

Domestic fungal viable propagules and sensitization in children with IgE mediated allergic diseases

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In order to study the possible relationship between domestic fungal exposure and sensitization to fungi, the homes of 20 children with allergic diseases (mainly bronchial asthma) were inspected for visible fungal growth and airborne viable fungal propagules were sampled. Inclusion criterion was sensitization to at least one of the tested fungi as shown by proof of specific IgE in serum. Twelve of 19 homes (63%) revealed a considerable amount of viable fungal propagules and/or visible fungal growth. *Penicillium*, *Cladosporium*, *Aspergillus*, *Mycelia sterilia* and yeasts were most prevalent. There was no clear-cut relationship to serologically proven sensitization.

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Allergic diseases, such as bronchial asthma and allergic rhinoconjunctivitis are very frequent in childhood. Typical trigger-factors for the occurrence of clinical symptoms include allergens, respiratory infections or physical exercise. Allergens rank among the most important factors in childhood. Typical clinically relevant allergens are pollen, pets and house-dust mites. While the role of these allergens as trigger-factors has become much clearer in recent years, little is known about the role of fungal spores for the pathogenesis of allergic diseases and as trigger-factors for clinical signs of allergic diseases.

Due to the fact that children spend most of the day indoors and because dampness in homes has increased over the last decade, a relation between increased symptoms and dampness has been proposed (1–3). It is tempting to speculate that fungi may play a more important role than hitherto assumed.

Allergen avoidance is the first line treatment for allergic diseases. However, despite current avoidance measures and improved pharmacological treatment facilities, some children do not show a satisfying state of relief of their symptoms. The aim of our study was therefore to investigate the extent of viable fungal propagules in homes of children with allergic diseases and to compare these results with the sensitization to fungi, in terms of proof of specific IgE in serum. It was hoped to be able to improve symptoms by allergen avoidance measures in case of high domestic exposure to fungi.

Material and methods

Twenty children (16 boys, four girls) aged 5 to 16 years (mean 9 years) from our outpatient department were enrolled in the study. Eighteen children suffered from mild to moderate bronchial asthma, as defined by the American Thoracic Society (4). Eight children fulfilled criteria of atopic dermatitis defined by the criteria of Sampson (5) and Seymour et al. (6), modified from the criteria of Hanifin and Rajka (7). Four children suffered from allergic rhinoconjunctivitis. Total IgE ranged from 7 kU/l to 3200 kU/l (mean 785 kU/l).

Study design

Inclusion criteria were: (1.) children with bronchial asthma, allergic rhinoconjunctivitis or both and in one case atopic dermatitis only; (2.) a positive proof of specific IgE (PCS-class ≥ 2) to one of the tested fungi. Specific IgE to six common fungi, known as important allergens, was investigated: *Cladosporium herbarum*, *Alternaria tenuis*, *Aspergillus fumigatus*, *Penicillium notatum*, *Mucor racemosus* and *Fusarium moniliforme*. Blood was taken once for routine allergy diagnostic, and within 6 weeks domestic exposure to fungi was investigated by indoor air sampling and subsequent analysis of fungi. In order to validate indoor measurements, outdoor air sampling was also performed.

Table 1. Time of sampling, indoor and outdoor exposure to fungi (CFU/m³ and visible growth)

Pat.	Date	Outdoor conc. (CFU/m ³)	Indoor range (CFU/m ³)	Visible fungal growth	Predominant fungi outdoor	Predominant fungi indoor
1	Sep	588	63 - 175	-	Cla	Cla
2	Dec	378	65 - 200	+	Cla	Cla, Myc
3	May	525	440 - 860	+	Cla, Myc	Cla
4	May	525	90 - 259	-	Myc, Cla	Pen, Cla
5	Oct	2250	175 - 675	++	Cla, Myc, Pen	Cla, Myc, Yea, Aur
6	Jul	1625	250 - 350	-	Cla	Cla, Myc
7	Nov	325	88 - 1652	-	Cla	Pen, Pae
8	Sep	1012	188 - 712	-	Cla, Myc	Cla, Myc
9	Sep	2375	177 - 515 (3750*)	+	Cla, Myc	Pen, Cla, Myc
10	Dec	328	< 13 - 76	-	Cla, Bot	Cla, Myc, Asp
11	Feb	77	1076 - > 4000	++	Cla	Asp, Pen, Cla, Alt
12	Mar	263	38 - 63	+	Cla, Bot	Cla, Yea
13	Mar	226	76 - 158	-	Myc, Bot, Asp	Cla
14	Mar	226	76 - 158	-	Myc, Bot, Asp	Cla
15	Sep	3281	1372 - 1813	++	Cla, Myc	Pen, Cla, Asp, Yea
16	Nov	788	51 - 775	-	Cla, Myc	Yea, Cla
17	Nov	525	64 - 225	-	Cla	Cla, Yea, Myc
18	Dec	493	52 - 214	-	Cla	Cla, Pen
19	Feb	338	64 - 233	+	Myc, Cla, Yea	Cla, Yea
20	Apr	575	63 - 89	-	Cla, Myc	Cla, Myc

Legend: CFU = colony forming units, Asp = *Aspergillus*, Aur = *Aureobasidium pullulans*, Bot = *Botrytis*, Cla = *Cladosporium*, Pae = *Paecilomyces*, Pen = *Penicillium*, Myc = *Mycelia sterilia*, Yea = yeasts, - = no visible growth, + = visible growth, ++ = heavily contaminated, * = weekend-house (Pen)

Methods

Total and specific IgE was determined by a Fluorescence-Enzyme-Immuno-Assay (FEIA) using the CAP-System (Kabi-Pharmacia, Freiburg, Germany) (8). Besides the fungi mentioned above, a panel of 6 common inhalant allergens (timothy, birch, mugwort, house-dust mite, cat and dog) and 6 nutritional allergens (milk, egg, fish, soy, wheat and peanut) was investigated by the same assay system.

In each home, at least three rooms were investigated for airborne viable fungal propagules: the children's room, bathroom and living room. In cases of suspected fungal growth in other rooms, these were also investigated.

Air was sampled by a Reuter centrifugal air sampler (RCS, Biotest, Frankfurt, Germany) in 19 homes in Berlin. To prevent overloading of the culture media, the sampling period was only 2 min (corresponding to 80 l sampled air). The fungal spores were impacted on two strips coated with 3% malt extract agar and Czapek Dox agar with 40% saccharose (for xerophilic fungi), respectively. Penicillin and streptomycin were added to prevent bacterial growth. Air sampling was performed approximately 1.90 m above the floor. Before sampling, the windows had been closed for 3–4 h. Outdoor airborne fungal spores were collected corresponding to indoor sampling.

In addition, homes were inspected for visible fungal growth, e.g. on walls, window-frames and plants. With sterile swabs, material from suspected

spots was smeared on agar plates containing the same culture media. The strips and plates were incubated at 22°C for up to 10 days. The number of colonies was counted and the results expressed as colony forming units (CFU) per m³ air. Identification usually up to genus level was done using standard mycological techniques.

Results

Data of the indoor and outdoor samplings and of the inspection of visible fungal growth are shown in Table 1. The values for xerophilic fungi are included. Results of outdoor air measurements varied widely (77–3281 CFU/m³) with highest values in summer and autumn. For indoor sites, the minimum and maximum concentration of CFU of the investigated rooms is given. The results showed that in the homes of patient 1, 6, 10, 13 and 14 (brothers), 17, 18 and 20 1.) no fungal growth was visible and additionally 2.) the maximum concentration of viable fungal propagules was distinctly below those of corresponding outdoor levels. The homes of these eight children were classified as not fungally contaminated.

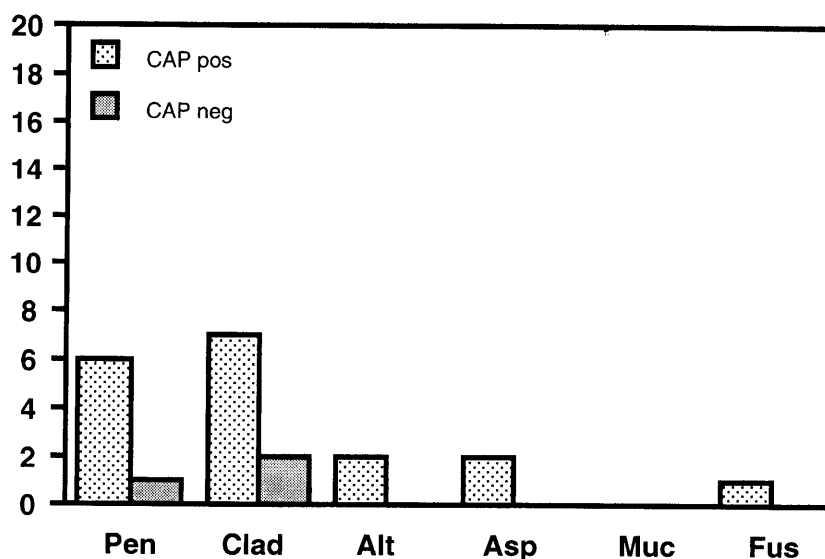
The homes of Patients 2, 3, 5, 7, 8, 9, 11, 12, 15, 16 and 19 were classified as contaminated due to elevated CFU values and/or visible fungal growth. In case of patient 4, the CFU values seemed not to be elevated but as there was a high proportion of *Penicillium* sp., the home was classified as contaminated

Fig. 1. Numbers of patients exposed (A) and not exposed (B) to fungi and differentiation between positive (CAP > 2) and negative (CAP < 1) sensitization.

Pen = *Penicillium*,
Clad = *Cladosporium*,
Alt = *Alternaria*,
Asp = *Aspergillus*,
Muc = *Mucor*,
Fus = *Fusarium*

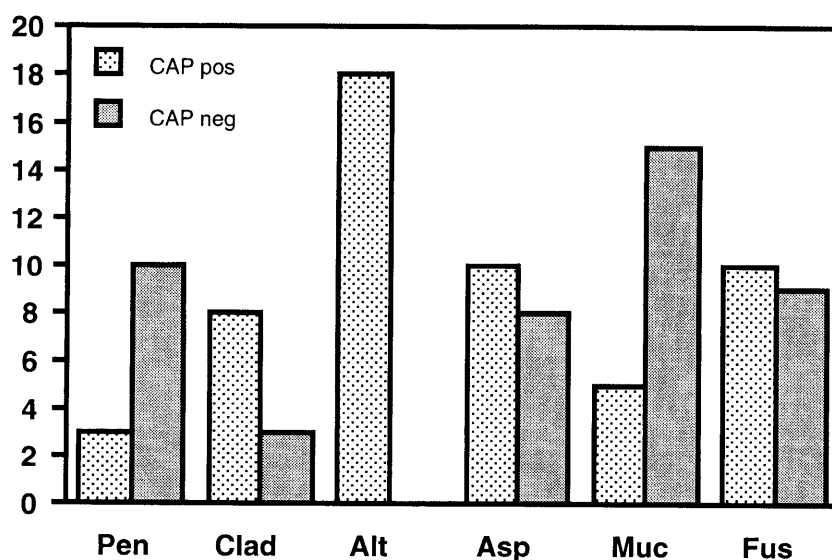
A

Number of patients with indoor exposure



B

Number of patients without indoor exposure



as well. Remarkably, homes of patients 2, 12, and 19 showed visible fungal growth but a relatively low concentration of airborne spores.

Predominant outdoor fungi were *Cladosporium* and *Mycelia sterilia*, while in fungally contaminated homes *Penicillium*, *Cladosporium*, *Aspergillus*, *Mycelia sterilia* and yeasts were prominent.

Concerning localization of fungal exposure, as expected, the room with a high risk of fungal growth was the bathroom. In the highly contaminated homes of patients 11 and 15, other rooms also showed a high concentration of viable fungal propagules with visible growth of fungi.

The clinical data of all patients and fungal exposure are given in Table 2. With the exception of patient 20, all children were also sensitized to common inhalant allergens, such as house-dust mites, pollens or animal dander. In terms of fungi, all children (except patients 5, 14 and 20) showed a polyvalent sensitization to several fungi.

In general, a close correlation between fungal exposure and specific IgE to fungi was not evident in this study. Indeed, in some cases a remarkable discrepancy was noted: for example patient 5 was solely sensitized to *Alternaria tenuis* but in his home *Cladosporium* was found to a great extent. Discrep-

Table II. Clinical data and indoor exposure of all patients

Pat.	Age (years)	Diag.	Tot-IgE (kU/l)	Cladosp.		Altern.		Aspergill.		Penicill.		Mucor		Fusarium	
				PCS	Exp	PCS	Exp	PCS	Exp	PCS	Exp	PCS	Exp	PCS	Exp
1	11	A	82	3	no	3	no	1	no	1	no	1	no	1	no
2	11	A, AD	821	4	yes	3	no	3	no	2	yes	2	no	3	no
3	6	A	617	2	yes	4	no	2	no	1	no	0	no	0	no
4	10	A, AD	2660	4	no	4	no	5	no	3	yes	3	no	4	no
5	8	A, AD	750	0	yes	4	no	0	no	0	no	0	no	0	no
6	9	A, AR	368	4	no	3	no	1	no	1	no	0	no	0	no
7	9	A, AR	463	3	no	4	no	3	no	3	yes	2	no	3	no
8	5	A	58	0	yes	2	no	0	no	1	no	0	no	0	no
9	5	A, AD	536	4	yes	3	no	2	no	3	yes	1	no	2	no
10	8	A	174	3	no	4	no	2	no	2	no	1	no	1	no
11	11	A	183	2	yes	3	yes	2	yes	0	yes	0	no	3	no
12	13	A	181	3	yes	3	no	2	no	0	no	0	no	3	no
13	11	AD	185	0	no	4	no	0	no	0	no	0	no	2	no
14	8	A, AD	65	0	no	3	no	0	no	0	no	0	no	0	no
15	11	A	1719	4	yes	2	yes	3	yes	2	yes	1	no	2	yes
16	7	A, AD, AR	1960	4	no	6	no	4	no	3	no	3	no	3	no
17	8	A, AD,	157	2	no	3	no	0	no	0	no	0	no	0	no
18	12	A	3200	4	no	4	no	3	no	2	no	2	no	3	no
19	8	A	1514	4	yes	2	no	3	no	2	yes	0	no	3	no
20	16	AR	7	0	no	2	no	0	no	0	no	0	no	0	no
mean			785	2.5		3.3		1.8		1.3		0.8		1.6	

Legend: Exp = exposure, no = no visible fungal growth and fungal concentration distinctly below outdoor levels, yes = visible fungal growth and/or fungal concentration above outdoor levels, A = asthma, AD = atopic dermatitis, AR = allergic rhinitis, PCS = Pharmacia CAP-system

ancies between exposure and specific IgE were striking for *Alternaria* (Fig. 1), thus 18 patients not exposed in their homes were all sensitized to this fungus. For *Cladosporium*, *Aspergillus* and *Fusarium*, there was also a remarkable discrepancy between the number of patients not exposed and sensitization. On the other hand, an association may be suggested for *Penicillium*, as 6/7 exposed patients were sensitized and 10/13 not exposed patients were not sensitized to this fungus. A similar association was also evident for *Mucor*: 15/20 not exposed patients were not sensitized.

Discussion

In accordance with other authors, we did not find a striking correlation between sensitization and domestic exposure (9). Fungi are ubiquitous and sensitization may occur in the outdoor environment. However, in accordance with our data, some fungi such as *Penicillium* and *Aspergillus* are considered as typical indoor fungi (9,10). It should be born in mind that extracts of fungal *species* were used for serological tests, while the sampled fungi were identified mainly by *genus*. Studies suggest that species share cross-reactive epitopes (11,12). In our study, fungi were identified by genus and not by species, because of the unwarranted expenditure.

Twelve out of nineteen homes were classified as fungally contaminated. These included those with

visible growth; other criteria were absolute numbers (CFU/m³), and also a predominant concentration of a single genus.

The results obtained from air sampling of viable fungal propagules and from the inspection of visible fungal growth did not correlate in all cases. This may be because (1.) airborne spores were not viable, or (2.) spore production did not occur when sampling was performed (e.g. due to diurnal variation). Apart from this, air sampling involves inherent problems such as the sampling device and culture media (10). To overcome some of these problems, the same sampling procedure was used throughout the whole study period.

Moreover, outdoor spore concentrations are known to influence spore load in homes. For example, high levels of indoor spore concentrations of patient 6 may be due to high outdoor levels. By relating indoor levels to outdoor results, seasonal variation is unlikely to influence the results.

In fungally contaminated homes, the genera *Penicillium*, *Cladosporium*, *Aspergillus*, *Mycelia sterilia* and yeasts were most frequently isolated. A Danish study found prevalence of the same fungi (13). It is of particular interest that *Cladosporium*, which forms the main part of outdoor spore load, not only influences the indoor air quantitatively, but also contributed to visible growth, e.g. on damp walls and silicone sealings. Especially at times of year when there are high spore loads, it is difficult to de-

cide whether CFU of *Cladosporium* reflects outdoor levels or contamination indoors.

Patients with very high total IgE (> 1000 kU/l) ($n = 5$) more often showed specific IgE to fungi (mean 18.4 kU/l) than those ($n = 15$) with lower IgE (9.0 kU/l). This is a well known phenomenon (especially in patients with atopic dermatitis) for other allergens (e.g. nutritional) and may reflect the production of clinically irrelevant specific IgE to environmental allergens.

Sensitization to fungi in children who do not show sensitization to other inhalant allergens seems to be rare. There was only one patient (No. 20) with this constellation. Most of the children were broadly sensitized and had typical clinical symptoms of allergic diseases. Moreover, sensitization to fungi was polyvalent in most of our children ($n = 17$). These results are in concordance with other groups (14).

Most of our children were sensitized to *Alternaria tenuis*, *Cladosporium herbarum* and *Aspergillus fumigatus*. It is of particular interest that a comparable Italian study, using skin prick testing, also revealed the prevalence of these fungi (15). Furthermore, we found that specific IgE to *Alternaria tenuis* and *Cladosporium herbarum* was highest (mean 3.3 kU/l and 2.5 kU/l, respectively). This is especially interesting for *Alternaria tenuis*, because with two exceptions no patient had a exposure to this fungus. On the other hand, specific IgE to *Penicillium notatum* was low (mean 1.3 kU/l), but five children were exposed to *Penicillium* sp. One might hypothesize that the sensitizing capacity of fungi varies.

There may be several reasons for the discrepancy between fungal exposure and sensitization: Firstly, sensitization may be much more likely to occur outside the home, which may be true for *Cladosporium* and *Alternaria*. Secondly, air samples may not reflect exposure over time. However, the aim of our study was to investigate correlation of spore loads with specific IgE to fungi at a given time-point and not to evaluate spore loads and/or sensitization over time. Thirdly, there is no standardization of fungal extracts (12, 16) and commercial extracts (as used in the specific IgE assay) may not be adequate.

Although correlation between exposure and sensitization was not striking in this study, proof of fungal exposure as contamination (e.g. in terms of adverse health effects by fungal toxins) and as a possible source of sensitization in homes of allergic children should lead to avoidance measures being adopted against fungal growth.

Future studies should include isolation of fungi from the patient's homes and testing sensitization with allergen extracts derived from these fungi.

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