

Allergy Review Series II

An update on allergens

Basidiomycete allergens

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Fungi have long been known to produce allergic reactions, but microfungi are usually considered to be the principal cause of mold allergy. Microfungi are the barely visible, microscopic molds that commonly inhabit leaf surfaces, decaying plant litter, and soil. Basidiomycete fungi number over 20 000 species, including mushrooms, puffballs, tree decay “conks” and “brackets”, and certain plant parasites. Most of these are considered macrofungi. Although microfungi unquestionably are important allergen sources, evidence now indicates that spores of macrofungi, such as basidiomycetes, also occur in high numbers in the environment and probably cause as many respiratory allergies as microfungi.

In fact, some early work on fungal allergens centered on basidiomycetes. Two early studies using fungal extracts concerned rusts and smuts; these are plant-parasitic basidiomycetes (1, 2). Both studies showed sensitization to basidiomycete extracts. One of the first aerobiology studies that used a volumetric sampler identified mushroom spores as potentially important aeroallergens (3). Moreover, some 30 years ago, basidiospores were implicated in epidemic occurrences of allergic asthma (4). In view of the early recognition of their allergenic potential, the known exposure, and the implication of causing disease, it is surprising that so little attention has been paid to basidiomycetes as allergens, in comparison with microfungi.

This review will emphasize the more recent work on basidiomycete allergens, including the production

of recombinant allergens. Basidiomycete allergens that have been purified and/or produced as recombinant allergens are summarized in Table 1.

Prevalence/variability of airborne basidiospores

Basidiospores are often a major portion of the airborne spore load in air-sampling surveys in various parts of the world. Basidiospores were 18–51% of the total spore catch in one 4-year study (5); in other studies, four of the top seven categories and three of the top seven categories were basidiomycetes (6, 7). In another study, *Coprinus* and other basidiomycetes (unidentified) were the most numerous spore types after *Cladosporium* and unidentified ascospores (8). Clearly, there is a significant exposure to basidiomycetes, and this may be an underestimate since basidiospores are often underestimated in aerobiology studies.

Temporal considerations

Basidiospore concentrations vary according to daily and seasonal factors, as well as weather-related factors. Different groups of basidiomycetes have distinct diurnal patterns of spore release (9). Some species release spores before dawn, while others release spores for a period shortly after dawn. However, samplers located farther from source areas will detect the peaks later in the day. Many basidiomycetes are seasonal. In temperate zones,

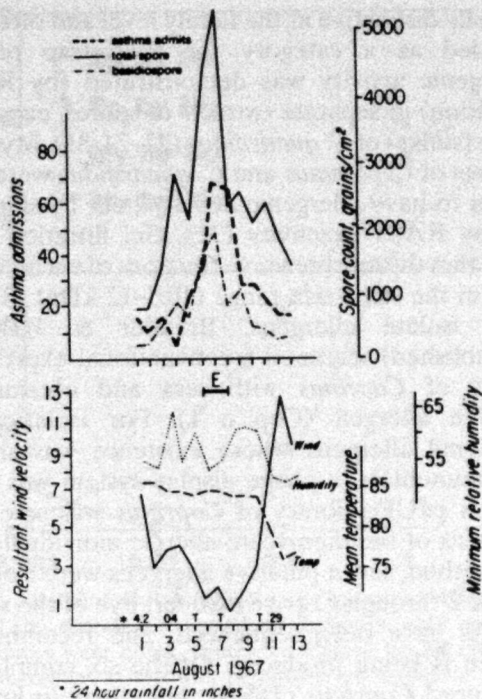


Fig. 1. Relationship between climatic conditions, airborne spore counts, and New Orleans asthma epidemic; during periods when asthma admissions were elevated at emergency room of local hospital, total spore and basidiospore levels were significantly elevated (upper graph), whereas wind velocity was decreased and temperature and humidity were elevated (lower graph). (Used with permission of *Journal of Allergy*, 1971.)

there is typically a spring peak of basidiospores and a larger autumn peak; these coincide with the spring and autumn flush of mushrooms. However, there are usually a few species fruiting whenever temperatures are above freezing. Weather patterns also influence basidiospore concentration, especially rain, since many basidiomycetes need moisture to fruit. Indeed, an early link between basidiospore exposure and asthma was recognized by an association between changing weather patterns and asthma epidemics (Fig. 1) (10, 11).

Geographic variability

Spore-trapping studies that have separated basidiospores as a group were summarized previously

Table 1. Purified basidiomycete allergens that have been sufficiently well characterized to warrant designation

Fungal taxa	Allergen designation	References
<i>Psilocybe cubensis</i>	Psi c 1	Reese (unpublished)
<i>P. cubensis</i>	Psi c 2 (cyclophilin)	19
<i>Coprinus commatus</i>	Cop c 1	20
<i>C. commatus</i>	Cop c 2	Brander & Helbling (unpublished)

(12). This compilation showed that basidiospores were important parts of the airborne spore mix in Finland, Mexico, and New Zealand (13–15). Basidiospores are also prevalent in cities such as London and in rural areas (16, 17). It is reasonable to assume that basidiospores are common in all areas except those with snow cover, or with prevailing oceanic winds (Table 2).

Indoors/outdoors

Demonstrating the presence of basidiomycetes indoors with air samplers is problematic. Many basidiomycetes do not sporulate readily in culture; therefore, culture-based samplers detect these fungi only as “sterile” or nonsporulating isolates. If wood decay fungi are sporulating, then the basidiospores can often be recognized on spore-trap samples (18–20). In the Gulf South of the USA, basidiomycete antigens were detected in settled house dust from about 25% of the houses surveyed (21). However, it is possible that the basidiomycete antigens detected were from spores that infiltrated from outside air. This is a research area that deserves additional attention.

Prevalence of sensitization (skin test reactivity)

Skin test surveys have compared the prevalence of sensitization among basidiomycete species and compared basidiomycetes with molds as groups (22, 23). In general, 25–30% of subjects with respiratory allergy are sensitized (skin prick test [SPT] reactive) to some species in the basidiomycete panel. Most of these 25–30% of subjects react to some species, such as *Psilocybe cubensis*, but only 5% of subjects are sensitized to other species, such as *Pisolithus tinctorius*. Likewise, at least 15% of allergic subjects are sensitized to *Alternaria alternata*, but less than 7% may react to *Cladosporium herbarum*. Generally similar results have been found in a number of skin test surveys; one study had 12 centers in Europe and the USA, and another study was in Argentina (24–26). The study in Argentina

Table 2. Basidiospore air-sampling surveys

Site location	Percentage of total or rank	Peak season
Harpden, UK	34%	Aug/Sept
London, UK	no. 2	Aug/Sept
Cardiff, UK	18–51%	Late summer/autumn
Hamilton, Canada	4th of top 7	July/Aug/Sept
Turku, Oulu, and Kevo, Finland	12.8–48.5%	July/Aug/Sept
Auckland, New Zealand	33.7–62.0%	Late summer/autumn
Southern California, USA	no. 2	Autumn

Table 3. Basidiomycete sensitivity in atopic children

Disease	Buenos Aires ¹	
	<i>n</i>	%
Rhinitis	2/8	25
Asthma	8/19	42.1
Rhinitis/asthma	12/26	46.1
All asthma	20/45	44.4%
Córdoba ²		
All asthma	13/50	26.0%
Total all asthma	33/95	34.7%

¹Máspero, et al. (1995) (26).²Baena-Cagnani, et al. (1994) (25).

was composed of only children; 34% of the subjects were sensitized to at least one basidiomycete (Table 3). Clearly, sensitization to some species is more prevalent than to other species.

Basidiomycete allergens that have been characterized

Boletus

A single, preliminary characterization of *Boletus* allergens has been reported. *Boletus* extracts were resolved by SDS-PAGE. Pooled sera from six SPT-reactive subjects bound IgE to several bands in the range of 34–50 kDa (27).

Calvatia

Skin test reactions to *Calvatia* spore extracts were prevalent in three different studies (22–24). This indicates that sensitization to *Calvatia* allergens is not unusual. Specimens of the lilac puffball, *C. cyathiformis*, may contain up to 50 g of essentially pure spores. This is very useful when conducting large-scale extractions. Consequently, *Calvatia* was used as a model species for allergen purification, and the allergens have been reasonably well studied. Immunoprints with a panel of 19 sera from SPT-positive subjects revealed at least 21 different allergens (28). Two of these allergens reacted with 68% and 63%, respectively, of the sera in the panel. The most reactive allergen had an estimated isoelectric point (pI) of 9.3 and was particularly labile (29). A number of the *Calvatia* allergens were later shown to be cross-reactive with allergens from three of four other species of *Calvatia* (30).

Coprinus

The genus *Coprinus* contains the “inky cap” mushrooms that occur seasonally in some lawn and meadow habitats. The spores are also morpho-

logically distinctive at the family level and often are included as a category for spore-trap results. Allergenic activity was demonstrated (by RAST inhibition) in separate extracts of spores, caps, and stipes (stalks) of *C. quadrifidus* (22–24, 31). Mycelial extracts of *C. comatus* and *C. quadrifidus* were also shown to have allergenic activity both by skin test and by RAST reactivity (32). Gel filtration indicated that the most reactive fractions of each extract were in the same size range (10.5–12 kDa) (31).

To isolate allergens, Brander & Helbling (unpublished) screened a conventional expression library of *Coprinus* with sera and obtained a putative allergen (Cop c 1). For isolation of additional allergens whose existence was shown in immunoblots, a phage display system was used (33). A pJUFO library of *Coprinus* was screened with sera of basidiomycete-allergic individuals. By this method, seven putative allergens were isolated (Cop c 2 through Cop c 8). So far, five of the seven cDNAs have been sequenced, and recombinant protein is being produced. Of the six completely sequenced *Coprinus* cDNAs, homology to known sequences could be found for only one protein. Tests with IgE ELISA and recombinant allergens revealed that Cop c 1 is a minor allergen, whereas Cop c 2 is recognized by 18 of 94 sera tested.

Ganoderma

Ganoderma species are wood-rot fungi that produce large, tough, woody caps on the sides of stumps and fallen logs. *Ganoderma* spores are also very distinctive and are readily identified in spore-trap analyses. These spores are quite common in most inhabited regions of the world; hence, there is a large potential exposure. Four different laboratories have published studies on *Ganoderma* allergens.

The earliest detailed study was in Canada and showed skin test reactivity that was confirmed by RAST (34). Sensitization did not correlate with sensitization to other common inhalant allergens, and polyclonal serum raised against *Ganoderma* did not react to other allergens either. In New Zealand, respiratory symptoms were broadly correlated with the seasonal fluctuations of *Ganoderma* spore count levels; skin test results here also indicated that subjects sensitized to *Ganoderma* were often not sensitized to the other fungi tested (35, 36). Blotting studies have shown that at least 16 allergens are present in *G. meredithae* extracts when screened against a panel with as few as six sera or by crossed immunoelectrophoresis (CIE) (37, 38). Blotting inhibition studies with *Ganoderma* and other basidiomycetes indicated that *Ganoderma* had little cross-reactivity even among basidiomycetes (39).

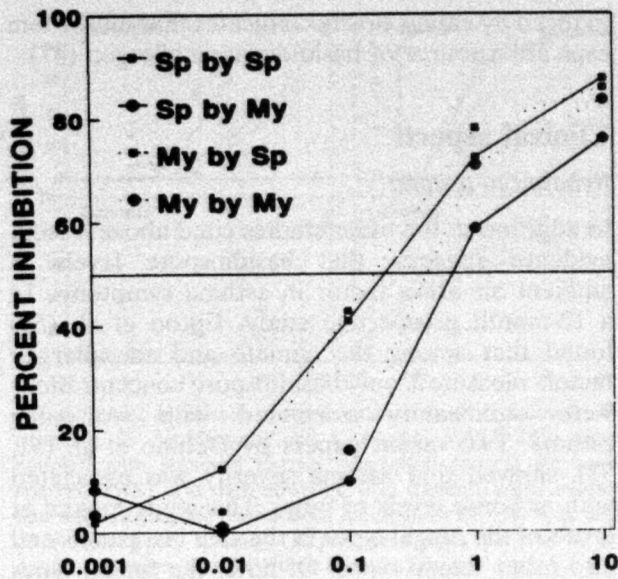


Fig. 2. Inhibition of *P. cubensis* spore (Sp) and mycelium (My) RAST by spores and mycelium. Although only moderate differences were noted, spores were more potent inhibitors than mycelium. (Used with permission of *Journal of Allergy and Clinical Immunology*, 1993.)

Pleurotus

The oyster mushroom produces rather nondescript spores. Analysis of a spore trap sample that contains *Pleurotus* spores would include these in the broad category of "colorless basidiospores". Hence, the exposure to *Pleurotus* is not well known. However, oyster mushrooms (and related species) are common throughout the forested portions of the temperate zone; hence, exposure is certainly not rare. *P. ostreatus*, considered a choice edible mushroom, is also cultivated; thus, there is the potential for occupational exposure. *Pleurotus* releases spores as soon as the cap begins to expand, in contrast to cultivated button mushrooms (*Agaricus*), which are generally harvested before the caps open and release spores. At least five allergens were shown by CIE in spore extracts of *Pleurotus*. In more recent immunoprint studies, at least 13 allergens were shown in mycelial extracts (40, 41). Allergens were also shown to be rapidly released from intact spores (42). Current efforts to clone cDNA of basidiomycete allergen genes include *P. ostreatus* as one of the model species (43).

Psilocybe

In temperate regions, the exposure of most people to *P. cubensis* allergens is undoubtedly minimal; thus, most reactivity is due to exposure to cross-reactive allergens of related species. *P. cubensis*

occurs from the Gulf South of the USA through the Caribbean to northeast Brazil. Yet a significant percentage of mold-sensitized patients in Europe (24) react to extracts of spores collected in Louisiana (USA). A number of related species occur in lawns and pastures throughout much of the temperate zone.

P. cubensis allergens are probably the best studied among basidiomycetes to date. Spore and mycelial extracts have been thoroughly characterized; there are 13 and 11 allergens, respectively, in these extracts (44). RAST inhibition showed that common epitopes were present, although the spore extract had greater inhibitory potency than did the mycelial extract (Fig. 2) (45). Monoclonal antibodies have been prepared against the major 48-kDa allergen. Another, 16-kDa allergen was determined to be homologous with cyclophilin (a cytosolic isomerase) by cDNA sequence analysis (46).

Cross-reactivity

Numerous studies have shown cross-reactivity among fungal allergens. Most of these have studied conidial fungi, and some have compared yeast allergens. Only three well-controlled studies have been reported with basidiomycete allergen extracts.

These three studies all used allergen extracts to inhibit IgE-specific immunochemical assays, either RAST or blotting. RAST inhibition was first used to detect the cross-reactivity of various basidiomycete allergen extracts (47). These cross-reactivities were confirmed with subsequent IgE immunoblots that were cross-inhibited with specific extracts (39). This second study included six species, and the results were consistent with the general taxonomy of basidiomycetes. Four species from related orders were cross-reactive, but species from two unrelated basidiomycete orders showed only minimal cross-reactivity with the other four species or with each other. This indicates broad cross-reactivity.

Another RAST inhibition study sought relations between basidiomycete and conidial fungi (48). In this study, there were minimal although detectable cross-reactivities between the groups, but far stronger reactions between species within each group.

These patterns of cross-reactivity are known to be important in pollen allergy and food allergy, among others. Presumably, these cross-reactions are also significant in fungal allergy. The limits and extent of these patterns must be determined, as they have been for grass pollens, if fungal allergy is to be properly managed.

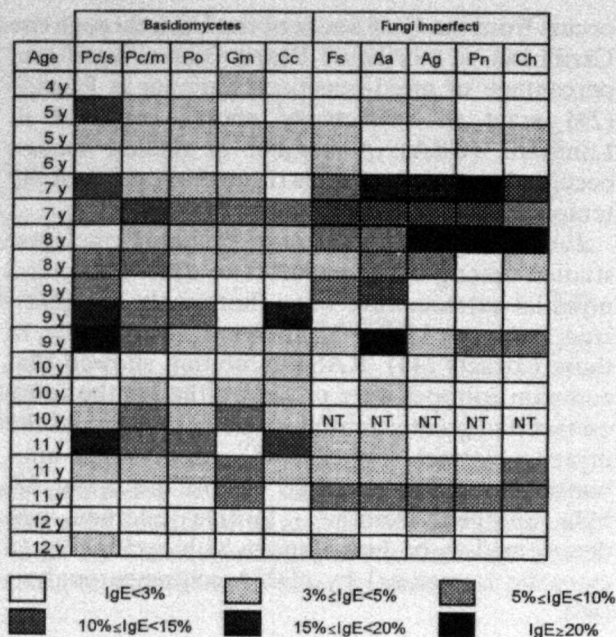


Fig. 3. Fungal specific IgE antibody reactivity of patients presenting at pediatric emergency room with acute exacerbations of asthma. Most significant aeroallergen to which patients reacted was house-dust mite (results not shown). Exactly 19/42 subjects reacted to basidiomycetes and/or Fungi Imperfecti. Basidiomycetes species tested were *P. cubensis* spore (Pc-s), *P. cubensis* mycelium (Pc-m), *Pleurotus ostreatus* (Po), *Ganoderma meredithae* (Gm), and *Calvatia cyathiformis* (Cc). Reactivity was also tested to Fungi Imperfecti *Fusarium solani* (Fs), *Alternaria alternata* (Aa), *Eurotium* as *Aspergillus glaucus* (Ag), *Penicillium chrysogenum* as *P. notatum* (Pn), and *Cladosporium herbarum* (Ch). Degrees of IgE antibody reactivity are indicated by intensity of shading.

Source materials of fungal allergens

There are only limited data available that directly compare different source materials of any fungi. Among the basidiomycetes, comparisons of different source materials have been made only for *Coprinus* and *Pleurotus*. In *Coprinus*, caps, spores, and stalks were compared. Although each of these materials contained allergens, the caps and stalks actually contained greater allergenic (RAST inhibition) activity than did spores. *Pleurotus* extracts from washed mycelium (broth-cultured), caps, and spores likewise each contained allergens. CIE analysis indicated that spore extracts contained allergens that were not detected in the other two extracts, although the total allergenic activity of the spore extract was no greater.

Only a few, limited studies have been reported that consider fruiting body tissues as a source of allergen extracts. These have shown that fruiting bodies of the basidiomycetes *Pleurotus*, *Ganoderma*, and *Coprinus* all contain allergens (31, 40, 49). Additionally, the recent report of anaphylaxis

induced by eating *Boletus* indicates that mushroom caps are a source of basidiomycete allergen (47).

Clinical aspects

Relation to asthma

In addition to the older studies cited above, recent evidence suggests that basidiospore levels in ambient air are a factor in asthma symptoms. In a 12-month prospective study, Epton et al. (50) found that among the climate and aeroallergen factors measured, only basidiospore concentrations were significantly associated with worsening asthma. Two recent papers by Delfino et al. (51, 52), showed that asthma severity was associated with airborne levels of fungi. This study looked at levels of the fungal types in the skin test panels and also other fungal types. Of note, the fungal types that were not in the test panel were a more significant associate of asthma severity than the levels of fungal types that were in the skin test panel. This indicates that the fungi typically included in test panels do not include all the clinically relevant types of fungi.

A study of asthma patients presenting to a pediatric emergency room in New Orleans also suggests that allergen test panels should include other extracts. Of patients presenting with acute asthma (physician-diagnosed), 95% of those between the ages 4 and 14 years ($n=43$) were sensitized to environmental allergens (53). Although mite was the most important allergen, 37% of the subjects reacted to at least one fungal extract (mold or basidiomycete) (Fig. 3). However, almost one-third of the fungal reactors were not sensitized to both molds and basidiomycetes. This again indicates that test panels should include extracts from both groups of fungi.

Nasal challenges

To demonstrate the clinical relevance of the *Pleurotus* extracts used in recent Swiss studies, nasal challenges were conducted. Challenges were made on 12 skin-test reactive subjects with respiratory allergies (nine asthmatic and three rhinitic) (43). Nasal airflow was measured by active anterior rhinomanometry at a reference pressure of 150 Pa, according to guidelines of the European Committee on the Standardization of Rhinomanometry. Three concentrations of spore extract were used (0.1, 1.0, and 10.0 mg protein/ml). Reductions of nasal airflow of 40% were considered positive reactions. Six controls, including two pollen-allergic patients, were unaffected, but all 12 *P. ostreatus*-reactive subjects had a

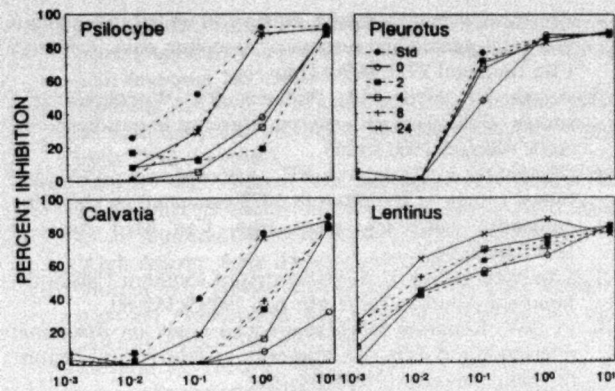


Fig. 4. RAST inhibition of spore extracts from *P. cubensis*, *P. ostreatus*, *C. cyathiformis*, and *L. edodes*. RAST disks were coupled with standard extract (disrupted spores) for each species. RAST was then inhibited with standard (Std) extract and with eluates harvested from incubations of 0, 2, 4, 8, and 24 h from each species. (Reproduced with permission of *Journal of Allergy and Clinical Immunology*.)

significant reduction in nasal airflow (mean 73%) with subjective symptoms.

Occupational sensitization

The cultivation of the common "button" mushroom (*Agaricus*) for food involves composting organic material for the mushrooms to grow on, and, separately, the culture and harvest (before spore release) of the mushrooms on the compost. Hypersensitivity pneumonitis (HP) that develops among *Agaricus* workers is often due to reactivity to the actinomycetes in the compost rather than to the basidiomycetes. However, workers raising other species of mushrooms, such as oyster mushrooms (*Pleurotus* species) or shiitake (*Lentinus edodes*), develop HP to the mushroom spores rather than to actinomycetes (54). Substrate materials for oyster and shiitake mushroom cultivation are not composted, but these caps open and release spores from the earliest growth stages. Consequently, high levels of mushroom spores are released when the caps are large enough for harvest. This became an intense exposure issue when the traditional oriental method of outdoor cultivation was brought indoors in Europe and North America in the last 20 years.

Occupational exposure can also be anticipated for workers using basidiomycete enzymes commercially. Specific ligninase applications have been proposed for decolorization of dye wastes, soil remediation of pollutants, and biopulping. These are all three very large, or potentially large industries, and hence any one of these applications would expose many workers to basidiomycete enzymes. Since numerous other fungal enzymes are allergenic, it is reasonable to expect that

ligninases are, too and, when used, will sensitize workers.

Allergen release from intact spores

Little is known about the allergen-release kinetics of spores. This is unfortunate, because the way in which allergen is released probably affects local allergen concentration at the point of spore deposition in the airways.

Studies of allergen release from *Cladosporium cladosporioides* conidia (55), *Alternaria alternata* mycelium (56, 57), and four basidiospores (42) indicate that differences occur among different types of fungi. Four basidiospores tested represented three different spore types. *Lentinus edodes* and *Pleurotus ostreatus* both have thin-walled, hyaline spores. *Calvatia cyathiformis* spores have very thick walls; although the walls are not heavily melanized, the spore walls are very strongly hydrophobic. *Psilocybe cubensis* has very thick walls that are strongly melanized, but the spores are rather easily wetted. When these four spore types were extracted without homogenization, and compared to homogenized extracts as a control, the *Lentinus* and *Pleurotus* spores released essentially all of their allergenic activity very quickly, within the first hour. *Calvatia* and *Psilocybe*, however, never released as much allergen as homogenized spores and required 24-h elutions to release what allergen was released (Fig. 4). These differences probably affect the concentration of allergen at the point of deposition in the airways and may affect which species are effective.

Food allergies to mushrooms

Available data on food allergies to mushrooms are largely anecdotal. Various mushrooms have long been cultivated or collected for food. These species are well known not to be poisonous, yet some occasionally cause gastrointestinal illness. In most cases, this is probably food intolerance, but two recent, well-documented cases of food allergy to mushrooms have been reported. One was allergy to shiitake (*Lentinus edodes*) and the other was an anaphylactic reaction to cèpe (*Boletus edulis*) (47, 58). Other cases of allergic reactions to cèpe have also been reported (Helbling, unpublished). This suggests that some basidiomycete allergens may be important food allergens.

Conclusions

Basidiomycete fungi clearly can sensitize atopic subjects and induce respiratory allergies. Furthermore, the prevalence of sensitization to basidio-

mycetes is comparable to that of conidial fungi, and exposure to either can induce asthmatic responses. However, there are tens of thousands of basidiomycete species, and only a few have been studied. Even so, allergens from enough species of basidiomycetes have been characterized to show clearly that a broad range of basidiomycetes are allergenic.

Among fungal allergic subjects, there is overlap between the population of subjects sensitized to basidiomycetes and to conidial fungi (24). There are also subjects who are sensitized only to basidiomycetes or only to conidial fungi. This suggests that the overlap is due to concurrent sensitization rather than to cross-reactivity. This suggestion is supported by one study (39), but, clearly, more work is needed to define the limits of allergenic cross-reactivity among groups of fungi.

The clinical importance of basidiomycete allergy was recently emphasized by studies correlating asthma severity with environmental factors (51, 52). Symptom scores and airborne spore counts correlated for the types of fungi not on the skin test panel rather than the types that were on the test panel. This is significant, since exposure to basidiomycetes can occur outdoors, and they are often a major portion of the airborne spore load. The available evidence, although scant, also suggests that indoor exposure to basidiomycete allergens is important.

Studies are needed to add well-defined and characterized basidiomycete allergens to fungal screening panels. These allergens should have well-described patterns of cross-reactivity. Such panels would aid accurate diagnosis and could greatly improve the determination of fungal allergy prevalence. Whether such allergens can be useful for specific immunotherapy depends on the degree of characterization attained for these extracts, and the development of new therapeutic strategies based on the current understanding of the pathomechanism of allergic disease.

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