



Effects of *Aspergillus fumigatus* colonization on lung function in cystic fibrosis

Jennifer J. Speirs^{a,b,c}, Cornelis K. van der Ent^a, and Jeffrey M. Beekman^{a,b,c}

Purpose of review

Aspergillus fumigatus is frequently isolated from cystic fibrosis (CF) patients and is notorious for its role in the debilitating condition of allergic bronchopulmonary aspergillosis (ABPA). Although CF patients suffer from perpetual microorganism-related lung disease, it is unclear whether *A. fumigatus* colonization has a role in causing accelerated lung function decline and whether intervention is necessary.

Recent findings

A. fumigatus morbidity appears to be related to cystic fibrosis transmembrane conductance regulator-dependant function of the innate immune system. *A. fumigatus*-colonized patients have a lower lung capacity, more frequent hospitalizations and more prominent radiological abnormalities than noncolonized patients. Treatment with antifungal agents can be of value but has several drawbacks and a direct effect on lung function is yet to be shown.

Summary

A. fumigatus appears to have an important role in CF lung disease, not exclusive to the context of ABPA. However, a causal relationship still needs to be confirmed. Study observations and trends indicate a need to further elucidate the mechanisms of *A. fumigatus* interactions with the host innate immune system and its role in CF lung morbidity.

Keywords

allergic bronchopulmonary aspergillosis, *Aspergillus fumigatus*, colonization, cystic fibrosis, lung function

INTRODUCTION

The cystic fibrosis (CF) lung contains a complex community of microorganisms that exacerbate perpetual lung disease. Antimicrobial strategies are, therefore, an important aspect of CF care, but, despite intensive therapeutic interventions, it remains challenging for CF patients to clear infections. The role of bacteria has been elaborately addressed and the ability for specific pathogens to claim a particularly prominent role is due to a combination of pathogen-specific virulence factors and cystic fibrosis transmembrane conductance regulator (CFTR) related and unrelated host susceptibility factors. This paradigm of an altered pathogen–host interaction is becoming the cornerstone of CF lung disease and is also relevant to fungi. The presence of fungi in the CF lung has until recently played a modest but potentially understated role in CF disease but is a valid concern. The fungus *Aspergillus fumigatus* is a prevalent isolate with the potential to cause severe disease in particular patients; however, in the majority, the significance of *A. fumigatus* remains unclear. This review

will address recent literature on fungal colonization in CF and immune mechanisms involved in *A. fumigatus* recognition, and aims to formulate a conclusion regarding the role of *A. fumigatus* in pulmonary deterioration.

COLONIZATION

Colonization refers to the presence of potentially pathogenic microorganisms in laboratory cultures of the nasopharynx or respiratory tract without apparent clinical symptoms. Colonization of the

^aDepartment of Paediatric Pulmonology, ^bDepartment of Immunology and ^cDepartment of Paediatrics, Center for Molecular and Cellular Intervention, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, The Netherlands

Correspondence to Professor Doctor Cornelis K. van der Ent, Department of Pediatric Pulmonology, University Medical Center Utrecht, Lundlaan 6, 3584 EA Utrecht, The Netherlands. Tel: +31 88 7553201; e-mail: K.vanderEnt@umcutrecht.nl

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KEY POINTS

- Lower pulmonary function and exacerbations are associated with *A. fumigatus* colonization in asthma and CF.
- The causal relationship between *A. fumigatus* and lung function remains unclear and requires further research.
- Novel tools are required for improved assessment of lung damage.
- Antifungal eradication therapy has important clinical limitations.

nasopharynx is not uncommon, but the lower airways are thought to be sterile. Interestingly, recent literature suggests the likelihood of a lung microbiome with flora extending, in decreasing amounts, from the nasopharynx to the lower airways [1[■],2[■]]. This novel concept may influence the future opinion on colonization in respiratory health and disease. The most important characteristic defining a commensal, colonizing or invasive organism is its virulence. Virulent microorganisms cause disease by evasion and suppression of host immune mechanisms. It remains unclear whether under homeostatic conditions the presence of pathogenic microorganisms such as *A. fumigatus* actively contributes to inflammation, deterioration and long-term morbidity. *A. fumigatus* is the most frequently cultured fungal pathogen from sputum of CF patients, but the prevalence of colonization is variable. Some areas report a prevalence of up to 58% in CF patients that may be increasing [3,4]. It is, therefore, of great importance to elucidate the exact role of *A. fumigatus* in CF lung disease and define strategies for intervention when appropriate.

ASPERGILLUS FUMIGATUS

A. fumigatus is ubiquitously present and produces abundant amounts of airborne conidia (1–100 conidia m⁻³) [5]. These spores are very small in size (3–5 µm) and penetrate deep into the lungs. The inert conidia are mechanically protected from environmental pressure by an external hydrophobic rodlet layer that evades immune recognition and prevents the initiation of an inflammatory response [6]. A rapid asexual growth cycle can be initiated within hours under nutrient-rich conditions at 37°C and induces the swelling and the subsequent germination of hyphae from the conidium. The combination of these, and possibly other *A. fumigatus*-specific, characteristics likely

contribute to its virulence and association with human disease.

INNATE RESPONSES

In healthy individuals, beating cilia and macrophages of the innate immune system rapidly eliminate conidia via noninflammatory mechanisms. Failure to remove conidia, as in CF, results in rapid germination that reveals inflammatory substrates such as inner cell wall-derived β-glucans that are recognized by innate mechanisms. These substrates bind to fungal pattern-recognition receptors such as the C-type lectin dectin-1 and Toll-like receptors on a variety of host cells. Recent studies highlight the role of dectin-1 in cell surface recognition of fungal β-glucan antigens for efficient uptake of conidia and for intracellular recognition and inflammatory activation at the acidified phagolysosome in macrophages [7[■]]. These interactions are crucial for clearance and initiation of inflammatory responses and for shaping the adaptive immune response.

Airway epithelial cells are the first cellular barrier for pathogens in the airways. The CFTR protein is apically expressed in epithelial cells and dysfunction here has been shown to influence the interaction with pathogens and subsequent inflammatory events [8–10]. Chaudhary *et al.* [11[■]] recently investigated the interaction between murine airway epithelial cells and germinating *A. fumigatus* conidia by performing in-vitro and in-vivo stimulations of CFTR-competent and deficient epithelial cells. They observed that CFTR-competent cells had the ability to ingest and eliminate conidia, preventing germination of conidia more efficiently than CFTR-deficient cells. However, it is difficult to determine whether decreased clearance of conidia is attributable to decreased detection and binding by epithelial cells or whether the uptake and killing are hindered, especially considering that epithelial cells from CFTR-deficient mice had higher baseline levels of apoptosis before and after *A. fumigatus* challenge. Furthermore, significantly increased levels of macrophage inflammatory protein-2 and chemokine ligand-1 were measured in bronchoalveolar lavage fluid that was associated with a greater influx of macrophages and polymorphonuclear cells in CFTR-deficient mice after challenge with *A. fumigatus*. These data point out that loss of CFTR can modify interactions between epithelial cells and germinating conidia, resulting in their reduced clearance and enhanced inflammation. In addition to possible defects in epithelial cells, impaired clearance of conidia by CF innate immune cell subsets may further contribute to the high colonization rate of CF patients with *A. fumigatus*.

Data indicate that the bacterial killing capacity of alveolar macrophages is reduced in a CFTR-dependent manner. CFTR-deficient macrophages showed normal phagocytosis but impaired intracellular killing and elevated proinflammatory cytokine responses upon bacterial challenges *in vitro*, and this was further supported by adoptive transfer experiments *in vivo* [12,13]. As it was observed that lysosomal acidification was reduced in CFTR-deficient cells, it is presumable that CF macrophages also show impaired killing of *A. fumigatus*, but specific CFTR-dependent *A. fumigatus* effects on macrophages should still be confirmed. Bhatia *et al.* [14²²] also showed that *A. fumigatus* induces alternatively activated macrophages, independent of dectin-1, but partially via T helper cell type 2 (Th-2) 2-associated signal transduction and transcription protein 6 signalling. This subtype appears to be important for efficient fungal clearance. Whether this subtype is reduced in CF, possibly influencing the immune response to *A. fumigatus* and colonization rates, is not known.

ADAPTIVE IMMUNITY

A. fumigatus causes an abnormal adaptive response in a select group of CF and asthmatic patients (see Fig. 1). The majority of CF patients colonized with *A. fumigatus* do not show obvious *A. fumigatus*-related symptoms, yet 10% develop allergic bronchopulmonary aspergillosis (ABPA) [15]. ABPA is a Th-2-mediated hypersensitivity reaction that leads to significant clinical deterioration, requiring treatment with systemic corticosteroids [16,17]. It is unclear why CF patients develop allergic disease, in contrast to the invasive disease that develops in immunocompromised patients. Galactomannan levels, which are used as a read out for invasive disease, are not particularly high nor do levels differ between ABPA and non-ABPA patients [18²³]. The allergic component may stem from *A. fumigatus* itself, based on evidence that *A. fumigatus* elicits a Th-2-skewing effect on CF leukocytes, and most studies thus far have not been able to clearly demonstrate a general Th-2 skewing activity against pathogens other than *A. fumigatus* in CF [19,20]. The

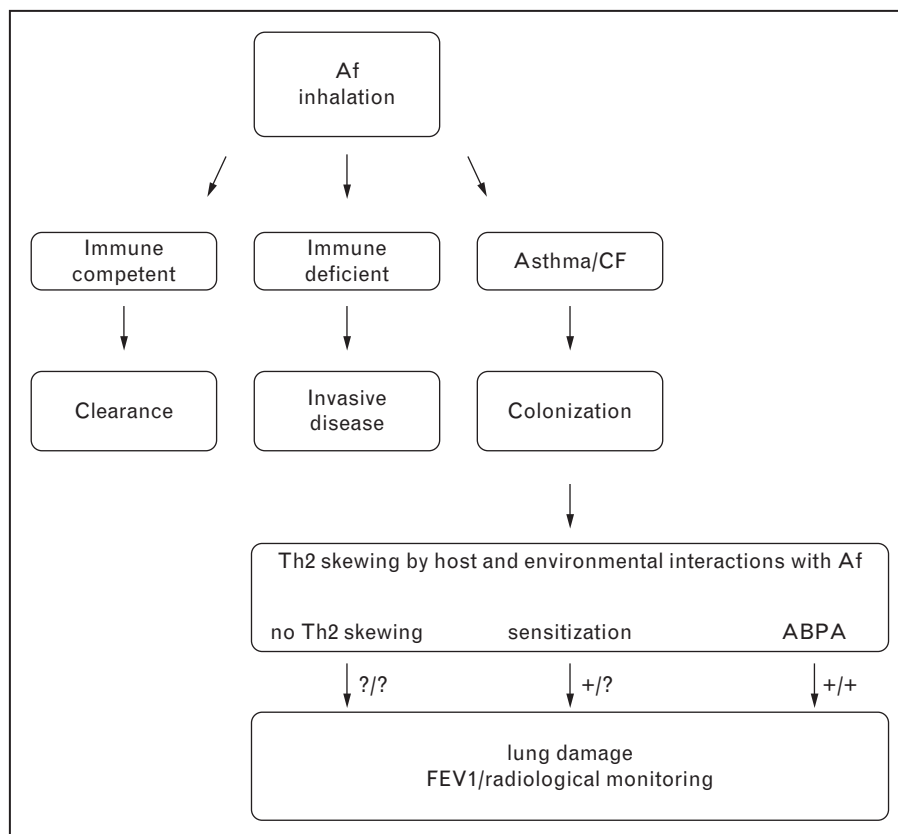


FIGURE 1. Mechanisms of *Aspergillus fumigatus*-related cystic fibrosis lung disease. Th2 skewing occurs in asthma or cystic fibrosis (CF) patients and *Aspergillus fumigatus*, thereby contributing to ongoing inflammation leading to tissue damage visible by radiographic imaging in sensitized and allergic bronchopulmonary aspergillosis (ABPA) patients. In the case of colonized patients without symptoms, it is unclear to what extent lung damage occurs and whether intervention is necessary. Af, *Aspergillus fumigatus*; FEV1, forced expiratory volume in one second.

fungal load in patients may be an important factor initiating sensitization and Th-2 hypersensitivity. However, other host-accountable factors, independent of fungal load, may also be important determinants for the induction of *A. fumigatus* Th-2 responses. For instance, data suggests that low-affinity binding of *A. fumigatus* antigen and the human leukocyte antigen DR subtypes 2 and 5 are significantly associated with ABPA development [21]. HLA type is, however, not fully responsible as half of all sensitized patients without ABPA have these types. As CFTR gene mutations are clearly associated with ABPA, it is tempting to speculate that lack of CFTR itself is also an intrinsic factor that contributes to Th-2 skewing by alterations of the cellular interactions underlying Th-2 differentiation. A recent study demonstrated that CFTR-deficient lymphocytes are prone to develop into Th-2 cells upon in-vivo challenge with *A. fumigatus* [22].

The cause of ABPA remains puzzling but seems to be the result of interactions between *A. fumigatus* and CFTR-dependent host-accountable factors that can influence *A. fumigatus* load and Th-2 skewing. This in turn explains the broad phenotypical variation in clinical outcome and *A. fumigatus*-related morbidity. Until now, it is not fully understood which patients are prone to ABPA and what role *A. fumigatus* has in CF disease in the absence of ABPA.

ASPERGILLUS FUMIGATUS-RELATED DISORDER IN CYSTIC FIBROSIS

Few studies have addressed the role of *A. fumigatus* on lung function in CF, and they have not shown significant lung function decline in patients colonized with *A. fumigatus*. Recently de Vrankrijker *et al.* [23²²] investigated a cohort of 259 CF patients for *A. fumigatus* colonization and found 61 (23.5%) to be colonized according to the criteria of having more than 50% positive sputum cultures in a given year. A retrospective cohort analysis on 163 patients was performed over a 5-year period and patients were grouped according to the number of years in which they met criteria for colonization. This longitudinal analysis did not find any differences between the groups regarding lung function decline during the study period. There were, however, significant differences in the baseline demographics, which are important confounding factors. Those who were colonized the longest were older, they had the lowest baseline forced expiratory volume in one second (FEV₁), they used more antibiotics and had lower BMI scores. This highlights the limitations of cross-sectional and retrospective studies in which one cannot distinguish between cause and

result: are the feeble patients more susceptible to colonization or does colonization lead to feeble patients? A causal relationship is not proven, but this study suggests that *A. fumigatus* may influence lung function and risk of exacerbations. Similarly, previously published data from a retrospective cohort study showed that chronic *A. fumigatus* colonization in CF was found to be associated with lower clinical status [24]. In this cohort, 37 (16%) of the 230 patients were considered chronically infected according to the criteria of having two or more positive sputum cultures per year. *A. fumigatus* and CF-related diabetes both had a significant association with hospitalization; however, after correction for baseline differences, only a trend towards an increased risk for pulmonary exacerbations was shown. Interestingly, in colonized patients with asthma (and without ABPA), it appeared that immunoglobulin E (IgE) sensitization to *A. fumigatus* was associated with lower lung function and that, together with neutrophil differential cell count, these were important predictors of lung function [25]. Although these are not CF patients, it is interesting to find similarities in associating factors, and it remains intriguing to understand possible common mechanistic pathways, especially as there is an increased frequency of CF carriers among asthma patients who suffer from ABPA [26,27].

An important and perhaps surprising consideration is that lung function may not be the best end point to utilize in short-term studies. McMahon *et al.* [28²³] used high-resolution computed tomography to evaluate patients with and without *A. fumigatus* to study radiological abnormalities in addition to lung function. They found that radiological abnormalities such as bronchiectasis, especially in the upper right lobes, were significantly worse and involved more pulmonary segments in *A. fumigatus*-positive patients, whereas no difference in lung function was detected. This indicates that *A. fumigatus*-positive patients do have visible abnormalities that are not detected by lung function testing, and they, therefore, may deserve radiological screening and monitoring or even treatment for eradication.

ASPERGILLUS FUMIGATUS-RELATED INTERVENTION

Recently a randomized, placebo-controlled pilot attempted to evaluate the effects of antifungal treatment on lung function in CF patients colonized with *A. fumigatus* [29²⁴]. This study used exacerbations requiring antibiotic therapy as primary endpoints and FEV₁ and quality of life as a secondary endpoint. A beneficial effect of using itraconazole

in colonized patients was not observed, but this study was limited by the failure to reach therapeutic levels of itraconazole, which may explain the lack of any measurable effect on long-term lung function. To date, there are few other studies investigating fungal eradication in colonized patients. Antifungal agents have been shown to have positive short-term effects in patients with ABPA and severe asthma with fungal sensitization [30]. In patients with ABPA, the mainstream intervention is systemic glucocorticoids, intended to decrease inflammation and progressive lung injury, but unfortunately with notorious side effects. The use of adjuvant antifungal agents, however, led to a reduction in IgE, eosinophilia and steroid use, and improvements in lung function were seen after 6 months in one study. Unfortunately, after 12 months, these effects were no longer distinguishable [31]. In another study, a positive effect on physiologic criteria and corticosteroid dose but not on radiographic infiltrates was seen [32].

The optimum duration of treatment remains uncertain, and in these short-term studies it remains difficult to evaluate the usefulness of itraconazole in the clinic, considering the challenges of obtaining therapeutic levels and avoiding adverse side effects. For this reason, the use of other azoles such as voriconazole may have additional potential [33]. However recent studies of (multi) azole-resistant *A. fumigatus* strains seed caution for the widespread use of azoles [34[•]–37[•]]. Furthermore, the lack of short-term improvement may be due to irreversible lung damage, and long-term trials are necessary to investigate this.

Delhaes *et al.* [38^{••}] provide an additional and relevant concern regarding the use of antimicrobial agents and highlight that the complexity of the microbiotic environment in CF lungs exceeds the information that we currently rely on. Using new detection techniques of high-throughput pyrosequencing, they observed that the majority of fungal species obtained by pyrosequencing were not detected in cultures. The various fungal subspecies that colonize the lower airways are far more diverse than previously anticipated [38^{••},39[•]]. The use of antimicrobial agents, both antibiotics and antifungal agents, decreases the diversity of microorganisms, and this seems to be unbeneficial for clinical condition. A lower clinical status, reflected in FEV₁, forced vital capacity and BMI, was related to a lower degree of species diversity. The importance of microbial diversity could reflect the competition and interaction between different organisms. Communal partners and local conditions create the potential for infection, but when one or more species are depleted this most likely creates an

opportunity for the most virulent, as has been shown for bacterial colonization. Furthermore, interactions between microorganisms are also highly complex, and it seems that an antagonistic interaction exists between *Pseudomonas aeruginosa* and *A. fumigatus*. If *P. aeruginosa* indeed prevents filamentation and biofilm formation of *A. fumigatus* by means of soluble mediators then it would be interesting to investigate the role of antibiotic use and development of *A. fumigatus*-related morbidity [40].

Other alternatives include anti-IgE therapy, which is relevant for patients who suffer from severe allergic disease; however, it has yet to form a prominent role in the clinical setting of ABPA. For the treatment of severe allergic asthma, omalizumab has shown efficacy for up to 1 year. Beneficial effects early on in ABPA treatment and in addition to corticosteroid use have been reported only in the form of case reports [41–44]. Whether anti-IgE therapy will be relevant in the future for a select group of *A. fumigatus*-colonized patients in order to prevent the development of ABPA is rather speculative.

CONCLUSION

The direct role of *A. fumigatus* on lung function in colonized CF patients is difficult to determine. Accumulating evidence depicts a clear association between older patients, bacterial colonization, lower lung function and the chronic presence of *A. fumigatus*. However, there is, as yet, no evidence directly supporting an isolated and causative effect of *A. fumigatus* colonization on these factors or increased morbidity. As depicted in Fig. 1, there are also host-specific and environmental factors that will affect the response to *A. fumigatus*. Advice for *A. fumigatus* eradication in colonized patients without ABPA using antifungal agents remains disputable due to the lack of data supporting this and the concern for increasing resistance. The observation of radiological abnormalities in the absence of lung function decline is, however, a concerning matter, and prospective studies should be performed to evaluate the significance of these abnormalities on a long-term basis in order to evaluate whether early intervention is necessary. Furthermore, radiological imaging may be of superior predictive value, in short-term studies, for long-term lung function. This too should be further investigated in long-term prospective studies. Due to the abundance of possible interfering and confounding factors, it is challenging to isolate the effects attributable to *A. fumigatus*, and agreements need to be made on concepts such as colonization and how this should be defined. These differences, which affect study

design, will influence patient selection and possibly outcome of analysis and they limit the comparison between studies.

It is nevertheless apparent that *A. fumigatus* and other fungal species most likely play a greater role in CF lung disease than previously thought, and not only in the context of ABPA. Insight into how *A. fumigatus* interacts with the CF lung environment will shed light on CF disease, and greater insight regarding antifungal mechanisms within CF goes hand in hand with understanding antifungal responses in other diseases and with defining conditions in which eradication therapies should be pursued.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 647).

1. Charlson ES, Bittinger K, Haas AR, *et al.* Topographical continuity of bacterial populations in the healthy human respiratory tract. *Am J Respir Crit Care Med* 2011; 184:957–963.
- Molecular techniques show that microbial species are present in the lungs of healthy individuals, challenging the notion that the lungs are sterile. The microbial composition of the lower lungs is, however, not distinguishable from the upper airways and is far lower in biomass.
2. Beck JM, Young VB, Huffnagle GB. The microbiome of the lung. *Transl Res* 2012. [Epub ahead of print]
- Review on studies demonstrating bacteria in the lower respiratory tract. These findings include indications that there are important interactions between microbiota of different organ systems and that lung immunity is affected by gastrointestinal microbiota.
3. Pihet M, Carrere J, Cimon B, *et al.* Occurrence and relevance of filamentous fungi in respiratory secretions of patients with cystic fibrosis—a review. *Med Mycol* 2009; 47:387–397.
4. Skov M, McKay K, Koch C, Cooper PJ. Prevalence of allergic bronchopulmonary aspergillosis in cystic fibrosis in an area with a high frequency of atopy. *Respir Med* 2005; 99:887–893.
5. Leenders AC, van Belkum A, Behrendt M, *et al.* Density and molecular epidemiology of *Aspergillus* in air and relationship to outbreaks of *Aspergillus* infection. *J Clin Microbiol* 1999; 37:1752–1757.
6. Amanianda V, Bayry J, Bozza S, *et al.* Surface hydrophobic prevents immune recognition of airborne fungal spores. *Nature* 2009; 460:1117–1121.
7. Faro-Trindade I, Willment JA, Kerrigan AM, *et al.* Characterisation of innate fungal recognition in the lung. *PLoS One* 2012; 7:e35675.
- Pattern-recognition receptors such as Dectin-1 are important at the surface and for intracellular recognition of fungal conidia at the acidified phagosome in innate immune cells.
8. Pier GB. Role of the cystic fibrosis transmembrane conductance regulator in innate immunity to *Pseudomonas aeruginosa* infections. *Proc Natl Acad Sci U S A* 2000; 97:8822–8828.
9. Pier GB, Grout M, Zaidi TS. Cystic fibrosis transmembrane conductance regulator is an epithelial cell receptor for clearance of *Pseudomonas aeruginosa* from the lung. *Proc Natl Acad Sci U S A* 1997; 94:12088–12093.
10. Schroeder TH, Lee MM, Yacono PW, *et al.* CFTR is a pattern recognition molecule that extracts *Pseudomonas aeruginosa* LPS from the outer membrane into epithelial cells and activates NF-kappa B translocation. *Proc Natl Acad Sci U S A* 2002; 99:6907–6912.

11. Chaudhary N, Datta K, Askin FB, *et al.* Cystic fibrosis transmembrane conductance regulator regulates epithelial cell response to *Aspergillus* and resultant pulmonary inflammation. *Am J Respir Crit Care Med* 2012; 185:301–310.
- This study indicates that CFTR-mediated epithelial cell function plays an important role in the innate response to *A. fumigatus* and CF lung disease. In CFTR-deficient mice, epithelial cells show reduced uptake and killing of conidia and an exaggerated inflammatory response.
12. Bruscia EM, Zhang PX, Ferreira E, *et al.* Macrophages directly contribute to the exaggerated inflammatory response in cystic fibrosis transmembrane conductance regulator-/- mice. *Am J Respir Cell Mol Biol* 2009; 40:295–304.
13. Di A, Brown ME, Deriy LV, *et al.* CFTR regulates phagosome acidification in macrophages and alters bactericidal activity. *Nat Cell Biol* 2006; 8:933–944.
14. Bhatia S, Fei M, Yarlagadda M, *et al.* Rapid host defense against *Aspergillus fumigatus* involves alveolar macrophages with a predominance of alternatively activated phenotype. *PLoS One* 2011; 6:e15943.
- This study adds to our knowledge on fungal immunity. Although Dectin-1 and Toll-like receptors (TLR) are necessary for fungal recognition, alternative activation of macrophages, which is defined by arginase-1 expression, is not dependant on Dectin-1 or TLR-mediated signalling. These alternatively activated macrophages seem to be important and have a protective role against *A. fumigatus*.
15. Mastella G, Rainisio M, Harms HK, *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis. A European epidemiological study. *Epidemiologic Registry of Cystic Fibrosis. Eur Respir J* 2000; 16:464–471.
16. Kraemer R, Delosea N, Ballinari P, *et al.* Effect of allergic bronchopulmonary aspergillosis on lung function in children with cystic fibrosis. *Am J Respir Crit Care Med* 2006; 174:1211–1220.
17. Stevens DA, Moss RB, Kurup VP, *et al.* Allergic bronchopulmonary aspergillosis in cystic fibrosis—state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin Infect Dis* 2003; 37 (Suppl 3):S225–S264.
18. Warren TA, Yau Y, Ratjen F, *et al.* Serum galactomannan in cystic fibrosis patients colonized with *Aspergillus* species. *Med Mycol* 2012; 50:658–660.
- Galactomannan levels which reflect invasive infection are not elevated in colonized patients. This adds to our knowledge and understanding of colonization being different from infection.
19. Allard JB, Poynter ME, Marr KA, *et al.* *Aspergillus fumigatus* generates an enhanced Th2-biased immune response in mice with defective cystic fibrosis transmembrane conductance regulator. *J Immunol* 2006; 177:5186–5194.
20. Hartl D, Griesse M, Kappler M, *et al.* Pulmonary T(H)2 response in *Pseudomonas aeruginosa*-infected patients with cystic fibrosis. *J Allergy Clin Immunol* 2006; 117:204–211.
21. Chauhan B, Santiago L, Kirschmann DA, *et al.* The association of HLA-DR alleles and T cell activation with allergic bronchopulmonary aspergillosis. *J Immunol* 1997; 159:4072–4076.
22. Mueller C, Braag SA, Keeler A, *et al.* Lack of Cfr in CD3+ lymphocytes leads to aberrant cytokine secretion and hyper-inflammatory adaptive immune responses. *Am J Respir Cell Mol Biol* 2010; 44:922–929.
23. de Vrankrijker AM, van der Ent CK, van Berkhout FT, *et al.* *Aspergillus fumigatus* colonization in cystic fibrosis: implications for lung function? *Clin Microbiol Infect* 2011; 17:1381–1386.
- This is one of the few studies that address the relationship between *A. fumigatus* colonization and lung function. It is a retrospective study that found that *A. fumigatus* colonization in patients is associated with lower clinical condition. However, colonized patients did not show a steeper lung function decline during the study period but rather maintained the lower lung function and clinical status that were measured at baseline.
24. 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; 137:171–176.
25. Fairs A, Agbetile J, Hargadon B, *et al.* IgE sensitization to *Aspergillus fumigatus* is associated with reduced lung function in asthma. *Am J Respir Crit Care Med* 2010; 182:1362–1368.
26. Lebecque P, Pepermans X, Marchand E, *et al.* ABPA in adulthood: a CFTR-related disorder. *Thorax* 2010; 66:540–541.
27. Miller PW, Hamosh A, Macek M Jr, *et al.* Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. *Am J Hum Genet* 1996; 59:45–51.
28. McMahon MA, Chotirmall SH, McCullagh B, *et al.* Radiological abnormalities associated with *Aspergillus* colonization in a cystic fibrosis population. *Eur J Radiol* 2012; 81:e197–202.

Using high-resolution computed tomography and pulmonary function testing, this study analysed the differences between CF patients colonized and not colonized with *A. fumigatus*. Their interesting finding is that colonized patients did not differ in lung function testing, but there were more pulmonary abnormalities visible on thorax imaging.

29. Aaron SD, Vandemheen KL, Freitag A, *et al.* Treatment of *Aspergillus fumigatus* in patients with cystic fibrosis: a randomized, placebo-controlled pilot study. *PLoS One* 2012; 7:e36077.

Other than investigating whether colonized patients have lower pulmonary function, one can investigate whether treatment of *A. fumigatus* improves lung function. This study did not observe beneficial effects on lung function after treatment, but this may be due to subtherapeutic pharmacological levels and/or due to irreversible lung damage.

30. Wark PA, Gibson PG, Wilson AJ. Azoles for allergic bronchopulmonary aspergillosis associated with asthma. *Cochrane Database Syst Rev* 2004;CD001108.
31. Pasqualotto AC, Powell G, Niven R, Denning DW. The effects of antifungal therapy on severe asthma with fungal sensitization and allergic bronchopulmonary aspergillosis. *Respirology* 2009; 14:1121–1127.
32. Stevens DA, Schwartz HJ, Lee JY, *et al.* A randomized trial of itraconazole in allergic bronchopulmonary aspergillosis. *N Engl J Med* 2000; 342:756–762.
33. Chishimba L, Niven RM, Cooley J, Denning DW. Voriconazole and posaconazole improve asthma severity in allergic bronchopulmonary aspergillosis and severe asthma with fungal sensitization. *J Asthma* 2012; 49:423–433.
34. Burgel PR, Baixench MT, Amsellem M, *et al.* High prevalence of azole-resistant *Aspergillus fumigatus* in adults with cystic fibrosis exposed to itraconazole. *Antimicrob Agents Chemother* 2012; 56:869–874.
Of patients with CF who had been previously exposed to itraconazole, 4.6% had isolates of *A. fumigatus* that were less susceptible to itraconazole, as well as other azoles.
35. van der Linden JW, Snelders E, Kampinga GA, *et al.* Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands, 2007–2009. *Emerg Infect Dis* 2011; 17:1846–1854.
A large prospective multicenter surveillance study in the Netherlands in which all clinical *A. fumigatus* isolates were screened for itraconazole resistance; 5.3% of the isolates were found to be resistant and patients suffering from invasive aspergillosis, secondary to oncologic disease, were more likely to harbour resistant strains. Of concern is that 64% of patients with a resistant strain were naive for azoles and had a high fatality rate.
36. Morio F, Aubin GG, Danner-Boucher I, *et al.* High prevalence of triazole resistance in *Aspergillus fumigatus*, especially mediated by TR/L98H, in a French cohort of patients with cystic fibrosis. *J Antimicrob Chemother* 2012; 67:1870–1873.
In this cohort of CF patients, a prevalence of *A. fumigatus* resistance of 8% was found, as well as insight into the mutations that underlie the development of this resistance.
37. Mortensen KL, Jensen RH, Johansen HK, *et al.* *Aspergillus* species and other molds in respiratory samples from patients with cystic fibrosis: a laboratory-based study with focus on *Aspergillus fumigatus* azole resistance. *J Clin Microbiol* 2011; 49:2243–2251.
In a Danish cohort, a large number of sputum samples from CF patients were analyzed for resistance and genotype. A prevalence for resistance of 4.5% was found, which is consistent with other recent studies.
38. Delhaes L, Monchy S, Frealle E, *et al.* The airway microbiota in cystic fibrosis: a complex fungal and bacterial community-implications for therapeutic management. *PLoS One* 2012; 7:e36313.
This study sheds light on the importance of microbial diversity in the CF lung and the potential interaction between microorganisms. Using pyrosequencing techniques, they show that the microbial community appears to be far more diverse than previously thought and that high diversity is favourable for CF patients.
39. Guss AM, Roeselers G, Newton IL, *et al.* Phylogenetic and metabolic diversity of bacteria associated with cystic fibrosis. *ISME J* 2011; 5:20–29.
In addition to the classical, CF-associated microorganisms, far more diverse genera are represented in the CF lung.
40. Mowat E, Rajendran R, Williams C, *et al.* *Pseudomonas aeruginosa* and their small diffusible extracellular molecules inhibit *Aspergillus fumigatus* biofilm formation. *FEMS Microbiol Lett* 2010; 313:96–102.
41. van der Ent CK, Hoekstra H, Rijkers GT. Successful treatment of allergic bronchopulmonary aspergillosis with recombinant anti-IgE antibody. *Thorax* 2007; 62:276–277.
42. Kanu A, Patel K. Treatment of allergic bronchopulmonary aspergillosis (ABPA) in CF with anti-IgE antibody (omalizumab). *Pediatr Pulmonol* 2008; 43:1249–1251.
43. Lebecque P, Leonard A, Argaz M, *et al.* Omalizumab for exacerbations of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *BMJ Case Rep* 2009; 2009. doi: bcr07.2008.0379.
44. Zirbes JM, Milla CE. Steroid-sparing effect of omalizumab for allergic bronchopulmonary aspergillosis and cystic fibrosis. *Pediatr Pulmonol* 2008; 43:607–610.