

Diagnosis and Immunotherapy of Mould Allergy

I. Screening for Mould Allergy

H.-J. MALLING,¹ B. AGRELL,⁵ S. CRONER,² S. DREBORG,^{4,5} T. FOUCARD,⁴
M. KJELLMAN,² A. KOIVIKKO,³ A. ROTH⁵ and B. WEEKE¹

¹ University Hospital, Copenhagen, Denmark, ² University Hospital, Linköping, Sweden,

³ University Hospital, Turku, Finland, ⁴ Academic Hospital, Uppsala, Sweden

and ⁵ Pharmacia Diagnostics AB, Uppsala, Sweden

In order to screen for mould allergy, extracts of five common atmospheric moulds (*Cladosporium*, *Alternaria*, *Penicillium*, *Aspergillus* and *Mucor*) from various manufacturers were investigated in 130 patients (5–60 years old) with clinical symptoms indicating possible mould allergy. The patients were screened by skin prick test (SPT) and radioallergosorbent test (RAST). SPT seemed to be more sensitive than RAST as a diagnostic screening procedure (80% positive reactions to one or more species compared to 50%). With a partially purified, standardized preparation of *Cladosporium herbarum* more positive reactions were obtained than with crude extracts without evidence of any unspecific reactions. The difference between commercial and standardized extracts is most probably a result of a variation of both the biological potency of allergenic determinants and the allergenic composition. A considerable number of negative RAST reactions with standard discs were found in patients with positive skin reactions to partially purified *Cladosporium*, but RAST seemed to be more sensitive than SPT with the other commercial mould extracts. Based on the screening, a very convincing tendency to IgE-reactivity to other moulds was found in patients reacting to *Cladosporium*, the most common cause of mould allergy. The results confirm the inadequacy of most mould extracts used in diagnostic procedures and strengthen the value of using standardized extracts.

Key words: diagnosis; immediate hypersensitivity; mould allergy; radioallergosorbent test; skin prick test.

Accepted for publication 22 August 1984

CLINICAL ASPECTS

The frequency of clinical allergy to mould spores, based on the diagnostic ability of commercial mould extracts, might be considerably underestimated. In diagnostic procedures the value of a partially purified and standardized extract is documented. With the use of this extract the skin prick test seems to be more sensitive as a screening procedure than the radio-allergosorbent test.

Respiratory allergy to moulds has been known for many years (13), but, as pointed out by Nilsson & Aas (11) and Aas et al. (2), a specific diagnosis can be difficult to obtain. This is probably because the commercial extracts available for diagnostic procedures have been unreliable (2). Investigations of mould allergy have only been carried out in children. In contrast to

other inhalant allergens, mould extracts tend to have a low allergenic activity and vary considerably from batch to batch (13) because of their complexity and heterogeneity. Furthermore, there may be toxins and irritants which elicit unspecific false positive reactions without clinical implication.

In recent years investigations using highly

specific qualitative and quantitative immunological procedures (quantitative immunoelectrophoresis, crossed radioimmuno-electrophoresis, etc.) on mould allergen extracts have been carried out by Aukrust (5, 6). When a partially purified, lyophilized extract of *Cladosporium* earlier studied by Aukrust et al. (2, 5, 6) was made available we decided to study mould allergy further.

The present study was initiated in order to screen patients, with a suspected clinical allergy to moulds, as candidates for further studies, i.e. to document the diagnostic capacity as well as the therapeutic efficacy of immunotherapy with a new generation of mould extracts. The diagnostic capacity of mould extracts in adults was also of interest.

MATERIAL AND METHODS

Patients

A total of 130 patients, 68 children from three paediatric clinics in Finland and Sweden and 62 adults from one clinic in Denmark, were included. Patient data are shown in Table 1. The patients were screened on the basis of a clinically suspected mould allergy defined either as start or worsening of asthma and/or rhinoconjunctivitis during the peak mould season (June to September), or aggravation of symptoms in mould-rich environment (high temperature and humidity) recovering in dry environment.

Table 1

Clinical data of patients participating in the mould allergy screening

	Center	Number	Sex		Age (years)
			♀	♂	
Children	1	35	14	21	7-15
	2	15	5	10	7-15
	3	18	6	12	5-16
Adults	4	62	30	32	16-60

Allergen extracts

Commercial extracts from five common atmospheric moulds were obtained from three different producers (A, B, and C). The mould panel consisted of *Cladosporium herbarum*, *Alternaria tenuis*, *Mucor racemosus*, *Penicillium notatum* and *Aspergillus fumigatus*. Extracts from two of the manufacturers were dispensed in 50% glycerol and another panel of aqueous extracts was used. All extracts were tested in the highest concentration available.

A partially purified, standardized, lyophilized gen[®], Nyco, Oslo, Norway, and Pharmacia, Uppsala, Sweden) in two concentrations (1,000 and 5,000 BU/ml) was tested simultaneously. 5,000 BU/ml) was tested simultaneously.

Histamine chloride 1 mg/ml (5.43 mmol/l) and albumin diluent were used as positive and negative controls respectively.

Skin prick testing

The skin prick tests (SPT) were carried out as single tests on the volar side of the forearm as previously described (9, 12). The wheal reaction was recorded at maximum size, i.e. for histamine after approximately 10 min and for allergen after 15 min. The outline of the wheal (not flare) was marked and the result transferred by means of tape to a record sheet. Wheal reactions were evaluated in relation to a histamine reference (1 mg/ml) as recommended by the Nordic Society of Allergology (16). The mean diameter (1/2 longest + 1/2 perpendicular) was used as an expression of the wheal area and the reaction graded in the + - system (1, 16). SPT reactions - were regarded as "negative", +1 and +2 as "inconclusive" (positive), and +3 or larger as (unambiguous) "positive". Children were only tested with extracts from manufacturers A and B and Spectralgen.

Patient serum

Venous blood was drawn immediately before skin testing. Serum was separated and stored at -20°C until analyzed.

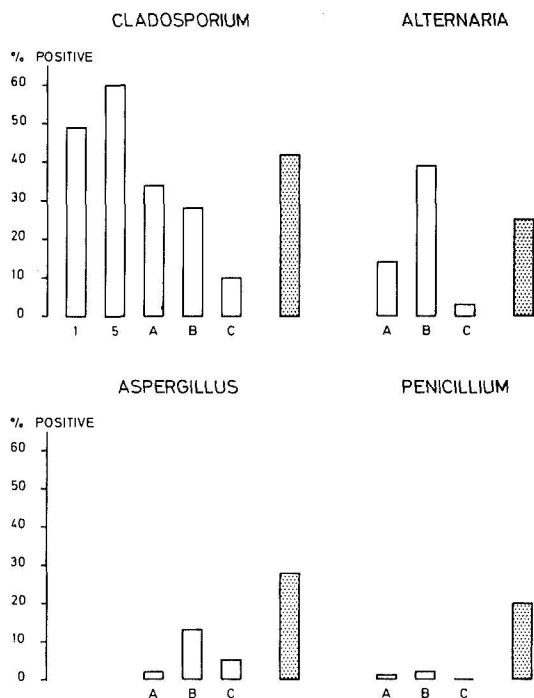


Fig. 1. Screening for mould allergy with various mould species. The number of unambiguous positive reactions, i.e. SPT $\geq +3$ (open columns) and RAST $\geq$ class 2 (hatched columns) are plotted as a function of different mould preparations. 1 and 5 represent Spectralgen 1,000 and 5,000 BU/ml respectively and A, B, and C are commercially obtained extracts from three different manufacturers.

Radioallergosorbent test (RAST)

RAST was performed in duplicate, with standard discs according to the procedure recommended by the manufacturer (Phadebas RAST®, Pharmacia, Uppsala, Sweden), against *Cladosporium*, *Alternaria*, *Aspergillus* and *Penicillium* and the result was expressed in RAST class 0–4. RAST class 0 was regarded as “negative”, class 1 as “inconclusive”, and class 2–4 as “positive”.

RESULTS

Eighty percent of the screened patients had a “positive” ($\geq +3$) SPT to at least one of the five different species of mould extracts. A “positive” RAST was found in 52% of the patients. As is evident from Fig. 1 there was a considerable difference in the number of “positive” reactions obtained in SPT and RAST with extracts of different moulds. Furthermore, mould extracts of the same species from different manufacturers showed wide variations in the number of “positive” SPT.

Based on SPT results *Cladosporium* seemed to be the most common cause of skin reactions (amounting to 60% of tested patients in the

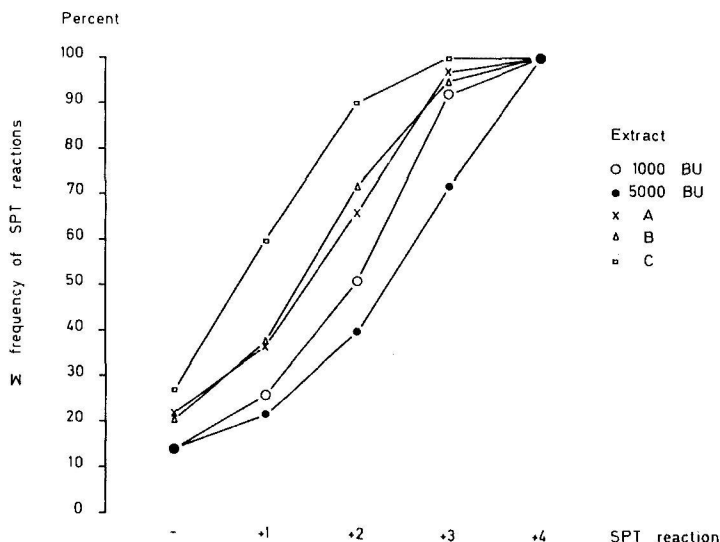


Fig. 2. The cumulated frequency of SPT reactions (- to +4) to *Cladosporium* Spectralgen 1,000 and 5,000 BU/ml and extracts from manufacturers A, B, and C.

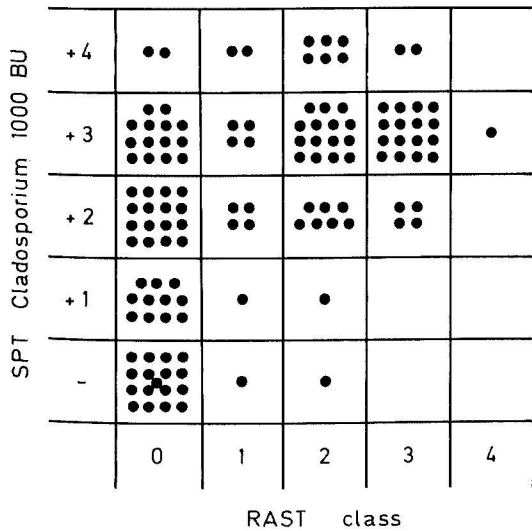


Fig. 3. The relation between the degree of reactivity to *Cladosporium* estimated by SPT (reaction - to +4) using Spectralgen 1,000 BU/ml and by RAST (Phadebas RAST class 0-4).

present study) followed by *Alternaria* (39%), *Mucor* (14%), *Aspergillus* (13%), and *Penicillium* (2%) based on tests with the most potent extract (results on *Mucor* not shown). The corresponding figures obtained with RAST were: *Cladosporium* 42%, *Aspergillus* 28%, *Alternaria* 25%, *Penicillium* 20%, (*Mucor* not tested) (Fig. 1).

The mould extracts from different manufacturers varied widely in their capacity to elicit SPT reactions. With *Cladosporium* Spectralgen 1,000 and 5,000 BU/ml a "positive" SPT was obtained in 49% and 60% of the patients, but with extract C only in 10%. The degree of reactions was expressed by relating the wheal area of the allergen to the area of a histamine reference. The cumulated frequency of reactions (- to +4) to *Cladosporium* is shown in Fig. 2. With extracts from manufacturer C 90% of the patients had a "negative" or "inconclusive" reaction. A shift towards more positive reactions was observed with manufacturers A and B. A further decrease of "negative" and "inconclusive" reactions and an increase of "positive" reactions was observed with Spectralgen 1,000 BU/ml and even more so with 5,000 BU/ml.

The relation between SPT and RAST was investigated with Spectralgen 1,000 BU/ml.

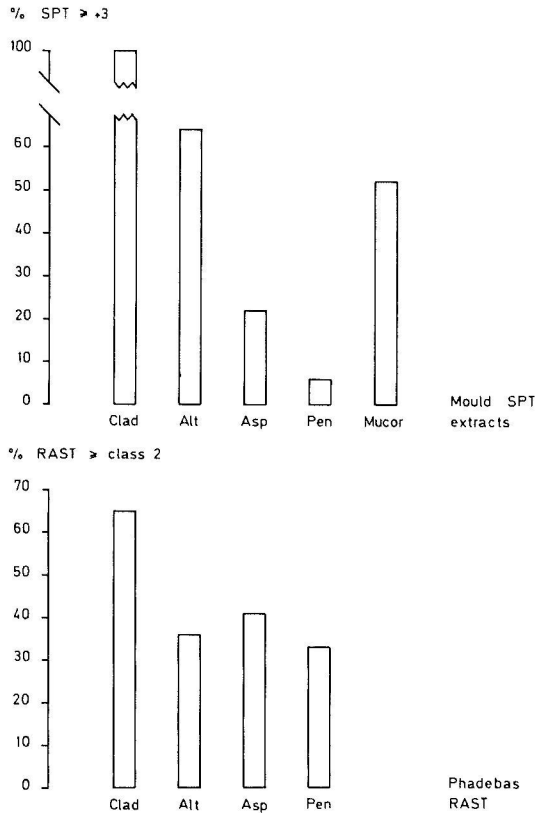


Fig. 4. The frequency of associated IgE-reactivity (SPT ≥ +3, RAST ≥ class 2) to *Alternaria*, *Aspergillus*, *Penicillium*, and *Mucor* in patients with a positive (SPT ≥ +3) reaction to *Cladosporium* 1,000 BU/ml.

Corresponding values of skin reactivity (+-system) were plotted against the RAST class (Fig. 3). It is evident that more than one third of clearcut "positive" SPT (≥ +3) had a "negative" or "inconclusive" RAST (class 0 or 1). On the other hand, about one fourth of the patients with a positive RAST (class 2-4) had an inconclusive SPT (+1 and +2), but only one was "negative" (-).

The degree of reactivity to other moulds in patients SPT-"positive" to *Cladosporium* was also investigated. Patients with a "positive" SPT reaction (≥ +3) to *Cladosporium* 1,000 BU/ml showed an associated "positive" SPT (≥ +3) with the other tested extracts: for *Alternaria* 64%, *Aspergillus* 22%, *Penicillium* 6%, and for *Mucor* 52%. Circulating specific IgE antibodies

as measured with RAST in patients with a "positive" SPT to *Cladosporium* 1,000 BU/ml were found in 62% (*Cladosporium*), 36% (*Alternaria*), 41% (*Aspergillus*), and 33% (*Penicillium*) (*Mucor* not tested) (Fig. 4). The frequency of associated "positive" reactions to other moulds was even higher when only patients with a "positive" RAST to *Cladosporium* were considered. "Positive" reactions were found in 46% with *Alternaria*, 52% *Aspergillus*, and 46% with *Penicillium*.

DISCUSSION

Airborne mould spores are found in far higher concentrations than pollen grains (7, 13). In spite of this only few patients are considered as having a clearcut allergy to moulds. This may partly be due to the poor quality of the mould extracts used for testing (2, 3, 4, 15), but there may also be a lower tendency to develop allergy to moulds than to pollen. Although mould allergy is, quantitatively, a minor problem, patients with clinical disease in the mould season tend to have severe symptoms. In contrast to pollen, patients reacting to *Cladosporium* very often have asthma, a phenomenon due probably to the small size of the mould spores (13).

The mould spore load is primarily caused by high outdoor concentrations in high temperature and humidity (7). Outdoor spores are impossible to eliminate and the only way to alleviate the symptoms of mould allergic patients is either by medical treatment or immunotherapy.

The clinical efficacy of specific mould immunotherapy has not been convincingly documented and this may partly be due to the quality of the extracts available for immunotherapy. Before immunotherapy is considered, a precise, reliable, and specific diagnosis is mandatory, and the problems involved in establishing this diagnosis have been clearly pointed out (2, 11, 16).

In the present study SPT and RAST were used as screening tests. Providing a potent and allergenically representative extract is used, these parameters could give very useful diagnostic information (2). To be used for screening a test must be easy to handle, inexpensive,

and highly sensitive. The specificity is less important than the sensitivity as the test must "catch" all patients suspected of allergy. Patients with a false positive reaction may be disclosed later by the use of more specific tests (16). This study is not an attempt to evaluate the results of SPT and RAST in relation to the "true" diagnosis, but only to investigate the difference in numbers between patients with positive reactions to mould extracts from different manufacturers.

The patients were included in this screening investigation on the basis of a case history of suspected mould allergy of clinical importance. The results of the SPT and RAST indicate that patients with a history compatible with mould allergy have a high degree of IgE antibody directed against mould allergens. In order to ensure that reactions of doubtful nature were not regarded as evidence of mould allergy, only wheals equal to or larger than the histamine reference were accepted as "positive" (1). About 80% of the patients tested had a "positive" SPT to one or more of the examined species and extracts. The very strict criterion was used because of the possibility of unspecific reactions due to irritant content (7, 13). Although with the use of a purified and standardized extract a +1 reaction seemed to be immunologically specific (2) the same criterion was used for all extracts.

The present study does not evaluate whether the positive SPT is elicited by toxins or irritants, although the high number of negative reactions would seem to indicate that this is not so important. However, there may be an individual difference in the susceptibility to unspecific reactions in some of the SPT-positive patients. But the finding that an increase of the allergen concentration from 1,000 to 5,000 BU/ml only results in a higher frequency of +4 reactions (Fig. 2), and does not convert a negative (−) reaction to a positive ($\geq +1$) does not support this possibility. The sensitivity of RAST in screening for mould IgE-antibodies was not as high as that of SPT as only 50% of the patients had a class 2 reaction, or higher, and only very few a class 4. The tendency towards low levels of circulating specific IgE estimated by RAST might be either a consequence of a genetically

determined true low level of circulating IgE or, what is more likely, because mould extracts insufficient in quality and composition were coupled to the RAST discs (2, 3, 4).

The use of the same extract for SPT and RAST gave a high degree of correlation (2). Although a complete accordance could not be expected, owing to a possible difference between skin mast cell-bound and circulating specific IgE (compartmentalization) (14), the tendency towards "negative" RAST values in patients with a convincingly "positive" SPT (+3 and +4) (Fig. 3) must be a consequence of the quality of the *Cladosporium* extract used for coupling to the allergosorbent discs. In the case of evidence of circulating IgE (RAST class 1–4) this might not be the only explanation, as the degree of skin reaction is not exclusively a consequence of cell-bound IgE molecules, but also reflects the releasability of skin mast cells and the local distribution of histamine receptors (10).

The problems involved in the production of mould allergen extracts are supported by the present study. The complexity and heterogeneity of various moulds have been intensively investigated (3, 5, 6, 8, 14), and the great difference in the number of "positive" SPT to individual moulds using extracts from different producers (Fig. 1) is a consequence of the inadequacy of commercial mould extracts. The quality of mould extracts is a result of their potency as well as their allergenic composition. The partially purified, standardized Spectralgen *Cladosporium* seemed to be the most potent extract. To be used as a screening extract all important allergenic determinants must be represented in sufficient concentrations (5). The difference in diagnostic ability is to a high degree a question of the difference in allergenic composition rather than the potency of those allergens present in the extract, as the reaction in some patients was of the same magnitude as with Spectralgen and in other patients positive to Spectralgen no reaction occurred at all.

Associated IgE-reactivity to *Alternaria*, *Aspergillus*, *Penicillium* and *Mucor* occurred much more frequently in *Cladosporium*-positive patients compared to the frequency of mould allergy in allergic persons as a whole (7, 13).

Co-reactivity to *Alternaria* and *Mucor* was found in more than 50% of the *Cladosporium* patients (Fig. 4). Almost all reactions to *Mucor* were found in the *Cladosporium* group. This association does not seem to be a coincidence, but is rather a reflection of a general tendency (genetically determined) to produce IgE antibodies against mould allergens. Cross-reactivity in the sense of partial identity between epitopes of the tested mould species has not been found (13).

Our findings confirm previous reports of the inadequacy of commercial mould extracts used for diagnosis in children (2). The general validity of this conclusion is supported by the similar frequency of positive and negative reactions in SPT and RAST in children and adults.

The difference in diagnostic capacity between the partially purified and standardized extract and extracts from three other manufacturers strengthens the need for both qualitative and quantitative control of the allergenic composition of extracts and raises doubts about the value of previous studies of the frequency of mould allergy.

ACKNOWLEDGEMENTS

This study was supported by grants from P. Carl Petersens Fond and Danmarks Astma- og Allergiforbund.

Nurses and laboratory workers of the involved departments are thanked for excellent technical assistance.

REFERENCES

1. Aas, K., Backman, A., Belin, L. & Weeke, B.: Standardization of allergen extracts with appropriate methods. *Allergy* 33, 130–137, 1978.
2. Aas, K., Leegaard, J., Aukrust, L. & Grimmer, Ø.: Immediate type hypersensitivity to common moulds. *Allergy* 35, 443–451, 1980.
3. Agawal, M. K., Jones, T. & Yunginger, J. W.: Immunochemical and physicochemical characterization of commercial *Alternaria* extracts: a model for standardization of mold allergen extracts. *J. Allergy Clin. Immunol.* 70, 432–436, 1982.
4. Aukrust, L. & Aas, K.: The diagnosis of immediate type allergy to *Cladosporium herbarum*. *Allergy* 33, 24–29, 1978.
5. Aukrust, L.: Crossed radioimmuno-electrophoretic studies of distinct allergens in two extracts of *Cladosporium herbarum*. *Int. Arch. Allergy Appl. Immunol.* 58, 375–390, 1979.

6. Aukrust, L. & Borch, S. M.: Partial purification and characterization of two *Cladosporium herbarum* allergens. *Int. Arch. Allergy Appl. Immunol.* **60**, 68–79, 1979.
7. Gravesen, S.: Fungi as a cause of allergic disease. *Allergy* **34**, 135–154, 1979.
8. 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.* **55**, 1–12, 1977.
9. Malling, H.-J., Andersen, C. E., Boas, M.-B., Holgersen, F., Munch, E. & Weeke, B.: The allergy pricker. *Allergy* **37**, 563–567, 1982.
10. Nelson, H.: Diagnostic procedures in allergy. I. Allergy skin testing. *Ann. Allergy* **51**, 411–417, 1983.
11. Nilsson, D. & Aas, K.: Immunological specificity and correlation of diagnostic tests for bronchial allergy to *Cladosporium herbarum*. *Acta Paediatr. Scand.* **65**, 33–38, 1976.
12. Pepys, J.: Skin tests in diagnosis. In Gell, P. G. H., Coombs, R. R. A. & Lachmann, P. J. (eds.): *Clinical aspects of immunology*, 3rd ed. Blackwell, London, 1975.
13. Salvaggio, J. & Aukrust, L.: Mold-induced asthma. *J. Allergy Clin. Immunol.* **68**, 327–346, 1981.
14. Slavin, R. G.: Skin tests in the diagnosis of allergies of the immediate type. *Med. Clin. North Am.* **58**, 65–69, 1974.
15. Yunginger, J. W., Jones, R. T. & Gleich, G. J.: Studies on *Alternaria* allergens. II. Measurement of the relative potency of commercial *Alternaria* extracts by the direct RAST and by RAST inhibition. *J. Allergy Clin. Immunol.* **58**, 405–413, 1976.
16. Østerballe, O., Malling, H.-J. & Weeke, B.: Specific diagnosis of exogenous bronchial asthma in adults. Comparison between case history, intracutaneous test, RAST, histamine release from basophil leucocytes, and bronchial provocation. *Allergy* **34**, 175–185, 1979.
17. Northern Society of Allergology: Standardization of allergen extracts. *Acta Allergol.* **28**, 219–220, 1973.

Address:

Hans-Jørgen Malling
Laboratory of Clinical Allergology
Medical Dept. TA 7511
State University Hospital
Tagensvej 20
DK-2200 Copenhagen N
Denmark

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.