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 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 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.)

Key words: Fungi, allergic disease, asthma, environment, exposure, abatement

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 causal 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-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.

Typically, 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.
producers might be assexual or anamorphic fungi.

The fungi in the ascomycetes grouping 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, although their presence can indicate that indoor amplification has occurred, most commonly as a result of moisture problems.

The fungi of the basidiomycetes group 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.

The deuteromycetes (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 available in print only
FUNGAL PREVALENCE

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.

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

A relationship also exists between indoor fungal accumulations and outdoor weather. Higher indoor levels are noted when outdoor fungal levels increase. Table II lists the distribution of indoor and outdoor allergenic fungi 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.

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, Herfordshire, 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., 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 II. Distribution of indoor and outdoor allergenic molds

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Indoor</th>
<th>Indoor summer</th>
<th>Indoor winter</th>
<th>Outdoor summer</th>
<th>Outdoor summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillium species</td>
<td>0-4737</td>
<td>0-7900</td>
<td>0-480</td>
<td>0-95</td>
<td>15,000</td>
</tr>
<tr>
<td>Cladosporium species</td>
<td>12-4637</td>
<td>0-160</td>
<td>0-160</td>
<td>11-430</td>
<td>600,000</td>
</tr>
<tr>
<td>Botrytis species</td>
<td>0-54</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>12,000</td>
</tr>
<tr>
<td>Yeasts</td>
<td>0-5</td>
<td>0-74</td>
<td>0-78</td>
<td>0-790</td>
<td>10,000</td>
</tr>
<tr>
<td>Aspergillus species</td>
<td>0-306</td>
<td>0-76</td>
<td>0-19</td>
<td>0-11</td>
<td>15,000</td>
</tr>
<tr>
<td>Alternaria species</td>
<td>0-282</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7500</td>
</tr>
<tr>
<td>Rhizopus species</td>
<td>0-24</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Nonsporulating mycelium</td>
<td>0-14,194</td>
<td>0-1700</td>
<td>0-200</td>
<td>19-9300</td>
<td>—</td>
</tr>
<tr>
<td>Epicoccum species</td>
<td>0-155</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fusarium species</td>
<td>0-47</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7500</td>
</tr>
</tbody>
</table>

‡Studies carried out in European homes.
†Studies carried out in Finnish homes.
*Studies carried out in Southern California homes.

### 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 immunochromed 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→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→3)-glucan. **β(1→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

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


and a carbonyl sulfide–like compound. 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. They have served as tracers for suspected microbial problems in buildings where occupants complained of poor indoor air quality and respiratory irritation. 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 teratogenic 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, the fumonisins, ochratoxin, and other mycotoxins produced by Aspergillus and Penicillium species. Pieckova and Jesenska 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 IMMUNOAASSAYS

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, such exposures are often transient, and brief collections may not accurately represent the integrated exposure over time. House dust, on the other hand, is considered to be a reservoir of spores and allergenic materials. 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). 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. Initially, assays were based on RAST inhibition with polyclonal antibodies derived either from laboratory animals or human sera containing specific IgE. 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. In addition, there may be differences between allergens isolated from spores and mycelia. 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. 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. Several 2-site enzyme-linked immunoassays to Alt a 1 have been described that could be used to detect Alt a 1 in both indoor and outdoor environmental samples. Alt a 2 is another major *Alternaria* species allergen that has been molecularly cloned. 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. 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. 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. 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. 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, as well as *Saccharomyces cerevisiae* and *Candida albicans*. A polyclonal antibody–based immunoassay has been described for *Cladosporium* species.

*Aspergillus* species

*Aspergillus fumigatus* is an important indoor allergen from which several allergens have been molecularly cloned. Asp f 1 is a cytotoxin homologous with mitogillin. 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 bind 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 dismutate that is important in ABPA. An ELISA has been described for detection of *Aspergillus* species in environmental samples. An mAb-based assay has been described for measurement of Asp f 1.

*Penicillium* species

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

**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 Kendrick 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, 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, 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

<table>
<thead>
<tr>
<th>Ambient outdoor air exposure</th>
<th>Ambient indoor air exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria alternata*</td>
<td>Rhodotorula rubra†</td>
</tr>
<tr>
<td>Stemphylium botryosum</td>
<td>Sporobolomyces salmonicolor†</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>Chaetomium globosum</td>
</tr>
<tr>
<td>Cladosporium herbarum</td>
<td>Cladosporium sphaerospermum†</td>
</tr>
<tr>
<td>Cladosporium cladosporioides*</td>
<td></td>
</tr>
<tr>
<td>Any one or as a mix:</td>
<td></td>
</tr>
<tr>
<td>- Curvularia inaequalis</td>
<td></td>
</tr>
<tr>
<td>- Curvularia lunata</td>
<td></td>
</tr>
<tr>
<td>- Curvularia specifera</td>
<td></td>
</tr>
<tr>
<td>- Drechslera sorokiniana</td>
<td></td>
</tr>
<tr>
<td>- Helminthosporium solani</td>
<td></td>
</tr>
<tr>
<td>- Epicoccum nigrum*</td>
<td></td>
</tr>
<tr>
<td>- Penicillus chrysogenum†</td>
<td></td>
</tr>
<tr>
<td>- Aspergillus flavus</td>
<td></td>
</tr>
<tr>
<td>- Aspergillus terreus</td>
<td></td>
</tr>
<tr>
<td>Fusarium roseum</td>
<td>Either one or mixed:</td>
</tr>
<tr>
<td>- Monilia sitophila*</td>
<td>- Fusarium roseum,†</td>
</tr>
<tr>
<td>- Sporobolomyces salmonicolor</td>
<td>- Fusarium moniliforme†</td>
</tr>
<tr>
<td>- Saccharomyces cerevisiae</td>
<td>- Gliocladium roseum,†</td>
</tr>
<tr>
<td></td>
<td>- Verticillium albo-atrum†</td>
</tr>
<tr>
<td></td>
<td>- Phoma herbarum†</td>
</tr>
</tbody>
</table>

*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 study,53 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 al. found a correlation between asthma-related emergency department visits in children and the presence of high levels of the ascospore Leptosphaeria, whereas Nelson et al. 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. 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.

Fungi may have played a secondary causative role in the outbreaks of asthma in Barcelona, Spain, which were initially attributed to soybean hull sensitivity. 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’Holleren et al. 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 al. 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.

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. 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 al. 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 study that showed that increased humidity was associated with increased airborne spore counts, along with the presence of pets and dampness.

Douwes et al. 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. 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, to air inhalation, to pets, to animals,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 watered; (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.

**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.86 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.

**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 maintenance9 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 immunosassays 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.

REFERENCES


