

Results of skin test and RAST in allergy to a clinically potent allergen (castor bean)

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Summary

In order to investigate once more the usefulness of the RAST procedure in the diagnosis of reaginic hypersensitivity, we studied a series of patients showing a well known sensitivity to castor bean, which is a potent antigen, and compared the results with those given by patients with no clinical allergy to this antigen as confirmed by negative skin testing. The latter group included patients with no contact, a remote contact or a close contact with castor bean. Castor bean allergy seems to be a good model in the testing of human reaginic hypersensitivity. We observed in the castor bean positive group of forty-one patients a 95% correlation between RAST positivity and skin test positivity with only two negative RAST scores, and in the castor bean negative group of ninety-four patients, a 97% correlation, with three RAST positive scores. For its specificity and its good sensibility (although lesser than skin testing), RAST can thus be recommended as a diagnostic tool as a complement to skin testing. Moreover, for potent allergens, RAST offers a risk-free diagnostic alternative.

Introduction

Since the introduction by Wide, Bennich & Johansson [1] of an *in vitro* method for measuring circulating IgE antibody, which promised to become a valuable tool in the diagnosis of type I hypersensitivity, the RAST (radio-allergo-sorbent test) has become widely used all over the world. A large number of allergens such as animal danders, pollens, moulds, foods, bee venom, green coffee and penicillin were studied in clinical investigations where the results of RAST and skin tests have been compared [2–14]. Depending on the type of allergen used and the selection of the patient material studied, correlation ranging from 50 to 100% has been found. However, the technique has been modified and improved recently and sometimes its usefulness has been questioned. [15–21].

We have investigated the usefulness of skin tests and RAST in measuring reactivity

to a clinically very potent allergen; castor bean antigen is probably one of the most potent allergens known which is capable of inducing an immediate hypersensitivity in humans. Castor bean allergy seems to us, therefore, a very interesting human model for the study of reaginic hypersensitivity. Castor bean allergy has been studied for years by one of us in collaboration with Layton [22–25] and has been used as a model for the evaluation of *in vitro* techniques of detection of specific IgE [26, 27]. Recently new aspects of castor bean allergy have been investigated [28–30]. Our aim was to study the problems of sensitivity to this allergen, using RAST as a complement to skin testing in the diagnosis of clinical allergy.

Materials and methods

Different groups of patients clinically allergic to or in contact with castor bean were studied. Venous blood was drawn before hyposensitization to any allergen. The serum samples were lyophilized and sent to Sweden by airmail for the RAST assays.

The serum samples were reconstituted to their initial volume by adding distilled water and were kept at +4°C during the period of analyses. It has been shown that IgE is not altered by the process of lyophilization.

(1) RAST procedure

Allergen-coated paper discs were prepared in the following way. One mg of isolated ricin-free castor bean allergen (preparation C.B.W.U., which stands for castor-bean western utilization and is a ricin-free allergen [32] kindly provided by Dr L.L. Layton, U.S. Department of Agriculture, Albany, California U.S.A.) or 0.5 ml 1/10 w/v of green coffee beans, was coupled to 100 CNBr-activated discs as previously described for micro-cellulose particle [32]. All allergen discs were controlled not to have an usually high tendency to bind IgE non-specifically by testing in RAST against a serum without any known IgE antibody activity and having an IgE level of approximately 5000 U/ml. The background was less than 1% of added anti-IgE ¹²⁵I-activity (T%).

RAST was performed essentially as described [32]. Fifty µl of undiluted patients' serum was incubated with one allergen-coated filter paper disc for 3 hr at room temperature. Excess of serum was washed away by twice repeated addition and aspiration of saline. In a second step, we added ¹²⁵I-labelled immunosorbent purified anti-IgE (De 2) [32, 33], corresponding to approx. 80 000 counts per minute (cpm) for incubation overnight. After washings as above, the cpm activity of the discs was determined in a gamma-counter and expressed in parts per thousand (‰) of a reference. The reference system consisted of a serum with a high concentration of IgE antibodies to birch pollen allergen tested in RAST against discs to which that allergen has been coupled in excess [32]. Results of more than 3‰, which correspond approximately to the double non-specific background activity of a normal serum, were reported as positive.

(2) Skin testing procedure

Skin tests were performed with the castor bean extract, i.e., scratch tests using a 1/100 000 dilution as a screening test. The castor bean antigen used was also the C.B.W.U.

The results of skin testing have been recorded in cm, the first figure corresponding to the average diameter of the weal, the second one to the average diameter of the flare.

The skin reactions were evaluated after 15–20 min. A positive skin reaction was taken when a weal of at least 0.5 cm was observed.

In the first group of castor bean allergic patients, an end point titration was performed: the end point titration consists of carrying out skin tests on a patient with solutions more and more diluted until there is no more skin reaction to the allergen.

Results

(a) *Castor bean allergic patients*

(1) As shown in Table 1, all fourteen patients in the first group were clinically afflicted with asthma (very often severe) and/or rhinitis or spasmodic cough. Some patients were also allergic to other airborne allergens. All those patients were selected on the ground of a strong cutaneous reactivity to castor bean. The patients lived either in the vicinity of the loading docks of the harbour in Marseilles or in the countryside where castor bean pomace is used as a fertilizer. The scratch tests using a ricin-free purified castor bean extract at a 10^{-5} dilution were strongly positive. A skin test titration was done in twelve cases and in some cases scratch tests were positive up to a 1/1000 000 000 dilution of the C.B.W.U. extract (Table 2). With a 10^{-10} dilution all the fourteen patients failed to react. The agreement between skin testing and RAST positivity was 100% in this first group.

(2) A second group of twenty-seven patients who were skin test positive to castor bean to a lesser degree than the first group has been also investigated; all those patients showed a respiratory allergy, asthma and/or allergic rhinitis; almost all of them were also reactive to other airborne allergens (house dust, household insects, moulds, pollens). All of them but two gave a positive RAST score to castor bean. It should be

Table 1. First group: Castor bean allergy

No.	Age	Sex	Clinical history	Other allergies	Skin test 1/100 000 C.B.W.U.	Specific IgE % U.L.
1	25	M	Asthma	House dust, grass pollens	2–6*	600
2	39	M	Asthma	House dust, household insects Moulds	1.5–6	6000
3	47	M	Asthma	House dust	2–4	3130
4	35	M	Asthma	House dust	1.5–6	210
5	22	F	Asthma	House dust	2–6	270
6	60	M	Asthma	House dust	2–4	2020
7	23	M	Asthma	House dust, grass pollens	3–8	840
8	52	M	Asthma	House dust, green coffee	3.5–5	700
9	63	M	Asthma	House dust, grass pollens	2–4	115
10	58	F	Asthma	House dust	1–4	140
11	40	M	Allergic rhinitis	House dust	4.5–6	460
12	42	M	Asthma	0	1.5–6	1365
13	46	M	Asthma	House dust, household insects	2–4	700
14	27	M	Spasmodic cough and allergic rhinitis	House dust	2–4	16.5

* The first figure indicates the mean diameter in cms of the weal, the second one the mean diameter in cms of the flare.

Table 2. Skin test titration on twelve patients

Dilution of C.B.W.U.	CB ⁻⁵	CB ⁻⁶	CB ⁻⁷	CB ⁻⁸	CB ⁻⁹
Number of Patients	12	11	8	6	3

CB⁻⁵ correspond to a 1/100 000 dilution, CB⁻⁶ correspond to a 1/1 000 000 dilution, CB⁻⁷ correspond to a 1/10 000 000 dilution, CB⁻⁸ correspond to a 1/100 000 000 dilution, CB⁻⁹ correspond to a 1/1 000 000 000 dilution.

noted that of the two patients whose sera gave a RAST score under 3‰, one was 0.4‰ and the other 1.2‰. In this second group of castor bean allergic patients, end point titers were not determined during skin testing (Table 4).

(b) Castor bean non-allergic patients

In order to investigate all the possibilities of allergy to castor bean, we selected four groups of patients (Table 3). All these patients were asthmatic or had a typical allergic rhinitis. Almost all of them were allergic to airborne allergens (house dust, mites, moulds, household insects, pollens).

(i) Group 1 corresponds to twenty patients having no known contact with castor bean: all of them had skin tests negative to castor bean. In all the cases, RAST with castor bean had been completely negative. In one case, the RAST score was 1.4‰.

(ii) Group 2 corresponds to fifteen patients having a close contact with castor bean, most of them being farmers handling castor bean pomace. In two cases, we found a positive RAST although the result of skin testing to castor bean was negative.

(iii) Group 3 corresponds to twenty-one patients having a remote contact i.e., patients living in Marseilles and in the vicinity of the harbour where the handling of castor bean

Table 3. RAST on castor bean skin tests negative patients

		Rast scores					
		Negative	Under 0·30	A	B	C	D
Group 1							
No known contact with castor bean (20 patients)		19	1	0	0	0	0
Group 2							
Close contact with castor bean (15 patients)		18	0	0	1	0	1
Group 3							
Remote contact with castor bean (21 patients)		17	3	0	1	0	0
Group 4							
Control group (38 patients)		35	3	0	0	0	0
Total 94							

RAST Classes: A, 0.30–0.70; B, 0.70–3.5; C, 3.50–17.5; D, > 17.5.

Table 4. RAST on castor bean skin test positive patients

	Negative	Rast scores				
		Under 0·30	A	B	C	D
First group (14 patients)	0	0	0	0	1	13
Second group (27 patients)	0	2	2	1	5	18
Total 41						

occurs from time to time. All of them gave negative skin tests to castor bean. We found only one patient showing a RAST positive.

(iv) Finally (group 4) control tests were performed on a group of thirty-eight patients allergic to house dust and household insects, and no RAST positivity to castor bean was found. It must be added that in three cases a very low RAST score (0·2%, 0·3%, 1%) was found and that these RAST patients were living at this time in Marseilles and could therefore have had a remote contact with the castor bean antigen.

We took at random those patients among people living in the South of France only on the basis of skin tests negative to castor bean and skin tests positive to house dust and/or household insects.

Discussion

In vitro diagnosis of atopic allergy using RAST is now a routine procedure in many countries. More than 180 different allergens coupled to discs are commercially available. In addition, hundreds of other more or less well characterized allergen preparations have been applied in RAST. The results obtained with such crude extracts are not always satisfactory.

In order to evaluate the clinical importance of circulating IgE-antibody, comparative studies have been performed with RAST and conventional diagnosis procedures such as skin and provocation tests. Figures of agreement of 75–100% have been reported [34–36]. The different results can be explained on the basis of the selection of the patients for the studies as well as on the type of *in vivo* procedure used; parts of the disagreement can be explained by quantitative and qualitative differences in the allergen preparation used *in vivo* and *in vitro*. An allergen extract is in essence a very crude mixture of all water soluble substances in the material regarded to be responsible for the symptoms of allergy. A classical, straightforward example is allergy to grass pollen. Grass pollens are easily obtained as pure preparations, the allergen components are readily water soluble, and the allergen extract thus produced are usually both potent and specific in both *in vivo* diagnosis tests and RAST. However, despite the seemingly good quality of grass pollen extracts, it has been shown that the allergenic proteins represent only a few per cent of solid substances of the extract. Thus, the majority of the protein constituents are non-allergenic substances, some of which have been shown to have irritating effects on the skin, to possess enzymatic activity, and so on. From what has been said of simple pollen allergen extracts, it is obvious that allergenic extracts of such uncharacterized material as house dust are likely to contain even less allergen-specific material.

The impurities in the allergen extracts will have negative influence on both RAST

and skin tests, but in different ways. The activated paper discs used in RAST will bind any protein components in the allergen preparation, and it is therefore necessary that the allergen used represents a substantial part of the active protein. In the skin test, the non-allergenic material can have non-specific irritating effect leading to false positive results. In the case of castor bean, if a non-ricin-free allergen is used, false positive reactions can occur due to the toxic and irritative properties of the ricin. The C.B.W.U. has been utilized by us for years and on several hundred patients with a very good specificity and no untoward local or general reactions at a 1/100 000 dilution as a screening test in our routine procedure [22, 24-26]. This is the reason why we have used the human castor bean allergy model since, on the one hand, castor bean is a very strong allergen and, on the other hand, it was available in purified form (C.B.W.U. from Dr L.L. Layton).

The pure and potent castor bean allergen preparation has had an excellent specificity in both *in vitro* and *in vivo* testings, a good correlation being observed between skin testing and RAST. On the one hand, the sera of a few patients who were skin test negative (i.e., two out of ninety-six cases) gave positive RAST scores. These two positive were found in the group of fifteen patients having a close contact with castor bean and there is the possibility of a real allergy to castor bean with specific castor bean IgE in the blood in absence of skin reactivity. On the other hand, considering patients having a well known clinical allergy to castor bean who were skin tests positive, we found out of two series: (a) a 100% correlation with RAST positivity in the first group of fourteen patients who were strongly positive to castor bean; (b) a less good correlation, i.e., twenty-five out of twenty-seven, in a second group of patients having a less important clinical allergy to castor bean. The correlation between skin test positive and RAST positive was 97%. The specificity of the RAST was good and its sensitivity lesser than the sensitivity of skin tests. From our study in the special case of castor bean allergy, RAST seems to be a very useful test, since it confirmed the results of case history and skin tests with a 97% correlation.

In cases which do not seem to clearly constitute allergy to castor bean from skin testing, we found 3% of RAST positive to castor bean (Table 5). In this group, since the

Table 5. Total patients

	Rast		% Positivity
	positive	negative	
Clinical allergy to castor bean	41	39	95
No clinical allergy to castor bean	94	3	97

specificity of the RAST to castor bean allergen does not seem to be questioned, we can say that its sensitivity is greater in the diagnosis of castor bean allergy than skin testing owing probably to the fact that IgE are, in these cases, mostly found in the blood rather than in the skin.

So, when a potent and purified antigen is used the RAST procedure is almost as accurate as skin testing; besides, in a few cases, its use raised the possibility of the occurrence of a true reaginic hypersensitivity with no response to skin testing. A possible advantage of a RAST-based diagnosis in the case of a potent allergen is that

RAST is absolutely free of risks.

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