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Review Article

Review Article

Pathophysiological aspects of *Aspergillus* colonization in disease

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Abstract

Aspergillus colonization of the lower respiratory airways is common in normal people, and of little clinical significance. However, in some patients, colonization is associated with severe disease including poorly controlled asthma, allergic bronchopulmonary aspergillosis (ABPA) with sputum plugs, worse lung function in chronic obstructive pulmonary aspergillosis (COPD), invasive aspergillosis, and active infection in patients with chronic pulmonary aspergillosis (CPA). Therefore, understanding the pathophysiological mechanisms of fungal colonization in disease is essential to develop strategies to avert or minimise disease. *Aspergillus* cell components promoting fungal adherence to the host surface, extracellular matrix, or basal lamina are indispensable for pathogen persistence. However, our understanding of individual differences in clearance of *A. fumigatus* from the lung in susceptible patients is close to zero.

Key words: *Aspergillus*, colonization, aspergillosis.

Introduction

Aspergillosis affects more than 14 million people worldwide, with allergic bronchopulmonary aspergillosis (ABPA, >4 million), severe asthma with fungal sensitization (>6.5 million), and chronic pulmonary aspergillosis (CPA, ~3 million) being considerably more prevalent than invasive aspergillosis (IA, >300000).[1] Other common conditions include *Aspergillus* bronchitis, *Aspergillus* rhinosinusitis (many millions), otitis externa, and *Aspergillus* onychomycosis (10 million).[2] Alterations in the composition and function of the lung microbiome and mycobiome have been associated with an increasing number of chronic pulmonary diseases such as COPD, cystic fibrosis, chronic rhinosinusitis and asthma.[3]

Aspergillus fumigatus is a ubiquitous saprophytic fungus to which humans are constantly exposed.[4] Its small spore size facilitates deposition throughout the airways with many spores reaching the alveoli. In the healthy host, conidia are rapidly cleared from the lung by immune defences.[5] However, in some immunocompromised patients, those with cavities in their lungs or asthmatics, *Aspergillus fumigatus* can evade the host immune response and colonize or invade the airway leading to disease.[6] Host and fungal factors contributing to invasive disease have been widely studied in recent years. However, the pathophysiology of ABPA and CPA, where fungal colonization and infection of the respiratory airways by *A. fumigatus* is a key factor, has been a neglected research topic. For the purpose of this review we define fungal colonisation as the presence of noninvasive actively growing fungi in the respiratory airways. Fungal colonization does not necessarily involve direct host damage or disease and it is especially important in patients with *Aspergillus* bronchitis.[7] However, the features of the host-pathogen interaction that allow fungal colonization are unknown. One hypothesis is that host lung defences are ineffective as fungal germination and persistence occur, or alternatively they might be very effective

because no disease ensues. Here we review the current knowledge about *Aspergillus* lung colonization and its importance in disease.

Fungal exposure and disease

Respiratory exposure to airborne fungi is constant.[8] Humans inhale many litres of air every minute, and with each breath we aspirate many fungal spores, fragments, and allergens.[9] The capability of the spores and fungal particles to penetrate into the lung depends on the spore size, the degree of airway branching, and the tubule diameter of the lung structures.[10] Fungal species with a spore size higher than 5 μm, such as *Alternaria* spp. or *Cladosporium* spp., are mainly deposited in the upper respiratory airways, whereas fungal species with a small spore size such as *Aspergillus*, can be deposited throughout the entire lung including the distal alveolus.[11] The capability of fungal species to cause disease is driven by the virulence and quantity of the inhaled fungus and the immune status of the host.[9] For primary pathogenic fungi like *Coccidioides* spp., *Blastomyces* spp., *Histoplasma* spp., or *Cladophialophora* spp., that can cause disease in the healthy host, exposure is a major driver of disease.[12] However, infections with other more prevalent environmental fungi only appear in some people with an impaired immune system.[13]

The initial lung response to fungal species is driven by the antifungal potential of the lung epithelium.[14] In the upper respiratory airway a mucus layer on top of ciliated epithelial cells captures and reverses the direction of the inhaled fungus and hyphal fragments. Additionally a subset of specialized epithelial cells contributes to the antimicrobial potential of the lung epithelium by secreting mucus, surfactants, and antimicrobial peptides and attracting more specialized immune cells contributing to fungal clearance.[9,14] Moreover, alveolar epithelial cells and alveolar macrophages contribute to the clearance of small spore size fungi and fragments by phagocytosis.[15,16]

Exposure to fungus is inescapable, but disease is not. Abnormal functioning of lung defences will facilitate pathogen persistence, colonization and the development of disease.

Colonization in the clinical spectrum of pulmonary aspergillosis

From a biological point of view, fungal colonization involves the establishment of fungal species in a particular niche without immediately causing disease.[17] However, fungal colonization is the antecedent for local airway infection in both chronic and allergic fungal disease[6] and also in invasive disease. The definition of clinical criteria to evaluate fungal colonization of the respiratory airways differs between experts (mostly because testing is of variable sensitivity), but it normally includes the presence of a single or serial positive tests for the detection of fungal species in a lower airways clinical sample (sputum or bronchoalveolar lavage) (Fig. 1).[18,19] Current culture methods to detect fungal colonization are insensitive, compared with molecular methods[19,20] leading to an underestimated prevalence. Therefore, distinction between disease and colonization is likely to be biased in one direction or another by detection methodology. The impact of fungal colonization of the respiratory airways differs across the clinical spectrum of disease. However, there is increasing evidence suggesting *Aspergillus* colonization should be used as a parameter to predict risk of disease in susceptible patients.[21]

Figure 1. High volume culture of sputum from a patient with *Aspergillus* colonization and probably *Aspergillus* bronchitis. With permission and thanks to Prof Malcolm Richardson, NHS Mycology Reference Laboratory, Manchester. This Figure is reproduced in color in the online version of *Medical Mycology*.

Aspergillus colonisation in Invasive Aspergillosis

Invasive aspergillosis (IA) is an opportunistic fungal infection in which lung tissue invasion by *A. fumigatus* or other species occurs.[6] IA is a growing health concern due to the broad and increasing range of susceptible patients, insensitivity of diagnostic testing and high mortality that can reach 94%.[22–24] The highest risk patient groups include immunocompromised, neutropenic patients, those receiving a solid organ transplant, individuals with AIDS, COPD, liver failure, and severe influenza.[24–27] Diagnostic microbiological criteria comprise the direct or indirect detection of *A. fumigatus* in sterile clinical samples. The significance of *Aspergillus* colonization of the lower respiratory airways in many patients is unclear as they may be temporary carriers, especially of *A. niger* complex species.[28] However, several publications indicate fungal colonization to be an important risk factor for the development of IA.[29–31] Lass-Florl et al. have reported fungal colonization as a possible source of endogenous spread in critically ill patients[32] leading to reduced short-term

survival.[33,34] Moreover, a retrospective study recently published by Barberán et al.[35] has shown that 18% of colonized patients develop IA, and this is especially significant in patients with COPD. In leukemia, isolation of *Aspergillus* from the nose has a 90% correlation with invasive pulmonary aspergillosis.[36] Entering the hospital for chemotherapy for leukemia with a positive nasal culture carries a poor prognosis because of the high likelihood of invasive pulmonary aspergillosis.[31] In liver transplant recipients, respiratory cultures positive for *Aspergillus* had a sensitivity of 79% and specificity of 68% for IA.[37] Altogether, this suggests asymptomatic colonisation of the respiratory tract needs close follow-up as it can lead to clinical disease especially in immunocompromised individuals.[38–40]

Aspergillus persistence in chronic pulmonary aspergillosis

Chronic pulmonary aspergillosis (CPA) is a slowly destructive pulmonary disease mainly caused by *A. fumigatus*, although other *Aspergillus* species can be involved.[6] This syndrome is characterised by progressive cavitation, fibrosis and pleural thickening.[41] CPA mainly occurs in patients with an altered lung structure such as those with underlying cavitating disease. In those patients, *Aspergillus fumigatus* can colonise and grow into a cavity damaging the surrounding parenchyma. The evolution and clinical presentation of CPA varies considerably. Some immunocompromised patients (e.g., with AIDS, corticosteroid treatment, or diabetes) develop subacute invasive aspergillosis (or chronic necrotising pulmonary aspergillosis) characterized by an evolution of symptoms and disease over 1–3 months, usually with enlarging cavities or nodules with or without cavitation. Histology in subacute invasive aspergillosis shows hyphae present in the lung parenchyma with necrotic tissue or inflammation.[42] On the other hand, chronic cavitary pulmonary aspergillosis appears in those patients not severely immunocompromised typically complicating other conditions like tuberculosis. In this case, the evolution of the disease is slow and *A. fumigatus* growth is confined to the wall of a lung cavity, with a local chronic inflammatory reaction. A fungal ball (aspergilloma) may appear in the cavity as fungal growth detaches from the cavity wall. Granulomas are occasionally found together with a chronic inflammatory cell infiltrate leading to pleural fibrosis.[6,42] CPA is thought to affect more than 3 million people worldwide with a mortality of up to 80% over 5 years.[1,41] The use of undiluted or high volume of sputum culture has been described as a useful tool to support the diagnosis of CPA as bronchoscopy is not always recommended in those patients.[43,44] Positivity rates of respiratory cultures of patients with CPA ranges from 17% to 32% in the small number of available studies.[19,45,46] However, the use of more sensitive tools such as real-time polymerase chain reaction (PCR) allows the correlation of low Ct values with pulmonary infection. In a study by Denning et al., 71.4% of patients with CPA had sputum positive *Aspergillus* PCR results.[19] As CPA mainly affects patients with a history of tuberculosis, COPD, and lung surgery, early intervention is required for cases in which *Aspergillus* species are isolated from respiratory samples.[47]

Aspergillus colonization in allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitization (SAFS)

Bronchial colonization by *A. fumigatus* in patients with asthma and cystic fibrosis drives the development of ABPA.[17] This Th2 hypersensitivity reaction to *A. fumigatus* antigens is characterised by immunoglobulin E (IgE) production, eosinophilia, mast cell degranulation, and bronchiectasis.[48,49] ABPA manifests as poorly controlled asthma alongside other symptoms including fatigue, persistent cough, and mucus plugs that can present with "pneumonia."[6] Histopathological examination reveal chronic inflammation, eosinophilia and fungal hyphae in mucous plugs.[50] Moreover, fungal colonization of the respiratory airways in some patients with severe asthma is linked to the development of fungal sensitization.[51] Although these diseases are not usually fatal, the global burden probably exceeds 10 million adults worldwide, and probably many children.[1,52,53] Overall, fungal colonization in *Aspergillus*-allergic diseases leads to decreased FEV-1 values, more rapid pulmonary function decline, airway obstruction and bronchiectasis.[54–59] The percentage of *Aspergillus* recovery from sputum cultures in patients with fungal allergic diseases varies from 0 to 60% of ABPA patients and it less than 10% in patients with SAFS.[19,55,60] However, using more sensitive approaches such as *Aspergillus*-specific real-time PCR, more than 70% of patients with ABPA and SAFS showed positive signals in several studies.[19,54,55] In addition, *A. fumigatus* PCR become negative after beneficial itraconazole treatment in some patients with cystic fibrosis and SAFS illustrating a plausible benefit of antifungal treatment on those patients.[61–63]

Importance of *Aspergillus* colonization in *Aspergillus* bronchitis

Aspergillus bronchitis is a chronic superficial infection of the lower respiratory airways that affects nonimmunocompromised patients. Patients with symptoms of chronic pulmonary disease, microbiological evidence of *Aspergillus* in the airways (culture or PCR) and *Aspergillus*-specific immunoglobulin G (IgG) antibodies that do not fulfil the diagnostic criteria for CPA, ABPA or invasive aspergillosis may have *Aspergillus* bronchitis (Fig. 1).[7] Chrdle et al.[7] reported repetitive identification of *Aspergillus* spp. in respiratory samples by culture or real-time PCR as key features for diagnosis of *Aspergillus* bronchitis. These patients present with breathlessness, repetitive infections that do not clear well with antibiotics (despite often growing bacteria in sputum) and occasionally severe mucus plugging. Bronchoscopic examination reveals mucus with ulceration and superficial hyphal invasion can be detected in biopsies.[7] Although *A. fumigatus* is the most

common species causing *Aspergillus* bronchitis, *A. niger*, *A. terreus*, and *A. flavus* have been described as causative agents.[7,64] Coinfection with bacterial pathogen is common in *Aspergillus* bronchitis. Moreover, a recent study has estimated the prevalence of *Aspergillus* bronchitis in 9% of cystic fibrosis colonised patients.[65] Other comorbidities include COPD, asthma, and the use of inhaled corticosteroids.[7]

Aspergillus colonization in COPD

A. fumigatus colonisation of the airways in patients with COPD manifests as positive sputum cultures and may proceed to severe CPA in some cases.[47] Some studies have reported high rates of positive sputum cultures in this patient group.[66,67] Moreover, fungal colonization of the airways in patients with COPD is associated with worse lung function, as it leads to *Aspergillus* sensitization.[68] Patients with COPD are moreover at risk of developing invasive aspergillosis (IA)[66] and CPA.[47] It is likely, but not proven, that colonized patients are greatest risk of developing IA when they have a COPD exacerbation. Moreover corticosteroid treatment of patients with COPD has been described as a risk factor for the development of invasive aspergillosis.[69]

Aspergillus factors promoting persistence

A. fumigatus spores can bind host cells, extracellular matrix, and basal lamina components. In the healthy host, basal lamina constituents are not exposed to inhaled fungus, but in some patients with an altered lung structure such as asthmatics or cystic fibrosis patients, fungal spores can adhere to collagen and fibronectin fibres in the basal lamina facilitating its persistence and the development of fungal disease.[70–75] *Aspergillus* adherence to the human lung lumen is a major mechanism of colonization.[14,74] Thus, the main mechanism leading attachment are described below.

The first *A. fumigatus* surface protein described to mediate adherence to host cells was RodA.[76] RodA assembles into a multilayer system on the outer conidia surface. This conidial hydrophobin protein mediates albumin and collagen binding.[77–79] In addition, unmasked rodlet structure of *Aspergillus* conidia contributes to mediate immune evasion early after inhalation.[80] Similarly, the fungal allergen Asp f 2 binds laminin in vitro, although its impact during infection has not been addressed so far.[81] Several studies indicate that negatively charged carbohydrate motifs on the conidial surface contribute to host adhesion.[82] *A. fumigatus* carbohydrates can bind host ficolins, a family of soluble lectin-like opsonin involved in innate immunity.[74] This interaction promotes complement activation, facilitates adhesion to the surface of A549 alveolar epithelial cells leading to interleukin (IL)-8 release.[18,83–85]

It is known that adherence of *Candida albicans* to host cells and subsequent biofilm formation is mediated by GPI-anchored cell wall proteins.[86] Recently, the importance of a putative GPI-anchored protein, cspA, in mediating *A. fumigatus* binding to the host has been evaluated in an *in vitro* model of infection using A549 alveolar epithelial cells.[87] CspA present in swollen spores, germinating conidia, and hyphae can bind laminin *in vitro*, but it is not a virulence driver in a murine model of invasive aspergillosis model.[88] Moreover, adhesion of *A. fumigatus* CspA deficient mutants to the extracellular matrix is only 50% reduced compared to the parental strain suggesting multiple conidial adhesion factors in *A. fumigatus* to mediate this process.[87,88] Transcriptomics and proteomic analysis of clinical and environmental *A. fumigatus* strains have demonstrated the existence of putative proteins that might act as adhesins, although further confirmation is required.[89,90] The most studied has been CalA, a protein required for spore germination and hyphal formation.[90] Recombinant CalAp binds laminin, murine epithelial cells, and spleen cells *in vitro*. However, an *A. fumigatus* ΔCalA mutant was able to bind laminin and A549 epithelial cells similarly to the parental strain indicating a limited role of CalA in mediating adhesion.[90] A recent study indicates CalA induces endocytosis by interacting with integrin $\alpha 5\beta 1$ on host cells.[91]

The exopolysaccharide galactosaminogalactan (GAG) is a secreted and hyphal constituent of *A. fumigatus* that require deacetylation to mediate adherence to the host extracellular matrix and epithelial cells.[92–95] GAG deacetylation is controlled by agd3. Deletion of agd3 is associated with loss of adherence and augments the positive charge on the surfaces of hyphae.[92]. GAG is composed of variable number of galactose residues linked to N-acetylgalactosamine units which are synthesised through the activity of the protein products of a co-ordinately regulated five-gene cluster.[96] Degradation of GAG by hydrolytic enzyme therapy impairs fungal invasion of tissues in a model of invasive aspergillosis.[97] The uncommon organisation of the GAG biosynthetic pathway is reminiscent of a bacterial operon. The *A. fumigatus* glucose-4 epimerase (uge3) regulates the synthesis of UDP-galactose and UDP-N-acetylgalactosamine.[98] *A. fumigatus* uge3 deficient strains show reduced capability to adhere and cause cell cytotoxicity in A549 alveolar epithelial cell monolayers.[94] The transcription factor SomA is a major regulator of genes required for *A. fumigatus* adhesion including medA,[99] a developmental regulator, and uge3. *A. fumigatus* strains lacking somA function are associated with reduced GAG expression, impaired adherence and biofilm formation.[100] PtaB regulates the expression of uge3 but also agd3.[100,101] A recent study indicates PtaB inversely regulates *A. fumigatus* biofilm formation and conidiation.[101]

Finally, *A. fumigatus* extracellular proteases are among the most common fungal allergens. They are secreted during active fungal growth and lead to epithelial cells desquamation and the release of pro-inflammatory cytokines.[14,102–105] Several authors postulated that this is a key fungal mechanism to facilitate pathogen persistence in the respiratory airways. In addition, *A. fumigatus* DHN-melanin is recognised by the C-type lectin

receptor MelLec (CLEC1a), and this is required for antifungal immunity in a mouse model of invasive infection. A single nucleotide polymorphism in MelLec has been described to increase the risk of invasive aspergillosis in stem-cell transplant recipients by diminishing the IL-8 and IL-1β response,[106] which might contribute to pathogen persistence.

Overall, *A. fumigatus* adherence drivers have been observed using *in vitro* models of immortalized cell lines and murine models of invasive disease in which colonization is a minor pathophysiological mechanism of disease. The lack of well-established animal models to study chronic forms of aspergillosis has constrained the investigation of *A. fumigatus* factors promoting its persistence in the context of ABPA and CPA.

Host factors promoting *A. fumigatus* colonization

The anti-*Aspergillus* function of the lung is achieved by the presence of airway epithelial cells, macrophages, and dendritic cells that are the first line defenses to recognize and react to inhaled fungi.[9,14,77] Consequently, after fungal exposure, airway epithelial cells contribute to the cytokine milieu facilitating the recruitment of leukocytes to the site of infection and orchestrating the downstream inflammatory response leading fungal clearance.[15,16,107] Immunocompromised patients such as those on acute immunosuppression with neutropenia are at risk of developing invasive fungal disease such as invasive pulmonary aspergillosis.[6] However, the mechanisms leading to fungal persistence in nonimmunosuppressed patients, such as those with CPA or ABPA, are likely to be different due to the more complex immune environment.

ABPA complicates asthma and cystic fibrosis.[108,109] An altered mucociliary clearance response of the lungs of these susceptible patients probably facilitates fungal growth and mucosal colonization.[110] However, ABPA affects less than 4% of at-risk patients, and genetic susceptibility factors might be important.[17] There are an increasing number of genetic association studies in host response genes including HLA-DR,[111,112] IL-4R,[113] SP-A2,[114] IL-10,[115] TLR9,[116] IL-13, or TLR3,[117] which suggests underlying abnormalities in both adaptive and innate responses. However, some of these associations are not strong, might be related to disease progression and a very low number have been described as colonisation risk factors. Brouad et al.[115] described the -10822GG genotype in the IL-10 promotor region as a predisposing factor of *Aspergillus* colonization and ABPA development in cystic fibrosis patients. Moreover, patients colonized by *A. fumigatus* showed high serum levels of IL-10 demonstrating an immune response against actively growing fungus. In addition, two polymorphisms in the collagen region of SP-A2 might facilitate *A. fumigatus* adhesion leading increased levels of total immunoglobulin E (IgE) antibodies and eosinophilia in those patients.[114] As described above, phagocytosis is important in *A. fumigatus* clearance. ABPA-associated genetic variants in the early endosome antigen 1 (EEA1) results in an exaggerated cellular response to the presence of *A. fumigatus* colonising the airways of those patients.[118]

Our knowledge about the pathophysiology of CPA is limited and focused on lung abnormalities in susceptible patients. Genetic susceptibility factors to CPA are poorly understood although disease susceptibility might be polygenic. Genetic variants in TLR1, Dectin-1, PLAT, VEGFA, DENNDB, IL-1β, IL-1RN, and IL-15 have been described as risk factors for CPA.[119–121] These genetic variants might indicate a reduced ability of the human host to efficiently remove *A. fumigatus* from the respiratory airways resulting in persistent inflammation and fibrosis. Moreover, *A. fumigatus* persistence in the respiratory airways from patients with CPA leads to increased levels of secreted pro-platelet basic proteins, a neutrophil attractant.[122]

Kolwijck and van de Veerdonk have recently suggested that changes in the composition of the normal flora in the respiratory airways of patients susceptible to aspergillosis might lead to filamentous fungal colonization of the airways and the development of disease.[123] In a retrospective study in patients with COPD, *A. fumigatus* was isolated in 17% of sputum samples. Moreover, co-isolation of *A. fumigatus* and *Pseudomonas aeruginosa* were associated with a higher risk of fungal colonization,[124] probably related to higher GAG production.[97] However, this was not associated with worse disease outcome. The altered structure of the lung epithelium in patients with COPD due to cigarette smoking can promote pathogen adhesion.[125] In addition, H1N1 influenza infection has been described as a driver of alterations in the lung microbiome composition promoting invasive aspergillosis.[126,127]

Antibiotic misuse and corticosteroid treatment in patients with ABPA have been associated with fungal colonisation. Noverr et al.[128] demonstrated using a murine model of allergic airway disease that antibiotic exposure has an impact in the gastrointestinal enteric bacteria composition promoting the development of the *A. fumigatus*-mediated allergic response. Since the gut microbiome modulates the mucosal response of distant organs, changes in the microbiome composition are likely to influence the anti-*Aspergillus* response.[129] In the same way, long-term antibiotic therapy in cystic fibrosis and chronic granulomatous disease patients is strongly associated with high risk of *A. fumigatus* colonization.[130,131] Fungal colonization of the respiratory airways is universal, and low fungal burdens can be found in healthy subjects.[19,32] A recent study by Fraczek et al. has identified the composition of the lung mycobiome in patients with allergic fungal disease, ABPA and SAFS.[20] Fungal burdens varied between individuals, but in general patients with well-characterized fungal disease or asthma had higher fungal burdens than healthy controls. Corticosteroid treatment is a known risk factor for the development of invasive aspergillosis, but it is also frequently used to treat asthmatic patients. This study has revealed for the first time to our knowledge that patients on corticosteroid treatment without antifungal therapy had higher

fungal loads in the lungs.[20] Corticosteroid treatment in asthmatics might locally impair *A. fumigatus* phagocytosis and promote its persistence in the upper respiratory airways. Corticosteroids have no impact on the IL17A response in asthma,[132,133] a mechanisms thought to be important in fungal defense.[134]

Modelling airway colonization

The study of the pathophysiology of invasive aspergillosis has been supported by the development of murine models of disease. These reproducible animal models mimic the clinical course and symptoms of invasive disease in humans (reviewed in[135]). However, the lack of airway fungal colonisation models has constrained the study of the more common clinical manifestations of aspergillosis where colonization is a key factor including ABPA, CPA, and *Aspergillus* bronchitis. This might be due to (i) the available models use conidia as infecting particle while cell wall composition and secreted factors differs for hyphae,[136] and (ii) it is very difficult to model in mice (or other species) the wide variety of underlying disease phenotypes in patients in which fungal colonization is a pathophysiological factor.

Most of the *Aspergillus* hyphae airway colonization models have been developed using immunocompetent mice.[137,138] These models are based on the induction of airway colonization by intratracheally infected mice with conidia embedded in agar beads. This model allows hyphae to persist in the lungs of mice for up to 28 days without developing invasive disease and correlates with a Th2 response early in the course of colonization.[137] However, adapting these models for ABPA or CPA where the natural host has another underlining disease is a challenge.

In addition, several *in vitro* models to study *Aspergillus*-host interaction have been developed in the last years. Most of them are focused in the co-culture of immortalized lung cell lines with *Aspergillus fumigatus* conidia or gremlins for a fixed period of time.[16,85,97,102,103,118] These models have been very useful to determine the initial steps of the interaction between *Aspergillus fumigatus* and the host and the cell biology underlining these processes described in this review. However, *Aspergillus fumigatus* growth in these *in vitro* systems cannot be controlled, and growing hyphae induce cell dead after a long incubation time. The available *in vitro* models therefore represent an infection process instead of colonization. Further works are need it in order to develop an *in vitro* colonization model in which growing hyphae do not damage the cells by the use for example of a two compartment system using trans-well inserts.

A clear understanding of the pathophysiological mechanisms underlying *Aspergillus* colonization in patients with allergic and chronic forms of pulmonary aspergillosis is essential to minimize the impact of these prevalent infectious diseases. Colonization is clearly an important prerequisite to subsequent fungal infection. Inhalation of air containing *Aspergillus* spores is inescapable, and the development of disease will be determined by the immune status of the host. However, genetic factors appear to be important as not all at risk patients develop disease. Even though the nature of CPA and ABPA suggests these diseases to be multigenic, most of the genetic-association studies describing genetic risk factors for the development of aspergillosis have been performed using preselected groups of genes in a limited number of patients, and most of them lack functional validation. This approach limits the applicability of the results and focuses future research on specific, mundane, aspects of the immune response of patients masking other potential pathways leading to disease. Moreover, the lack of animal models that can faithfully reproduce ABPA and CPA significantly narrows the study of the pathophysiological mechanisms leading to fungal colonization, a key driver of pathogen persistence in those diseases. It is of utmost importance to characterize genetic factors that contribute to the mycobiome composition in patients with fungal disease in order to develop tools for stratification of patient therapy.

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Declaration of interest

S.G. declares no conflicts of interests. D.W.D. and family hold founder shares in F2G Ltd, a University of Manchester spin-out antifungal discovery company, and in Novocyt, which markets the Myconostica real-time molecular assays; he has a patent for fungal infection assays that has been externally licensed; he acts or has recently acted as a consultant to Astellas, Sigma Tau, Basilea, Scynexis, Cidara, Biosergen, Quintilles, and Pulmocide; and in the last 3 years he has been paid for talks on behalf of Astellas, Dynamiker, Gilead, Merck, and Pfizer. He is a longstanding member of the Infectious Disease Society of America Aspergillosis Guidelines group, the European Society for Clinical Microbiology and Infectious Diseases Aspergillosis Guidelines group and the British Society for Medical Mycology Standards of Care committee. P.B. is a founder of Alergenetica SL. and Syngenics Ltd.

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