



REVIEW

The link between fungi and severe asthma: a summary of the evidence

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ABSTRACT: There is current evidence to demonstrate a close association between fungal sensitisation and asthma severity. Whether such an association is causal remains to be confirmed, but this is explored by means of a detailed literature review. There is evidence from two randomised controlled trials that, in the example of allergic bronchopulmonary aspergillosis (ABPA), treatment with systemic antifungal therapy can offer a therapeutic benefit to ~60% of patients. ABPA is only diagnosed if a combination of clinical and immunological criteria is achieved. It is not known whether such cases are a discrete clinical entity or part of a spectrum of the pulmonary allergic response to fungi or fungal products.

This paper describes the epidemiological evidence that associates severity of asthma with fungi and discusses possible pathogenetic mechanisms. Many airborne fungi are involved, including species of *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium*, and exposure may be indoors, outdoors or both. The potential for a therapeutic role of antifungal agents for patients with severe asthma and fungal sensitisation is also explored.

Not only are many patients with severe asthma desperately disabled by their disease, but, in the UK alone, asthma accounts for 1,500 deaths per yr. The healthcare costs of these patients are enormous and any treatment option merits close scrutiny. Within this report, the case for the consideration of a new term related to this association is put forward. The current authors propose the term "severe asthma with fungal sensitisation". However, it is recognised that enhanced and precise definition of fungal sensitisation will require improvements in diagnostic testing.

KEYWORDS: Aspergillosis, asthma clinical/basic investigations, asthma epidemiology, asthma immunology, fungi

Asthma is common in the developed world and increasing in frequency, despite better living conditions. In 2002, there were 15,960,496 adults with self-reported asthma in the USA [1]. Asthma ranked 8th in terms of visits to the doctor in the USA, with 17 million such visits in 2002. Considerable research is currently being directed towards understanding the role of genetic susceptibility to allergy and asthma [2]. The putative role of house dust mite allergy as the dominant exogenous precipitant for asthma, especially in childhood, has been questioned by recent randomised cohort mite avoidance studies in adults [3]. Thus far, the role of fungi as a primary exogenous driver of asthma has been incompletely explored, possibly because exposure is universal but highly variable in time and intensity and hard to measure. This paper reviews the substantial body of epidemiological

and clinical data supporting a major role for fungal sensitisation as a driver for severe asthma.

SEARCH STRATEGY AND SELECTION CRITERIA

Papers linking fungi and asthma, its pathogenesis, occupational asthma and thunderstorm asthma were identified from Medline searches, the historical section of the *Aspergillus* website (www.aspergillus.man.ac.uk) and the current authors' own files. Allergens and their homologues were identified from published literature, GenBank and www.allergome.org.

EARLY DESCRIPTIONS

The first case recorded in western literature of deterioration of asthma on mould exposure was possibly that of an "asthmatic who fell into a violent fit, by going into a wine cellar where the

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must was fermenting" in 1698 [4, 5]. In the 1870s, Charles Blackley, a Manchester physician, induced hoarseness, aphonia and an attack of "bronchial catarrh" by inhaling fungi from straw (bristle mould (*Chaetomium elatum*) and *Penicillium glaucum*) [6]. In 1924, asthma attributed to wheat rust (*Puccinia graminis*) exposure was reported by CADHAM [7]. At the same time, it was determined that asthma was more prevalent in the humid parts of the Netherlands [8], and there was reported relief with the use of filtered air [9]. A single feather-allergic patient was not helped by substitution of feather pillow with kapok, as mould grew on the second pillow. A remarkable frequency (50%) of mould skin sensitivity principally to *Mucor*, *Penicillium* and *Aspergillus* spp. was then described in Dutch asthmatics [9]. In 1928, in Germany, HANSEN [10] found 15% of his asthmatic patients had positive skin tests to *Aspergillus* or *Penicillium* and that inhalation challenge reproduced symptoms. In Spain, JIMINEZ-DIAZ *et al.* [11] demonstrated in 1928 that "house dust" sensitivity was often due to moulds. COHEN *et al.* [12] showed that cotton and kapok-stuffed mattresses, pillows and furniture were potent sources of "house dust antigen", and that removal of the offending articles abolished asthmatic symptoms in those in whom mould allergy was demonstrated. Cases of asthma attributed to *Alternaria*, *Aspergillus fumigatus* and *Trichophyton* spp. were described in 1930 [13–15]. A patient with *A. fumigatus*-related asthma was treated with a 1:5,000 extract of the fungus subcutaneously, but this caused severe exacerbations of asthma, and treatment had to be discontinued [14]. Subsequent investigations focused on understanding mould and pollen distributions, developing higher quality skin-testing reagents (to attribute allergy to single species of fungus) and desensitisation (so-called "mould therapy") [16, 17]. Allergic bronchopulmonary aspergillosis (ABPA) was first described in 1952 [18].

FUNGAL ALLERGY AND ASTHMA SEVERITY

While most asthma patients have mild symptoms, which are well controlled with anti-inflammatory and bronchodilator therapy, a minority has severe airway inflammation and airflow obstruction requiring multiple hospital admissions. The reasons for these differences in asthma severity are complex and not fully understood. There are many different phenotypes of "severe" asthma, including brittle asthma, and the differentiation of these more severe phenotypes is somewhat subjective [19–21]. Even the definition of asthma severity is complex. It usually relies on the expression of symptoms, although it is clear that many of the symptoms expressed in this group are not caused by either airway inflammation or bronchospasm. Many authors use the term "severe" to represent those with symptoms that are definitely related to asthma, and the term "difficult" is reserved for those who use healthcare resources with symptoms that are only indirectly associated with asthma, such as vocal cord dysfunction and dysfunctional breathing [21].

Much evidence indicates that atopy (especially to mould allergens) is related to asthma severity [22–31]. Amongst those with persistent asthma requiring specialist referral, 20–25% have skin-test reactivity to *Aspergillus* or other fungi [24, 32–34]. Mould sensitivity has been associated with increased asthma severity and death, hospital admission and intensive

care admissions in adults and with increased bronchial reactivity in children [24–31, 35, 36]. Severity of asthma was strongly linked to *Alternaria* skin-test positivity in the Tucson cohort, where house dust mite allergy is uncommon [27]. Admission to intensive care for asthma was strongly associated ($p=0.005$) with skin-test reactivity to one or more fungi (*Alternaria tenuis*, *Cladosporium cladosporoides*, *Helminthosporium maydis* and *Epicoccum nigrum*) in 87 patients [31]. A link between *Alternaria* sensitivity and severe asthma was proposed by NEUKIRCH *et al.* [37]. A cross-sectional study of 1,132 adults with asthma found that sensitisation to *Alternaria alternata* (odds ratio (OR) 2.03) or *Cladosporium herbarum* (OR 3.2) is a significant risk factor for severe asthma (both 2.34) in several European countries and also in Australia, New Zealand and Portland (OR, USA) [30]. No such association was found for pollens or cats, although sensitisation to house dust mite was slightly more frequent in those with severe asthma (OR 1.61) [30]. Adults with severe asthma (defined as an admission to hospital with acute asthma at least twice) were more likely to be skin-test positive for at least one of the moulds *A. fumigatus*, *Penicillium notatum*, *C. herbarum* or *A. alternata* or the yeast *Candida albicans* (76%) versus adults with moderate or mild asthma (16–19%; $p<0.0001$) [35]. Another study in which severity of asthma was defined by forced expiratory volume in one second (FEV₁) also showed a doubling of the frequency of mould-positive radioallergosorbent tests (RASTs) in the moderate and severe groups (31–35%) compared with mild and no asthma controls (17–19%; $p=0.01$) [38].

Temporal relationship with loss of asthma control

Skin-test reactivity to fungal allergens, such as *Alternaria* species, has been reported to be especially common in patients with life-threatening asthma [24]. Asthma deaths in young adults in England and Wales (UK) are most common in the months of July, August and September, which coincides with peak levels of mould spores in the outdoor air in the UK [39–41]. Asthma deaths, hospital admissions, respiratory symptoms and peak expiratory flow rates can be adversely affected by high fungal spore concentrations in outdoor air [25, 41–43]. The risk of an asthmatic patient dying increased by 2.16 times if exposed to $>1,000$ spores·m⁻³ compared with $<1,000$ spores·m⁻³ [25].

Evidence for a temporal relationship between high environmental spore counts and asthmatic attacks is strong. Airborne spore levels may be up to 1,000 times higher than pollen levels [44]. The data of TARGONSKI *et al.* [25] provide strong evidence that asthma deaths in Chicago (IL, USA) are more likely to occur on days when local mould spore counts are high. High mould spore counts have been associated with asthma admissions in New Orleans (LA, USA; adults), Derby (UK) and Mexico City (adults and children), and Ottawa (Canada; children) [44–48]. In the Canadian study there was a positive association with spore counts, but not pollen counts, and emergency asthma attendance at hospital [46]. Asthma symptoms are increased in California and Pennsylvania on days when spore (*Cladosporium* spp., *Alternaria* spp. and *E. purpurascens*) counts are high [42, 43].

The seasonal (summer–autumn) peak of asthma admissions occurs when ambient air counts of moulds are high, in contrast to snowy conditions when spore counts are very low. Asthma

admissions and deaths coincide with the summer–autumn peak of ambient mould spores [39–41, 49–51]. In Cardiff (UK), maximal levels of *Cladosporium* spp. were reported in July, *Alternaria* spp. and hyaline basidiospores in August, uredospores in September and coloured basidiospores in October [41], and similar results were seen in Derby [45] and Copenhagen (Denmark) [52]. However, these data were collected retrospectively, and prospective data are required to confirm the associations demonstrated.

Thunderstorm asthma

In the medical literature, an association of thunderstorms with increased acute asthmatic attacks was first noted by PACKE and AYRES [53] in 1985. They described a concurrent increase in airborne spores of the fungi *Didymella exitialis* and *Sporobolomyces*. Since then, many episodes of thunderstorm asthma have been noted in several parts of the world [24, 45, 54–58]. The hypothesis is that increased humidity, coupled with higher winds, triggers increased spore production and dissemination. Local investigations have implicated other fungi, such as *Alternaria* [24, 46], grass pollens [54, 55, 59] or both [58]. In some studies that noted a correlation with grass pollens, which may also be produced and disseminated in greater numbers under thunderstorm conditions, fungal spore counts and patient sensitisation to fungi were not measured [54, 55]. In those studies in which both were measured, fungal spores may be more highly associated with asthma than pollen [46]. Thunderstorm asthma was positively correlated with a doubling of ambient fungal spores [45]. In North America, the central prairies generate huge quantities of *Alternaria* spores. A well-documented “spore storm” occurred on October 6–7, 1937, throughout the eastern USA when huge air masses travelled rapidly to the Atlantic seaboard, conveying several tons of mould spores across several hundred miles [60]. A number of pollen allergens (such as Hor v 4; gi:55859461, Jun a 2; gi:9955724, Pla a 2; gi:49523393, Cry j 2; gi:577695, Bet v 7; gi:21886602, and Tri a25; gi:8980490) have homologues in fungi. This raises the interesting possibility that pollen and fungal allergens could potentially cross-sensitise in a synergistic manner, giving rise to the reactions observed in thunderstorm asthma, which is clearly a complex interaction [59].

OCCUPATIONAL ASTHMA

Only a few case reports exist for an occupational fungal exposure and asthma association. These few reports implicate the fungi *Pleurotus cornucopiae*, *Lycopodium clavatum*, *Dictyostelium discoideum*, *Rhizopus nigricans* and *Neurospora* spp. [61–66].

Numerous examples of extrinsic allergic alveolitis (or more correctly, bronchioloalveolitis) caused by fungi are described, although the relevance of this to asthma is unknown.

IMPLICATED FUNGI

Many different fungi have been implicated in both exacerbations of asthma and chronic allergic sinusitis, including some basidiomycete fungi and *Botrytis* spp., the causative agent of blight [22–33, 36, 67–70]. These data reflect the prevailing airborne species locally, what is identifiable microscopically from spore shape or culturable. In most instances, the attribution is to genera if based on air sampling, and species if based on immunological tests, although cross-reactivity within and between genera occurs. In North America,

Alternaria spp. appears to predominate in outside air and, in Europe, *Cladosporium* spp. and *A. fumigatus* are perhaps more important. Sensitisation to *C. albicans*, while not an aeroallergen, is seen in up to 10% of individuals with mild stable asthma and 33% of patients with severe asthma [35]. *Trichophyton* spp., which causes superficial skin and nail infection, has also been implicated [67]. In studies from the USA and Europe, there was a significant relationship between basidiospore reactivity and the presence of atopy, asthma, and asthma and rhinitis ($p < 0.005$), but not rhinitis alone [70]. *Stachybotrys chartarum* is a common fungus in the context of damp housing, but has not clearly been implicated in allergic conditions [71]. A single antigen (cellulase) has been identified from *S. chartarum*, which is a protein found in many fungi [72].

The reactivity of asthmatic patients to multiple mould allergens could be due to genuine sensitisation to a variety of fungi, or it could be due to cross-reactivity between fungal allergens. HEMMANN *et al.* [73] suggest that *Aspergillus* and *Candida* allergens may share immunoglobulin (Ig)E-binding epitopes. Indeed, numerous fungal allergens are described (table 1), and many are similar proteins. Multiple mould sensitisation skin-test reactions are usually due to sensitivity to multiple antigens, whether cross-reactive or not [74, 75]. Few fungi out of the >1 million species thought to exist worldwide have been subjected to the antigenic scrutiny that *Aspergillus* and a few other common airborne fungi have, and it is likely that sensitisation to other fungi will be discovered in the future.

TESTING FOR FUNGAL ALLERGY AND SENSITISATION

While skin testing for fungi has been in use since the 1920s and much has been published on the subject, some general statements can be made. First, reagents available from manufacturers differ [75]. Secondly, skin-test responses may vary over time in the same individual, even if performed in an identical manner with the same reagent [76]. Thirdly, there is substantial variation in the pattern of skin-test positivity to different allergens [77]. Fourthly, while there is a reasonable correlation between skin-test reactivity and a specific IgE RAST test, there is enough discordance to require both to be performed to identify all patients who are sensitised to fungi [78, 79]. Some clinics test for a wide range of fungal sensitivity; others test for a limited number or only for *A. fumigatus* sensitivity. Less commonly tested examples include *Botrytis* spp., *Rhizopus* spp. and *Fusarium* spp. Such variations in testing protocols will alter the number of identified patients.

Therefore, additional work may be required to identify the optimal testing protocol and reagents to define patients as sensitised to fungi or not. As discussed previously, some of the reactions may be due to cross-reactivity between similar proteins shared by different fungi.

PATHOGENESIS

It is not known why certain mould allergens should produce more severe airway disease than other common allergens. Fungi are very common in the environment, and respiratory exposure to airborne spores is almost constant. The most common airborne fungi include *C. herbarum*, *A. alternata* and *A. fumigatus*, although a multitude of other species are readily found. Other fungi such as *Candida* and *Trichophyton* are

TABLE 1 Approved fungal allergens

Protein classification	Named allergen
Protein synthesis/secretion	
Elongation factor 1 β [#]	Pen c24 (A2K12; <i>A. fumigatus</i>)
Eukaryote initiation factor-2 α -kinase [#]	Alt a2
Cyclophilin [#]	Asp f11, Cand a CyP, Psi c2, Sac c CyP, Mala s6
HSP70 [#]	Asp f12, Alt a3, Cla h HSP70, Pen c19, Mala s10
Cold shock protein	Cla h8
Disulphideisomerases [#]	Alt a4
Acid ribosomal protein P1 [#]	Alt a12, Cla h12
Acid ribosomal protein P2 [#]	Asp f8, Alt a6, Cla h4, Fus c1
Stress response	
Peroxisomal membrane protein [#]	Asp f3, Cand a2, Cand b2, Mala f2, Mala f3 Mala s1
Thioredoxin	Cop c2, Fus c2
YCP4 protein, similar to flavodoxins	Alt a7, Cla h5
MnSOD [#]	Asp f6, Sac c MnSOD, Mala s11
Proteases/toxins	
Ribotoxin	Asp f1
Vacuolar serine protease, cerevisin [#]	Asp f18, Asp fl18, Asp n18, Pen c2, Pen c18, Pen ch18, Pen o18, Rho m2
Acid protease	CAAP
Alkaline serine protease, oryzin	Asp f13, Asp fl13, Pen b13, Pen c13, Tri r2, Asp fl1, Asp o13, Cur l1, Epi p1, Pen c1, Tri r4, Tri t4
Aspartic protease [#]	Asp f10
Metalloprotease	Asp f5
Enzymes of gluconeogenesis from lipid	
Aldehyde/alcohol dehydrogenase	Alt a10, Cla h3, Cand a1
Malate dehydrogenase	Mala f4
Enolase [#]	Asp f22, Alt a5, Alt a11, Cand a enolase, Cla h6, Pen c22, Rho m1, Sac c enolase
Glycosidases	
β -glucanase (family 16)	Asp f2 Asp f9, Asp f16, Asp f17, Tri t1, Asp n1
Glycosyl hydrolase (cellulase)	Sta c cellulase, Asp f4, Asp f7, Asp n hemicellulase
β -Xylosidase	Asp n14
3-Phytase B	Asp n25
Glucoamylase	Asp n glucoamylase
TAKA-amylase A	Asp o21, Asp o2
N-Acetyl glucosaminidase	Pen ch20
β -Galactosidase	Asp o lactase
Glycoprotein Ag-54	Cla h2
Others	
Nuclear transport factor 2 [#]	Alt a NTF2, Cla h NTF2
Lipase [†]	The l1
Hydrophobin (conidia)	Cla h HCh-1
Leucine zipper protein	Cop c1
Unknown function	Fus c3, Fus s45kD, Fus s1, Cop c3, Cop c5, Cop c7, Cop c6, Cop c4, Psi c1, Alt a70kD, Alt a1, Alt b1, Alt a8, Alt a9, Cla h1, Cla h7, Cla h9

Alt a: *Alternaria alternata*; Alt b: *Alternaria brassicola*; Asp f: *Aspergillus fumigatus*; Asp fl: *Aspergillus flavus*; Asp n: *Aspergillus niger*; Asp o: *Aspergillus oryzae*; Cand a: *Candida albicans*, Cand b: *Candida boidinii*; Cla h: *Cladosporium herbarum*; Cop c: *Coprinus comatus*; Cur l: *Curvularia lunata*; Epi p: *Epicoccum nigrum*; Fus c: *Fusarium culmorum*; Fus s: *Fusarium solani*; Mala f: *Malassezia furfur*, Mala s: *Malassezia sympodialis*; Pen b: *Penicillium brevicompactum*; Pen c: *Penicillium citrinum*; Pen ch: *Penicillium chrysogenum*; Pen o: *Penicillium oxalicum*; Psi c: *Psilocybe cubensis*; Rho m: *Rhodotorula mucilaginosa*; Sac c: *Saccharomyces cerevisiae*; Sta c: *Stachybotris chartarum*; The l: *Thermonyces lanuginosus*; Tri r: *Trichophyton rubrum*; Tri t: *Trichophyton tonsurans*; CAAP: *Candida albicans* alkaline protease; CyR: cyclophilin; HCL-1: hydrophobin; HSP: heat shock protein; MnSOD: manganese superoxide dismutase; Ag: antigen; NTF: nuclear transport factor. [#]: proteins with significant levels of mammalian homology (better than 1E⁻³⁰, basic local alignment search tool (BLAST) score >200, percentage identity >30%); [†]: as used in detergents.

natural skin or gut inhabitants. One key difference is that other allergens, such as house dust mites, cat dander or grass pollen are sources of allergenic protein, but fungi have the additional ability to actively germinate and infect the host skin or attempt

to colonise the respiratory tract. Thus, it is possible that fungi have a much greater impact on an individual in terms of triggering host defences against pathogens and producing nonallergen toxins and enzymes that may play an accessory

role in triggering allergy. Many potent allergenic proteins have been described in *Aspergillus* and other fungi (table 1) [74]. All fungal antigens described are expressed during hyphal growth (often within hours of germination) [80]. There is considerable overlap between fungal allergen function and sequence, and the current authors discuss here the allergens from *A. fumigatus* that have recently been sequenced, but suggest that allergens from *Alternaria* and *Cladosporium* are likely to act in the same way. It is noted that both *Alternaria* and *Cladosporium* produce other allergens with unknown function.

Fungal allergens

Allergens can be grouped into several categories (table 1): proteases; glycosidases; components of protein production; oxidative stress response proteins; and enzymes involved in gluconeogenesis or the pentose phosphate shunt. The first two groups suggest secreted enzymes that have a direct effect on the host. The latter three groups are suggestive of metabolism in spores germinating in a hostile environment. Oxidative attack is a known mechanism of macrophage defence and stress proteins typical of oxidative and heat stress are common allergens. Additionally, the latter three groups contain many proteins, primarily producing energy and biosynthetic intermediates through gluconeogenesis, with high levels of homology to mammalian proteins. Thus, the allergen proteins produced by fungi are typical of those that might be induced in large amounts during germination on the respiratory epithelium. In this case, exposure to fungal spores is likely to result in exposure to the whole group of fungal allergens simultaneously rather than as individual allergens, which appears to be the case for other major allergens, such as pollen proteins or animal danders.

Protease action appears to be an important step, possibly the first, in initiating an allergic response in the lung. Many allergens are proteases, including Der p1 [81] and some fungal antigens are also proteases (e.g. Asp f5, Asp f10, Asp f13, Asp f15, Asp f18; table 1). It may be that a similar pathogenic role can be postulated. Studies by KHERADMAND *et al.* [82] have demonstrated that the intrinsic protease activity in allergenic preparations of *A. fumigatus* promote eosinophil-driven allergic airway disease in mice. These proteases are critical to the initiation of allergic airway disease, since allergens that lack intrinsic protease activity do not develop strong IgE responses to ovalbumin. It is proposed that proteases can enhance the ability of other proteins present in the inoculum to become allergens. This "bystander" effect may enhance the allergenic potential of the other fungal proteins and increase the allergenicity of the organism as a whole.

KAUFFMAN and coworkers [83, 84] showed that fungal proteases (those associated with *A. fumigatus*, *Alternaria* spp., etc.) directly impact the airway epithelium as evidenced by morphological and synthetic changes. *Aspergillus* proteases [85] caused cell shrinkage, cell desquamation and interleukin (IL)-6 and IL-8 generation. The mechanism of protease action is not clear at present, but it is speculated that fungal proteases injure epithelial cells in a receptor-specific manner through protease-activated receptor type-2 [84], or by degrading the tight junctions between epithelial cells to allow increased allergen access to subepithelial cell layers.

Some of the allergen genes from *A. fumigatus* are predicted to produce secreted glycosidases (specifically, family 16 glucanases and cellulases). The role of these carbohydrate-degrading enzymes in the pathogenesis of allergy is not clear. Family 16 glucanases appear to possess cell wall localising regions and are likely to be involved in cell wall remodelling during pathogenic development or stress response. It should be noted that several innate immune responses are triggered by fungal cell wall glucans, and it would be interesting to investigate whether the allergenic glucanases have any impact on this process.

The final group of allergens comprises enzymes and proteins involved in defence against oxidative stress. These include protein chaperones, such as heat shock protein and cyclophilin, and also enzymes that are directly involved in preventing oxidative damage, such as manganese superoxide dismutase (MnSOD), peroxiredoxin and thioredoxin. It seems certain that these proteins will be produced in abundance in germinating spores that are under attack from host macrophages. The high degree of homology of these proteins to human counterparts is striking and may allow these proteins to directly interact with components of the host immune response.

Therefore, fungi are a source of proteins that can damage airways, enhance bystander-type reactions and simultaneously act as allergens. This combination of properties makes them a very potent threat to an asthmatic lung. Direct exposure to fungal allergens and airborne hyphae could be very important [86]. The evidence suggests the hypothesis that these allergens act in concert, possibly with other nonallergen proteins or toxins, in order to produce an enhanced host response.

Fungi and bronchiectasis

There is also recent evidence of higher than expected rates of bronchiectasis in patients attending severe asthma clinics [87]. It is uncertain whether the presence of bronchiectasis leads to uncontrolled symptoms and, therefore, dictates their definition as severe, or whether uncontrolled asthma may indeed be a risk factor for the development of bronchiectasis. The presence of bronchiectasis in this cohort of patients (or other pulmonary defects) could allow colonisation by fungi, particularly the thermotolerant *A. fumigatus*. Such a scenario would allow persistence of the fungal antigen stimulus *in situ*, and might contribute to fungal sensitisation in atopic individuals. Fungal colonisation could then act as a continuing driver of disease.

Chronic exposure to fungi as a driver for asthma?

Another possible explanation of the causative role of fungi in severe asthma might be long-term colonisation of atopic individuals by fungi. Fungi are well known to colonise and cause diseases in skin, nails, sinuses or airways. Many of these low-level infections, such as thrush or athlete's foot, are long term and recurrent; thus, exposure to these fungi, while not as directly damaging as respiratory infection, may provide a chronic source of allergen exposure. The association of *Trichophyton* with asthma [67] and improvement in asthma with antifungal treatment [88] both suggest a possible link between chronic infection and asthma. Resolution or improvement of chronic fungal sinusitis may improve asthma [89–91]. It may be that mould-associated asthma is more common after years of mucosal inflammation due to asthma. As well as

providing a source of chronic infection, fungal spores or degraded material may persist in resident pulmonary macrophages. In mice, the allergic lung provides a permissive environment for the accumulation of fungal material within immune cells that reside in the lung. Previously sensitised mice (to *A. fumigatus* or other allergens such as cockroach antigens) accumulate intact spores or partially degraded fungal material within lung mononuclear cells [92], although the persistence may rely on the high inoculum used or the genetic predisposition of these mice to disease. These mice manifest chronic allergic airway hyperreactivity and inflammation, unlike nonallergic mice, which do not accumulate any fungal material in macrophages. Elimination of this fungal material from these mice reverses these major features. Experimental strategies that have been successfully employed to eliminate fungal material from the lungs of *Aspergillus*-sensitised mice include genetic deletion of CC chemokine receptor (CCR)1 [93], CXCR2 [94] or CCR4 [95], or eradication of IL-13 [96] or RANTES/CCL5-responsive cells [97]. All of these approaches also result in resolution of allergic airway inflammation and airway hyperreactivity, and enhancement of the pulmonary innate immune response through macrophages and/or neutrophils. Interestingly, the importance of the innate immune response directed against *Aspergillus* in the allergic lung is highlighted by the observation that *A. fumigatus*-sensitised mice that are deficient in CCR2 (the receptor for CCL2) exhibit markedly enhanced allergic airway disease and are highly susceptible to *Aspergillus* growth in the airways [98]. How chemokine–chemokine receptor interactions regulate innate pulmonary and acquired immune responses against *Aspergillus* is not well understood. The enhanced innate immune responses observed in CCR4^{-/-} mice appear to be related to the expression and/or activation of triggering receptor expressed on myeloid cells (TREM)-1 [99], and *Aspergillus* appears to strongly upregulate the expression of TREM-1 [100]. Recognition of *Aspergillus* is *via* the Toll-2 receptor [101]; Toll-2 activation can lead to pulmonary inflammation in the experimental setting [102].

Possible role of nonprotein cell components in asthma

It may be that it is not whole fungal hyphae or spores that induce the abnormal immune response, but fungal cell wall components such as glucan or chitin instead [103, 104]. Glucan can also be used as a surrogate marker for fungal biomass. In guinea pigs, β 1,3-D-glucan causes pulmonary eosinophilia [103], and exposure to larger amounts of environmental β 1,3-D-glucan is associated with lower tumour necrosis factor- α concentrations in stimulated blood monocytes [104].

Homology of fungal allergens to human proteins: a role for cross-reactivity in asthma?

Another possible driver for chronic asthma is the probability that some fungal antigens generate “auto-immune” cross-reactive responses. For example, Asp f6 (a manganese-dependent superoxide dismutase) is closely related to the human enzyme and generates a self-perpetuating allergic response [105, 106]. Skin-test cross-reactivity to both human and fungal *A. fumigatus* MnSOD and P2 proteins has been demonstrated in an asthmatic allergic to *A. fumigatus* [106]. The main hypothesis to explain how allergens trigger auto-reactive responses in humans is that the fungal proteins have

significant homology to their human paralogs so that an immune response directed at the fungal protein will also target human counterparts. Comparison of *A. fumigatus*-approved allergens to human proteins shows that Asp f3, f6, f8, f10, f11, f12, f17, f18 and f22 have strong homology to human proteins. In general, allergens with a high degree of homology to human proteins can be classified as cytoplasmic components that may be involved in protein folding or oxidative stress responses. Two secreted protease allergens, Asp f10, and f18, have significant homology to human proteins. Comparison of the *A. fumigatus* allergens with allergenic proteins from other fungal species shows that many of the highly conserved allergens are present in these species (table 1).

Volatile organic compounds

In addition, a potentially important issue is the production of volatile organic compounds (VOCs) by most fungi. Although detectable in, for example, compost heaps [107], the exposure in other environments, such as a bedroom, is uncertain. VOCs are clearly irritants of the respiratory mucosa [108]. Building-related VOCs may persist for some time, but these need to be distinguished from those generated in the home by fungi.

Fungi, therefore, have the tools to induce and maintain asthma in several ways. They are present in the air and are capable of colonising the human body over long periods. They can damage airways by production of toxins, proteases and enzymes, as well as by production of VOCs. They actively produce a wide range of allergenic proteins. As fellow eukaryotes, the similarity of their proteins to those of humans may itself induce auto-reactive responses. The question of which, if any, of these tools are used to operate and perpetuate the mechanisms of allergy and asthma remains to be determined.

AGE

In children with asthma, sensitisation to common allergic fungi (*A. fumigatus*, *Penicillium* spp., *Alternaria* spp. and *Cladosporium* spp.) is relatively uncommon in the UK and in Finnish schoolchildren [109] compared with Arizona [26] and Australia, where up to 31% of asthmatic children and up to 23% of nonasthmatic controls react to at least one fungal allergen [29, 31, 110]. Sensitisation rates may then decline with age [111]. Loss of fungal sensitisation could relate to “maturing” of the immune system and, by inference, its documentation in childhood is not a marker for disease in children.

INDOOR MOULD EXPOSURE

Many fungi are found in great numbers in the outdoor environment but are less common inside. The opposite is also true, with much variation from building to building [110]. In addition to external fungal exposure triggering asthma, indoor mould exposure may also contribute to asthma severity, although the data are less strong. A general practice survey in south Wales compared 76 adults with asthma on a register of 9,500 patients, and documented RAST test results and mould in their homes [112]. Those asthmatics with visible mould had a higher frequency of *P. notatum* RAST positivity compared with controls and asthmatics without mould at home, but, overall, 54% of asthmatics were RAST positive [112]. Sensitisation to indoor allergens was strongly associated with asthma in Quebec [34]. Many patients report respiratory symptoms in damp and mouldy houses, and a review of nine

population-based studies found that seven reported one or more positive association between fungal levels and health outcomes [113]. However, measurements and personal assessment of dampness, although apparently associated with worse asthma [114–117], are unreliable. Water damage plus mould, but neither alone, was associated with breathlessness in a large Swedish study [115]. TASKINEN *et al.* [109] found that the prevalence of asthma was similar (4.8%) amongst children attending a school with moisture and mould problems compared with a control school, but asthma symptoms, such as wheeze and cough, were more common in the damp mouldy school as were emergency visits to hospital (OR 2.0; $p < 0.01$). Only visible mould growth at home (and smoking) was found to be important in one study of children with asthma [116]. *A. fumigatus* and other fungi are found in substantial quantities in homes in normal bedding (pillows) [118] but there have been no studies examining any correlation between this observation and asthma occurrence or severity. There are few longitudinal data assessing any trends in household fungal flora, whilst housing has changed over the last few decades and asthma has increased. One study addressed changes in apartment bedrooms before and after installation of insulated windows and central heating in Dresden (Germany) [119]. Both house dust mite antigen and *A. fumigatus* concentrations increased after the installation, whereas other moulds remained unchanged. House dust mites consume fungi as part of their diet, and house dust mite faeces may provide a carbon and nitrogen source for fungal growth. Recently, in Australia, MATHESON *et al.* [120] found that adults whose domestic exposure to *Cladosporium* had doubled over a

2-yr period had 52% greater odds of reporting an attack of asthma in the previous 12 months.

ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS/MYCOSIS

A. fumigatus in particular is a major respiratory allergen, which causes the vast majority of known cases of allergic bronchopulmonary mycosis [121–125]. This disease may represent one of the extreme manifestations of mould allergy. The definition of ABPA is shown in table 2 [126, 127]. Genetic influences are also important in this area and, for example, some patients with ABPA have a defect in surfactant A2 [128]; human leukocyte antigen (HLA) restriction in ABPA (DR2 and DR5) is also more frequent in asthmatics who have *Aspergillus* allergy, but the presence of HLA DQ2 (especially DQB1*0201) provided protection from ABPA [123].

Using recombinant *A. fumigatus* antigens, KURUP and CREMERI [74] have demonstrated IgE serological responses to different antigens in ABPA and asthmatic, *Aspergillus*-allergic patients. The pathogenetic significance of this is uncertain, but it has considerable diagnostic utility in determining the particular asthma phenotype. Four of these antigens (recombinant (r)Asp f1, rAsp f2, rAsp f4 and rAsp f6) are now commercially available as RASTs.

ANTIFUNGAL THERAPY

The potential utility of systemic antifungal therapy for ABPA was first shown in the early 1990s [129], nebuliser therapy with nystatin having failed [130]. Two randomised placebo-controlled trials of itraconazole confirmed these results [131,

TABLE 2 Definition of allergic bronchopulmonary aspergillosis (ABPA) and proposed definition of severe asthma with fungal sensitisation (SAFS), with some additional features

Feature	ABPA*	SAFS (proposed)
Clinical features		
Asthma	Any severity	Severe [†]
Pulmonary infiltrates (history)	Yes, which resolve with corticosteroids	No
Eosinophilia	Yes, if not on systemic corticosteroids	Not studied, but not required
Central bronchiectasis	Yes, but many patients with early disease do not have this feature	No
Thick mucous plugs	Yes, usually	Unknown
Chronic rhinosinusitis, with or without nasal polyps	Occasional	Sometimes
Fungal features		
<i>Aspergillus</i> precipitins positive (2 × asthma control)	Yes (almost all cases)	No
<i>Aspergillus</i> IgG test positive (2 × asthma control)	Yes	No
<i>Aspergillus</i> prick test positive (>3 mm)	Yes	Yes or no [‡]
Other fungal skin tests positive (>3 mm)	No [§]	Yes or no [‡]
Serum IgE elevated (>1000 IU·mL ⁻¹)	Yes (may be only >500 IU·mL ⁻¹ , especially if on corticosteroids)	No (<1000 IU·mL ⁻¹)
<i>Aspergillus</i> -specific RAST test positive (2 × asthma control)	Yes	Yes or no [‡]
Other fungal RAST test positive	No [§]	Yes or no [‡]
Airways colonised by <i>Aspergillus fumigatus</i>	Yes	Unknown

Ig: immunoglobulin; RAST: radioallergosorbent test. #: as defined by RICKETTI *et al.* [126] and PATTERSON *et al.* [127]; †: typically British Thoracic Society level 4 or equivalent; ‡: at least one fungal skin or RAST test positive (better and more specific tests may emerge in the future); §: there are rare instances of bronchopulmonary mycosis due to other fungi, with typical clinical features.

132], and a Cochrane review concluded that therapy was beneficial [133]. Antifungal therapy is now commonly used in ABPA, except for cystic fibrosis (CF) patients in whom the diagnosis is less secure and itraconazole absorption poor. Reduced sputum eosinophils, cationic protein and systemic immune activation were demonstrated in the itraconazole arm of one of these studies [132]. The treatment of fungus-allergic patients with difficult asthma, who do not have ABPA, has not been systematically studied. Fluconazole was used to treat cutaneous fungal infection in 11 asthmatic patients; improvement in asthma was noted in all patients, together with a reduction in steroid requirement and bronchial hypersensitivity to *Trichophyton* [88]. Emerging evidence suggests that treatment with nasal amphotericin B douches benefits patients with allergic fungal sinusitis, not only in terms of their nasal symptoms but also their asthma [90]. Thus, those colonised or infected by fungi appear to benefit from antifungal therapy.

In a retrospective audit conducted by the current authors, patients with allergy to *Aspergillus* (RAST positive) and severe asthma, but not fulfilling the criteria for ABPA were treated with itraconazole for several months. Falls in hospital admissions (pre 1.63, post 0.4 per yr; $p < 0.05$) and steroid courses (pre 2.15, post 0.43 per yr; $p = 0.07$) were demonstrated, but not in the total and specific IgE [134]. These patients were not demonstrated to be colonised by fungi, although the search was not thorough and current mycological methods are insensitive.

Despite the two randomised trials showing some benefit of itraconazole in ABPA, the existing evidence for a potential benefit of antifungal therapy in asthmatics without ABPA is very limited. The mechanism of any observed effect would need to be explored, as therapy might reduce or eliminate airway colonisation but cannot prevent environmental contact. Whether benefit can be demonstrated in those not colonised or infected with fungi will need to be studied. Alternative mechanisms of action could be the promotion of endogenous or exogenous steroid activity by itraconazole [135], or some direct effects on the immune system. There is clearly enough evidence to merit further prospective studies of antifungal therapy for severe asthma patients with fungal sensitisation, but it is premature to speculate on the outcome of such studies.

PROPOSED TERMINOLOGY

The current authors propose a new label for those patients who have persistent severe or brittle asthma (despite standard treatment) and evidence of fungal sensitisation, as defined by positive prick testing, or fungus or fungal antigen-specific blood IgE testing, and do not meet the criteria for ABPA. The term severe asthma with fungal sensitisation (SAFS) is proposed. This avoids any notion of direct causality, but allows identification of patients who are sensitised to one or more fungi as determined by skin or blood IgE testing. It is suggested that the term should be applied primarily in adulthood, since sensitisation may disappear in late childhood. This label will help in clinical practice and studies but may need more precise definition once better testing for sensitisation is available.

SIZE OF THE PROBLEM

In 2002, there were 15,960,496 adults with self-reported asthma in the USA [1]. Depending on the definition, up to 20% of

patients with asthma can be defined as severe [30], although others have estimated lower proportions [19]. In this group, 35–70% will have evidence of fungal allergy [30, 32–35, 37] depending on how many fungi are tested for and whether both RASTs and skin tests are taken into account. This suggests that SAFS affects 1,117,000–2,234,000 in the USA, and a similar number in Europe. An alternative estimate would be to use the study by ZUREIK *et al.* [30], comprising data from multiple countries in which 20.7% had severe asthma and 22.1% had positive skin tests to *A. alternata* and/or *C. herbarum* [30]. Using these assumptions, the number of cases in the USA would be 730,000.

CONCLUSIONS

The evidence linking asthma severity, including unheralded death, with the intensity of fungal exposure and/or reactivity is strong. The current authors suggest that a phenotype of severe asthma may exist where there is a link with sensitisation to fungal allergens. It remains to be clarified whether this observed association is caused by colonisation of the airways with allergenic fungi or an extreme response to exogenous fungi. If proven, the idea of severe asthma associated with fungal sensitisation may be part of a disease spectrum akin to allergic bronchopulmonary aspergillosis. This suggests that severe asthma with fungal sensitisation is one phenotype of severe asthma, and is separate from allergic bronchopulmonary aspergillosis. Clinically validated definitions need to be drawn up, especially given the variability in skin-test reagents, different fungi that patients are allergic to, and different definitions of asthma severity. Antifungal therapy for severe asthma with fungal sensitisation should be studied in controlled trials.

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