

Improvement of the radioallergosorbent test (RAST) sensitivity by using an antibody specific for the determinant D ϵ 2

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Summary

A technical modification was introduced by the manufacturer (Pharmacia) of a commercially available RAST, with the use of an anti-IgE antibody specific for the domain ϵ 2 of the IgE molecule. Results of the previous and of the new RAST were compared in 100 patients suffering from bronchial asthma or chronic rhinitis. When the same serum was assayed by both techniques, the new RAST technique disclosed a higher positivity in 26% of the comparisons. The new RAST improved the overall correlation between RAST and skin-tests by 6%: individually by 12% for house dust, 8% for *Dermatophagoides pteronyssinus* and 15% for animal danders. However, the level of correlation was decreased by 8% for the moulds. In patients with a total IgE concentration larger than 100 PRIST units, the new RAST allowed the demonstration of specific IgE in a number of cases twice as large as the previous technique. Thus, the modification of the RAST provides a more sensitive test, the results of which better correlate with those of other diagnostic methods.

Introduction

In diseases of possible allergic aetiology, like bronchial asthma and chronic rhinitis, the demonstration of one or more allergens responsible for symptoms may be beneficial to the patients, since it may lead to environmental control or, in some cases, allow specific immunotherapy. Provocation tests are considered as most reliable in detecting a responsible allergen (Aas, 1974). Results obtained by provocation tests have frequently been used as 'absolute criteria' in studies evaluating other diagnostic means, like case history, skin tests, total IgE concentration (Johansson, 1967) and detection of specific IgE by the radioallergosorbent test (RAST) (Berg, Bennich & Johansson, 1971; Eriksson, Ahlstedt & Belin, 1976; Osterballe, Malling & Weeke, 1979). Unfortunately, provocation tests are time-consuming and may be hazardous which limits their use in clinical routine. Several studies showed that combining the results of case history, skin tests and RAST provided information very close to that derived from

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provocation tests (Ahlstedt *et al.*, 1974; Berg & Johansson, 1974; Eriksson, 1977). Any improvement of one of these methods may improve the accuracy of diagnosis and thus make the provocation tests less necessary.

In 1977, the commercially available Phadebas RAST® (Pharmacia, Diagnostics AB, Uppsala, Sweden) was modified by the introduction of an antibody more specific for the IgE molecule, with activity against the determinant De2 of the heavy chain of IgE (Kurvits, 1981). Background radioactivity was decreased, which was supposed to result in an augmentation of the test sensitivity.

The purpose of the present study was to estimate the advantages of this modification in clinical routine. We compared the results simultaneously obtained by both techniques on the same serum sample of patients with bronchial asthma and chronic rhinitis, and investigated the influence of this technical modification on the correlations between RAST and other diagnostic tests.

Materials and methods

Patients

We studied 100 adults, sixty-three with bronchial asthma and thirty-seven with chronic rhinitis seen in succession at an out-patient clinic. Their mean age was 39 years (16–85 years) and their average age at onset of disease 29 (11–75 years). Asthmatic patients suffered from perennial attacks of reversible bronchial obstruction. In none of them could the criteria of chronic bronchitis be assessed. Patients with chronic rhinitis had perennial bouts of nasal obstruction, rhinorrhea and sneezing. Patients with seasonal symptoms during the period of pollinosis were excluded from the study, since the demonstration of allergens in these cases is not difficult, and makes a detailed investigation unnecessary.

Methods

History. A systematic and detailed history was taken on each patient by a trained allergist.

Skin-tests. Prick-tests were performed on the forearm with: house dust (total extract), *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* mixed moulds (three preparations), cat danders, dog danders and horse danders (Beecham Pharmaceutical, Bencard Allergy Unit, Clarendon-Rd., Worthing, Sussex, BN 14, 8QH, England). Skin reactions were graduated from 0 to ++++ according to the recommendation of the Standardization Committee of the Northern Society of Allergy. 0 corresponded to a reaction similar to the control prick-test and +++ to a reaction identical to that evidenced by the injection of histamine (1 mg/ml) (Aas & Belin, 1972). In the correlations with the other diagnostic methods, only +++ to ++++ results were considered as positive.

Total IgE. The total concentration of IgE was measured in serum by the paper radioimmunosorbent test (Phadebas PRIST, Pharmacia Diagnostics AB, Uppsala, Sweden). According to indications given by the manufacturer, values larger than 100 PRIST units (approx. 200 ng/ml) were considered as indicative of a probable allergic state.

Specific IgE. were measured on the same serum sample, taken on the day of investigation, by the radioallergosorbent test, and by simultaneously using the former

and the new antibody. In both cases, the test was carried out according to the indications given by the manufacturer. The following allergens were tested: total house dust extract (two preparations), *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, five individual moulds (*Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Mucor racemosus* and *Alternaria tenuis*) and animal danders (cat, dog and horse). Results were estimated from 0 to + + + +, according to the amount of labelled antibody bound to the solid phase. With the previous technique, one + results are considered as negative. With the new technique, one + results are considered, according to the indication of the manufacturer, as indicative of low concentration of specific IgE. This interpretation is justified by a calibration of the test achieved with lower known amounts of IgE. However, for our correlations with the skin-tests, and with the total IgE, only + + to + + + + results were considered as positive, in both previous and new RAST.

Results

The results of 445 determinations of specific IgE, obtained in a serum sample, simultaneously assayed by the RAST, are given for each allergen in Table 1. In 71.9% of the tests, results were identical for both techniques. In 26.4%, the new technique showed a higher concentration of specific IgE, and, in 1.6% a lower concentration. Table 1 shows that the new technique gave + instead of 0 in 10.3% of the tests; + + instead of 0 in 3.6%; and + + instead of + in 5.8% of the tests.

Table 2 shows the overall correlation between skin-tests on one hand, and previous or new RAST on the other hand, without any reference made to individual allergens. Only clear-cut positive results for skin-tests and RAST were considered, as defined under 'Materials and methods'. The results were concordant, i.e. either skin-test pos-RAST pos or skin-test neg/RAST neg, in 74% of the cases when previous RAST was used, and 80% of the cases when new RAST was used. With the new RAST, the number of discordant results skin-test pos/RAST neg decreased clearly, from 24 to 16%, most of these cases becoming skin-test pos/RAST pos. However, the new technique was responsible for a slight increase in discordant results skin-tests neg/RAST pos, from 2 to 4%.

The details of this comparison, considered for each individual allergen, are given in Table 3. The extent to which the correlation was modified by the new RAST depended upon the allergen considered. *Dermatophagoides farinae* and the group of moulds were the only allergens with which the new technique of RAST did not improve the correlation with the skin-tests. With the moulds, the correlation was even poorer; this might be explained by the fact that the new RAST showed specific IgE to moulds in five patients, who were skin-test neg/RAST neg by the previous technique. The largest improvement of correlation obtained by using the new RAST was observed when using dog and horse dander. However, one should stress that the number of comparisons for these allergens was smaller.

Table 4 shows that the proportion of patients with specific IgE for at least one allergen, and whose total IgE concentration was higher than 100 PRIST units was 16% with the previous technique and 31% with the new one. The use of the RAST induced only a slight increase in the proportion of positives among patients with low values of total IgE. Thus, the total correlation between high total IgE concentration and RAST positivity increased by 15%, when the new technique was used.

Table 1. Comparison of the results simultaneously obtained by the previous (P) and the new (N) RAST on the same serum samples

		Discordant results													
		P < N							P > N						
		Nb of comparisons		Identical results		P	N	P	N	P	N	Increase in positives due to new RAST (%)		P	N
Allergens						0	+	0	+	+	+	+	+	+	+
House dust															
h ₁		82	60	12	4	4		4					24	2	2
h ₂		84	61	7	3	3		3					27	0	0
<i>D. pteronyssinus</i>		84	56	7	0	0		7					31	2	2
<i>D. farinae</i>		74	59	4	2	2		2					19	1	1
Moulds		47	33	6	4	4		1					26	2	2
Cat dander		30	21	5	1	1		3					30	0	0
Dog dander		26	21	1	2	2		2					19	0	0
Horse dander		18	9	4	0	0		4					50	0	0
Total		445	320	46	16	16		26						7	7
Percentage		100	71.9	10.3	3.6	3.6		5.8					26.4	1.6	1.6

Table 2. Comprehensive correlation between skin-tests and RAST (previous and new), calculated on 359 comparisons, considering all the allergens on the whole

Skin tests	Previous RAST (%)		New RAST (%)	
	Positive	Negative	Positive	Negative
Positive	10	24	16	16
Negative	2	64	4	64
Concordance*	74 %		80 %	

* The concordance is given by the addition of the proportion of cases skin-tests pos/RAST pos and those skin-tests neg/RAST neg.

Table 3. Correlation between skin-tests and RAST (previous and new), obtained for each allergen

Allergens	n	Concordance (%)*		
		Previous	New	Variation
House dust	81	60	72	+12
<i>D. pteronyssinus</i>	84	80	88	+8
<i>D. farinae</i>	74	89	89	0
Moulds	47	92	84	-8
Cat dander	30	57	69	+12
Dog dander	25	68	82	+14
Horse dander	18	42	61	+19
Total	359	74	80	+6

* Defined under Table 2.

Table 4. Correlation between the concentration of total IgE and the presence of specific IgE, as demonstrated by previous or new RAST, in eighty-one patients

Percentage of patients with:	Previous RAST		New RAST	
	Positive*	Negative	Positive*	Negative
IgE > 100 PRIST units	16	30	31	13
IgE < 100 PRIST units	10	44	12	44
Concordance†	60%		75 %	

* Positivity for one allergen at least.

† The concordance is given by the addition of the proportion of cases with IgE > 100 PRIST units/RAST pos. and those with IgE < 100 PRIST units/RAST neg.

Discussion

Our results indicate that the use of the more specific anti-IgE antibody clearly improved the sensitivity of the RAST: in 26% of the comparisons made on the same serum samples, a higher frequency of positive RAST results was obtained with the new antibody. In 19% of these 26%, results which were considered as negative with the previous RAST (0 or one +), became clearly positive with the new technique (2+ or more). If, according to the manufacturer, the (1+) results obtained with the new technique can be also considered as clearly positive the net improvement is 26%.

The new technique also improved by 6% the overall correlation between skin-tests and RAST. This overall improvement of the correlation is relatively low, but corresponds to the average calculated from values which were very different according to the individual allergens. The correlation was improved, for instance, by 12, 14 and 19% for the three animal danders studied. Several authors (Berg & Johansson, 1974; Eriksson, 1977; Wüthrich & Arrendal, 1979) mentioned the poor correlation between skin-tests to animal danders and RAST. Thus, the improvement of the correlation obtained with the new RAST technique constitutes progress, with regards to the investigation of these allergens. For house dust, one of the allergens most frequently tested in this study, the correlation between skin-tests and RAST was 60% with the previous and 72% with the new RAST. On the whole, these figures are similar to those obtained by others: 59% for instance by Aas & Johansson (1971) and 68% by Virchow, Roth & Moller (1976). Surprisingly the degree of correlation of skin-tests/RAST decreased when the new technique was applied to the moulds: the new technique detected specific IgE in five patients, which were skin-test neg/previous RAST neg. One might argue that, in our patients, the skin-tests might have been false negative, since performed with mixtures of moulds. Thus, the better correlation obtained with the previous RAST might be artificial.

The overall correlation of 80%, that we observed between skin-tests and RAST (new antibody), is similar to values observed by others (Ahlstedt *et al.*, 1974; Apold *et al.*, 1974; Berg & Johansson, 1974; Pepys, Roth & Carrol, 1975). However, in our study, and unlike other reports, the results were obtained on patients with non-seasonal symptoms and with allergens other than pollens, and, in most of these studies, pollens gave the best correlation scores.

Finally, this study shows that, in patients with elevated total IgE, the new RAST technique detects specific IgE twice as often as the previous one, without detecting specific IgE more frequently in patients with low total IgE. Thus, the correlation between elevated total IgE and the presence of specific IgE, which was 60% with the previous technique, is 75% with the new one. The level of this correlation is satisfactory, if one considers that total IgE can be elevated for non allergic reasons (Wüthrich & Kopper, 1974), and on the other hand, patients allergic to a small number of allergens may lack an elevation of total IgE (Wide, Bennich & Johansson, 1967).

In conclusion, these results show that the use of a more specific anti-IgE antibody increased the sensitivity of RAST, even in patients with non seasonal conditions, and improved the correlation with the skin-tests.

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