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Aspergillus infections in cystic fibrosis

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Aspergillus;
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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.

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Aspergillus bronchitis

Shoseyov et al. described a group of six CF patients (aged 10–30 years) experiencing respiratory exacerbations not responding to appropriate antibiotic treatment who had sputum cultures positive for *Aspergillus* spp. and responded to antifungal treatment.[15] These patients did not fulfil the criteria for ABPA and the disease described, which they defined as *Aspergillus* bronchitis, appears clinically distinct from other manifestations of aspergillosis in CF. In the proposed novel diagnostic classification system of *Aspergillus* disease in CF using molecular detection methods combined with immunological disease markers, a distinct sub-group of CF patients was found characterized by the presence of high fungal burden without evidence of a hypersensitivity response.[16] Surprisingly, they found that 30% of their adult CF patients fell within this category termed *Aspergillus* infection/bronchitis. *Aspergillus* bronchitis has been described in immunocompetent hosts with structural lung abnormalities with a clinical presentation including productive cough, tenacious sputum, breathlessness and haemoptysis; symptoms indistinguishable from a bacterial exacerbation in CF.[16,33] CF patients with *Aspergillus* bronchitis may benefit from antifungal therapy as this study showed a significant clinical improvement with the use of antifungals.[33]

Aspergillus sensitization and ABPA

Allergic bronchopulmonary aspergillosis is the most well-characterized and well-recognized *Aspergillus* disease in CF but reported prevalence varies significantly (range 2–25%) and is likely to be underdiagnosed.[6,34–36] Comparable ABPA prevalences in paediatric and adult CF patients were found based on national CF registry data although huge variations were observed between countries with a prevalence as low as <1% in Serbia up to 18.6% in Switzerland.[6]

ABPA is the result of a Th2-mediated hypersensitivity response to *Aspergillus* allergens seen in CF and asthma patients. It manifests clinically with respiratory exacerbations, especially wheeze, positive antibodies against *Aspergillus* and characteristic radiological abnormalities.[17] Over time it can result in bronchiectasis, lung fibrosis and ultimately respiratory failure. Risk factors for developing ABPA include increasing age, reduced lung function and chronic airway infection.[35] Diagnosis can be difficult due to the similarity in clinical symptoms and radiological abnormalities to those found during bacterial exacerbations.[17,35] In addition, a recent review of clinical ABPA studies showed that half of the studies used criteria other than the previously published consensus statement for diagnosing and management of ABPA suggesting a lack of consensus in current practice and accounting for the ongoing variation in reported prevalence.[36]

Different from ABPA, patients can become sensitized to *Aspergillus* spp., with serological evidence of hypersensitivity but without apparent clinical exacerbation.[16,37] Baxter et al. showed to be able to distinguish *Aspergillus* sensitization from ABPA by the absence of raised serum IgG and a negative galactomannan, suggesting a distinct pathophysiological process.[16] Around a third of adult CF patients are estimated to be affected by either ABPA or *Aspergillus* sensitization, both of which have shown to significantly and independently reduce lung function,[37–40] highlighting again the importance of appropriate identification of *Aspergillus* disease in CF patients. Despite the high prevalence of ABPA in adolescence, no attempt has been made to validate this classification in children and there are no corresponding paediatric estimates of the overall prevalence of *Aspergillus* disease in paediatric CF. In addition, there is no consensus on appropriate diagnostic criteria for ABPA in children and to date this has received barely any attention.

The current recommended first line treatment for ABPA in CF is oral prednisolone.[17,41] Corticosteroids appear to show substantial clinical and radiological benefit although this has never been validated in randomized controlled trials.[42] Corticosteroids may interfere with antifungal host defence by impairing antifungal defence mechanisms in phagocytes.[12] Concerns regarding the potential adverse effects of corticosteroid use may in part explain the observation that only 56% of CF patients with ABPA are prescribed oral steroids.[34,41] Itraconazole is recommended as rescue therapy in CF patients with ABPA and is widely used in clinical practice.[17] Observational studies have shown a consistent improvement in disease symptoms, frequency of exacerbations, improvement in lung function and biomarkers, and a reduction in steroid use. The suggestion has been made that itraconazole is beneficial but all studies performed are of low quality, many studies have shown significant adverse effects of treatment, and two separate Cochrane reviews have found insufficient evidence to support the use of antifungal agents for the treatment of ABPA in CF patients.[42,43] Voriconazole and posaconazole are potentially good alternatives but studies are lacking. The use of voriconazole is hampered by drug–drug interactions, the need for careful therapeutic drug monitoring, and the risk of photosensitivity and the development of skin cancers.[44]

The recombinant humanized monoclonal anti-IgE antibody, Omalizumab, has shown benefit in allergic asthma and has been proposed as a potential treatment for ABPA in CF.[45] In eight case reports, 13 children with CF and ABPA received Omalizumab resulting in improved FEV1, fewer respiratory symptoms and decreased corticosteroid use.[46] The only randomized controlled trial in CF was prematurely terminated due to an inability to enrol and maintain trial participants.

Separate disease entities versus continuum of disease

Taken together the clinical disease entities, the mycological and immunological classification as recently proposed by Baxter et al.,[16] two alternative models of the development of *Aspergillus* disease in CF can be postulated.[6] One model represents sequential development of mild-moderate *Aspergillus* disease to severe disease, e.g. ABPA following *Aspergillus* sensitization and development of *Aspergillus* bronchitis following persistent colonization. The other model considers the four disease entities as separate entities and each entity can progress in terms of severity and lead to progressive lung damage. In the study

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of Armstead et al. no differences were found in the median age at presentation for the different *Aspergillus* disease entities suggesting the first model was unlikely.[6] Additional supportive data in favour of the first model is the result of a longitudinal study of *Aspergillus* sensitization in which no association was observed between patients with sensitization and those who developed ABPA.[47]

Antifungal immunity in cystic fibrosis

The role of CFTR in the observed alterations in immune responses in CF-cells is far from understood and a major question is to what extend the immune dysfunction in non-epithelial cells contributes to the progressive lung disease. The pathophysiology of *Aspergillus* infections in the CF-lung, except for ABPA,[48] is not known and the consequences of the absence of a functional CFTR protein on the specific host—fungus interaction is not understood. CFTR protein has been found in cells of both the innate and adaptive immune system[49,50] and has shown to play a critical role for their normal function.[51] Cystic fibrosis lung disease is characterized by early, non-resolving activation of the innate immune system, with excessive neutrophil recruitment followed by excessive expansion of lymphocyte populations and failure of normal counter-regulatory mechanisms.[5,52] Furthermore, the CF lung is characterized by the abundancy of pro-inflammatory mediators such as interleukin (IL)-1, tumour necrosis factor α (TNF)-α, IL-8 and IL-17, and reduced amounts of the anti-inflammatory IL-10.[53—56] Neutrophilia and markers of excessive airway inflammation have been identified in the BAL fluid of CF infants as young as 4 weeks old, even in the absence of apparent infection, and do support an intrinsic dysregulation of inflammatory processes.[57]

A small number of functional studies implicate that the absence of a functional CFTR-protein in phagocytes leads to inadequate intraphagosomal chloride transport resulting in intracellular alkalization, alteration in degranulation, an impaired production of reactive oxygen species and increased release of pro-inflammatory cytokines resulting in a diminished killing of bacteria.[58,59] An increased pro-inflammatory response in CFTR-deficient phagocytes upon bacterial stimulation has repeatedly been observed.[60,61] However, no study so far has investigated the antifungal activitiy and inflammatory response of CFTR-deficient phagocytes against *A. fumigatus*. Indirectly it has been shown that pulmonary infection with *Aspergillus* species was associated with significant inflammatory responses in BAL-fluid in young children with CF.[29] In addition, 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]

Recently, major steps have been made in the development of new drugs, CFTR modulators, which have an interesting potential to decrease the progressive lung disease by either potentiating the gating properties of the defective CFTR-protein or correcting the translocating of the CFTR-protein to the cell surface.[62] Treatment with the CFTR potentiator Ivacaftor has shown significant improvement in lung function, reduction in the frequency of exacerbations, improved weight gain, and improvement in quality of life measures and it is now licensed for use in both North America and Europe.[63,64]

Interestingly, the results of a recent study looking at the effect of Ivacaftor use on specific microbial colonization showed a 23% reduction in cultures positive for *Pseudomonas* spp., with an even more pronounced effect of a 53% reduction in *Aspergillus* colonization (OR = 0.47, p = 0.039).[65] This may be related to a reduction in inflammation, and improved immune function of the epithelial cells or a direct effect on immune cells present in the lung environment. Support for the latter explanation can be found in recent work demonstrating that Ivacaftor can restore neutrophil degranulation and improve neutrophil bactericidal activity suggesting this therapy may have far-reaching benefits.[66]

Summary

A. fumigatus is frequently detected in respiratory secretions of both adults and children with CF. Once present in the airways, *Aspergillus* can exacerbate lung inflammation, establish infection and trigger hypersensitivity responses. Based on clinical and immunologic parameters, at least 3 *Aspergillus* disease phenotypes can be characterized and are each associated with progressive lung disease. Therefore, detection of *Aspergillus* spp. in the CF lung is not simply reflecting colonization without doing harm. However, underlying pathophysiology is not well understood and consequently clear management strategies are lacking. An important question which needs to be answered is which CF patients will benefit from antifungal therapy. Long-term antifungal treatment with mould-active azoles, the only class of antifungals with an oral formulation, is not without risk for toxicity and adverse events, and should be carefully balanced against its benefit. Research into the role of the CFTR-protein in immune cells has just started and specific host—fungus interaction data are not available yet. The development of CFTR potentiators and correctors may have a dramatical impact on the quality of life and survival of CF patients. This impact may well go beyond the effect on epithelial cells and may correct the impaired immune responses and hyperinflammation by CF immune cells.

Overall, improved understanding of disease pathogenesis and new therapeutic options targeting the underlying CFTR mutation show considerable promise for CF patients. To improve the quality of life and the life expectancy of CF-patients a better understanding of the role of the CFTR-deficient phagocyte is urgently needed.

Conflict of interest

None.

References

1. O'Sullivan BP, Freedman SD. Cystic fibrosis. *Lancet* 2009;**373**:1891—904.
2. Davies JC, Ebdon AM, Orchard C. Recent advances in the management of cystic fibrosis. *Arch Dis Child* 2014;**99**:1033—6.

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3. Siobhan C, Rebecca C, Vian RH. *UK Cystic Fibrosis Registry 2014 Annual Data Report*. UK: The Cystic Fibrosis Trust; 2015.
4. Cohen TS, Prince A. Cystic fibrosis: a mucosal immunodeficiency syndrome. *Nat Med* 2012;**18**:509–19.
5. Grasemann H, Ratjen F. Early lung disease in cystic fibrosis. *Lancet Respir Med* 2013;**1**:148–57.
6. Armstead J, Morris J, Denning DW. Multi-country estimate of different manifestations of aspergillosis in cystic fibrosis. *PLoS One* 2014;**9**:e98502.
7. Chaudhary N, Datta K, Askin FB, Staab JF, Marr KA. Cystic fibrosis transmembrane conductance regulator regulates epithelial cell response to *Aspergillus* and resultant pulmonary inflammation. *Am J Respir Crit Care Med* 2012;**185**:301–10.
8. Luong ML, Chaparro C, Stephenson A, Rotstein C, Singer LG, Waters V, et al. Pretransplant *Aspergillus* colonization of cystic fibrosis patients and the incidence of post-lung transplant invasive aspergillosis. *Transplantion* 2014;**97**:351–7.
9. Bonvillain RW, Valentine VG, Lombard G, LaPlace S, Dhillon G, Wang G. Post-operative infections in cystic fibrosis and non-cystic fibrosis patients after lung transplantation. *J Heart Lung Transp* 2007;**26**:890–7.
10. Pihet M, Carrere J, Cimon B, Chabasse D, Delhaes L, Symoens F, et al. Occurrence and relevance of filamentous fungi in respiratory secretions of patients with cystic fibrosis–a review. *Med Mycol* 2009;**47**:387–97.
11. Liu JC, Modha DE, Gaillard EA. What is the clinical significance of filamentous fungi positive sputum cultures in patients with cystic fibrosis? *J Cyst Fibros* 2013;**12**:187–93.
12. Latge JP. *Aspergillus fumigatus* and aspergillosis. *Clin Microbiol Rev* 1999;**12**:310–50.
13. Speirs JJ, van der Ent CK, Beekman JM. Effects of *Aspergillus fumigatus* colonization on lung function in cystic fibrosis. *Curr Opin Pulm Med* 2012;**18**:632–8.
14. Chotirmally SH, McElavaney NG. Fungi in the cystic fibrosis lung: bystanders or pathogens? *Int J Biochem Cell Biol* 2014;**52**:161–73.
15. Shoseyov D, Brownlee KG, Conway SP, Kerem E. *Aspergillus* bronchitis in cystic fibrosis. *Chest* 2006;**130**:222–6.
16. Baxter CG, Dunn G, Jones AM, Webb K, Gore R, Richardson MD, et al. Novel immunologic classification of aspergillosis in adult cystic fibrosis. *J Allergy Clin Immunol* 2013;**132**:560–6.
17. Agarwal R, Chakrabarti A, Shah A, Gupta D, Meis JF, Guleria R, et al. Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. *Clin Exp Allergy* 2013;**43**:850–73.
18. Milla CE, Wielinski CL, Regelmann WE. Clinical significance of the recovery of *Aspergillus* species from the respiratory secretions of cystic fibrosis patients. *Pediatr Pulmonol* 1996;**21**:6–10.
19. McMahon MA, Chotirmall SH, McCullagh B, Branagan P, McElvaney NG, Logan PM. Radiological abnormalities associated with *Aspergillus* colonization in a cystic fibrosis population. *Eur J Radiol* 2012;**81**:e197–202.
20. de Vrankrijker AM, van der Ent CK, van Berkhout FT, Stellato RK, Willems RJ, Bonten MJ, et al. *Aspergillus fumigatus* colonization in cystic fibrosis: implications for lung function? *Clin Microbiol Infect* 2011;**17**:1381–6.
21. Jubin V, Ranque S, Stremler le Bel N, Sarles J, Dubus JC. Risk factors for *Aspergillus* colonization and allergic bronchopulmonary aspergillosis in children with cystic fibrosis. *Pediatr Pulmonol* 2010;**45**:764–71.
22. Amin R, Dupuis A, Aaron SD, Ratjen F. The effect of chronic infection with *Aspergillus fumigatus* on lung function and hospitalization in patients with cystic fibrosis. *Chest* 2010;**137**:171–6.
23. Borman AM, Palmer MD, Delhaes L, Carrere J, Favennec L, Ranque S, et al. Lack of standardization in the procedures for mycological examination of sputum samples from CF patients: a possible cause for variations in the prevalence of filamentous fungi. *Med Mycol* 2010;**48**:S88–97.
24. Coburn B, Wang PW, Diaz Caballero J, Clark ST, Brahma V, Donaldson S, et al. Lung microbiota across age and disease stage in cystic fibrosis. *Sci Rep* 2015;**5**:10241.
25. El-Dahr JM, Fink R, Selden R, Arruda LK, Platts-Mills TA, Heymann PW. Development of immune responses to *Aspergillus* at an early age in children with cystic fibrosis. *Am J Respir Crit Care Med* 1994;**150**:1513–8.
26. Fillaux J, Bremont F, Murris M, Cassaing S, Tetu L, Segonds C, et al. *Aspergillus* sensitization or carriage in cystic fibrosis patients. *Pediatr Infect Dis J* 2014;**33**:680–6.
27. Cimon B, Symoens F, Zouhair R, Chabasse D, Nolard N, Defontaine A, et al. Molecular epidemiology of airway colonisation by *Aspergillus fumigatus* in cystic fibrosis patients. *J Med Microbiol* 2001;**50**:367–74.
28. Delhaes L, Monchy S, Frealle E, Hubans C, Salleron J, Leroy S, et al. The airway microbiota in cystic fibrosis: a complex fungal and bacterial community–implications for therapeutic management. *PLoS One* 2012;**7**:e36313.
29. Gangell C, Gard S, Douglas T, Park J, de Klerk N, Keil T, et al. Inflammatory responses to individual microorganisms in the lungs of children with cystic fibrosis. *Clin Infect Dis* 2011;**53**:425–32.
30. Tirouvanziam R. Neutrophilic inflammation as a major determinant in the progression of cystic fibrosis. *Drug News Perspect* 2006;**19**:609–14.
31. Pillarisetti N, Williamson E, Linnane B, Skoric B, Robertson CF, Robinson P, et al. Infection, inflammation, and lung function decline in infants with cystic fibrosis. *Am J Respir Crit Care Med* 2011;**184**:75–81.
32. Aaron SD, Vandemheen KL, Freitag A, Pedder L, Camerom W, Lavoie A, et al. Treatment of *Aspergillus fumigatus* in patients with cystic fibrosis: a randomized, placebo-controlled pilot study. *PLoS One* 2012;**7**:e36077.
33. Chrdle A, Mustakim S, Bright-Thomas RJ, Baxter CG, Felton T, Denning DW. *Aspergillus* bronchitis without significant immunocompromise. *Ann N Y Acad Sci* 2012;**1272**:73–85.
34. Mastella G, Rainisio M, Harms HK, Hodson ME, Koch C, Navarro J, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis. A European epidemiological study. Epidemiologic Registry of Cystic Fibrosis. *Eur Respir J* 2000;**16**:464–71.
35. Thia LP, Balfour Lynn IM. Diagnosing allergic bronchopulmonary aspergillosis in children with cystic fibrosis. *Paediatr Respir Rev* 2009;**10**:37–42.
36. Maturu VN, Agarwal R. Prevalence of *Aspergillus* sensitization and allergic bronchopulmonary aspergillosis in cystic fibrosis: systematic review and meta-analysis. *Clin Exp Allergy* 2015;**45**:1765–78.
37. Fillaux J, Bremont F, Murris M, Cassaing S, Rittie JL, Tetu L, et al. Assessment of *Aspergillus* sensitization or persistent carriage as a factor in lung function impairment in cystic fibrosis patients. *Scand J Infect Dis* 2012;**44**:842–7.
38. Wojnarowski C, Eichler I, Fartner C, Götz M, Renner S, Koller DY, et al. Sensitization to *Aspergillus fumigatus* and lung function in children with cystic fibrosis. *Am J Respir Crit Care Med* 1997;**155**:1902–7.
39. Kraemer R, Delosea N, Ballinari P, Gallati S, Crameri R. Effect of allergic bronchopulmonary aspergillosis on lung function in children with cystic fibrosis. *Am J Respir Crit Care Med* 2006;**174**:1211–20.
40. de Almeida MB, Bussamra MH, Rodrigues JC. Allergic bronchopulmonary aspergillosis in paediatric cystic fibrosis patients. *Paediatr Respir Rev* 2006;**7**:67–72.

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41. Moss RB. Allergic bronchopulmonary aspergillosis and *Aspergillus* infection in cystic fibrosis. *Curr Opin Pulm Med* 2010;**16**:598–603.
42. Elphick HE, Southern KW. Antifungal therapies for allergic bronchopulmonary aspergillosis in people with cystic fibrosis. *Cochrane Database Syst Rev* 2014;**11**:CD002204.
43. Moreira AS, Silva D, Ferreira AR, Delgado L. Antifungal treatment in allergic bronchopulmonary aspergillosis with and without cystic fibrosis: a systematic review. *Clin Exp Allergy* 2014;**44**:1210–27.
44. Hilliard T, Edwards S, Buchdahl R, Francis J, Rosenthal M, Balfour-Lynn I, et al. Voriconazole therapy in children with cystic fibrosis. *J Cyst Fibros* 2005;**4**:215–20.
45. Brodlie M, McKean MC, Moss S, Spencer DA. The oral corticosteroid-sparing effect of omalizumab in children with severe asthma. *Arch Dis Child* 2012;**97**:604–9.
46. Tanou K, Zintzaras E, Kaditis AG. Omalizumab therapy for allergic bronchopulmonary aspergillosis in children with cystic fibrosis: a synthesis of published evidence. *Pediatr Pulmonol* 2014;**49**:503–7.
47. Hutcheson PS, Knutsen AP, Rejent AJ, Slavin RG. A 12-year longitudinal study of *Aspergillus* sensitivity in patients with cystic fibrosis. *Chest* 1996;**110**:363–6.
48. Eickmeier O, Rieber N, Eckrich J, Hector A, Graeppler-Mainka U, Hartl D. Immune response, diagnosis and treatment of allergic bronchopulmonary aspergillosis in cystic fibrosis lung disease. *Curr Pharm Des* 2013;**19**:3669–78.
49. Zhou Y, Song K, Painter RG, Aiken M, Reiser J, Stanton BA, et al. Cystic fibrosis transmembrane conductance regulator recruitment to phagosomes in neutrophils. *J Innate Imm* 2013;**5**:219–30.
50. Johansson J, Vezzalini M, Verze G, Caldrer S, Bolognin S, Buffelli M, et al. Detection of CFTR protein in human leukocytes by flow cytometry. *Cytom A* 2014;**85**:611–20.
51. Rieber N, Hector A, Carevic M, Hartl D. Current concepts of immune dysregulation in cystic fibrosis. *Int J Biochem Cell Biol* 2014;**52**:108–12.
52. Ratner D, Mueller C. Immune responses in cystic fibrosis: are they intrinsically defective? *Am J Respir Cell Mol Biol* 2012;**46**:715–22.
53. Dubin PJ, McAllister F, Kolls JK. Is cystic fibrosis a TH17 disease? *Inflamm Res* 2007;**56**:221–7.
54. Noah TL, Black HR, Cheng PW, Wood RE, Leigh MW. Nasal and bronchoalveolar lavage fluid cytokines in early cystic fibrosis. *J Infect Dis* 1997;**175**:638–47.
55. Muhlebach MS, Stewart PW, Leigh MW, Noah TL. Quantitation of inflammatory responses to bacteria in young cystic fibrosis and control patients. *Am J Respir Crit Care Med* 1999;**160**:186–91.
56. Bonfield TL, Panuska JR, Konstan MW, Hilliard KA, Hilliard JB, Ghnaim H, et al. Inflammatory cytokines in cystic fibrosis lungs. *Am J Respir Crit Care Med* 1995;**152**:2111–8.
57. Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW. Early pulmonary inflammation in infants with cystic fibrosis. *Am J Respir Crit Care Med* 1995;**151**:1075–82.
58. Painter RG, Bonvillain RW, Valentine VG, Lombard GA, LaPlace SG, Nauseef WM, et al. The role of chloride anion and CFTR in killing of *Pseudomonas aeruginosa* by normal and CF neutrophils. *J Leuk Biol* 2008;**83**:1345–53.
59. Del Porto P, Cifani N, Guarnieri S, Di Domenico EG, Mariggiò MA, Spadaro F, et al. Dysfunctional CFTR alters the bactericidal activity of human macrophages against *Pseudomonas aeruginosa*. *PLoS One* 2011;**6**:e19970.
60. Bruscia EM, Zhang PX, Satoh A, Caputo C, Medzhitov R, Shenoy A, et al. Abnormal trafficking and degradation of TLR4 underlie the elevated inflammatory response in cystic fibrosis. *J Immunol* 2011;**186**:6990–8.
61. Mueller C, Braag SA, Keeler A, Hodges C, Drumm M, Flotte TR. Lack of cystic fibrosis transmembrane conductance regulator in CD3+ lymphocytes leads to aberrant cytokine secretion and hyperinflammatory adaptive immune responses. *Am J Respir Cell Mol Bio* 2011;**44**:922–9.
62. Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, et al. Rescue of CF airway epithelial cell function in vitro by a CFTR potentiator, VX-770. *Proc Natl Acad Sci USA* 2009;**106**:18825–30.
63. Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Drevinek P, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med* 2011;**365**:1663–72.
64. Davies JC, Wainwright CE, Canny GJ, Chilvers MA, Howenstine MS, Munck A, et al. Efficacy and safety of ivacaftor in patients aged 6 to 11 years with cystic fibrosis with a G551D mutation. *Am J Respir Crit Care Med* 2013;**187**:1219–25.
65. Heltshe SL, Mayer-Hamblett N, Burns JL, Khan U, Baines A, Ramsey BW, et al. *Pseudomonas aeruginosa* in cystic fibrosis patients with G551D-CFTR treated with ivacaftor. *Clin Infect Dis* 2015;**60**:703–12.
66. Pohl K, Hayes E, Keenan J, Henry M, Meleady P, Molloy K, et al. A neutrophil intrinsic impairment affecting Rab27a and degranulation in cystic fibrosis is corrected by CFTR potentiator therapy. *Blood* 2014;**124**:999–1009.

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