

ORIGINAL ARTICLE

Allergic Memory of Patients Sensitized to Castor Bean After a Long Stimulation-Free Period

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We have taken advantage of the temporary exposure of Marseilles population to castor bean seed proteins to follow 26 allergic patients more than 20 years after sensitization. Skin tests, specific immunoglobulin E (IgE) antibody assays, and specific immunoblots were performed. Skin test reactivity to *Ricinus Communis* and specific IgE concentrations decreased progressively and almost completely disappeared after 20 years. Specific IgE concentration displayed a fairly exponential decrease, with a half-life of 4.7 years. Thus, in the absence of any antigenic stimulation, directly by castor bean, or indirectly by cross-reactivity to other *Euphorbiaceae*, especially latex, IgE sensitization is bound to disappear.

Keywords allergy, castor bean, *Euphorbiaceae*, IgE, immunoblot

INTRODUCTION

The nature of the immunological memory, which is the hallmark of the immune system, remains a controversial issue. Several hypotheses have been proposed to account for the persistence of immunological memory: lymphocyte differentiation into a “memory cell” with a very long life span, decrease of the threshold of lymphocyte stimulation allowing their activation by cross-reacting environmental antigen, or antigen sequestration causing a continued lymphocyte stimulation (1–6).

Specific immunoglobulin (Ig) G or IgM antibodies may persist in serum 20 or 30 years after immunization in the absence of antigenic stimulation, but the natural history of human IgE-mediated allergy remains less known. It depends partly on the age, the way of allergen penetration, the persistence of the exposure to the allergen or cross-reacting-antigens, and the allergen concentration. A major issue is to determine how long specific IgE and clinical manifestations persist when allergen exposure stops and whether the allergen specificity is constant over the time.

In general, with stinging insect allergy (hymenoptera, mosquitoes, etc.) and with drug allergy (e.g., penicillin), specific IgE disappear after a certain period of time, when there is no more contact with the allergen. As far as food allergy is concerned, in the absence from the diet of the offending allergen, clinical sensitization disappears in almost all cases.

Knowledge is less developed concerning respiratory allergies. Randomized controlled trials have shown that the decrease of house dust mite allergen exposure improved

symptoms and bronchial responsiveness in both asthmatic children and adults sensitized to mite. However, the follow-up period was, at best, limited to one year (7–10). Occupational asthma is also a good model for studying the persistence of IgE sensitization after the eviction of the offending antigen. Malo et al. have shown that in occupational asthma caused by snow crab processing, the mean half-life of specific IgE was 20 months (11–13). Venables et al., studying phthalic anhydride asthma, obtained the same results after one year (14). These studies have shown that asthma persists for years in about 50% of cases, and possibly indefinitely after avoidance of exposure. However, once again, patient follow-up was limited to 4 years.

We had the opportunity to study an outbreak of castor bean allergy and to follow patients many years after allergen avoidance (15–17). *Ricinus communis* (castor bean) belongs to the *Euphorbiaceae* family, which contains *Hevea brasiliensis* and *Mercurialis annua*. Castor bean seeds contain a very interesting oil, which has many industrial, cosmetic, and pharmaceutical applications. To date, India and Brazil are the main producers of castor bean in the world. It was established since the beginning of our studies that there was an extensive cross-reactivity between the seed and pollen of *Ricinus Communis* and also between *Ricinus Communis* and other members of the *Euphorbiaceae* including latex (18–20). We were also able to purify the two major allergens of castor bean, Ric c 1 and Ric c 3 (21).

From World War II to 1980, there was in Marseilles an important outbreak of asthma due to the inhalation of the proteins of castor bean seed in relation to the pollution by two oil processing factories and by the harbor, where tons of castor bean seeds and pomace were handled very often without any protection (22).

The reason of this outbreak was the fact that until 1937 oil was extracted by a mechanical process that yielded pomace or oil cake containing about 6 to 8% oil. The cakes were

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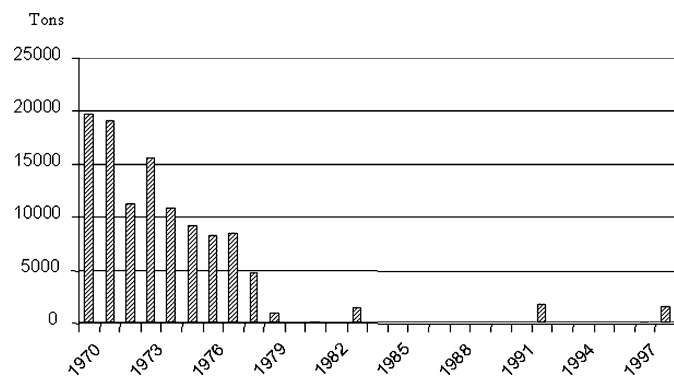


FIGURE 1.—Traffic (tons) of castor bean in Marseilles harbor.

therefore wet and nonpowdery. Afterwards, a solvent extraction process was used that yielded a cake containing only 1% oil. This much drier and more powdery cake was very dusty and hence much more dangerous.

However, around 1980 the outbreak was over because on the one hand the factories were compelled to close down in 1975, and, on the other hand, there was only sporadic importations of seeds and pomace in Marseilles harbor (Figure 1). Moreover, since 1980, contrary to the previous period in which the asthma outbreak was observed, the castor bean seed pomace was imported in closed containers and not in bulk. In addition, according to results reported by Panzani et al., the analysis of dust samples collected in the warehouses of Marseilles harbor in 1994 and 1999 showed that there was no trace of castor bean allergens in dust (22).

Sera of patients were taken and lyophilized during the outbreak and many years after, i.e., from 1963 to 1998. This article describes the outcome of the castor bean allergy, which became a good model to study the natural history of a very potent antigen. Respiratory castor bean allergy is a good model of IgE mediated allergy, and contrastly, as it is often related or assimilated to an occupational disease, there is the possibility that the absence of antigen may lead to a disappearance of clinical symptoms and IgE reactivity (23).

MATERIALS AND METHODS

Patients

Within a cohort of more than 200 patients, we had the opportunity of selecting 26 subjects whose blood was collected for 20 years or more. All patients lived close to the harbor or the castor bean factories, but none of them was in direct contact with castor bean.

All patients provided their informed written consent.

According to clinical history (Table 1), we observed 1 case of vesicular polymorph erythema, 4 cases of allergic rhinitis, and 21 cases of asthma, 1 of them associated to urticaria. Among them, 11 were mono-sensitized and 15 were polysensitized (mostly house dust mites, less often grasses or *Parietaria judaica* pollens or molds: *Alternaria alternata*).

The diagnosis of castor bean respiratory allergy was assessed on the basis of the following criteria:

1. A careful clinical history of subjects having asthma only in Marseille or in areas of the countryside contaminated by castor bean pomace.

TABLE 1.—Patient's clinical data.

Patients	Date of birth	Gender	Clinical history*	Skin test at the first advice**	Other allergens***	Years (19–) of taking of blood	Location
1	1943	M	Vesicular Polymorph erythema	2+ 6+	0	68,70,72,86,90,98	Alps
2	1952	M	A	4+ 6+	0	71,86	Marseilles
3	1954	F	A	4+ 8+	DPF HI	71,77,83	Marseilles
4	1919	M	A	3+ 8+	0	68,75,86	Vaucluse
5	1954	F	A	2+ 5+	0	74,86	Marseilles
6	1968	F	A	3+ 8+	0	74,86	Marseilles
7	1941	M	A U	1+ 4+	GR	72,86	Vaucluse
8	1949	F	A	3+ 5+	0	71,74	Marseilles
9	1931	F	A R	1+ 4+	DPF Cy	83 91,92	Marseilles
10	1951	M	A R	Doubtful	Cy GR Par	83,85,91	Marseilles
11	1923	F	A R	4+ 3+	GR Par	72,98	Marseilles
12	1915	M	A	4+ 8+	DPF HD	81,85,89,92	Marseilles
13	1912	M	A	3+ 6+	GR Par	71,72,89	Marseilles
14	1949	F	A	3+ 5+	0	73,85	Marseilles
15	1935	F	A	4+ 6+	0	67,89	Marseilles
16	1933	M	A	2+ 5+	0	76,89	Marseilles
17	1951	F	A	3+ 5+	GR DPF	74,98	Marseilles
18	1951	M	A R	2+ 3+	0	73,83	Avignon
19	1921	F	A	3+ 6+	DPF Alt	73,85,98	Marseilles
20	1931	M	A	3+ 8+	HD	73,98	Marseilles
21	1926	M	A	2+ 4+	0	74,89	Marseilles
22	1922	M	A	4+ 8+	HD DPF	85,98	Marseilles
23	1943	F	A	3+ 6+	HD	75,89	Marseilles
24	1929	M	A	2+ 4+	DPF	68,89	Drôme
25	1955	M	A	3+ 6+	DPF	73,85	Marseilles
26	1953	M	A	5+ 8+	DPF Alt	66,85	Marseilles

*A: asthma; AR: allergic rhinitis; U: urticaria.

** Skin tests were performed with a 1/1000 dilution of the purified allergen CB W U. First number corresponds to the average diameter (in centimetres) of the weal, the second one to the average diameter of the flare.

*** GR: grasses; Par: *Parietaria judaica*; Cy: *Cupressus sempervirens*; Alt: *Alternaria alternata*; DPF: house dust mites; HI: household insects; HD: house dust.

2. All patients were skin tested by prick with an extract of castor bean prepared by L.L. Layton at the U.S. Department of Agriculture (Albany, California). This extract, called C.B.W.U, was partially purified and did not contain ricin, the toxin of castor bean. It was used from the beginning of our studies until recently.

Poly-sensitization was defined by a panel of skin tests with the most common aero-allergens in our area:

- Pollens: grasses, *Olea europea*, *Cupressus sempervirens*, *Parietaria judaica*
- Mites: *Dermatophagoides pteronissinus* and *farinae*
- *Alternaria alternata*
- Cat dander
- Household insects: cockroach, house fly, spider, silverfish
- Green coffee

These extracts were provided by two laboratories: Berkeley Biologicals (California USA), and Stallergens (France).

Latex skin test was not carried out because at the beginning of the study, this allergen was unknown.

For obvious reasons, no nasal or bronchial provocation test was performed even for the cases in which a local production of IgE was suspected (24).

Six controls were included, consisting in subjects having no contact with castor bean antigens.

Serum Collection

All sera were lyophilized and kept at +4°C until the inclusion of the last sample. We know by experience and in Coombs's opinion that after freeze-drying, specific IgE remain unaltered even after 30 years or more.

Specific IgE Antibody Assays (CAP-FEIA)

The circulating *Ricinus communis* and *Hevea brasiliensis* latex-specific IgE concentrations were measured with an immuno-enzymo-fluorimetric method (UNICAP 100; Pharmacia, St Quentin en Yveline, France). The sensitivity threshold was 0.35 KU/L.

Specific Immunoblots

The test provided by DPC (la Garenne-Colombe, France) allows the qualitative analysis of *Ricinus communis* specific IgE in the serum. The assay was performed in accordance with the manufacturer's instructions. A 10-fold serum dilution was incubated with the membrane strips. After washings, the alkaline phosphatase labeled anti-IgE antibodies, then the chromogenic substrate were added. The reaction was stopped by distilled water washing.

Blots were scanned and analyzed with the Bioscan software provided by DPC. This program determines the approximate molecular weight of the allergens recognized by specific IgE. All sera from the same patient were treated and analyzed in the same experiment and with the same lot of reagents.

The immunoblot inhibitions were carried out with purified Ric c 1 and Ric c 3 extracts (a gift supplied by Pr Zeppezeaur M) at 5 and 2.5 mg/mL concentrations, in accordance with the DPC instructions.

Statistical Analysis

Results were expressed as the mean $\pm$ SEM. Data were compared using Student's *t* test.

RESULTS

Clinical Evolution of Patients

Among the 26 patients, 20 were free of any respiratory symptom 1 or 2 years after the closing down of the two castor oil processing factories and the fall of castor bean importations (seed and pomace) in the harbor in 1979. However, 4 of them had mild attacks of asthma or allergic rhinitis in 1983 and 1992, periods in which a small quantity of castor bean was unloaded in the harbor. The symptoms of the 2 other patients improved greatly after 1980, but had from time to time mild attacks of asthma or allergic rhinitis; All these patients were poly-sensitized.

The evolution of skin test reactivity followed the same pattern, but with a delay of one or several years after the symptom disappearance. However, it was not possible to statistically evaluate the differences because the castor bean extracts used over the years came from different laboratories. For 2 patients (10 and 15) who had still specific IgE to castor bean, the skin reactivity remained almost the same as it was at the beginning.

Specific IgE

The study was carried out in a retrospective way, among patients whose serum was taken at different times, over a maximum period of 32 years (from 1966 to 1999) (Figure 2). Four patients (patients 4, 5, 11, 24) out of 26 did not have specific IgE, but clinical manifestations and positive skin tests allowed us to class them in the castor bean allergic group. Two patients had a maximum specific IgE concentration lower than 0.70 KU/L (class 1); 4 patients had values ranging between 3.5 and 17.5 KU/L (class 3); 12 patients had values ranging from 17.5 to 50 KU/L (class 4). Lastly, for 4 patients, the maximum values were higher than 100 KU/L (class 6). As a control, castor bean-specific IgE were not detectable in the serum of 6 non-allergic patients.

Twenty patients had a maximum concentration of specific IgE sufficient to study their kinetics (specific IgE $\geq$ class 3). The castor bean-specific IgE concentration decreased with time for 19 patients out of 20. This reduction was evaluated over 5-year periods (Table 2). The castor bean-specific IgE concentration displayed a fairly exponential decrease, with a half-life of 4.7 years (Figure 3).

However, specific IgE concentration of patient 10 does not appear to decrease in course of time (14 KU/L [1983]; 17KU/L [1985]; 20 KU/L [1991]. Unexpectedly, this patient had at the beginning a doubtful skin test.

Poly-sensitization does not seem to influence *Ricinus communis* specific IgE concentration owing to the fact that the mean of specific IgE concentration is not different between the groups of mono-sensitized or poly-sensitized patients (37 $\pm$ 58 KU/L vs 51 $\pm$ 50 KU/L, *p* = 0.6). Because cross-reactivity was described between *Ricinus communis* and latex, we performed CAP-FEIA to latex. Except for 3 patients (patient 3: 1.2 KU/L [1983]; patient 5: 0.36 KU/L [1986]; patient 13: 0.67 KU/L [1989]) latex-specific IgE were not detectable.

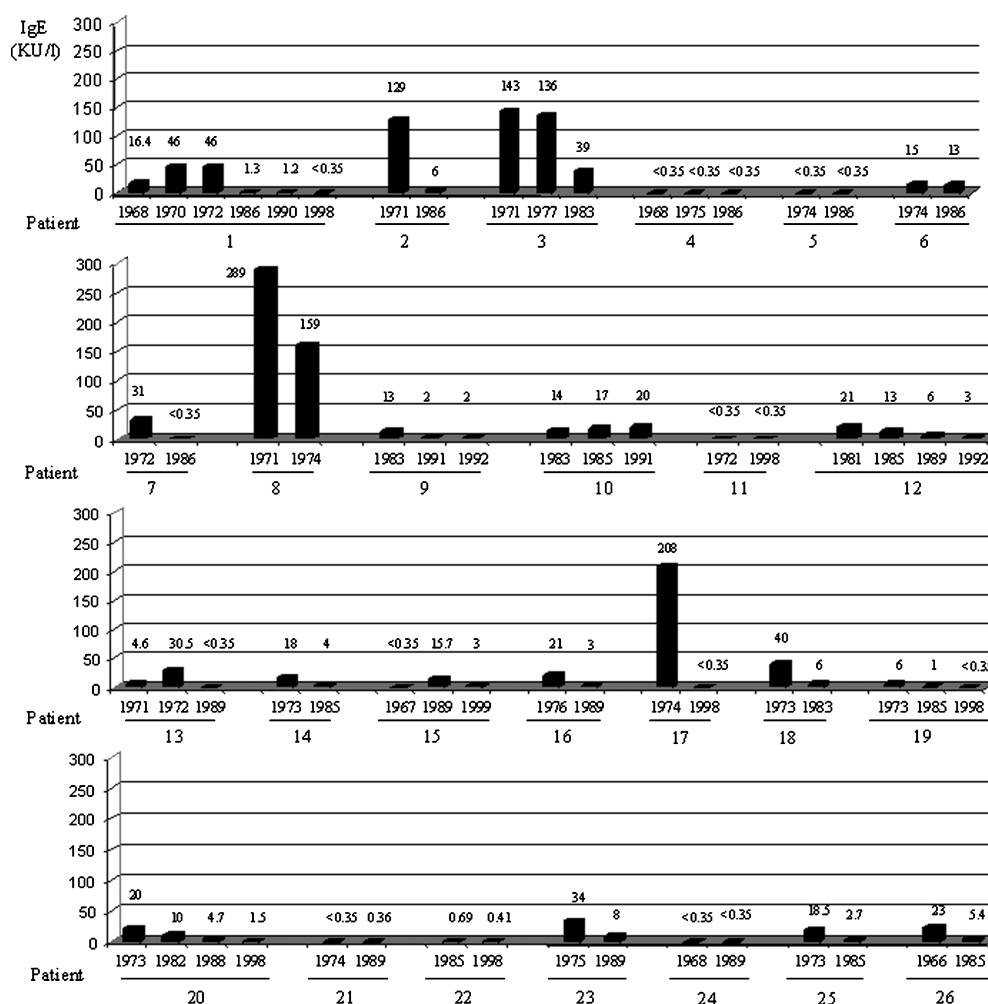


FIGURE 2.—Evolution of castor bean-specific IgE concentrations in allergic patients.

Immunoblots

The blot analysis gave the approximate molecular weight of the allergens recognized by specific IgE. These values ranged between 11 and 65 kDa. The band profiles were different from one patient to another, but they were preserved for the samples obtained from the same patient (Figure 4). In the course of time, the band intensity decreased or even disappeared.

Two major allergens of *Ricinus communis* were described: Ric c 1 (11 kDa) and Ric c 3 (12 kDa) (21). Inhibition tests performed with purified Ric c 1 and Ric c 3 allowed us to check the presence of specific IgE towards these major aller-

gens. Fourteen patients of 26 displayed specific IgE towards both Ric c 1 and Ric c 3. Eight patients had only Ric c 3-specific IgE. Lastly, 1 patient had only Ric c 1-specific IgE (data not shown). Patients 4, 11, and 24 did not have positive immunoblots. In agreement with these results, their castor bean-specific CAP-FEIA were also negative. Immunoblot analysis displayed that the bands corresponding to Ric c 1, Ric c 3, 20 kDa, 22 kDa, 31 kDa, 51 kDa, and 56 kDa persisted more often than the others in course of time.

DISCUSSION

Thirty years ago, castor bean allergy was an important public health problem in Marseilles. This gave us the opportunity to follow 26 castor bean allergic patients during 10 to 32 years. The majority of patients were free of any respiratory symptom 1 or 2 years after the end of castor bean exposure. Four patients having an obvious clinical history of castor bean allergy had a positive skin test but negative CAP-FEIA; this fact is more likely correlated to a local production of IgE, an "entopy" according to the word coined by Powe (24). Entopy corresponds to patients who have negative skin test and RAST but positive specific IgE in their nasal secretions.

TABLE 2.—Decrease of castor bean specific IgE concentration in time.

Intervals of time (years)	% of castor bean-specific IgE decrease		No. of patients
	Mean	Range	
0 to 5	45,6	44,8–46,4	2
6 to 10	58,7	4,9–85	5
11 to 15	77	11,2–100	11
16 to 20	92,8	76,6–100	4
>20	98,7	94,9–100	4

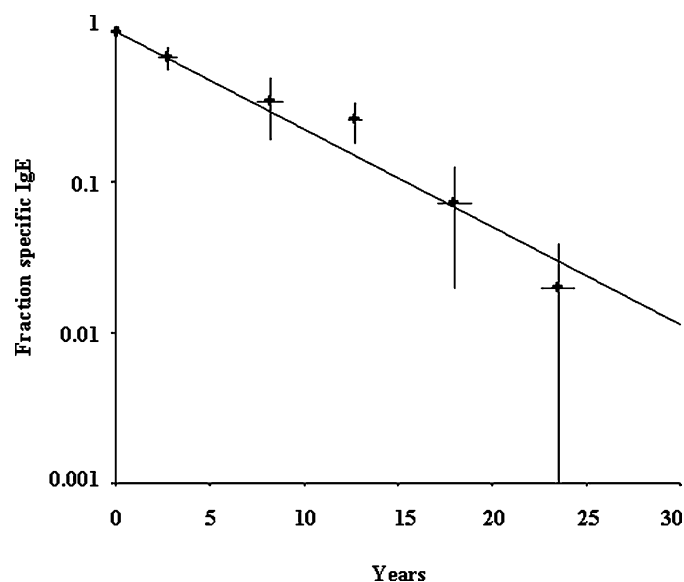


FIGURE 3.—Kinetics of specific IgE decrease: Each point represents a average of 4 to 11 determinations. Standard error of the mean is shown for both time period (horizontal bars) and IgE concentration (vertical bars). The IgE concentration follows an exponential law $([IgE]/[IgE]_0 = \exp(-0.15 t))$, where t is expressed in years), for about 25 years.

Castor bean-specific IgE concentrations displayed a fairly exponential decrease with a half-life of 4.7 years. These results were strengthened by the disappearance of the bands on immunoblots. However, castor bean-specific IgE of patient 10 did not decrease over the time until 1991. Unfortunately, we were unable to obtain a more recent sample. Castor bean-specific IgE increase after 1980 is difficult to explain. Indeed, with the disappearance of castor bean pollution, no new case of respiratory allergy to castor bean was found in Marseilles and all mono-sensitized patients recovered without any other symptom left. Furthermore, we were unable to find even traces of castor bean in samples of dust collected in 1994 and 1999 at the place on the docks where castor bean was stocked. The possibility of contact with castor bean pomace used as fertilizer for market gardening can also be discarded.

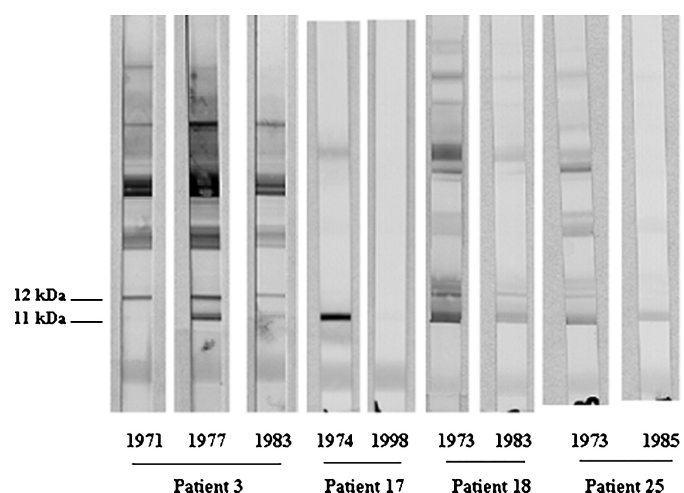


FIGURE 4.—Evolution of IgE profiles obtained with immunoblots.

Conversely, castor bean-specific IgE increase could be related to the exposition to cross-reactive allergens, especially among the Euphorbiaceae family. Contact with *Mercurialis annua* or castor bean pollen (which is known to cross-react with castor bean seed) is unlikely because these plants are not cultivated up until now in our country.

Euphorbia Lathyris (Euphorbe de Provence) is the main natural Euphorbiaceae of our country, but yields a negligible quantity of pollen. Concerning Euphorbiaceae used as ornamental plants, we never found any case of allergy to them. As far as latex allergens are concerned the answer is less easy with the growing number of latex allergy cases and since some hevein like domains are panallergens. Latex CAP-FEIA performed among the 26 patients were negative except in three cases. Patient 5 never had castor bean-specific IgE. Castor bean-specific IgE of patient 13 became undetectable in 1989. Concerning patient 3, castor bean-specific IgE decreased dramatically from 143 KU/L in 1971 to 39 KU/L in 1983. So, sensitization to latex seems not to be related to the persistence of castor bean allergy. The possibility of pan-allergy to unrelated species seems unlikely because there is also no report, as far as we know, on the finding in castor bean seeds of other plant panallergens such as profilins, chitinases, pathogenic-related proteins, chitin-binding proteins and calcium-binding proteins, which are rather widely distributed among the vegetal kingdom. For all these reasons, except an exposition to castor bean dust during the sporadic importations after 1980, we have no hypothesis to explain the castor bean-specific IgE increase of patient 10.

Similarly, Anto et al. in Barcelona (Spain) reported several outbreaks of asthma related to the spreading and the inhalation of soybean dust from a harbour where this material was unloaded (25). Patients were followed during 8 years. Extensive measures of protection with filters were undertaken and after 2 years there was a great improvement among the patients, but no complete remission. Soybean-specific IgE remained present in 55% of patients. Still, we have no information about their levels.

Castor bean allergy is a very strong IgE-mediated allergy. As far as we know, there is no other retrospective study concerning the natural history of human castor bean hypersensitivity. Moreover, the new European program to develop castor bean culture needs to know this allergy better (cf: <http://www.nf-2000.org>). It is also tempting to speculate that this observation might be extended to some other cases of IgE-mediated human respiratory allergy.

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