

Allergy to castor bean

I. Its relationship to sensitization to common inhalant allergens (atopy)

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*The IgE response to castor bean (*Ricinus communis*) was studied in 96 castor bean-allergic patients from Marseilles, France. All had positive skin tests to castor bean. The IgE response to grass, cat, dust mite, olive, and *Parietaria* was also measured, and a positive RAST to one or more of these allergens was taken to indicate atopic status. Castor bean-specific IgE antibodies, measured by RAST, were found in 87 (91%) of the castor bean-allergic patients, in two of 13 atopic Marseilles residents living close to the castor bean mills, in three of 42 allergic subjects who had no known contact with castor bean, and in none of a control group of 111 Marseilles blood donors. Very high levels of castor bean-specific IgE (maximum class 4 readings on the Phadebas RAST score) were found in 54 (56%) of the castor bean-allergic patients, but the level of IgE antibody to castor bean was not significantly different in atopic and nonatopic subjects. The frequency of a positive serological test (RAST) for atopy in castor bean-allergic subjects (32%) was very similar to that found in the local population (36%). These data indicate that castor bean is an extremely potent sensitizer for both atopic and nonatopic individuals. The magnitude of the specific IgE antibody response is not related to the atopic status of the patient and may be a function of the physiochemical characteristics of the allergen itself. (J ALLERGY CLIN IMMUNOL 1988;82:62-6.)*

The history of respiratory allergy to castor bean (*Ricinus communis*) spans at least 70 years, with the first reported case described by Alilaire in 1914.¹ Since that time, outbreaks of asthma caused by castor bean hypersensitivity have been reported in towns near to oil-processing mills, for example, Toledo, Ohio,² Johannesburg, South Africa,³ Marseilles, France,⁴ Port Sudan, Sudan,⁵ and Ancona, Italy.

In 1955, in an outbreak of castor bean allergy in Johannesburg, Ordman³ found that more than half of the exposed population were sensitized. No evidence was available, either in that study or the study carried out in the Sudan,⁶ that might indicate whether the high rate of sensitization is related to the potency of the antigen or to host factors, such as an associated atopic state or a high level of parasitic infestation.

Our present aim was to investigate the role of atopy in castor bean allergy using freeze-dried serum samples collected from a group of castor bean-allergic

patients from Marseilles who had developed allergic symptoms in an outbreak of castor bean allergy 15 years previously. Atopy was defined by the presence of specific IgE antibodies to one or more of the most prevalent inhalant allergens in the south of France (dust mite, cat, and grass, *Parietaria*, and olive pollens) measured by RAST.

MATERIAL AND METHODS

Selection of patients

Four groups of patients were studied (Table I). These groups were classified according to their exposure and skin reactivity to castor bean dust. Group A comprised 96 individuals who were all scratch test positive to a 1/100,000 dilution of castor bean extract (kind gift of Dr. Layton, Western Regional Research Laboratory, Albany, California). A positive scratch test was defined as a wheal diameter of >1 cm. Fifteen of the 96 patients were exposed to castor bean through their occupation, but most patients (81) were exposed to castor bean through living close to the castor oil mills situated 9 miles north of Marseilles. All of these patients complained of symptoms of asthma and allergic rhinitis believed to be due to sensitization to castor bean. The age range was 5 to 67 years (mean 34 years); 36 were female and 60 were male.

Groups B and C were scratch test negative to castor bean extract. Group B consisted of individuals with asthma who lived within a 10-mile radius of the castor oil mills and

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TABLE I. Patient groups

Patient group (and No.)	Clinical symptoms	Exposure to castor bean	Skin prick test
A (96)	Asthma/allergic rhinitis	Close contact	100%
B (13)	Asthma	Close contact	All negative
C (42)	Asthma	No contact	All negative
D (111)	Unselected Marseilles population (blood donors)	Not known to have contact	Not tested

would also have been heavily exposed to castor bean dust. All were skin test positive to one or more inhalant allergens. Of the 13 individuals, four were female and nine were male, with an age range of 17 to 60 years (mean 43.9 years).

Group C consisted of 42 subjects with asthma who were skin test positive to one or more inhalant allergens and who lived outside the Marseilles region and would not be expected to have any contact with castor bean. Their ages ranged from 9 to 70 years (mean 32.1 years); 21 were female and 21 were male.

Group D were 111 unselected blood donors from the Marseilles region. Their clinical history and skin tests to castor bean and common inhalant allergens were not documented. The age range was 22 to 60 years (mean 40 years); 81 were male and 30 female.

Blood was drawn from each patient by venipuncture, and the serum was (10 ml) separated, lyophilized, and stored at 4° C until it was reconstituted with 10 ml of distilled water. Sera were collected and skin tests were performed on patients 15 years previously when industrial hygiene at the oil mills resulted in a high level of air pollution in the region surrounding the oil mills.

Preparation of castor bean extract

Castor bean seeds (variety Impala) were purchased from Thompson and Morgan Ltd., Ipswich, U.K. The seeds (20 gm) were ground to a fine powder and extracted in 100 ml of phosphate buffer for 3 hours at room temperature in a fume cupboard. The resultant aqueous slurry was centrifuged at 2000 g for 10 minutes in sealed containers to remove the debris, and the upper fatty layer was skimmed from the top. Some of the supernatant was heated in a boiling-water bath for 1 hour to detoxify the castor bean toxin, ricin.

The toxicity of the heated extract was tested with six hooded Lister rats. Three rats were injected intravenously with 100 times the median lethal dose of the heated extract and three rats with the unheated extract. The unheated extract proved fatal in all three rats within 24 hours, whereas the heated extract was not toxic.

Specific IgE antibody assays

IgE antibodies to castor bean and the other inhalant allergens were measured by RAST as described previously.^{7, 8} Cyanogen bromide-activated Whatman 541 (Whatman Ltd., Maidstone, England) paper disks, diameter

TABLE II. Specific IgE binding to castor bean extract

Dilution of undiluted extract	Unheated (%)	Heated (%)
Undiluted	16	21
1/10	15	17
1/100	12	11
1/1000	8	7
1/10000	6	4
Negative (1/10)	1	0.5

Specific IgE binding to castor bean extract coupled to paper disks expressed as percent counts per minute bond demonstrating no significant difference between the heated and unheated extracts.

6 mm, were coated with 10 ml of castor bean extract. Grass pollen-, dust mite-, and cat epithelium-coated disks (20 µg of protein per gram of disks) were prepared with extracts kindly provided by Mr. B. Overall, Beechams Pharmaceuticals, Betchworth, Surrey, U.K. *Parietaria* and olive-pollen disks were purchased from Pharmacia, Milton Keynes, U.K. Serum samples were assayed at 1/10 dilution in horse serum, and results were adjusted for dilution and expressed in arbitrary units per milliliter by reference to an "in-house" grass-pollen standard curve. Phadebas RAST standards were also run, and a positive RAST was defined by a class 1 or greater RAST score (mean + 3 SD of 20 negative control sera, that is, 22 ± 4 arbitrary units per milliliter).

RESULTS

To determine the optimum conditions for preparation of the castor bean allergen-coated paper disks, two extracts, heated (nontoxic) and unheated (toxic) were coupled to paper disks in tenfold dilutions from undiluted to 1/100,000. These disks were then used to measure castor bean-specific IgE antibodies in a reference serum pool. The reference serum was diluted 1/10 in horse serum, and the results were expressed in percentage counts bounds. There was no significant difference between the two extracts, and for all subsequent experiments, the heated extract was used undiluted (Table II).

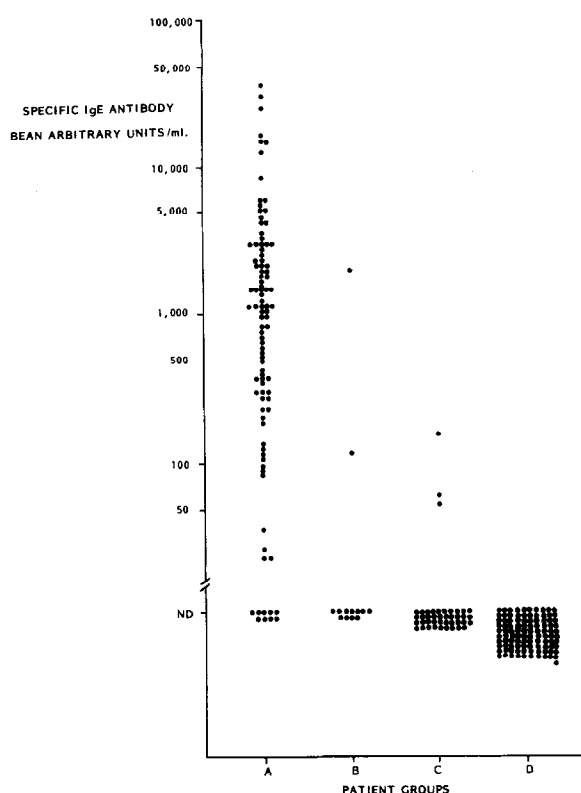


FIG. 1. IgE antibodies to castor bean were found in most (91%) of castor bean-allergic patients (A) but rarely in exposed (B) and unexposed (C) atopic control groups and never in unselected blood donors (D).

The castor bean extract was compared with two other preparations with serum from patients who were skin prick positive to castor bean (group A). Disks prepared from the heated extract were compared with disks purchased from Pharmacia. Results were expressed in arbitrary units per milliliter by reference to an "in-house" grass-pollen standard curve. There was reasonable agreement ($r = 0.61$; $p < 0.01$) between the levels of specific IgE obtained with the two types of disks (results not presented).

The RAST disks prepared from the heated extract were also used to measure the specific IgE in a group of patients from the Sudan who were previously studied for castor bean allergy in 1981.^{5, 6} The results obtained in 1981, with an extract prepared from *R. communis* (type Pacific), were similar to those obtained with the recently prepared extract ($r = 0.82$; $p < 0.01$) (results not presented).

IgE antibodies to castor bean

Four groups of patients were studied. Their clinical history, exposure to castor bean dust, and skin test

response to castor bean are presented in Table I. Specific IgE antibody to castor bean (Fig. 1) was detected in 87 (91%) of group A. The levels of specific IgE antibody measured were very high; 54 (56%) gave a class 4 RAST score, findings that are similar to those reported previously^{5, 6} in dockworkers from Port Sudan.

IgE antibody levels were substantially higher in the castor bean-allergic group (A) compared with all the other groups (Students *t* test, $p < 0.001$). A few of the patients who were skin test negative to castor bean had a positive RAST. In patients who were exposed to high levels of dust (group B), two of 13 (15%) had a positive RAST, and in those who had no known contact with castor bean (group C), three of 42 (7.1%) had a positive RAST.

Total serum IgE

The mean (geometric) serum IgE level of the castor bean-allergic group (A) was 115 IU/ml, which was similar to the level of atopic, exposed patients (B: mean serum IgE, 109 IU/ml) and atopic, nonexposed patients (C: mean serum IgE, 125 IU/ml). These differences were not significantly different (Students *t* test). All had significantly higher levels of serum IgE than the control group of blood donors (D) whose mean serum IgE was 21 IU/ml (Fig. 2) ($p < 0.01$, analysis of variance, multiple *t* test).

Atopy and castor bean allergy

All serum samples were assayed for specific IgE antibodies to five common inhalant allergens prevalent in the Marseilles region of southern France: grass, olive and *Parietaria* pollens, cat epithelium, and dust mite. A positive RAST is defined as class 1 or greater (Phadebas RAST), and atopy, as a positive RAST to one or more of the five common inhalant allergens tested (Table III). Groups B and C were all subjects with asthma or rhinitis whose atopic status was confirmed by a high proportion (at least 50%) having a positive RAST to one or more of the inhalant allergens tested. There was no statistically significant difference (Student's *t* test) in the frequency of atopy between group A subjects, in which 31 of 96 (32%) were atopic, and blood donors from Marseilles (group D), in which 40 of 111 (36%) were atopic. However, IgE antibodies to dust mite were far more common in the castor bean-allergic group (10.4%) compared with the blood donor control group (1.0%). We also studied the possible relationship between the magnitude of the specific IgE response to castor bean and atopy (Fig. 3) but found that there was no statistically significant difference (Student's *t* test).

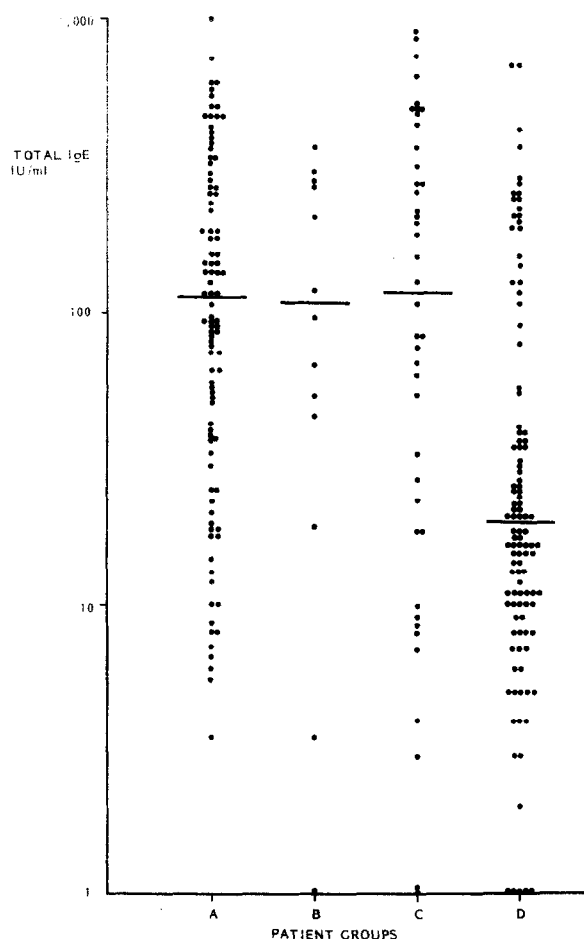


FIG. 2. Serum IgE levels were similar in the castor bean-allergic patients (A) and exposed (B) and unexposed (C) atopic control groups, and were significantly higher in all three groups compared with the unselected blood donors (D).

DISCUSSION

The improvement of industrial hygiene in castor oil-processing mills in Marseilles has made prospective studies of castor bean allergy impractical because of the low frequency with which sensitization occurs today. However, the availability of 15-year-old freeze-dried serum samples has allowed us to carry out this retrospective study. The castor bean-allergic group, described here, was drawn from Marseilles residents whose asthma and rhinitis were associated with a positive skin test to castor bean. Exposure to the dust usually occurred through occupation, in dockworkers, farmworkers (with the pomace as a fertilizer), or oil-mill workers, but also occurred in those living close to the oil mills situated just north of Marseilles.

Eighty-seven of 96 subjects (91%) had a positive castor bean RAST, and of these subjects, 54 (56%)

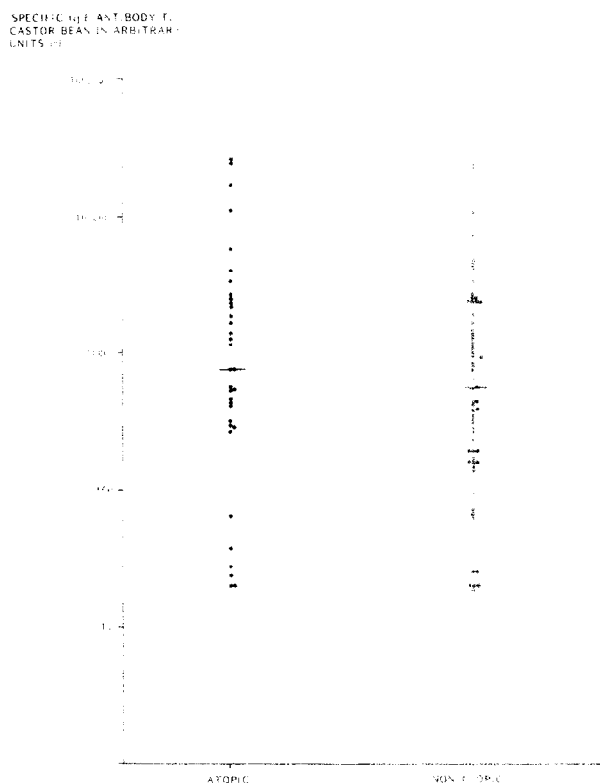


FIG. 3. The relationship between atopy and the magnitude of the IgE response to castor bean. There was no significant difference in the amount of specific IgE antibody to castor bean between atopic and nonatopic individuals.

had a class 4 RAST score. This compares with 24% of atopic individuals from Marseilles who had a class 4 RAST score to a common inhalant allergen (grass pollen), confirming the high potency of the castor bean allergens that have now been characterized as the 2S albumin, the crystalloid protein, and a protein doublet of molecular weights 47 and 51 kd.⁹ The specific IgE antibody response was substantial, a finding confirmed by RAST absorption studies in which the proportion of total IgE protein specific for castor bean varied between 9% and 64% (mean 38%),¹⁰ and this may partially account for the higher level of serum IgE in castor bean-allergic patients (mean 115 IU/ml, Fig. 2) than in unselected blood donors (mean 21 IU/ml). A positive skin prick test to one or more of five common allergens can be used to identify atopic individuals.¹¹ Atopy may also be defined by the presence of circulating specific IgE antibodies with RAST to at least one common allergen in which a positive RAST is indicated by class 1 or greater on the Phadebas RAST score.¹² We studied the IgE response to the five most common inhalant allergens in the south of France in castor bean-allergic patients

TABLE III. Inhalant allergen RAST

Patient group	No.	Grass pollen	Cat epithelium	Dust mite	<i>Parietaria judaica</i>	Olive pollen	% Atopic
A	—96	19.8	3.1	10.4	11.5	8.3	32
B	—13	23	7.6	30.7	0	23	53
C	—42	21	4.7	40.4	16.2	14.2	66.6
D	—111	7.2	0	1.0	16.2	18.0	36

The frequency of a positive RAST to inhalant allergens in patients groups A to D. There was no significant difference in frequency of atopy, defined as one or more positive RASTs, between Group A, the castor bean-allergic individuals (32%), and group D, unselected Marseilles blood donors (36%).

and compared the frequency of atopy with an unselected group of blood donors also from Marseilles. There was no significant difference in frequency of atopy between castor bean-sensitive patients (32%) and unselected blood donors (36%), but there were, however, differences in the frequency of positive RAST to individual allergens in the two groups. IgE specific antibodies to dust mite and grass pollen were more frequently found in the castor bean-sensitive patients, whereas IgE antibodies to *Parietaria* and olive were more prevalent in the control population of blood donors. These results indicate that nonatopic and atopic individuals were equally likely to become sensitized to castor bean, which agrees with previous studies in which the ability of castor bean dust to sensitize the population at large was demonstrated.^{3,4} Furthermore, there was no significant difference between the levels of castor bean-specific IgE in atopic and nonatopic subjects, indicating that atopy does not enhance the IgE response to castor bean.

The ability to sensitize nonatopic individuals is a property of other occupational allergens. Laboratory animal urine can sensitize nonatopic workers,¹³ particularly after long-term exposure,¹⁴ and sensitization to platinum salts can develop in nonatopic platinum refinery workers, although this is less likely than in atopic subjects.¹⁵ In our study, it was not possible to relate the degree or length of exposure to castor bean dust to the level of severity of clinical symptoms or sensitivity induced, but neither could we find evidence that the presence of atopy predisposed to castor bean sensitization.

Since atopy does not appear to have an important role in determining which individuals become sensitized, we believe that the potent IgE-provoking capacity of castor bean may be due to the physicochemical nature of the allergen itself or to other IgE-promoting factors contained in the dust. Once stimulated, the IgE response to castor bean in nonatopic subjects was of sufficient magnitude¹⁰ to be correlated

with, and partially explain, the significantly raised levels of serum IgE found in these subjects.

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