

Skin testing with extracts of fungal species derived from the homes of allergy clinic patients in Toronto, Canada

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Summary

Airborne fungi in the homes of patients with allergic rhinitis or asthma, from a Toronto Allergy Clinic population, were isolated, quantified and identified to species. Allergen extracts were prepared from sixteen of these isolated species and used for skin-prick testing of twenty-six patients. Fourteen of the total patients reacted to one or more of these extracts at 1:10 (w/v) concentrations. The most common positive skin responses (8/14 to 6/14) were found for *Cladosporium cladosporioides*, *Alternaria tenuis*, *C. sphaerospermum*, and *Fusarium* sp. The two *Cladosporium* species were also most commonly isolated in homes, but *A. tenuis* and *Fusarium* sp. were found only in 4% and <1% of the air samples, respectively. *Epicoccum purpurascens* and *C. herbarum*, which were isolated on approximately 10% of the plates, showed fewer skin reactions compared with the above. Positive skin-test response to the other ten study extracts ranged from 5/14 for two species of *Aspergillus* and *Phoma glomerata*, to 1/14 for *Penicillium viridicatum*; of these species, *Aspergillus fumigatus* was isolated in 3% of the home samples, the others were less than 1%. The findings suggest that fungal antigens from species found in homes are commonly associated with skin sensitization in an allergy clinic population with upper or lower respiratory allergy. No specific relationships were found, however, between the prevalence of fungal species in the home environment and their prevalence as skin-test allergens.

Introduction

Fungal spores have been recognized as important inhalant allergens since 1925 [1] and have been implicated as contributing to symptoms of both rhinitis and asthma [2-7]. Spores are common components of the air but high exposure to these allergens has

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been reported in agriculture [2], during frost-free seasons [8], and in domestic indoor situations such as homes and offices [6,7,9-13]. The latter instances are of special concern because we spend over 50% of our time in the home [14].

The literature on the mycoflora in the air of residential buildings has often proposed that spore assessments, using air sampling techniques, can have direct application for determining the exposure of occupants, and for the management of atopic patients [8,11-13,15-17]. However, very few of the aerobiological studies have been supported by clinical data. Such information is essential for determining the relationships between airborne concentrations of spores and/or spore types, and the response of patients to these allergens.

The present study was undertaken to examine the mycoaeroflora in the homes of patients with allergic rhinitis and/or asthma, and to compare the environmental findings with the skin reaction of the patients to extracts of several of the species isolated.

Subjects and methods

Subjects

Thirty patients with allergic rhinitis or asthma from the Allergy Clinic of the Toronto General Hospital agreed to take part in the study. Informed consent was given by all patients for sampling of their homes, between July and September 1985, for fungal spores [18]. Twenty-six of these patients then returned for skin testing with extracts prepared from the fungi identified from the thirty homes. The other four patients had moved or found it inconvenient to return for skin testing. The patients, eighteen female and eight male, had a mean age of 41.2 years. Fifteen patients had both rhinitis and asthma, eleven rhinitis alone and one asthma alone. Eighteen patients had perennial symptoms with seasonal exacerbation. Four patients had only seasonal symptoms attributed in part to fungal allergens, and four had perennial symptoms with no seasonal variation. Twenty-three of the twenty-six patients were atopic on the basis of skin testing to a group of sixteen common allergen extracts. Common fungal allergen extracts used in skin tests, referred to subsequently as 'commercial' extracts, were *Alternaria tenuis* w/v 1/20 *Cladosporium herbarum* w/v 1/20 and *Aspergillus* mix, (*A. amstelodami*, *A. niger*, *A. terreus*) w/v 1/20 (Bencard Allergy Service, Mississauga, Canada). Other common allergen extracts used were cat fur w/v 1/20, dog hair w/v 1/20, horse dander w/v 1/125, house dust w/v 75%, *Dermatophagoides farinae* w/v 1%, feathers w/v 1/20, mixed tree pollen (ash, beech, birch, elm, hazel, maple, oak, poplar, sycamore, willow) 25 000 PNU/ml, mixed grass pollen 25 000 PNU/ml, Eastern ragweed pollen 25 000 PNU/ml, egg white w/v 1/10, milk w/v 1/10, mixed nuts w/v 1/10 and salt water fish w/v 1/10 (Bencard Allergy Service, Canada). The three skin-test negative patients gave a history of seasonal exacerbation of their perennial upper or lower respiratory symptoms. Nine patients, including the four patients with seasonal symptoms alone, had positive skin-test response to at least one of the three commercial fungal extracts included in skin testing: four to *C. herbarum*, five to *Aspergillus* mix and eight to *Alt. tenuis*. Eighteen patients had air conditioning in their homes, six with central units and twelve with window units.

Environmental studies

The homes and the immediate environment were inspected for sources of moulds, and information regarding the conditions of each home was collected using a short questionnaire. The respirable fraction of the airborne fungi was collected using a two-stage Andersen impactor [18], trapping particles less than 8 μm in diameter in the first stage. The air sampler was operated at 28.3 litres/min for 3–5 min in each location. Rose Bengal agar supplemented with streptomycin was used as the collection medium [19]. In all cases, samples were taken approximately 150 cm above the floor in two locations inside the house, and in one ambient location near the home. After sampling, the plates were covered and allowed to incubate at 23°C in the dark. The plates were examined daily for fungal growth. Numbers of colony-forming units (c.f.u.) were recorded after up to 7 days' incubation and colonies were identified either directly on the collecting media or after they were sub-cultured on other types of media. The media used included Czapek agar, Czapek-yeast-extract agar, and malt extract agar for identification of species of *Penicillium* [20] and *Aspergillus* [21], Hagem's agar for other moulds [22] and Weitymans agar for induction of fruiting bodies production and sporulation in isolates, which initially occurred as sterile mycelia only [22]. The fungi were identified using various taxonomic manuals [20–23] according to proper mycological procedures.

Skin tests

All subjects were asked to avoid antihistamines for at least 4 days prior to skin testing (6 weeks if Astemizole was used). Skin testing was performed using the prick (puncture) test method. The weal diameter at 15 min was recorded as negative if absent or equal to the weal produced by diluent alone. Positive responses are taken as mean weal diameters greater than those produced by diluent. Positive responses of 3 mm or more weal diameter after subtracting the size of the control weal are recorded as a subgroup of the total positive responses.

Allergen extracts of 'home' fungi used for skin tests

Allergen extracts were prepared by Omega Laboratories Ltd (Montreal, Canada) from sixteen species of fungi isolated from air in homes. Their standard method of preparation was used. Briefly, several isolates of each species were cultured in Czapek broth for 3 weeks at 20°C with light. The mycelium mass was defatted with acetone, evaporated, dried and weighed. The fungi were then extracted over 3 days in sterile water (1/5 w/v). The liquid was prefiltered and filtered with a 0.22 μm filter before glycerine was added to obtain a 50% glycerine solution. The final concentration was 1/10 w/v.

The extracts used for skin testing were prepared from *Alt. tenuis*, *A. fumigatus*, *A. niger*, *A. flavus*, *C. cladosporioides*, *C. sphaerospermum*, *C. herbarum*, *Penicillium chrysogenum*, *P. viridicatum*, *P. roqueforti*, *P. rubrum*, *Epicoccum purpurascens*, *Fusarium* sp., *Phoma glomerata*, *Stigmina negundis* and *Stachybotrys chartorum*. All were used in a concentration of 1:10 (w/v).

Results

Results of the home survey of fungal spores have been previously reported [24]. Results of skin tests are summarized in Table 1. Fourteen of the twenty-six patients (54%) had

Table 1. Skin reactions of allergy patients to various allergens of home and commercial sources

Source	Allergen extracts	No. patients with positive skin reactions*		
		Per species	Per genera	Per source
Home	<i>Cladosporium cladosporioides</i>	8 (4)†		
	<i>Cladosporium sphaerospermum</i>	7 (3)	10 (5)	
	<i>Cladosporium herbarum</i>	4 (2)		
Home	<i>Aspergillus fumigatus</i>	5 (1)		
	<i>Aspergillus niger</i>	3 (0)	8 (2)	
	<i>Aspergillus flavus</i>	5 (1)		
Home	<i>Penicillium chrysogenum</i>	4 (0)		14 (9)
	<i>Penicillium viridicatum</i>	1 (0)		
	<i>Penicillium roqueforti</i>	4 (1)	7 (1)	
	<i>Penicillium rubrum</i>	3 (0)		
Home	<i>Alternaria tenuis</i>	7 (5)		
	<i>Epicoccum purpurascens</i>	5 (2)		
	<i>Fusarium</i> sp.	6 (2)		
	<i>Phoma glomerata</i>	5 (3)		
	<i>Stigmina negundis</i>	4 (1)		
	<i>Stachybotris chartorum</i>	4 (2)		
Commercial	<i>Cladosporium herbarum</i>		4 (1)	
	<i>Aspergillus</i> mix		5 (1)	9 (5)
	<i>Alternaria tenuis</i>		8 (5)	
Common commercial allergens excluding fungi				23 (21)

* A reaction was recorded where weal was 1–2 mm greater than control.

† Data in parentheses represent weals 3 mm or more greater than control.

positive skin tests to one or more of the extracts prepared from fungi identified in the homes. The commonest responses were found to be *Cladosporium* species, with ten patients (38%) responding to at least one of them. Of these, most had positive skin tests to *C. cladosporioides* (8/10) and fewest had positive tests to *C. herbarum* (4/10). Half of the observed skin-test responses to *Cladosporium* species were small (mean weal diameter 1–2 mm greater than the control). Eight patients (31%) had positive skin-test responses to *Aspergillus* species (three to five patients reacting to each one of the three species). All but two test weals to *Aspergillus* species were 2-mm diameter or less. Seven patients (27%) were skin-test positive to at least one *Penicillium* species, most commonly *P. chrysogenum* and *P. roqueforti* (4/7 to each). However, only one skin-test weal to *Penicillium* was greater than 2 mm. Of the remaining six fungal extracts, positive skin-test responses were found in at least four patients with each extract, most frequently to *Alt. tenuis* (seven patients) and *Fusarium* species (six patients).

The commercial fungal extracts are not directly comparable with the home extracts. Nevertheless, it is of interest that the rate of positive skin-test response to the commercial extracts of *Cladosporium* and *Alternaria* was similar to the rate obtained with extracts of the same species isolated from the homes. *Cladosporium herbarum* from both sources was positive in 15% of patients, and *Alt. tenuis* in 31% and 27%, respectively. Commercial *Aspergillus* species were not tested individually at the same time as the three species isolated from homes. Nevertheless, earlier skin tests with a

Table 2. Frequencies of positive skin reactions of airborne fungal species

Fungal species	Positive skin reaction (%)*	Frequency of isolation (%)†	
		Indoor	Outdoor
<i>Cladosporium cladosporioides</i>	57	34	37
<i>Alternaria tenuis</i>	50	4	9
<i>Cladosporium sphaerospermum</i>	50	19	18
<i>Fusarium</i> sp.	43	<1	<1
<i>Phoma glomerata</i>	36	<1	<1
<i>Epicoccum purpurascens</i>	36	9	3
<i>Aspergillus fumigatus</i>	36	3	<1
<i>Aspergillus flavus</i>	36	<1	<1
<i>Cladosporium herbarum</i>	29	10	7
<i>Stachybotrys chartorum</i>	29	<1	<1
<i>Penicillium roqueforti</i>	29	<1	<1
<i>Stigmina negundis</i>	29	<1	<1
<i>Penicillium chrysogenum</i>	29	<1	<1
<i>Aspergillus niger</i>	21	<1	<1
<i>Penicillium rubrum</i>	21	<1	<1
<i>Penicillium viridicatum</i>	7	<1	<1

* Percentage of the population (fourteen individuals) reacting to house fungi extracts, including responses 1–2 mm greater than control responses.

† Percentage of the total respirable colony forming units/m³ air.

commercial *A. fumigatus* extract had been positive in 15% of these patients, while the extract from homes in this study was positive in 19%. The commercial *Aspergillus* mix was positive in only 19% of patients, while the three separate extracts from the homes were positive in a total of 31% of patients. The same proportions of patients with rhinitis and with asthma had positive skin tests to fungal isolates from the home (44% of each). Three patients had taken short-acting antihistamines between 14 hr and 4 days before skin testing, but each had at least one positive skin-test response.

A comparison between frequencies of isolation and positive reactions to the different species is shown in Table 2. *Cladosporium cladosporioides* and *C. sphaerospermum*, which were isolated most frequently, were also associated with highest rate of positive skin reaction (57% and 50%, respectively). *Alternaria tenuis* had a comparable allergenic capacity (50% positive skin reaction rate) but was found in only 4% of the indoor air samples. A similar positive skin-test reaction rate was found for *Fusarium* sp., although these organisms were recovered at negligible concentrations from sampled indoor air. There were relatively low rates of skin reaction to *E. purpurascens* (36%) and *C. herbarum* (29%), both of which were found in approximately 10% of the indoor samples examined. Most of the other species studied were isolated very infrequently from home air, and showed moderate to low rates of skin responses.

Further analysis confirmed that there was no consistent relationship between patients with positive skin tests and prevalence of specific fungi in the home. Examination of the individual cases showed that eight patients had a positive skin-test response to the extract of at least 1 fungus predominating in their home at the time of sampling. However, twelve patients had positive responses to fungi that were more

predominant in outdoor air than in their homes at the time of sampling. The eight patients with positive skin tests to fungi predominant in their homes exhibited thirteen such skin-test responses to; *Cladosporium* extracts (five patients); *Alternaria* and *Aspergillus* (three patients to each); and *Penicillium* extracts (two patients). Nine patients had a total of twelve positive skin tests to fungal species not isolated from their home, one to *C. herbarum*, two to *Alt. tenuis* and three each to *Aspergillus* species, *Penicillium* species and *S. chartarum*.

Discussion

Our findings have shown no clear correlation between fungal species found in a home with specific sensitization of residents to that fungal extract. It is possible that exposure of a high concentration of fungal spores outside the home or in the work environment may have sensitized those patients with positive skin tests to fungi absent or present in low levels in their own homes. Similar findings have been reported in relation to *Aspergillus* sensitization, in which the indoor prevalence of *Aspergillus* was 3% in a population, of whom 70% showed allergic sensitization to *Aspergillus* [15]. Similarly, sensitization to *Ganoderma* extracts has been found to be less frequent than sensitization with less prevalent fungi [25]. This study suggests that speciation of fungi from homes of patients with positive skin tests to fungal extracts, and symptoms compatible with sensitization, is unlikely to be helpful.

Nevertheless, inclusion of extracts of additional fungi in routine skin testing may be of value. Although we were not comparing standardized, comparable extracts in our study, five patients (19%) who were skin-test negative to the three 'commercial' fungal extracts did react to one or more of the study extracts on skin testing. However, only two patients (7%) had definite positive skin-test responses (3 mm or greater weal diameter) to these extracts and negative responses to the three usual extracts.

The skin-test responses in the population selected for this study may not accurately reflect those in the general allergic population, as our sample was biased towards those with perennial symptoms and those with asthma. Nevertheless, such a population is commonly found in an allergy clinic, and our results are relevant to this setting.

Use of three species of *Aspergillus* and three species of *Cladosporium* identified more skin-test positive subjects than use of a single 'commercial' preparation of these fungi. There were differences in response to each species of *Aspergillus*, *Cladosporium* and *Penicillium*, so that omission of any one species would have resulted in failure to detect some positive responses to that fungal genus. Therefore, the potential exists for clinical sensitivity to an individual species of fungus without crossreactivity with other species from the same genus, as has been suggested by previous studies [26,27].

Of the seventy-five positive skin-test responses to the home fungal extracts, fifty-three (71%) were small responses, 1–2 mm weal diameter. This is comparable to the skin-test response reported to *Ganoderma* fungal antigen [25] in which 79% of positive skin-test responses were 1–2 mm weal diameter. In that study, also conducted in Southern Ontario, positive prick tests with *Alternaria*, *Cladosporium* and *Aspergillus* were weak in 44% of the *Ganoderma*-positive subjects, whereas they were weak in 59% of our positive responses to 'commercial' extracts of these same three fungi.

The clinical significance of these weakly positive skin-test responses to fungal extracts is not clear, but the work of Cockcroft *et al.* [28] suggests that in the presence of marked bronchial hyperreactivity, even relatively weak allergic responses may produce significant bronchoconstriction. It is unlikely that the small positive skin-test responses

were due to an irritant effect of the extracts as these 1–2 mm diameter responses occurred in less than half the patients tested, with no uniformity of response to different extracts in individual patients.

Our 'home' extracts were not 'standardized' by assay of protein nitrogen units or skin-test titration. However, all patients who had a definite (3 mm weal, or greater) skin-test positive response using the *Alternaria* extract from the home (w/v 1:10) also reacted to the 'commercial' extract (w/v 1:20) and vice versa, so that it would appear likely that the extracts used were comparable in strength to usual fungal allergen extracts used in clinical practice.

In summary, in some allergic patients with rhinitis or asthma who are skin-test negative to fungal extracts normally used in skin testing, skin tests with further fungal extracts such as individual species of common indoor or outdoor fungi, may identify specific fungal sensitivity. However, in view of the poor relationship between skin-test responses and the individual's home fungal prevalence, it is not known whether home environmental control measures will significantly alter allergic symptoms related to these fungi. It could, however, be important in determining treatment for the patient, such as hyposensitization therapy.

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