

Assessment and Control of Fungal Allergens

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Fungal sensitivity is a significant cause of allergic disease. Understanding the role fungi play in allergic disease, and how to best control exposure among those with allergy, can have important clinical ramifications.

Introduction

One of the more significant causes of allergic disease is sensitivity to fungi (sometimes referred to as molds). Understanding the classification and the ubiquity of fungi, as well as their allergenic properties, can ultimately help control not only the fungi, but also the allergic and asthmatic symptoms that they may cause.

Fungi play an important role in many human diseases, such as hypersensitivity pneumonitis, allergic bronchopulmonary mycoses, allergic fungal sinusitis, allergic rhinitis, and allergic asthma. Fungi belong to their own separate kingdom of multicellular eukaryotes, and are classified into "true fungi" and fungi imperfecti. The true fungi include zygomycetes (eg, *Rhizopus*), ascomycetes and basidiomycetes (eg, rust, smut, Ganoderma) [1]. Fungi imperfecti are asexual forms of ascomycetes and among the best-studied fungal allergens and include *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria* and *Epicoccum*. Fungi are obligate parasites or saprophytic decomposers, and grow where carbon sources are available and enough moisture is present.

Fungal allergenic activity has been most associated with spores, though other parts of the fungus, such as mycelial fragments, may also contribute to allergenicity. In the northern United States, fungal spores appear with spring warming and increase with warmer weather. Fungal counts peak in the late summer and recede with the first snow. In the southern United States, fungal spores are present all year, with peaks in the summer or early fall. Fungi tend to thrive in areas where they receive adequate moisture, though they can survive in hot, dry climates. On hot, dry, windy days, dry spores from outdoor fungi like

Alternaria and *Cladosporium* tend to reach their peak levels. Fungal growth is enhanced by damp conditions, and fungi often grow outdoors on leaves, in soil, and plant litter. Spore release from fungi that thrive in moist environments is highest early in the morning when relative humidity tends to be the highest. Other spores are released in the late afternoon, when it tends to be less humid and windier.

Though fungal allergen exposure is typically considered to occur with exposure to the outdoors, indoor exposure also occurs, as outdoor fungi invade through open windows, doors and small cracks. Some species may be more prevalent indoors than outdoors, such as *Penicillium* and *Aspergillus* [2]. Indoor spaces that have been flooded or have excessive moisture problems are at risk for fungal contamination.

Prevalence

IgE-mediated fungal allergy can be demonstrated by skin testing with extracts prepared from fungi, or by in vitro assays such as radioallergen sorbent test (RAST) or enzyme-linked immunoassays (ELISA). Though commercial extracts are not standardized and give variable results, surveys from across the world show that fungal sensitivity is common, particularly among people with asthma. A large-scale epidemiological study in the US showed that 3.6% of the population is sensitized to the fungus *Alternaria alternata* [3].

In people with allergic rhinitis or asthma, there is increased prevalence of skin test reactivity to fungi. Up to 80% of asthmatic patients in the US have also been shown to have positive reactivity to one or more fungi [4]. Of 1286 asthmatic children living in inner cities of the US, 38.3% had positive skin test to *Alternaria*, compared with 35.8% with sensitivities to cockroach and 34.6% to dust mites.

Role of Fungal Exposure in Asthma

Increased emergency room visits secondary to asthma have been correlated with increased fungal spore counts. High levels of the ascospore *Leptosphaeria* have been associated with increased emergency room visits for children with asthma [5•]. Other allergen sensitivities, including sensitivity to *Alternaria alternata*, have been associated with emergency room visits in children with asthma.

Increased spore counts have also been associated with epidemic asthma (an excessive outbreak of symptomatic asthma requiring emergency medical care). In New Orleans, 40 patients with asthma were admitted in a 24-hour period when the total basidiomycete spore counts were high [6]. This occurred from June to December, with peaks in September and November. Similar events have occurred in England and New Zealand when fungal spore counts were high [7]. This also occurred in England in association with ascospores.

In addition to increased emergency room visits, sensitivity to *Alternaria* has also been associated with severe, life-threatening asthma. In a study by O'Holleran [8], 10 of 11 older children and young adults who presented with respiratory arrest due to asthma were sensitized to *Alternaria*. The arrests occurred during peak of *Alternaria* season, and correlated with increased spore count. The adjusted odds ratio (189; CI 6.5–5535.8) for potentially life-threatening asthma attack was highly correlated with *Alternaria* sensitivity [8].

Fungal Sensitivity and Asthma

A role for fungal sensitization as a risk factor for asthma has been shown in epidemiologic studies. Sensitization to fungi, particularly to *Alternaria*, is associated with asthma. In fact, in people with *Alternaria* sensitivity, the odds ratio for those having self-reported asthma is 2.3 (95% CI, 1.5–3.4) [9]. In coastal Australia, with increased humidity, house-dust mites have been correlated with the presence of asthma, compared with the less humid inner part of the country, where *Alternaria* is more associated with asthma. Throughout the US, skin test responsiveness to *Alternaria* has been associated with asthma in young children [10–12,13•].

It has been more difficult to directly correlate exposure to fungal spores with the development of asthma in sensitized patients. Some studies [4,15] have associated fungal exposure with asthma symptoms. Symptom scores were correlated with total outdoor spore count, and symptoms increased by 0.1–0.3 for every 1000 spores per cubic meter of air, along with increased need for inhaled bronchodilators [15].

Though counts of *Cladosporium* tend to be much higher than *Alternaria*, *Alternaria* is more antigenic. Skin tests with various fungal antigens have shown positive reaction to *Alternaria* most often [16]. This may reflect the variable potency and composition of fungal allergenic extracts used for diagnostic testing.

Fungi in the Indoor Environment

Indoor fungal spores usually come from both indoor and outdoor sources. In northern climes, during cold or snowy weather, there is little contribution from outdoor spores. There are various ways to count fungal spores in the

environment. The most common involves a Burkard trap (Burkard Manufacturing Co., Ltd., Rickmansworth, Hertfordshire, England), where air is drawn into the device at 10 l/min, and a continuous particle deposit is developed onto a tape-coated drum, giving a 7-day record. Cultures may also be performed to collect viable spore or fungal elements from the air or dust. Multiple methods of collecting samples should be utilized to decrease the error associated with one sampling method [17••]. It may in fact be more effective to utilize methods that span a period of time, as opposed to one moment, to adequately assess overall fungal exposure in the environment.

Although a large contribution to indoor molds comes from outdoor sources, there are some species that predominate in the winter when there are little to no measurable fungal counts in northern climates. Cultures of air samples in Michigan homes have been shown to contain *Cladosporium*, *Alternaria*, *Epicoccum*, *Fusarium*, *Penicillium*, and *Aspergillus* during frost-free months [18]. *Cladosporium*, *Penicillium*, *Aspergillus*, *Geotrichum*, pigmented yeast and other filamentous genera predominated in the winter [32]. Similar results were obtained from 12 homes in Wisconsin, where *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus fumigatus* were recovered in the warmer months [19]. Of note, only *Penicillium* and *Aspergillus* remained significantly elevated in the winter [19].

Several species of fungi tend to predominate in studies of indoor fungi in both the United States and Europe during frost-free periods. *Cladosporium*, *Alternaria*, *Epicoccum*, *Fusarium*, and to a lesser degree *Penicillium* and *Aspergillus* are found during warmer months. In the winter, when the outdoor molds counts drop, *Penicillium* and *Aspergillus* are most commonly found indoors.

Most studies have relied on cultured fungi from the environment, or from air-sampling devices like the Burkard spore traps. Few studies have measured total allergenic activity or specific fungal allergenic protein levels. Evidence for *Penicillium*, *Alternaria* and *Cladosporium* allergens has been found in homes in the Kansas City, MO, area in a limited study [20].

Penicillium and *Aspergillus* are often found in basements, crawl spaces and bedding. These two fungi are also commonly found growing on stored grain, fruits and vegetables. *Aspergillus* prefers growing on substrates with a moisture count ranging from 12% to 16%. *Penicillium* is often seen in basements as a green mildew. *Rhizopus* can be found as the black mold on bread and will also grow on vegetables, preferring onions.

Indoor plants have been shown to have only a slight impact on indoor fungal spore numbers, including *Alternaria*, *Penicillium*, *Cladosporium* and *Epicoccum*. Though the number of spores have been shown to increase in greenhouses with agitation like watering or fanning [21], laboratory studies have not shown excessive numbers of fungal spores on indoor plants [22].

People have also been exposed to fungal allergens in work environments. Symptoms including coughing, wheezing, and shortness of breath have been associated with exposure to fungal allergens, like *Alternaria*. Canadian office workers with sensitization to *Alternaria*, most often with positive skin tests, experienced symptoms of coughing, wheezing, or shortness of breath associated with increased spore counts [23]. Fungi were collected either by culture or by volumetric personal samplers.

It is quite difficult to associate indoor fungal exposure to asthma, though in children, damp conditions conducive to fungal growth have been shown to have significant health effects [24]. Skin testing and in vitro IgE testing have not borne out allergic reactions to fungi as a cause of asthma symptoms, although further research is necessary. Verhoeff and Burge [17••], as well as Peat *et al.* [24] have reviewed the association between fungi at home, and health risks. Respiratory disease was associated with damp homes in these studies. Fungal levels and adverse health outcomes were associated with each other in multiple cross-sectional studies [18].

Fungal Allergens

Alternaria alternata

Ninety percent of *Alternaria*-allergic patients have IgE antibodies to Alt a 1, which has been purified, and subunits of it have been molecularly cloned [25,26]. Alt a 2 is another major *Alternaria* allergen, which has been molecularly cloned and differs from Alt a 1 [27•]. Other minor allergens have been identified, including Alt a 6, which is a P₂ ribosomal protein [28,29]. Other allergens include Alt a 7, which is homologous with YCP4 yeast protein, and Alt a 10, which is an alcohol dehydrogenase [28,29].

Cladosporium herbarum

Major allergens associated with *Cladosporium herbarum* include Cla h 1 and Cla h 2. Another protein, Cla h 3, has been molecularly cloned [30], and shown to be another P₂ protein associated with RNA in the cytosol. Approximately 30% of patients with *Cladosporium* allergy have IgE binding to an hsp 70 heat shock protein that has been cloned from *Cladosporium*.

Enolases

Approximately 50% of patients with *Alternaria* and *Cladosporium* allergy have IgE binding to enolases. These proteins have been obtained from *Cladosporium*, *Alternaria*, *Saccharomyces cerevisiae* and *Candida albicans* [31••], indicating that they are conserved proteins found in a number of fungi.

Aspergillus

A major allergen, mitogillin, has been cloned from *Aspergillus fumigatus* [32,33]. The allergen is known as Asp f 1 and is a cytotoxin. At least 50% of *Aspergillus*-sensitive

subjects react to skin tests with this protein [34]. Mitogillin is excreted into culture media only during active growth of *Aspergillus* [33]. Asp f 3 is a peroxisomal membrane protein that binds IgE from *Aspergillus*-allergic asthmatic patients at a rate of approximately 72% [35]. Asp f 5 is a metalloprotease [36,37•]. Both Asp f 3 and Asp f 5 are secreted proteins and recognized by patients with sensitivity to *Aspergillus* with or without ABPA (37). A diagnostic sensitivity of 97% for *Aspergillus* allergy has been shown, using a combination of Asp f 1, Asp f 3 and Asp f 5.

Another *Aspergillus* allergen, Asp f 2, has been demonstrated to have IgE binding in sera of patients with allergic bronchopulmonary aspergillosis (ABPA) [38]. Asp f 4 also shows IgE binding in some patients with ABPA [37•]. Asp f 6 binding is often seen in patients with ABPA [37•]. Asp f 4 and Asp f 6 are nonsecreted proteins and are recognized in patients with ABPA [37•]. In the future, in vitro testing for IgE antibodies to these cloned proteins may be especially useful in the diagnosis of ABPA.

Penicillium

Approximately 93% with sensitivity to *Penicillium* sensitivity have IgE binding to a 33 kDa major allergen that has been found to be an alkaline serine proteinase [38,40]. Another *Penicillium* allergen has been identified as a heat shock protein (hsp) 70 [41].

Asthma and Fungal Cell Wall Components

(1→3)-β-D-glucans are glucose polymers found in fungal, plant and bacterial cell walls. These polymers may produce airway inflammation [42], and indoor exposure to (1→3)-β-glucans has been reported to increase, and even cause symptoms of dry cough and wheezing in children exposed to mold growth in school buildings [43], although more research is needed. Extracellular polysaccharides are stable carbohydrates secreted or shed during mold growth. They have antigenic specificity and some extracellular polysaccharides from *Aspergillus* and *Penicillium* have shown to be cross-reactive. They may be markers for fungal exposure, but currently there are no pathogenic roles known for these carbohydrates.

Assessment of Fungal Allergen Exposure

Spore counts have been traditionally determined by using volumetric samplers, like Burkard spore traps. These determine the number of spores per cubic meter of air over a period of time and can be used indoors and outdoors. After the spores are collected, they are identified on microscopic characteristics. This can be difficult, as many spores, including *Aspergillus*, *Penicillium*, ascospores and basidiomycetes, may not be able to be identified based on morphological factors.

Cultures have also been used to determine fungal exposure. An Andersen sampler is often used to collect air

samples (primarily in the indoor environment), and then the fungus is grown on culture media and quantified as colony forming units per cubic meter of air sampled. Problems arise when the fungal material is not viable, and cannot be grown. This technique is also time intensive and can be expensive. Dust samples can be collected and cultures can be obtained. This has the same problems inherent in air sampling. One must realize that these studies analyze exposure at a single point in time. Also, it is important to assess indoor and outdoor levels at the same time to determine the predominant exposure at a given point in time. It is not necessary to perform fungal sampling if visible fungal growth is occurring in an indoor environment, since remediation (cleaning, removal of contaminated material), and humidity control should be performed.

Other assays are being developed to assess actual allergen levels, since asthma due to fungal sensitivity is the result of allergen exposure. An assay for the *Alternaria* allergen, GP70, has been developed that detects levels greater than $2\mu\text{g/mL}$, though it has not been applied to dust or airborne samples [44]. An assay for Alt a 1 has also been developed [45•], which may eventually be applicable in quantifying this important allergen in environmental samples. Assays for fungal allergens will be quite useful to quantify airborne exposure levels.

In general, it has been more difficult to obtain accurate levels of indoor fungal loads. There is significant variability in indoor exposure levels because of the variety of different ventilation systems, as well as the contributions of fungal levels to the indoor environment.

Fungal Avoidance

If one has fungal allergy and is exposed to high fungal levels, such as farmers or those working with moldy materials, avoidance measures play an important role in controlling symptoms. Fitted facemasks may be helpful for outdoor exposure. As fungi are often found in damp places outdoors, thriving on plants and their decaying parts, lawn mowing and raking can increase exposure and symptoms.

Indoor fungal exposure is often dependent on outdoor fungal counts and tends to occur in dark, humid, poorly ventilated areas such as basements. Other areas like bathrooms with water sources are often home to fungi. Humidifiers can contribute to fungal growth, with their freestanding water. Any source of foundation leaks need to be repaired to help decrease fungal growth, and water-damaged carpeting must be removed as well. Kitchens and bathrooms require ventilation. Moldy materials such as clothing and books need to be thrown away. A 5% to 10% chlorine bleach with detergent solution or approved fungicidal solution should be used to clean washable surfaces like bathroom tiles and kitchen counters. If the person doing the cleaning has a fungal allergy, a fitted face-

mask should be worn. During times of high humidity, it is advised to run air-conditioning to keep the humidity less than 50% to help limit fungal growth [46].

High-efficiency particulate (HEPA) cleaners or electrostatic air cleaners may help decrease fungal spores in the air, but there is no proof that they help in the treatment of asthma. To further reduce indoor airborne fungal growth and humidity, it is advised to avoid drying clothes indoors unless the dryer is vented outdoors, as well as decreasing indoor plants, and having no unheated rooms in the winter as well as unheated outside wall closets [47]. These common-sense methods may help decrease fungal exposure, but they have not been studied extensively to ultimately determine their effectiveness.

Immunotherapy for Fungal-induced Asthma

There have been few clinical trials regarding immunotherapy as a treatment of fungal-induced asthma because of problems identifying exposure to fungal allergens as well as lack of suitable standardized reagents for treatment. There have been a few double blind, placebo-controlled trials of immunotherapy with *Cladosporium* and *Alternaria* extracts [48], which showed benefits in the treatment of fungus-induced asthma.

Conclusions

Fungal sensitivity is a major risk factor in the development of asthma. Though the prevalence of fungal sensitivity in asthma is not completely understood, in some studies nearly 80% of asthmatic patients may be sensitized to at least one fungus. To fully assess fungal exposure requires not only measurement of airborne spores and culture techniques, but also identification of the offending organisms. Decreasing the growth of fungi in the indoor environment may help reduce allergic symptoms. Immunotherapy may be of benefit as well in selected cases.

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