

CASE-CONTROL STUDY OF SERUM
IMMUNOGLOBULIN-E ANTIBODIES
REACTIVE WITH SOYBEAN IN EPIDEMIC
ASTHMA

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Summary Since 1981, twenty-six asthma outbreaks have been identified in Barcelona, all coinciding with the unloading of soybean in the harbour. Serum from patients with epidemic asthma and individually matched controls with non-epidemic asthma was assayed for immunoglobulin-E (IgE) antibodies against soybean antigens by means of a radioallergosorbent test. In 64 of 86 cases (74.4%) there was a reaction with commercial soybean antigen extracts, compared with only 4 of the 86 controls (4.6%) (odds ratio = 61; lower 95% confidence limit = 8.1). The statistical significance was greater for reactions with extracts of soybean dust taken from Barcelona harbour (odds ratio, unquantifiably high; lower 95% confidence limit = 11.7). No other serological covariate (total serum IgE levels or specific IgE levels against the commonest airborne allergens or legumes) confounded the association between serum anti-soybean IgE antibodies and epidemic asthma. These results support a causal relation between the release of dust during unloading of soybean at the harbour and the occurrence of asthma outbreaks, suggesting an underlying allergic mechanism.

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Introduction

SINCE 1981, twenty-six asthma outbreaks have occurred in the city of Barcelona. These outbreaks were characterised by sudden rises in emergency-room admissions for asthma.¹⁻³ Striking space clustering, hourly time clustering, and simultaneous time-space clustering strongly suggested that the outbreaks were of the point-source type.³ The asthma cases were clustered near the harbour and near an industrial area.³ A review of harbour unloading activities showed that all twenty-six outbreaks had coincided with the unloading of soybean, which in turn showed a significant association with epidemic asthma (unpublished).

We studied the relation between epidemic asthma and serum immunoglobulin-E (IgE) antibodies reactive with soybean antigens. We tested the hypothesis that epidemic asthma patients have higher levels of anti-soybean IgE antibodies than individually matched controls with non-epidemic asthma. Positive results would reinforce the evidence that asthma outbreaks had been caused by dust released during unloading of soybean in the harbour.

Patients and Methods

This study had a matched case-control design. 86 patients with epidemic asthma were matched individually to 86 patients with non-epidemic asthma. Cases and controls were selected from the medical records of emergency-room admissions for asthma at four large urban hospitals, which cover 90% of all emergencies in Barcelona. An emergency-room admission for asthma was defined as a visit during which any asthma-related diagnosis was recorded. To take account of differences in terminology, a panel of chest physicians drew up a list of expressions used that would qualify for inclusion as asthma.³ The identification of asthma admissions was made in all the participating hospitals by a specially trained physician from the coordinating centre. All records were reviewed and their completeness checked.

On Sept 4 and 7, 1987, two large asthma epidemics occurred, causing 157 emergency-room admissions for asthma (daily mean asthma emergency-room admissions for 1985-86 = 5.6 [SD = 4.7]). Serum samples were taken from 86 of these patients at the emergency room, who were considered as cases in this study; the concentration of patients—which led to the collapse of some services for several hours—precluded the collection of samples from the remaining 71 patients. Controls were taken from emergency-room admissions for asthma seen in the period October, 1987, to January, 1988, when no epidemic days occurred. Potential controls previously seen for asthma on an epidemic day were excluded. An

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TABLE I—DISTRIBUTION OF CASES AND CONTROLS ACCORDING TO SERUM IgE LEVELS AGAINST SOYBEAN ANTIGENS

IgE (PRU/ml)	Cases (n = 86)			Controls (n = 86)		
	S	SD1	SD2	S	SD1	SD2
< 0.35	22	13	11	82	81	82
≥ 0.35	64	73	75	4	5	4
0.35–0.69	22	7	9	1	1	0
0.70–3.49	30	21	25	2	1	1
3.50–17.49	11	28	30	1	2	2
≥ 17.50	1	17	11	0	1	1

S = commercially prepared soybean antigens; SD1 and SD2 = our two soybean dust extracts.

“epidemic day” was defined objectively as a day with an unusually large number of asthma emergency-room admissions and hourly time clustering of asthma cases.^{3,4} A further requirement was that both cases and controls be resident in Barcelona and aged over 14 years, since no substantial rise in admissions of asthma patients under that age occurred on most of the epidemic days.³

Blood samples were taken from cases and controls at the time of their admission to the emergency room and were stored at -20°C until analysis.

Cases and controls were matched individually by age (±10 years), sex, and area of residence. The city was divided into two parts (high and low epidemicity), according to whether the ratio of the rate of emergency-room admissions for asthma on epidemic days over non-epidemic days was greater or less than 10. Matching was done sequentially from the alphabetic registry of included cases.

Total serum IgE was determined by paper radioimmunosorbent test (Pharmacia Diagnostics AB, Uppsala, Sweden) and specific IgE levels to the antigens soybean, *Dermatophagoides pteronyssinus*, *Parietaria officinalis*, and *Aspergillus fumigatus*, prepared by the same company, were measured by radioallergoabsorbent test (RAST).

In addition, two soybean dust extracts were prepared. The first was prepared from dust collected in the hold of a ship which had come from the United States and had been unloaded during the outbreaks on Sept 4 and 7. The second was prepared from dust collected from the bottom of a harbour silo, containing soybean unloaded on non-epidemic days from a ship that had come from Brazil. Both extracts were coupled to cyanogen-bromide-activated paper discs and prepared at the Karolinska Hospital (Stockholm, Sweden).

In 36 repeater case-control pairs (a repeater case is a patient seen on at least two epidemic days), specific IgE antibodies reactive with commercially prepared antigens of peanut, pea, and white bean (Pharmacia) were determined. These assays could not be carried out in three, six, and six pairs respectively.

Total IgE titres of 150 IU/ml or above were taken as positive. RAST results were expressed as Phadebas RAST units (PRU). PRU were determined by referring the number of bound counts per ml to the curve obtained for the four reference materials (birch allergen-IgE antibody of 17.5, 3.5, 0.7, and 0.35 PRU/ml) provided by Pharmacia. Specific allergen IgE antibodies were taken as positive when titres above 0.34 PRU/ml were found.

Each case-control pair was analysed with the same kit and on the same day to control for batch effect.⁵ The same technician carried out the tests under blind conditions.

The association between serum IgE levels and epidemic asthma was calculated by the maximum likelihood estimate of the odds ratio, conditional to the number of discordant pairs.⁶ Approximate tests of significance and confidence intervals were based on McNemar’s corrected chi-square test.⁶ The odds ratio adjusted estimation to determine the effect of immunological covariates was based on conditional logistic regression analysis equations calculated by the maximum likelihood technique.⁷⁻⁹ Confidence intervals were calculated by the method proposed by Breslow.⁹ Because of the strong multicollinearity among immunological covariates, several conditional logistic regression models were constructed to adjust soybean IgE levels individually for immunological covariates.

Results

Although the median age was slightly higher in cases than in controls (55.0 vs 52.5 years), matching by ±10 years resulted in very similar mean ages in the two groups (51.71 [SE 15.42] vs 50.37 [16.04] yr). 41 pairs were male and 45 female, and 44 came from areas of high epidemicity and 42 from areas of low epidemicity. Serum from 64 of the 86 epidemic cases (74.4%) was reactive with commercially prepared soybean antigen; in assays with our soybean dust extracts there were even more positive reactions (first extract 73 [84.9%]; second extract 75 [84.2%]). Only 4 (4.6%) of the controls were reactive to commercially prepared soybean antigen and 5 (5.8%) and 4 (4.6%), respectively, to our two soybean dust extracts (table I).

Among epidemic cases, there was a correlation between levels of antibody against commercial soybean antigen and those against soybean dust extract 1 ($r=0.6$, $p<0.001$) and soybean dust extract 2 ($r=0.6$, $p<0.001$). The correlation between reactions to dust extracts 1 and 2 was greater ($r=0.8$, $p<0.001$).

The odds ratio for the relation of epidemic asthma with IgE antibodies against commercial soybean antigen was 61, with a lower confidence limit of 8.1. The relation between serum IgE antibodies reactive with soybean dust extract 1 and epidemic asthma was stronger (odds ratio unquantifiably high; lower 95% confidence limit, 11.7; table II). When more restrictive cutoff points of positive reaction were used (0.7 and 3.5 PRU/ml), the odds ratios remained significant (table II).

The mean total serum IgE level was 204.2 (SD 776.2) IU/ml in the cases and 95.5 (371.5) IU/ml in the controls. 53 cases (61.6%) and 33 controls (38.4%) had total serum IgE levels of 150 IU/ml or above and 8 (9.3%) and 1 (1.2%), respectively, had levels of 1000 IU/ml or above. Total serum IgE levels were significantly associated with epidemic

TABLE II—ODDS RATIO AND LOWER 95% CONFIDENCE LIMIT (CL) FOR EPIDEMIC ASTHMA IN RELATION TO LEVELS OF IgE ANTIBODY AGAINST COMMERCIAL SOYBEAN ANTIGEN AND SOYBEAN DUST EXTRACT 1, WITH DIFFERENT CUTOFF POINTS OF POSITIVE REACTION

Cutoff point	Commercial soybean antigen					Soybean dust extract 1				
	Discordant pairs*		Odds ratio	Lower CL	χ^2	Discordant pairs*		Odds ratio	Lower CL	χ^2
	+/-	-/+				+/-	-/+			
0.35	61	1	61.0	8.1	56.1†	68	0	†	11.7	66.0†
0.75	42	1	42.0	5.5	37.0†	63	1	63.0	8.3	58.1†
3.50	12	1	12.0	1.4	7.7§	42	0	†	7.1	40.0†

*Total pairs = 86. +/- case +, control -; -/+ case -, control +.
†Unquantifiably high.
‡p < 0.001; §p < 0.01.

TABLE III—ODDS RATIO AND 95% CONFIDENCE INTERVALS (CI) FOR EPIDEMIC ASTHMA IN RELATION TO IgE ANTIBODY LEVELS REACTIVE WITH DIFFERENT ANTIGENS

—	Discordant pairs		Odds ratio	95% CI	χ^2	p
	+/-	-/+				
Total IgE	34	14	2.4	1.2–5.0	7.52	<0.01
<i>D pteronyssinus</i>	27	8	3.8	1.4–9.0	9.26	<0.001
<i>P officinalis</i>	18	2	9.0	1.7–111.6	11.25	<0.001
<i>A fumigatus</i>	6	1	6.0	0.6–269.9	2.28	NS
Allergy*	33	8	4.1	1.7–10.7	14.04	<0.001

*Defined as levels of specific IgE reactive with the three antigens listed ≥0.35 PRU/ml.
NS = not significant.

TABLE IV—ODDS RATIO AND 95% CI FOR EPIDEMIC ASTHMA IN RELATION TO IgE ANTIBODY LEVELS REACTIVE WITH COMMERCIAL SOYBEAN ANTIGEN, ADJUSTED FOR EFFECT OF ALLERGY-RELATED VARIABLES

Variable of adjustment	Commercial soybean antigen	
	Adjusted odds ratio	95% CI
Allergy	51.9	5.9–372
<i>D pteromyssinus</i>	53.9	7.4–383
<i>P officinalis</i>	56.8	7.6–424
<i>A fumigatus</i>	57.9	8.1–485
Total IgE	82.1	10.3–651

TABLE V—ODDS RATIO AND 95% CI FOR EPIDEMIC ASTHMA IN RELATION TO IgE ANTIBODY LEVELS REACTIVE WITH LEGUMES IN REPEATER CASE-CONTROL PAIRS

—	Pairs			Odds ratio	95% CI	χ^2
	n	+/-	-/+			
Pea	30	2	3	0.6	0.0–7.5	0
Peanut	33	5	2	2.5	0.3–37.9	0.57
White-bean	30	2	1	2.0	0.0–> 1000	0
Legumes*	31	6	2	3.0	0.4–43.6	1.12
Soybean†	36	24	1	24.0	3.0–> 1000	19.36†

*Allergy to legumes was defined as levels of specific IgE reactive with pea, peanut, or white bean ≥ 0.35 PRU/ml.
†Commercial soybean antigen.
‡p < 0.001.

asthma (odds ratio 2.4), as were levels of specific IgE antibodies against *Dermatophagoides* and *Parietaria* (odds ratio 3.8 and 9.0, respectively; table III).

When the association between epidemic asthma and IgE antibody levels against commercial soybean antigen was adjusted for the effect of allergy-related variables (IgE specific antibodies against *Dermatophagoides*, *Parietaria*, and *Aspergillus* and total IgE levels), the relation between anti-soybean IgE levels and epidemic asthma remained similar or even stronger (table IV).

In the study of serum IgE antibodies against legumes in the subgroup of repeater case-control pairs positive values for pea, peanut, and white bean were found in 10.0%, 15.6%, and 10.0%, respectively, of cases, and 11.0%, 5.5%, and 5.5% of controls. None of these differences was significant (table V). The association of IgE antibodies against commercial soybean antigen and epidemic asthma was independent of the reactivity with legumes (soybean odds ratio adjusted for hypersensitivity to legumes = 36.2, 95% CI = 3.4–358; p = 0.003).

Discussion

This study reveals a strong association between serum IgE antibodies against commercially prepared soybean antigen and epidemic asthma, which was independent of confounding allergy-related variables. There was an even stronger association for the antigen extract obtained from soybean dust collected at the harbour of Barcelona.

The selection of cases and controls was based on a diagnosis of asthma made in emergency rooms, and it could be argued that diagnoses were not wholly reliable. However, cases and controls were submitted to the same selection procedure, based on the criteria set down by the asthma emergency admission monitoring system of Barcelona.³ Moreover, total serum IgE levels of 150 IU/ml or above were found in 38.4% of the controls, which is similar to the overall prevalence of this feature reported in asthmatic patients.¹⁰

The cases included in the study represented only 55% of all eligible admissions and were not randomly selected. However, there were no differences in demographic variables or in patterns of discharge or referral from emergency services between those included and those not included in the study, and there were no other differences between these groups which could explain the differences in serum IgE titres between cases and controls.

Misclassification of IgE reactivity seems improbable, since RAST tests have been proved reliable,⁵ and all tests were carried out under blind conditions, on the same day, with the same kit, and by the same technician.

It could be claimed that soybean IgE binding is due to a crossreaction between soybean and other legumes, which could be explained by non-immune IgE binding¹¹ or by the recognition of different protein legumes, particularly peanut, as the same.^{12–14} However, Barnett and Howden¹⁵ concluded that non-specific IgE interaction does not interfere with the RAST of legumes. Crossreaction can be discounted because only 5 (17.2%) soybean-positive repeater cases showed RAST positivity to peanut. In addition, the prevalence of soybean IgE antibodies in our cases (over 74%) seems difficult to explain by the reported non-specific reaction figures, ranging from 6/1000 in a normal population to 30% in an atopic population.¹¹ On the other hand, legumes were not found to be associated with epidemic asthma, and the relation between serum soybean IgE antibodies and epidemic asthma was independent of the confounding legume effect.

Soybean allergy by ingestion is a well-known clinical feature. Some studies of allergic children concluded that soybean is a weak antigen,¹⁶ whereas others considered it a potent immunogen.^{17,18} Although some cases of severe gastrointestinal reaction^{19,20} or anaphylaxis^{17,21–24} after soybean ingestion have been reported, the rarity of such adverse reactions has been attributed to an efficient gastrointestinal barrier to soybean allergen.¹⁸

Respiratory soybean exposure can cause a severe clinical response.²⁵ There have been several reports of asthma after soybean inhalation,^{25–30} including cases in soybean flour mill workers,^{26,27} a woman who inhaled soybean dust during the preparation of a soyloid bath (and who had an attack after eating soybean pancakes),²⁸ a previously non-allergic patient in whom immediate and late-onset asthma developed after soybean flour inhalation,²⁹ and a farmer harvesting a soybean crop.³⁰ At present, soybean is considered an occupational aeroallergen causing asthma.^{31–33} The low number of cases of soybean asthma reported previously contrasts with the large number (687) in the asthma epidemics in Barcelona. This disparity could be explained by the existence of local factors in Barcelona which allowed soybean dust to reach the population and cause the occurrence in epidemic form of an otherwise rare disorder. However, an underestimation of the number of soybean asthma cases in other places cannot be ruled out. Although castor-bean dust has been reported to be a specific cause in asthma epidemics,^{34–36} the causes of most other asthma epidemics remain controversial.^{37–39}

Our results indicate an underlying type I allergic mechanism for epidemic asthma in Barcelona. The fact that the different soybean antigens showed similar RAST reactivity in the epidemic cases suggests that a structural soybean antigen, rather than a contaminant, was the allergen causing the epidemic asthma. Studies are in progress to characterise the specific parts of soybean which have an

antigenic role in epidemic asthma. Preliminary results show that epidemic asthma patients reacted specifically with low molecular weight proteins from the dust and hull of soybean. We conclude that the association between serum anti-soybean IgE levels and epidemic asthma supports the evidence that soybean dust released during unloading of soybean in the harbour was the cause of these asthma outbreaks.

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EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTORS ASSOCIATED WITH LACK OF RESPONSE TO ENDOCRINE THERAPY IN RECURRENT BREAST CANCER

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Summary Epidermal growth factor receptors (EGFR) and oestrogen receptors (ER) were analysed in 221 patients with primary operable breast cancer by means of radioligand assays. After median follow-up of 24 months (range 3-60 months), there had been recurrences in 99 patients, of whom 72 (median age 56 years, range 32-77 years) received tamoxifen alone as first-line treatment for recurrence. 20 patients (28%) showed a response to this therapy and 52 (72%) did not. Of 32 ER-positive tumours, 12 (37.5%) showed an objective response to tamoxifen compared with only 2 of 40 (5%) ER-negative tumours ($p < 0.005$). Of 35 EGFR-positive tumours, 3 (8.5%) achieved an objective response compared with 11 of 37 (30%) EGFR-negative tumours ($p < 0.05$). Only 1 of 28 EGFR-positive, ER-negative tumours achieved an objective response. Including patients whose disease remained stable for more than 6 months with the responders, however, EGFR status was a better predictor of response to tamoxifen; 15 of 37 EGFR-negative patients and 5 of 35 EGFR-positive patients responded ($p < 0.01$).

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