A review of *Alternaria alternata* sensitivity

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Summary

Sensitivity to fungi is a major risk factor for the development of asthma. However, the prevalence of fungal sensitivity in asthma is not completely understood. Nonetheless upward of 80% of asthmatic patients may be sensitized to one or more fungi. Fungal exposure occurs primarily from outdoors sources, but can occur in the indoor environment as well. Assessment of fungal exposure requires a multifaceted approach including measurement of airborne spores and culture techniques to identify the relevant organisms. Preventing intrusion of outdoor fungal spores into the indoor environment may be helpful in reducing allergic symptoms. Methods to abate indoor fungal growth include reduction of indoor humidity and removal of water sources. Patients with fungal sensitivity should be advised to avoid exposure as much as possible. For patients who have failed to respond to environmental control measures and appropriate medications, it may be reasonable to consider specific immunotherapy.
The application of molecular biology techniques to the study of allergens has enhanced the researcher's ability to produce *Alternaria* allergens in quantity, to determine their biological relevance, as well as to evaluate mechanisms of *Alternaria* sensitivity. We look forward to new developments and improved treatments through modulation of the immune response with molecularly produced and well characterized fungal allergy.

Key words *Alternaria,* Asthma, Allergens, Sensitivity

Revisión sobre la sensibilidad a *Alternaria alternata*

Resumen

La sensibilidad a los hongos es un factor de riesgo importante para el desarrollo del asma. Sin embargo, la prevalencia de la sensibilidad a los hongos en el asma no se conoce adecuadamente. En cualquier caso, más del 80% de los pacientes asmáticos pueden estar sensibilizados frente a uno o varios hongos. La exposición primaria a los hongos ocurre a partir de fuentes externas, pero puede ocurrir en el entorno interior también. La comprobación de la exposición a los hongos requiere una aproximación múltiple que incluye la medición de esporas en el aire y técnicas de cultivo para identificar los organismos relevantes. Evitar la entrada de esporas fúngicas del exterior al ambiente interior puede ayudar en la reducción de los síntomas alérgicos. Los métodos para evitar el crecimiento fúngico en interiores incluyen la reducción de la humedad y la eliminación de fuentes de agua. Se debería aconsejar a los pacientes con sensibilidad a los hongos que eviten la exposición a éstos en la medida de lo posible. La inmunoterapia específica puede ser valorada en aquellos pacientes que no respondan a las medidas de control ambiental.
La aplicación de técnicas de biología molecular al estudio de los alérgenos ha mejorado la capacidad para producir cantidades importantes de alérgenos de *Alternaria*, determinar su relevancia biológica, así como evaluar los mecanismos de sensibilidad a *Alternaria*. Esperamos que se produzcan nuevos desarrollos y tratamientos mejorados a través de la modulación de la respuesta inmune con alérgenos bien caracterizados producidos molecularmente.

Palabras clave *Alternaria,* Asma, Alérgenos, Sensibilidad

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Sensitivity to the fungus *Alternaria alternata* is a common cause of asthma. Epidemiological studies from a variety of locations worldwide indicate that *Alternaria* sensitivity is closely linked with the development of asthma [2,4]. In addition, up to 70 % of mold-allergic patients have skin test reactivity to *Alternaria* [1], and *Alternaria* sensitivity has been shown to be a risk factor for asthma [2-4]. Importantly *Alternaria* sensitivity can also lead to severe [21] and potentially fatal asthma [7]. *Alternaria* spores are found in atmospheric survey thoughtout the United States and in many areas is the predominant spore type [5,8]. Fungal exposure differs from pollen exposure in quantity (airborne spore count are often 1,000-fold greater than pollen counts) and duration (*Alternaria* exposure occurs for months, whereas ragweed exposure occurs for weeks). This prolonged intense exposure mimics that of other allergens such as cat dander and dust mite which likely contributes to both the chronicity and severity of asthma in *Alternaria* sensitive subjects [9].

HISTORY AND EPIDEMIOLOGY

Allergic reactions attributable to mold sensitivity have long been recognized. In 1726, Sir John Floyer [7] noted asthma in patients that had just visited a wine cellar. In 1873, Blackley [8] suggested that *Chaetomium* and *Penicillium* were associated with asthma attacks. In 1924 van Leeuwen [9] noted the relationship of climate to asthma and found a correlation between the appearance of mold spores in the atmosphere and asthma attacks. At the same time Cadman [10] reported the first documented case of asthma due to wheat rust. Over the following ten years, case reports appeared attributing the source of mold allergy to the home or to occupational settings. In the late 1930's Prince *et al.* [11] and Feinberg [12] reported that outdoor air was a significant source of mold spores and demonstrated that many of their patients had positive skin test reactivity to mold extracts.

The role of mold sensitivity in a variety of allergic diseases was established in provocation studies. In 1941 Harris [13] exposed patients to 1 g of *Alternaria* powder dispersed in a 700 cubic feet room, which provoked asthma and rhinitis symptoms in 10-12 patients with positive skin tests to *Alternaria* who had a history compatible with sensitivity to the fungus. It has also been demonstrated that inhalation of either *Alternaria* or *Penicillium* spores in quantities comparable with those encountered by natural exposure can induce both immediate and late phase asthma in sensitive individuals [14].

Fungal allergen exposure is generally considered to arise from the outdoor environment, but indoor exposure to fungal allergens also occurs. Much of the indoor environmental exposure is a reflection of fungal spores emanating from the outdoor environment that invade interiors through open windows, doors and cracks. Certain species such as *Penicillium* and *Aspergillus* may be recovered in indoor environments at rates higher than outdoors [15].

Surveys conducted in various parts of the world indicate that fungal sensitivity is common, particularly among asthmatic individuals. In the general population of the United States, a large-scale epidemiological study indicated that 3.6% of the population were sensitized to *A. alternata* [16]. In one Scandinavian study, 4% showed positive skin test to *Alternaria*, while in reports from the United States up to 80 % of asthmatics patients demonstrated positive reactivity to one or more fungi [17]. D'Amato *et al.* [18] studied the skin test reactivity to *Alternaria* and *Cladosporium* inpatients throughout Europe presenting with suspected respiratory allergy. The frequency of positive skin test varied from country to country. Approximately 3% of the patients in Portugal had a positive skin test to either *Alternaria* or *Cladosporium*, while in Spain 20 % demonstrated positive skin tests to these fungi [18].

Lehrer *et al.* [19] studied basidiomycetes skin test reactivity in symptomatic patients residing in the United States and various European countries. Of these subjects, 25-33 % reacted to one or more basidiomycete species which suggested that there was a strong association between sensitivity to this fungus and the presence of allergic respiratory diseases [19]. In a large-scale epidemiological study of children with asthma residing in inner cities of the United States, the most common sensitizer was *Alternaria;* 38.3 % of 1286 asthmatic children had positive skin test to this allergen [20]. *Alternaria* sensitivity and exposure to airborne fungal spores have been associated with severe episodes of potentially fatal asthma [21]. Furthermore, the risk of death from asthma has also been correlated with the presence of fungal spores in the atmosphere [22].

Although fungal sensitivity is well recognized, it is not always possible to establish a correlation between mold spore counts in the atmosphere and the presence of allergic symptoms. This could be due to the use of inadequate air sampling techniques and equipment. Aerosampling methods, which quantitate the amount of allergen in the atmosphere (as opposed to morphologic identification and quantitation of fungal spores by microscopy) may shed further light on this problem. Agarwal *et al.* [23] was able to detect *Alt-a1* (one of the major allergens of *Alternaria*) in the atmosphere. This correlated well with mold spore counts and with the total *Alternaria* allergen activity in the atmosphere. Peat *et al.* [24] extensively revised the literature regarding the relationship between dampness and mold in the home and respiratory health. Although the studies did not employ standardized methodology for measuring exposure or health outcomes, a number of studies in children evaluated cough and wheeze in a more consistent fashion. Analysis of the data obtained from the studies indicates that the risk for children having respiratory symptoms in a home that is damp or has fungal growth has an odd ratio in the range of 1.5-3.5. This is similar to those observed in environments where children are exposed to environmental tobacco smoke or outdoor air pollutants [24].

Although correlations between indoors fungal exposure and asthma are somewhat tenuous, damp conditions that would be conducive to fungal growth have also been shown to have significant health effects, at least in children [24]. Allergic reactions to fungi as a cause of these symptoms have not been established by appropriate skin testing or in vitro IgE tests, however. Some symptoms that patients experience in indoor environments may be related to exposure to mycotoxins such as trichothecne [26] or to other agents such as 1-3 ß-D-glucan [25].

Verhoeff and Burge [26] also reviewed the association between fungi in homes and health risks. These authors came to the same conclusions as Peat and coworkers concerning a positive association between damp homes and respiratory morbidity of the occupants [26]. Dampness and fungal problems have been reported to occur in 20-50 % of modern homes [26]. Poorly maintained heating, ventilation and air-conditioning systems are often sources of exposure to fungi. In seven of nine cross-sectional studies evaluated by Verhoef and Burge [26], one or more positive associations were found between fungal levels and adverse health outcome.

Fungal sensitivity and exposure to airborne fungal spores have been associated with severe episodes of asthma. O'Hallaren *et al.* [21] reported on 11 patients aged 1-25 years who presented respiratory arrest due to asthma.

There were two fatalities in the group. Ten of the 11 patients were sensitized to *Alternaria*. These patients developed their difficulty during the peak of the *Alternaria* season, and there was a correlation between the *Alternaria* spore counts and the time of their presentation to the emergency room. The adjusted odds ratio (189; CI = 6.5 -5535.8) for the severe and potentially fatal attack of asthma was highly correlated with *Alternaria* sensitivity [21]. Thus, sensitivity to *Alternaria* is recognized as a major risk factor for severe and potentially fatal asthma.

IDENTIFICATION AND PURIFICATION OF *ALTERNARIA* ALLERGENS

The identification and purification of *Alternaria* allergens is important in the standardization of allergenic extracts used in the diagnosis of allergic disease as well as developing ways to improve treatment of these common diseases. The purification of allergens that consist largely of proteins or glycoproteins by physiochemical methods has proven to be difficult due to the complex composition of fungal extracts and batch to batch variability. Furthermore, amino acid sequence data are limited. The determination of both primary and tertiary structures of proteins is important not only for the understanding of their biologic activities but also for determining the immune response to these allergens. Yunginger *et al.* [27], using conventional biochemical methods, such as ion-exchange chromatography and gel filtration, isolated a major allergenic fraction Alt a1. Kroutil and Bush detected several *Alternaria* allergens with Western blotting techniques [28]. Several other investigators have reported isolation and partial purification of *Alternaria* allergens [29,32,37].

The advent of molecular biology, however, and its application to the study of allergens during the past decade has led to an ever expanding base of knowledge. Through the use of these techniques, the cDNA sequences and deducted amino acid sequences for a number of *Alternaria* allergens have been obtained [30,35,37]. To date three major *Alternaria* allergens have been identified:

(a) *Alt a1*. Alt a1 is a major allergen that is recognized by IgE antibodies in 80-90 % of *Alternaria* allergic individuals [27]. Subunits of the protein that bind IgE have been cloned [29,30] but a full-length DNA sequence has not been reported. Recombinant Alt a1 is available commercially (Biomay, Vienna, Austria). Skin testing of *Alternaria*-sensitive individuals (defined by allergic symptoms and the presence in serum of IgE antibodies to crude *Alternaria* extracts) demonstrated that 85.7 % had positive skin tests to recombinant Alt a1, while none of ten controls reacted [32]. The Alt a1 protein is a two-chain dimer linked by disulfide bonds with a molecular weight of approximately 30 kDa [33]. The biological function of this protein has not been elucidated.

(b) *Alt a 2.* Alta 2 [34] is 25 kDa protein that binds IgE from 60 % of *Alternaria*-allergic subjects. A full DNA sequence has been identified, and the complete protein's amino acid sequence has been deduced. Using PCR techniques *rAlt a2* sequence has been found in six other *Alternaria* strains [34,35]. These findings suggest that Alt a2 is a conserved protein in *A alternata*.

(c) *Enolase*. Enolase has been identified as an *Alternaria* allergen that is recognized by approximately 50% of sera tested [31,36]. However skin testing with recombinant enolase in seven *Alternaria* allergics only produced positive test in two individuals [32]. Enolases have also been cloned from both *Cladosporium herbarium*, and *Candida albicans* indicating that it might be a highly conserved fungal protein [36].

Three additional minor *Alternaria* allergens have also been cloned:

(a) *Alt a6.* Alta 6 is a 53 kDa, P2 ribosomal protein [33,37] that binds IgE from the sera of 8% of *Alternaria*-sensitive individuals [37].

(b) *Alt a7.* Alt a7 is 22 kDa homologous to YCP4 yeast protein and binds IgE in 7% of the *Alternaria* sensitive people [37]. However, skin testing with recombinant Alt a7 did not elicit a positive result in seven *Alternaria*-sensitive subjects [32].

(c) *Alt a10.* Alt a10 is an 11 kDa aldehyde dehydrogenase that binds IgE in 2 % of sensitive patients.

A given allergen is considered major when there is IgE binding by radioimmunoelectrophoresis in 50 % or more individuals sensitive to that allergen and minor if the IgE binding is present in less than 50% of allergic individual [38]. Thus, although a number of *Alternaria* allergens have been identified, Alt a 1 and r Alt a 2 appear to be the most clinically significant as major allergens.

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