Immunol Allergy Clin N Am
23 (2003) 501–518

IMMUNOLOGY
AND ALLERGY
CLINICS OF
NORTH AMERICA

Indoor fungal exposure

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The presence of fungal growth in buildings has long been considered undesirable, largely because of its propensity to discolor interior finishes, degrade building materials, and produce offensive odors that reduce perceived indoor air quality. In addition to these cosmetic and structural effects and perceptions, it has been questioned whether fungal growth is also detrimental to the health of building occupants. During the past few decades, several studies have indicated important associations between fungal growth in buildings and health effects, and much has been written about the assessment of indoor environments for fungal exposure. This article summarizes the current state of this information.

Fungal agents and human disease

Fungi are complex organisms that cause a number of different kinds of illness. A prime example of this diverse effect is represented by *Aspergillus fumigatus*. This single organism is an opportunistic infectious agent that produces allergens and mycotoxins. Colonization of the bronchopulmonary system by *Aspergillus fumigatus* results in pulmonary aspergillosis that can take several forms, such as nonpathogenic saprophytic colonization, pulmonary aspergilloma, hypersensitivity-induced aspergillosis (*Aspergillus* asthma, allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis), and invasive pulmonary aspergillosis [1].

Infectious diseases

Although infection by dermatophytic fungi (eg, *Trichophyton*, *Microsporum*) is reasonably common, causing superficial infections of the skin, hair, nails and mucous membranes, most fungi cannot cause serious infectious disease except in severely immune compromised hosts. Among the fungi, *Aspergillus fumigatus, Candida albicans,* and *Cryptococcus neoformans* are the most common life-threatening opportunistic human pathogens [2]. The incidence of infection by

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doi:10.1016/S0889-8561(03)00061-4

other taxa, such as yeasts, Zygomycetes, *Fusarium* spp, *Acremonium* spp, *Paecilomyces*, and *Trichoderma*, is increasing [2,3]. Pneumonia caused by *Pneumocystis carinii* colonization frequently has been associated with individuals with HIV infection [4]. Invasive fungal infections are becoming more common because of the increase in aggressive cancer drug treatments, organ transplants, and HIV infections. For most fungal infections, the source of exposure is unknown. For some cases, the exposure likely is related to: (1) outdoor exposure (*Histoplasma capsulatum*, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, *Coccidioides immitis*), (2) exposure to another infected host (*Pneumocystis carinii*), (3) commensal organisms (*Candida albicans*), or (4) indoor fungal growth (*Aspergillus fumigatus*). *Histoplasma capsulatum* and *Cryptococcus neoformans* are fungi that commonly are found outdoors and are associated with bird droppings or bat guano. Fungal infections can occasionally result from an indoor exposure when cleaning out attics and garages or handling pets.

The most important fungal pathogen with a common indoor source is *Aspergillus fumigatus.* It also commonly is found outdoors, particularly in association with composting operations [5]. Although *Aspergillus fumigatus* accounts for 80% to 90% of human aspergillosis, a few other species of *Aspergillus* are also capable of invasive disease (eg, *A flavus*, *A terreus*, *A niger*, *A nidulans*) [6].

Hypersensitivity diseases

Probably the most common adverse response to fungal exposure is the development or exacerbation of hypersensitivity disease. Fungal antigen exposure can precipitate changes in the immune system of genetically predisposed individuals and induce the production of antigen-specific antibodies that mediate the diseases of allergy, asthma, allergic fungal sinusitis, allergic bronchopulmonary mycoses, and hypersensitivity pneumonitis.

IgE-mediated fungal allergy and asthma

Fungal allergy is characterized by the production of fungal specific IgE antibodies that mediate the allergic response. Symptoms include rhinitis, sinusitis, conjunctivitis, and asthma. Fungal allergy is relatively common, and an estimated 6% to 10% of the general population and 15% to 50% of the atopic population are sensitized to fungi [7]. These values are likely underestimates because of the lack of high-quality skin test materials to determine sensitization and the practice of using one or two fungal extracts in skin testing to represent all fungal allergy. It is unknown whether initial sensitization results from indoor exposure, outdoor exposure, or both. Symptoms are certainly exacerbated by indoor exposures [7].

Asthma can be associated with both allergic (IgE-mediated) and nonallergic disease mechanisms. Symptoms include chest tightness, wheezing, shortness of breath, and cough. Fungi are unequivocally associated with the exacerbation of asthma episodes, but their role in disease onset remains unclear [7]. Since an estimated 40% of the asthmatic population shows fungal sensitivity [7], it is likely that a significant proportion of asthma episodes are caused by fungal

exposure. Evidence exists for an association between skin test reactivity to *Alternaria* allergens and potentially fatal asthma [8].

Hypersensitivity pneumonitis

Hypersensitivity pneumonitis (HP; allergic alveolitis) is characterized in part by the production of antigen-specific IgG and is an acute recurrent pneumonia with fever plus other symptoms. This rare disease can result from fungal antigen sensitization and exposures in high concentration, most commonly encountered in occupational settings, although residential exposures can occasionally cause HP [9].

Hypersensitivity related to fungal colonization

Allergic mycoses result when a fungus colonizes mucous within the respiratory tract, leading to a constant allergen challenge. Allergic fungal sinusitis results when fungi colonize mucous within the sinuses. Many fungi have been implicated in this disease, and dark-spored fungi are especially common. *Aspergillus flavus* commonly is associated with *Aspergillus* sinusitis [6]. Allergic bronchopulmonary aspergillosis is a disease that occurs in asthmatics, in which the fungus colonizes mucous in the lung. *A fumigatus* is the most common offender, but other fungi also may be involved. Aspergilloma occurs within cavities in the lung that have resulted from other disease processes (eg, tuberculosis). In this case, a large fungus ball forms, again resulting in constant systemic allergen exposure.

Allergens

Fungi produce a number of proteins and glycoproteins that are antigenic (up to 60 from a single species), some of which are also allergenic. Fungal allergens range in molecular weight from roughly 10 to 70 Kd [10,11]. Allergen production varies by isolate, strain, environmental conditions, and growth substrates. Even under highly controlled conditions, considerable variability exists in allergen production [11].

Allergens are contained in mycelium and spores. Although there are some spore-specific allergens, mycelium with few or no spores is used almost exclusively in the preparation of skin test materials [12]. Allergens typically are released quickly from fungal material; however some allergens require spore germination for release (eg, *A fumigatus*).

There are an estimated 1.5 million species of fungi [13], but skin test material has been produced for only a couple dozen. Harvest of appropriate allergens for skin test material is hampered by the fact that many fungi do not grow reliably, if at all, in culture. For fungi that can be grown in culture, allergen production may differ from that produced on natural substrates or building materials, providing allergens that are qualitatively and quantitatively different from allergens that are encountered in ambient exposures. Fungal allergens are not necessarily unique to a single species, and allergenic cross reactivity can be found in variously related taxa [14,15]. Skin prick testing with fungal extracts has produced inconsistent results and a net underestimate of the prevalence of fungal allergy [16].

Toxic and irritant effects

Fungi contain or produce many agents that can have irritant or toxic effects. Glucans, which are structural components of the fungal cell wall, can cause irritant-type health effects in high concentration and have been implicated in building-related symptoms [17,18]. Glucans may have a role in opportunistic fungal infections or the development of HP [19].

Mycotoxins

Fungi produce several secondary metabolites (those not needed for primary growth and reproduction) that are known to be toxic to humans and animals. These mycotoxins are small, are less than 1 Kd, and are nonproteinaceous compounds that are produced and released to inhibit other microbes. Toxins can be found in mycelium and spores and are released onto growth substrates [20]. Many fungal species produce mycotoxins, and toxin production varies by species, strain, and environmental growth conditions [21].

Health effects of mycotoxins. According to animal studies, mycotoxin exposure through ingestion of food contaminated with fungi is known to cause health effects. With such exposures, these compounds have been shown to be immunogenic, immunosuppressive, cytotoxic, carcinogenic, and neurotoxic [19]. Human health effects have also been documented for large airborne exposures in agricultural and occupational settings [19]. Great debate has been generated in the past decade as to whether fungal mycotoxins could cause health effects through inhalation of airborne spores in indoor environments. A well-known case of a severely water-damaged house, with extensive fungal contamination, documented the presence of mycotoxins and health complaints consistent with mycotoxicosis [22]. A study of an outbreak of pulmonary hemorrhage in infants in Cleveland initially associated symptoms with water-damaged homes, fungal growth, and, in particular, mycotoxins from *Stachybotrys* [23]. A scientific task force from the Centers for Disease Control and Prevention later discounted these associations because of problems of study quality; the initial conclusion of mycotoxin effects from *Stachybotrys* were deemed unproven [24]. Several reviews of the literature point to the same conclusion: Unequivocal evidence is lacking for any association between mycotoxic health effects and fungal exposure in indoor environments [25–29]. The debate continues, however [30].

Microbial volatile organic compounds

Microbial volatile organic compounds (MVOCs) are responsible for the musty smell that is characteristic of fungal growth. They are produced at certain stages in the growth of the fungus as products of primary and secondary metabolism. Similar to the variations in production of allergens and toxins, the production of MVOCs varies with fungal species and strain, environmental conditions, and growth substrate [31]. MVOCs may be associated with some building-related symptoms such as headache, dizziness, and inability to concentrate [31].

Attempts are being made to use MVOCs as markers of active fungal growth, or growth of specific taxa, in indoor environments [32]. The profiles of MVOCs that are produced by specific taxa on various materials are being characterized [33,34]. Investigators are searching for unique volatile organic compounds that are produced by microorganisms; however, the existence of unique compounds is under debate [35,36]. Perhaps a more attainable goal is to find profiles of MVOCs that may be associated with fungal contamination; however, many of the known MVOCs can be generated by other nonbiologic substances [37]. Indoor air contains a mixture of volatiles from biologic and nonbiologic sources. These methods will be useful in elucidating the relationships between MVOCs and health effects, but will not substitute for thorough visual building examinations or exposure measures for other fungal agents.

Fungi, dampness, and health effects

Several studies have shown associations between reported indoor dampness and several health outcomes [38–40]. A review of the relevant literature (61 studies) by Bornehag et al [41] concluded that almost all studies showed that dampness is associated with respiratory symptoms. Some studies found associations with symptoms of tiredness, headache, and upper airway infections. However, the mechanism of interaction has not been clearly elucidated. Although an increased likelihood exists for the presence of fungal growth in damp conditions, there may be other health-relevant effects of indoor dampness, such as increased exposure to house dust mites or release of volatiles from building materials. The health effects that are associated with dampness may be caused by chemical, allergenic, or microbiologic agents.

Sources

Outdoor air

The primary source of fungi that colonize indoor environments is spore-laden outdoor air. In temperate regions, fungal spores are present in outdoor air almost year-round. Total spore concentrations show marked seasonality and peak in the fall when abundant nutritional sources, such as decaying leaves, are available. During the fall, outdoor spore concentrations are vastly higher than typical indoor concentrations, peaking at 70,000 spores/m^3 or more. Exposure to airborne spores indoors must be considered in the context of other possible exposures to spores in outdoor air.

Spores infiltrate buildings from outdoor air through openings such as air intakes, windows, and cracks. Spores are deposited on surfaces either by settling, impaction, or electrostatic forces. It is quite reasonable that deposited spores can be found on all indoor surfaces.

Indoor spore concentrations frequently are compared with outdoor concentrations at the time of sampling to take into account the background influx from

outdoor sources. In noncontaminated buildings, indoor concentrations generally should be qualitatively similar and quantitatively lower than outdoor concentrations. During the winter months at higher latitudes, however, there may be extended periods of snow cover when outdoor concentrations are very low. In these circumstances, indoor concentrations may be higher than outdoor levels, but this situation would not signal a problem of fungal contamination in the building. Comparison of indoor-to-outdoor concentration ratios that does not account for seasonality of outdoor concentrations can be misleading.

Fungal growth

When conditions on surfaces become favorable for germination and growth to occur, a sufficient bank of viable spores is available to take advantage of the opportunity. Recent studies have shown that new building materials carry their own microbial populations that, under the appropriate conditions, can flourish [42]. Inadvertent incorporation of fungal spores into or onto building materials can occur during many stages of processing or storage. Characteristics of materials, such as wicking ability or drying rate, have an impact on the latent period before fungal growth after wetting conditions. Further work is needed on the variability of natural fungal populations of various building materials and the characteristics of building materials that may deter fungal growth.

Indoor fungal habitats and reservoirs

The primary factor that controls fungal growth is the availability of moisture. If sufficient moisture is available, virtually any surface can become colonized by fungi. Nutrient sources are plentiful in indoor environments, and fungi can use many normal building materials and furnishings as growth substrates. Ceiling tiles and wallboard are particularly favorable substrates. Point sources of moisture on these substrates can support localized growth of fungi. Extensive areas of wetting can support large areas of growth on wetted substrates and can cause colonization of new areas because of increased humidity and subsequent condensation on cool surfaces that are distant from the original moisture source.

The amount of spores available for airborne dispersal is related to the extent of fungal contamination and depends on spore productivity of the fungal taxa, the ease of aerosolization of the respective spores, and the stage of development of the colonizations. Establishment of a quantified area of fungal contamination that relates to a certain concentration of airborne spores within a space has not been determined, and such a task would be fraught with difficulties. Therefore, no standard can be established for the size of an area of contamination that poses a health risk. The extent of fungal contamination has been used, however, as a guideline for appropriate remediation methods [43].

Spores remain airborne for various lengths of time, depending on settling velocity, before being deposited on interior surfaces. Surfaces, such as carpeting or ducts and filters in heating, ventilating, and air conditioning (HVAC) systems,

act as reservoirs for accumulated spores, which in turn can become secondary sources for re-aerosolization or new sources if moisture conditions in these locations are sufficient for growth.

Exposure considerations

To induce health effects, individuals must be exposed to fungal-disease agents in clinically relevant quantities (sufficient doses). Detection of a specific type of fungus or types of fungi in an environment or documentation of a certain degree, or location, of fungal contamination is insufficient evidence that true exposure at clinically relevant levels has taken place [44]. Health effects caused by fungi are known to occur when clear routes of exposure are established by way of inhalation, contact, or ingestion.

Inhalation

For an inhalation exposure to occur, spores or other fungal components must become airborne in the location of the individual, or must travel to that location, and be present in the breathing space. The quantity of spores that are disseminated depends on the extent of fungal contamination, plus temporal and ontological variations in spore production, release, and dispersal. Initiation of spore production is governed by complex relationships between light and available resources [45]. In terms of exposure, variation in the number of spores produced between taxa is important. *Penicillium* and *Aspergillus* are abundant spore producers, whereas *Ulocladium* produces fewer spores. A useful measure of relative productivity might be the amount of spores per unit area of growth, but this measure also would differ depending on the myriad of growth substrates and conditions; therefore, no such measure is available. Although productivity is an important factor for exposure, it does not take into account other factors such as the differences in amount of disease-causing agent (eg, allergen) per spore between types.

Spore release from either fungi can occur by active or passive means. The greatest challenge for a spore to become airborne is to cross the laminar-boundary air layer to become incorporated in more turbulent air currents. Several fungal types have evolved elaborate mechanisms to propel spores across this barrier of still air. Some types use turgor pressure to squirt or blast spores out into the air. A number of types propel spores into the air through centrifugal force upon the drying and twisting of the spore-bearing structures [46]. Alternatively, spores can be picked up passively by air movement, whether induced by convective currents in an unoccupied room [47] or by greater turbulence caused by human activities [48].

Probably the most effective method of aerosolizing spores in indoor environments is by mechanical disturbance, such as banging, scraping, touching, or vibrating any contaminated surface. Many human activities (eg, walking, sitting) produce sufficient disturbance in areas of direct contact to generate

significant airborne spore concentrations [48–50]. For some types of fungi, spore release is relatively easy, and the slightest movement or vibration can release large amounts of spores. For other types, such as *Stachybotrys*, in which spores are produced in a slime ball, release is much more difficult, particularly when the mycelium and substrate are wet. On drying, however, spore release is induced more easily.

Tremendous spatial variability in spore concentrations is possible when a localized source of fungi is present. A home without indoor fungal growth has a more homogeneous pattern of airborne fungal concentrations than does a home with an indoor source of fungi. Spores and fungal components disperse passively in indoor environments, as they do outdoors, and follow the flow of air through a building. Concentrations close to a source (eg, within the same room) may be magnitudes higher than concentrations elsewhere in the building.

Spores can travel far from source areas with sufficient turbulence to keep them airborne. Spores also can become incorporated into intake air streams of ventilation systems, particularly when HVAC units draw air from the surrounding air space. HVAC systems frequently are located in basements or in poorly maintained mechanical rooms that are more likely to have fungal contamination. Proper maintenance of ventilation source areas is a key component to maintaining good indoor air quality.

Airborne concentrations of fungal spores show a high degree of temporal variability. Fungi are keenly tuned to diurnal rhythms in atmospheric conditions (in natural environments or within a building) to optimize timing of release of spores and ensure dispersal. In buildings, diurnal rhythms in human activities add an additional flush of spores to the air. Periods of low ventilation rates and human activity at night provide conditions in which spores settle onto surfaces. Day-to-day variation in conditions and activities causes large fluctuations in airborne spore concentrations.

The process of spore deposition onto surfaces removes agents from the zone of possible inhalation exposure. Accumulations on these surfaces over time, however, generate potential reservoirs of spores that may be subject to re-aerosolization processes through mechanical disturbance. Spore viability is lost over time, but it may take a much longer time for deposited fungal agents to lose their ability to induce health effects. Therefore, the current fungal contamination status as well as the history of fungal contamination within an indoor environment are important for characterizing fungal spore exposure.

Contact

Another significant mode of fungal exposure is through contact. Physical contact with spores or mycelium is known to cause dermatitis with some fungal types (eg, *Stachybotrys*). Contact with sufficient fungal material to induce symptoms is most likely to occur during remediation efforts, highlighting the importance of the use of personal protective equipment. In addition, mechanical disturbance of spores through direct contact often causes the release of spores

into the air. Great care is needed during remediation to prevent the aerosolization of massive amounts of spores and hyphae [43].

Ingestion

Large exposures to fungal disease agents are known to occur through ingestion of food or other substances that are contaminated by fungal growth. Although many foods are processed with the aid of fungi (eg, bread, beer, wine, cheese) with no ill effects, toxic effects are well documented and have been described for numerous fungal types, including several mushroom species. Mushroom hunters are well aware of the care needed in correctly identifying edible species. Regulation and inspections of food crops have provided fresh and processed foods that are relatively safe from fungal contamination. Improperly stored fruits, vegetables, and grains in basements or out buildings are common sources of foods that can become contaminated by fungi and produce health effects on ingestion. Much of the current knowledge on the toxicity of spores and fungal types comes from studies of exposure through ingestion, in which the exposure to toxins can be immense.

Measures of exposure

An abundance of fungal spores in indoor air or on surfaces frequently is used as an indicator of potential building contamination and may signal the potential exposure of individuals to fungal-disease agents. Various methods for sampling and quantifying fungal spores are described elsewhere in this issue (see articles by Muilenberg and Horner); however, some discussion of sampling as it relates to exposure assessment is warranted.

Accurate fungal exposure assessment requires appropriate temporal and spatial measures that reflect the characteristics of exposure for the individuals of concern. For contact or ingestion exposures, the assessments may be straight forward; however, assessment of inhalation exposure poses particularly difficult issues because of the transient nature and high temporal and spatial variability of fungal aerosols. In addition, the limitations of sampling devices, protocols, and techniques mean that compromises to ideal sampling plans must be made [51].

Personal exposure monitoring provides the best measures of exposures. When monitors are worn for long periods of time, the sample reflects the many environments to which a person has been exposed. Personal monitoring is seldom done, but new technologies may make this type of assessment more common [52].

Airborne fungal exposure assessment is performed using air, surface, or dust sampling to detect fungal spores or their products. Each collection method represents an accumulation of fungi in the samples over different time periods and conditions, making comparisons between sampling types challenging. Some characteristics that differ between these types of collections and their relation to exposure assessment are outlined (Table 1). The advantage of air sampling for a defined period of time is that it is known with confidence that all recovered

Table 1
Comparison of characteristics of air, surface, and dust samples

Characteristic	Air	Surface	Dust
Fungi accumulate in sample over known period of time	Yes	No	No
Represents accumulation of fungi over longer-term exposure period	No	Possibly	Assumed
Affected by current activity levels in the space	Yes	No, unless cleaning	No, unless vaccuming
Represents accumulation of fungal spores from air and from fungal growth on the surface	No	Possibly	Carpets, yes; hard floor, no

particles were airborne and could have been involved in exposure. The disadvantage is that the period of sampling is usually short because of limitations in samplers and protocols and does not characterize airborne spore concentrations for which longer-term exposures need to be assessed. Surface and dust accumulations of spores may provide integrated samples over longer periods, but the length of those periods are unknown. It is not known at what points in time these areas were also reservoirs of fungal growth; therefore, these measures may reflect a certain proportion of the fungal agent that may never have been airborne.

Source sampling is another type of measurement of fungi in indoor environments with the loosest association to exposure. Identification of fungi that contaminate an indoor space by any of a number of methods highlights only the potential for aerosolization and cannot be related to any implied exposure. In combination with air or surface sampling, however, sources of airborne spores sometimes can be identified.

The profile of fungi recovered from all collection areas, and the profile to which exposure is assumed, is highly dependent on the analytical method used. Culture-based techniques are used widely to enumerate the culturable and viable fungal spore component of the aerosol. This method is important for assessing infectious organisms. Techniques that use direct microscopic examination of spores are useful for assessing the total spore load in a space. The relative advantages and disadvantages of these techniques are outlined (Table 2). In any sampling plan, the relative merits of the methods must be weighed against the inherent compromises. The increased potential for more precise taxonomic identifications with culture methods does not outweigh the considerable resulting selective bias [53], particularly if taxa are differentiated only to genus level or if species information is not used in some meaningful manner in data analysis. Although culturable sampling has been the standard in the past, it quickly is being replaced by spore trap sampling. Culturable sampling rarely should be the sole method of analysis; however, used together these methods can give the most complete picture of airborne fungal spore concentrations.

By far the most appropriate assessments to be made are direct measurements of the specific fungal disease–causing agents, because the quantity of such agents in spores varies by fungal type, growth conditions, ontogeny, and many other

Table 2
Relative merits of culturable and total-spore analytical methods

Method	Advantages	Disadvantages
Total	Estimate total spore load	Less precise taxonomic identification
Culturable	Ability to determine species for some types	Live spores are subject to sampling stress Requires 5–7 days for growth Recovers only a small fraction of the total load; missing are: Nonculturable fungi including many ascomycetes and basidiomycetes Dead spores Spores not able to grow on the chosen media Fungi not able to compete with others

factors. Direct measurement techniques are available for a number of fungal-disease agents, such as allergens, mycotoxins, MVOCs, and glucan. When inhalation exposure is the pathway of concern, these measurements also should reflect quantities in air. With the exception of MVOCs, these direct measurements typically use source sampling, and some of the advantage of direct agent assessment is lost.

Direct detection of allergens has been limited by the low number of major allergens that have been characterized and for which antibody assays have been developed. Monoclonal assays (detection of one specific allergenic protein) are widely available only for Alt a 1 (*Alternaria alternata*) and Asp f 1 (*Aspergillus fumigatus*), and a polyclonal assay (which detects a suite of proteins) has been developed for *Cladosporium cladosporioides*. A number of other assays have been developed in the food industry, but have not been applied to indoor environmental analyses. Although detection of specific allergens is a good indication of exposure, the measures most frequently are made from collected dust samples, which only loosely relate to airborne quantities. Although detection of specific allergens is more clinically relevant for a particular fungal type, it does not capture the total spectrum of fungal exposure, and it cannot be assumed that all fungi vary in concert with any of these specific allergen measures.

A number of chemical detection methods have been developed, mainly in the food industry, for quantifying mycotoxins. These methods are used primarily on surface or bulk samples to determine the presence of toxin-producing fungi or the amount of toxin in a substrate [54]. These tests rarely have been used in conjunction with air sampling, but combining these methods would provide the best evidence of possible mycotoxin exposure [55]. Hinkley et al [21] examined mycotoxin production in various isolates of *Stachybotrys* and found that only one third of isolates were capable of producing macrocyclic tricothecenes. This study offers compelling evidence that one should not rely simply on the presence of the organism to infer mycotoxin presence or exposure.

Detection of biomarkers gives the most direct indication of exposure. Fungal agents (eg, mycotoxins) in bodily fluids can provide evidence and degree of

exposure [56,57]. Alternatively, some compounds produced in response to fungal agents can be detected (eg, antibodies or metabolic products) [58]. Mycotoxin biomarker assays have been developed with sensitivities expected for exposure from food, but relatively few of these assays are sensitive enough to quantify indoor exposures.

Presence of serum IgG or IgE to specific fungal antigens is theoretically a good indicator of fungal exposure; however, it provides no information on when or where that exposure was received. Because outdoor, home, work, school, and recreational environments contribute to the total exposure, controls and cases in exposure studies often have elevated levels of fungal specific antibodies. Because fungal allergens are commonly cross-reactive between taxa, interpretation of positive antibody results is difficult. Serologic tests for fungal exposure are not sensitive enough to indicate current exposures in specific environments [59], and further studies are needed to determine the interpretive value of these tests. Although the presence of serum antibodies to a particular taxon's antigens may be an indication of exposure to that organism, this information cannot be used to infer exposure to the organisms' mycotoxins or other agents.

Fungal populations

In the United States, no regulations exist for an unacceptable level of airborne fungi or their agents. Obstacles that prevent such a determination are the variation in human susceptibility to disease agents, lack of standardized protocols in measurement, and lack of data in relation to health effects (as outlined earlier). Recently, legislation has been passed in California that proposes to set such limits.

Airborne fungal populations

The most common fungal genera found in indoor air are *Cladosporium*, *Penicillium*, and *Aspergillus* [60]. There are noticeable differences between the mycoflora of indoor environments and the profile of fungal concentrations in outdoor air. The most common species of *Cladosporium* in outdoor air are *C cladosporoides* and *C herbarum*, whereas the most common species indoors is *C sphaerospermum*. The difference likely results from the presence of a nutrient composition in indoor materials that is preferred by *C spherospermum*. Other common indoor taxa when water damage and contamination are present include *Ulocladium*, *Stachybotrys, Chaetomium*, *Aspergillus versicolor*, and many other common taxa.

The quantity of fungal spores in indoor air has been shown to vary with housing characteristics, but temperature, humidity, season and the presence of cats seem to have a larger impact on fungal concentrations [61]. For large office buildings, season, humidity and CO_2 are important in predicting fungal concentrations [62]. Indoor fungal concentrations also vary geographically; southern areas of the United States register higher indoor values compared with the rest of the country [60].

Criteria that define whether the air in an indoor space is unduly populated with fungal components are needed. Several groups (governmental, academic, public, and private organizations) have attempted such definitions with varying results [63]. These determinations require arbitrary decisions on what defines an unacceptable level. Ideally, the decisions should be based on good exposure, dose, and response data; however, little information exists. Some information exists on the characteristic levels of fungi in complaint and noncomplaint buildings, and such studies will form the basis of decisions until good data are acquired (Environmental Protection Agency Building Assessment Survey and Evaluation [BASE] and National Institute for Occupational Safety and Health).

The interpretation of air sampling results remains a complex mental algorithm of juggling qualifications and is the bane of the inexperienced consultant (and trial lawyer). Sample results must be kept in context of time of day, location of sampling, human activity, season, profile of fungi, presence of taxa (that are indicators of high moisture or building contamination or that are known to cause health effects), and many other issues that might affect results. Broad generalizations can be made based on zones of consensus from the more formal guidelines and regulations of the various regulatory and professional groups. Based on culturable sampling data, total airborne fungal concentrations (not a single taxon) greater than 100 colony-forming units (CFU)/m^3 might be considered low and may not indicate any building problem, whereas total concentrations greater than 1000 CFU/m^3 might be considered high if outdoor air is eliminated as a direct source (eg, open window in summer) and would warrant further investigation. For any single taxon, concentrations greater than 100 CFU/m^3 might indicate a problem in a building, and concentrations greater than 1000 CFU/m^3 definitely would indicate contamination if outdoor air can be eliminated as a direct source. In between these boundaries, all of the various qualifications are required for interpretation. Airborne fungal concentrations should be qualitatively similar to outdoor air levels (possibly assessed by rank order) and quantitatively lower than outdoor air (possibly assessed by indoor–outdoor concentration ratio). Because regulatory guidelines are based largely on experience in building investigation or research [63], the terms used here refer to building contamination rather than to any implied health hazard. The previous broad guidelines do not apply to pathogenic organisms or when immune compromised individuals may be exposed.

Dustborne fungal populations

Fungal populations that are recovered from settled dust represent a different spectrum of fungi relative to airborne populations [64]. Yeasts frequently are recovered in dust samples [65] but are seldom seen in air. This difference may represent growth on skin scales, the predominant organic substrate, in dust. Although dust samples are believed to represent long-term accumulations of airborne spores, there may be an over-representation of types with the hardiest spores (because the most common method of dust analysis is by culture). Conversely, because airborne spore concentrations are highly variable, short-

term air samples may not reflect what is accumulating in dust over a longer period of time.

Total CFU recoveries from dust are commonly in the range $10^4$to 10^6 CFU/g, whereas single-taxon recoveries are normally below 10^5 CFU/g [66]. The most common taxa are yeasts, *Eurotium*, *Penicillium*, *Alternaria*, *Aureobasidium*, and *Cladosporium*. Nonsporulating fungal colonies (ie, nondifferentiable taxa) commonly are observed from air and dust. There has been no discussion in the literature as to what represents low, moderate, or high levels of dustborne fungi, as have been discussed for airborne fungi. Levels of dustborne fungi are related to housing characteristics, such as housing type (lower levels in apartments), carpeting (higher levels), and presence of dogs (higher levels for *Alternaria*) [64].

A limited number of studies have examined fungal agents in dust. Measurable levels of *Alternaria* and *Cladosporium* allergens have been detected in dust from homes of patients with allergies (Alt a 1 and Alt a 70-kD concentrations of 3–1000 U/g of dust) [67]. *Aspergillus fumigatus* allergen (Asp f 1) levels in dust have been investigated in offices and homes and found to be undetectable in most samples, even if culturable *A fumigatus* was present, because the allergen is only present after spore germination [68,69]. When they were detectable, levels were low (<0.17 μg/g of dust) [69]. Doewes et al [70] measured $\beta(1 \rightarrow 3)$-glucans in house dust, with values ranging from 180 to 6500 μg/g of dust. Engelhart et al [54] detected low concentrations (2–4 ng/g of dust) of sterigmatocystin, a mycotoxin produced by *Aspergillus versicolor*, in carpet dust.

Analysis of fungal data

In epidemiologic studies, investigators tend to group fungal measurements into total fungal counts (without any taxon-specific determinations) or other broad fungal categories (ie, basidiospores) [71]. This tendency may result from the lack of expertise in making more taxonomically detailed determinations or from a requirement to limit the number of variables in a statistical analysis. Both limitations may be valid; however, overly broad groupings may have weakened the ability to distinguish significant relationships between health effects and specific fungi. It is well known that immunologic responses and resulting health effects are allergen specific and that specific fungal taxa vary widely in the composition and concentration of allergens that they carry. Comprehensive studies on the health effects resulting from exposure to specific fungi are needed, as are studies on the cross-reactivity of fungal taxa. Because of the specialized skills that are required to correctly identify fungal taxa, detailed determinations are labor intensive and expensive. Technologies that quickly and accurately can make specific differentiations (such as polymerase chain reaction techniques) can do so for only a limited number of taxa, although the list is continually expanding [72]. Where detailed data are available, epidemiologic studies should include extensive exploratory analysis of datasets to determine which specific taxa may be important for showing significant health associations. Much may be learned by keeping (or increasing) the specificity of the fungal data in these analyses.

Future directions

Of prime importance for the accurate determination of the prevalence of fungal allergy is continued work on the standardization and characterization of fungal allergens and the production of stable, reliable skin test material. Development of more appropriate measures of fungal exposure is needed to solidify the links between exposure and health effects. These new measures would facilitate studies of the exposure, dose, and response relationships that are necessary for setting threshold limits and regulating fungal levels. Examination of health associations with total (nonculturable) airborne fungal concentrations may provide a better picture of fungal health impacts than has culturable sampling. The debate over mycotoxin exposure and impact would be aided greatly by refinement of biomarker and serologic tests with studies of their sensitivity, specificity and positive and negative predictive value [73]. Determination of natural fungal populations incorporated in and likely to grow on various building materials could highlight the changes needed in the production and selection of materials and in construction practices to deter fungal growth.

Summary

Fungi affect humans in complex ways and are capable of eliciting a number of disease responses, such as infectious, allergic, and irritant and toxic effects. Fungal exposure is unequivocally associated with exacerbations of asthma, although the role of fungi in causing the disease is yet to be determined. The association between home dampness and respiratory health effects is strong, and fungal exposure is suspected to be associated with this linkage. Fear of toxin exposures has generated debate over the possible toxic health effects of airborne fungi; however, several recent reviews discount the health impacts of mycotoxin through indoor exposures. Nevertheless, fungal contamination of indoor environments is undesirable. Knowledge of sources and characteristics of fungal spore release and dispersal are important for understanding the processes of exposure. Environmental monitoring for fungi and their disease agents are important aspects of exposure assessment, but few guidelines exist for interpreting their health impacts. Much work is needed in isolating, characterizing and standardizing fungal disease agents to properly assess the prevalence of fungal health effects.

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