

The role and abatement of fungal allergens in allergic diseases

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Sensitivity to a variety of fungi is known to be a factor in allergic rhinitis and asthma. In this review methods for measuring exposure to fungi in the indoor environment are evaluated. A variety of markers for the presence of fungi are also described in addition to their known relationship to either toxic or adverse immunologic effects. Key studies documenting the clinical effects of different types of fungi are also reviewed, as well as a description of abatement methods that either have been successful or need further investigation. Although many studies have shown an association between exposure to fungi and allergic disease, in many cases a direct cause-and-effect relationship has not been established. Improved knowledge of the epidemiology and mechanisms behind fungal-induced human disease will hopefully establish this causal link and suggest methods for reducing morbidity. (*J Allergy Clin Immunol* 2001;107:S430-40.)

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Fungi have long known associations with human diseases. Although many studies have described environmental conditions that lead to fungal growth, there is no consensus on the most clinically relevant methods for measuring personal exposure. Lists of observed airborne and dust-containing fungi have been described for a variety of environments, including residences, workplaces, and schools; however, their clinical relevance is rarely clear. With even less information available on how much exposure one must have to fungi and their metabolites to actually become ill,^{1,2} optimal standardized methods for measuring levels of fungi in indoor environments is critical. In this review we will describe methods for measuring fungal exposure and critically evaluate their usefulness. Although the amount of exposure that causes illness undoubtedly will relate to individual sensitivity, it currently is not feasible to assess this directly because fungal emanations, such as mycotoxins, are too toxic for challenge experiments. As a result, we must rely on studies of natural exposure and abatement to support causality. Methods for reduction of fungal exposure and our current knowledge of their effect on spore counts and antigen contents are also addressed in this review.

Abbreviations used

ABPA: Allergic bronchopulmonary aspergillosis
EPS: Extracellular polysaccharide
VOC: Volatile organic compound

FUNGI

Fungi, also commonly called molds, comprise a taxonomic kingdom with many members ubiquitous in nature. They generally have semirigid walls, are nonmotile, and, lacking chlorophyll, must obtain carbohydrates from their environment.³ To do this, fungi facilitate aerobic decay of nonliving organic matter, although some species have evolved symbiotic relationships with living organisms. Many fungi reproduce sexually by means of meiosis by producing spores that form in or on specialized structures (ie, sacs called asci). Fungi that reproduce in this manner are referred to as *perfect fungi*. These same species also may reproduce asexually by means of mitosis alone, and this is referred to as the *imperfect* stage. For many genera, the sexual or asexual stages have not been identified or both may be known but not recognized as stages of one taxon. This makes classification of the fungi confusing at times, especially for the nonmycologist, and leads to predictable changes in nomenclature over time. For the most part, fungi that are known to cause human allergic diseases are in asexual stages and are somewhat artificially grouped as *fungi imperfecti*.

Many species of fungi may act as pathogens by means of invasive growth in human tissues, especially those of immunocompromised patients,⁴ and they may cause inflammatory conditions through a variety of immunologic mechanisms.⁵ Examples of the latter include hypersensitivity pneumonitis, allergic bronchopulmonary mycoses, allergic fungal sinusitis, allergic rhinitis, and allergic asthma. In addition, some fungi produce toxic metabolites that directly cause acute, chronic, or both types of human diseases.

TAXONOMY OF THE COMMON FUNGAL GROUPS

More than 80 genera of the major fungal groups have been associated with respiratory tract disorders (Table I),⁶ with the most commonly identified belonging to 3 distinctive fungal groups: ascomycetes, basidiomycetes, and deuteromycetes (imperfect fungi). Many mycologists hesitate to give the deuteromycetes separate group status because many species are biologically members of the ascomycetes (or basidiomycetes) where the sexual stage is not known. A better way to refer to this group of spore

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TABLE I. Allergenic fungi

Table available in print only

producers might be as asexual or anamorphic fungi.

The fungi in the ascomycetes grouping^{3,7} are those species that produce spores by means of meiosis in a sac, or ascus, often in groups of 8. The spores are generally hydrophilic, although spore production is able to survive periods of relative desiccation. Once exposed to atmospheric moisture, the asci swell and the spores are ejected forcefully. Hence, ascomycetes tend to release spores during periods of high humidity or rainfall or in microenvironments with high water content. These spores are morphologically diverse, making visual identification possible for some genera. Ascospores found indoors often represent infiltration from outdoors,⁸ although their presence can indicate that indoor amplification has occurred, most commonly as a result of moisture problems.

The fungi of the basidiomycetes group^{3,7} comprise diverse forms, including mushrooms, puffballs, rusts, and smuts. Conditions required for their dissemination vary between species. At times, basidiospores can dominate the outdoor air flora. Indoors, the presence of basidiospores may reflect significant moisture problems and rotten wood or most often infiltration of outdoor air.⁸

The deuteromycetes³ (also called fungi imperfecti or asexual fungi) replicate asexually. Their spores, called conidia, generally form on specialized hyphae (conidiophores), although sexual stages may form in other growth

conditions. Many taxa of this group have a distinctive appearance, making identification possible, at least to the (form) genus level. Many species that are known to be allergenic are in this easily identified group. Indoor amplification of deuteromycetes, such as *Cladosporium*, *Penicillium*, and *Aspergillus* species, is fairly common and occurs when excessive moisture is present.

ECOLOGY OF FUNGAL GROWTH

Even among deuteromycetes, fungi differ in conditions for optimal growth. Some are xerophilic, growing in relatively dry environments (*Wallemia* species, certain *Penicillium* and *Aspergillus* species). Others, such as *Stachybotrys* (a major mycotoxin source but uncertain as an allergenic species), are hydrophilic, requiring substrates with high water content. Some important allergenic fungi grow on leaf surfaces (*Alternaria*, *Cladosporium*, *Epicoccum*, and *Aureobasidium* species). Many fungi use wood or cellulose as nutritive sources. Saprophytes grow on decaying vegetation containing cellulose and other substrates, whereas others attack living plant tissues. All fungi require oxygen for growth in addition to sources of carbohydrate and water. Optimal growth temperatures among species vary but are commonly between 18°C and 32°C.

TABLE II. Distribution of indoor and outdoor allergenic molds

Fungus	Range (spores/m ³)				
	Indoor*	Indoor summer†	Indoor winter†	Outdoor summer†	Outdoor summer‡
<i>Penicillium</i> species	0-4737	0-7900	0-480	0-95	15,000
<i>Cladosporium</i> species	12-4637	0-160	0-160	11-430	600,000
<i>Botrytis</i> species	0-54	—	—	—	12,000
Yeasts	0-5	0-74	0-78	0-790	10,000
<i>Aspergillus</i> species	0-306	0-76	0-19	0-11	15,000
<i>Alternaria</i> species	0-282	—	—	—	7500
<i>Rhizopus</i> species	0-24	—	—	—	—
Nonsporulating mycelium	0-14,194	0-1700	0-200	19-9300	—
<i>Epicoccum</i> species	0-155	—	—	—	—
<i>Fusarium</i> species	0-47	—	—	—	7500

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*Studies carried out in Southern California homes.

†Studies carried out in Finnish homes.

‡Studies carried out in European homes.

SEASONAL AND WEATHER PATTERNS OF FUNGAL PREVALENCE

Outdoors, in northern areas of the United States, fungal spores appear in late winter, when snow cover begins to dissipate. As the weather warms, especially in the months of May and June, the spores become more prevalent. Peak spore counts occur in the months of late summer (July-October), with numbers falling thereafter. Once snow covers the ground (or the ground is frozen), airborne spores decrease, until the cycle begins again in late winter.

In the southern areas of the country, although fungal spores may be abundant throughout the year, peak spore levels occur during the summer or early fall months of June through October each year.⁹

Wet and dry weather conditions also affect outdoor spore prevalence. *Fusarium* species, *Phoma* species, ascospores, and basidiospores predominate during periods of wet weather.¹⁰ Spores from *Cladosporium*, *Alternaria*, *Epicoccum*, *Helminthosporium*, and *Drechslera* species predominate during dry weather, especially when it is windy.¹⁰

A relationship also exists between indoor fungal accumulations and outdoor weather.¹¹ Higher indoor levels are noted when outdoor fungal levels increase. Table II lists the distribution of indoor and outdoor allergenic fungi¹² from studies conducted in Southern California, Finland, and England. In Japan, indoor fungal counts of *Cladosporium*, *Aspergillus*, *Wallemia*, and *Penicillium* species peak in October and increase with increasing outdoor temperature and humidity.¹³

ASSESSMENT OF FUNGAL EXPOSURE

Volumetric air samplers

Exposure to fungi (and presumably to fungal allergens) has been traditionally assessed by microscopically identifying and measuring the number of fungal spores in air samples or by using semiquantitative cultures obtained from air or settled dust samples (Table III).

Total spore counts or individual genera that can be

microscopically identified can be collected by using volumetric samplers (those which sample a known volume of air), such as the Rotorod (Sampling Technologies, Inc, St Louis Park, Minn), Burkard sampler (Burkhard Manufacturing Co Limited, Rickmansworth, Hertfordshire, England), and Sampler MK-3 (Allergenco/Blewstone Press, San Antonio, Tex). In general, suction samplers, such as the Burkard, are more efficient over a broader range of particle sizes, especially smaller units, than impaction samplers, such as the Rotorod.

Volumetric samplers can be used to sample at repeated intervals because they can be programmed to obtain samples on specific schedules. Indoor spore counts equal to or greater than 1000/m³ most likely represent indoor fungal contamination. One study in which a volumetric sampler was used is that of Garrett et al,¹⁴ in which 80 homes with 148 children in Australia were studied. Samples for airborne total and viable spores were collected from several rooms in each house. Higher airborne levels corresponded with increased respiratory symptoms in the children.

Airborne spores can be collected with suction samplers in which air is sucked onto an adhesive-coated microscope slide where particles impact and are captured, sucked onto membranes, or sucked into small fluid volumes for subsequent culture or microscopic tally or onto culture plates for determination of viable colony-forming units. Colony counts on the order of 1000 to 10,000/m³ likely represent indoor fungal contamination. The last approach frequently makes use of either a multistage Andersen sampler, which separates spores by size, or a single-stage Andersen sampler, which detects viable colonies on a single plate. The culture method may undersample certain taxa, such as *Stachybotrys* species, which grow slowly or not at all on commonly used substrates, such as malt extract or Sabourauds media. Other fungal types, such as *Wallemia* species, grow well only on selected media, such as DG18, whereas some, such as many *Penicillium* species, grow well and quickly on most media. The culture methodology for assessing fungal exposure, however, is expensive and time consuming. Such methods have been used in assessing indoor fungal exposure.

TABLE III. Methods for measuring indoor fungal exposure

Marker	Advantages	Disadvantages	Human health implications
Visual identification of air samples	Quick, tells about airborne exposures, sensitive—does not rely on viability	Cannot identify species, labor intensive, spores may be intermittently airborne	Inhaled spores can cause human disease
Culture of air samples	Can identify species, assay for mycotoxins	Very labor intensive, takes a long time, not very sensitive—only detects spores with growth potentiation media used	Inhaled spores can cause human disease, including infection for viable spores
Questionnaires	Inexpensive, easy to do	Not specific, few validated surveys	NA
Allergen immunoassays	Very sensitive, specific for certain genera	Monoclonal assays may be too specific	Measures allergenic potential of the exposure
Ergosterol	Reflects total fungal biomass	Cannot be used to identify individual taxa	No known effect
EPSs	Can be used to identify some genera		No known effect
β (1 $\rightarrow$ 3)-glucan	Good marker of total fungal biomass	Nonfungal sources can affect results	Has direct inflammatory effect
VOCs	Variably specific for individual taxa	Nonfungal sources can affect results	Has direct irritant effect
Mycotoxins	Reveals toxic potential of the exposure	Assays are difficult to reliably reproduce	Carcinogenic, teratogenic, variable neurotoxicity and organ toxicity

NA, Not applicable.

Gorny et al,¹⁵ for example, sampled 60 apartments in Poland with a 6-stage Andersen sampler. The most common fungi recovered included *Penicillium* species, *Aspergillus* species, and yeasts.

Personal bioaerosol samplers¹⁶ are also available, providing integrated collections or separating particles into respirable and nonrespirable fractions for variable periods; several preserve particle viability. Total counts can be determined after fungal propagules are collected on a filter or on a sticky surface, such as the Air-O-Cell cassette (Zefon, Inc, St Petersburg, Fla), by means of microscopic analysis.

Although some fungal spore species can be easily identified by appearance, others, such as those of *Penicillium* and *Aspergillus* species, are not distinctive, making analysis of colonial growth essential. This is one advantage of using viable or cultured sampling techniques along with microscopic tallies. Immunofluorescence also can help with fungal identification, but its use is not widespread.

Questionnaires

Questionnaires are a very low-tech but useful method of assessing indoor fungal presence. When the indoor environments of persons who responded to questionnaires were examined, several factors correlated with spore counts, including reports of visible growth of mold, musty smell, damp spots, and moisture or water damage. These conditions also were correlated with eye irritation and increased rates of respiratory infections.¹⁷

In addition to visually observing fungal growth on surfaces or in air samples, a variety of biochemical and immunochemical markers of fungal presence have been developed. These may offer an indication of the extent of contamination that may be present in an indoor environment, particularly when specific assays for all potentially relevant species may not be available. Such markers have

been described in the medical literature and include ergosterol, extracellular polysaccharides (EPSs), β (1 $\rightarrow$ 3)-glucan, volatile organic compounds (VOCs), and fungal mycotoxins (Table III). Although these markers do not measure fungal allergen exposure, they can indicate the presence and relative extent of fungal growth. Their use in quantitating fungal exposure and its relationship to human allergic disease is still largely investigational, however.

Ergosterol. Ergosterol is a primary cell membrane sterol of most fungi. Because ergosterol is not found in vascular plants, its relative specificity for fungi makes it a good marker for total fungal biomass. The quantity of ergosterol produced by a particular fungal isolate depends on its surface area and growth conditions. Levels of ergosterol correlate well with total spore counts.¹⁸ They are not helpful indicators of individual species, however.¹⁹

Extracellular polysaccharides. EPSs are stable carbohydrates that are secreted or shed during fungal growth. These polysaccharides have antigenic specificity, usually at the genus level. EPSs, which frequently are measured with immunoassays, may serve as a marker for fungal exposure, as was shown by Douwes et al.²⁰ They identified EPSs from both *Aspergillus* and *Penicillium* species in household dust by means of enzyme immunoassay, and their findings correlated with culturable fungus recoveries. At present, there is no evidence for a direct pathogenic role for EPSs in fungal-related disease.

β (1 $\rightarrow$ 3)-glucan. β (1 $\rightarrow$ 3)-glucan is a glucose polymer found in filamentous fungal cell walls and in other sources, including yeasts, some bacteria, and many plants. Although glucans have variable molecular weights, they are a helpful marker of total fungal biomass.²¹ Glucans can be measured with membrane filter sampling by using the *Limulus* amoebocyte lysate assay with factor G.

Volatile organic compounds. Fungi produce a variety of VOC metabolites, including alcohols, aldehydes, ketones,

TABLE IV. Some relevant allergens from fungi by molecular cloning

Fungal species	Allergen designation	Molecular size (kd)	Reactivity with patient sera
<i>Aspergillus fumigatus</i>	Asp f 1	18	IgE/IgG binding with ABPA sera
	Asp f 2	34	IgE binding with ABPA having central bronchiectasis
	Asp f 6	26.7	IgE binding
	Asp f 12	65	IgE/IgG binding with ABPA sera
<i>Alternaria alternata</i>	Alt a 1	28-29.2	IgE binding
	Alt a 2	25	IgE binding
	Alt a 3	hsp 70	IgE binding
	Alt a 6	11	IgE binding
	Alt a 7	22	IgE binding
	Alt a 10	53	IgE binding
<i>Cladosporium herbarum</i>	Cla h 1	13	IgE binding
	Cla h 2	23	IgE binding
	Cla h 3	11.1	IgE binding
<i>Candida albicans</i>	Can a ?	40	IgE binding

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hsp, Heat shock protein.

and a carbonyl sulfide-like compound.²² These are often perceived as causing moldy, earthy, or musty smells. Although many VOCs have diverse fungal sources, some are specific for individual species and can serve as markers for their presence.²³ They have served as tracers for suspected microbial problems in buildings where occupants complained of poor indoor air quality and respiratory irritation.²⁴ When using VOCs as a marker for the presence of fungi, it must be remembered that many of these compounds have other sources, such as traffic, off-gassing from building materials, and human activities.

Mycotoxins. Mycotoxins are biologically active, non-volatile, low-molecular-weight metabolites produced during fungal growth. Approximately 400 of these fungal metabolites are considered to be toxic. It is likely that all fungi produce mycotoxins when growth conditions are suitable. Mycotoxins can be carcinogenic, neurotoxic, and teratogenic²⁵ but are not directly related to allergic disease. Most cases of airborne exposure have been described among farm workers, persons processing moldy materials, or persons living in homes with excessive mold growth. Because of the potential adverse health effects on humans, there is a great deal of concern about mycotoxin exposure.

Fungal mycotoxins can be measured with HPLC and some enzyme immunoassays. Although these methods generally were developed to measure mycotoxins in foods, they also can be used to detect toxins in environmental samples. The more common agents measured include macrocyclic tricothecenes,²⁶ the fumonisins, ochratoxin, and other mycotoxins produced by *Aspergillus* and *Penicillium* species.²⁷ Pieckova and Jesenska²⁸ measured a number of these markers (mycotoxins, glucans, and VOCs) to identify *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Stachybotrys*, and *Wallemia* species. Although mycotoxins can be used to detect the presence of certain fungi in the environment, it is more common for mycotoxin levels to be measured once fungi have been already identified. Thus fungi may be used as a marker for the possible presence of mycotoxins rather than the other way around.

FUNGAL ALLERGEN-SPECIFIC IMMUNOASSAYS

The most direct way to assess the role of fungal allergen exposure as a determinant of allergic rhinitis and asthma is by measurement of allergenic concentrations in airborne or settled dust samples. This technology, as applied to fungal allergen exposure, is not as highly developed as that for house dust mite, cat, or cockroach allergens.

Allergen-specific immunoassays are generally used to measure the concentration of fungal sensitizers in airborne or settled dust samples. Although exposure to spores and allergens occurs as a result of airborne particles,²⁹ such exposures are often transient, and brief collections may not accurately represent the integrated exposure over time.³⁰ House dust, on the other hand, is considered to be a reservoir of spores and allergenic materials.³¹ By measuring fungal antigens and allergens in such samples, an alternative estimate of the total fungal exposure can be obtained.

Several fungal allergens have been purified and molecularly cloned (Table IV).^{9,12} The purification of fungal allergens is important for the development of immunoassays to quantitate fungal allergen exposure. Assays generally are performed by using either polyclonal sera obtained from fungal extract-immunized animals or by using mAbs in a 2-sited assay, as has been done with other allergens.³² Initially, assays were based on RAST inhibition with polyclonal antibodies derived either from laboratory animals or human sera containing specific IgE.³³ The advantages of the polyclonal antibody-based assays are that they are relatively inexpensive to develop, and they can detect the presence of fungi even if they are not producing an allergen. This is important because fungi may change their antigenic characteristics depending on the environmental conditions under which they grow.³⁴ In addition, there may be differences between allergens isolated from spores and mycelia.³⁵ An important disadvantage of polyclonal antibodies is that they are directed only toward antigens recognized by the source species, and they can be variable in composition and potency.

A number of mAb-based immunoassays have been developed that rely on binding to 2 different sites on the

molecule to be assayed. An advantage of this approach is its increased sensitivity and specificity. Monoclonal antibody-based assays, on the other hand, are more expensive to produce but can be made to be specific for a single allergenic protein. In addition, they are consistent and can be produced in quantities as large as desired. All of these assays are only as good as the antigens used to produce them.³⁶ Unfortunately, in some instances an mAb-based assay may be too specific to detect the presence of fungi that may not produce the exact protein that the assay is designed to detect. A combination of assays may therefore be necessary to more accurately assess fungal exposure.

PURIFIED FUNGAL ALLERGENS

Alternaria species

A number of *Alternaria* species allergens have been isolated. Alt a 1 is a major allergen with a molecular weight of 29 to 30 kD.^{9,14,37,38} Several 2-site enzyme-linked immunoassays to Alt a 1 have been described^{39,40} that could be used to detect Alt a 1 in both indoor and outdoor environmental samples.⁴¹

Alt a 2 is another major *Alternaria* species allergen that has been molecularly cloned.⁴² Several minor *Alternaria* species allergens have been identified as well: Alt a 3 is a heat shock 70 protein; Alt a 6 is a P₂ ribosomal protein; Alt a 7 is homologous with YCP4 yeast protein; and Alt a 10 is an alcohol dehydrogenase. These proteins appear to be cytoplasmic housekeeping proteins that are conserved among several fungal species. A double mAb assay for a 70-kD glycoprotein allergen known as GP70 has also been described.⁴³ Although GP70 has not been given a standard nomenclature designation, it accounted for 13% of the dry weight of an *Alternaria* species extract and elicited a positive skin test reaction in 87% of *Alternaria* species-sensitive patients tested.⁴³ When GP70 and Alt a 1 were measured simultaneously in air samples, substantial discrepancies were observed over time, suggesting that these allergenic glycoproteins are released under differing conditions.⁴¹ This points out a possible disadvantage of mAb-based assays that might be circumvented if more broadly reactive assay reagents were used in prevalence studies.

Cladosporium species

Three *Cladosporium herbarum* allergens have been purified.⁹ Cla h 1 and Cla h 2 are major allergens with molecular weights of 13 and 23 kD, respectively. Cla h 3 is a ribosomal P₂ protein similar to Alt a 6. Enolases are highly conserved major allergens found in *Cladosporium* and *Alternaria* species,⁴⁴ as well as *Saccharomyces cerevisiae* and *Candida albicans*. A polyclonal antibody-based immunoassay has been described for *Cladosporium* species.⁴¹

Aspergillus species

Aspergillus fumigatus is an important indoor allergen from which several allergens have been molecularly cloned.^{45,46} Asp f 1 is a cytotoxin homologous with mitogillin.⁴⁷ The protein is secreted into the surrounding

media only during active growth of the organism. Asp f 2 is a major allergen in patients with allergic bronchopulmonary aspergillosis (ABPA). Asp f 3 is a secreted peroxisomal membrane protein that is important in allergic asthma. Asp f 4 also binds IgE from sera of patients with ABPA. Asp f 5 is another secreted protein important in allergic asthma. Asp f 6 is a nonsecreted manganese superoxide dismutase that is important in ABPA. An ELISA has been described for detection of *Aspergillus* species in environmental samples.⁴¹ An mAb-based assay has been described for measurement of Asp f 1.⁹

Penicillium species

Only a limited number of allergens from *Penicillium* species have been characterized.^{9,14} A 33- to 34-kD alkaline serine proteinase has been isolated from *Penicillium citrinum* that is cross-reactive with similar allergens from *Aspergillus* species.⁴⁸ A heat shock 70 protein has also been identified as an allergen.⁴⁹

RELATIONSHIP BETWEEN FUNGI AND ALLERGIC SYMPTOMS

Several studies have demonstrated the relationship between increased spore counts and fungal antigen levels with the presence of allergic symptoms. Li and Kendrick⁵⁰ established a descending order for spore counts by location in 15 homes in Ontario, Canada. Overall counts were highest in living rooms, followed by family rooms, kitchens, bathrooms, and bedrooms. The highest diversity of spore types was found in the kitchens. Although the presence of damp conditions and carpets increased spore counts, forced-air heating systems, dehumidifiers, air filters, and air conditioners reduced fungal levels. Allergic symptoms were higher in persons living in damp residences.

In the homes of 20 children with known allergies,⁵¹ 12 (63%) of 19 homes had increased fungal levels. Most commonly identified were *Penicillium* species, *Cladosporium* species, *Aspergillus* species, *Mycelia sterilia*, and yeasts.

When the homes of 46 individuals (20 atopic and 26 nonatopic) living in Taiwan were evaluated,⁵² those with atopy had higher fungal levels than control subjects in summer but not in winter. This finding was true for *Aspergillus* species, *Cladosporium* species, *Penicillium* species, and yeast. No relationship between dampness and spore counts were found, although dampness did relate to allergic symptoms. The presence of *Cladosporium* species also correlated with asthma.

FUNGAL SENSITIVITY SKIN TESTING

IgE-mediated sensitivity to fungi is demonstrated by means of skin testing with extracts prepared from fungi, by means of in vitro assays (eg, the RAST), and by means of ELISA. Although sensitivity does not necessarily reflect disease, it does help to determine its frequency and how that frequency relates to respiratory symptoms. How-

TABLE V. Useful fungal allergenic extracts

Ambient outdoor air exposure	Ambient indoor air exposure
<i>Alternaria alternata</i> *	<i>Rhodotorula rubra</i> †
<i>Stemphylium botryosum</i>	<i>Sporobolomyces salmonicolor</i> †
<i>Botrytis cinerea</i>	<i>Chaetomium globosum</i>
<i>Cladosporium herbarum</i>	<i>Cladosporium sphaerospermum</i> †
<i>Cladosporium cladosporioides</i> *	
Any one or as a mix:	<i>Aspergillus fumigatus</i> †
<i>Curvularia inaequalis</i>	<i>Aspergillus niger</i>
<i>Curvularia lunata</i>	<i>Aspergillus repens</i>
<i>Curvularia spicifera</i>	<i>Aspergillus sydowii</i>
<i>Drechslera sorokiniana</i>	One or mixed:
<i>Helminthosporium solani</i>	<i>Mucor mucedo</i> , <i>Rhizopus nigricans</i>
<i>Epicoccum nigrum</i> *	<i>Stachybotrys atra</i> (=chartarum)
<i>Penicillium chrysogenum</i> *	<i>Paecilomyces variolata</i>
<i>Aspergillus flavus</i>	<i>Acremonium strictum</i>
<i>Aspergillus terreus</i>	
<i>Fusarium roseum</i>	Either one or mixed: <i>Fusarium roseum</i> ,† <i>Fusarium moniliforme</i> †
<i>Monilia sitophila</i> *	<i>Gliocladium roseum</i> ,† <i>Verticillium albo-atrum</i> †
<i>Sporobolomyces salmonicolor</i>	<i>Phoma herbarum</i> †
<i>Saccharomyces cerevisiae</i>	

Modified from *Allergy Asthma Proc.* 1999;20:289-92.

*Also common indoors.

†Also notable outdoors.

ever, many of the extracts currently available for testing are unstandardized materials and, unfortunately, provide variable results. Until standardized extracts are available commercially, the true prevalence of sensitivity to fungi in asthma will be difficult to establish.⁵³ Some clinically relevant fungal allergenic extracts are listed in Table V.⁵⁴

Nevertheless, international studies indicate that fungal sensitivity is common among asthmatic subjects. A general population epidemiologic survey in the United States,⁵⁵ for example, indicated that 3.6% of the population was sensitized to the fungus *Alternaria alternata*. In a study of 981 4-year-old children on the Isle of Wight, Tariq et al⁵⁶ reported that 0.5% of children reacted to *Alternaria* species and 2.9% reacted to *Cladosporium* species extracts. A Scandinavian study⁵³ showed that 4% of patients had positive test responses when given a fungal-based skin test. In the United States up to 80% of subjects with confirmed asthma had a positive reaction to one or more fungi.⁵⁷

Individuals presenting with symptoms of allergic rhinitis or asthma had positive skin test reactions to fungi as well. D'Amato et al⁵⁸ studied the fungal skin test reactivity in various European countries among patients with suspected respiratory allergy. Approximately 3% of the patients in Portugal and 20% of the patients in Spain had a positive skin test reaction to either *Alternaria* or *Cladosporium* species. Of symptomatic patients residing in the United States and various European countries, 25% to 33% of the subjects reacted to one or more basidiomycete species.⁵⁹ Epidemiologic studies have now established a role for fungal sensitization in the development of symptomatic asthma. An accumulating body of evidence suggests that fungal sensitization, particularly to fungi of the genus *Alternaria*, is associated with asthma. In a large-scale study of children with asthma residing in inner cities

of the United States,⁶⁰ 38.3% of the 1286 asthmatic children had a positive skin test reaction to *Alternaria* species. Gergen and Turkeltaub⁶¹ reported that in individuals with *Alternaria* species sensitivity, the adjusted odds ratio for such a person having self-reported asthma was 2.3 (range, 1.5-3.4; $P = .05$). In one study covering 2 diverse climatic regions of Australia, Peat et al⁶² examined house dust mite and *Alternaria* species allergen exposure in children. Along the coastal areas, where humidity is high, house dust mite antigen was highly correlated with the presence of asthma. In the less-humid inner area of the country, *Alternaria* species were more likely to be associated with asthma. In another study of children residing in the desert southwest of the United States,⁶³ skin test responsiveness to *Alternaria* species at age 6 was associated with both persistent asthma and the onset of new asthma, whereas at age 11 years, those correlations did not occur. This finding is consistent with previous reports,⁵ which found an age-related decline in fungal sensitization in asthmatic subjects. Henderson et al⁶⁴ demonstrated that *Alternaria* species sensitivity in school-aged children in North Carolina was associated with recurrent wheezing. Perzanowski et al,⁶⁵ conducting a similar study, found a positive association between *Alternaria* species sensitivity and asthma in school-aged children in Los Alamos, New Mexico, and in Charlottesville, Virginia, but not in the surrounding county.

It is more difficult to causally relate exposure to fungal spores and the development of asthma symptoms in sensitized individuals. Bruce et al⁶⁶ indicated that in about 50% of subjects, asthma symptoms were correlated with the presence of *Alternaria* species sensitivity by skin test. A San Diego, California, study⁶⁷ of children aged 9 to 18 years found that fungal exposure was significantly associated with asthma symptom severity. In

this study asthma symptom scores increased by 10% to 30% for every 1000 spores per cubic meter of air. The need to use inhaled bronchodilators was similarly correlated. Interestingly, there was a better correlation with increments in non-skin tested fungi (basidiospore and ascospore) spore counts compared with fungi to which the patients had a demonstrable positive skin test response. Although the results of such studies demonstrate the limitations in assessing exposure-response relationships, it appears that fungal spores do play a role in symptoms of asthma. The ability to test for sensitivity to many of the fungal spores is currently limited, and as a consequence, exact assessments cannot be made.

FUNGAL EXPOSURE

Fungal exposure and emergency department visits for asthma

Exposure to fungal spores has been associated with emergency department visits for asthma, especially in children, as 2 studies have confirmed. Rosas et al⁶⁸ found a correlation between asthma-related emergency department visits in children and the presence of high levels of the ascospore *Leptosphaeria*, whereas Nelson et al⁶⁹ reported an association between emergency department visits for asthma in children and sensitivity to a variety of allergens, including *Alternaria* species.

Fungal exposure in epidemic asthma

Epidemic asthma is defined as an excessive outbreak of symptomatic asthma requiring emergency medical care. For example, a high volume of hospital admissions (40 over 24 hours) for asthma occurred during a period of time in which there were high basidiospore counts in the air in New Orleans.⁷⁰ These epidemics occurred during the months of June through December, with peaks occurring in September and November. High fungal spore counts have also been documented during outbreaks of asthma in England and New Zealand.^{7,71}

Fungi may have played a secondary causative role in the outbreaks of asthma in Barcelona, Spain, which were initially attributed to soybean hull sensitivity.⁷² The investigators demonstrated that the patients who had asthma episodes on exposure to soybean hulls were sensitized to these proteins and also to *Aspergillus* and *Penicillium* species.

Fungal sensitivity and exposure in severe and fatal asthma

Fungal sensitivity and exposure to airborne fungal spores has been associated with life-threatening episodes of asthma. O'Holleran et al⁷³ reported on 11 young patients between the ages of 11 and 25 years who experienced respiratory arrest caused by asthma. Two fatalities occurred in this small group. Ten of the 11 patients were sensitized to *Alternaria* species. These patients experienced their difficulty during the peak of *Alternaria* species prevalence, and there was a correlation between the high *Alternaria* species spore counts and the times of their emergency department visit. The adjusted odds ratio

(189; 95% CI, 6.5-5535.8) for a severe and potentially fatal attack of asthma was highly correlated with *Alternaria* species sensitivity.

The risk of death from asthma has also been correlated with the levels of fungal spores in the atmosphere. Targonski et al⁷⁴ reviewed death certificates that named asthma as the cause of death in the Chicago, Illinois, area and reported that the adjusted odds ratio for death on a day when the fungal spore counts exceeded 1000/m³ of air were 2.3 times greater than when the spore counts were less than that amount (95% CI, 1.3-3.56). The odds ratio for death was 1.2 (range, 1.07-1.34; *P* = .05) for every increase in the fungal spore count by 1000/m³. On the basis of these observations, fungal sensitivity, which carries an increased risk for the development of asthma, also plays a role in asthma epidemics, acute severe life-threatening episodes of the disease, and asthma deaths.

Indoor fungal exposure studies

Fungal allergen exposure is generally considered to arise from outdoor environments. However, many species invade homes through open windows and doors or cracks in walls. *Penicillium* and *Aspergillus* species are recovered at greater rates from within buildings than from the outdoor air, although they are abundant in soil.¹⁰

Many studies have looked at conditions in the indoor environment that encourage fungal growth. For the most part, fungal growth favors homes that are damp, have high humidity levels, or have cold surfaces onto which moisture can condense.⁷⁵ For that reason, microclimates, such as a damp basement or a humid bathroom within an otherwise dry house, can generate spores that can spread to the rest of the house.

Airborne fungal spore concentrations are often associated with musty odor, water intrusion, high indoor humidity, limited ventilation, and failure to remove indoor mold growth. *Cladosporium* species have been associated with visible mold or condensation.

In homes in Melbourne, Australia, Dharmage et al⁷⁶ noted that detectable amounts of fungi were lower in rooms where a ceiling fan was operating, where no mold was visible, that were frequently vacuumed, that had a solid fuel fire, that had windows closed when sampling occurred (to avoid outdoor infiltration), and that were in homes that lacked pets. Mold levels were dramatically increased in homes that had more than one cat residing. In this study high ergosterol levels were associated with infrequent vacuuming, pets, visible mold, and old carpets. This finding confirmed the results of an earlier study⁷⁷ that showed that increased humidity was associated with increased airborne spore counts, along with the presence of pets and dampness.

Douwes et al²⁰ measured EPSs of *Aspergillus* and *Penicillium* species by using an enzyme immunoassay. They found that *Aspergillus* and *Penicillium* species EPS levels were readily detectable (40 to 46,513 nanogram equivalent per gram of dust) in 161 house dust extracts, with the highest concentrations in living room floor dust. In addition, *Aspergillus* and *Penicillium* species EPS lev-

els were 2 to 3 times higher on carpeted floors than on smooth floors. The EPS levels also were significantly correlated with total culturable fungi ($r = 0.3-0.5$), house dust mite allergens ($r = 0.3-0.5$), occupant-reported home dampness, and respiratory symptoms.

In addition to homes, the work environment can be a source of exposure to fungal allergens. The term *sick building* refers to a structure whose building materials, ventilation systems, or location appears to affect the health and well-being of its inhabitants. Symptoms experienced by the occupants are often nonspecific and transient and do not appear to have an allergic basis in most instances.⁷⁸ Such buildings may support high levels of fungal growth.^{79,80} However, the cause-and-effect relationship between exposure and symptoms of the occupants is often speculative, and the mechanism of illness is often unclear.

ABATEMENT TO REDUCE FUNGAL EXPOSURE

On the basis of the literature, a strong direct correlation exists among fungal exposures, sensitivity to fungi, and development of allergic diseases. It is reasonable to believe that if that exposure were reduced, the severity and incidence of the resulting illness would be reduced also. Although this has been demonstrated for several types of exposure, such as to that to dust mites,^{81,82} to date, no controlled trials have addressed fungal exposure, and the recommendations are therefore largely empiric.

Exposure abatement in buildings heavily contaminated with fungi can be very difficult (eg, flooded buildings), and it may be necessary to completely raze the structure and start to rebuild. When damage is more localized, it may be possible to remediate the problem. Indoor fungal exposure occurs in 2 ways: through infiltration of spores from outside and through the growth of fungi indoors. Successful abatement strategies need to consider both sources of contamination.

Reduction of spore infiltration

Spore infiltration from outside can be reduced by closing doors and using air conditioning for cooling. This can also reduce indoor fungal allergen levels at times when outdoor spore levels are high.⁹ Use of a window air conditioner with the vent closed has been shown to effectively exclude spore infiltration in one study.⁸³ The paradox to this practice is that interior fungal concentrations are decreased from indoor sources when ventilation (ie, opening windows) is increased.⁸⁴ Although reducing the ingress of outdoor air can reduce spore infiltration, it also may accentuate concentrations of nonparticulate fungal metabolites, such as VOCs. Therefore a compromise of providing adequate filtered outdoor air ventilation throughout the year is desirable to reduce spore exposure while also allowing fungal metabolites to dissipate.

Control of moisture

Because indoor fungal growth is dependent on moisture and a carbon source, a fundamental principle for

reducing or eliminating its growth is controlling available moisture.⁸⁵ Small steps taken in the average home can significantly control moisture: (1) maintain indoor relative humidity at no greater than 50%; (2) seal all leaks to prevent water accumulation; (3) increase bathroom and kitchen ventilation by using exhaust fans; (4) vent clothes dryers to the outside; (5) reduce the excessive number of live indoor plants that must be watered⁸⁶; (6) use air conditioning during the summer months and at other times of high humidity levels; (7) heat all rooms in the winter and add heating to outside wall closets; (8) use a dehumidifier in the basement or other areas of dampness; and (9) use a sump pump in basements that are prone to flooding.⁸⁷

Cleaning and removing contaminated materials

Carpets, wallpaper, paneling, and heating or air-conditioning systems are known to harbor fungal spores. Fungal levels are higher in the presence of old wall-to-wall carpets.⁸⁴ Frequent vacuuming can reduce fungal spore levels. However, if contamination is extensive or if family members are extremely sensitive, more effective and permanent control of fungal growth might be gained by replacing carpeting with hardwood, tile, or other types of firm flooring materials.

Washable wallpaper and paneling can be treated with a 5% bleach and detergent solution. Caution should be exercised when applying this solution. To ensure safety, respiratory protection may be necessary. Treatment of washable surfaces with a commercial fungicidal compound or an antimicrobial product active for molds or mildew is also an option, although not all products are equally effective, and some are irritating or otherwise toxic.⁸⁸ Wallpaper or paneling may have to be removed to reduce a severe fungal contamination. Contaminated air ducts and filters may be cleaned to reduce fungal exposure as well.⁸⁹

Use of air filters

Air filters can be used to remove particles from air, the most effective being the high-efficiency particle air filter.^{90,91} An electrostatic filter is less effective in removing spores from air in indoor environments.⁹² Electronic air cleaners and negative ionizers have also been shown to have a modest effect on spore counts.⁹³ A study has evaluated the effectiveness of air filters to reduce fungal exposure and found them to be as good as or superior to mattress covers used to reduce dust mite and animal dander exposure.⁹⁴

Proper maintenance of the heating, ventilation, and air-conditioning system

A contaminated heating, ventilation, and air-conditioning system can circulate spores through a building. Such contamination can be reduced by proper maintenance⁸⁹ with regular inspection and cleaning.

Use of personal protection

A person who is highly sensitive to fungal spores should

use a well-fitted particulate mask that will retain particles as small as 1 μm or less when involved in activities (eg, cleaning) that can disperse spores into the air. Individuals who have a severe fungal allergy should avoid any activity, such as handling compost, vacuuming, or cleaning fungal contamination, that would put them at increased risk.

CONCLUSIONS

Fungal allergen exposure is associated with the development and severity of asthma in sensitized individuals. The contribution of indoor fungal allergen exposure to allergic diseases is still not completely clear. Methods to assess fungal allergens by using immunoassays are still in their infancy. More traditional methods of exposure assessment with spore counts and quantitative cultures suggest that indoor fungal exposure indeed contributes to allergic airway disease. The presence of fungal growth in the home or office implies a problem with excessive dampness in the environment. Measures to decrease the infiltration of fungal spores from the outdoor environment, control indoor moisture problems, and clean or remove contaminated materials may improve the health of individuals with fungal-induced allergic diseases.

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