

Specific IgE antibodies to 16 widespread mold genera in patients with suspected mold allergy

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A panel of 55 sera from patients with suspected mold allergy from two different geographic areas, A and B, was investigated for specific IgE antibodies to 16 individual molds using Phadebas RAST® technique. The molds investigated were *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium*, *Mucor*, and *Candida* from the former RAST panel and ten additional new mold genera.

The RAST screening revealed that 73% of patients had specific IgE antibodies to at least one of the 16 molds. Eighteen percent were negative to the six molds of the previous RAST panel but had specific IgE antibodies to at least one of the ten new molds.

The highest frequency of IgE antibodies in the A group could be assigned to *Cladosporium*, followed by *Botrytis* and *Helminthosporium*. In group B, sensitivity to *Botrytis* and *Phoma* showed the highest frequencies.

This study revealed that the relative importance of mold genera may vary greatly between patient groups from different geographic areas. Some of the species of the expanded RAST panel seem to be at least as important as *Alternaria* and *Cladosporium*.

Multiple sensitivities seem to be caused by sensitization by many mold species rather than by cross-reactivity.

INTRODUCTION

Since 1873 it has been known that inhalation of airborne mold spores produces respiratory allergic symptoms.¹ Further evidence was presented by several European workers in the mid-1920s.² The independent studies by Prince et al³ and Feinberg and Little⁴ in the 1930s gave convincing documentation and added much knowledge to the concept.

The reported incidence of mold sensitization in atopic patients ranges from 5% to more than 29% in different studies.⁵

Mold allergy has been difficult to diagnose due to many factors such as the absence of a clear seasonal pattern for molds, the poor quality of mold allergen extracts, and difficulties encountered in the identification of molds. Further, of the thousands of mold genera occurring in the environment, there are still many to be investigated with respect to their role in allergy. In more recent literature, approximately 85 genera have been associated with

allergy: *Alternaria*, *Cladosporium*, *Aspergillus* and *Penicillium* are among the most frequently reported.⁶

The aim of this study was to investigate patients with clinical history compatible with mold allergy for specific IgE antibodies against an expanded panel of widespread molds.

MATERIALS AND METHODS

Allergens

Freeze-dried source material of the following 16 individual mold species were obtained from Allergon AB, Sweden. Also given is their Phadebas RAST® codes.

Penicillium notatum, m1
Cladosporium herbarium, m2
Aspergillus fumigatus, m3
Mucor racemosus, m4
Candida albicans, m5
Alternaria alternata, m6
Botrytis cinerea, m7
Helminthosporium halodes, m8
Fusarium moniliforme, m9
Stemphylium botryosum, m10
Rhizopus nigricans, m11
Aureobasidium pullulans (*Pullularia*), m12
Phoma betae, m13
Epicoccum purpurascens, m14

Trichoderma viride, m15

Curvularia lunata, m16

The molds were grown on a completely synthetic culture medium under well-defined culturing conditions. The different mold batches contained both mycelium and conidia (spores).

Extraction of the source material was performed under agitation at 4 to 8 °C in 0.1 M phosphate buffer, pH 7.4, for one hour at a concentration between 1/20 to 1/150 wt/vol, which was individually optimized for each mold. The extract suspension was clarified by centrifugation at 19,000 rpm for 30 minutes and filtered. For the immunoblotting experiments, the crude extracts were desalted in a column filled with Sephadex G-25 (Pharmacia AB) and then freeze-dried.

RAST Assays

The IgE antibodies in the patient sera were measured using Phadebas RAST® (Pharmacia Diagnostics AB) standard procedure in duplicate with the former Phadebas RAST mold panel (m1 to m6) and ten new mold discs (m7 to m16) of an extended panel. The results were expressed in Phadebas RAST® units

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(PRU/mL) an arbitrary unit that refers to a reference curve with a cut-off at 0.35 PRU/mL. All procedures were performed according to the manufacturer's instructions for use.

Patient Characteristics

Fifty-five patients with suspected mold allergy were referred to our laboratory for specific IgE measurements. Thirty-four patients were from Scandinavia, mostly from Southern Sweden and Denmark (named group A) and 21 patients from USA, mostly from Alabama and Pennsylvania (named group B). The mold allergy diagnosis for all 55 patients was suspected according to the following criteria: history including asthma or rhinoconjunctivitis caused by molds and positive skin test to one or several molds. The skin test techniques used were skin prick tests in the Scandinavian group and intradermal testing in the USA group. A few of the Scandinavian patients also reacted during a provocation test to one or several molds. Serum from one healthy non-atopic volunteer spiked with myeloma IgE to 500 kU/L. Five non-atopic volunteers were used as negative controls.

RAST Inhibition

The RAST inhibition experiments were performed essentially as previously described by Yman et al⁷ using 30 μ L of serial dilutions of the mold extracts 1:20 (wt/vol). The allergen extracts were incubated with 20% patient serum for two hours in triplicate at room temperature. The RAST discs used as allergosorbents were from the same batches as in the RAST screening experiment.

Nitrocellulose Immunoblotting Experiments

The mold allergens were separated by SDS gradient PAGE and transferred to a nitrocellulose membrane by electrophoretic blotting. Specific IgE antibodies directed against the molds were utilized as probes to detect the allergens. The bands were localized in the nitrocellulose mem-

brane by means of enzyme-labeled anti-IgE.

The SDS gradient PAGE gel was molded in Biorad Protein Dual Slab Cell equipment, according to the operating instructions. The electrophoresis was carried out essentially as described by Neville⁸, run at 6 mA/gel for 18 to 20 hours. The electrophoretic blotting, performed in a Trans-blot cell (Biorad), and the immunologic detection using enzyme-labeled anti-IgE (Phadezym RAST[®]) and a chromogenic substrate, were performed as earlier described by Bengtsson et al¹⁰ and Yman et al.⁹

The nitrocellulose immunoblotting inhibition experiments were performed as follows: the allergens to be studied were transferred onto nitrocellulose strips as described above. Before serum incubation of the strips, the serum (0.5 mL) was inhibited with 0.5 mL 1:20 (wt/vol) of the allergen extract to be studied.

RESULTS

Specific IgE Measurements

A RAST[®] screening was performed on the 55 samples of patient sera, using the former Phadebas RAST[®] mold panel (m1 to m6) and ten new molds (m7 to m16). The screening revealed that 40 patients (73%) had specific IgE antibodies to at least one of the 16 molds. Thirty patients had detectable IgE antibodies against the traditional mold panel (m1 to m6) and 40 patients against the expanded panel of 16 molds (Fig 1).

Comparing the two patient groups A and B, the corresponding figures for the A group were 22 and 26, respectively. In the B group, the 16 mold panel detected antibodies in 14 patients as compared with eight for the traditional range.

Figure 2a shows the frequency of positive tests with the 16 different genera in the A group. *Cladosporium*,

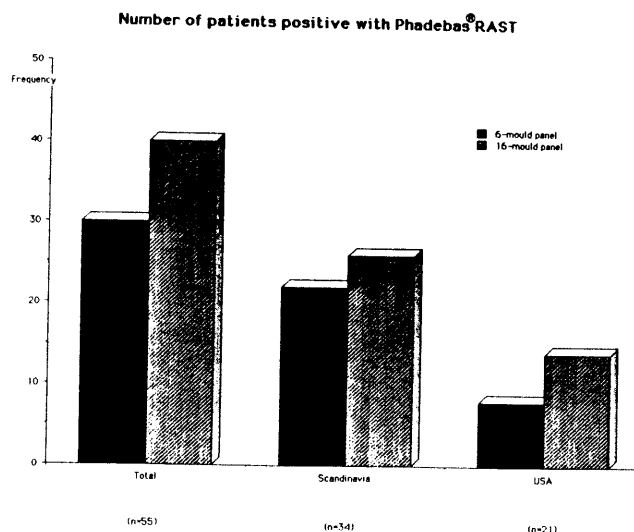


Figure 1. RAST screening of 55 patients sera using the former Phadebas RAST[®] mold panel m1 to m6 and the ten new molds, m7 to m16.

ium, which displays the highest frequency, gave positive test results in 17 out of 34 patients. *Botrytis*, *Helminthosporium*, *Epicoecum* and *Stemphylium* were also very frequent as sensitizing agents. *Alternaria*, *Aspergillus* and *Penicillium* were ranked as Nos. 7, 10, and 12 in the A group.

Figure 2b shows the frequency of positive tests in the B group. *Botrytis* and *Phoma* were the most frequent molds in this patient group, positive in 11 out of 21 patients. Response to *Stemphylium*, *Epicoecum* and *Helminthosporium* was also very frequent in the B group. It can be noted that *Alternaria* and *Cladosporium* have low frequencies in this group, positive in 4 and 3 patients, respectively. *Aspergillus* and *Penicillium* were ranked as No. 13 and No. 15.

All negative controls showed specific IgE values well below the cut-off value.

Allergen Composition Studies

Comparison of IgE antibody concentrations measured with different mold genera shows various degrees of positive concordance between species. *Botrytis* and *Helminthosporium*, for example, agreed in 75% of the cases while *Alternaria* and *Botrytis* did so in only 38%, as is shown in Figure 3.

To investigate whether the incidence of positive concordance between certain molds in the RAST screening was caused by cross-reacting allergens, the allergen composition of different mold genera was studied. Patterns of specific IgE antibodies to individual allergenic components were detected on nitro-

cellulose strips after separation of the proteins by SDS gradient PAGE applying the enzyme labeled anti-IgE and a chromogenic substrate.

Figures 4 and 5 show IgE antibody specificities of 22 sera against *Helminthosporium* and *Botrytis*, respectively. The IgE responses differ greatly between different patient sera. Both Figures show sera with IgE antibodies to just a few components as well as sera with a broad spectrum of IgE antibodies. The specificity patterns of the individual sera against *Helminthosporium* and *Botrytis* have a few similarities but vary greatly in most cases.

For further study of possible cross-reacting agents between these mold genera, RAST inhibition and immunoblotting inhibition experiments were performed. Figure 6 shows inhibition experiments where

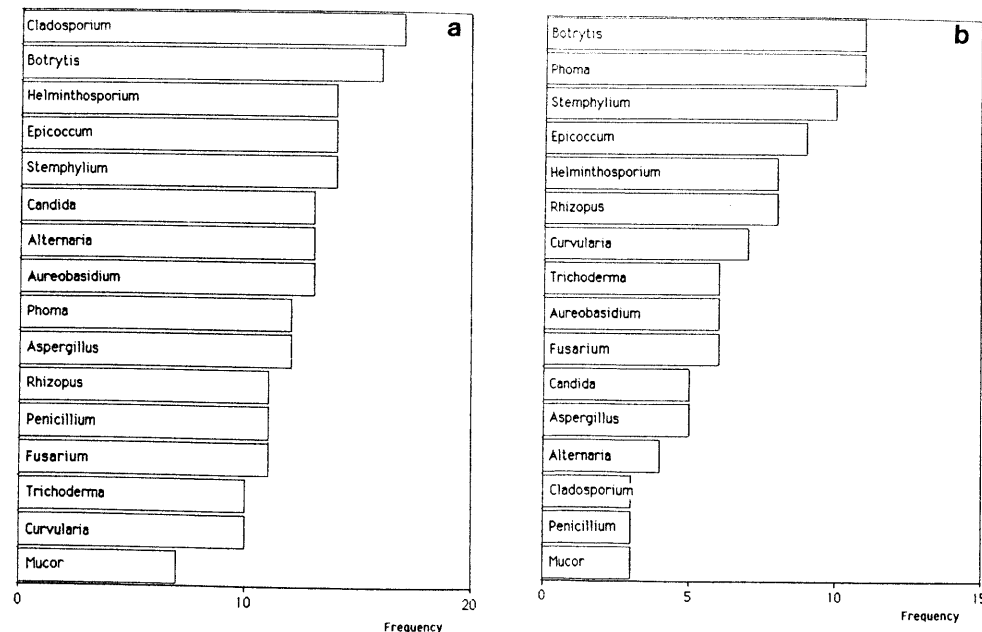


Figure 2a. Frequency of positive tests with the 16 different mold genera in group A.

Figure 2b. Frequency of positive tests with the 16 different mold genera in group B.

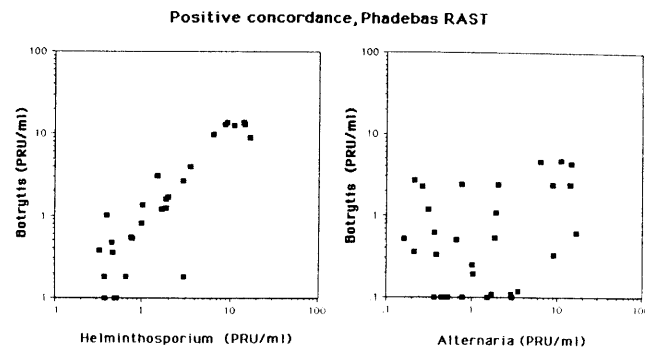


Figure 3. Degree of positive concordance, Phadebas RAST[®], expressed in PRU/mL between *Botrytis*—*Helminthosporium* and *Botrytis*—*Alternaria*.

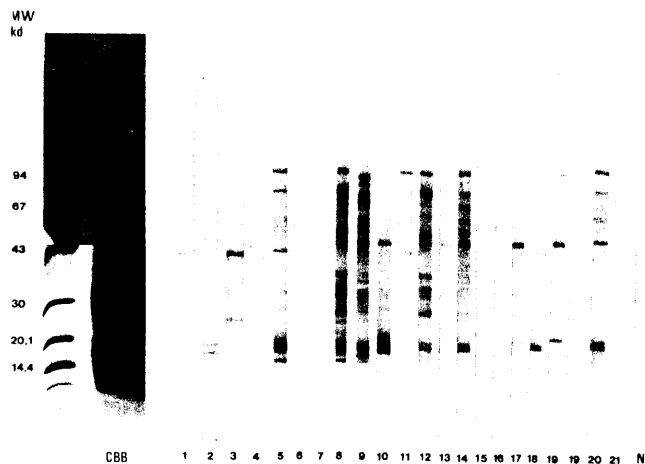


Figure 4. IgE antibody specificities of 22 sera against *Helminthosporium*. N is a negative serum and CBB is a Coomassie Brilliant Blue-stained gel.

the binding of specific IgE antibodies to *Helminthosporium* allergens on nitrocellulose strips was inhibited by *Helminthosporium* and *Botrytis* allergen extracts, respectively. The immunoblotting results clearly show that the binding to a few *Helminthosporium* components was inhibited by *Botrytis* but that most of the allergens are species specific as seen by these particular patient sera.

The RAST inhibition results confirmed these findings (Fig 6). Inhibition experiments were performed with *Alternaria* on the nitrocellulose strips and with *Alternaria* and *Botrytis* extracts as inhibitors, using 3 positive patient sera (Fig 7).

Using serum No. 1, binding of IgE to *Alternaria* was to a certain extent inhibited by *Botrytis*. When using sera Nos. 2 and 3, however,

the results indicate only species-specific components. The RAST inhibition in this case also confirmed the immunoblotting findings.

DISCUSSION

The presence and type of airborne fungal spores in both indoor and outdoor air have been studied and surveys from all parts of the world have been published demonstrating large differences. It has also been generally assumed that the genera most common in air are also the most frequent inhalant allergens.¹¹ It is therefore reasonable to assume that when studying the frequencies of specific IgE antibodies to different molds in distant geographic areas, differences can be expected. *Cladosporium* was reported the most commonly found outdoor air-borne fungal spore in most of the temperate zones of the world to which Scandinavia is exposed. Patients from the group A came from this area. *Alternaria* was reported to be the most common genus in the outdoor air on the US East Coast and Midwest, an area with a subtropical climate. The patients from group B came from this area. *Aspergillus* and *Penicillium* are the dominant genera indoors. Influencing factors, often independent of the outdoor climate, are for instance temperature and degree of humidity.^{11,12}

In the present study, 55 patients from two different geographic areas, groups A and B, with suspected mold allergy were screened for specific IgE antibodies to a widespread panel of 16 molds. Approximately 75% of the total patient panel had specific IgE antibodies to one or more of the molds. This was an increase of 33% when using 16 molds instead of the 6 molds in the former Phadebas RAST[®] panel. For group A, the new panel of molds increased the number of positive patients by 18% and in group B by 75%.

The frequencies of the 16 molds in the two patient groups revealed

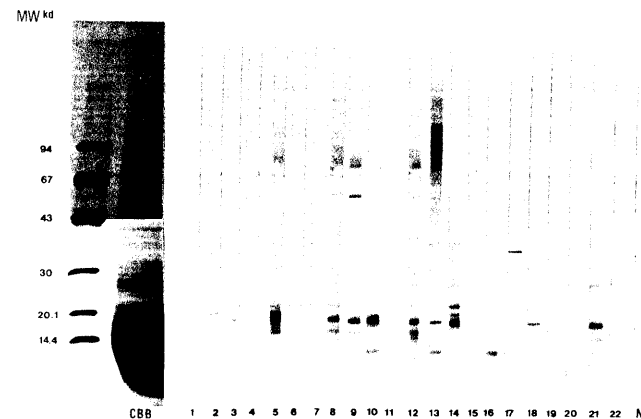


Figure 5. IgE antibody specificities of 22 sera against *Botrytis*. N is a negative serum and CBB is a Coomassie Brilliant Blue-stained gel.

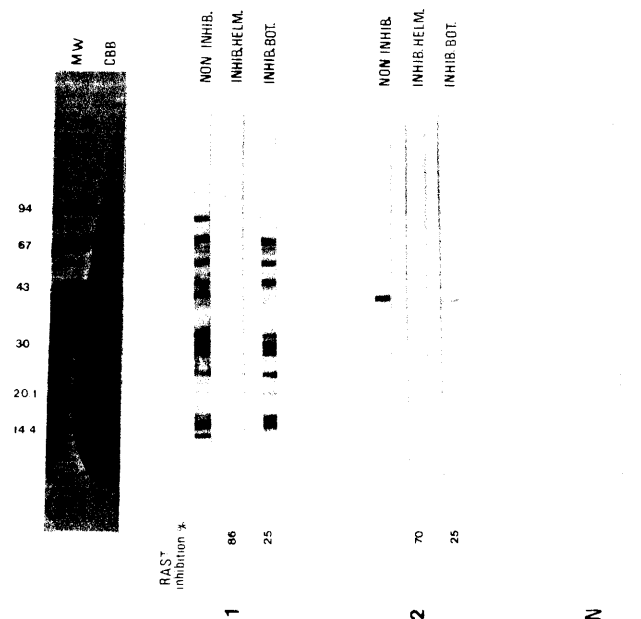


Figure 6. Inhibition of specific IgE antibodies to *Helminthosporium* by *Helminthosporium* and *Botrytis*, respectively. CBB is a Coomassie Brilliant Blue-stained gel; 1, 2 and 3 are positive sera; and N is a negative serum.

some similarities. Specific IgE antibody to *Botrytis* was very high in both groups (No. 1 in group B and No. 2 in group A) closely followed by *Helminthosporium* (No. 5 and 3, respectively) and *Epicoccum* (No. 4 in both groups).

Considerable differences were also found, e.g., for *Cladosporium*, ranked as No. 1 in group A and only as No. 14 in group B. Another example is *Alternaria*, ranked as No. 13 and 7, respectively in the two groups. *Phoma* and *Curvularia* also show large differences in ranking between the two groups.

Although the patient groups were small and certainly not representative of their respective geographic areas, it is evident that the groups differ. The reasons behind this difference might be factors such as climate, housing and other local factors.

The genera *Botrytis*, *Phoma*, *Helminthosporium*, *Epicoccum*, and *Stemphylium*, often neglected when testing for mold allergy, all showed high frequencies in one or both patients groups.

Mold allergic individuals are often sensitive to several mold species. This clinical observation had led to the assumption that the degree of cross-reactivity is high among mold species, much in analogy with grass pollen reactivity. Cross-reactivity between allergens of different mold genera have been found, such as that between *Alternaria*, *Stemphylium* and *Curvularia*.¹³ *Epicoccum* and *Fusarium* were shown to exhibit incomplete cross-reactivity.¹³ Gravesen and Nyholm have demonstrated great similarities between *Alternaria* and *Ulocladium*.¹⁴ In other studies little or no cross-reactivity between mold genera has been found. Karr and co-workers¹⁵ found no cross-reactivity between *Aspergillus fumigatus* and *Alternaria alternata*. Salvaggio and Aukrust found a single antigen but no allergens in common between *Alternaria alternata* and *Cladosporium herbarum*.¹⁶

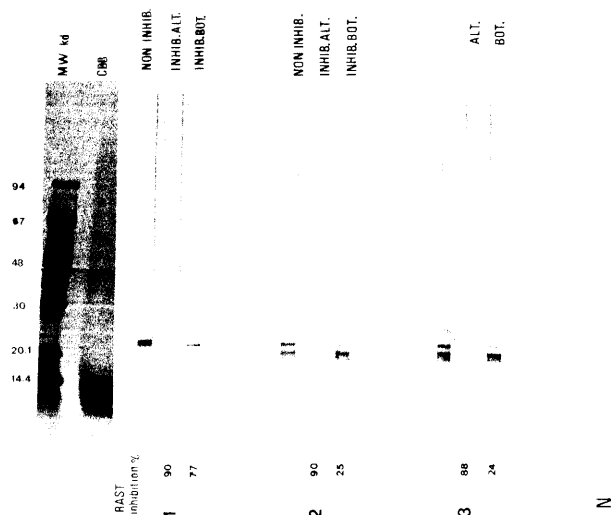


Figure 7. Inhibition of specific IgE antibodies to *Alternaria* by *Alternaria* and *Botrytis*, respectively. CBB is a Coomassie Brilliant Blue-stained gel; 1, 2 and 3 are positive sera; and N is a negative serum.

In our study we found a few cross-reacting components in different mold genera but mostly species-specific allergens were detected.

We found that an expanded Phadebas RAST[®] mold panel detects more mold-sensitive patients than the traditional panel of six allergens. The results also indicate that some mold genera, eg, *Botrytis*, *Phoma*, *Helminthosporium* and *Epicoecum* are at least as important as *Alternaria* and *Cladosporium*. The relative importance of the mold genera differs greatly between patient groups. It is also obvious that the appearance of specific IgE antibodies to many mold genera in one patient can sometimes be explained by cross-reacting components, but probably the patient more often displays a parallel and independent sensitivity to different mold species.

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