

Allergic Fungal Airway Disease

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■ Abstract

Fungi are ubiquitous and form their own kingdom. Up to 80 genera of fungi have been linked to type I allergic disease, and yet, commercial reagents to test for sensitization are available for relatively few species. In terms of asthma, it is important to distinguish between species unable to grow at body temperature and those that can (thermotolerant) and thereby have the potential to colonize the respiratory tract. The former, which include the commonly studied *Alternaria* and *Cladosporium* genera, can act as aeroallergens whose clinical effects are predictably related to exposure levels. In contrast, thermotolerant species, which include fungi from the *Candida*, *Aspergillus*, and *Penicillium* genera, can cause a persistent allergenic stimulus independent of their airborne concentrations. Moreover, their ability to germinate in the airways provides a more diverse allergenic stimulus, and may result in noninvasive infection, which enhances inflammation. The close association between IgE sensitization to thermotolerant filamentous fungi and fixed airflow obstruction, bronchiectasis, and lung fibrosis suggests a much more tissue-damaging process than that seen with aeroallergens.

This review provides an overview of fungal allergens and the patterns of clinical disease associated with exposure. It clarifies the various terminologies associated with fungal allergy in asthma and makes the case for a new term (*allergic fungal airway disease*) to include all people with asthma at risk of developing lung damage as a result of their fungal allergy. Lastly, it discusses the management of fungi-related asthma.

Key words: Thermotolerant fungi. Allergic fungal airway disease (AFAD). ABPA. SAFS. Asthma. Allergic fungal rhinosinusitis (AFRS). Immune responses. Diagnosis. Treatment.

■ Resumen

Los hongos son obicuos y forman su propio reino. Hasta 80 géneros de hongos han sido asociados con la enfermedad alérgica tipo I; sin embargo, los reactivos comerciales para testar las sensibilizaciones a los mismos, se encuentran disponibles para un número relativamente pequeño de especies. En cuanto al asma, es importante distinguir entre especies incapaces de crecer en temperatura ambiente y aquellas que son termotolerantes y que, por lo tanto, tienen una capacidad potencial para colonizar el tracto respiratorio. Los primeros, que incluyen los géneros comúnmente estudiados *Alternaria* y *Cladosporium*, pueden actuar como aeroalérgenos y sus efectos clínicos están relacionados con los niveles de exposición. En contraste, las especies termotolerantes, que incluyen hongos del género *Candida*, *Aspergillus* y *Penicillium*, pueden causar un estímulo alérgico persistente independiente de su concentración en el aire. Además, su capacidad para germinar en las vías aéreas da lugar a estímulos alérgicos más diversos y puede dar como resultado infecciones no invasivas que facilitan la inflamación. La estrecha asociación entre sensibilización dependiente de IgE a hongos filamentosos termotolerantes y la obstrucción del flujo aéreo, bronquiectasias y fibrosis pulmonar sugiere que esto lleva a un proceso de daño tisular mucho mayor que el producido por aeroalérgenos. Esta revisión ofrece una visión general de los alérgenos de los hongos y los patrones de las enfermedades clínicas asociadas a su exposición. Trata de aclarar la terminología variada asociada con la alergia a hongos en asma, ofreciendo un nuevo término (enfermedad alérgica de las vías aéreas por hongos) para englobar a todos los pacientes asmáticos con riesgo de desarrollar daños pulmonares como resultados de su alergia a hongos. Por último, se discute el manejo del asma relacionada con hongos.

Palabras clave: Hongos termotolerantes. Enfermedad alérgica de las vías aéreas por hongos. Aspergillosis broncopulmonar alérgica. Asma severa con sensibilización a hongos. Asma. Rhinosinusitis por alergia a hongos. Respuesta immune. Diagnóstico. Tratamiento.

Introduction

Fungi are eukaryotes that form their own kingdom. Around 100 000 fungal species have been named [1], although there are estimated to be from 1.5 to 3 million species worldwide [2,3]. According to the Assembling the Fungal Tree of Life (AFTOL) project, the fungal kingdom can be divided into as many as 8-10 phyla. Among these, the Ascomycota and Basidiomycota groups together form the Dikarya, which represent approximately 98% of described fungal species [4]. These ubiquitous organisms have adapted to a variety of ecological habitats. They are involved in the degradation of decomposing organic matter in nature [5], but are also used in industry, for example in the production of food, antibiotics, and enzymes [6].

Most fungi, such as the plant pathogens *Cladosporium* and *Alternaria* species, are mesophilic, growing at an optimum temperature of 18°C to 22°C. They rarely cause infection but can be encountered as allergens. Thermotolerant fungi are able to grow in the environment and at body temperature and are therefore capable of acting as allergens, commensals, and opportunistic pathogens [7]. Fungal pathogens have evolved independently and repeatedly throughout the kingdom [8]. They can damage their host through infection, ranging from superficial cutaneous infections to life-threatening invasive mycoses, by producing toxins, or by inducing allergic reactions [9]. The few species that have been detected in the upper and lower respiratory airways [10,11] include yeasts, particularly *Candida* species, *Penicillium*, and *Aspergillus* species, with the latter constituting the most prominent cause of fungal lung infections.

The incidence of allergic disease, which affects around 1 in 3 people in the UK [12], has increased in developed societies over the last 50 years and now represents a considerable challenge to the healthcare industry [13]. Everybody inhales a complex mixture of hyphal fragments, fungal spores, and yeasts daily [14]. The species' composition varies depending on the day and season, with the highest concentrations in late summer and early autumn, when >50 000 fungal spores per cubic meter of air per day can be present [14]. Spores frequently exceed the concentration of pollen grains by 100 to 1000-fold [9]. Frequency also varies between the outdoor and indoor environment, with the indoor fungal spore concentration comprising around 16% of the outdoor concentration in noncontaminated housing [15]. The majority of airborne spores are produced by members of the Ascomycota and Basidiomycota, with asexually produced conidia comprising 30% to 60% of total airborne spores [9]. Larger spores (>10 µm) are usually deposited in the nasopharynx and associated with hay fever symptoms, although most are smaller, ranging from 2 µm to 10 µm, including those from *Aspergillus* and *Penicillium* species [9,16]. Small spores and fragments of larger spores can reach the lower airways [9,17]. The quantity of fragments of some fungi can exceed their respective number of spores [18,19].

Both the upper and lower airways try to remove fungi by mechanical means such as sinus turbulence or the mucociliary escalator and by immunological means such as engulfment and digestion by alveolar phagocytes. The immune response

thereby represents a balance between pro-inflammatory reduction of fungal burden and anti-inflammatory reduction of host tissue damage [20]. Most fungal allergens are released after spores germinate [21], as the spores are covered by a protective hydrophobin layer that enables evasion of the immune system [22]. Symptoms triggered by fungal allergens range from rhinitis to allergic bronchopulmonary aspergillosis (ABPA), a complication that usually accompanies other lung diseases such as asthma. Thermotolerant fungi play a special role, as they are able to colonize the lower airways, thereby representing a persistent allergen source.

Although the importance of sensitization to fungi in human health has been evaluated, its contribution to allergic airway diseases remains largely understudied [23,24].

Allergic Immune Responses to Fungi

The defence against pathogens in humans is based on a combination of innate and adaptive immune responses. Traditionally, the latter discriminates between type 1 helper T cell (T_H), T_H2 , T_H17 , and regulatory T-cell responses, which are dependent on the pathogens and cytokines involved. T_H2 responses are usually directed against parasites, whilst the antifungal response is predominantly mediated by T_H1 and T_H17 cells [25]. Allergic reactions represent a deranged T_H2 immune response against normally harmless molecules [26] that is commonly associated with the production of allergen-specific IgE antibodies. This humoral type I immune response represents 1 of 4 classified hypersensitivity responses [27]. IgE antibodies are produced after the first contact with the allergen, which leads to an asymptomatic stage called atopy. After re-exposure to the allergenic source, allergen-specific IgE antibodies bound to high-affinity receptor FcεRI of innate effector cells such as basophils and mast cells are crosslinked, leading to the immediate release of anaphylactogenic mediators [26] and peripheral blood eosinophilia [28]. These cells reside mostly near skin and mucosal surfaces such as the respiratory and gastrointestinal tracts, where the subsequent allergic reactions occur. The reactions can manifest as IgE-associated atopic dermatitis [29], allergic rhinitis or rhinosinusitis, allergic asthma, and food allergy [23]. A sensitized individual does not have to experience symptoms, which may depend on the level of exposure and other contributing factors during exposure, such as the individuals' health condition and their immune system [30,31].

Type I hypersensitivity reactions have been observed to about 80 fungi, predominantly to species from the Ascomycota [24]. The prevalence of sensitization to fungi is unclear, although estimates suggest that 3% to 10% of the general population, 12% to 42% of atopic patients, and up to 66% of patients with severe asthma may be sensitized to fungi [9,10,32]; yet, not all sensitized individuals will develop allergic symptoms [5]. Prevalence varies with age [33,34] and can differ between countries [35]. Surveys based on skin prick testing (SPT), which is relatively insensitive for some fungal allergens, may underestimate the prevalence of IgE sensitization.

Type IV hypersensitivity reactions (delayed-type) may also play a role in allergic fungal airway disease. Type IV responses are T cell-mediated and induce apoptosis of target

cells [27]. Both type I and IV hypersensitivity responses are observed in ABPA [36,37].

Fungal allergens can be secreted, cytoplasmic, or structural proteins [24,38]. Based on known allergens, predicted allergens, and the results of IgE binding to phage libraries, it has been suggested that 0.5% to 1% of fungal proteins may be allergens [39]. Although more than 80 fungal genera have been associated with allergy [9], the World Health Organization (WHO) and the International Union of Immunological Societies (IUIS; www.allergen.org, October 2016) list only 111 allergens from 29 fungal species. The majority of allergenic proteins are proteases, glycosidases, and stress response proteins or result from protein synthesis/secretion and gluconeogenesis [40]. The allergen profile can differ between fungal spores and germinated hyphae, and germination increases the amount of detectable allergens [21].

A person can be cosensitized to multiple allergens [41]. The fungi most associated with fungal polysensitization are *Aspergillus fumigatus*, *Cladosporium herbarum*, *Penicillium chrysogenum*, and *Saccharomyces cerevisiae*, whilst monosensitization occurs most commonly in individuals sensitized to *Alternaria alternata* [42]. The extent to which polysensitization is due to cross-sensitization between fungal allergens as opposed to primary sensitization is unclear [43]. An argument for the latter would be that fungal sensitization is often associated with sensitization to other airborne allergens [42]. While a few allergens are unique, such as the major allergens from *A. fumigatus* (Asp f 1) and *A. alternata* (Alt a 1) [38], the same or very similar epitopes can be shared between different organisms, which can include self-antigens [38,44]. These epitopes often derive from proteins with similar functions produced by various species and are known as orthologs [39]. This has been observed between closely related species such as *P. chrysogenum* and *Penicillium citrinum* and distantly related species such as *Candida boidinii* and *A. fumigatus* [45-47]. Cross-reactivity between fungal allergens may result in false-positive results in sensitization tests and contribute to exacerbation of allergic symptoms in conditions such as ABPA. Nevertheless, the clinical significance of cross-reactivity between fungal allergens requires further investigation [38].

Another factor to consider with fungal polysensitization is genetic predisposition, as suggested by an Italian study, where 82.9% of children sensitized to fungus had a family history of fungal sensitization [48]. However, few studies have explored the genetic basis of fungal lung disease other than some that link human leukocyte antigen genotypes with ABPA [49,50].

Diagnosis of Fungal Allergy

It is unclear how much exposure to fungi over which time frame is necessary to trigger sensitization. Similarly, the role of genetic factors and immunoregulatory elements has not been clearly defined, since not everyone becomes sensitized. In general, until a person is known to be allergic to a specific component, he/she has to go through a process of anamnesis and allergen-reactivity tests. The SPT is most commonly used for the diagnosis of sensitization. While not as sensitive as intradermal tests [51], it has a lower rate of false positives [31].

In addition, blood tests for specific IgE are available, with the immunoassay capture (ImmunoCAP) system being a commonly used platform [23].

In 2004, extracts for 75 fungal species were available from 7 different US manufacturers [52]; however, none of these have been approved by the United States Food and Drug Administration [23]. Fungi are not usually included in a standard SPT or specific IgE test panel because of a low index of suspicion that they are clinically relevant. Individuals with fungal allergies are often not aware of fungi as a potential allergen source, since they are frequently cosensitized to other aeroallergens such as grass pollens, which peak at similar times. If included, the most commonly tested fungi are *A. alternata* and *C. herbarum*, which are recommended in European Community Respiratory Health Survey [35]. However, for a more complete understanding of fungal sensitization, the panel should also include *A. fumigatus*, *P. chrysogenum*, *Candida albicans*, *Malassezia* species, *Trichophyton* species, and *Saccharomyces cerevisiae* [7,10]. Most sensitization assays are aimed at fungi within the Ascomycota, in particular conidia-producing anamorphs (asexual forms) and yeasts [9]. The prevalence of sensitization to basidiospores (sexual spores from the Basidiomycota) is thought to be similar, although it is not often tested for, and allergic reactions to ascospores (sexual spores from the Ascomycota) are understudied [9]. Non-Dikarya fungi such as *Rhizopus* and *Mucor* species have also been implicated in asthma [10]. However, the number of species described is far higher, and many potential allergens are not well characterized.

Discrepancies between SPT and specific IgE make it difficult to ascertain an individual's fungal sensitization profile. Studies comparing SPT and specific IgE are often discordant in both asthma [32] and rhinitis [53]. The SPT has a high negative predictive value, whilst the ImmunoCAP is more sensitive, so some authors suggest using both [32]. It is important to note that sensitization patterns change with age [54-56], suggesting that measurement of sensitization has to be repeated over time. A possible reason for the problem of discordance is the fact that fungal extracts are not standardized and differ between SPT and IgE testing. Extracts vary between companies and even between batches from the same company. The choice of the fungal strain, culture conditions, protein source (spores, hyphae or secreted proteins), and extraction protocols used can all influence the allergen content and antigenicity of fungal extracts [9,52].

Conditions Related to Fungal Allergy

Allergic Fungal Rhinitis and Allergic Fungal Rhinosinusitis

The nose is a key portal of entry for fungi into the respiratory system, with the result that fungi are often detected in nasal mucus culture [57]. Up to 40% of the population has allergic rhinitis with symptoms such as sneezing or clear rhinorrhea, which results in sleeping problems and decreased quality of life.

Allergic fungal rhinosinusitis (AFRS) accounts for 5%-10% of all cases of chronic rhinosinusitis in immunocompetent

patients and is caused by fungal colonization of a sinus with impaired mucociliary clearance [40]. AFRS represents 6%-9% of all cases of chronic rhinosinusitis requiring surgery [58]. Only around 50% of AFRS patients are asthmatic [59]. AFRS is mostly associated with *Aspergillus* species, followed by *Bipolaris*, *Curvularia*, and *Alternaria*. Other fungi, such as *Rhizomucor* and *Fusarium*, have rarely been implicated [60,61]. AFRS was first observed in a patient diagnosed with ABPA in 1976 who had characteristic fungal mucus plugs (composed of a thick eosinophilic secretion) in the paranasal sinuses [62]. This is why AFRS is sometimes described as the upper airway version of ABPA, even though the conditions rarely co-occur [63,64]. AFRS causes nasal airway obstruction, unilateral chronic sinus infection, thick dark mucous rhinorrhea, impaired postnasal drainage, and facial pain and pressure, as well as orbital and facial distortion in advanced disease [65,66]. The major diagnostic criteria for AFRS are sensitization to several fungi and other allergens, nasal polyposis, abnormalities revealed by computed tomography, eosinophilic mucus, presence of fungi without tissue invasion, and positive fungal stain of sinus contents [40,65,67]. Other characteristics include paranasal sinus mucocoeles, high-attenuation sinus contents, bone remodelling (sinuses, orbit, and skull base) [68], higher total IgE [59], and T cell-mediated eosinophilic inflammation [40]. The underlying immune response of AFRS involves type I and IgG-mediated type III and type IV hypersensitivity responses, leading to impaired drainage of mucus through the sinonasal passages and subsequent fungal growth and inflammation.

Asthma

Asthma is a chronic disease that affects >300 million people worldwide [69]. It represents a large burden for health systems, with £500 billion per year being spent in the UK alone [70]. Patients experience variable airflow obstruction and a range of symptoms including breathlessness, airway inflammation, and reduced expiratory volume in 1 second (FEV₁) [69]. The symptoms are the result of pathophysiological abnormalities, including changes in the resistance to airflow and airway hypersensitivity responses of the airway smooth muscle cells, leading to contraction [71], increased cough reflex, mucus hypersecretion, and lung damage, which is expressed as fixed airflow obstruction, bronchiectasis, lung fibrosis [7,72], subepithelial membrane thickening, and smooth muscle hypertrophy and hyperplasia [73-75]. These abnormalities are independent of each other, thus resulting in a highly heterogeneous disease. Consequently, various endotypes, including allergic asthma, have been defined [76,77].

Several studies in both pediatric and adult populations have associated outdoor and indoor fungal exposure with asthma requiring hospital admission [78-81], decreased lung function, increased use of asthma medication, and greater risk of cough [82-89].

IgE-mediated fungal sensitization is frequently present in early-onset atopic eosinophilic asthma [7]. Sensitization to fungi, particularly *Alternaria* species, has been associated with life-threatening acute asthma attacks [90] and asthma-related deaths [91], and fungi have been considered to cause asthma [92-94].

Severe asthma is present in 5%-10% of asthmatics, which means that patients require high doses of medication to treat their symptoms or experience symptoms despite optimal treatment [95]. Fungal exposure has been associated with asthma severity [96]. Almost 40% of children with asthma are sensitized to fungi, and prevalence is as high as 60% in children with severe asthma [97], which may persist into adulthood. The prevalence of fungal sensitization in adults with severe asthma can reach 70% [98], although it can be as high as 76% in those requiring multiple hospital admissions [96]. Patients with severe asthma are also more commonly cosensitized to multiple fungi [32]. People with moderate to severe asthma who are sensitized to *Aspergillus* have impaired lung function, as shown by reduced FEV₁, more severe airway obstruction, and the need for higher corticosteroid doses [99,100]. A 22% reduction in lung function was associated with fungal sensitization and fungus-positive sputum culture in patients with moderate to severe asthma [101]. The majority of recovered fungi were isolates of *A.fumigatus*, although more than 20 other taxa were also detected [101]. Tissue damage and inflammation were also more frequent in *Aspergillus*-sensitized asthmatics, as more cases of bronchiectasis and higher eosinophil counts and IgE levels have been detected [99,100]. Culture of *Aspergillus* was significantly more frequent in patients with moderate-to-severe asthma who were IgE-sensitized to *A.fumigatus* (63%) than nonsensitized asthmatics (31%) and healthy controls (7%) [100]. These studies indicate that fungal colonization plays a role as a continuous allergic stimulus.

A.fumigatus is by far the most common thermotolerant fungus associated with all forms of fungal lung disease, although other *Aspergillus* species such as *A.niger*, *A.flavus*, and *A.nidulans* can also play a role [102]. Besides *Aspergillus* species, *C.albicans*, *Bipolaris* species, *Schizophyllum commune*, *Curvularia* species, and *Pseudallescheria boydii* have been involved in asthma, although most results are from case reports where disease was confirmed by culture. A convincing case for a causal association is not always made. Clinically relevant fungal allergy is usually present alongside other respiratory diseases such as cystic fibrosis (CF) and asthma, but can occur in their absence [31].

Evidence of fungal allergy complicating airway disease is found in about 7-9% of CF patients [10] and 0.7%-3.5% of patients with asthma [103]; however, these findings depend on the criteria used (see below). Fungal allergy plays a significant role in more than a quarter of patients with severe asthma. Fungal allergy was defined as an endotype of asthma by an EAACI Task Force [77].

The Semantics of Fungal Allergy-Associated Airway Disease

The term *allergic bronchopulmonary aspergillosis* (ABPA), or *allergic bronchopulmonary mycosis* when another fungal genus is involved, has dominated both the literature and conceptual approaches to fungal allergy in relation to asthma and CF. The term was first coined in the 1950s in a small case series describing a pattern of severe lung damage associated with fungal sensitization [104]. Further case series, mainly from the UK and USA, were published in subsequent decades,

culminating in the 1970s in a description of the immunological and radiological features that characterized these patients, such as eosinophilia, fleeting lung shadows, bronchiectasis, high total IgE, and raised *A fumigatus*-specific IgE and IgG. These features became established in the literature as a firm set of diagnostic criteria. However, these criteria were based on a relatively small number of patients and lacked the statistical underpinning that would be expected in modern practice. The problem with the criteria is that they are largely based on a florid immune response to fungal sensitization characterized by high levels of total IgE and specific IgG. An IgE of >417 IU/L (or 1000 IU/L in some reports) is given great prominence [7,105]. However, total IgE is not a very specific or sensitive marker of fungal associated lung disease. While levels of IgE of >1000 IU/L almost invariably denote the presence of IgE sensitization to thermotolerant fungi, they can also be due to yeasts such as *Candida* or skin commensals such as *Malassezia* or *Trichophyton*. Such sensitization is common, particularly if there is a history of eczema, which often accompanies asthma. Even where the raised IgE is due to *Aspergillus* or *Penicillium* sensitization, patients may have normal lung function with little evidence of the lung damage that is the hallmark of allergy to thermotolerant fungi. In addition, many patients with an IgE of <417 IU/L who are sensitized to fungi do have evidence of lung damage. The restrictive nature of the criteria for ABPA has meant that they do not make much sense to the practicing clinician, and, as a result, the term ABPA has tended to be used rather loosely to describe asthma where the clinician believes fungal allergy may be playing a part. As a result, the term has lost clarity if not credibility. A number of attempts have been made to revise the criteria over subsequent decades, but the problem is an element of circularity where the criteria for ABPA have been used as the gold standard starting point for revision [105]. An alternative approach was taken by the group in Manchester, who recognized the problem of placing considerable emphasis on total IgE and using the term *severe asthma with fungal sensitization* to refer to anyone with severe asthma and IgE sensitization to any fungi with an IgE concentration of less than 1000 IU/L [32]. However, again, this definition was based on the arbitrary cutoff of 1000 IU/L, which is problematic in a condition where total IgE varies considerably over time. In addition, it includes people who are sensitized to nonthermotolerant fungi and yeasts, which are not obviously associated with lung damage in the same way as thermotolerant filamentous fungi. Moreover, many patients with fungal lung disease do not necessarily have asthma as it is usually defined (fungal allergy has a heterogeneous presentation), and if they do have asthma, this may not fit the criteria for severe asthma. The starting point for any discussion about the clinical relevance of fungal allergy should be what distinguishes it from asthma without fungal allergy. The evidence points towards lung damage with fixed airflow obstruction, bronchiectasis, and other radiological abnormalities (eg, fleeting shadows, mucus plugging, and lung fibrosis) as being particular features of fungal allergy. The question then should be, what are the best biomarkers of the presence of lung damage? We studied a population of 431 patients with generally severe asthma enriched by patients with fungal allergy to determine the

relationship between immunological markers of sensitization, lung function, and radiological abnormalities [106]. The best biomarker was positive specific IgE to *A fumigatus* or *P chrysogenum*. This was independent of atopy. Total IgE was associated with fleeting lung shadows (present in a fraction of patients) and tree-in-bud shadowing on the computed tomography scan, but no other features of lung damage. We concluded that total IgE was not helpful in determining who was at risk from developing lung damage due to fungal allergy. However, an unbiased cluster analysis revealed a small population (~10%) with a florid immune response to fungi and very high total IgE, poor lung function (postbronchodilator FEV₁, 63% predicted), and high rates of bronchiectasis (80%). This group all met the criteria for ABPA and, presumably, represent those patients from whom the concept of ABPA was derived. Nevertheless, equal numbers of patients who fitted the criteria for ABPA were found in the other clusters, thus emphasizing the difficulty of separating ABPA from the general population of people with allergic sensitization to fungi in any statistically meaningful way. The conclusion from this study was that the only useful biomarker for predicting risk of developing lung damage in asthma associated with fungal allergy is positive IgE to thermotolerant filamentous fungi [106]. While a small subgroup of patients with a florid immune response are at higher risk of developing lung damage, separating them from the much larger body of patients with fungal allergy by using the term ABPA makes it difficult to appreciate the full spectrum of fungal allergy-related lung disease. To describe patients with airway disease who are IgE-sensitized to thermotolerant fungi mainly of the *Aspergillus* and *Penicillium* genera, we prefer the inclusive term *allergic fungal airway disease* (AFAD), which can be qualified in terms of severity and also the underlying condition (eg, asthma, CF) if appropriate [7].

Treatment of Allergic Fungal Airway Disease

Therapeutic strategies against AFAD include allergen avoidance, antifungal medications, surgery, and immunotherapy.

Allergen Avoidance

Allergen avoidance is difficult, as fungi are ubiquitous in nature. Avoidance is further complicated by a lack of allergenic thresholds for most fungi, the exception being *Alternaria* and *Cladosporium*, where 100 spores for *Alternaria* and 3000 spores for *Cladosporium* per cubic meter of air is known to evoke allergic symptoms [107]. These thresholds are frequently exceeded outdoors during the late summer and autumn. Gardening, particularly composting and collection of dead and rotting vegetation, is a potentially important source of high levels of fungal spores, and gardeners with fungal allergy should wear masks during these tasks. Various occupations such as industrial composting and farming also bring people into contact with high levels of fungal spores, and many such industries have avoidance strategies in place. The risk of fungal exposure within buildings can be avoided by moving from the building [108] or decreased by active interventions

such as removing visible mold or water damaged materials and application of fungicides [109-113]. The effectiveness of these strategies remains controversial, and, as a result, general guidelines for mold avoidance have not yet been established.

Asthma Treatment

Many patients with AFAD have severe asthma requiring intensive treatment, usually at the top stages of the British Thoracic Society guidelines. Sometimes this involves frequent courses of high-dose oral corticosteroids or continuous oral corticosteroids. However, as a group, acute severe exacerbations are not a particularly prominent feature of AFAD, and periods of poor control tend to be more chronic, coming on over days and weeks rather than hours and often lasting for months. Because of the frequency of bronchiectasis, exacerbations may be caused by bacterial bronchitis, which can be stubborn to treat. Omalizumab has occasionally proven successful in patients with ABPA [114-116], although no clinical trials of this treatment have been performed specifically in patients with AFAD. In patients undergoing pulmonary rehabilitation of severe fixed airflow obstruction, a program of exercise, education, and support provided by clinically trained staff is appropriate.

Antifungal Therapy

The key difference between the management of asthma with and without fungal complications is the potential role of antifungal therapy. Since fungal airway colonization is thought to be the underlying trigger for AFAD, there is a strong rationale for treatment with antifungal agents. However, in both clinical practice and in the small number of controlled clinical trials that have addressed this question, the outcome of treatment is often disappointing. Four randomized-controlled trials have assessed antifungal treatment in asthma associated with AFAD. Three used itraconazole [117-119], the fourth used voriconazole [120]. Although the disease names and inclusion criteria were slightly different in each study, the patient population was essentially the same in all 4. There was a modest benefit at best in the 3 itraconazole studies and no improvement with voriconazole. The benefits seen with itraconazole may well have been due to its corticosteroid-enhancing effects [121], which are less prominent with voriconazole. The minor benefit of triazole antifungal agents may be due in part to the difficulty in eradicating the fungi from the airways owing to lack of penetration of the drug into the bronchial lumen [122,123]. In the EVITA3 study [120], 40% of patients still had at least 1 positive sputum culture while taking voriconazole. In addition, rates of positive sputum culture returned to baseline values within 2 months of stopping the intervention. Triazoles are more likely to be effective when there is active infection. AFAD can manifest as fungal bronchitis in which there is production of large amounts of mucopurulent sputum with heavy growth of filamentous fungi. Relatively few patients with AFAD experience this problem, but it is these patients who anecdotally do well with antifungal therapy. Selection for treatment with antifungals therefore requires careful identification of patients with active bronchitis reflecting heavy fungal colonization, as shown by culture of airway secretions. As methods for sputum fungal culture are

not standardized or quantified and often insensitive [124], fungal bronchitis is not well recognized or characterized. Fungal bronchitis, however, is a common problem in all airway diseases irrespective of IgE sensitization and often involves yeast as well as filamentous fungi.

Treatment strategies for AFRS were extensively reviewed by Ryan et al [65]. AFRS is usually treated with surgery to remove the polyps and eosinophilic mucin, clear the affected sinuses, and create access for topical intranasal medication. If the sinuses are not completely cleared, inflammation will continue and resistance to anti-inflammatory drugs develops. Patients can take nasal irrigations and intranasal corticosteroids to reduce inflammation and maintain sinus drainage. AFRS patients, who were followed for 7 years, had to undergo an additional 2 surgeries and repeated courses of oral corticosteroids (yearly, on average), thereby showing persistent polypoid mucosal edema and increased total IgE. The advantage of antifungal treatment with itraconazole has not been unambiguously demonstrated. While itraconazole improved endoscopic appearance and reduced the use of oral corticosteroids and the frequency of the relapse phase (30%) in some studies, it was noneffective or even worsened the conditions in 63% of AFRS patients [65]. Local treatment with fluconazole nasal spray was beneficial, resulting in the lowest recurrence rate (10%), whereas a recurrence rate of 66.7% was recorded in patients receiving itraconazole. However, the clinical benefit of antifungal treatment requires further investigation.

Immunotherapy

Immunotherapy can cure allergic diseases, relieve symptoms, and reduce medication usage. The WHO guidelines for safe and effective immunotherapy recommend the use of well-defined vaccines for carefully selected patients [125]. However, fungal extracts are not standardized [9,52], and immunotherapy is not recommended for patients with asthma, since it very frequently leads to adverse reactions [30]. In their review, Twaroch et al [30] found only a limited number of controlled immunotherapy trials, inconsistent results, and a small clinical benefit of *A alternata* and *C herbarum* extracts in patients with rhinoconjunctivitis and/or mold-induced asthma. Subcutaneous immunotherapy with *C herbarum* extract resulted in decreased bronchial, conjunctival, and skin reactivity, as well as increased peak expiratory flow and decreased medication score. However, symptoms were not significantly different between the treatment and placebo groups. Similar results were obtained with subcutaneous or sublingual immunotherapy using extracts from *Alternaria* species. An increase in IgG levels (including IgG4), which is generally considered favorable in immunotherapy, was common. Production of IgE probably derives from the activation of regulatory T cells (Treg), which is crucial for tolerance. The noninflammatory isotype is thought to prevent antigen-binding of IgE and therefore subsequent mast cell and basophil activation. Owing to its poor binding property, serum level itself is not an indicator of its protective function; similarly, a decrease in IgE levels is not an indicator of a decreased response to allergens. Instead, it is more likely that the IgE/IgG ratio in serum shows a predominance of Tregs over T_H2 cells [126].

The use of recombinant fungal allergens in immunotherapy has not yet been assessed, although hypoallergenic derivatives of Asp f 2 [127] and Alt a 1 were shown to efficiently block IgE binding in sera of fungus-sensitized patients [128]. Similar results were obtained with mutated Alt a 13 allergen, which also resulted in reduced IL-4 production by T cells and by Alt a 13-treated peripheral blood mononuclear cells [129]. These studies are a good basis for further clinical trials using recombinant allergens, although more clinically relevant allergens need to be evaluated in the population before widespread use of vaccines can be implemented [30].

Conclusion

IgE sensitization to fungi is common in asthma, particularly in its more severe manifestations, affecting both children and adults. The clinical outcomes of allergy to aeroallergens such as *Alternaria* and *Cladosporium* are predictable from their spore levels and cause short-term allergic manifestations similar to those induced by grass pollen. The clinical impact is relatively modest considering the very high levels of spores in the summer and autumn, possibly because the spore envelope is relatively nonallergenic and the spores in some cases are too large to access the lower airways. Certain climatic conditions such as summer thunderstorms disrupt spores, rendering them more potent. In contrast, thermotolerant fungi such as *Aspergillus* and *Penicillium* species can colonize the lung, where they create more persistent allergenic stimuli and, when colonization is considerable, an infective component. This leads to progressive lung damage due in part to chronic obstruction of the airways with viscid mucus. All asthmatics who are IgE-sensitized to these fungi are at risk of developing lung damage, although a subset with a florid immune response are at the greatest risk. Present knowledge reveals no criteria other than IgE sensitization to thermotolerant filamentous fungi for selection of this or any other subgroup in a clinically useful way. Therefore, we prefer an inclusive term such as AFAD to identify asthmatics at risk from fungal allergy, as opposed to terms such as ABPA or severe asthma with fungal sensitization, which exclude significant numbers of relevant patients. Treatment of AFAD is similar to that of severe asthma, with the exception that triazole antifungals have a place in the treatment of accompanying fungal bronchitis.

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Conflicts of Interest

Professor Wardlaw has received funding from Pfizer for a clinical trial in fungal allergy, from GSK for advisory work on fungal allergy, and from Pulmocide for advisory work on fungal allergy. Dr Pashley has received funding for advisory work from Pulmocide.

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