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Aspergillus infections in cystic fibrosis

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Summary Patients with cystic fibrosis (CF) suffer from chronic lung infection and airway inflammation. Respiratory failure secondary to chronic or recurrent infection remains the commonest cause of death and accounts for over 90% of mortality. Bacteria as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex have been regarded the main CF pathogens and their role in progressive lung decline has been studied extensively. Little attention has been paid to the role of *Aspergillus* spp. and other filamentous fungi in the pathogenesis of non-ABPA (allergic bronchopulmonary aspergillosis) respiratory disease in CF, despite their frequent recovery in respiratory samples. It has become more apparent however, that *Aspergillus* spp. may play an important role in chronic lung disease in CF. Research delineating the underlying mechanisms of *Aspergillus* persistence and infection in the CF lung and its link to lung deterioration is lacking. This review summarizes the *Aspergillus* disease phenotypes observed in CF, discusses the role of CFTR (cystic fibrosis transmembrane conductance regulator)-protein in innate immune responses and new treatment modalities.

Introduction

Cystic fibrosis (CF), caused by a mutation in a gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR) protein, is the most common fatal genetically inherited disease in Caucasian populations affecting 1 in 2400 live births.[1] Around 2000 CFTR mutations have now been identified of which the single amino acid deletion, F508del, is the most common and accounts for around 70% of disease.[2] Over a generation CF has transformed from a disease of early childhood mortality to a disease with a current median survival of 28 years and projected survival into the fifth decade for a child born today.[3]

The absence of the CFTR-protein, which acts as an ATP-driven chloride channel in the cell membrane, leads to defective ion fluxes and intracellular calcium homoeostasis. In airway epithelial cells this leads to thickened mucus impairing an efficient mucociliary clearance of inhaled pathogens and results via a cycle of infection and excessive inflammation in progressive airway damage and ultimately respiratory failure.[4,5] Mortality and morbidity of CF patients is almost exclusively due to chronic lung infections and airway inflammation. Prevention and treatment of airway infection as a means of intervening in this destructive cycle has been the mainstay of clinical management but, despite this, respiratory failure secondary to chronic or recurrent infection remains the commonest cause of death and accounts for over 90% of mortality.[1] Research has traditionally

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focused on the role of bacterial pathogens, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Burkholderia cepacia* complex, in the pathogenesis of respiratory decline. Despite the frequent isolation in respiratory samples, little attention has been paid to the role of *Aspergillus* spp. and other filamentous fungi in the pathogenesis of non-ABPA (allergic bronchopulmonary aspergillosis) respiratory disease in CF. It has become more apparent however, that *Aspergillus fumigatus* may play an important role in the CF lung as well.[6] Current literature delineating the underlying mechanisms of *A. fumigatus* persistence in the CF lung and its link to lung deterioration is lacking. It has been shown that CF airway epithelial cells display reduced uptake and killing of *A. fumigatus*.[7] The observation that CF patients have a higher risk of developing invasive aspergillosis after lung transplantation than non-CF transplant patients suggests impaired antifungal effector mechanisms of CF immune cells.[8,9] This review summarizes the *Aspergillus* disease phenotypes observed in CF, discusses the role of CFTR-protein in innate immune responses and new treatment modalities.

Aspergillus phenotypes in cystic fibrosis

Aspergilli are saprophytic, spore-forming, filamentous fungi found ubiquitously in the environment. *A. fumigatus* is the most prevalent species causing human disease and is the species most frequently isolated from the respiratory secretions of CF patients.[10,11] *A. fumigatus* disperses in the environment by releasing small, hydrophobic, airborne spores (conidia) which we all inhale hundreds of every day.[12] Their small size (2–4 μm) allows them to reach the terminal alveoli of the lung where, in the healthy host, they are rapidly cleared by resident alveolar macrophages and the mucociliary escalator without triggering a significant inflammatory response.[13] Failure of mucociliary clearance of inhaled conidia leads to persistence in the CF lung environment. This can result in several distinct clinical phenotypes; *Aspergillus* can persist without obvious respiratory decline (*Aspergillus* colonization)[14]; development of localized infection, mucosal inflammation and worsening respiratory disease without obvious allergic responses (*Aspergillus* bronchitis)[15,16]; or trigger an IgE-mediated hypersensitivity response either with or without respiratory exacerbation, airway inflammation, and the development of bronchiectasis and fibrosis (*Aspergillus* sensitization and ABPA respectively).[6,16,17]

Aspergillus colonization

The reported prevalence of *Aspergillus* colonization in CF patients ranges from 10 to 57% with the frequency of isolation increasing with increasing age and worsening respiratory function.[18–22] Reported rates vary considerably across all age ranges.[6,11,17] While this may be due in part to differences in the definition of colonization and geographical differences in exposure, differences in sample obtainment, storage, and laboratory processing likely represent the greatest determinants of variation in reported prevalence.[14,23] The prevalence of *Aspergillus* colonization in paediatric CF patients is not well established. Recent work by Coburn et al. to characterize the lung microbiota in a large cohort of CF patients (aged 0–60 years) found *Aspergillus* spp. were significantly more likely to be detected in sputum samples from adults than children (33.5% versus 17.1% respectively, $p = 0.011$).[24] This is in line with a reported mean age of 12.3 years at time of first isolation of *A. fumigatus*.[10] However immune responses to *Aspergillus* spp. have been demonstrated at much earlier time points. El-Dahr et al. showed that specific IgG to *A. fumigatus* antigens was already present in 41% of children by 4 years-of-age and in 98% by 10 years-of-age.[25] The median age for first detection of specific IgE to *A. fumigatus* showed to be as early as 5.5 years (range 4.2–9.7 yrs).[26] As positive serology tends to follow isolation in respiratory samples, the burden of *Aspergillus* spp. in the paediatric lung is underestimated.[27] Smaller volume sputum samples and reliance on non-invasive sampling techniques, such as cough swabs, in children unable to expectorate sputum may limit mycological detection.[11,23] Additionally, only 60% of fungi identified in respiratory secretions using molecular techniques were recoverable on mycological culture suggesting a reliance on standard, culture-based, mycological detection methods may further underestimate fungal burden.[28]

A large retrospective cohort study of paediatric non-ABPA CF patients in Canada showed that two or more respiratory samples positive for *A. fumigatus* in any given year was associated with a significant reduction in FEV1 ($p = 0.0001$) and a significant increase in pulmonary exacerbations requiring hospitalization (relative risk (RR) = 1.94, $p = 0.0002$) compared with paediatric CF patients without *A. fumigatus* in collected respiratory samples.[22] Although, others have failed to show an association between lung function or radiological abnormalities and *Aspergillus* colonization.[18–20] A retrospective cohort analysis assessing the effect of *A. fumigatus* colonization on lung function in adults and children with CF showed that colonized patients had a worse lung function at baseline.[20] It remains an open question whether this poorer lung function at baseline was due to the *Aspergillus* colonization or because of more severe respiratory disease. A study investigating inflammatory responses in bronchoalveolar lavage (BAL) fluid of paediatric CF patients (<7 years-of-age) with and without *Aspergillus* colonization showed significantly increased neutrophil counts, increased free neutrophil elastase activity and increased IL-8 levels in those colonized.[29] The observation of this increased inflammatory response is important as neutrophilic inflammation in the CF lung is associated with more severe lung disease and is thought to play a significant role in the pathogenesis of respiratory failure.[5,30,31]

Given the potential contribution of *Aspergillus* colonization to pulmonary inflammation and lung function decline, antifungal therapy may have a role in the management of *Aspergillus* colonization in CF patients. A double blind, randomized, placebo-controlled trial of itraconazole treatment in *A. fumigatus* colonized non-ABPA CF patients did not show any clinical benefit.[32] However the study population was small ($n = 35$) and 43% of patients failed to achieve adequate itraconazole levels. It remains therefore to be seen if clinical benefit can be achieved with adequate antifungal drug exposures.