

Mite allergens did not cause asthma epidemics in Barcelona, Spain

Rosa Codina, MB,^a Enrique Fernández-Caldas, PhD,^b

Walter L. Trudeau, BA,^b Maria José Rodrigo, MD,^a Ferran Morell, MD,^c

and Richard F. Lockey, MD^b Barcelona, Spain, and Tampa, Fla.

The inhalation of soybean dust has been identified as the main cause of the Barcelona asthma outbreaks.¹⁻³ However, no studies have examined the possibility and implications of soybean seeds and dust being contaminated with mites and mite allergens during these epidemics. It is possible that mites may have been implicated in the asthma outbreaks, because seeds can be contaminated during transport and storage.⁴

The purposes of this study were to evaluate the presence of mites in soybean hull and dust, to measure Der p 1 and Der f 1 allergen levels in these samples, and to determine the prevalence of specific IgE to the storage mites *Blomia tropicalis*, *Aleuroglyphus ovatus*, *Chortoglyphus arcuatus*, and to the house dust mites *Dermatophagoides pteronyssinus* and *D. farinae* (Df) in two groups of patients with asthma and in a control group.

METHODS

Patient population

The patient population consisted of three groups. Group 1 was comprised of 14 asthmatic epidemic patients (AEPs) (mean age, 44.5 years); group 2 was comprised of 31 asthmatic nonepidemic patients (ANEPs) (mean age, 35.6 years), and group 3 was comprised of 28 nonasthmatic, nonallergic control subjects (mean age, 40.5 years).

Allergenic extracts

Standardized extracts of *D. pteronyssinus* and *D. farinae* were purchased from Greer Laboratories (Lenoir,

Abbreviations used

AEP: Asthmatic epidemic patient

ANEP: Asthmatic nonepidemic patient

N.C.). The three storage mite species were cultured in the laboratory according to previously described methods.^{5,6} Antigenic extracts from storage mites and soybean hull and dust were prepared by defatting the raw materials with anhydrous ether at 37° C in a Soxhlet apparatus (Corning Ltd., Stone, Staffordshire, England) for 4 hours. Materials were then dried and extracted (1:20 wt/vol) in 0.1 mol/L of ammonium bicarbonate, pH 9, overnight at 4° C. Extracts were centrifuged at 18,000 rpm and dialyzed overnight at 4° C against deionized water in a 3500 molecular weight cutoff membrane (Spectra/Por; Spectrum Medical Industries, Inc., Los Angeles, Calif.) for 2 days. Total protein content was determined by bicinchoninic acid assay (Pierce Chemical Co., Rockford, Ill.).

Mite counts

Mite contamination in soybean hull and dust samples collected in Barcelona during the asthma epidemic outbreaks was determined by one of the authors using light microscopy techniques just after the unloading of soybean into the silos of Barcelona Harbor.

Measurement of Der p 1 and Der f 1 allergen levels

Der p I and Der f I allergen levels were determined by an ELISA monoclonal antibody method.⁷

Specific IgE determination

Specific IgE determinations to the five mite species were performed by direct microtiter plate assay with methods previously described.^{5,6} Each well of the Immunolon4 Microtiter Removawell strips (Dynatech Laboratories, Alexandria, Va.) was coated with 1 µg of antigen in 100 µl 0.2 mol/L sodium carbonate/bicarbonate buffer, pH 9.2, and 100 µl of serum (diluted 1:2) was added to each well. The results were expressed as the mean of the percentage of the total counts bound, and results were considered positive when a serum bound more than 1% of the total counts added.

From the ^aServei de Bioquímica (U. de Immunología), Hospital General Universitari Vall d'Hebron, Barcelona; ^bDivision of Allergy and Clinical Immunology, Department of Internal Medicine, University of South Florida College of Medicine, and the James A. Haley Veterans Hospital, Tampa; and ^cServei de Pneumologia, Hospital General Universitari Vall d'Hebron, Barcelona.

Reprint requests: Maria José Rodrigo, MD, Servei de Bioquímica (U. de Immunologia), Hospital General Universitari Vall d'Hebron, Pg Vall d'Hebron, s/n, 08035 Barcelona, Spain.

J Allergy Clin Immunol 1996;98:236-8.

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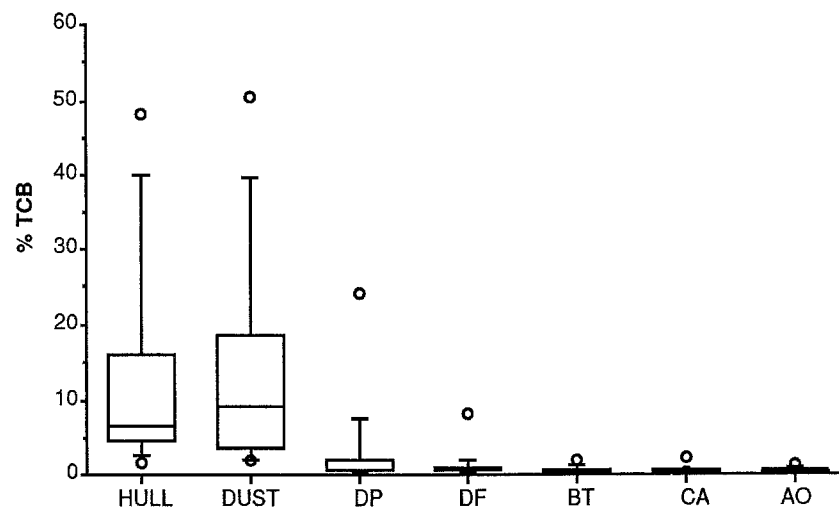


FIG. 1. Box plot of RAST results to soybean hull and dust and to *D. pteronyssinus* (DP), *D. farinae* (DF), *B. tropicalis* (BT), *C. arcuatus* (CA), and *A. ovatus* (AO) in 14 AEPs. TCB, Total counts bound.

TABLE I. Specific IgE to five mite species (percent total counts bound)

Mite species	Group		
	AEP	ANEP	Control
<i>D. pteronyssinus</i>	2.80 ± 6.30*	32.30 ± 20.0†	0.125 ± 0.06
<i>D. farinae</i>	1.10 ± 2.10*	24.10 ± 19.7†	0.125 ± 0.06
<i>B. tropicalis</i>	0.46 ± 0.52*	2.30 ± 3.50†	0.126 ± 0.06
<i>A. ovatus</i>	0.40 ± 0.33*	1.20 ± 1.50†	0.122 ± 0.04
<i>C. arcuatus</i>	0.42 ± 0.52*	1.80 ± 3.60†	0.108 ± 0.03

Values are expressed as means ± SD.

* $p < 0.05$ versus control group.

† $p < 0.001$ versus control group.

RESULTS

The morphologic analysis of the soybean hull and dust samples revealed the absence of mites in these samples. Der p 1 and Der f 1 allergen levels were below the lower limits of detection.

Specific IgE results to the five mite species are shown in Table I.

Positive specific IgE binding results (in percentages) for *B. tropicalis*, *A. ovatus*, and *C. arcuatus* in the ANEP group were 64%, 68%, and 55% and 21%, 29%, and 35% in the AEP group, respectively.

Fig. 1 shows a box plot view of the positive RAST results to different allergens in AEPs. Higher values of specific IgE binding are observed for hull and dust extracts. In 14 AEPs no significant correlations were obtained between specific IgE to soybean hull and dust extracts and specific IgE to the five mite extracts. IgE values for the five

mite species were much higher in ANEPs than in AEPs.

DISCUSSION

The results of this study suggest that ANEPs have greater reactivity than AEPs to mite allergens.

RAST results, together with the lack of correlation between specific IgE values to the five mite extracts and soy extracts in AEPs, the lack of mite contamination on visual inspection of soybean raw material, and the undetectable levels of Der p 1 and Der f 1, rule out mites as possible etiologic allergens in the Barcelona asthma outbreaks. These data indirectly support soybean allergens as the primary cause of these epidemics.^{3,8}

We thank Christine O'Hara and Andrew Campbell, MPH, for their editorial assistance.

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