

Sensitization to soybean hull allergens in subjects exposed to different levels of soybean dust inhalation in Argentina

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Background: Soybean hulls (SHs) have been identified as the source of aeroallergens responsible for soybean asthma outbreaks. However, the prevalence of sensitization to SH allergens in subjects from Argentina, a country where soybeans are produced, is unknown.

Objective: The purpose of this study was to determine the prevalence of sensitization to SH by in vivo and in vitro tests in subjects with asthma or allergic rhinitis and in control subjects from Argentina who have been exposed to different levels of soybean dust inhalation (SDI).

Methods: Exposure to SDI is defined as follows: (1) direct = occupational, (2) indirect = proximity to soybean fields or grain elevators, and (3) urban = urbanized areas without a known source of SDI. Two groups were studied. Group 1 consisted of 365 subjects with asthma or allergic rhinitis and group 2 (control group) of 50 healthy individuals. Subjects from both groups were classified according to their exposure to SDI. All subjects completed standard questionnaires. Prick skin tests (STs) with an SH extract and with common allergens were performed on all subjects. Specific IgE and IgG4 to SH were measured in sera of 51 of 56 subjects from group 1 who had a positive ST to SH and in all sera from group 2.

Results: Fifty-six (15.3%) subjects from group 1 and no subjects from group 2 had a positive ST to SH (wheal SH/wheal histamine ≥ 0.5). In group 1, positive STs to SH were 38.7%, 20.3%, and 8.2% in subjects with direct, indirect, and urban exposures, respectively ($P < .001$). Monosensitization to SH is absent in all subjects from group 1. The percent of subjects with positive STs to mites, pollen, and molds was highest in those with a positive ST to SH versus those with a negative ST to SH ($P < .01$). Asthmatic patients with a positive ST to SH, compared with those exclusively sensitized to mites, had a higher frequency of daily or weekly symptoms (59.4% vs 25.7%, respectively, $P < .001$) and a higher percent of glucocorticoid dependence (52.8% vs 34%, respectively, $P < .01$).

Percent positive IgE in group 1 and group 2 were 39.2% and 10% ($P < .001$) and percents positive IgG4 were 27.4% and 12%, respectively (not significant). In subjects from group 1 and group 2 with direct exposure percents positive IgE were 58.3% and 13.3% ($P < .001$) and percents positive IgG4 were 75% and 20%, respectively ($P < .02$). IgG4 in group 1 was significantly higher in subjects with direct exposure compared with subjects with indirect or urban exposure.

Conclusion: This study demonstrated that there was (1) a high prevalence of sensitivity to SH in subjects with asthma or allergic rhinitis from Argentina and (2) an association between sensitivity to SH and severity of asthma and level of exposure to SDI. (*J Allergy Clin Immunol* 2000;105:570-6.)

Key words: Soybean-induced asthma, soybean hull allergens, sensitization, exposure

Asthma associated with exposure to soybean aeroallergens was first reported by Duke¹ in 1934. Subsequently, allergic and respiratory symptoms associated with occupational and nonoccupational exposure to soybeans were reported in isolated cases.²⁻⁵

Dust discharged into the air during unloading of soybeans from ships was identified as the etiologic agent of asthma outbreaks in Barcelona, Cartagena, and Tarragona, all cities in Spain.⁶⁻⁸ Asthma outbreaks that took place in New Orleans, La, were attributed to the same etiology.⁹ Soybean hulls (SHs) were identified as the source of aeroallergens that cause soybean epidemic asthma and both skin test (ST) and specific IgE determination to SH allergens was recommended to demonstrate sensitization for soybean-induced asthma.¹⁰ In addition, a significant correlation between specific IgE and IgG4 to SH allergens was demonstrated in subjects affected by soybean epidemic asthma and it was suggested that IgG4 acts as an anaphylactic antibody.¹¹

The first epidemiologic investigation of the relationship between soybean dust inhalation (SDI) and respiratory symptoms in the soybean industry was performed by Zuskin et al.^{12,13} Irritant, rather than immunologic, mechanisms were suggested to cause airway diseases.

Economically, soybean is the most important bean in the world, representing one of the richest and cheapest sources of protein. Modern agriculture produces large quantities of soybeans, which are handled many times from harvest to final consumption with grain dust production. Dust levels have been measured in grain elevators and levels up to 1000 mg/m³ have been reported, and approximately 10% of this airborne dust is $<10 \mu\text{m}$ and therefore it can reach the lower airways.¹⁴ It is logical to assume that a large number of subjects exposed to SDI

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Abbreviations used

ABTS:	2,2'-Azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid)
HR:	Histamine rate
OD:	Optical density
OR:	Odd ratio
RAST:	Radioallergosorbent test
SDI:	Soybean dust inhalation
SH:	Soybean hull
ST:	Skin test
TBS:	TRIS-buffered saline solution
TCB:	Total counts bound
TTBS:	Tween-TRIS-buffered saline solution

are at risk to become sensitized and have respiratory symptoms. In 1998 Argentina was the fourth largest producer of soybeans, and the crop represented approximately 10% of the total world production.¹⁵ However, the prevalence of sensitization to SH allergens in subjects exposed to SDI in Argentina has not been studied.

The purpose of this study was to (1) evaluate the prevalence of sensitization to SH allergens by ST in subjects with asthma or allergic rhinitis and in control subjects from Argentina exposed to different levels of SDI, (2) study the clinical characteristics of subjects with asthma or allergic rhinitis and a positive ST to SH, and (3) determine the prevalence of specific IgE and IgG4 to SH allergens in subjects with asthma or allergic rhinitis and a positive ST to SH and in controls.

MATERIAL AND METHODS

Patient population

Four hundred fifteen subjects from two main soybean producer regions in Argentina (south of Santa Fe and northwest of Buenos Aires) were recruited through the participation of 8 regional health services.

Two groups were studied. Group 1 consisted of 365 subjects with asthma or allergic rhinitis, 178 (48.8%) male subjects and 187 (51.2%) female subjects, with an age range from 5 to 70 years (24.9 ± 15.3 years). These individuals were recruited among subjects being treated for asthma or allergic rhinitis in allergy departments and who were compliant with medications and appointments. Control subjects from group 2 had no chronic or acute illnesses and were recruited from subjects seeking health care in other departments. They consisted of 50 nonsmokers without any respiratory symptoms and negative STs to common allergens and a normal total IgE value. Twenty-two (44%) subjects from group 2 were male and 28 (56%) female, with an age range from 12 to 70 years (40 ± 15.4 years). All subjects gave informed consent and completed standard questionnaires.

The level of exposure to SDI was defined as follows: (1) direct exposure, subjects with an occupational exposure to SDI, which includes farmers, grain elevator workers, and individuals working in soybean processing factories; (2) indirect exposure, subjects living in rural areas (fewer than 2000 inhabitants) near grain elevators or soybean fields; and (3) urban exposure, subjects living in cities or towns without a known source of SDI. Thirty-one of 365 (8.5%) from group 1 and 15 of 50 subjects (30%) from group 2 had direct exposure, 133 of 365 (36.4%) from group 1 and 5 of 50 subjects

(10%) from group 2 had indirect exposure, and 201 of 365 (55%) from group 1 and 30 of 50 subjects (60%) from group 2 had urban exposure.

Serum samples were obtained from 51 of the 56 subjects from group 1 who had a positive ST with an SH extract and from all subjects from group 2. Serum samples were kept at -20°C until specific IgE and IgG4 determinations were performed. The demographic characteristics of the two groups of subjects are included in Table I.

Allergen extracts

Allergens from SH were extracted 1:20 (wt/vol) with 0.2 mol/L ammonium bicarbonate, pH 9, overnight at 4°C . The supernatant was isolated by filtration under vacuum with use of a $0.44\text{ }\mu\text{m}$ cellulose nitrate filter (Nalgene Company, Rochester, NY) and sterilized through a $0.22\text{ }\mu\text{m}$ filter (Millipore, Bedford, Mass). Total protein content was measured by the bicinchoninic acid protein assay (Pierce Chemical, Rockford, Ill). Allergen extracts of *Der-matophagoides pteronyssinus*, *Blomia tropicalis*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, and *Chortoglyphus arcuatus* were prepared from mite cultures grown in the laboratory with use of analogous methods. Mold (*Alternaria* spp, *Cladosporium* spp, *Mucor* spp, *Penicillium* spp, and *Aspergillus* spp), pollen (tree mix, weed mix, and grass mix), and epithelia (cat and dog) allergen extracts were commercially purchased (IPI Asac Argentina).

Skin tests

STs were performed on all subjects from groups 1 and 2 by the prick technique. Extracts were applied on the forearm and a prick lancet (Hollister Stier) used to prick the skin. Negative (saline solution) and positive (histamine chloride, 1 mg/mL) controls were used. Each skin test was assessed at 15 minutes. The estimated averages of the largest and orthogonal wheal diameters obtained with the SH extract and with the positive control were recorded. The histamine rate (HR) was calculated as the ratio of SH wheal diameter compared with the histamine wheal diameter. Results were considered positive when the HR was greater than or equal to 0.5. For mite, mold, pollen, and dander allergen extracts, a wheal diameter greater or equal than 3 mm was regarded as positive.

Specific IgE determinations

Specific IgE determinations to SH allergens were performed by RIA as follows: SH proteins were adsorbed to wells of Immulon 4 Removawell strips (Dynatech Laboratories, Chantilly, Va). Each well received 1 μg of protein in 100 μL of 0.1 mol/L sodium carbonate-bicarbonate buffer, pH 9.6. The wells were incubated overnight at room temperature in a humid box and washed four times with 0.1 mol/L phosphate buffer, pH 7.5, containing 1% Tween 20 (washing buffer). After 100 μL of undiluted serum was added to each well, the wells were again incubated overnight and then washed four times with washing buffer. One hundred microliters of rabbit iodine 125-labeled antihuman IgE (Sanofi Diagnostics Pasteur, Chaska, Minn), diluted to 1:2 in 0.1 mol/L phosphate buffer, pH 7.5, and containing 1% Tween 20 and 1% BSA (diluent buffer), were then added to all wells, which were incubated overnight and again washed, and the counts per minute were counted in a gamma counter. The results were expressed as the percentage of total counts bound (%TCB).

Specific IgG4 determinations

Specific IgG4 determinations to SH allergens were performed by an amplified ELISA method based on the biotin-streptavidin complex as follows: SH proteins were adsorbed to wells of high-binding microtiter plates (Costar, Cambridge, Mass) by incubating 1 μg of protein in 100 μL of 0.1 mol/L sodium carbonate-bicarbonate

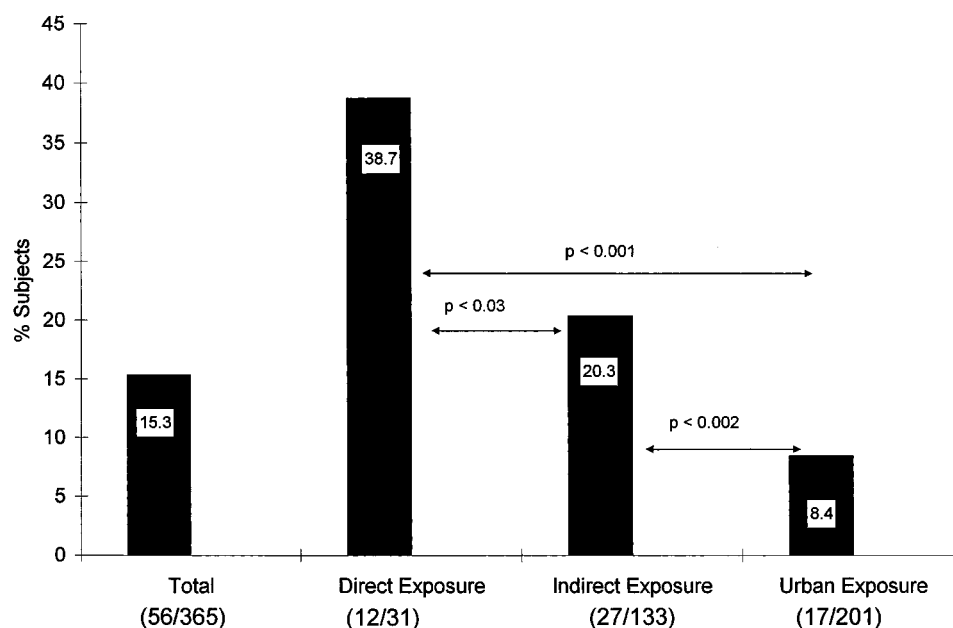


FIG 1. Prevalence of positive skin tests to soybean hulls in group 1.

TABLE I. Demographic characteristics of the subjects from groups 1 and 2

	Group 1 (n = 365)	Group 2 (n = 50)
Sex (No. [%])		
Female	187 (51.2)	27 (54.0)
Male	178 (48.8)	23 (46.0)
Age (y)		
Mean $\pm$ SD	24.9 $\pm$ 15.3	40.8 $\pm$ 14.9
Range	5-70	12-70
Exposure (No. [%])		
Direct	31 (8.5)	15 (30.0)
Indirect	133 (36.4)	5 (10.0)
Urban	201 (55.1)	30 (60.0)
Positive ST to SH according to level of exposure (No.)		
Direct	12 (38.7)	0
Indirect	27 (20.3)	0
Urban	17 (8.4)	0
Symptoms (No. [%])		
Rhinitis	173 (47.4)	0
Asthma	59 (16.2)	0
Asthma + rhinitis	133 (36.4)	0

buffer, pH 9.6, overnight at room temperature in a humid box. Then the wells were washed four times with washing buffer and blocked with 100 μ L per well of 0.1 mol/L phosphate buffer, pH 7.5, containing 1% Tween 20 and 1% BSA (blocking and diluent buffers) for 1 hour at room temperature. The plates were washed four times between steps, and the assay was performed in duplicate by incubating for 1 hour at 37°C 100 μ L of each serum diluted in diluent buffer to 1:10. A monoclonal biotin conjugated antihuman IgG4 (Sigma Chemical, St Louis, Mo) was diluted in diluent buffer to 1:15,000 and incubated for an hour at 37°C. A solution of streptavidin-peroxidase (Sigma Chemical) diluted to 1:10,000 in diluent

buffer was added and the plates were incubated for 30 minutes at 37°C (100 μ L/well) after which a substrate working solution of 0.1 mmol/L 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) (Sigma Chemical) in 0.1 mol/L citrate buffer, pH 4.2, was incubated for a minimum of 5 minutes (100 μ L/well) until the color was developed. The reaction was stopped with 100 μ L of 2 mmol/L sodium azide. Optical density (OD) at 414 nm was measured with a microplate reader (Dynatech). Results were expressed as OD units at 414 nm.

Statistical analysis

The statistical analysis was performed with the Statview program (Abacus Concepts, Berkeley, Calif). To ascertain whether the values were normally distributed, the Kolmogorov-Smirnov test was used. The Spearman rank correlation coefficient was calculated. The chi-square test was used to study the association between qualitative variables. A *P* value less than .05 was considered significant.

RESULTS

Skin tests, symptoms, and exposure to SDI

All subjects from group 2 had a negative ST to SH. Fifty-six of 365 subjects (15.3%) from group 1 had a positive ST to SH, and this prevalence was significantly correlated with the level of exposure to SDI (Fig 1). The prevalence of a positive ST to SH was higher in male subjects than in female subjects (odds ratio [OR] 1.61, 0.87 to 3.01), although not significant, and in subjects with a higher mean age (30.5 $\pm$ 16.9 years vs 23.9 $\pm$ 14.8 years, *P* < .01).

Monosensitization to SH was absent in all subjects from group 1. The percent of positive STs to mites, pollen, and molds is significantly higher in subjects with a positive ST to SH than in subjects with a negative ST to SH (Table II).

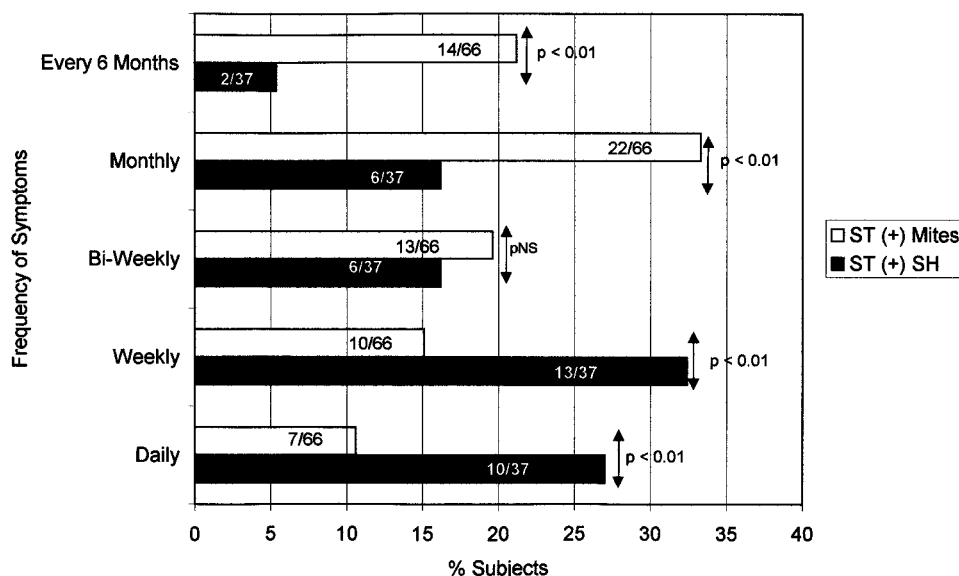


FIG 2. Frequency of symptoms in group 1.

Thirty-seven of the 56 (66%) with a positive ST to SH and 155 of the 309 subjects (50.1%) with a negative ST to SH have asthma, with or without rhinitis ($P < .05$). Sixty-six of the 155 subjects (42.5%) with a negative ST to SH are monosensitized to mites.

In the group of subjects with a positive ST to SH, 8 of 12 (66.7%) with direct exposure and 6 of 44 subjects (13.6%) with indirect or urban exposures reported respiratory symptoms after SDI ($P < .001$). Seven of 12 (58%) with direct exposure and 7 of 44 subjects (16.7%) with indirect or urban exposures had exacerbations of respiratory symptoms during the soybean harvesting season ($P < .01$), especially when visiting soybean-producer areas.

Clinical characteristics of asthma in subjects with a positive ST to SH

Nine of 37 subjects (24.3%) with asthma and a positive ST to SH and 66 of 66 subjects (100%) with asthma and monosensitized to mites had urban exposure. Subjects with a positive ST to SH, compared with those exclusively sensitized to mites, have the following characteristics: (1) a higher frequency of perennial asthma symptoms with spring and fall exacerbations ($P < .01$), (2) a higher frequency of daily or weekly symptoms during the greatest severity periods (59.4% vs 25.7%, $P < .001$), and (3) a higher percent of inhaled glucocorticoid-dependent subjects (52.8% vs 34%, $P < .01$) (Fig 2).

Specific IgE and IgG4 to SH allergens

Specific IgE and IgG4 values did not follow a normal distribution. Therefore the reference values for specific IgE and IgG4 were established as the percentile 97.5 of the results found in the subjects with urban exposure from group 2 (controls without a known exposure to SDI). The reference value for specific IgE is <1.19 percent TCB and for specific IgG4 <0.030 OD units. All values greater than or equal to these are considered positive.

TABLE II. Skin sensitivity (No. [%]) to common aeroallergens in subjects from group 1

Allergen	Positive ST to SH (n = 56)	Negative ST to SH (n = 309)
Mites	49 (87.5)	217 (70.2)*
Pollen	29 (51.8)	93 (30.1)*
Molds	22 (39.3)	78 (25.2)†
Danders	10 (17.9)	44 (14.2)

Chi-square test, comparison of subjects with positive versus negative ST to SH.

* $P < .01$.

† $P < .05$.

Percent positive results for specific IgE in groups 1 and 2 are 39.2% and 10.0% ($P < .001$) and for specific IgG4 27.4% and 12.0%, respectively (not significant) (Figs 3 and 4). Percent positive IgE and IgG4 in group 1 increased to 58.3% and 75%, respectively, in subjects with direct exposure (Figs 3 and 4).

Although percent specific IgE in group 1 is higher in subjects with direct exposure because of the wide SD, the values do not significantly differ according to different levels of exposure to SDI. However, percent specific IgG4 is significantly higher in subjects with direct exposure compared with indirect or urban exposures (Figs 3 and 4). Specific IgE and IgG4 in group 2 do not significantly differ according to different levels of SDI (Figs 3 and 4). The correlation between specific Ig and ST results in group 1 is significant for IgG4 ($r = 0.47$, $P = .0009$) but not for IgE.

DISCUSSION

Since the 1980s, the farming, processing, and exportation of soybeans are of growing importance in Argentina and many other countries. Therefore a large number of subjects living in rural areas are exposed to SDI and have

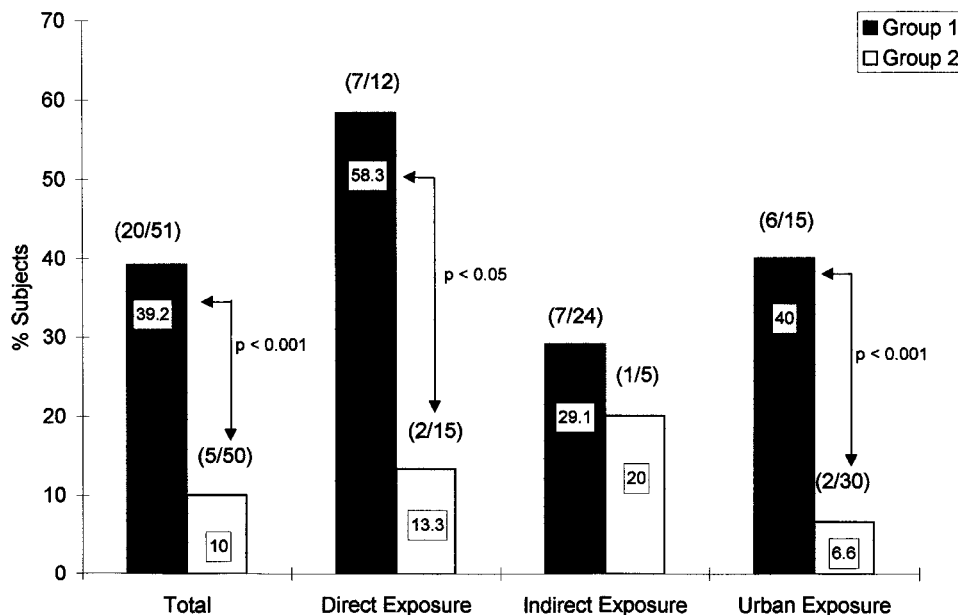


FIG 3. Percent positive specific IgE in groups 1 and 2.

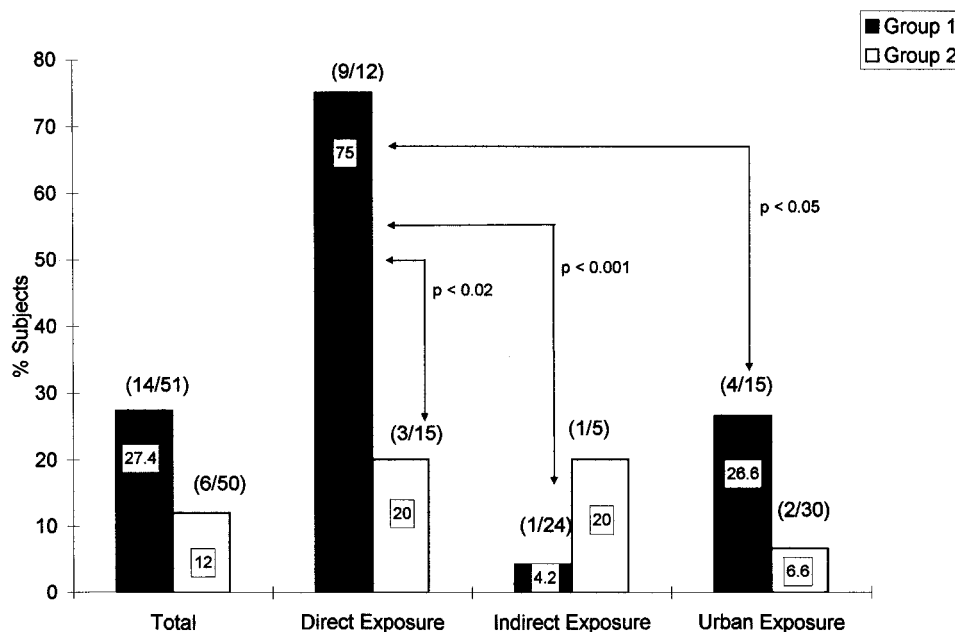


FIG 4. Percent positive specific IgG4 in groups 1 and 2.

a high risk for sensitization and for development of allergic respiratory diseases. This is the first study that examines the prevalence of sensitization to SH allergens in a rural population of subjects with asthma or allergic rhinitis and control subjects repeatedly exposed to SDI.

The prevalence of positive skin test to SH is 15.3% in the group of subjects with asthma or allergic rhinitis, and it is significantly associated with the level of exposure to SDI. It is also higher in men than in women (22.7% vs 12.4%), perhaps because men are mostly involved in the harvesting,

transporting, and loading and unloading of soybeans in grain elevators. Subjects with a positive ST to SH also have a higher mean age than subjects with a negative ST to SH (30.5 vs 23.9 years). These findings suggest that there may be an association between level and duration of exposure to SDI and sensitization to soybean aeroallergens.

Zuskin et al¹³ performed a study of subjects working in a soybean-processing factory. Positive intradermal soybean extract STs were attributed to an irritant reaction rather than an immunologic mechanism. In the current

study all subjects from the control group had a negative skin prick test to SH, thus ruling out an irritant skin test reaction. Intradermal skin tests as performed with non-standardized extracts by Zuskin et al have a low specificity and might produce false-positive results.

Monosensitization to SH allergens is absent in the study subjects. This indicates that both a genetic atopic background and exposure to SDI are necessary for sensitization to SH. Soriano et al¹⁶ described an association of soybean epidemic asthma with specific HLA class II alleles.

The prevalence of positive STs to pollen, molds, and mites is significantly higher in subjects with asthma or allergic rhinitis with a positive ST to SH compared with those with a negative ST to SH. Similar findings have been described in subjects affected by soybean epidemic asthma.¹⁷ The majority of subjects in this study with a positive ST to pollen are from rural areas and are exposed to outdoor allergens. Molds that contaminate soybeans could be responsible for the cosensitization to molds in soybean-sensitized subjects.¹⁸ Sensitization to the storage mites, *B tropicalis*, *C arcuatus*, and *T putrescentiae* accounts for a high percent of positive STs to mite allergens in this study. However, it remains unknown whether these mite species contaminate soybeans.

Most subjects with a positive ST to SH have asthma, with or without rhinitis, and the majority, especially those with an occupational exposure to SH, report symptoms after SDI. However, no individual had near-fatal or fatal asthma, which is characteristic of soybean epidemic asthma.¹⁷ Subjects affected by soybean epidemic asthma had a sudden exposure to high levels of SDI, whereas in this study subjects were repeatedly exposed to different levels of fresh and stored soybean allergens. Fresh SH versus stored SH is less allergenic.¹⁹ These different forms of exposure could account for these differences.

Clinical differences, such as frequency and seasonality of symptoms and use of medication, between asthmatic subjects with a positive ST to SH and those exclusively sensitized to mites are demonstrated in this study. Only 26% of subjects with a positive ST to SH, but all subjects monosensitized to mites, live in urbanized areas. These findings indicate that differences in clinical presentation of the disease may be the result of differences in environmental factors.

Specific IgE and IgG4 to SH are detected in 39.2% and 27.4%, respectively, in subjects in this study with symptomatic asthma or allergic rhinitis and a positive ST to SH. They increase to 58.3% and 75%, respectively, in individuals with an occupational exposure to soybeans. The prevalence of specific IgE to SH allergens in subjects affected by the asthma epidemics in Barcelona and Cartagena, Spain, has been reported to be lower than 100%.^{6,7} This has been attributed to a misclassification of some individuals included in the group of asthmatic epidemic subjects.^{6,7} Soybean dust may also be an irritant in soybean-induced asthma¹³ and may explain the presence of irritant positive ST in the absence of specific IgE. Skin test results indicate that this phenomenon is not present in the group of individuals in this study. In addition,

specific IgE and IgG4 levels to SH allergens increase proportionately to the degree of exposure to SDI, and the differences are significant for IgG4, which indicates that an immunologic mechanism is probably responsible for soybean-induced asthma in subjects who are repeatedly exposed to SDI. Failure to observe significant differences in IgE levels based on type of exposure is probably related to the small sample size, a limitation of this study. The presence of low-affinity specific Ig to SH, which has been suggested to be present in sera of subjects with a positive ST to SH from Argentina,²⁰ could explain negative Ig values in individuals with a positive ST to SH.

The presence of specific IgG4 to SH in soybean asthma epidemics has been previously reported.¹¹ The mechanism of production of IgG4 is similar to that for IgE (eg, IL4 stimulates switching by human B cell to both IgE and IgG4) and the switch from IgE to IgG involves an initial switch to IgG4.²¹ However, whether IgG4 plays a role in type I hypersensitivity or whether it is just an epiphenomenon remains unknown. The results of the current study demonstrate a significant correlation between specific IgG4 and IgE levels and specific IgG4 and positive STs to SH. These facts, together with the higher levels of specific IgG4 found in subjects with a direct exposure to SDI, indirectly support the hypothesis that IgG4 plays a role in soybean-induced asthma.

Approximately 6% of healthy individuals living in urbanized areas without a known source of soybean dust release had positive IgE and IgG4 to SH in this study. These values are similar to those found by Zuskin et al¹³ and Sunyer et al,⁶ both of whom reported 5% positive results in their unexposed control groups.

In conclusion, this in vivo and in vitro study demonstrated that in subjects with asthma or allergic rhinitis from rural areas in Argentina the degree of exposure to SDI correlates with the skin test reactivity and levels of specific IgE and IgG4 to soybean aeroallergens and with the severity of asthma symptoms. Sensitization to soybean aeroallergens should be suspected in exposed subjects from other parts of the world.

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REFERENCES

1. Duke WW. Soybean as a possible important source of allergy. *J Allergy* 1934;5:300-2.
2. Olsen AM, Prickman LE. Hypersensitivity to soybeans. *Proc Mayo Clin* 1934;11:465-8.
3. Wightman HB. Soybean sensitivity with a case report. *J Allergy* 1938;9:601-3.
4. Peters GA. Bronchial asthma due to soybean allergy: report of a case with audiovisual documentation. *Ann Allergy* 1965;23:270-2.

5. Bush RK, Cohen M. Immediate and late onset of asthma from occupational exposure to soybean dust. *Clin Allergy* 1977;7:369-73.
6. Sunyer J, Antó JM, Rodrigo MJ, Morell F. Clinical Toxicologic-epidemiological Committee. Case-control of serum immunoglobulin-E antibodies reactive with soybean in epidemic asthma. *Lancet* 1989;1:179-82.
7. González R, Zapatero L, Caravaca F, Carreira J. Identification of soybean proteins responsible for respiratory allergies. *Int Arch Allergy Appl Immunol* 1991;95:53-7.
8. García-Ortega P, Rovira E, Bartolomé B, Martínez A, Mora E, Richart C. Epidemia de asma alérgica a polvo de soja: estudio clínico e inmunológico de los pacientes afectados. *Med Clin* 1998;110:731-5.
9. White MC, Etzel RA, Olson DR, Goldstein LE. Reexamination of epidemic asthma in New Orleans, Louisiana, in relation of the presence of soy at the harbor. *Am J Epidemiol* 1997;145:1-7.
10. Morell F, Codina R, Rodrigo MJ, Sunyer J, Antó JM, Reed CE. Diagnosis of soybean-induced asthma. *J Allergy Clin Immunol* 1995;96:320-4.
11. Codina R, Calderón E, Lockey RF, Fernández-Caldas E, Rama R. Specific immunoglobulins to soybean hull allergens in soybean asthma. *Chest* 1997;111:75-80.
12. Zuskin E, Skuric Z, Kanceljak B, Pokrajak D, Schachter EN, Witek TJ. Respiratory symptoms and ventilatory capacity in soybean workers. *Am J Ind Med* 1988;14:157-65.
13. Zuskin E, Kanceljak B, Schachter EN, Witek TJ, Marom Z, Goswami S, et al. Immunological and respiratory changes in soybean workers. *Int Arch Occup Health* 1991;63:15-20.
14. Farrant JP, Moore CF. Dust exposure in the Canadian grain industry. *Am Ind Hyg Assoc J* 1978;39:177-94.
15. Oilseed statistics. In: Golbitz P, editor. *Soya and oilseed bluebook*. Bar Harbor (ME): Soyatech; 1999. p 329-72.
16. Soriano JB, Ercilla G, Sunyer J, Real F, Lázaro C, Rodrigo MJ, et al. HLA class II genes in soybean epidemic asthma patients. *Am J Respir Crit Care Med* 1997;156:1394-8.
17. Sunyer J, Antó JM, Sabriá J, Rodrigo MJ, Roca J, Morell F, et al. Risk factors of soybean epidemic asthma: the role of smoking and atopy. *Am Rev Respir Dis* 1992;145:1096-102.
18. Codina R, Lockey RF. Possible role of molds as secondary etiologic agents of the asthma epidemics in Barcelona, Spain. *J Allergy Clin Immunol* 1998;102:318-20.
19. Codina R, Oehling AG, Lockey RF. Neoallergens in heated soybean hull. *Int Arch Allergy and Immunol* 1998;117:120-5.
20. Jaén C, Codina R, Lockey RF, Arduoso L, Crisci C, Bertoya N. Identification of the soybean hull allergens involved in sensitization to soybean dust in a rural population from Argentina [abstract]. Proceedings of the 18th annual meeting of the EAAACI; 1999 July 3-7; Brussels, Belgium. Brussels: The Association; 1999.
21. Snapper CM, Finkelman FD. Immunoglobulin class switching. In: William EP, editor. *Fundamental immunology*. 3rd ed. New York: Raven Press; 1993. p 837-64.

Correction

The following correction applies to the article by Sicherer et al (Sicherer SH, Muñoz-Furlong A, Burks AW, Sampson HA. Prevalence of peanut and tree nut allergy in the US determined by a random digit dial survey. 1999;103:559-62). An acknowledgment of financial support was omitted. It should have read: The study was also possible through the generous financial support of the Finkelstein family.