Mycopathologia (2018) 183:119–137
https://doi.org/10.1007/s11046-017-0184-y

Fungal Pathogens in CF Airways: Leave or Treat?

A. Singh · A. Ralhan · C. Schwarz · D. Hartl · A. Hector

Received: 29 March 2017 / Accepted: 22 July 2017 / Published online: 2 August 2017

Abstract Chronic airway infection plays an essential role in the progress of cystic fibrosis (CF) lung disease. In the past decades, mainly bacterial pathogens, such as *Pseudomonas aeruginosa*, have been the focus of researchers and clinicians. However, fungi are frequently detected in CF airways and there is an increasing body of evidence that fungal pathogens might play a role in CF lung disease. Several studies have shown an association of fungi, particularly *Aspergillus fumigatus* and *Candida albicans*, with the course of lung disease in CF patients. Mechanistically, in vitro and in vivo studies suggest that an impaired immune response to fungal pathogens in CF airways renders them more susceptible to fungi. However, it remains elusive whether fungi are actively involved in CF lung disease pathologies or whether they rather reflect a dysregulated airway colonization and act as microbial bystanders. A key issue for dissecting the role of fungi in CF lung disease is the distinction of dynamic fungal–host interaction entities, namely colonization, sensitization or infection. This review summarizes key findings on pathophysiological mechanisms and the clinical impact of fungi in CF lung disease.

Keywords Cystic fibrosis · Fungal colonization · *Aspergillus fumigatus* · *Candida albicans* · *Pneumocystis jirovecii* · *Exophiala dermatitidis*

A. Singh · A. Ralhan · D. Hartl · A. Hector (✉)
Department of Pediatrics, Pediatric Infectiology, Immunology and Cystic Fibrosis, Children's Hospital, University of Tübingen, Hoppe-Seyler-Str. 1, 72076 Tübingen, Germany
e-mail: Andreas.Hector@med.uni-tuebingen.de

C. Schwarz
Department of Pediatric Pneumology and Immunology, Cystic Fibrosis Center Berlin/Charité, University of Berlin, Berlin, Germany

Introduction

Cystic fibrosis (CF) is the most common lethal inherited disease in Caucasians. Although CF is a multiorgan disease, the lung disease remains the main cause for morbidity and mortality in CF patients [1, 2]. Lung disease develops very early in life and is characterized by chronic progressive inflammation and infection [3–5]. In the past decades, marked effort has been put into the development of antibacterial drugs, especially those directed against *Pseudomonas aeruginosa*, the most common pathogen in CF airways besides *Staphylococcus aureus* [6–15], which resulted in a decrease in the prevalence of *P. aeruginosa* in CF patients [16–18]. At the same time, the prevalence of other pathogens such as fungi has increased over the past decades [19–21], although there was one study from the UK reporting a decrease in the prevalence of *Aspergillus* species between 1985 and 2005 [22]. Some hypotheses have been proposed to explain the increase in fungal pathogen recovery from the CF respiratory secretions:

(a) prolonged use of inhaled antibiotics [23–27] or inhaled corticosteroids [28] and (b) increasing age of the patients [26, 29]. A simple and very important explanation might be the significant improvement of sample processing and microbiological detection methods over the past decades, using improved culture methods [30–33] or non-culture methods such as PCR [20, 34], genotyping [35] or oligonucleotide arrays [36]. The detection methods will be discussed in detail in separate reviews of this thematic special issue (see "Challenges in laboratory detection of fungal pathogens in the airways of cystic fibrosis patients" and "Towards the standardization of mycological examination of sputum samples in cystic fibrosis—results from a French multicenter study"). In this review, we will focus on the clinical impact of fungal pathogens and the cellular pathomechanisms in CF lung disease.

Although the clinical relevance of fungi in CF lung disease is yet a matter of further investigations, lately, an increasingly appreciated role of fungal pathogens has been attributed especially to the most commonly isolated fungi in CF, the *Aspergillus* and *Candida* species [37, 38]. The airways are constantly challenged by fungal spores, and the human immune system has evolved a plethora of defense mechanisms to clear fungal pathogens effectively [39–43]. CF airways, however, might be prone to fungal colonization/infection because of several factors: (a) The underlying CFTR defect leads to defective mucociliary clearance and airway mucus thickening resulting in persistence of pathogens and recurrent infections [2, 44]; (b) CF patients display a local immune dysfunction within the airways either primary due to the CFTR defect [45–47] or secondary due to the chronic overwhelming inflammatory and proteolytic processes [38, 48–51]; (c) certain modifier genes were found to be associated with increased risk of fungal colonization in CF, especially by *A. fumigatus* [52–54]; (d) CF patients with low lung function, representing an advanced stage of the CF lung disease with, e.g., significant bronchiectasis, have greater risk of colonization by *A. fumigatus* [19, 55].

Epidemiology of Fungal Pathogens in the CF Airways

The most commonly isolated fungi include the filamentous molds of *Aspergillus* species, particularly *A. fumigatus*, and the yeast fungi of *Candida* species, mainly *C. albicans*. The prevalence among CF patients given in past studies differs significantly, ranging from 6 to 60% for *A. fumigatus* [30, 33, 56–61], 35–93% for *C. albicans* [27, 30, 57, 58, 61–64], 3–17% for *Scedosporium* spp. [58, 61, 65–68], 1–19% for *Exophiala dermatitidis* [30, 58, 61, 69–71] and 7 to 38% for *Pneumocystis jirovecii* [72–75]. The variety of fungal prevalence in these studies might be due to: (a) different detection methods used; (b) geographical differences [58]; or (c) microbiome changes due to the application of inhaler in addition to systemic antibiotics over years. Nagano et al. [20] showed that fungi were detected in only 18% of the patients using conventional microbiological culture, while 78% of the patients were tested positive using fungal-specific culture media and 100% using a molecular method. In another study, Borman et al. [58] identified significant differences between CF centers throughout Europe regarding their microbiological methods and detection rates of fungi and concluded that standardization in microbiological laboratories is indispensable. A recent monocentric study from a German reference center for fungal infections examined the prevalence of airway fungi in CF patients over a 5-year period between 2009 and 2013. The most common pathogens were *C. albicans*, *C. dubliniensis* and *C. glabrata* among the yeasts (38, 12 and 9%, respectively), and *A. fumigatus* and *S. apiospermum* (29 and 2%, respectively) as well as the yeast-like *E. dermatitidis* (3%) among the filamentous fungi [61].

Recent mycobiome studies in airway samples from CF patients reported a high variability of fungal pathogens, and the results differ from studies utilizing conventional media-based culture methods [76–79]. These studies showed that fungal microbiota in the lower airways of CF patients are more diverse and complex than previously thought, especially when it comes to cross-kingdom synergy and interactions [76, 80]. This might necessitate novel methods to identify the fungal and bacterial colonization/infection in CF airways as well as different strategies for antimicrobial treatment in CF patients in future.

The epidemiology of fungal pathogens and the microbiome of CF lung disease are discussed in detail in separate reviews within this thematic special issue (see "Organization of patient management and epidemiology of fungal infections in cystic fibrosis" and "The airway mycobiome in cystic fibrosis").

Aspergillus Species and Their Role in CF Lung Disease

Colonization of the Respiratory Tract by *Aspergillus* Species

The most common filamentous fungi isolated from respiratory secretions from CF patients are *Aspergillus* species, particularly *A. fumigatus*. *Aspergillus* species are saprophytic spore-forming fungi that are ubiquitously found in the environment. The spores (also called conidia) which are very small (2–4 μm in diameter) are inhaled by hundreds every day and directly enter the small airways [81]. In healthy airways, they are instantly cleared without provoking inflammatory responses. However, in CF airways, the spores are able to persist and germinate (hyphae), thus eliciting an immune response with leukocyte infiltration and mucus accumulation [82]. A number of studies have reported a negative association of fungi with the lung function in CF patients. In a cross-sectional study with 7010 CF patients from the European Epidemiologic Registry of Cystic Fibrosis (ERCF), colonization by *Aspergillus* species was associated with slightly impaired lung function [5–10% forced expiratory volume in one second (FEV_1)] [83]. Amin et al. [59] conducted a retrospective study in 230 Canadian children with CF between 1999 and 2006 and found significantly lower FEV_1 in children chronically infected by *A. fumigatus* compared to uninfected individuals. At the same time, these patients were at higher risk of pulmonary exacerbations requiring hospitalization. In another Canadian prospective study between 2005 and 2008 with 446 adult CF patients, the prevalence of *Aspergillus* species was twice as high in patients with frequent exacerbations per year compared to those with less than one exacerbation per year [84]. Noni et al. [55] performed a prospective case–control study in Greece with 80 pediatric CF patients and found a significant impact of chronic colonization by *A. fumigatus* on lung function decline over a 7-year period. Interestingly, vice versa, lower FEV_1 at baseline 4 years before study enrollment was the only factor associated with acquisition of chronic *A. fumigatus* colonization [55]. In a retrospective study with 45 CF children from UK, Saunders et al. [29] concluded that chronic colonization by *A. fumigatus* might be associated with worse lung function, especially in children with severe lung disease. Importantly, the Australian Respiratory Early Surveillance Team for Cystic Fibrosis (AREST CF) study with 56 CF newborns from Australia highlighted the significant impact of early airway infection within the first 2 years of life as predictors of lung function and found *Aspergillus* species among the harmful pathogens [5]. In our retrospective study comprising 770 CF patients from three CF centers in Germany and Austria, *A. fumigatus* colonization was associated with a more rapid decline in FEV_1 when compared to CF patients who had never had positive culture for *A. fumigatus*. In this study, *A. fumigatus* was also the only risk factor for acquisition of chronic *P. aeruginosa* infection in these patients [85]. Consistently, in a past study, Amin et al. [59] found a significant interaction between *A. fumigatus* and *P. aeruginosa* on the course of lung function. An Irish study by McMahon et al. [86] in 36 adolescent and adult CF patients reported significant changes in the severity of bronchiectasis using computed tomography in the *Aspergillus*-colonized subgroup compared to non-colonized individuals, although Bhalla scores and other radiological parameters as well as FEV_1 were not different. A very interesting study from the AREST CF consortium in Australia with 215 pediatric CF patients found increased inflammatory responses, namely increased neutrophil cell count, neutrophil elastase and interleukin (IL)-8 levels, in bronchoalveolar lavage samples from *Aspergillus*-positive CF children compared to never-infected CF children, while colonization by *Candida* species had no effect [87]. However, other studies could not show any association between colonization by *A. fumigatus* and lung function or radiological abnormalities. In a study from the Netherlands in 259 adult CF patients, de Vrankrijker et al. [26] did not find an independent association between chronic *Aspergillus* colonization and lung function or radiological changes over a 5-year period. Similarly, colonization by *Aspergillus* species did not correlate with lung function or computed tomography imaging in a cross-sectional study with 104 adult CF patients [24].

Other *Aspergillus* species such as the transient pathogens *A. flavus*, *A. niger*, *A. nidulans*, *A. tubingensis* and the chronic colonizer *A. terreus* are frequently detected in CF airways, yet their clinical impact remains elusive [88–90].

Aspergillus Bronchitis

Aspergillus bronchitis was first described by Shoseyov et al. [91] in 2006, when six CF patients experienced respiratory exacerbations without clinical improvement despite appropriate antibiotic treatment, had positive sputum cultures for *Aspergillus* species and responded to antifungal treatment. Importantly, these patients did not fulfill the criteria for allergic bronchopulmonary aspergillosis (ABPA). In 2013, Baxter et al. [92] proposed a novel classification for *Aspergillus*-related lung disease in CF using *Aspergillus*-specific PCR and measurement of sputum galactomannan alongside with detection of total IgE and specific anti-*A. fumigatus* IgE and IgG serum antibodies (Table 1). By this way, the enrolled 130 adult CF patients were subdivided into four groups: (1) non-diseased (37.7%)—either positive or negative PCR, no specific anti-*A. fumigatus* antibodies, negative galactomannan; (2) ABPA (17.7%)—positive PCR, elevated total IgE and specific anti-*A. fumigatus* IgE and IgG, positive galactomannan; (3) *Aspergillus* sensitized (AS) (14.6%)—either positive or negative PCR, elevated specific anti-*A. fumigatus* IgE (but not IgG), negative galactomannan; and (4) *Aspergillus* bronchitis (30%)—positive PCR, elevated specific anti-*A. fumigatus* IgG (but not IgE), positive galactomannan. Notably, 30% of these patients fell into the group with *Aspergillus* bronchitis. Furthermore, in this 2-year prospective study, FEV_1 dropped significantly faster in the CF patients with ABPA, AS and *Aspergillus* bronchitis compared to the non-diseased CF patients [92]. This classification might be helpful to discriminate *Aspergillus* colonization from *Aspergillus* bronchitis, and AS from ABPA, and, more importantly, might be a useful tool to identify those patients who would benefit from antifungal therapy.

Aspergillus Sensitization

The prevalence of AS in CF varies from 20 to 65%. In a recent review, Maturu and Agarwal [93] performed a meta-analysis on 41 studies and calculated the pooled prevalence to be 39%. Interestingly, studies using cutaneous testing showed higher prevalence (43%) compared to studies using serological testing (33%) [93]. AS is defined by T helper cell type 2 (Th2) immune response and a subsequent increase in IgE-mediated immune response to *Aspergillus* species without fulfilling the diagnostic criteria of ABPA. The role of AS in CF lung disease and its association with poorer lung function are well established by a number of studies [60, 92, 94–98]. In the above-mentioned study by Baxter et al. [92], CF patients with AS (elevated IgE, normal IgG, negative galactomannan, PCR positive or negative) experienced a significantly higher FEV_1 decline over the course of 2 years compared to the group of non-diseased patients. Furthermore, Baxter et al. [60] reported that sensitization to *Aspergillus*, but not to *Candida*, was associated with greater FEV_1 decline over a 2-year period and with increased numbers of pulmonary exacerbations in a cohort of 55 CF adults. Interestingly, there was no association between *Aspergillus* or *Candida* colonization and the development of serological sensitization. In another retrospective study over two 5-year periods from 1996 to 2000 (19 sensitized and 19 non-diseased CF children) and 2001–2005 (24 sensitized and 23 non-diseased CF children), AS was associated with changes in pulmonary function only in the first period. The AS patients in the second cohort had significantly higher baseline FEV_1 values compared to those from the first cohort, and this was associated with significantly more oral antifungal treatment in the second cohort [98].

Table 1 Classification of *Aspergillus*-related lung disease in CF, modified from Baxter et al. [92]

	Aspergillus colonization	ABPA	Aspergillus sensitization	Aspergillus bronchitis
Aspergillus-specific PCR	±	+	±	+
Sputum galactomannan	–	+	–	+
Total IgE	–	+	+	–
Specific anti-A. fumigatus IgE	–	+	+	–
Specific anti-A. fumigatus IgG	–	+	–	+

ABPA allergic bronchopulmonary aspergillosis

Allergic Bronchopulmonary Aspergillosis (ABPA)

ABPA is the most well-characterized and well-recognized *Aspergillus*-related disease in CF. The given prevalence varies significantly among the studies, ranging from 3 to 25% with a pooled prevalence of 9% in the meta-analysis of Maturu and Agarwal [93], being higher in adults (10.1%) compared to children (8.9%). ABPA is caused by an exaggerated hypersensitivity reaction to antigens of various fungi, mostly *A. fumigatus*, in individuals with chronic lung diseases such as asthma and CF. Th2-associated immune response with elevated cytokine levels for IL-4, IL-5 and IL-13 results in upregulation of low-affinity IgE receptors on B cells, which subsequently leads to an increase in total IgE and specific anti-*Aspergillus* IgE and IgG, causing type I and type III hypersensitivity reactions [99–101]. Without early diagnosis and sufficient treatment, ABPA becomes chronic and persistent inflammation results in bronchiectasis and severe lung fibrosis [40, 102, 103]. The role of ABPA in lung function decline, and unstable disease was demonstrated in a number of studies [92, 97, 104–107]. Kraemer et al. [97] demonstrated a significant negative effect of ABPA on FEV_1 and forced expiratory flow at 50% (FEF_{50}) in a retrospective study with an observational period of 28 years in 122 mostly pediatric CF patients. In a prospective study in 50 CF adults, Chotirmall et al. [105] described a mean transient decline of 6.7% in predicted FEV_1 in patients with ABPA followed by a normalization after treatment. Another study in 56 adult CF patients analyzed the factors associated with the time until the next pulmonary exacerbation (TUNE) and found ABPA aside of CF-related diabetes to be correlated with shorter TUNE [107]. A study from Germany found a significant association of pet ownership with ABPA and increased numbers of pulmonary exacerbations in 109 CF patients [108]. In the aforementioned study by Baxter et al. [92], CF patients with ABPA had a more pronounced FEV_1 decline over a 2-year period compared to the non-diseased individuals.

However, the optimal method to distinguish between AS and ABPA in CF is still a matter of debate. For example, different variable cutoff values for specific anti-*Aspergillus* IgE and IgG have been proposed [92, 98, 101, 109]. Furthermore, novel markers such as thymus- and activation-regulated chemokine (TARC)/CCL17 [100, 110], galactomannan in sputum or *Aspergillus* recovery by PCR have been introduced as diagnostic tools for *Aspergillus*-related disease entities in CF [92]. Other studies have suggested using the basophil activation test (BAT) by measurement of CD203c expression on basophils upon stimulation with *Aspergillus* antigen as a diagnostic tool for ABPA [111] or discriminating AS from ABPA [112, 113]. Recommendations from the Cystic Fibrosis Foundation Consensus Conference in 2001 were published in 2003 for diagnostic and screening criteria as well as treatment [114]. Nevertheless, a large part of the studies on ABPA in CF published after issue of this consensus paper used different diagnostic criteria [93]. This might be due to a need for improved diagnostic and monitoring techniques for ABPA in CF [92, 115].

Clinical Impact of Other Fungal Pathogens in the CF Airways

Colonization by Non-*Aspergillus* Filamentous Fungi

Molds belonging to the *Scedosporium* genus and *Lomentospora prolificans* (formerly *Scedosporium prolificans*) are the second most common filamentous fungi detected in the airways of CF patients [65]. Normally, *Scedosporium/Lomentospora* infections include sinusitis, pneumonia, lung fungus ball, chest abscesses or disseminated infections in immunocompromised patients [116]. While there are case studies reporting detrimental effects of *Scedosporium/Lomentospora* infections in CF patients without previous lung transplantation, the clinical impact of these pathogens is not fully understood. One case study reported on the development of a lung mycetoma, respiratory distress and suggestive cerebral involvement due to neurological symptoms within 18 months after initial isolation of *S. apiospermum* in a young male CF patient. After neurological symptoms occurred, the clinical state deteriorated dramatically, resulting in coma and death within a few days [117]. Another case report showed a rapid clinical impairment within 2 years in a young female CF patient who had excellent exercise tolerance before the acquisition of *S. apiospermum*. After numerous therapeutic approaches including systemic and inhaled antimycotics, intravenous antibiotics, corticosteroids and

immunoglobulins, in the end, lung transplantation was required [118]. In a third study, a 11-year-old CF male presented with respiratory distress and reduced physical tolerance. After isolation of *S. apiospermum* for the first time and insufficient response to intravenous voriconazole and corticosteroid therapy, respiratory distress worsened with a significant decline in FEV_1, necessitating therapeutic bronchoscopy. Bronchoalveolar lavage cultures were positive for *S. apiospermum* only, which showed resistance to voriconazole. The authors suspected a plastic bronchitis due to *S. apiospermum* [119]. Despite these case reports, systematic studies have not found any significant impact of *Scedosporium/Lomentospora* species on lung function. Blyth et al. [67] did not find any association between FEV_1 and colonization by these fungi in their prospective study with 69 adult CF patients. This was confirmed in another recent prospective study with 161 adolescent and adult CF patients showing no difference in FEV_1 between colonized and non-colonized patients. In the latter study, colonization by *Scedosporium/Lomentospora* species was associated with younger age, less colonization with *Haemophilus influenzae* and mucoid *P. aeruginosa* [120]. Interestingly, in both prospective studies, CF patients colonized by these fungi tended to have higher FEV_1 values compared to the non-colonized individuals, although not statistically significant. Nevertheless, colonization by *Scedosporium/Lomentospora* species was associated with higher risk of ABPA [33, 120] or was even linked to symptoms of allergic bronchopulmonary disease similar to ABPA but independently from *Aspergillus* species [65].

Exophiala dermatitidis grows as a black yeast at 37 °C and as a filamentous fungus at room temperature. In CF airways, *E. dermatitidis* may be present transiently or chronically, but usually without any clinical signs. A single case study from Germany reported on a young CF girl who presented with respiratory distress symptoms due to pneumonia with parahilar mottles in the X-ray, which is atypical for CF [121, 122]. In two prospective studies from a Swedish cohort with 98 CF patients aged more than 12 years, *E. dermatitidis* or increased serum levels of IgG against *E. dermatitidis* were associated with pancreatic insufficiency or more frequent colonization by non-tuberculous mycobacteria, increased blood inflammation markers, more frequent intravenous antibiotic treatment and lower FEV_1, respectively [70, 123]. Although the clinical impact of this pathogen is an ongoing matter of research, its presence in the airways should be considered as a CF biomarker and isolation in context with bronchiectasis should dispose the physician to search for an atypical manifestation of CF (see *Exophiala* colonization and infection as a surrogate marker for cystic fibrosis in the elderly in this special issue).

Other filamentous fungi such as *Rasamsonia argillacea* (formerly *Geosmithia argillacea*, but first reported in CF as *Penicillium emersonii*) or *Acrophialophora fusispora* are almost exclusively isolated from CF patients [124]; however, there have been very limited studies on the clinical relevance of these fungi in CF [125, 126].

Colonization by Yeasts

Chronic colonization by *Candida* species was similarly associated with aggravated CF lung disease. In the aforementioned cross-sectional study from the ERCF, colonization by *Candida* species was associated with slightly impaired lung function (5–10% FEV_1) in CF patients [83]. In a prospective longitudinal study over 11 years from Ireland in 89 adult CF patients, intermittent and chronic colonization by *C. albicans* was a significant predictor for hospitalization due to pulmonary exacerbation and accelerated the decline in FEV_1 and body mass index (BMI) [62]. Another prospective longitudinal study over a 6-year period in 91 pediatric and adult CF patients reported that baseline FEV_1 was significantly lower and annual decline was significantly higher in patients chronically colonized by *C. albicans* compared to non-colonized patients, while intermittently colonized individuals had intermediate values. However, in this study, no difference was observed in BMI between the groups, although BMI <20 kg/m^2 was an independent predictor for chronic *C. albicans* colonization. Furthermore, colonization by *A. fumigatus* was significantly associated with chronic *C. albicans* colonization and was also an independent risk factor for chronic *C. albicans* colonization [64]. In our previously mentioned longitudinal study, we did not find any negative association between *C. albicans* colonization and lung function or BMI [85].

Other common members of the *Candida* species include *C. dubliniensis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. lusitaniae* and *C. tropicalis*. Very little

is known about the impact of these pathogens in CF lung disease. For *C. glabrata*, the above-mentioned study by us showed a more rapid FEV_1 decline over time in patients colonized by this pathogen compared to those patients who have never had positive culture [85]. In two prospective studies with 54 or 20 pediatric and adult CF patients, both conducted to investigate the prevalence of *C. dubliniensis* in CF airways, all patients colonized by *C. dubliniensis* were clinically stable over the study period of 7 months or 2 years, respectively [127, 128].

Colonization by *Pneumocystis jirovecii*

Pneumocystis jirovecii, initially described as a protozoan in the early twentieth century, was classified officially as a fungus in 1999. While *P. jirovecii* pneumonia (PJP) plays a major role in morbidity and mortality in hematologic malignancies and in immunodeficient and immunosuppressed patients [129, 130], the role of this pathogen in CF lung disease is unknown. A recent case report described a pulmonary *P. jirovecii* infection in a female CF toddler that was successfully treated with cotrimoxazole [131]. A recent prospective longitudinal study over 1 year in 111 adult CF patients found that *P. jirovecii* was more likely isolated when patients experienced an acute pulmonary exacerbation. However, pulmonary exacerbation with positive *P. jirovecii* detection was milder as suggested by lower pulmonary exacerbation score and lower serum C-reactive protein levels [132]. In a prospective multicenter study from France in 104 adult CF patients, colonization with *P. jirovecii* was associated with the absence of *P. aeruginosa* and with higher FEV_1 values [75]. Furthermore, no PJP was observed and patients were stable in two prospective studies with 95 or 88 CF patients colonized by *P. jirovecii* over a 2-month or a 1-year follow-up period, respectively [72, 73]. Although it has been shown that *P. jirovecii* might induce inflammatory changes and airway obstruction in chronic obstructive pulmonary disease [133, 134], there is no evidence until now that it might play a significant role in CF lung disease. A possible explanation might be that CF airways are more frequently colonized by some less pathogenic *P. jirovecii* genotypes compared to the genotypes found in immunosuppressed patients suffering from PJP [135].

Treatment of Fungal Colonization and Infection in CF Lung Disease

Although a number of case studies on treatment of CF patients with fungus-related lung diseases have been published, almost no randomized, controlled trials have been conducted to date. *Aspergillus* colonization and infection are treated with antimycotics, while ABPA is mostly treated with corticosteroids and sometimes with adjunctive itraconazole or voriconazole. Furthermore, the successful use of the anti-IgE drug omalizumab in CF-ABPA has been reported in several case reports [136–147]. Only one double-blind, randomized, placebo-controlled study was initiated for omalizumab, but has been prematurely terminated due to the inability to recruit participants into the study [148]. For antifungal therapy, the only completed randomized, controlled trial to date was conducted by Aaron et al. [21] in a total of 35 adolescent and adult CF patients with *A. fumigatus* colonization (non-ABPA), 18 receiving itraconazole 5 mg/kg/d and 17 receiving placebo over 24 weeks. No beneficial effect was observed after treatment with itraconazole. On the contrary, the time to first pulmonary exacerbation was shorter in the treatment group (77 days) compared to the placebo group (134 days), without reaching statistical significance. However, this study had two major limitations—firstly, the number of patients were too small, and secondly, itraconazole blood levels were subtherapeutic in 43% of the treated patients [21]. A French multicenter, open-label trial on the impact of itraconazole and voriconazole in *Aspergillus*-infected CF patients has been completed, recently, but the results have not been published, until now (clinicaltrials.gov). In a single-center open-label study, 13 CF patients with *A. fumigatus* colonization (non-ABPA, non-AS with total serum IgE < 190 IU/ml, negative cutaneous test to *Aspergillus* antigens) were treated with itraconazole over 6 weeks, resulting in a significant decrease in airway fungal burden, improved respiratory symptoms and mosaic pattern changes in computed tomography as well as preserved lung function and a decrease in episodes of pulmonary exacerbations in the follow-up period of up to 12 months. The authors concluded that *A. fumigatus* eradication by itraconazole resulted in reduced levels of the fungal metabolite gliotoxin that was responsible for down-regulation of the nuclear vitamin D receptor.

Decreased vitamin D receptor expression led to increased production of IL-5 and IL-13 and hence to an enhanced Th2-associated immune response. Therefore, the beneficial effect of high-dose vitamin D_3 supplementation (4.000 IU/day) in AS patients with history of ABPA as shown by Nguyen et al. [149] might be hampered by *A. fumigatus* gliotoxin and might necessitate concurrent elimination of *A. fumigatus* in order to permit vitamin D receptor expression [34]. Hilliard et al. [150] reported on a retrospective study in 21 pediatric CF patients receiving voriconazole over various durations (1–50 weeks), with diverse clinical indications and either as a monotherapy or in combination with an oral corticosteroid. Thirteen patients had ABPA, and 7 of the remaining 8 patients had a history of *A. fumigatus* colonization or previous ABPA. In the group with ABPA, voriconazole (monotherapy and in combination with corticosteroids) resulted in significant clinical and serological improvement, while it had no effect in the group without ABPA [150]. In another retrospective study encompassing 21 CF patients with ABPA over a 5-year period, treatment with itraconazole (5–10 mg/kg/day for 6–54 months in 14 patients or continuously in 7 patients) as monotherapy had no significant effect on lung function, while in combination with corticosteroids, FEV_1 and FVC significantly increased. Total IgE decreased in 42% of the patients receiving monotherapy and in 56% of the patients on combination therapy [151]. In the aforementioned study by Shoseyov et al. [91], six CF patients with *Aspergillus* bronchitis received itraconazole or voriconazole after antibacterial therapy had been ineffective, and azole treatment improved lung function, nutritional status and serological markers. The study by Kanthan et al. [98] comparing two 5-year periods (1996–2000 and 2001–2005) in a pediatric CF cohort with AS noted that the patients received significantly more antifungal treatment in the 2001–2006 period compared to the first 5-year period, which was associated with better lung function. Furthermore, several case studies on antimycotics reported the safe use of nebulized amphotericin B for ABPA [152, 153] or nebulized voriconazole for *S. apiospermum* [154] in CF. However, there are insufficient data on safety, necessity and effectivity of antifungal treatment in CF lung disease to date, both for ABPA [155] and for other fungus-related disease entities. More randomized, controlled studies are urgently needed to answer the questions on when (in colonized, bronchitis or sensitized patients?), which compound, route of administration (inhaled or systemically?) and how long (monitoring the fungal burden or clinical improvement?). Importantly, the increasing azole resistance (around 5–8% azole-resistant strains have been reported [156–158]) and drug–drug interactions due to inhibition of cytochrome P450 enzymes (increased serum concentration of corticosteroids and other cytochrome P450-dependent drugs such as Ivacaftor) [159] have to be taken into account when considering eradication therapy of fungal pathogens.

Treatment of fungal infections in CF and azole resistance in *A. fumigatus* will be discussed in detail in separate reviews of this thematic special issue (see "Progress in treatment and prevention of fungal infections in CF" and "Azole resistance in *Aspergillus fumigatus* in patients with cystic fibrosis: a matter of concern?").

Pathophysiology of Fungi in CF

Figure 1 gives an overview of the pathogen recognition receptors on innate immune cells involved in fungal detection.

Interaction of Fungi with Other Microbes in CF

Yeasts

CF lungs consist of a complex microbial community, and resulting biofilms contain different microbes [79, 160]. Initially, it was believed that biofilms are traditionally monomicrobial in nature. However, there is growing evidence that these biofilms in CF lung provide a niche to many microbes to thrive [161, 162]. *Candida albicans* is one of the major players involved in polymicrobial communities in CF lungs. This yeast can often be found to grow with bacteria in these biofilms, and these pathogen–pathogen interactions lead to unprecedented events in terms of virulence of the pathogens. *P. aeruginosa* and *S. aureus* are the most prevalent opportunistic pathogens in individuals with CF lung disease followed by *H. influenzae* and other species [6, 7, 9, 14, 15]. Several studies have demonstrated that *C. albicans* and *P. aeruginosa* interact with each other in the lung and also cause synergistic infections [163, 164]. Studies show that *P. aeruginosa*

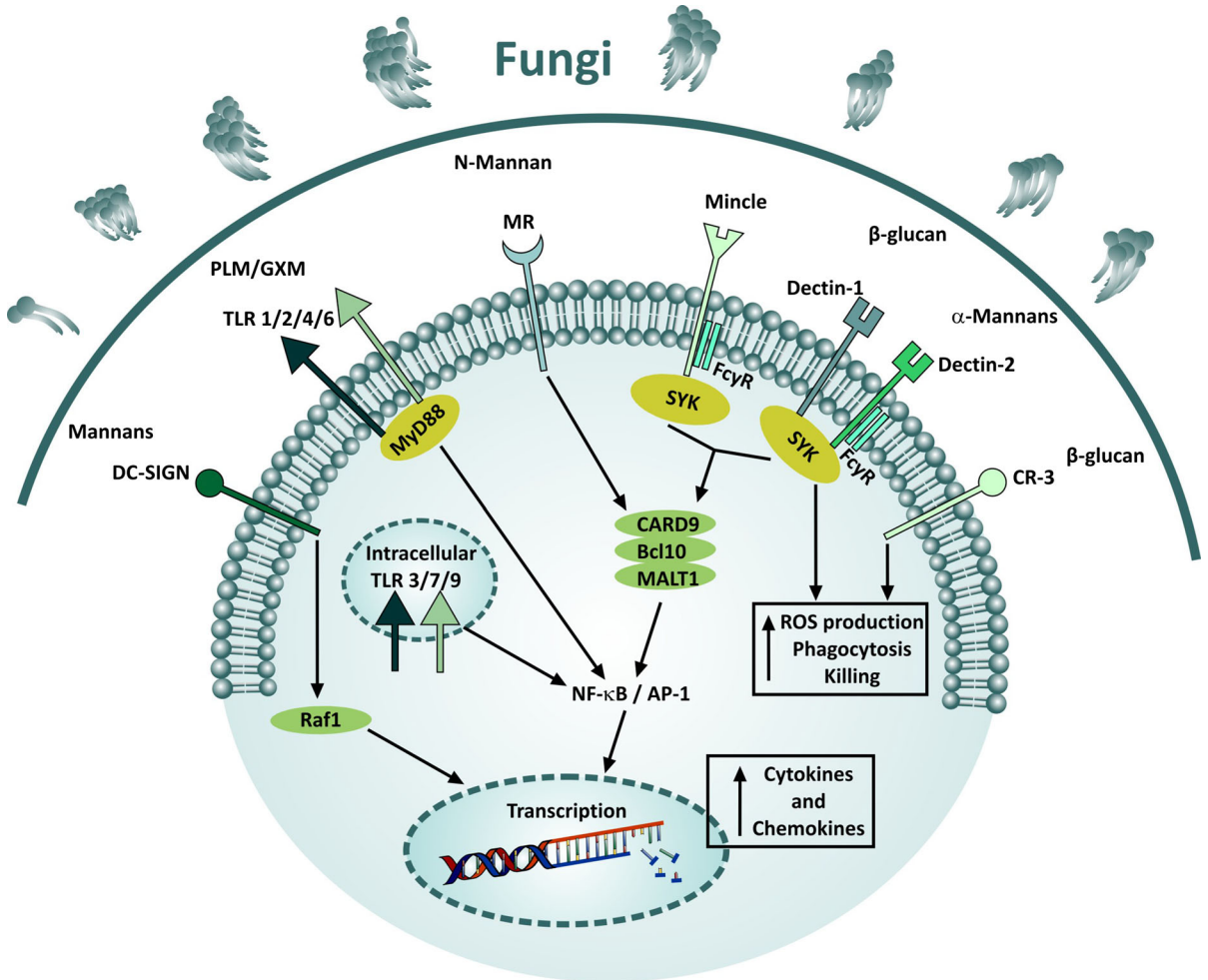

Fig. 1 Fungal recognition by innate immune cells. Fungal pathogens are detected by a variety of pattern recognition receptors (PRRs). Through receptor recognition, signaling pathways are activated leading to increased production of cytokines and chemokines and to engagement of different antifungal immune mechanisms. *AP-1* activator protein 1, *Bcl10* B cell lymphoma/leukemia 10, *CARD9* caspase recruitment domain-containing protein 9, *CR-3* complement receptor 3, *DC-SIGN* dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin, *GXM* glucuronoxylomannan, *MALT1* mucosa-associated lymphoid tissue lymphoma translocation protein 1, *Mincle* macrophage inducible Ca2+-dependent lectin, *MR* mannose receptor, *MyD88* myeloid differentiation primary response gene 88, *NF-κB* nuclear factor kappa-light-chain-enhancer of activated B cells, *PLM* phospholipomannan, *Raf1* rapidly accelerated fibrosarcoma 1, *ROS* reactive oxygen species, *SYK* spleen tyrosine kinase, *TLR* Toll-like receptor

can form a dense biofilm on *C. albicans* filaments leading to the killing of fungi. This phenomenon was dependent on morphogenetic changes as only hyphal form of *Candida* was affected by killing and there was no effect on the yeast form [163, 165]. Secreted mediators-based signaling between both the organisms also occurred, validating an important role of *P. aeruginosa* pigments in interaction with *Candida* quorum-sensing molecules. Hyphal formation by *Candida* also helped in acquiring the nutrition for *P. aeruginosa* [165]. As an example, Chen et al. [166] reported that *Candida* ethanol stimulated the biofilm formation and adhesion of *P. aeruginosa* through inhibition of locomotion. This effect was accompanied by enhanced production of antifungal phenazine derivatives by *P. aeruginosa*, molecules that, in turn, were shown to promote ethanol production within fungi [166]. McAlester et al. [167] reported *C. albicans* farnesol inhibiting the swarming capability of *P. aeruginosa* in CF isolates. Using a combination of clinical and genomic methods,

Kim et al. [168] showed how pathogens and their interactions adapt to the CF environment. The study reported that *C. albicans* isolates from CF patients could go through adaptive mutation in *NRG1* (*Pro-neuregulin*-1), which led to filamentation without any environmental stimulus. Interestingly, these filamentous forms of *C. albicans* were resistant to *P. aeruginosa*-mediated factors, which in general are known to suppress the filamentous growth of the fungus [168].

Staphylococcus aureus is the other most prevalent bacteria in CF lung especially during early onset of the disease. Due to close proximity and niche in the CF lung, *Candida* and *Staphylococcus* also interact with each other at various physical and biochemical levels [169]. Using a mouse model, Carlson et al. [170] showed enhanced mortality of the mice after coinfection with *C. albicans* and *S. aureus.* Physical interaction between *C. albicans* and *S. aureus* led to synergistic effect on biofilm formation, followed by degradation of *S. aureus* biofilm by *C. albicans* farnesol [171, 172]. Apart from *C. albicans*, *C. dubliniensis* are also reported to show an interesting effect in CF environment. A study by Wahab et al. [128] reported that the presence of *C. dubliniensis* in the lower airways of CF patients might be due to their increased adherence in comparison with other *Candida* species. Since *C. dubliniensis* was isolated along with co-colonizing bacteria like *P. aeruginosa* and *S. aureus*, authors speculated that it might hint toward unknown survival strategies of *C. dubliniensis* and coexistence with other pathogens in CF lungs [128].

Aspergillus spp.

Some studies shed light on the interaction of *A. fumigatus* with *P. aeruginosa*. Briard et al. [173] showed that *A. fumigatus* and *P. aeruginosa* could interact at a distance through volatile compounds-mediated communication and, interestingly, *A. fumigatus* growth was enhanced in response to these compounds [173]. On the contrary, Mowat et al. [174] demonstrated that small diffusible and heat-stable molecules of *P. aeruginosa* could potently inhibit filamentous growth of *A. fumigatus*, making it an interesting phenomenon for the CF lung. In an earlier study, Briard et al. [175] demonstrated interactions between *A. fumigatus* and *P. aeruginosa*, leading to stimulatory or antagonistic effects. They analyzed the functions of the four *P. aeruginosa* phenazines and their mode of action on *A. fumigatus*. All four phenazines showed *A. fumigatus* growth inhibitory effects. The inhibition of *A. fumigatus* involved production of reactive oxygen species (ROS), specifically O_2^-, and the reactive nitrogen species (RNS) $ONOO^-$. They also found that subinhibitory concentrations of pyocyanin (PYO), phenazine-1-carboxylic acid (PCA) and phenazine-1-carboxamide (PCN) could enhance *A. fumigatus* [175]. In CF, Shirazi et al. [176] demonstrated that biofilm filtrates of *P. aeruginosa* strains isolated from CF patients could inhibit preformed *A. fumigatus* biofilms through apoptosis and non-CF isolates were less inhibitory. Complex and adaptive fungi–bacteria interaction was demonstrated by Moree et al. [177] who showed that reported inhibitors of *A. fumigatus*, phenazines secreted by *P. aeruginosa*, were used as siderophores by *A. fumigatus.* Another mechanism of fungi killing was described showing bacteriophage Pf4 produced by *P. aeruginosa* inhibiting *A. fumigatus* biofilm by iron sequestration [178]. The role of IL-10 in CF during infection with *A. fumigatus* and *P. aeruginosa* was addressed by Casaulta et al. [179] using an in vitro model. Using peripheral blood mononuclear cells (PBMCs) isolated from CF patients, the authors demonstrated that they secreted large amounts of IL-10 upon exposure to *A. fumigatus* antigens or heat-inactivated *P. aeruginosa* which controlled T cell response. Blockage of IL-10 led to a significant increase in T cell proliferation and interferon-γ production [179].

Other Fungi

For other fungal species co-colonizing the CF airways, evidence is still scarce in terms of their interactions with other microbes. A study by Kaur et al. [180] showed that *P. aeruginosa* inhibits the growth of *Scedosporium aurantiacum.* They also demonstrated that *P. aeruginosa* biofilm formation is important, but not crucial for inhibiting the growth of *S. aurantiacum* in a lung-mimicking model [180]. A recent study by Schwarz et al. [102] presented a novel association of ABPA and *Pseudomonas* as a new potential risk factor for *Scedosporium/Lomentospora* infections. It was also speculated that allergy might be involved in inducing immunological host reactions which eventually lead to a less effective response to *Scedosporium/Lomentospora* infections [102].

Immune Recognition of Fungi

Yeasts

Cell wall is one of the most important factors responsible for the recognition of *Candida* by the host immune system. The outer layer of *Candida* cell wall is composed of β-(1,3)-glucan polysaccharide fibrils, which are covalently linked with chitin (a β-(1,4)-linked polymer of *N*-acetyl glucosamine) and β-(1,6)-glucans. The outer layer is made of *N*- or *O*-linked mannosylated proteins termed as mannans. These cell wall polysaccharides or pathogen-associated molecular patterns (PAMPs) are recognized by various pattern recognition receptors (PRRs) present on the host cells [181]. Major PRRs that are involved in recognition of fungal pathogens by the host are mainly classified among the Toll-like receptors (TLRs) and C-type lectin receptors (CLRs). These TLRs and CLRs are expressed on the surface of different cell types that participate in antifungal host response like airway epithelial cells, macrophages, neutrophils and monocytes [182–184]. The expression of TLR1–TLR6 and TLR9 have been reported by Greene et al. [185] on CF tracheal epithelial cell lines, and no difference was found on TLR expression between both CF and non-CF cell lines. On the other hand, Muir et al. [186] also demonstrated the presence of TLR1–TLR10 in CF bronchial epithelial cell lines and an increased TLR2 expression in the CF conditions. While membrane-bound mannoprotein-recognizing TLRs such as TLR2, TLR4 and TLR6 have been described as the most important TLRs for recognition of *Candida* species [187], there is also evidence suggesting that the intracellular TLRs that recognize cytoplasmic nucleic acids, namely TLR3 and TLR9, may also have a role in anti-*Candida* host defense [188]. Also, TLR9 has been found to be expressed on the surface of primary airway epithelial cells and in the context of CF, sinonasal epithelial cells isolated from patients with CF-associated chronic rhinosinusitis display an elevated level of TLR9 [189]. A recent study in murine knockout models demonstrated that TLR9, and also nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and mannose receptor (MR), recognized fungal chitin resulting in the production of the anti-inflammatory cytokine IL-10 [190]. These findings also hint toward a broader role of TLR9 in anti-*Candida* immune response. *Candida* cell wall β-(1,3)-glucan is exposed in bud scars that are revealed during the process of cell division or hyphal transition [191]. This process facilitates *Candida* recognition by the host and may provide the hint to the host of a transition from fungal colonization to infectious form [192]. The importance of dectin-1 in the control of fungal infections is highlighted by its role in fungal killing and by the susceptibility of dectin-1-deficient mice to *C. albicans* infection [193–195]. However, the role of dectin-1 in fungal host defense remains a topic of debate. While Taylor et al. [195] showed that *dectin-1*$^{-/-}$ mice had increased susceptibility to disseminated candidiasis, another report found that *dectin-1*$^{-/-}$ deficiency was dispensable to *C. albicans* infection [196]. Furthermore, in human, dectin-1 deficiency has been studied in a family and it was found that it can lead to susceptibility to certain fungal infections including chronic mucocutaneous and recurrent vulvovaginal candidiasis [197]. Apart from dectin-1, other CLRs such as dectin-2, mannose receptor (MR) and Mincle also play a role in recognition of *C. albicans.* Dectin-2 recognizes the α-mannan of *C. albicans* and is mainly expressed on macrophages, dendritic cells (DCs) and neutrophils and modulates T helper cell responses [198]. MR recognizes N-linked mannans present on the fungal cell wall. MR is primarily present on macrophages and was shown to be responsible for further IL-17 induction [199]. Mincle is reported to be expressed on monocytes and neutrophils. It has been shown that Mincle-deficient mice are highly susceptible to systemic candidiasis [200]. However, the ligand for Mincle is still unknown.

Aspergillus spp.

Using various in vitro studies, both TLR2 and TLR4 signaling pathways have been associated with *Aspergillus*-mediated proinflammatory cytokine production and NF-κB translocation [201, 202]. Studies showed that blocking of TLR4 resulted in decreased TNFα production by adherent monocytes [203]. There is also evidence suggesting that morphology-based antifungal immune response takes place during *Aspergillus* pathogenesis via TLR2 and TLR4 as *Aspergillus* conidia and hyphae showed a different effect [204]. Further studies suggested that intracellular TLRs, TLR3 and TLR9 also play an important role during *Aspergillus* host defense. A TLR3-mediated

mechanism was reported for protection against *A. fumigatus* in epithelial cells [205, 206]. Similarly, TLR9 could recognize *Aspergillus* DNA and was actively recruited during phagocytosis of *A. fumigatus* conidia [207].

Inflammatory cytokines are produced in CF airways by continuous activation of NF-κB and TLR-independent neutrophil recruitment. Also, in CF, TLR2 and TLR5 are highly expressed on apical surfaces [208]. During infection in CF, further NF-κB is activated due to TLR signaling. Now impaired TLR4 signaling in CF further prevents the activation of immune response resulting in failure in pathogen clearance and recurrent chronic infections [209]. The CLR dectin-1 recognizes β-(1,3)-glucans and is involved in the recognition of *A. fumigatus* [210]. Human airway epithelium and other immune cells express dectin-1 [211], making it an important candidate for *Aspergillus* recognition. Binding of dectin-1 to *Aspergillus* germ tubes leads to augmentation of TLR2-mediated proinflammatory cytokine production [212]. Apart from germ tubes, swollen *Aspergillus* conidia are also recognized by dectin-1 due to enhanced surface expression of β-(1,3)-glucan [210]. There are ample studies showing dectin-1-deficient mice being extremely susceptible to pulmonary aspergillosis [213–215]. Apart from dectin-1, there is some evidence of involvement of other CLRs, namely the dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), MR and dectin-2 in anti-*Aspergillus* host defense. DC-SIGN is expressed on DCs as well as macrophages and has been shown to be involved in binding and phagocytosis of *A. fumigatus* [216]. While the MR has been shown to induce production of proinflammatory cytokines by *A. fumigatus* conidia [204], dectin-2 has been found to mediate the release of inflammatory lipid mediators from murine bone marrow-derived DCs [217]. However, the function and importance of these CLRs in anti-*Aspergillus* host immune response needs to be elucidated.

Along with TLRs and CLRs, the secreted receptor pentraxin 3 (PTX3) has been also shown to play an important role in CF conditions. Polymorphisms in the *PTX3* gene were found to correlate with the risk of *P. aeruginosa* infections in CF patients [218], and PTX3 was found to be localized with *P. aeruginosa* in sputum of CF patients [219]. Paroni et al. [219] also explored the therapeutic potential of PTX3 in CF during *P. aeruginosa* infection, by using a CF mouse model. Administration of PTX3 led to diminished inflammation in CF by a decrease in inflammatory cytokine levels and improved bacterial clearance by enhancing the phagocytosis capability of CF neutrophils [219]. Some studies shed light on the relevance of PTX3 and fungi in CF. Garlanda et al. [220] reported that conidia of *A. fumigatus* induced the PTX3 secretion in the lungs during pulmonary *Aspergillus* infection. It further binds to galactomannans of the conidial wall and facilitates the phagocytosis by macrophages [220]. Hamon et al. [50] reported decreased levels of PTX3 in the respiratory secretions of CF patients, even though PTX3 level in the CF serum was high. This decrease in PTX3 was attributed to proteolytic cleavage by elastases and *Aspergillus* protease enzymes [50]. This leads to an interesting speculation that lower PTX3 levels in CF lung might correlate with recurring fungal colonization. There is also evidence regarding involvement of the serum opsonin, H-ficolin, in modulation of the host immune response. H-ficolin binding to *A. fumigatus* led to enhanced activation of the lectin complement pathway and lung epithelial cell association with fungal colonization [221].

To summarize, there is growing evidence suggesting that patients with CF have an impaired immune response toward pathogens. Studies reporting the lack of PTX3 in the lung of CF patients, higher expression of TLR2 on apical surfaces of CF epithelial cells and defect in appropriate execution of TLR4 signaling-mediated immune response in CF hint toward some potential mechanisms responsible for recurrent fungal colonization and infection in the CF environment. In the last decade, there has been significant progress in the field of analyzing, processing and handling the CF patient samples in terms of diagnosis and identification of microorganisms colonizing the CF airways. Further studies to dissect the function of fungal PRRs and the impact of colonizing fungi on patient's health are necessary to uncover novel mechanisms and broaden our understanding on the relevance of fungal abundance in CF lung disease.

Conclusions

Novel detection methods have revealed that CF airways harbor a plethora of microbial species, including a vast

variety of fungi [76–79]. In this review, the possible role of a range of fungi, including filamentous molds such as *Aspergillus* species, *Scedosporium species*, *L. prolificans* and *E. dermatitidis*, as well as yeasts such as *Candida* species and other fungi such as *P. jirovecii*, in CF lung disease has been discussed. There is an increasing body of evidence, at least for certain fungi such as *Aspergillus* and *Candida* species, supporting a harmful role in CF lung disease rather than being mere bystanders [5, 55, 59, 62, 64, 84, 85]. However, while a few studies have proposed that fungi are associated with aggravation of CF lung disease [60, 92, 222], multicenter studies corroborating these findings are lacking. Until now, there is only one published prospective, randomized, controlled study that examined the effect of antifungal therapy on pulmonary outcome in CF patients [21]. Consequently, prospective, randomized, controlled studies on antifungal treatment in CF patients that take different fungal disease entities into account are urgently needed.

Acknowledgements This work was supported by the German Research Foundation (DFG to A.H.; DFG, SFB/CRC685 at Tübingen to D.H.), Christiane-Herzog-Stiftung (Christiane-Herzog-Award to A.H.) and funding of the University of Tübingen (Junior Research Group Program/IZKF to A.H.). We would like to thank Mr. Peter-Michael Weber for his excellent work on the illustration.

References

1. Hamutcu R, Rowland JM, Horn MV, et al. Clinical findings and lung pathology in children with cystic fibrosis. Am J Respir Crit Care Med. 2002;165:1172–5.
2. Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. N Engl J Med. 2005;352:1992–2001.
3. Sly PD, Gangell CL, Chen L, et al. Risk factors for bronchiectasis in children with cystic fibrosis. N Engl J Med. 2013;368:1963–70.
4. Wielputz MO, Puderbach M, Kopp-Schneider A, et al. Magnetic resonance imaging detects changes in structure and perfusion, and response to therapy in early cystic fibrosis lung disease. Am J Respir Crit Care Med. 2014;189:956–65.
5. Ramsey KA, Ranganathan S, Park J, et al. Early respiratory infection is associated with reduced spirometry in children with cystic fibrosis. Am J Respir Crit Care Med. 2014;190:1111–6.
6. Jensen T, Pedersen SS, Garne S, et al. Colistin inhalation therapy in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection. J Antimicrob Chemother. 1987;19:831–8.
7. Ramsey BW, Pepe MS, Quan JM, et al. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group. N Engl J Med. 1999;340:23–30.
8. Quittner AL, Buu A. Effects of tobramycin solution for inhalation on global ratings of quality of life in patients with cystic fibrosis and *Pseudomonas aeruginosa* infection. Pediatr Pulmonol. 2002;33:269–76.
9. McCoy KS, Quittner AL, Oermann CM, et al. Inhaled aztreonam lysine for chronic airway *Pseudomonas aeruginosa* in cystic fibrosis. Am J Respir Crit Care Med. 2008;178:921–8.
10. Retsch-Bogart GZ, Quittner AL, Gibson RL, et al. Efficacy and safety of inhaled aztreonam lysine for airway *pseudomonas* in cystic fibrosis. Chest. 2009;135:1223–32.
11. Konstan MW, Flume PA, Kappler M, et al. Safety, efficacy and convenience of tobramycin inhalation powder in cystic fibrosis patients: the EAGER trial. J Cyst Fibros. 2011;10:54–61.
12. Schuster A, Haliburn C, Doring G, et al. Safety, efficacy and convenience of colistimethate sodium dry powder for inhalation (Colobreathe DPI) in patients with cystic fibrosis: a randomised study. Thorax. 2013;68:344–50.
13. Conole D, Keating GM. Colistimethate sodium dry powder for inhalation: a review of its use in the treatment of chronic *Pseudomonas aeruginosa* infection in patients with cystic fibrosis. Drugs. 2014;74:377–87.
14. Elborn JS, Flume PA, Cohen F, et al. Safety and efficacy of prolonged levofloxacin inhalation solution (APT-1026) treatment for cystic fibrosis and chronic *Pseudomonas aeruginosa* airway infection. J Cyst Fibros. 2016;15:634–40.
15. Flume PA, VanDevanter DR, Morgan EE, et al. A phase 3, multi-center, multinational, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of levofloxacin inhalation solution (APT-1026) in stable cystic fibrosis patients. J Cyst Fibros. 2016;15:495–502.
16. Lee TW, Brownlee KG, Denton M, et al. Reduction in prevalence of chronic *Pseudomonas aeruginosa* infection at a regional pediatric cystic fibrosis center. Pediatr Pulmonol. 2004;37:104–10.
17. Hansen CR, Pressler T, Hoiby N. Early aggressive eradication therapy for intermittent *Pseudomonas aeruginosa* airway colonization in cystic fibrosis patients: 15 years experience. J Cyst Fibros. 2008;7:523–30.
18. Crull MR, Ramos KJ, Caldwell E, et al. Change in *Pseudomonas aeruginosa* prevalence in cystic fibrosis adults over time. BMC Pulm Med. 2016;16:176.
19. Sudfeld CR, Dasenbrook EC, Merz WG, et al. Prevalence and risk factors for recovery of filamentous fungi in individuals with cystic fibrosis. J Cyst Fibros. 2010;9:110–6.
20. Nagano Y, Elborn JS, Millar BC, et al. Comparison of techniques to examine the diversity of fungi in adult patients with cystic fibrosis. Med Mycol. 2010;48(Suppl1):S166–76.
21. 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.

22. Millar FA, Simmonds NJ, Hodson ME. Trends in pathogens colonising the respiratory tract of adult patients with cystic fibrosis, 1985–2005. J Cyst Fibros. 2009;8:386–91.
23. Burns JL, Van Dalfsen JM, Shawar RM, et al. Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. J Infect Dis. 1999;179:1190–6.
24. Bargon J, Dauletbaev N, Kohler B, et al. Prophylactic antibiotic therapy is associated with an increased prevalence of *Aspergillus* colonization in adult cystic fibrosis patients. Respir Med. 1999;93:835–8.
25. Hodson ME, Gallagher CG, Govan JR. A randomised clinical trial of nebulised tobramycin or colistin in cystic fibrosis. Eur Respir J. 2002;20:658–64.
26. 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;17:1381–6.
27. Noni M, Katelari A, Kaditis A, et al. *Candida albicans* chronic colonisation in cystic fibrosis may be associated with inhaled antibiotics. Mycoses. 2015;58:416–21.
28. Noni M, Katelari A, Dimopoulos G, et al. Inhaled corticosteroids and *Aspergillus fumigatus* isolation in cystic fibrosis. Med Mycol. 2014;52:715–22.
29. Saunders RV, Modha DE, Claydon A, et al. Chronic *Aspergillus fumigatus* colonization of the pediatric cystic fibrosis airway is common and may be associated with a more rapid decline in lung function. Med Mycol. 2016;54:537–43.
30. Bakare N, Rickerts V, Bargon J, et al. Prevalence of *Aspergillus fumigatus* and other fungal species in the sputum of adult patients with cystic fibrosis. Mycoses. 2003;46:19–23.
31. Valenza G, Strasen J, Schafer F, et al. Evaluation of new colorimetric vitek 2 yeast identification card by use of different source media. J Clin Microbiol. 2008;46:3784–7.
32. Baumann K, Carnicer M, Dragosits M, et al. A multi-level study of recombinant *Pichia pastoris* in different oxygen conditions. BMC Syst Biol. 2010;4:141.
33. Paugam A, Baixench MT, Demazes-Dufeu N, et al. Characteristics and consequences of airway colonization by filamentous fungi in 201 adult patients with cystic fibrosis in France. Med Mycol. 2010;48(Suppl 1):S32–6.
34. Coughlan CA, Chotirmall SH, Renwick J, et al. The effect of *Aspergillus fumigatus* infection on vitamin D receptor expression in cystic fibrosis. Am J Respir Crit Care Med. 2012;186:999–1007.
35. Lott TJ, Kuykendall RJ, Reiss E. Nucleotide sequence analysis of the 5.8S rDNA and adjacent ITS2 region of *Candida albicans* and related species. Yeast. 1993;9:1199–206.
36. Bouchara JP, Hsieh HY, Croquefer S, et al. Development of an oligonucleotide array for direct detection of fungi in sputum samples from patients with cystic fibrosis. J Clin Microbiol. 2009;47:142–52.
37. Eickmeier O, Rieber N, Eckrich J, et al. Immune response, diagnosis and treatment of allergic bronchopulmonary aspergillosis in cystic fibrosis lung disease. Curr Pharm Des. 2013;19:3669–78.
38. Tang AC, Turvey SE, Alves MP, et al. Current concepts: host–pathogen interactions in cystic fibrosis airways disease. Eur Respir Rev. 2014;23:320–32.
39. Alekseeva L, Huet D, Femenia F, et al. Inducible expression of beta defensins by human respiratory epithelial cells exposed to *Aspergillus fumigatus* organisms. BMC Microbiol. 2009;9:33.
40. Hartl D. Immunological mechanisms behind the cystic fibrosis-ABPA link. Med Mycol. 2009;47(Suppl 1):S183–91.
41. Balloy V, Chignard M. The innate immune response to *Aspergillus fumigatus*. Microbes Infect. 2009;11:919–27.
42. Morton CO, Bouzani M, Loeffler J, et al. Direct interaction studies between *Aspergillus fumigatus* and human immune cells; what have we learned about pathogenicity and host immunity? Front Microbiol. 2012;3:413.
43. Chotirmall SH, Al-Alawi M, Mirkovic B, et al. *Aspergillus*-associated airway disease, inflammation, and the innate immune response. Biomed Res Int. 2013;2013:723129.
44. Chmiel JF, Davis PB. State of the art: why do the lungs of patients with cystic fibrosis become infected and why can't they clear the infection? Respir Res. 2003;4:8.
45. Painter RG, Valentine VG, Lanson NA Jr, et al. CFTR Expression in human neutrophils and the phagolysosomal chlorination defect in cystic fibrosis. Biochemistry. 2006;45:10260–9.
46. Bonfield TL, Hodges CA, Cotton CU, et al. Absence of the cystic fibrosis transmembrane regulator (Cftr) from myeloid-derived cells slows resolution of inflammation and infection. J Leukoc Biol. 2012;92:1111–22.
47. Ng HP, Zhou Y, Song K, et al. Neutrophil-mediated phagocytic host defense defect in myeloid Cftr-inactivated mice. PLoS ONE. 2014;9:e106813.
48. Hartl D, Latzin P, Hordijk P, et al. Cleavage of CXCR1 on neutrophils disables bacterial killing in cystic fibrosis lung disease. Nat Med. 2007;13:1423–30.
49. Iannitti RG, Carvalho A, Cunha C, et al. Th17/Treg imbalance in murine cystic fibrosis is linked to indoleamine 2,3-dioxygenase deficiency but corrected by kynurenines. Am J Respir Crit Care Med. 2013;187:609–20.
50. Hamon Y, Jaillon S, Person C, et al. Proteolytic cleavage of the long pentraxin PTX3 in the airways of cystic fibrosis patients. Innate Immun. 2013;19:611–22.
51. Ralhan A, Laval J, Lelis F, et al. Current concepts and controversies in innate immunity of cystic fibrosis lung disease. J Innate Immun. 2016;8:531–40.
52. Brouard J, Knauer N, Boelle PY, et al. Influence of interleukin-10 on *Aspergillus fumigatus* infection in patients with cystic fibrosis. J Infect Dis. 2005;191:1988–91.
53. Iannitti RG, Napolioni V, Oikonomou V, et al. IL-1 receptor antagonist ameliorates inflammasome-dependent inflammation in murine and human cystic fibrosis. Nat Commun. 2016;7:10791.
54. Hector A, Chotirmall SH, Lavelle GM, et al. Chitinase activation in patients with fungus-associated cystic fibrosis lung disease. J Allergy Clin Immunol. 2016;138(1183–9):e4.
55. Noni M, Katelari A, Dimopoulos G, et al. *Aspergillus fumigatus* chronic colonization and lung function decline in cystic fibrosis may have a two-way relationship. Eur J Clin Microbiol Infect Dis. 2015;34:2235–41.
56. 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.
57. Valenza G, Tappe D, Turnwald D, et al. Prevalence and antimicrobial susceptibility of microorganisms isolated from sputa of patients with cystic fibrosis. J Cyst Fibros. 2008;7:123–7.
58. Borman AM, Palmer MD, Delhaes L, 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(Suppl 1):S88–97.
59. Amin R, Dupuis A, Aaron SD, et al. The effect of chronic infection with *Aspergillus fumigatus* on lung function and hospitalization in patients with cystic fibrosis. Chest. 2010;137:171–6.
60. Baxter CG, Moore CB, Jones AM, et al. IgE-mediated immune responses and airway detection of *Aspergillus* and *Candida* in adult cystic fibrosis. Chest. 2013;143:1351–7.
61. Ziesing S, Suerbaum S, Sedlacek L. Fungal epidemiology and diversity in cystic fibrosis patients over a 5-year period in a national reference center. Med Mycol. 2016;54:781–6.
62. Chotirmall SH, O'Donoghue E, Bennett K, et al. Sputum *Candida albicans* presages FEV(1) decline and hospital-treated exacerbations in cystic fibrosis. Chest. 2010;138:1186–95.
63. Muthig M, Hebestreit A, Ziegler U, et al. Persistence of *Candida* species in the respiratory tract of cystic fibrosis patients. Med Mycol. 2010;48:56–63.
64. Gileles-Hillel A, Shoseyov D, Polacheck I, et al. Association of chronic *Candida albicans* respiratory infection with a more severe lung disease in patients with cystic fibrosis. Pediatr Pulmonol. 2015;50:1082–9.
65. Cimon B, Carrère J, Vinatier JF, et al. Clinical significance of *Scedosporium apiospermum* in patients with cystic fibrosis. Eur J Clin Microbiol Infect Dis. 2000;19:53–6.
66. Williamson EC, Speers D, Arthur IH, et al. Molecular epidemiology of *Scedosporium apiospermum* infection determined by PCR amplification of ribosomal intergenic spacer sequences in patients with chronic lung disease. J Clin Microbiol. 2001;39:47–50.
67. Blyth CC, Middleton PG, Harun A, et al. Clinical associations and prevalence of *Scedosporium* spp. in Australian cystic fibrosis patients: identification of novel risk factors? Med Mycol. 2010;48(Suppl 1):S37–44.
68. Sedlacek L, Graf B, Schwarz C, et al. Prevalence of *Scedosporium* species and *Lomentospora prolificans* in patients with cystic fibrosis in a multicenter trial by use of a selective medium. J Cyst Fibros. 2015;14:237–41.
69. Horré R, Schaal KP, Siekmeier R, et al. Isolation of fungi, especially *Exophiala dermatitidis*, in patients suffering from cystic fibrosis. A prospective study. Respiration. 2004;71:360–6.
70. Kondori N, Lindblad A, Welinder-Olsson C, et al. Development of IgG antibodies to *Exophiala dermatitidis* is associated with inflammatory responses in patients with cystic fibrosis. J Cyst Fibros. 2014;13:391–9.
71. Kirchhoff L, Olsowski M, Zilmans K, et al. Biofilm formation of the black yeast-like fungus *Exophiala dermatitidis* and its susceptibility to antiinfective agents. Sci Rep. 2017;7:42886.
72. Sing A, Geiger AM, Hogardt M, et al. *Pneumocystis carinii* carriage among cystic fibrosis patients, as detected by nested PCR. J Clin Microbiol. 2001;39:2717–8.
73. Respaldiza N, Montes-Cano MA, Dapena FJ, et al. Prevalence of colonisation and genotypic characterisation of *Pneumocystis jirovecii* among cystic fibrosis patients in Spain. Clin Microbiol Infect. 2005;11:1012–5.
74. Pederiva MA, Wissmann G, Friaza V, et al. High prevalence of *Pneumocystis jirovecii* colonization in Brazilian cystic fibrosis patients. Med Mycol. 2012;50:556–60.
75. Hernandez-Hernandez F, Fréalle E, Caneiro P, et al. Prospective multicenter study of *Pneumocystis jirovecii* colonization among cystic fibrosis patients in France. J Clin Microbiol. 2012;50:4107–10.
76. Delhaes L, Monchy S, Fréalle E, et al. The airway microbiota in cystic fibrosis: a complex fungal and bacterial community-implications for therapeutic management. PLoS ONE. 2012;7:e36313.
77. Willger SD, Grim SL, Dolben EL, et al. Characterization and quantification of the fungal microbiome in serial samples from individuals with cystic fibrosis. Microbiome. 2014;2:40.
78. Kramer R, Sauer-Heilborn A, Welte T, et al. Cohort study of airway mycobiome in adult cystic fibrosis patients: differences in community structure between fungi and bacteria reveal predominance of transient fungal elements. J Clin Microbiol. 2015;53:2900–7.
79. Losada PM, Chouvarine P, Dorda M, et al. The cystic fibrosis lower airways microbial metagenome. ERJ Open Res. 2016;2:00096-2015.
80. Nguyen LD, Viscogliosi E, Delhaes L. The lung mycobiome: an emerging field of the human respiratory microbiome. Front Microbiol. 2015;6:89.
81. Latgé JP. *Aspergillus fumigatus* and aspergillosis. Clin Microbiol Rev. 1999;12:310–50.
82. 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;185:301–10.
83. Navarro J, Rainisio M, Harms HK, et al. Factors associated with poor pulmonary function: cross-sectional analysis of data from the ERCF. European Epidemiologic Registry of Cystic Fibrosis. Eur Respir J. 2001;18:298–305.
84. de Boer K, Vandemheen KL, Tullis E, et al. Exacerbation frequency and clinical outcomes in adult patients with cystic fibrosis. Thorax. 2011;66:680–5.
85. Hector A, Kirn T, Ralhan A, et al. Microbial colonization and lung function in adolescents with cystic fibrosis. J Cyst Fibros. 2016;15:340–9.
86. 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.
87. Gangell C, Gard S, Douglas T, et al. Inflammatory responses to individual microorganisms in the lungs of children with cystic fibrosis. Clin Infect Dis. 2011;53:425–32.
88. Cimon B, Zouhair R, Symoens F, et al. *Aspergillus terreus* in a cystic fibrosis clinic: environmental distribution and patient colonization pattern. J Hosp Infect. 2003;53:81–2.

89. Rougeron A, Giraud S, Razafimandimby B, et al. Different colonization patterns of *Aspergillus terreus* in patients with cystic fibrosis. Clin Microbiol Infect. 2014;20: 327–33.
90. Gautier M, Normand AC, L'Ollivier C, et al. *Aspergillus tubingensis*: a major filamentous fungus found in the airways of patients with lung disease. Med Mycol. 2016;54:459–70.
91. Shoseyov D, Brownlee KG, Conway SP, et al. *Aspergillus* bronchitis in cystic fibrosis. Chest. 2006;130:222–6.
92. Baxter CG, Dunn G, Jones AM, et al. Novel immunologic classification of aspergillosis in adult cystic fibrosis. J Allergy Clin Immunol. 2013;132(560–6):e10.
93. 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.
94. Baxter CG, Rautemaa R, Jones AM, et al. Intravenous antibiotics reduce the presence of *Aspergillus* in adult cystic fibrosis sputum. Thorax. 2013;68:652–7.
95. Nicolai T, Arleth S, Spaeth A, et al. Correlation of IgE antibody titer to *Aspergillus fumigatus* with decreased lung function in cystic fibrosis. Pediatr Pulmonol. 1990;8:12–5.
96. Wojnarowski C, Eichler I, Gartner C, et al. Sensitization to *Aspergillus fumigatus* and lung function in children with cystic fibrosis. Am J Respir Crit Care Med. 1997;155: 1902–7.
97. 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;174:1211–20.
98. Kanthan SK, Bush A, Kemp M, et al. Factors effecting impact of *Aspergillus fumigatus* sensitization in cystic fibrosis. Pediatr Pulmonol. 2007;42:785–93.
99. Knutsen AP, Bellone C, Kauffman H. Immunopathogenesis of allergic bronchopulmonary aspergillosis in cystic fibrosis. J Cyst Fibros. 2002;1:76–89.
100. Hartl D, Latzin P, Zissel G, et al. Chemokines indicate allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. Am J Respir Crit Care Med. 2006;173: 1370–6.
101. Felton IC, Simmonds NJ. *Aspergillus* and cystic fibrosis: old disease—new classifications. Curr Opin Pulm Med. 2014;20:632–8.
102. Cockrill BA, Hales CA. Allergic bronchopulmonary aspergillosis. Ann Rev Med. 1999;50:303–16.
103. Greenberger PA. Allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol. 2002;110:685–92.
104. Cortese G, Malfitana V, Placido R, et al. Role of chest radiography in the diagnosis of allergic bronchopulmonary aspergillosis in adult patients with cystic fibrosis. Radiol Med. 2007;112:626–36.
105. Chotirmall SH, Branagan P, Gunaratnam C, et al. *Aspergillus*/allergic bronchopulmonary aspergillosis in an Irish cystic fibrosis population: a diagnostically challenging entity. Respir Care. 2008;53:1035–41.
106. Cohen-Cymberknoh M, Blau H, Shoseyov D, et al. Intravenous monthly pulse methylprednisolone treatment for ABPA in patients with cystic fibrosis. J Cyst Fibros. 2009;8:253–7.
107. Sequeiros IM, Jarad N. Factors associated with a shorter time until the next pulmonary exacerbation in adult patients with cystic fibrosis. Chron Respir Dis. 2012;9:9–16.
108. Thronicke A, Heger N, Antweiler E, et al. Allergic bronchopulmonary aspergillosis is associated with pet ownership in cystic fibrosis. Pediatr Allergy Immunol. 2016;27:597–603.
109. Barton RC, Hobson RP, Denton M, et al. Serologic diagnosis of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis through the detection of immunoglobulin G to *Aspergillus fumigatus*. Diagn Microbiol Infect Dis. 2008;62:287–91.
110. Latzin P, Hartl D, Regamey N, et al. Comparison of serum markers for allergic bronchopulmonary aspergillosis in cystic fibrosis. Eur Respir J. 2008;31:36–42.
111. Gernez Y, Dunn CE, Everson C, et al. Blood basophils from cystic fibrosis patients with allergic bronchopulmonary aspergillosis are primed and hyper-responsive to stimulation by *aspergillus* allergens. J Cyst Fibros. 2012;11:502–10.
112. Mirkovic B, Lavelle GM, Azim AA, et al. The basophil surface marker CD203c identifies *Aspergillus* species sensitization in patients with cystic fibrosis. J Allergy Clin Immunol. 2016;137(436–43):e9.
113. Katelari A, Tzanoudaki M, Noni M, et al. The role of basophil activation test in allergic bronchopulmonary aspergillosis and *Aspergillus fumigatus* sensitization in cystic fibrosis patients. J Cyst Fibros. 2016;15:587–96.
114. 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):S225–64.
115. Accurso FJ. Update in cystic fibrosis 2006. Am J Respir Crit Care Med. 2007;175:754–7.
116. Guarro J, Kantarcioglu AS, Horré R, et al. *Scedosporium apiospermum*: changing clinical spectrum of a therapy-refractory opportunist. Med Mycol. 2006;44:295–327.
117. Borghi E, Iatta R, Manca A, et al. Chronic airway colonization by *Scedosporium apiospermum* with a fatal outcome in a patient with cystic fibrosis. Med Mycol. 2010;48(Suppl 1):S108–13.
118. Russell GK, Gadhok R, Simmonds NJ. The destructive combination of *Scedosporium apiospermum* lung disease and exuberant inflammation in cystic fibrosis. Paediatr Respir Rev. 2013;14(Suppl 1):22–5.
119. Padoan R, Poli P, Colombrita D, et al. Acute *Scedosporium apiospermum* endobronchial infection in cystic fibrosis. Pediatr Infect Dis J. 2016;35:701–2.
120. Schwarz C, Brandt C, Antweiler E, et al. Prospective multicenter German study on pulmonary colonization with *Scedosporium*/*Lomentospora* species in cystic fibrosis: epidemiology and new association factors. PLoS ONE. 2017;12:e0171485.
121. Haase G, Skopnik H, Kusenbach G. *Exophiala dermatitidis i*nfection in cystic fibrosis. Lancet. 1990;336:188–9.
122. Kusenbach G, Skopnik H, Haase G, et al. *Exophiala dermatitidis* pneumonia in cystic fibrosis. Eur J Pediatr. 1992;151:344–6.
123. Kondori N, Gilljam M, Lindblad A, et al. High rate of *Exophiala dermatitidis* recovery in the airways of patients

with cystic fibrosis is associated with pancreatic insufficiency. J Clin Microbiol. 2011;49:1004–9.
124. Pihet M, Carrère J, Cimon B, et al. Occurrence and relevance of filamentous fungi in respiratory secretions of patients with cystic fibrosis-a review. Med Mycol. 2009;47:387–97.
125. Marguet C, Favennec L, Matray O, et al. Clinical and microbiological efficacy of micafungin on *Geosmithia argillacea* infection in a cystic fibrosis patient. Med Mycol Case Rep. 2012;1:79–81.
126. Hong G, White M, Lechtzin N, et al. Fatal disseminated *Rasamsonia* infection in cystic fibrosis post-lung transplantation. J Cyst Fibros. 2017;16:e3–7.
127. Peltroche-Llacsahuanga H, Dohmen H, Haase G. Recovery of *Candida dubliniensis* from sputum of cystic fibrosis patients. Mycoses. 2002;45:15–8.
128. Wahab AA, Taj-Aldeen SJ, Kolecka A, et al. High prevalence of *Candida dubliniensis* in lower respiratory tract secretions from cystic fibrosis patients may be related to increased adherence properties. Int J Infect Dis. 2014;24:14–9.
129. Sokulska M, Kicia M, Wesolowska M, et al. *Pneumocystis jirovecii*-from a commensal to pathogen: clinical and diagnostic review. Parasitol Res. 2015;114:3577–85.
130. Goto N, Futamura K, Okada M, et al. Management of *Pneumocystis jirovecii* pneumonia in kidney transplantation to prevent further outbreak. Clin Med Insights Circ Respir Pulm Med. 2015;9:81–90.
131. Kaur R, Katariya P, Dhakad MS, et al. An unusual case of cystic fibrosis associated *Pneumocystis jirovecii* pneumonia in an infant. Case Rep Infect Dis. 2016;2016:9206707.
132. Green HD, Bright-Thomas RJ, Mutton KJ, et al. Increased prevalence of *Pneumocystis jirovecii* colonisation in acute pulmonary exacerbations of cystic fibrosis. J Infect. 2016;73:1–7.
133. Morris A, Sciurba FC, Lebedeva IP, et al. Association of chronic obstructive pulmonary disease severity and *Pneumocystis* colonization. Am J Respir Crit Care Med. 2004;170:408–13.
134. Calderon EJ, Rivero L, Respaldiza N, et al. Systemic inflammation in patients with chronic obstructive pulmonary disease who are colonized with *Pneumocystis jirovecii*. Clin Infect Dis. 2007;45:e17–9.
135. Montes-Cano MA, de la Horra C, Dapena FJ, et al. Dynamic colonisation by different *Pneumocystis jirovecii* genotypes in cystic fibrosis patients. Clin Infect Dis. 2007;13:1008–11.
136. van der Ent CK, Hoekstra H, Rijkers GT. Successful treatment of allergic bronchopulmonary aspergillosis with recombinant anti-IgE antibody. Thorax. 2007;62:276–7.
137. Zirbes JM, Milla CE. Steroid-sparing effect of omalizumab for allergic bronchopulmonary aspergillosis and cystic fibrosis. Pediatr Pulmonol. 2008;43:607–10.
138. Kanu A, Patel K. Treatment of allergic bronchopulmonary aspergillosis (ABPA) in CF with anti-IgE antibody (omalizumab). Pediatr Pulmonol. 2008;43:1249–51.
139. Lebecque P, Leonard A, Argaz M, et al. Omalizumab for exacerbations of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. BMJ Case Rep. 2009. doi:10.1136/bcr07.2008.0379.
140. Brinkmann F, Schwerk N, Hansen G, et al. Steroid dependency despite omalizumab treatment of ABPA in cystic fibrosis. Allergy. 2010;65:134–5.
141. Elmallah MK, Hendeles L, Hamilton RG, et al. Management of patients with cystic fibrosis and allergic bronchopulmonary aspergillosis using anti-immunoglobulin E therapy (omalizumab). J Pediatr Pharmacol Ther. 2012; 17:88–92.
142. Nathan N, Girodon E, Clement A, et al. A rare CFTR intronic mutation related to a mild CF disease in a 12-year-old girl. BMJ Case Rep. 2012. doi:10.1136/bcr-2012-006918.
143. Wong R, Wong M, Robinson PD, et al. Omalizumab in the management of steroid dependent allergic bronchopulmonary aspergillosis (ABPA) complicating cystic fibrosis. Paediatr Respir Rev. 2013;14:22–4.
144. Zicari AM, Celani C, De Castro G, et al. Anti IgE antibody as treatment of allergic bronchopulmonary aspergillosis in a patient with cystic fibrosis. Eur Rev Med Pharmacol Sci. 2014;18:1839–41.
145. Lehmann S, Pfannenstiel C, Friedrichs F, et al. Omalizumab: a new treatment option for allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. Ther Adv Respir Dis. 2014;8:141–9.
146. Emiralioglu N, Dogru D, Tugcu GD, et al. Omalizumab treatment for allergic bronchopulmonary aspergillosis in cystic fibrosis. Ann Pharmacother. 2016;50:188–93.
147. Nove-Josserand R, Grard S, Auzou L, et al. Case series of omalizumab for allergic bronchopulmonary aspergillosis in cystic fibrosis patients. Pediatr Pulmonol. 2017;52: 190–7.
148. Jat KR, Walia DK, Khairwa A. Anti-IgE therapy for allergic bronchopulmonary aspergillosis in people with cystic fibrosis. Cochrane Database Syst Rev. 2015;(11):CD010288.
149. Nguyen NL, Pilewski JM, Celedon JC, et al. Vitamin D supplementation decreases *Aspergillus fumigatus* specific Th2 responses in CF patients with *Aspergillus* sensitization: a phase one open-label study. Asthma Res Pract. 2015;1:3.
150. Hilliard T, Edwards S, Buchdahl R, et al. Voriconazole therapy in children with cystic fibrosis. J Cyst Fibros. 2005;4:215–20.
151. Skov M, Hoiby N, Koch C. Itraconazole treatment of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. Allergy. 2002;57:723–8.
152. Proesmans M, Vermeulen F, Vreys M, et al. Use of nebulized amphotericin B in the treatment of allergic bronchopulmonary aspergillosis in cystic fibrosis. Int J Pediatr. 2010;2010:376287.
153. Hayes D Jr, Murphy BS, Lynch JE, et al. Aerosolized amphotericin for the treatment of allergic bronchopulmonary aspergillosis. Pediatr Pulmonol. 2010;45:1145–8.
154. Holle J, Leichsenring M, Meissner PE. Nebulized voriconazole in infections with *Scedosporium apiospermum*—case report and review of the literature. J Cyst Fibros. 2014;13:400–2.
155. Elphick HE, Southern KW. Antifungal therapies for allergic bronchopulmonary aspergillosis in people with cystic fibrosis. Cochrane Database Syst Rev. 2016;11:CD002204.

156. Bader O, Weig M, Reichard U, et al. cyp51A-based mechanisms of *Aspergillus fumigatus* azole drug resistance present in clinical samples from Germany. Antimicrob Agents Chemother. 2013;57:3513–7.
157. 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;67:1870–3.
158. 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;49:2243–51.
159. Burgel PR, Paugam A, Hubert D, et al. *Aspergillus fumigatus* in the cystic fibrosis lung: pros and cons of azole therapy. Infect Drug Resist. 2016;9:229–38.
160. Surette MG. The cystic fibrosis lung microbiome. Ann Am Thorac Soc. 2014;11:S61–5.
161. Coburn B, Wang PW, Diaz Caballero J, et al. Lung microbiota across age and disease stage in cystic fibrosis. Sci Rep. 2015;5:10241.
162. Caverly LJ, Zhao J, LiPuma JJ. Cystic fibrosis lung microbiome: opportunities to reconsider management of airway infection. Pediatr Pulmonol. 2015;50:S31–8.
163. Hogan DA, Vik A, Kolter R. A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. Mol Microbiol. 2004;54:1212–23.
164. Williams P, Cámara M. Quorum sensing and environmental adaptation in *Pseudomonas aeruginosa*: a tale of regulatory networks and multifunctional signal molecules. Curr Opin Microbiol. 2009;12:182–91.
165. Hogan DA, Kolter R. *Pseudomonas–Candida* interactions: an ecological role for virulence factors. Science. 2002;296:2229–32.
166. Chen AI, Dolben EF, Okegbe C, et al. *Candida albicans* ethanol stimulates *Pseudomonas aeruginosa* WspR-controlled biofilm formation as part of a cyclic relationship involving phenazines. PLoS Pathog. 2014;10:e1004480.
167. McAlester G, O'Gara F, Morrissey JP. Signal-mediated interactions between *Pseudomonas aeruginosa* and *Candida albicans*. J Med Microbiol. 2008;57:563–9.
168. Kim SH, Clark ST, Surendra A, et al. Global analysis of the fungal microbiome in cystic fibrosis patients reveals loss of function of the transcriptional repressor Nrg1 as a mechanism of pathogen adaptation. PLoS Pathog. 2015;11:e100530.
169. Shirtliff ME, Peters BM, Jabra-Rizk MA. Cross-kingdom interactions: *Candida albicans* and bacteria. FEMS Microbiol Lett. 2009;299:1–8.
170. Carlson EC. Synergism of *Candida albicans* and delta toxin producing *Staphylococcus aureus* on mouse mortality and morbidity: protection by indomethacin. Zentralbl Bakteriol Mikrobiol Hyg A. 1988;269:377–86.
171. Jabra-Rizk MA, Meiller TF, James CE, et al. Effect of farnesol on *Staphylococcus aureus* biofilm formation and antimicrobial susceptibility. Antimicrob Agents Chemother. 2006;50:1463–9.
172. Kuroda M, Nagasaki S, Ito R, et al. Sesquiterpene farnesol as a competitive inhibitor of lipase activity of *Staphylococcus aureus*. FEMS Microbiol Lett. 2007;273:28–34.
173. Briard B, Heddergott C, Latgé J-P. Volatile compounds emitted by *Pseudomonas aeruginosa* stimulate growth of the fungal pathogen *Aspergillus fumigatus*. mBio. 2016;7:e00219-16.
174. 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.
175. Briard B, Bomme P, Lechner BE, et al. *Pseudomonas aeruginosa* manipulates redox and iron homeostasis of its microbiota partner *Aspergillus fumigatus* via phenazines. Sci Rep. 2015;5:8220.
176. Shirazi F, Ferreira JAG, Stevens DA, et al. Biofilm filtrates of *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patients inhibit preformed *Aspergillus fumigatus* biofilms via apoptosis. PLoS ONE. 2016;11:e0150155.
177. Moree WJ, Phelan VV, Wu C-H, et al. Interkingdom metabolic transformations captured by microbial imaging mass spectrometry. Proc Natl Acad Sci USA. 2012;109:13811–6.
178. Penner JC, Ferreira JAG, Secor PR, et al. Pf4 bacteriophage produced by *Pseudomonas aeruginosa* inhibits *Aspergillus fumigatus* metabolism via iron sequestration. Microbiology. 2016;162:1583–94.
179. Casaulta C, Schöni MH, Weichel M, et al. IL-10 controls *Aspergillus fumigatus*- and *Pseudomonas aeruginosa*-specific T-cell response in cystic fibrosis. Pediatr Res. 2003;53:313–9.
180. Kaur J, Pethani BP, Kumar S, et al. *Pseudomonas aeruginosa* inhibits the growth of *Scedosporium aurantiacum*, an opportunistic fungal pathogen isolated from the lungs of cystic fibrosis patients. Front Microbiol. 2015;6:866.
181. Erwig LP, Gow NAR. Interactions of fungal pathogens with phagocytes. Nat Rev Microbiol. 2016;14:163–76.
182. Hardison SE, Brown GD. C-type lectin receptors orchestrate antifungal immunity. Nat Immunol. 2012;13:817–22.
183. Netea MG, Brown GD, Kullberg BJ, et al. An integrated model of the recognition of *Candida albicans* by the innate immune system. Nat Rev Microbiol. 2008;6:67–78.
184. Romani L. Immunity to fungal infections. Nat Rev Immunol. 2011;11:275–88.
185. Greene CM, Carroll TP, Smith SGJ, et al. TLR-induced inflammation in cystic fibrosis and non-cystic fibrosis airway epithelial cells. J Immunol. 2005;174:1638–46.
186. Muir A, Soong G, Sokol S, et al. Toll-like receptors in normal and cystic fibrosis airway epithelial cells. Am J Respir Cell Mol. 2004;30:777–83.
187. Netea MG, Van De Veerdonk F, Verschueren I, et al. Role of TLR1 and TLR6 in the host defense against disseminated candidiasis. FEMS Immunol Med Microbiol. 2008;52:118–23.
188. Nahum A, Dadi H, Bates A, et al. The biological significance of TLR3 variant, L412F, in conferring susceptibility to cutaneous candidiasis, CMV and autoimmunity. Autoimmun Rev. 2012;11:341–7.
189. Melvin T-AN, Lane AP, Nguyen M-T, et al. Sinonasal epithelial cell expression of Toll-like receptor 9 is elevated in cystic fibrosis-associated chronic rhinosinusitis. Am J Rhinol Allergy. 2013;27:30–3.
190. Wagener J, Malireddi RKS, Lenardon MD, et al. Fungal chitin dampens inflammation through IL-10 induction

mediated by NOD2 and TLR9 activation. PLoS Pathog. 2014;10:e1004050.
191. Lowman DW, Greene RR, Bearden DW, et al. Novel structural features in *Candida albicans* hyphal glucan provide a basis for differential innate immune recognition of hyphae versus yeast. J Biol Chem. 2014;289:3432–43.
192. Davis SE, Hopke A, Minkin SC, et al. Masking of (1–3)-glucan in the cell wall of *Candida albicans* from detection by innate immune cells depends on phosphatidylserine. Infect Immun. 2014;82:4405–13.
193. Kennedy AD, Willment JA, Dorward DW, et al. Dectin-1 promotes fungicidal activity of human neutrophils. Eur J Immunol. 2007;37:467–78.
194. Marakalala MJ, Vautier S, Potrykus J, et al. Differential adaptation of *Candida albicans* in vivo modulates immune recognition by dectin-1. PLoS Pathog. 2013;9:15.
195. Taylor PR, Tsoni SV, Willment JA, et al. Dectin-1 is required for β-glucan recognition and control of fungal infection. Nat Immunol. 2007;8:31–8.
196. Saijo S, Fujikado N, Furuta T, et al. Dectin-1 is required for host defense against *Pneumocystis carinii* but not against *Candida albicans*. Nat Immunol. 2007;8:39–46.
197. Ferwerda B, Ferwerda G, Plantinga TS, et al. Human dectin-1 deficiency and mucocutaneous fungal infections. N Engl J Med. 2009;361:1760–7.
198. Saijo S, Ikeda S, Yamabe K, et al. Dectin-2 recognition of α-mannans and induction of Th17 cell differentiation is essential for host defense against *Candida albicans*. Immunity. 2010;32:681–91.
199. Chai LYA, van de Veerdonk F, Marijnissen RJ, et al. Anti-*Aspergillus* human host defence relies on type 1 T helper (Th1), rather than type 17 T helper (Th17), cellular immunity. Immunology. 2010;130:46–54.
200. Wells CA, Salvage-Jones JA, Li X, et al. The macrophage-inducible C-type lectin, mincle, is an essential component of the innate immune response to *Candida albicans*. J Immunol. 2008;180:7404–13.
201. Chai LYA, Kullberg BJ, Vonk AG, et al. Modulation of Toll-like receptor 2 (TLR2) and TLR4 responses by *Aspergillus fumigatus*. Infect Immun. 2009;77:2184–92.
202. Meier A, Kirschning CJ, Nikolaus T, et al. Toll-like receptor (TLR) 2 and TLR4 are essential for *Aspergillus*-induced activation of murine macrophages. Cell Microbiol. 2003;5:561–70.
203. Flo TH, Ryan L, Latz E, et al. Involvement of toll-like receptor (TLR) 2 and TLR4 in cell activation by mannuronic acid polymers. J Biol Chem. 2002;277:35489–95.
204. Chai LYA, Vonk AG, Kullberg BJ, et al. *Aspergillus fumigatus* cell wall components differentially modulate host TLR2 and TLR4 responses. Microbes Infect. 2011; 13:151–9.
205. de Luca A, Bozza S, Zelante T, et al. Non-hematopoietic cells contribute to protective tolerance to *Aspergillus fumigatus* via a TRIF pathway converging on IDO. Cell Mol Immunol. 2010;7:459–70.
206. Shin S-H, Lee Y-H. Airborne fungi induce nasal polyp epithelial cell activation and Toll-like receptor expression. Int Arch Allergy Immunol. 2010;153:46–52.
207. Kasperkovitz PV, Cardenas ML, Vyas JM. TLR9 Is actively recruited to *Aspergillus fumigatus* phagosomes and requires the N-terminal proteolytic cleavage domain for proper intracellular trafficking. J Immunol. 2010;185: 7614–22.
208. Cohen TS, Prince A. Cystic fibrosis: a mucosal immunodeficiency syndrome. Nat Med. 2012;18:509–19.
209. John G, Yildirim AÖ, Rubin BK, et al. TLR-4-mediated innate immunity is reduced in cystic fibrosis airway cells. Am J Respir Cell Mol. 2010;42:424–31.
210. Steele C, Rapaka RR, Metz A, et al. The beta-glucan receptor dectin-1 recognizes specific morphologies of *Aspergillus fumigatus*. PLoS Pathog. 2005;1:e42.
211. Sun WK, Lu X, Li X, et al. Dectin-1 is inducible and plays a crucial role in *Aspergillus*-induced innate immune responses in human bronchial epithelial cells. Eur J Clin Microbiol Infect Dis. 2012;31:2755–64.
212. Gersuk GM, Underhill DM, Zhu L, et al. Dectin-1 and TLRs permit macrophages to distinguish between different *Aspergillus fumigatus* cellular states. J Immunol. 2006; 176:3717–24.
213. Liu Z-C, Wang M, Sun W-K, et al. Up-regulation of Dectin-1 in airway epithelial cells promotes mice defense against invasive pulmonary aspergillosis. Int J Clin Exp Med. 2015;8:17489–97.
214. Werner JL, Metz AE, Horn D, et al. Requisite role for the dectin-1 beta-glucan receptor in pulmonary defense against *Aspergillus fumigatus*. J Immunol. 2009;182:4938–46.
215. Yang J, Lu Q, Liu W, et al. Cyclophosphamide reduces dectin-1 expression in the lungs of naive and *Aspergillus fumigatus*-infected mice. Med Mycol. 2009;48:1–7.
216. Serrano-Gómez D, Leal JA, Corbí AL. DC-SIGN mediates the binding of *Aspergillus fumigatus* and keratinophylic fungi by human dendritic cells. Immunobiology. 2005;210: 175–83.
217. Loures FV, Röhm M, Lee CK, et al. Recognition of *Aspergillus fumigatus* hyphae by human plasmacytoid dendritic cells is mediated by dectin-2 and results in formation of extracellular traps. PLoS Pathog. 2015;11: e1004643.
218. Chiarini M, Sabelli C, Melotti P, et al. PTX3 genetic variations affect the risk of *Pseudomonas aeruginosa* airway colonization in cystic fibrosis patients. Genes Immun. 2010;11:665–70.
219. Paroni M, Moalli F, Nebuloni M, et al. Response of CFTR-deficient mice to long-term chronic *Pseudomonas aeruginosa* infection and PTX3 therapy. J Infect Dis. 2013; 208:130–8.
220. Garlanda C, Hirsch E, Bozza S, et al. Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. Nature. 2002;420:182–6.
221. Bidula S, Sexton DW, Yates M, et al. H-ficolin binds *Aspergillus fumigatus* leading to activation of the lectin complement pathway and modulation of lung epithelial immune responses. Immunology. 2015;146:281–91.
222. Chrdle A, Mustakim S, Bright-Thomas RJ, et al. *Aspergillus* bronchitis without significant immunocompromise. Ann N Y Acad Sci. 2012;1272:73–85.