG. 2. SDS-PAGE stained with Coomassie brilliant blue; *1*, MM standards, high and low (Bio-Rad Laboratories, Richmond, Calif.); *2*, hulls from field-picked beans; *3*, field hulls (wintered over); *4*, Barcelona hulls; *5*, Barcelona dust; *6*, immature soybean pods; *7*, oat dust; *8*, corn dust; *9*, MM standards, high and low (Bio-Rad Laboratories).

TABLE II. Allergen reactivity in various grain dusts

	Allergens	
	Whole soybean	**LMM* soybean**
Corn	15,290†	1,022,770‡
Wheat	51,940	3,700,050
Oat	53,460	3,259,540
Barley	52,320	2,497,810
Rape seed	<2,550	9,000
Soybean	1,000,000	17,065,722,000

*Low molecular mass.
†Total mass units per gram of dust.
‡Low molecular mass units.

Soybean-plant parts

Soybean plants from a field near Rochester, Minn., were picked at four stages of maturity. In June, just as the plants became healthy seedlings, stems and leaves were collected. In July, when the plants flowered, stems, leaves, and flowers were collected. By mid-August the plants had well-developed pods. From the pods, hulls and beans were separated and collected along with leaves and stems. The mid-September plant was mature and ready for harvest. Stems, pods, hulls, and beans were collected at this stage.

Soybean dust and telae

Soybean dust and telae were obtained from an American ship that was docked in Barcelona and implicated in the asthma outbreaks.

Grain dust

Individual grain-dust samples of wheat, barley, and corolla (rape seed) were obtained through the courtesy of Dr. D. A. Enerson of the University of Alberta, Edmonton, Canada. Dusts from corn and oats were obtained locally by sifting a quarter bushel of each of the grains through a window screen onto butcher paper. None of these five grains had been kept in silos in which soybeans had ever been stored.

Antigen extracts

After ether defatting in a soxhlet apparatus for 4 hours, all soybean-plant parts and grain dusts were extracted 1:30 wt/vol in 100 mmol/L of NH_4HCO_3. After centrifugation, the solids were discarded, and the supernatants were di-

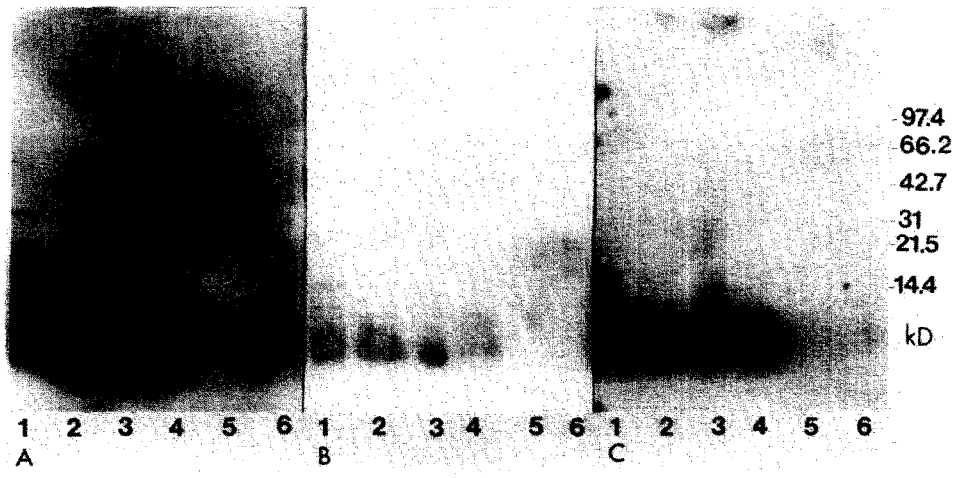

FIG. 3. IgE immunoblots with three individual sera, *A*, *B*, and *C*; *1*, hulls from field-picked beans; *2*, field hulls (wintered over); *3*, Barcelona hulls; *4*, immature soybean pods; *5*, oat dust; *6*, corn dust.

alyzed in 3500 dalton cutoff casings (Spectrum Medical Industries, Inc., Los Angeles, Calif.) against distilled water. The extracts were stored at −70° C or glycerinated and stored at −20° C.

MM characteristics of the corn, oat, and soybean-grain dusts were examined with Centricon microconcentrators (Amicon Corp., Danvers, Mass.). Equal volumes of each grain-dust extract were concentrated separately, first through a 10,000 dalton cutoff membrane at 3000 *g* for 55 minutes. The filtrate was recovered and concentrated through a 3000 dalton cutoff membrane at 6000 *g* for 1 hour. The retentate was saved, and low MM allergen content was assessed in corn, oat, and soybean-grain dusts.

Antigen reference standards

Low MM allergen(s). Soybean telae from beans collected in Barcelona from a shipment associated with an epidemic were used to make the whole extract. This extract had an absorbance at 280 nm, 1 cm of 11.2, and was arbitrarily assigned a potency of one million total mass units per milliliter. Twenty milliliters of whole extract were placed in a dialysis casing of 10,000 dalton cutoff. This casing was placed inside another casing of 3500 dalton cutoff that contained 300 ml of distilled water. After exhaustive dialysis against distilled water, the inner bag was removed, and the outer bag, containing components between 3500 and 10,000 daltons was concentrated by absorption with dry Sephadex beads to the original volume of whole extract. This concentrate had an absorbance at 280 nm, 1 cm of 0.8, and was also assigned an arbitrary potency of one million low MM units per milliliter.

RAST-inhibition

Separate competitive assays were developed to quantify the whole hull-extract allergen activity and low MM allergen activity. These assays were accomplished by absorbing the allergens to the surface of Immulon II microtiter plates (Dynatech Laboratories, Alexandria, Va.). The extracts were diluted 1:500 in 200 mmol/L of carbonate-bicarbonate buffer, pH 9.2. One hundred microliters of solution was added to each well and incubated overnight at room temperature. After wells were washed three times with 0.1 mol/L of phosphate buffer containing 1% Tween 20, 50 μl of each test extract was added separately to each well along with 50 μl of the three human sera (pooled) containing specific IgE antibodies. After overnight incubation, the wells were washed again, and 100 μl of radioiodinated rabbit antihuman IgE (20 ng per well) was added. The wells were incubated overnight, washed as before, and counted on a gamma counter. The plant and dust extracts were quantified in terms of each whole reference standard (10^6 total mass units per milliliter) by interpolation from the regression lines plotted for the allergen standards. The results were calculated with regression analysis on a Hewlett-Packard (Cupertino, Calif.) 9845B computer.

Protein determination

Protein content of each soybean-plant part and dust extracts was determined by the bicinchoninic acid method.[4]

SDS-PAGE

Extracts of fresh soybean telae, immature soybean pods, and soybean dusts from Barcelona, or dusts of oat and corn collected in the United States, were loaded onto a 5% to 20% SDS-polyacrylamide gradient gel with 2.7% crosslinkage. The gels were loaded with 100 μg of protein per lane and electrophoresed at 30 mA per gel. The gels were stained with Coomassie brilliant blue or used for immunoblotting.

Immunoblotting

After electrophoresis, gels were electroblotted onto 0.1 μm nitrocellulose in Tris-glycine-SDS-methanol buffer (continuous buffer, LKB, Bromma, Sweden) at 0.8

TABLE III. Low MM allergen in Centricon fraction of MM 10,000 to 3000

	LMM* U/ml of ultrafiltrate
Corn	468
Oats	9,826
Soybean	68,282

*Low molecular mass.

mA/cm.^{2} After the transfer the sample lanes and lanes containing the MM standards were removed and sequentially stained with amido black and India ink. The blotted samples were blocked overnight in PBS buffer containing 0.01% Tween 20 and 3% bovine serum albumin. The blots were then washed five times in PBS-Tween, incubated overnight with various RAST-positive sera diluted 1:10 in PBS-Tween, washed again, and incubated with 0.2 μCi of ^{125}I-labeled antihuman IgE. The membrane was finally washed again, dried, mounted, and autoradiographed with X-OMAT AR film (Eastman Kodak Co., Rochester, N.Y.).

RESULTS

The low MM allergen fraction was found in small amounts in all parts of the plant and at every stage of development with pods and telae being particularly rich sources (Table I), and the extract of soybean telae collected in Barcelona was the richest source of allergen reacting with IgE antibody in the sera of the patients.

Regression analysis of the various inhibition curve from the soybean-plant parts and grain dusts were similar (Fig. 1). A one-way analysis of covariance indicated no significant differences between slopes indicating antigenic similarity. That dust from corn, wheat, oats, and barley, but not from rape seed, contains small amounts in cross-reacting molecules is presented in Table II. On separation of the low MM components from equivalent extract volumes of corn, oat, and soybean-grain dusts, they were assayed for the specific low MM allergen found associated with soybean (Table III). These results indicate that at least part of the corn and oat allergens exist in an MM range similar to the soybean allergen, although in the latter source they are found in much greater quantities.

SDS-PAGE reveals bright, strong Coomassie brilliant blue staining for components >15,000 daltons and poor visualization of components <15,000 daltons when equivalent protein quantities are applied from extracts of various hulls, pods, grains, and dust (Fig. 2). Immunoblots with the same grain and plant antigens and individual sera from the patients with asthma, described earlier, demonstrate intense radiostaining of components <15,000 daltons. In particular, a broad band <14,000 daltons is common to all sera. Few, if any, high MM bands were reactive with these sera (Fig. 3). Where the Coomassie brilliant blue stain produced little protein staining with the corn- or oat-dust extracts, the immunoblot reveals a hint of radiostaining in the low MM region. This method is less sensitive than the RAST inhibition, and the amount of allergen present was too small to yield a strong reaction. The Barcelona dust extract was incubated with sera separately since this electrophoretic transfer required higher serum dilutions because of intense IgE binding in the low MM region (Fig. 4).

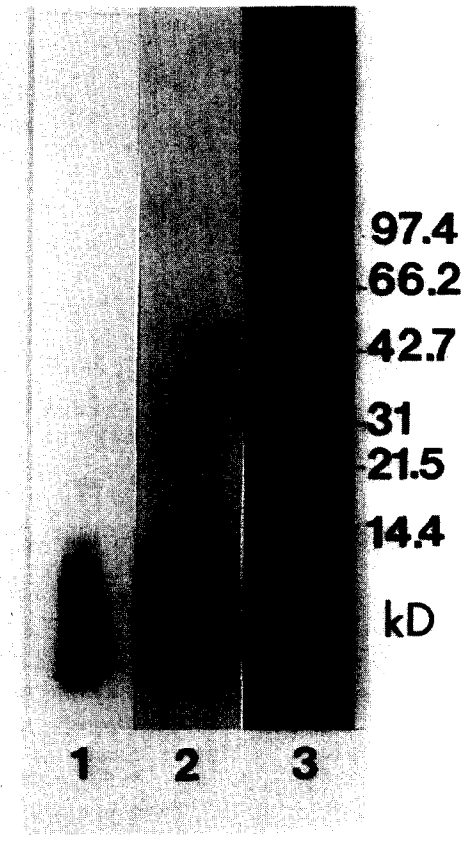

FIG. 4. IgE immunoblot of Barcelona dust and sera; *1*, serum A; *2*, serum B; *3*, serum C.

DISCUSSION

Asthma epidemics of Barcelona appeared to be caused by unloading of soybeans in the harbor.[1, 2] The characterization of allergens in soybean demonstrated that most of the allergens were proteins of <14 kd concentrated mainly in the hull.[3]

Investigations studying the implication of contaminating agents of the soybean seed in the etiology of this epidemic have been focused so far on the study of the three mite species that grow with stored seeds (*Blomia tropicalis*, *Aleuroglyphus ovatus*, and *Chor-*

toglyphus arcuatus).[5] These studies demonstrated that these species of mites did not take part in the etiology of the epidemics. Similarly, attempts to inhibit the soybean allergen with fungi, *Asperigillus fumigatus* and others, or insects were negative.

These results strongly suggest that the source of allergen is the soybean plant, specifically the telae, and not contaminating microorganisms, mites, or insects. The occurrence of cross-reacting allergen of similar low MM, albeit in much lower quantity, in cereal-grain dusts is not readily explained. The function of these proteins in the plant is unknown.

We also were unable to find a definitive explanation for the occurrence of epidemic sensitization in Spain and only isolated cases in the United States. In Barcelona, the dust was discharged directly into the air; in the United States, dust is confined by on-site, air-handling equipment. On epidemic days, moreover, once the dust in Barcelona was discharged, geographic and climatologic conditions were ideal for dust to hover over the city. Furthermore, mechanical abrading or milling as the soybeans move about in the hold of the ship and as it rocks with the waves crossing the Atlantic might help grind the soybean telae down into more respirable-sized particles. Also, the beans have been transferred two more times into and out of the hold; therefore, in Spain, more of the allergen might be in respirable particles than in the United States.

Although the sensitivity and severity of the disease in Barcelona subjects with asthma was presumably due to the unusually large exposure, the physician should suspect asthma from soybean whenever a patient with asthma is in contact with airborne soybean allergens. Additional studies are warranted to investigate the presence of this allergen at other loading or unloading ports handling grains, especially soybeans.

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