

The Diagnosis of Immediate Type Allergy to *Cladosporium herbarum*

Differences between Extracts and the Efficacy of Radioallergosorbent Test (RAST) Assays

L. AUKRUST and K. AAS

The Allergy Unit, Pediatric Research Institute, Rikshospitalet, University Hospital and the Allergy Institute, Voksentoppen, Oslo, Norway

The radioallergosorbent test (RAST) was used to demonstrate IgE antibodies to *Cladosporium herbarum* in 35 children. Freeze-dried samples of *Cladosporium herbarum* were obtained from two commercial producers, extracted and used for the preparation of two RAST disc series which were compared with the commercially available Phadebas *Cladosporium herbarum* discs. Marked qualitative differences were found.

Key words: *Cladosporium herbarum*; diagnosis; hyposensitization; moulds; radioimmunoassay.

Accepted for publication 22 August 1977

In clinical practice *Cladosporium herbarum* allergy is difficult to handle. Case histories are often of limited value for mould allergy in general. The biological activity of commercially available extracts is often low and may vary considerably from batch to batch. The diagnosis is often complicated by the presence of unspecific irritants causing false positive reactions (12). Furthermore, extracts of *Cladosporium herbarum* are very complex, e.g. in one extract 57 precipitates were demonstrated by means of crossed immunoelectrophoresis (10).

The radioallergosorbent test (RAST) has been shown to be a valuable tool for the diagnosis of IgE-mediated allergies (1, 3, 5, 7) since the reliability of RAST in predicting specific clinical allergy is equal to that of skin prick testing (SPT) under optimal conditions. Previous studies have demonstrated that SPT and RAST correlate fairly well when the same extracts are used in both tests (7, 8). The precision, sensitivity and reliability of both tests are, however, highly dependent upon the quality of the allergenic material

used (2, 5). The present study was undertaken to investigate to what extent differences in extract type and quality influence the diagnostic sensitivity and reliability of RAST in the diagnosis of allergy to *Cladosporium herbarum*.

MATERIAL AND METHODS

Cladosporium herbarum extracts

Lyophilized *Cladosporium herbarum* (mycelium and conidia) were obtained from two commercial suppliers, Allergon AB (Engelholm, Sweden) and the Allergologisk Laboratorium (Copenhagen, Denmark). Extracts were prepared as described in detail earlier, i.e. basically, by a 24-h agitation at +5°C in a 10% (w/v) solution followed by centrifugation, dialysis and lyophilization (10). The extracts were denoted Ch-Allergon and Ch-ALK, respectively. The protein content of the two preparations was approximately 28% and 20% of the dry weight, respectively, as measured according to Lowry *et al.* (9) using human serum albumin as standard.

Table 1

Non-hyposensitized patients. Total serum IgE levels and RAST results with three different series of *Cladosporium herbarum* RAST discs. X_1 and X_2 represent the highest (X_1) and lowest (X_2) of the two single determinations in each duplicate assay

Patients	Total serum IgE in $\text{U} \times \text{ml}^{-1}$	RAST results in per cent uptake of ^{125}I -anti-IgE					
		Ch-Allergon		Ch-ALK		Phadebas m2	
		$X_1 + X_2$ 2	$X_1 - X_2$ 2	$X_1 + X_2$ 2	$X_1 - X_2$ 2	$X_1 + X_2$ 2	$X_1 - X_2$ 2
K.M.	6200	51.8	(1.8)	49.3	(0.1)	16.4	(2.2)
N.A.H.	5600	7.1	(0.7)	5.6	(0)	2.6	(0.1)
A.J.Ø.	4000	13.0	(0.3)	8.2	(0.1)	14.1	(0.4)
G.I.	2000	43.2	(1.8)	7.3	(1.4)	17.0	(0)
N.M.F.	1680	40.6	(1.5)	41.1	(0.8)	9.4	(2.0)
B.L.	1520	36.2	(0.9)	29.5	(0)	3.5	(0.3)
T.A.	1155	42.5	(1.5)	1.3	(0)	4.7	(0.5)
D.H.	1120	14.7	(1.7)	3.0	(0)	5.0	(0)
T.V.B.	980	22.5	(0.5)	14.7	(0.5)	8.4	(0.2)
T.N.I.	920	4.5	(0.3)	3.8	(0.1)	2.3	(0.1)
B.R.S.	720	47.3	(0.8)	2.3	(0)	5.3	(0.2)
T.L.M.	560	26.7	(0.7)	25.0	(1.7)	4.5	(0.4)
O.J.H.	520	40.4	(0.6)	31.0	(0.9)	5.9	(0.8)
G.N.	500	10.9	(0.2)	5.4	(1.0)	1.1	(0.4)
T.M.	280	9.8	(0.5)	6.1	(0)	2.2	(0.1)
T.H.	280	36.3	(0.1)	30.5	(1.1)	0.9	(0.2)
S.R.B.	210	32.3	(0.7)	1.6	(0.4)	3.1	(0.3)
B.T.	200	6.4	(0.2)	6.2	(0.9)	0.7	(0.1)
G.V.	130	18.1	(0.7)	10.4	(0.6)	3.7	(0.2)
C.S.T.	90	4.0	(0)	0.3	(0)	0.1	(0)
Mean	1483	25.4		14.1		5.5	

Patient sera

Serum was obtained from 35 paediatric patients with suspected clinical allergy towards *Cladosporium herbarum*. They all had case histories suggestive of mould allergy, and 20 of them elicited positive SPT to a commercial *Cladosporium herbarum* extract from Allergologisk Laboratorium (Copenhagen, Denmark) at the time of venipuncture. Fifteen patients had received hyposensitization treatment with mould extracts for more than 1 year.

Radioallergosorbent test (RAST)

Phadebas® m2 *Cladosporium herbarum* discs were obtained from Pharmacia (Sweden).

The Ch-Allergon and Ch-ALK extracts were coupled to CNBr-activated paper discs according to Yman *et al.* (13) using $5 \text{ g} \times 1^{-1}$ (dry weight) solutions of the extracts. RAST assays were performed with $50 \mu\text{l}$ serum samples employing all three types of paper discs described. Results were expressed as uptake of ^{125}I -anti-IgE in per cent of the total amount applied, and in RAST classes as recommended by the Phadebas manufacturer. Although the Phadebas manufacturer recommends RAST class 1 to be considered positive we preferred to regard this as a border class (doubtful) between clearly positive and clearly negative, according to clinical practice (1, 12). All results were means of duplicate analyses.

Table 2

Hyposensitized patients. Total serum IgE levels and RAST results with three different series of *Cladosporium herbarum* RAST discs. X_1 and X_2 represent the highest (X_1) and lowest (X_2) of the two single determinations in each duplicate assay

Patients	Total serum IgE in $U \times ml^{-1}$	RAST results in per cent uptake of ^{125}I -anti-IgE					
		Ch-Allergon		Ch-ALK		Phadebas m2	
		$X_1 + X_2$	$X_1 - X_2$	$X_1 + X_2$	$X_1 - X_2$	$X_1 + X_2$	$X_1 - X_2$
		$\frac{2}{2}$	$\frac{2}{2}$	$\frac{2}{2}$	$\frac{2}{2}$	$\frac{2}{2}$	$\frac{2}{2}$
P.O.G.	5400	58.9	(0.5)	51.0	(0.8)	26.8	(1.6)
E.N.	3800	50.6	(1.0)	58.7	(0.6)	12.9	(1.8)
J.E.A.	3600	49.6	(1.5)	50.2	(0.4)	11.7	(1.2)
G.A.G.	3000	51.6	(0.3)	45.5	(1.0)	12.8	(0.4)
L.M.K.	2500	34.1	(1.4)	27.1	(0.8)	6.3	(0.5)
A.C.L.	1880	43.9	(1.9)	41.2	(0.5)	16.2	(0.3)
F.T.	1380	34.5	(2.1)	10.8	(0)	5.5	(0.5)
J.E.T.	1200	6.8	(0.3)	2.2	(0)	1.4	(0)
N.R.	1000	42.5	(0.6)	26.8	(0.2)	3.6	(0.8)
E.R.	780	30.9	(2.9)	16.7	(0.6)	2.8	(0.2)
F.H.	720	45.7	(0.3)	15.3	(0.6)	8.6	(0.1)
S.T.	580	18.6	(0)	6.5	(0.3)	7.2	(0.4)
K.S.J.	420	10.5	(2.0)	9.7	(0.1)	1.3	(0.2)
K.I.S.	210	20.0	(2.5)	11.2	(1.0)	4.7	(0.2)
T.N.	180	36.0	(2.0)	17.5	(0.7)	3.7	(0.3)
Mean	1790	35.3		25.7		8.4	

Radioimmunosorbent test (RIST)

Total IgE levels were estimated by means of Phadebas® RIST kits (Pharmacia, Sweden). Results were given in $U \times ml^{-1}$ (1 U corresponds to 2.4×10^{-9} g IgE).

RESULTS

Preliminary results demonstrated a $5 g \times l^{-1}$ dry weight allergen extract concentration to be optimal for coupling to the CNBr-activated discs. The RAST and RIST results are summarized in Tables 1 and 2. The results obtained with the Ch-Allergon and the Phadebas m2, the Ch-ALK and the Phadebas m2, and finally the Ch-Allergon and the Ch-ALK, respectively, are compared in Figs. 1-3.

The Phadebas m2 discs showed a

maximum isotope uptake of 26.8%, whereas both the Ch-Allergon and Ch-ALK discs gave rise to maximum uptakes of more than 50%. The mathematical means of the isotope uptakes obtained with the 35 sera were 29.6, 19.1 and 6.7% for the Ch-Allergon, Ch-ALK and Phadebas m2 discs, respectively. RAST class 2 and higher were found in 35, 29 and 23 patients with the Ch-Allergon, Ch-ALK and Phadebas m2 discs, respectively. With RAST class 1 included as "positive", the results were 35, 32 and 29 "positive" patients, respectively. Thirty-two of the patient sera gave higher uptakes of isotope on the Ch-Allergon discs than on the Ch-ALK discs whereas the reverse was never observed. Three sera, B.R.S., S.R.B. and T.A., were negative with the Ch-ALK discs while the isotope uptake exceeded 32% with the Ch-Allergon discs.

In the hyposensitized group of patients the average total serum IgE levels were 21%

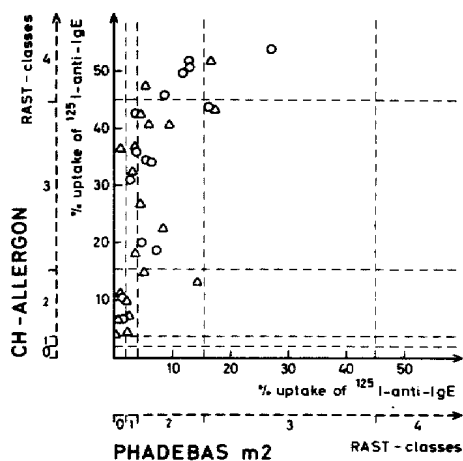


Fig. 1. Comparison of *Cladosporium herbarum* RAST assays using Ch-Allergen and Phadebas m2 paper discs. O, hyposensitized patients; Δ, non-hyposensitized patients. Broken lines indicate the RAST classes.

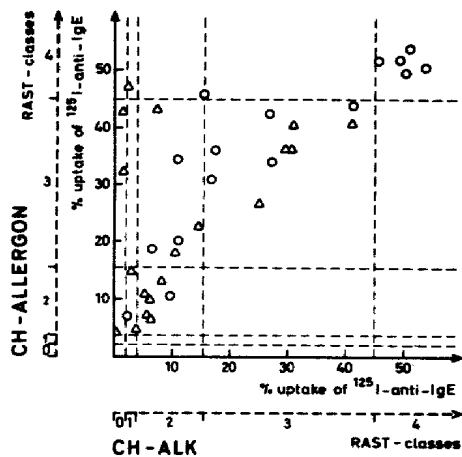


Fig. 3. Comparison of *Cladosporium herbarum* RAST assays using Ch-Allergen and Ch-ALK paper discs. O, hyposensitized patients; Δ, non-hyposensitized patients. Broken lines indicate the RAST classes.

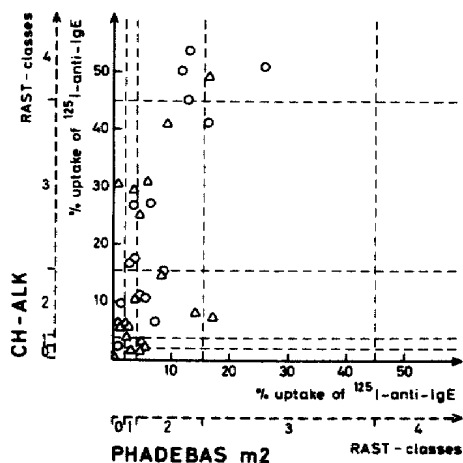


Fig. 2. Comparison of *Cladosporium herbarum* RAST assays using Ch-ALK and Phadebas m2 paper discs. O, hyposensitized patients; Δ, non-hyposensitized patients. Broken lines indicate the RAST classes.

higher than in the non-hyposensitized group. The hyposensitized group of patients also had higher serum levels of specific IgE against *Cladosporium herbarum*, i.e. 39, 82 and 53% higher on the Ch-Allergen, Ch-ALK and Phadebas m2 discs, respectively.

DISCUSSION

The results obtained with the commercial Phadebas m2 *Cladosporium herbarum* discs are in agreement with those obtained with Phadebas m2 discs in clinical routine (12). High positive *Cladosporium herbarum* RAST results are rarely found with these discs, and negative results do not indicate absence of clinical allergy towards *Cladosporium herbarum*.

The present results with the Ch-Allergen and the Ch-ALK discs show that it is possible to prepare *Cladosporium herbarum* RAST allergosorbents of considerably better quality than the Phadebas m2. In light of this, it is regrettable that, due to a patent, Phadebas m2 are the only commercially available *Cladosporium herbarum* RAST allergosorbents.

The results also demonstrate variations

among the different discs employed. This suggests qualitative differences between the allergosorbents. The observed differences were probably due to differences in the original fungal material, since the Ch-Allergon and Ch-ALK material were extracted identically according to a procedure which gives reproducible results (10). These data stress the importance of employing reproducible techniques for the growth of the mould as well as for the extract preparation. They also confirm previous reports in that the allergen extract quality is critical for the reliability of results and for the relevance of comparative work applying such material (2, 5).

The importance of the different *Cladosporium herbarum* allergens also varies from patient to patient (6). Difficulties have been observed earlier in preparing high quality *Cladosporium herbarum* RAST discs (10). The main reason for this may probably be that allergens important to the patient sera used were lacking in the extracts employed.

The definition of a major allergen has been based on the statistical term that all or the majority of patients are sensitive to this particular allergenic component (4). The data presented suggests that the Ch-ALK extract was deficient in at least one important *Cladosporium herbarum* allergen that was present in the Ch-Allergon extract, as illustrated by the higher isotope uptake obtained on the Ch-Allergon discs than on the Ch-ALK discs for some patients (Fig. 3). This shows that at least one essential *Cladosporium herbarum* allergen exists which may not be present in all crude extracts of the mould. At this stage it can not be excluded that this may even be a major allergen.

Sera from the hyposensitized group of patients generally showed higher isotope uptakes than the non-hyposensitized group. This seems reasonable considering the fact that the patients given hyposensitizing treatment are usually the ones suffering from the most pronounced form of *Cladosporium herbarum* allergy. Furthermore, it is well es-

tablished that there is a rise in specific IgE antibodies during the initial phase of hyposensitization. It appears from the results of this study, however, that the extracts used for hyposensitization may not always contain all the relevant allergens for all the patients in question. This is illustrated by the fact that patients F.H., S.T. and F.T. were hyposensitized with a commercial *Cladosporium herbarum* extract from the Allergologisk Laboratorium (Copenhagen, Denmark) produced from the same sort of original material as Ch-ALK. Although it is not known whether the Ch-ALK extract prepared for this study and the commercial *Cladosporium herbarum* extracts from the Allergologisk Laboratorium were identical, it is noticeable that the isotope uptake obtained with sera from these patients was three times higher with the Ch-Allergon as compared with the Ch-ALK discs. The possibility also exists that specific IgE antibodies against proteins in the Ch-ALK extract, though originally present in higher concentrations, were greatly reduced due to an effect of the hyposensitizing treatment. The latter explanation is doubtful, however, considering that high Ch-Allergon RAST results were found also in the non-hyposensitized group of patients.

The data suggest that when hyposensitizing treatment with *Cladosporium herbarum* is considered, it is of crucial importance to check that the extract to be used really contains the important allergens for the patient in question. This is illustrated by the three patients, T.A., B.R.S. and S.R.B. Hyposensitizing treatment of these patients with a Ch-Allergon type extract would seem relevant, while a Ch-ALK type extract would seem meaningless since no specific IgE antibodies against Ch-ALK were detectable in their sera. Hyposensitizing treatment with non-relevant allergens should be avoided. There is reason to suspect that a number of antigenic proteins may induce IgE antibody synthesis by excessive parenteral stimulation as observed even in non-allergic individuals (11).

In our hands the Ch-Allergen RAST discs were the most effective ones for diagnostic purposes in the patients studied. In spite of this, the possibility cannot be ruled out that even the Ch-Allergen extract and discs may be deficient in some *Cladosporium herbarum* allergens. Until further information is available, it is necessary to conclude that whichever *Cladosporium herbarum* extract is used, one should be aware of possible negative RAST assays, even when the serum of the patient contains *Cladosporium herbarum* specific IgE antibodies.

ACKNOWLEDGEMENTS

This work was supported in part by grants from The Research Foundation of The Norwegian Asthma and Allergy Society and from the H. Chr. Paulsen Research Fund, The Norwegian Association for Allergy and Immunopathology. The skilful technical assistance of Trine-Lise Almeland is acknowledged.

REFERENCES

1. Aas, K.: The radioallergosorbent test (RAST): Diagnostic and clinical significance. *An. Allergy* 33, 251-255, 1974.
2. Aas, K.: Clinical and experimental aspects of standardization and purification of allergens. *Int. Arch. Allergy Appl. Immunol.* 49, 44-54, 1974.
3. Aas, K.: Diagnosis of immediate type respiratory allergy. *Pediatr. Clin. North Am.* 22, 33-42, 1975.
4. Aas, K.: Common characteristics of major allergens. In Johansson, S. G. O., Standberg, K. & Uvnäs, B. (eds.): *Molecular and biological aspects of the acute allergic reaction*, pp. 3-22. Plenum, New York, 1976.

5. Aas, K. & Johansson, S. G. O.: The radioallergosorbent test in the *in vitro* diagnosis of multiple reaginic allergy. *J. Allergy Clin. Immunol.* 48, 134-142, 1971.
6. Aukrust, L. & Aas, K.: Unpublished observations.
7. Berg, T. & Johansson, S. G. O.: Allergy diagnosis with the radioallergosorbent test. A comparison with the results of skin and provocation tests in an unselected group of children with asthma and hay fever. *J. Allergy Clin. Immunol.* 54, 209-221, 1974.
8. Haynen, J., Apold, J., Hvatum, M., Oseid, S. & Aas, K.: The radioallergosorbent test in the *in vitro* diagnosis of multiple reaginic allergy. *Clin. Allergy* 4, 411-420, 1974.
9. Lowry, O. H., Rosebrough, N. J., Fair, A. L. & Randall, R. J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265-275, 1951.
10. Løwenstein, H., Aukrust, L. & Gravesen, S.: *Cladosporium herbarum* extract characterized by means of quantitative immunoelectrophoretic methods with special attention to immediate type allergy. *Int. Arch. Allergy Appl. Immunol.* In press.
11. Marsh, D. G.: Allergens and the genetics of allergy. In Seal, M. (ed.): *The antigens*, vol. 3, pp. 271-359. Academic Press, New York, 1975.
12. Nilsson, D. & Aas, K.: Immunological specificity and correlation of diagnostic tests for bronchial allergy to *Cladosporium herbarum*. *Acta Paediatr. Scand.* 65, 33-38, 1976.
13. Yman, L., Ponterius, G. & Brandt, R.: RAST-based allergen assay methods. *International WHO-IABC Symposium on Standardization and Control of Allergens Administered to Man*, (Geneva). *Dev. Biol. Stand.* 29, 151-165. Karger, Basel, 1975.

Address:

Lars Aukrust
The Allergy Unit
Pediatric Research Institute
Rikshospitalet
University Hospital
Oslo
Norway

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.