

Possible role of molds as secondary etiologic agents of the asthma epidemics in Barcelona, Spain

Rosa Codina, PhD, and Richard F. Lockey, MD Tampa, Fla.

Soybean dust discharged from unloading ships in the Barcelona and Cartagena harbors in Spain caused outbreaks of asthma.^{1,2} Soybean-induced asthma correlates with specific IgE and IgG4 to soybean hull allergens.³ The three main soybean hull allergens that cause soybean asthma have been identified and purified.^{2,4}

Soybean dust is an uncommon, very potent source of allergens. Atopy, as well as cigarette smoking and the presence of certain HLA class II molecules, are risk factors for this disease.^{5,6} It is possible that stored soybeans contaminated with mites, molds, and bacteria are partially responsible as etiologic agents for soybean-induced asthma outbreaks. Storage mites were found not to be responsible for the Barcelona asthma epidemics⁷; however, no studies have ruled out the possibility that mold-contaminated soybeans are also causative factors for soybean-induced asthma.

The purposes of this study are (1) to determine whether soybeans stored in the silos of the Barcelona harbor are contaminated with molds; (2) to measure specific IgE levels in three groups of subjects (asthmatic epidemic patients [AEPs], asthmatic nonepidemic patients [ANEPs], and nonallergic subjects) to soybean hull allergens and to seven mold species that frequently contaminate soybeans; and (3) to study the cross-reactivity among allergens from soybean hulls and the seven mold species.

METHODS

Patient population

Serum samples from three groups of subjects from Barcelona were kept at -20° C until analysis: group 1 consisted of 12 AEPs attended in an emergency department during one of the asthma outbreaks; group 2 consisted of 23 ANEPs attended in an emergency department during a nonepidemic day; and group 3 consisted of 29 nonallergic control subjects.

Allergen extracts

Soybean hull allergen extract was prepared from soybeans unloaded from a ship that caused one of the asthma outbreaks in Barcelona, Spain. The hulls were kept at -20° C until the extracts were prepared, according to protocols previously described.³ Allergen extracts of *Aspergillus flavus*, *A. nidulans*, *A. fumigatus*, *A. glaucus*, *Penicillium notatum*, *P. chrysogenum*, and *P. digitatum*,

Abbreviations used

AEP: Asthmatic epidemic patients
ANEP: Asthmatic nonepidemic patients
TCB: Total counts bound

which frequently contaminate stored soybeans, were purchased from Greer Laboratories (Lenoir, N.C.).

Mold isolation and identification

Soybean hull was seeded in liquid Saboureaud agar and incubated for 7 days at 25° C, after which a culture in potato-dextrose agar was prepared under the same conditions. Hyphae were isolated, spread onto a glass slide, stained with lactophenol blue, and observed at a magnification of 400×. Microcultures of pure colonies were prepared in a Petri dish containing potato agar and incubated for 7 to 15 days at 25° C. Identification of different mold species was determined by examining the macroscopic and microscopic characteristics of the colonies.

Specific IgE determination

Specific IgE determination to the soybean hull extract and to the seven mold species was performed by RAST as previously described.³ The results were expressed as percentage of total counts bound (TCB).

Cross-inhibition study

A cross-inhibition study for specific IgE binding was performed as previously described³ by using a pool of four serum samples from group 1 and the soybean hull and mold extracts as solid and fluid phases.

Statistical analysis

The statistical analysis was performed with a software program (Statview; Abacus Concepts, Inc., Cupertino, Calif.). The IgE reference values were established as the percentile 97.5 of the values found in group 3. Wilcoxon signed-rank test was used to compare mean specific IgE values, and Yates-corrected chi-square test was used to compare percentages of positive results. Spearman rank correlation was used to analyze the correlation between specific IgE to soybean hull and to the seven mold species.

RESULTS

The mold cultures obtained from soybean hull did not sporulate, and therefore it was possible to identify the species and the genus of only one mold. Six molds species were isolated: *A. flavus*, *Aspergillus* spp., and four species of *Penicillium* spp. The reference value for specific IgE to soybean hull extract is less than 0.04% TCB, and to *A. flavus*, *A. niger*, *A. fumigatus*, *A. glaucus*,

From the Division of Allergy and Immunology, Department of Internal Medicine, University of South Florida, and James A. Haley V.A. Hospital, Tampa.

Reprint requests: Rosa Codina, PhD, Division of Allergy and Immunology, Department of Internal Medicine, University of South Florida and James A. Haley V.A. Hospital, 13,000 Bruce B. Downs Blvd. (VAR 111D), Tampa, FL 33612-4745.

J Allergy Clin Immunol 1998;102:318-20.

1/54/90844

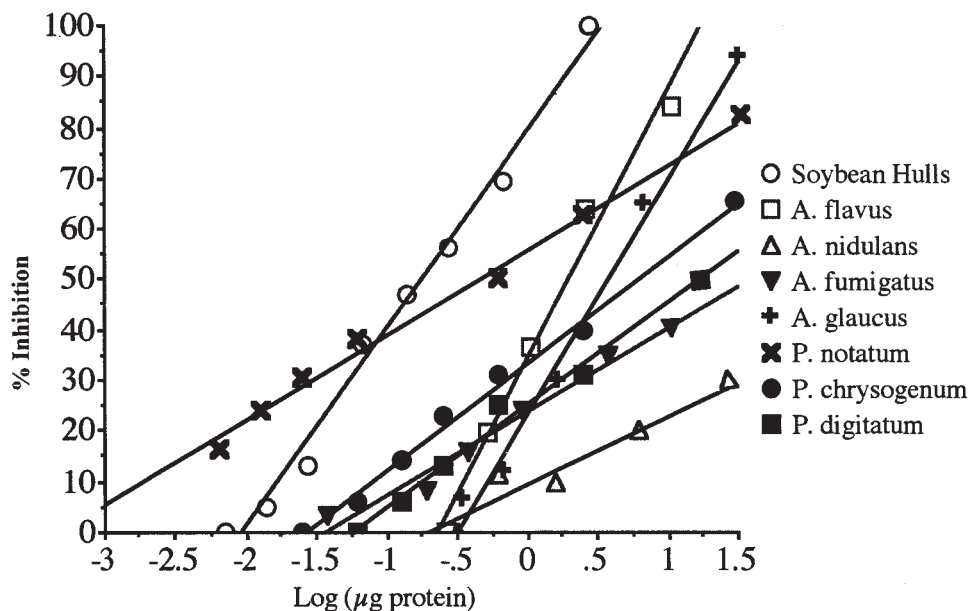


FIG. 1. Cross-inhibition study for specific IgE to soybean hulls and to the seven mold species using soybean hulls as solid phase.

TABLE I. Positive results (no. [%]) to the soybean hulls and to the seven mold extracts in the three groups of subjects

Allergen extract	Group no.		
	1 (AEP)	2 (ANEP)	3 (nonallergic subjects)
Soybean hulls (%)	12 (100)*(*)	1 (4.3)*	0 (0)
<i>Aspergillus flavus</i> (%)	8 (66.6)†(*)	3 (13.0)	3 (10.3)
<i>A. nidulans</i> (%)	6 (50)†(†)	1 (4.3)	2 (6.9)
<i>A. fumigatus</i> (%)	5 (41.6)	10 (43.5)‡	3 (10.3)
<i>A. glaucus</i> (%)	7 (58.3)†(†)	2 (8.7)	3 (10.3)
<i>Penicillium notatum</i> (%)	11 (91.6)*(*)	4 (17.4)	3 (10.3)
<i>P. chrysogenum</i> (%)	5 (41.6)‡	1 (4.3)	3 (10.3)
<i>P. digitatum</i> (%)	1 (8.3)	2 (8.6)	2 (6.8)

Comparisons of groups 1 and 2 with group 3. Comparisons of group 1 with group 2 (Yates-corrected chi-square test) are indicated in parentheses.

* $P < .001$.

† $P < .01$.

‡ $P < .05$.

P. notatum, *P. chrysogenum*, and *P. digitatum* extracts, it was less than 0.65% TCB, 1.19% TCB, 0.90% TCB, 0.75% TCB, 0.84% TCB, and 0.98% TCB, respectively.

The percentage of positive results for specific IgE to soybean hull and mold extracts are shown in Table I. In group 1 compared with groups 2 and 3, higher percentages of positive results are found for all extracts studied, except for *A. fumigatus* and *P. digitatum*. In group 1 compared with group 3, the mean values of specific IgE are significantly higher for all extracts except for *P. digitatum*, and in group 2 compared with group 3, means values are significantly higher for soybean hull and *A. fumigatus* only (Table II).

There is a significant correlation between specific IgE

to soybean hulls and to *A. flavus* and *A. glaucus* in group 1 only ($r = 0.758$ and $r = 0.801$ [$P < .01$], respectively). Cross-inhibition study indicates that the slopes of the inhibition curves found with soybean hulls, *A. flavus*, and *A. glaucus* extracts as fluid phases are similar (Fig. 1).

DISCUSSION

The results of this in vitro study demonstrate that soybean hulls are contaminated by *Aspergillus* and *Penicillium* species and that allergen extracts of soybean hulls and *A. flavus* and *A. glaucus* cross-react. Although specific IgE levels to mold allergens in the sera of AEPs are much lower than specific IgE to soybean hull allergens,

TABLE II. Mean and SD for specific IgE to soybean hulls and to the seven mold species in the three groups of subjects (%TCB)

Allergen extract	Group no.		
	1 (AEP)	2 (ANEP)	3 (nonallergic subjects)
Soybean hulls	13.60 ± 12.80*(*)	0.18 ± 0.09*	0.07 ± 0.04
<i>Aspergillus flavus</i>	2.90 ± 0.30†(*)	0.40 ± 0.43	0.52 ± 0.28
<i>A. nidulans</i>	1.21 ± 0.144*(*)	0.45 ± 0.10	0.49 ± 0.35
<i>A. fumigatus</i>	2.65 ± 2.87†	1.46 ± 0.94*	0.66 ± 0.34
<i>A. glaucus</i>	2.81 ± 0.37*(*)	0.89 ± 0.69	0.87 ± 0.56
<i>Penicillium notatum</i>	1.34 ± 0.76*†	0.61 ± 0.22	0.60 ± 0.20
<i>P. chrysogenum</i>	1.32 ± 1.76†	0.44 ± 0.23	0.51 ± 0.44
<i>P. digitatum</i>	0.80 ± 0.76	0.98 ± 0.69	0.72 ± 0.50

Comparisons of groups 1 and 2 with group 3. Comparisons of group 1 with group 2 (Wilcoxon signed-rank test) are indicated in parentheses.

†*P* < 0.01.

**P* < 0.05.

the sera of AEPs, compared with that of ANEPs and non-allergic individuals, contain higher levels of specific IgE to several *Aspergillus* and *Penicillium* species.

Aspergillus spp., particularly the *A. glaucus* group, and *Penicillium* spp., known as "storage" molds, may contaminate grains shortly after they are stored. These molds can cause damage to grains, produce mycotoxins, and raise the temperature of the grains through fermentation. In addition, *Aspergillus* and *Penicillium* species are allergenic and can also cause hypersensitivity pneumonitis.⁸

In conclusion, the results of this study demonstrate that soybean hulls are contaminated by allergenic mold species and that AEP sera contain low levels of specific IgE to these species. This suggests that molds may play a role in soybean-induced asthma.

We thank Adriana Rodriguez, MSc, from the Hospital Lorencita Villegas de Santos, Bogotá, Colombia, for her assistance with the mold culture and identification.

REFERENCES

1. Antó JM, Sunyer J, Rodríguez-Roisín R, Suárez-Cervera M, Vázquez L, Toxicoepidemiological Committee. Community outbreaks of asthma associated with inhalation of soybean dust. *N Engl J Med* 1989;320:1097-102.

2. González R, Polo F, Zapatero L, Caravaca F, Carreira J. Purification and characterization of major inhalant allergens from soybean hulls. *Clin Exp Allergy* 1992;22:748-55.
3. 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.
4. Codina R, Lockey RF, Swanson MC, Trudeau WL, Fernández-Caldas E, Rama R. Purification and characterization of a soybean hull allergen responsible for the Barcelona asthma outbreaks. II. Purification and sequencing of the *Gly m 2* allergen. *Clin Exp Allergy* 1997;27:424-30.
5. Sunyer J, Antó JM, Sabriá J, Rodrigo MJ, Roca J, Morell F, et al. Risk factors of soybean epidemic asthma. *Am Rev Respir Dis* 1992;145:1098-102.
6. Soriano JB, Ercilla G, Sunyer J, Real FX, 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.
7. Codina R, Fernández-Caldas E, Trudeau WL, Rodrigo MJ, Morell F, Lockey RF. Mite allergens did not cause asthma epidemics in Barcelona, Spain. *J Allergy Clin Immunol* 1996;86:236-8.
8. Mediavilla A, Angulo J, Rodero JM, Domínguez E, Galán C, Infante F. Fungal contamination of potential medical interest in Spanish grain stores. *J Invest Allergol Clin Immunol* 1996;6:196-201.