

Challenges associated with indoor moulds: Health effects, immune response and exposure assessment

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Assessment of the health effects of indoor moulds is complicated by the diversity of mould species found in homes and the limitations of current methods to determine exposure. Thus it is difficult to establish whether there is a relationship between mould exposure and disease. Allergic respiratory diseases are commonly caused by *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* spp. IgE-mediated sensitization to these moulds is a strong risk factor for asthma: IgG and IgE antibody responses to *Aspergillus fumigatus* are common in patients with other respiratory diseases, including allergic bronchopulmonary aspergillosis and cystic fibrosis. Several important mould allergens have been cloned with different biologic functions. These allergens can also serve as immunologic markers which may be associated with disease activity. Evidence for health effects associated with exposure to mould toxins e.g. from *Stachybotrys* spp. is less compelling. Recently, several new technologies have been introduced which could be applied to mould exposure assessment. Ion-charging devices can silently sample air within homes and have been successfully used to monitor animal allergens. Fluorescent multiplex array technology is being used to make quantitative measurements of five to ten allergens simultaneously on dust samples. The development of monospecific (monoclonal or polyclonal) antibodies to specific fungal antigens or allergens will facilitate more accurate assessments of the mould burden in homes, schools and commercial buildings. The application of these techniques in well-designed clinical studies will enable better understanding of the health effects of moulds.

Keywords mould allergens, asthma, toxic moulds, exposure assessment, health effects

Introduction

Considerable progress has been made over the past 25 years in defining the health effects of exposure to indoor allergens (dust mites, animal allergens, cockroaches) and other indoor biologics, such as endotoxin (see [1,2] for a review). However, the challenges associated with defining the health effects of indoor mould remain significant. Assessments of allergic sensitization and exposure to mould allergens are not as well established as for other indoor biologics.

Moulds are associated with diverse health effects, including allergic respiratory disease, infection (e.g. invasive aspergillosis), chronic sinus and pulmonary diseases, as well as more idiosyncratic disease states, such as chronic fatigue syndrome, lethargy, migraines etc. Many different mould species have been implicated in disease causality and this, in combination with the diverse array of potential health effects, has made it difficult to establish clear relationships between mould exposure and disease activity.

The 2nd Advances Against Aspergillosis conference focused on *Aspergillus* spp., the clinical importance of which is well established. However, data for other moulds is less complete. This paper will review the evidence supporting health effects for other moulds, especially as they relate to allergic diseases, and will

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also cover new technologies that are in the pipeline for improving air quality and environmental assessments of mould exposure in homes, the workplace and commercial buildings.

Health effects

The principal moulds associated with allergic (IgE-mediated) diseases, such as allergic rhinitis and asthma, are *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* spp. [3]. Sensitization to *Alternaria alternata* is a significant risk factor for wheezing and asthma in both children and adults (odds ratios 5–6.8) and is significantly associated with increased airway hyper-responsiveness. A seminal paper by O'Hollaren *et al* [4] showed that exposure to *Alternaria* was associated with respiratory arrest in children with asthma in the mid-western United States. Subsequent studies have shown that sensitization to *Alternaria* was particularly associated with asthma in dry arid climates, such as the desert regions of Arizona and Australia [5,6]. Most of these cases occur as a result of outdoor exposure to *Alternaria* spores. However, sensitization to *Alternaria* has also found to be common among children living in cities. A high prevalence of *Alternaria* sensitization (~35%) was found among poor inner-city children with asthma enrolled in the US National Cooperative Inner City Asthma Study (NCICAS). A recent follow up study found that *Alternaria* (as well as *Aspergillus*, *Cladosporium* and *Penicillium*) were the most prevalent mould spores found in inner-city homes in seven US cities, occurring in 70–80% of homes in Boston, the Bronx, Chicago, Dallas, New York, Seattle and Tucson [7]. The main risk factors for the presence of mould in those homes were dampness, cockroach infestation (a surrogate for dampness) and, surprisingly, the presence of cats.

Aspergillus fumigatus, and to a lesser extent other *Aspergilli*, cause allergic respiratory diseases, as well as more chronic respiratory conditions such as allergic bronchopulmonary aspergillosis (ABPA). *Aspergillus fumigatus* also causes a variety of opportunistic infections in patients with cystic fibrosis, aspergilloma and invasive infection of immunocompromised hosts [8]. Other fungi, including *Bipolaris* and *Curvularia* spp., have also been implicated in chronic allergic fungal sinusitis (see article by Dolen [this issue]). The association between these fungi and health effects is established by clinical symptoms, by measurements of specific IgG or IgE antibody responses, or other immunologic markers, and by culturing fungi from secretions or tissue samples. High-level exposure to airborne moulds can cause occupational lung diseases

such as hypersensitivity pneumonitis, though these conditions are rare.

Associations between mould exposure and other health effects are less well established. Media and public interest in “toxics” moulds was initiated by a series of cases of acute idiopathic pulmonary hemorrhage (AIPH) in infants in Cleveland, OH (1993–1994), which were associated with exposure to *Stachybotrys chartarum*. These observations aroused intense public concern and prompted further investigation into the toxigenic properties of *Stachybotrys* and other moulds. About 30% of *Stachybotrys* strains produce tricothecene and other toxins. Administration of *Stachybotrys* spores to the nasal passages and lungs of mice and rats was shown to cause inflammation and bleeding. However, the dose for “no effects” in rats was $3\text{--}5 \times 10^6$ spores/ml, which extrapolated to an exposure of $2\text{--}15 \times 10^6$ spores/m³ for humans, taking into account resting breathing rates of newborns or adults. A position statement by the American College of Occupational and Environmental Medicine concluded that these exposure levels were unlikely to be achieved in housing and that the evidence that indoor exposure to *Stachybotrys* caused toxic effects in humans was insufficient [9]. Subsequently, the US Center for Disease Control found that the apparent association of the Cleveland cases of AIPH with *Stachybotrys* was not causative. A recent Morbidity and Mortality Weekly report (a publication of the US Centers for Disease Control) suggested that those cases may have represented a inherited metabolic bleeding disorder (von Willebrand's disease) [10].

In the United States, the presumption that exposure to toxics moulds has adverse health effects has spawned a growing number of mould litigation claims. There are estimated to be ~10,000 mould-related lawsuits pending in the United States, some of which have resulted in multi-million dollar damages awarded against insurance companies and building contractors. These cases have also lead to the development of a huge mould remediation industry, which is largely unregulated. While a detailed discussion of the medico-legal aspects of toxic moulds is beyond the scope of the review, readers are referred to the work of Hutchinson and Powell [11] who critically analyzed the rise of mould litigation in the United States on behalf of the US Chamber Institute for Legal Reform.

Mould allergens and specific immune responses

Although there appears to be a general perception that moulds produce an array of ill-defined allergens and

antigens, there has been significant progress in defining important allergens from several mould species over the past 15 years. Molecular (cDNA) cloning has identified panels of allergens from *A. alternata*, *A. fumigatus*, *C. herbarum* and *Penicillium* spp. and several other fungal species (see: www.allergen.org for the official list of allergens authorized by the World Health Organization and International Union of Immunological Sciences sub-committee on Allergen Nomenclature). Major allergens have been identified from all of these species and over 25 allergens alone have been reported from *A. fumigatus*. The cloned allergens have a diversity of biologic functions and include serine and ribosomal proteases, enolase, heat shock proteins and cytotoxins. The major allergen of *A. alternata* is a heat stable, 28 kD dimeric protein, designated *Alt a 1*, which elicits IgG and IgE antibody responses in >80% of *A. alternata* sensitive patients with allergic rhinitis or asthma [12]. *Asp f 1*, a major allergen of *A. fumigatus*, is an 18kD cytotoxic ribonuclease expressed by *A. fumigatus* and *A. restrictus* which is homologous to mitogillin and α -sarcin. The allergen is not found in spores and is secreted in large amounts when *A. fumigatus* germinates. Approximately 85% of *A. fumigatus* sensitive patients produce IgE antibody to *Asp f 1*. Measurement of IgG anti-*Asp-f-1* antibody has been a useful marker of *A. fumigatus* colonization in patients with cystic fibrosis, ABPA and aspergilloma [8]. *Asp f 1* has also been used to assess T cell reactivity in patients with ABPA (see article by Knutsen [this issue]).

While a large number of fungal allergens and antigens have been cloned, the clinical and immunologic importance of many these allergens has not been fully established. Thus the direction of future research should be to establish which allergens are of clinical significance and can be used as markers of colonization or disease activity, which antigens may serve, for example, to monitor the effects of treatment with anti-fungal drugs, and which can serve as markers of environmental exposure.

New technologies for exposure assessment

Assessment of exposure to moulds has traditionally relied on spore counts (in outdoor air) and microscopic identification and mould culture (indoors, in dust and air samples). More recently, enzyme immunoassays using polyclonal antibodies have been developed to measure total antigen load in dust samples. Monoclonal immunoassays have been developed for specific allergens such as *Alt a 1* and *Asp f 1*. Generic tests for β -glucans, ergosterol, and extracellular polysaccharides have also been used to assess exposure to moulds (see

[3] for a review). Monoclonal immunoassays have been useful for comparing *Alt a 1* and *Asp f 1* levels in allergenic products used for diagnosis and treatment [12]. However, these assays have limited value for exposure assessment. The *Alt a 1* assay measures allergen on spores, but the spore concentrations in most house dust samples are too low to be detectable in the assay. The *Asp f 1* assay does not detect allergen in spores (the most common form of the mould found in dust) and the assay usually gives positive results only when germinated mould is present.

Nonetheless, antibody-based tests provide the best potential measures of exposure assessment for specific mould antigens or allergens. A polyclonal inhibition assay was recently used to measure *Alternaria* in a large, representative survey of housing in the United States [13]. However, the specificity of the assay was not validated and the apparently high prevalence of exposure to *Alternaria* suggested that some of the exposure could represent cross-reactivity with other mould species. These effects can be avoided by the use of monospecific antibodies with defined specificity. The limiting factor in measuring specific mould antigens has been that few such antibodies have been developed, especially for moulds that are commonly found in damp houses, such as *S. chartarum*, *Aspergillus versicolor* and *Penicillium chrysogenum*.

Several new technologies are being developed that could have significant impact on exposure assessment for moulds, most of which have been developed as a result of research on indoor allergens. Upright, silent ion-charging devices which sample the air and collect airborne antigens and spores on stainless steel plates have been used to monitor exposure to animal allergens (cat, dog, rat, mouse) in homes and in vivaria [14,15]. The plates provide a large surface area upon which airborne antigens collect and swabs from the plates can be analyzed for antigen, spores and products such as endotoxin and glucans. The devices themselves can quietly operate in homes for prolonged periods (8 h or more) without affecting the occupants.

Multiplex technology for measuring multiple antigens simultaneously also has great potential for measuring fungal allergen exposure. This technology is now routinely being applied to measure immune markers such as cytokines, growth factors and cell signaling molecules. Our group has focused on the application of fluorescent suspension array technology for allergen detection using the Luminex xMAP® system Luminex Corp, Austin, TX, USA. This platform uses polystyrene beads containing various concentrations of fluorophores to which monoclonal antibodies (or antigens) can be covalently coupled. The beads provide a solid phase that

is used in place of an ELISA-well to perform immunoassays. Each bead set contains a unique fluorescent dye and multiple beads can be incubated in a sample reaction. Theoretically, up to 100 different analytes can be assayed simultaneously, though panels of 10–20 analytes are the norm. We have recently developed a multiplex array for dust mite, cat and dog allergens and in principle the technology can also be applied to mould antigens [16]. The caveat being that monoclonal antibodies with well-defined specificity for the antigen are a pre-requisite for array development. Multiplex technology provides quantitative results and is ideally suited to systems where multiple antigens need to be measured. The ability to measure 10–20 mould antigens simultaneously would help to overcome the complexity of mould measurements because the number of antigens to be measured would be less of an obstacle.

Similar considerations apply to the use of lateral flow tests for mould toxins and antigens. Some of these tests are already commercially available and allow consumers to screen their homes for the presence of moulds, though there is little published data on the performance of most of these tests. Our group is currently working on the development of a credit-card-sized device for measuring several indoor allergens at once using lateral flow technology. In principle, this approach can be extended to mould antigens as suitable antibodies become available. These tests would enable an indoor air quality specialist or industrial hygienist to screen homes for the presence of four to six mould species on site and to provide advice about remediation procedures.

Conclusions

The medical effects of mould exposure were recently reviewed in a Position Statement of the American Academy of Allergy Asthma and Immunology [17]. Moulds cause adverse health effects through allergic hypersensitivity responses, infections and toxicity (ingested mycotoxins). There is little compelling evidence linking inhaled mycotoxins to health effects in humans or of mycotoxin mediated immune dysfunction. While a large number of mould antigens and allergens have been defined, there needs to be more critical appraisal of the clinical significance of these antigens, apart from the major allergens which have already been well characterized. Several promising new technologies are being developed for sampling and detection of airborne mould antigens. These technologies are dependant on demonstrating the clinical or immunologic importance of the antigen, whether it can be used as a marker of mould exposure, and whether monospecific antibodies can be that react with the antigen can be developed. Well-

defined clinical studies, coupled with precise exposure assessments, should result in a better understanding of the health effects of moulds and the risks to public health.

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