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Emerging concepts in mold allergy: What is the role of immunotherapy?

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Since Blackley¹ reported the development of "bronchial catarrh" after inhaling spores from *Penicillium* cultures in 1873, molds and mold spores have been known to cause upper respiratory tract allergic disease. Feinberg² and Prince et al.^{2a} were pioneers in documenting the occurrence of respiratory tract symptoms after exposure of clinic patients with allergy to mold in the 1930s.² More recently, epidemics of bronchial asthma have been associated with high concentrations of basidiospores in certain climates.³

Molds are actually fungi, and literally tens of thousands of fungal species are thought to exist. Many genera of Hyphomycetes (*Fungi Imperfecti*) class of fungi that serve as sources of allergens include *Cladosporium*, the spores of which usually outnumber all other airborne biologic particles in outdoor air; *Alternaria*, found throughout North America, particularly in great abundance in outdoor air in areas where grain is grown; *Drechilera*, airborne in highest concentrations in warm humid regions; *Epicoccum*, found in many temperate regions, grasslands and abundantly airborne during agricultural activities; *Fusarium*, an important plant pathogen, particularly prominent in air dur-

ing damp, humid weather; *Aspergillus* and *Penicillium*, which often colonize indoor substrates. Fungi of the basidiomycetes class including mushrooms, puffballs, bracket fungi, rusts, and smuts are found throughout the world and have also been shown to be significant outdoor aeroallergens.⁴

Many investigators have demonstrated that inhalation exposure to fungi induces the formation of specific IgE antibody demonstrable by both in vivo and in vitro procedures. The subject of allergy to fungi has been reviewed, and several problems that required investigation have been highlighted.⁵ Among these are the lack of well-characterized, purified, and standardized fungal allergens needed to establish the relevance of fungi in allergic respiratory disease and the complexity and toxicity of fungal extracts as compared with those from pollen. The apparent low degree of cross-reactivity among different fungi in the IgG system also poses problems. These problems remain essentially unsolved, and it is still difficult to determine the comparative relevance of environmental fungus exposure to dust mite and pollen allergens in causing rhinitis and asthma.

Among the most difficult problems associated with immunotherapy in which fungal materials are used are determination of individual exposure conditions and development and testing of appropriate diagnostic and therapeutic agents. These are discussed below.

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PROBLEMS IN DETERMINING EXPOSURE CONDITIONS

In determining exposure conditions, it is necessary to know the species of fungi to which individuals are exposed. Without such information, one cannot make logical decisions as to which materials should be used for skin testing and, subsequently, immunotherapy. Such information is also necessary to guide research efforts directed toward the identification of specific fungal allergens. Unfortunately, accurate information that defines either ambient or personal exposure patterns to fungal species is still not available.

Most allergen materials currently available as diagnostic and therapeutic agents have been chosen on the basis of conventional "settle-plate" or "settle-slide" sampling. Settling methods provide only a rough estimate of the larger spore types present in the air. Smaller spores are always underrepresented in such samples, especially in outdoor air where significant air movement occurs during sampling.⁶

The most commonly used sampler for ambient (outdoor) fungal spores in the United States is the rotorod.⁷ The rotorod, as commonly used, efficiently collects particles larger than 20 μm , which include most types of pollen grains. However, fungal spores range in size from 2 to more than 50 μm , with most spores being less than about 15 μm . The rotorod sampler collects less than 10% of the total fungal spores present in outdoor ambient air and less than 1% of small spores, such as those produced by most basidiomycetes and by the common species of *Penicillium* and *Aspergillus*. The Burkard spore trap is more efficient; it collects approximately 50% of spores 5 μm in diameter, including most basidiospores.

Recognition and identification of spores on rods and slides adds additional bias.⁸ In general, investigators trained to recognize pollen grains tend to use approximately $\times 400$ magnification, which is suitable for identification of pollen and large spores such as *Alternaria*. This magnification is, however, not optimal for many smaller fungal spores, including those of *Penicillium* and *Aspergillus* species and basidiospores, which often go unnoticed and unidentified. In addition, microscopic observation rarely allows identification of fungal species, and spores are usually grouped into generic (e.g., *Alternaria*, *Cladosporium*) or even larger categories (e.g., basidiospores, ascospores, rusts).⁹ Although pollen identification presents similar problems, field collections allow identification of local pollen-producing species.

However, field collections of fungi are much more difficult to obtain, and culture is usually necessary for species determinations.

Some investigators rely only on culture sampling for evaluating certain environments, especially indoor areas. Unfortunately, many fungal spores, including basidiospores, do not germinate, grow, and produce new spores in artificial culture. In addition, viability may not be a necessary condition for antigenicity, so that levels based strictly on numbers of viable spores may not be representative of allergen exposure. Several good culture-plate sampling devices are available.¹⁰ However, all such culture-plate devices collect only short-term "grab" samples over a maximum period of less than 10 minutes. Unless multiple samples are collected, these devices do not account for temporal variation in the air spora.

Finally, almost all current airborne fungal spore and pollen grain sampling is "ambient" rather than personal. Ideally, exposure to allergens should be evaluated with the use of sampling devices that accurately and specifically measure the full range of allergens to which an individual is actually exposed. Unfortunately, a great deal more research is necessary before such devices are made available.

PROBLEMS IN PRODUCTION OF STANDARDIZED FUNGAL ALLERGENS FOR USE IN DIAGNOSIS AND IMMUNOTHERAPY

Even with accurate information on human exposure, significant problems remain with respect to the production of relevant allergen materials for diagnosis and immunotherapy. Few if any well-standardized fungal allergens are currently available to the practitioner, and there may be a lack of correlation between in vivo skin test and RAST results with fungal allergens, possibly because of problems associated with coupling these allergens to cyanogen bromide-activated nitrocellulose discs or related materials, which are used in in vitro tests. The complexity, possible toxicity, and variability of fungal extracts add to these problems. In view of the crude nature and variability of uncharacterized ground-extracted fungal growth, it is not surprising that the quality of commercially available fungal extracts has generally been poor.¹¹⁻¹⁵ This is illustrated by studies by Yunginger et al.¹³ and Aas et al.,¹⁵ who skin-tested 80 patients with highly suspected fungal allergy with a variety of commercial extracts and found that the incidence of positive skin-test reactivity with *Cladosporium herbarum* varied from 12% to

65% depending on the commercial extract used. The same strains of a particular fungal species grown under identical culture conditions over similar time intervals can also produce extracts of varying potency.^{11, 12} This suggests that until defined allergens are available, standardized pools of a variety of strains should be used in skin testing to ensure the presence of a sufficient number of determinants from a representative group of strains of a particular species.

Some of these problems are related to the intrinsic variability of fungi. Most fungi have evolved as decay organisms and have developed the ability to adapt rapidly to changes in available food sources.¹⁶ This means that given a new food source, many fungi will "learn" to produce new enzymes that allow the new food to be digested and used. One mechanism for this phenomenon is a high rate of somatic mutation. *Alternaria* is one of the fungi that will produce morphologically obvious somatic mutations in culture. These changes in structure are reflected in the biochemistry of the organism and probably in its allergen profile. Because such mutations are common in nature, each new strain of a specific fungus is likely to be at least somewhat different than every other strain. Sometimes these differences are so large that major differences in allergen content occur.¹⁷

Similarly, changing conditions under which a single strain is cultured will often drastically affect patterns of allergen production. For example, allergen patterns are probably specific to the culture medium on which the fungus is grown; the level, wavelength, and pattern of lighting; and the length of time in culture before harvest. Variability can also result from extract preparation methods. Once the fungus is harvested, the processing of material can itself directly affect the allergen content of resultant extracts. For example, some fungi produce cellulolytic enzymes that will digest dialysis tubing and other cellulosic dialysis devices, changing the pore size and (possibly) causing loss of specific allergens.^{18, 19} It should also be noted that fungi possess large amounts of carbohydrate-containing materials, some of which are allergens. Extraction and purification methods that focus on proteins alone can result in loss of important carbohydrate-rich allergens.

Stability of extracts also poses concerns. In addition to cellulases, fungi produce a variety of other enzymes that can degrade proteins and glycoproteins, including allergens. Not only can

these enzymes degrade and digest dialysis tubing and fungal allergens, but there is some evidence that fungal enzymes will degrade other allergens (e.g., pollen allergens) when mixed extracts are used. The use of glycerin in skin test material and constant cold temperatures or lyophilization for material that cannot contain glycerin is essential to minimize these effects.

Finally, problems can also arise from the presence of metabolic products in fungal extracts. Although pollen extracts are made from the actual exposure units (pollen grains), entire cultures, which often include metabolites, are used to make fungal extracts. Such metabolites may be irrelevant for treating allergic symptoms that result from airborne spore exposures and may be antigenic and cause sensitization. For example, penicillin, a known sensitizer in human beings, is produced by *Penicillium chrysogenum*, one of the most common components in the indoor air spora and in commercially available *Penicillium* mixes. In addition, some fungi produce toxic materials that can cause effects ranging from acute dermatitis to immunosuppression and cancer.²⁰ Fortunately, the majority of these compounds are fat-soluble and of relatively low molecular weight and are therefore removed by defatting and/or dialysis.

The solution to most of these problems lies in the identification of specific fungal allergens that can be provided as well-defined mixtures. Several have already been isolated (e.g., *Alt a I*).²¹⁻²³ The introduction of molecular biology into allergy research has also led to the cloning and partial DNA sequencing of several allergens that may culminate in improved methods of therapy designed, for example, to eliminate allergen epitopes. By such means fungal allergens and the immune response elicited by them can also be more fully characterized. However, there are hundreds of thousands of different fungi to which we are exposed, and the task of determining prevalence of single allergens such as *Alt a I* and of identifying, cloning, and sequencing other important allergens is a formidable one.²⁴

In view of the above problems and pitfalls, the following currently practical solutions would seem reasonable.

1. Examine local prevalence patterns for fungi with the best available methods. If the rotorod must be used, develop skills in identifying fungal spores and use adequate magnification.

2. Limit the use of mold mixtures and always keep allergen materials cold to avoid degradation by fungal enzymes.
3. Use dialyzed or defatted fungal allergen materials for immunotherapy and accomplish dialysis without the use of cellulose-based membranes.

IMMUNOTHERAPY TRIALS

Controlled immunotherapy trials with relatively standardized purified and potent extracts of *Alternaria* and *Cladosporium* species have demonstrated efficacy. Studies by Malling et al.^{25, 26} in Scandinavia, for example, employed subjects who had positive skin prick test results to standardized *Cladosporium* extracts and exacerbations of symptoms during the peak of *Cladosporium* atmospheric concentrations in the late summer months. Individuals chosen for study also had positive results to bronchial provocation testing with *Cladosporium* when a double-blind placebo control protocol was used. Clinical efficacy was noted in 81% of the treated group versus 27% of the placebo group on the basis of the participants' subjective comments (symptom scores).^{25, 26} Dreborg et al.²⁷ also studied children with *Cladosporium*-induced asthma and rhinitis in a double-blind protocol with the same criteria as in the previous study. All study subjects had positive results to either conjunctival or bronchial challenge with *Cladosporium* allergen. The 16 patients in the treated group demonstrated a significant increase in tolerance to *Cladosporium* extract on conjunctival or bronchial challenge with *Cladosporium* after treatment. Symptom scores were also significantly lower in the treated group during the peak late summer *Cladosporium* season. In 1988, Cantani et al.²⁸ reported a 3-year prospective study of the effects of *Alternaria* immunotherapy on asthma and rhinitis in 79 children. Challenges were not used as an endpoint, but all individuals had highly positive reactions to *Alternaria* extracts as determined by skin test and RAST. Subjects were matched and equally divided among the placebo and treatment groups. Results indicated that with higher doses (above 80,000 PNU), 80% of the treatment group experienced a significant reduction in symptoms. A more recent study in 1990,²⁹ in which a double-blind placebo-controlled protocol with a standard *Alternaria* extract was used, also demonstrated significant reduction of symptoms in a group of subjects with rhinitis, asthma, or both after immunotherapy with a "rush" protocol. Another report

by Metzgar et al.³⁰ also demonstrated diminution of late asthmatic responsiveness during immunotherapy with *Alternaria* in subjects with asthma.

Potential dangers from allergenic extract immunotherapy with fungi must be considered. It has been theorized that fungi may be good inducers of IgG-precipitating antibody and that immunotherapy may increase the potential for developing high levels of precipitating antibody and perhaps subsequent immune complex-mediated lung injury. Indeed, the sudden development of severe anaphylactic reactions to crude fungal allergens after injections of doses that were previously tolerated in a large number of children undergoing allergenic extract immunotherapy has been reported,³¹ but there have been no confirmatory reports of this occurrence. In this prospective study involving 106 children, six patients had exacerbated adverse reactions. Reactions were very severe in three of the six children, and fungal extracts (including several *Alternaria* and *Cladosporium* species) were responsible for the more serious side effects.

In spite of studies indicating efficacy of allergenic extract immunotherapy with certain fungal species, it is clear that the question of immunotherapy for inhalant fungal allergy requires further study. Among the major problems that remain in considering studies of immunotherapy with mold extracts are extract variability and the fact that an extract of a particular species may not contain all of the important individual allergens. The complexity of fungal extracts used for therapeutic purposes, as compared with pollen extracts, also makes it likely that the number of different antigenic determinants to be handled by the patient's immune system will be quite high. This could lead to a lesser immune response than if a single purified or highly characterized allergenic determinant had been used. This consideration is highly speculative but is in accordance with the general concept of antigenic competition.³² Support for this concept has also been noted in immunotherapy studies with pollen extracts in which better effects were refined preparations as opposed to crude extracts.^{33, 34} Fungi also contain considerable amounts of high molecular weight (above 100,000 d) nonallergenic materials, which could further compound this problem.

CONCLUSIONS AND RECOMMENDATIONS

In spite of these limitations, clinical and anecdotal evidence suggest that many patients with broad patterns of wheal and flare skin reactivity to

crude fungal extracts and bronchial asthma or rhinitis experience improvement after empirical immunotherapy with fungal allergens. Several controlled studies²⁵⁻²⁸ tend to bear this out.

Considering all of the above observations collectively, it would seem prudent that immunotherapy in mold-sensitive patients might be reserved for those who demonstrate documented symptoms (rhinitis or asthma) after natural exposure during periods when high concentrations of fungal allergens are present in the atmosphere. These patients should demonstrate IgE antibody to well-characterized and standardized fungal allergens by in vitro or in vivo techniques (i.e., IgE skin test, RAST, ELISA, etc.), and there should be an inability to control symptoms by conventional pharmacotherapy or avoidance.

The use of fungus extract mixtures, which include species to which patients may not be reactive, should be avoided. In addition, the practitioner should keep in mind that diagnosis of IgE-mediated sensitivity and immunotherapy to most fungi requires further study and poses many unsolved problems. Among these are the facts that accurate information on the degree of exposure is often not available and that clinical effects of injecting complex mixtures of fungal metabolites are largely unknown. For these reasons, alternatives to immunotherapy should always be considered, including avoiding or minimizing exposure. Home and workplace areas should be kept as free as possible of fungal growth by maintaining a clean and dry environment. One of the most useful preventive measures that patients can take to reduce allergic symptoms to fungi is dehumidification. It is important to keep dehumidifiers free of contamination. Air-conditioning a home or office may reduce exposure to outdoor fungi and reduce indoor relative humidity. Air purifiers have not been demonstrated to be of great use in the management of mold allergy. If patients persist with questions about purchasing air cleaning devices, it is probably prudent to recommend cleaners with high efficiency, such as high efficiency particulate air filters, and to advise them to consider renting a device for several months to determine effectiveness before actual purchase.

Mold-sensitive patients who are farmers or who mow lawns, rake leaves, or do similar yard work are known to have exacerbation of symptoms, probably primarily related to fungus spore exposure, although disturbed pollen particles, algae, insect fragments, or related allergens may also be involved. When major exposure to fungal aller-

gens and similar dust allergens is anticipated, it is wise to advise patients to use simple filter masks, which often prove effective in reducing exposure to molds, while they are performing such activities.

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