



Review

Fungi in the cystic fibrosis lung: Bystanders or pathogens?☆



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ABSTRACT

Improvement to the life expectancy of people with cystic fibrosis (PWCF) brings about novel challenges including the need for evaluation of the role of fungi in the cystic fibrosis (CF) lung. To determine if such organisms represent bystanders or pathogens affecting clinical outcomes we review the existing knowledge from a clinical, biochemical, inflammatory and immunological perspective. The prevalence and importance of fungi in the CF airway has likely been underestimated with the most frequently isolated filamentous fungi being *Aspergillus fumigatus* and *Scedosporium apiospermum* and the major yeast *Candida albicans*. Developing non-culture based microbiological methods for fungal detection has improved both our classification and understanding of their clinical consequences including localized, allergic and systemic infections. Cross-kingdom interaction between bacteria and fungi are discussed as is the role of biofilms further affecting clinical outcome. A combination of host and pathogen-derived factors determines if a particular fungus represents a commensal, colonizer or pathogen in the setting of CF. The underlying immune state, disease severity and treatment burden represent key host variables whilst fungal type, form, chronicity and virulence including the ability to evade immune recognition determines the pathogenic potential of a specific fungus at a particular point in time. Further research in this emerging field is warranted to fully elucidate the spectrum of disease conferred by the presence of fungi in the CF airway and the indications for therapeutic interventions.

This article is part of a Directed Issue entitled: Cystic Fibrosis: From o-mics to cell biology, physiology, and therapeutic advances.

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Abbreviations: CF, Cystic Fibrosis; CFTR, Cystic Fibrosis Transmembrane Conductance Regulator; ABPA, Allergic bronchopulmonary aspergillosis; ISHAM, International Society for Human and Animal Mycology; NE, Neutrophil elastase; ECM, Extracellular matrix; HLA, Human leucocyte antigen; BAL, Bronchoalveolar lavage; AFLP, amplified fragment length polymorphism; MLST, Multilocus sequence typing; CT, Computed tomography; IPA, Invasive pulmonary aspergillosis; IA, Invasive aspergillosis; Th2, T-helper 2; Ig, Immunoglobulin; rAsp, Recombinant *Aspergillus* antigen; TARC, Thymus activation and regulated chemokine; FEV1, Forced expiratory volume in 1 second; CFU, Colony forming units; VDR, Vitamin D receptor; OX40L, OX 40 ligand; IL, Interleukin; PRR, Pattern recognition receptor; ASL, Airway surface liquid; ROS, Reactive oxygen species; SLPI, Secretory leucoprotease inhibitor; SP, Surfactant protein; MBL, Mannan binding lectin; TLR, Toll-like receptor; MR, Mannose receptor; CLR, C-lectin type lectin; ECA, Erythritol-chloramphenicol agar.

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1. Introduction

1.1. Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive disease. Its basis is a dysfunctional Cystic fibrosis transmembrane conductance regulator (CFTR) protein. Normally functioning as an apically based ion channel, CFTR facilitates chloride efflux from epithelial surfaces to maintain electrochemical balance, airway homeostasis, mucociliary clearance and luminal hydration. Encoded on chromosome seven, several hundred CFTR mutations are described with the most common being the F508 del mutation (Rowe et al., 2005).

The multi-system manifestations of disease are based on the level of CFTR activity which in turn depends on the type of genetic mutation (Table 1) (Mishra et al., 2005; Rowe et al., 2005). Airway manifestations result from impaired mucociliary clearance and the inability to mobilize thick secretions. This results in mucus impaction, microorganism colonization, recurrent infections, persistent inflammation and death from respiratory failure.

The evolving understanding of disease coupled with newer therapies, advancing research and more aggressive earlier management has increased life expectancies (Dodge et al., 2007). With increases in longevity, new challenges emerge including determining the role of fungal colonization and infection. This review will examine existing knowledge of this field from a clinical, biochemical and immunological perspective and attempt to decipher whether fungal isolation from the CF lung represents a situation of bystander or pathogen with concomitant effects on clinical outcomes in CF.

Table 1
Clinical manifestations of CF disease based on CFTR activity.

Level of CFTR activity (%)	Clinical manifestations
>5 ≤ 10	Congenital bilateral absence of the vas deferens (CBAVD)
=5	CBAVD + mild lung disease + pancreatic sufficient
<5	“Classic CF” CBAVD + moderate lung disease + varied gastrointestinal involvement
<1	Severe lung disease + pancreatic insufficiency

1.2. Fungi in cystic fibrosis

While CF lung disease is classically associated with *Staphylococcus aureus*, *Haemophilus influenzae* and *Pseudomonas aeruginosa*, an increasing recognition of fungal isolates has emerged. The respiratory tract communicates directly with the atmosphere and is constantly exposed to airborne environmental sources of fungi. In the context of chronic lung disease such as CF, it is the inability to effectively clear such inhaled particles that results in their persistence, colonization and potential infection within the airway. This spectrum of consequence from colonization to infection in the context of increasingly sensitive methods for fungal detection makes it likely that we have underestimated both their prevalence and importance in clinical practice over the last decade of CF care (Delhaes et al., 2012).

Fungi are generally divided into moulds or yeasts with the latter circumferentially shaped with a one-celled thallus. Moulds also known as filamentous fungi grow as branching cylindrical hyphae. The distinction between yeasts and filamentous fungi is however not an absolute one as some species for instance *Exophiala dermatitidis* grow as a yeast at body temperature but are filamentous at room temperature. Likewise, *Trichosporon* species as well as some *Candida* species may also produce true hyphae in particular culture conditions or in the host tissues.

The reported prevalence rates of fungi vary considerably between institutions because of differences in methodology used for their isolation. Thermo-tolerant filamentous fungi such as *Aspergillus fumigatus* are the most frequently isolated species however other important filamentous fungi include *Scedosporium* species and *E. dermatitidis* (Pihet et al., 2009; Sudfeld et al., 2010; Whittaker and Teneback, 2009). While *Aspergillus flavus*, *A. niger* and *A. nidulans* are commonly detected, these are transient in comparison to *A. fumigatus* and *A. terreus* as chronic CF colonizers (Cimon et al., 2003).

The major yeasts in the CF context are the genus *Candida* species, the most common of these being *Candida albicans*. Other members include *Candida glabrata*, *C. krusei*, *C. parapsilosis*, and *C. dubliniensis* however less is known of the consequences for yeast detection in comparison to filamentous fungi (Tavanti et al., 2005).

Owing to the size of their spores, fungal inhalation allows access to bronchioles and alveoli where they can undergo germination and hyphal growth. In the presence of a nutrient rich environment, fungi colonize and potentially infect the CF airway (Hope et al.,

2005). Fungi may then induce a multitude of manifestations ranging from invasive disease and chronic necrotizing pneumonia to a spectrum of hypersensitivity reactions including bronchitis, sinusitis and allergic broncho-pulmonary mycoses (ABPM) (Cimon et al., 2000; Hope et al., 2005).

What continues to remain a challenge is the clinical relevance of fungi in the context of CF. Advances in our understanding of the risk factors for acquisition, biodiversity as well as the biochemical and immunological signalling pathways associated with fungi in the CF airway are discussed below with focus on the most commonly isolated filamentous fungi *A. fumigatus* and yeast *C. albicans*. Other important and emerging fungi including bacterial–fungal interactions are also addressed.

1.3. Why do CF patients acquire fungi?

Patients with CF are at increased risks of fungal acquisition, colonization and infection for similar reasons they acquire bacteria. Abnormal mucociliary function and local immunogenic impairment promote fungal colonization whereas prolonged antibiotic and corticosteroid use facilitates fungal growth.

Defective anatomical barriers impair clearance of inhaled fungi. Owing to their small sizes (2–5 microns), some *Aspergillus* conidia directly enter small airways and alveolar sacs bypassing the large airways where they illicit immunological responses and proliferate. The significant protease burden including neutrophil elastase (NE) within the CF milieu directly damages airway epithelial cell surfaces exposing sub-epithelial extracellular matrix (ECM) proteins laminin and fibronectin. Such proteins facilitate adherence of airborne fungal spores and facilitate airway persistence. Damaged epithelial cell surfaces undergo a healing process including surface fibrinogen/fibrin deposition which itself facilitates fungal binding and colonization (Bouchara et al., 1999). Interestingly, laminin and fibronectin receptors have been detected on *Aspergillus* conidial surfaces (Gil et al., 1996; Tronchin et al., 1997). In addition to protease-mediated epithelial damage, fungi themselves damage epithelia comparable to that induced by bacterial infection. *A. fumigatus* possesses the capability to affect mucociliary clearance and inhibit phagocytosis through production of secondary metabolites and proteases such as alkaline protease (Tomee et al., 1997).

Emerging genetic and immunological data suggest that particular HLA-molecules play a role in the context of both susceptibility (HLA-DR2 and DR5) and resistance (HLA-DQ2) to allergic responses following fungal exposure. Polymorphisms in the interleukin-4 receptor α -chain and interleukin-10 gene also influence host responses to fungal airway exposure (Brouard et al., 2005; Chauhan et al., 2000; Knutsen et al., 2006).

Broad-spectrum antibiotics remain key to the treatment of bacterial-related exacerbations however prolonged use is associated with fungal isolation by facilitating growth. A large multi-centre double-blind placebo controlled trial of intermittent nebulized Tobramycin administration found a significantly increased rate of *Aspergillus* colonization in the Tobramycin treated cohort (Burns et al., 1998). An unconnected Tobramycin versus Colomycin comparative study corroborated these findings (Hodson et al., 2002). Such prospective data is further backed by retrospective cohort studies (Bargon et al., 1999; de Vrankrijker et al., 2011). The selective pressure applied by use of anti-bacterial therapies potentially reduces airway competition for limited nutritional resources favouring fungal proliferation. In contrast to this, recent work by Baxter et al. (2013c) showed a significant reduction in *Aspergillus* colonization following a course of intravenous antibiotics aimed against *P. aeruginosa*. This may reflect different responses to acute versus chronic antibiotic therapy. Besides host-derived factors, fungal related elements also have key roles. For instance, genotype studies of multiple and sequential isolates from

CF patients colonized with *A. fumigatus* illustrates an eclectic range of genotypes within a single sputum sample (Cimon et al., 2001; Neugeglise et al., 1997; Vanhee et al., 2008). As colonization is prolonged, a single genotype may dominate through selection however the factors directly influencing this process remain uncertain.

2. Fungal biodiversity in cystic fibrosis

Many different fungal species are isolated from the CF airway but this is significantly dependent on the methods used for isolation. Traditional methodologies such as fungal culture are the mainstay however emerging molecular based techniques and genotyping are increasingly being used for accurate fungal detection and classification. Non-culture based methods including the detection of fungal metabolites such as siderophores provides an alternative for the detection of fungal colonization in the CF airway.

2.1. Fungal culture

Differing yields between care centres for the various fungi isolated from airway samples are explained by different laboratory systems and sputum processing protocols. Differences include incubation temperatures, culture duration, culture number and media choice (Bakare et al., 2003; Bauernfeind et al., 1987; Paugam et al., 2010; Valenza et al., 2008) however in addition to sputum alone, specimen source also has influence. Whether sputum is spontaneously expectorated or induced influences detection as does the volume and dilution of sputum utilized for examination. Simple additional steps such as prolonging the incubation period for up to six weeks permits the recovery of more slower growing fungi such as *Exophiala* species. Methods suggested to improve the sensitivity of fungal culture include use of neat mucus plugs or homogenized sputum and improved fungal-selective media with novel combinations of antibiotics against *Pseudomonas*, *Staphylococcus* and *Burkholderia* (Masoud-Landgraf et al., 2013; Nagano et al., 2008, 2010; Pashley et al., 2012). To address this lack of standardization in the procedures for mycological examination of sputum samples from CF patients, a series of initiatives have been proposed by the International Society for Human and Animal Mycology (ISHAM) working group, fungal respiratory infections in cystic fibrosis (Fri-CF) (Borman et al., 2010).

2.2. Culture independent molecular methodology

The use of molecular-based fungal isolation techniques enhances detection rates and provides insight into the fungal microbiome that is restricted by culture-based methods. Polymerase chain reaction (PCR) based detection has greater sensitivity for fungal detection when compared to culture methods alone (Coughlan et al., 2012). Use of the highly preserved 18S ribosomal RNA subunit of the fungal makeup is one specific practice employed (Nagano et al., 2010).

Typing is a specialized technique analysing isolates based on both their phenotypic characteristics and genetic make-up. Occurring below species levels, it generates clone and strain specific fingerprints providing an insight unobtainable by simple culture alone (van Belkum et al., 2007). This methodology allows delineation between clinical and environmental isolates allowing for exploration of patient to patient transmission or environmental sources of pulmonary contamination. An abundance of methodologies are described including amplified fragment length polymorphism (AFLP) and multilocus sequence typing (MLST) that are reviewed elsewhere (Vanhee et al., 2010).

Molecular typing techniques for fungal species such as *A. fumigatus* illustrate that several genotypes can be present in a single sputum sample but over time one particular ‘chronically colonizing’

genotype is selected out (Cimon et al., 2001; de Valk et al., 2009). Isolation of different genotypes of fungi in separate specimens illustrates a capacity to clear isolates and perhaps be re-colonized by alternative strains however recurrent isolation of the same genotype is more concerning as it suggests an inability to clear the fungi from the airway with a potential impact on disease (Cimon et al., 2001).

Newer emerging detection techniques include high-throughput pyro-sequencing that possesses the ability to identify fungal species previously undescribed. Such advanced systems illustrate that fungal subspecies in CF are more diverse than previously recognized (Delhaes et al., 2012; Guss et al., 2011). The liberal use of antimicrobials reduces microbial diversity, a process disadvantageous for the clinical state. Diversity represents competition, interaction and symbiosis between organisms and where one is diminished it permits those remaining to exert virulence.

The use of oligonucleotide arrays has been successful for both detection and identification of fungi, even where multiple species co-exist in the sputum of CF patients (Bouchara et al., 2009). Recent work by Delhaes et al. investigated eight sputum samples from $n=4$ adult CF patients using conventional microbiology and pyro-sequencing techniques targeting the ITS2 locus and the 16 rDNA gene. Like bacteria, a reduced fungal biodiversity and species richness conferred poorer clinical status (Delhaes et al., 2012). Furthermore 60% of the fungal species or genera identified were not detectable by culture methods alone (Delhaes et al., 2012). Emerging work on the CF fungal microbiome has additionally described inverse correlations between the abundance of difficult to culture *Malassezia* species and pulmonary function however mechanisms to date remain uncertain (Harrison et al., 2013).

The airway and specifically fungal microbiome continues to evolve and with increased frequencies and antibiotic types administered in CF, molecular based techniques for fungal isolates are likely to predominate in the overall strategy employed for detecting emerging micro-organisms.

3. Filamentous fungi: *Aspergillus* species in cystic fibrosis

The most frequently isolated filamentous fungi are the *Aspergillus* species, in particular *A. fumigatus*. CFTR mutation, a hallmark of CF affects epithelial cell interactions with microbes such as *A. fumigatus*. Dysregulated immune and infectious processes ensue illustrating the critical role CFTR plays in mediating cell responses to *Aspergillus*. The fungi elicit aberrant pulmonary inflammation in CF where fungal spores (conidia) are normally ingested by airway epithelia and cleared but with abnormal epithelial barrier function, an impaired uptake and killing occur coupled with conidial-induced cellular apoptosis. Consequently, germinated (hyphal) forms of the fungus evoke detrimental inflammatory responses, exaggerated lymphocytic infiltration, mucus accumulation and acute lung injury resulting in a vicious cycle of infection and destruction (Chaudhary et al., 2012).

The major clinical manifestation in the context of CF is allergic broncho-pulmonary aspergillosis (ABPA) however aspergillomas or rarely invasive disease may occur. Non-ABPA *Aspergillus* colonization remains a key area of debate and ongoing research. We discuss below the relevant pathogenesis and therapy for each clinical manifestation and relate this to our understanding of the fungus from a molecular, cell biology and immunological perspective (Fig. 1).

3.1. Aspergilloma

Defined as an aggregation of consolidated hyphae within a lung cavity, aspergillomas are rarely observed in CF (Chotirmall

et al., 2013). They occur where a pre-existing thoracic cavity exists and are detected in the upper zones on chest radiography or by the 'air-crescent sign' on computed tomography (CT) (Abramson, 2001). While the majority show no symptoms, significant episodic haemoptysis can occur where the fungus invades through bronchial arteries. No association to ABPA has been clearly documented. Management strategies of choice are surgical resection to prevent bleeds or alternatively bronchial embolization although complications for the former and recurrence rates for the latter remain high (Akbari et al., 2005; Swanson et al., 2002). The optimal treatment approach (or approaches) to an aspergilloma remain unclear and further complicated by the poor penetration of systemic anti-fungal administration into the mass. Hence, in selected inoperable cases, CT-guided instillation of anti-fungal therapy such as amphotericin-B has shown potential however limited data is currently available for this particular approach in CF (Giron et al., 1998).

3.2. Invasive disease

Invasive disease occurs in two major forms: local spread into bronchial epithelium termed invasive pulmonary aspergillosis (IPA) or extension systemically called invasive aspergillosis (IA). The former is seen with higher frequency in CF although both forms are generally rare. IPA can occur in the context of CF where a patient acquires a genotype of *Aspergillus* with propensity for enhanced pathogenicity (Gibson, 2006; Kauffman, 2003). Two pathological forms of IPA are described in the literature distinguished by the presence of angio-invasion. Non-angioinvasive aspergillosis is the chief form in CF with the absence of neutropenia. A pyo-granulomatous inflammatory response occurs resulting in cavitation and tissue destruction (Stergiopoulou et al., 2007). IA occurs in the setting of immunosuppressive states following an inability to clear conidia permitting germination and bloodstream invasion. Angio-invasion is associated with mortality (Fukuda et al., 2003). Pre-transplant colonization with *Aspergillus* species is frequent among CF-lung transplant recipients and positive intra-operative *Aspergillus* culture quadruples risks of developing IA (Luong et al., 2013). Making a clinical diagnosis remains challenging as radiology lacks sensitivity and serological markers are non-specific. While galactomannan (an *Aspergillus* cell wall component) can be measured from serum, this is absent in CF patients colonized by *Aspergillus* so is of little relevance outside the setting of suspected IA which may occur albeit rarely in CF (Warren et al., 2012). Voriconazole remains frontline therapy with liposomal amphotericin-B occasionally considered. Protracted therapy is necessary until all active disease has subsided. Routine monitoring of serum levels for anti-fungal therapy is required with rates of systemic absorption variable between individuals (Herbrecht et al., 2002; Limper et al., 2011; Walsh et al., 2008).

3.3. Allergic bronchopulmonary aspergillosis (ABPA) and non-ABPA *Aspergillus* colonization

ABPA is a consequence of hypersensitivity responses to *Aspergillus* antigen exposure and is the commonest manifestation of *Aspergillus* in the CF airway. A T-helper 2 (Th2) cell mediated response ensues with release of IL-4 and IL-10 which may subsequently lead to production of immunoglobulin-G and -E (IgG and IgE) specific to *Aspergillus*. There is release of pro-inflammatory cytokines and eosinophils to bronchial tissue (Hartl et al., 2006). Precise pathophysiology is complex and not fully understood however inability to clear conidia and genetic variability may alter susceptibility to ABPA (Chauhan et al., 2000). Dendritic cells play a critical role through enhanced IL-10 release and vulnerability to IL-4. Recurrent episodes of ABPA result in bronchiectasis, fibrosis and airway remodelling. Colonization of the CF airway with the

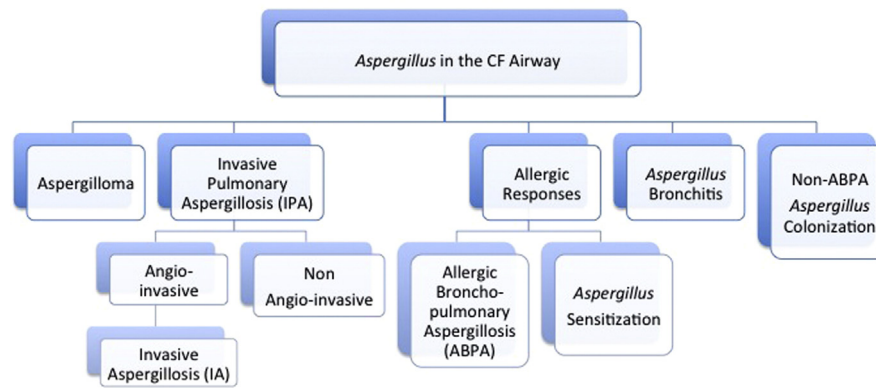


Fig. 1. Clinical manifestations of *Aspergillus* species in the airways of patients with cystic fibrosis.

major bacterial pathogens further increases risk of ABPA (Miller et al., 1996).

Comparison between data collated from various centres is difficult in view of clinical difficulties encountered when diagnosing CF-ABPA. Rates for both *Aspergillus* isolation and ABPA vary between centres with sputum culture ranging from 15% to 50% with ABPA rates of 1–10% (Pihet et al., 2009). ABPA prevalence at our centre approximates 12% agreeing with published values from other cohorts. Frequency of *Aspergillus* isolates from sputum however does not correlate with ABPA occurrence (Chotirmall et al., 2008). Difficulties in the diagnosis of ABPA occurs in CF because of overlapping clinical, radiological, microbiological and immunological features indistinguishable in most cases from exacerbations. It is common upon failure of antibiotic therapy that ABPA is considered. In an attempt to address this, an international consensus conference was convened that determined both classical and minimal diagnostic criteria (Chotirmall et al., 2008; Stevens et al., 2003).

Despite these guidelines, many confirmed ABPA cases do not fulfil required criteria prompting searches for potential biomarkers (Delhaes et al., 2010). Recombinant *Aspergillus* antigens (rAsp) have been suggested to permit differentiation between sensitization (rAsp 1 and 3) and ABPA (rAsp 4 and 6) however, their usefulness is debated (Casaula et al., 2005). Serum IgE against *Aspergillus* allergens is helpful in suggesting ABPA but does not differentiate sensitization (de Oliveira et al., 2007). *Aspergillus*-precipitins and anti-*Aspergillus*-IgG form part of the diagnostic criteria however have inherent problems including fluctuating levels, absence in some ABPA cases and do not delineate sensitization. Furthermore, this assay lacks standardization making comparison difficult (Hutcheson et al., 1996; Kurup, 2005; Kurup and Kumar, 1991). A potential advance occurred with the evaluation of thymus- and activation-regulated chemokine (TARC). This Th2-chemokine rises in early ABPA (before IgE) and correlates with rAsp 4 concentrations. TARC inverse correlation with FEV₁ and may also reflect treatment response (Hartl et al., 2006; Latzin et al., 2008). Another promising marker is the basophil surface marker CD203c. Blood basophils are normally primed and hyper-responsive to *A. fumigatus* allergen stimulation. Measured by flow cytometry, baseline CD203c levels are up-regulated in ABPA and stimulation with rAsp 1 causes further up-regulation of surface concentrations in CF ABPA (Gernez et al., 2012). Both these newer markers require further validation before being deemed a significant advance over presently available tests.

Acute ABPA flares are effectively treated with corticosteroids such as prednisolone that suppress the inflammatory response. Recommended protocols include commencement at 40 mg prednisolone for 2 weeks with taper over 12 weeks according to clinical response. An alternative strategy is use of

pulsed-methylprednisolone at 10–15 mg/kg/day for a three day period each month for up to ten months annually (Cohen-Cymerknoh et al., 2009). In these cases, anti-fungal therapy is also recommended. Itraconazole is preferred for this purpose due to a favourable side effect profile however voriconazole has also been suggested despite associations with photosensitivity in CF (Rondeau et al., 2012). Posaconazole is a newer agent but remains untested. Itraconazole however does have variable absorption. Interactions with certain foods necessitate serum monitoring to ensure adequate dosing. Developing azole resistance is also an emerging concern (Burgel et al., 2012). To date, no randomized controlled trials exist to examine anti-fungal therapy in the treatment of CF-ABPA (Elphick and Southern, 2012). Anti-IgE strategies have assisted in cases of chronic steroid dependence however these are mostly case reports (Tanou et al., 2013).

In addition to ABPA, *Aspergillus*-sensitization and persistent carriage within CF airways has an independent effect on pulmonary function (Fillaux et al., 2012). The relationship between *Aspergillus* and the development of allergic sensitization however remains unclear. Allergic sensitization is not associated with recovery of fungi from sputum and *Aspergillus* but not *Candida* sensitization has been associated with greater declines in lung function and pulmonary exacerbations (Baxter et al., 2013b). This work appears the clinical corollary of epithelial work revealing that *Aspergillus* infection modulates pro-inflammatory responses in CF epithelial cells while *C. albicans* does not (Reihill et al., 2011). Severe CF mutations, baseline FEV₁ > 70%, absence of *Pseudomonas* and prior azithromycin exposure are all predictors for *Aspergillus* sensitization (Fillaux et al., 2013). Recently, a novel immunological classification for aspergillosis in CF was proposed based on IgE and IgG levels, sputum galactomannan and the presence of PCR detectable *Aspergillus*. Four distinct sub-groups were defined including ABPA, *Aspergillus*-sensitized, *Aspergillus* bronchitis and those non-diseased. This improved classification improves clinical phenotyping and has potential for subsequent management in CF (Baxter et al., 2013a).

Colonization refers to the presence of potentially pathogenic organisms in culture specimens in the absence of clinical symptoms. Differentiating *Aspergillus* colonization and infection is vital. Isolating the fungi in greater than 50% of respiratory samples obtained over a year appears to strongly indicate colonization and infection only deemed significant in the presence of effects on lung function, respiratory exacerbations and little or no response to a course of broad spectrum antibiotics. These proposed definitions do have limitations.

An emerging area of both interest and controversy is the recognition of *Aspergillus* in CF sputum cultures in the absence of ABPA. Clinical concern persists over whether clinical disease results in this

setting. Early work assessing this area differed considerably in their findings however definitions used for colonization coupled to heterogeneity of study designs and duration, respiratory sampling and isolation techniques made such data difficult to compare (Bargon et al., 1999; de Vrankrijker et al., 2011; Jubin et al., 2010).

Work by our group illustrated an increased degree of radiological abnormalities in an *Aspergillus*-colonized cohort compared to a non-colonized group using a modified Bhalla scoring system focusing on lung structure. Interestingly, no effect on pulmonary function was observed suggestive that radiology may be a better means of measuring *Aspergillus*-related effects in the absence of ABPA (McMahon et al., 2012). Further retrospective clinical study showed that persistence of *Aspergillus* colonization represents a risk factor for hospital admissions in CF (Amin et al., 2010). These works together provide evidence for the potential clinical effects of *Aspergillus* colonization in the absence of ABPA. It therefore is logical to assess if eradication of the fungi provides any clinical benefit.

We conducted a prospective observational cohort study administering itraconazole to a non-ABPA, steroid naïve CF population with *Aspergillus* colonization ($n = 13$). A 6-week course of itraconazole at 400 mg daily is effective at reducing *Aspergillus* bioburden by colony forming unit (CFU) counts and PCR detection of *Aspergillus* DNA. Treatment effectively attenuates mosaic pattern perfusion on CT imaging and stabilizes pulmonary function while reducing exacerbations and improving quality of life (Coughlan et al., 2012). In agreement with others, we found PCR detection to be more sensitive than culture alone for detection of *Aspergillus* from airway samples however it is important to note that PCR detects both dead and living organisms along-with dormant spores. Sputum galactomannan however is produced in the logarithmic growth phase of the fungus and is a major biofilm component representing active clinically relevant disease. Galactomannan is however present in other fungal cell walls e.g. *Trichophyton*, *Malassezia*, *Fonsecaea* and *Candida* and not *Aspergillus* specific (Barnes, 2008). A combination of both PCR and galactomannan measures may be most practical to determine effects of therapy (Baxter et al., 2013c; Coughlan et al., 2012; Hope et al., 2010; Lousert et al., 2010).

We also investigated the effect of *Aspergillus* on vitamin D, its receptor (VDR) and the immune response in relation to *Aspergillus* species. Prior *in-vitro* work intimated links between *Aspergillus*, ABPA and vitamin D pathways. Kreindler et al. (2010) showed that vitamin D₃ treatment attenuated Th2 responses to *Aspergillus* by CD4⁺ T-cells obtained in CF-ABPA. Vitamin D receptor (VDR)-deficient mice fail to develop experimental allergic asthma with Th2 cytokine elevations of IL-5 and IL-13 (Wittke et al., 2004). Strengthening such links is work examining the OX40 ligand (OX40L) a co-stimulatory molecule involved in mediating Th2 allergic responses. Vitamin D treatment in animal models of CF-ABPA showed VDR binding of the promoter region of OX40L altering NF- κ B-p50 subunit binding and subsequently repressing its inflammatory and Th2 induced responses (Nguyen et al., 2013).

Aspergillus down-regulates VDR gene and protein expression *in-vitro* and *in-vivo*. Down-regulation was mediated by a heat inactivated small molecule virulence factor gliotoxin, an immune-evader and promoter of cellular apoptosis. Reduced gliotoxin and Th2 cytokine (IL-5 and IL-13) concentrations were detectable post-itraconazole treatment in PWCF with *Aspergillus* with reciprocal increases in VDR airway receptor expression (Coughlan et al., 2012). Despite our positive findings following therapy, the only double-blind placebo controlled trial to date found no benefit in lung function parameters from itraconazole treatment (Aaron et al., 2012). Randomized to oral itraconazole (5 mg/kg/day) or placebo, $n = 35$ patients were followed to 24 weeks. It is noteworthy that while the two studies share similarities there are also importance differences crucial to the interpretation of their findings. While both has small patient numbers and found no effect on

pulmonary function, the Canadian trial failed to achieve therapeutic itraconazole levels in a significant number of patients making interpretation of results difficult. Furthermore, no molecular detection for *Aspergillus* or radiological assessment of clinical benefit to treatment was performed in the Canadian work (Aaron et al., 2012). These were both areas of positive outcome in the Irish study. There appears now a definite need for a well powered multi-centre trial designed to definitively determine the effects of such a therapeutic approach. One important concern for future work remains the high prevalence of azole resistant *Aspergillus* strains in adults exposed to itraconazole. In addition to recent exposure prior exposure of up to a three year period may be relevant (Burgel et al., 2012). This suggests further need for caution in the use of itraconazole when treating adult patients with CF. In addition to prolonged exposure to azole-therapy, an alternative mechanism has been proposed for the acquisition of azole-resistance. This method involves the direct acquisition of an azole-resistant mutant resulting from the widespread use of azole fungicides in agriculture and must be considered in the context of CF where exposure to fungicides exists (Morio et al., 2012).

3.4. Innate and adaptive immunity against *Aspergillus* species

Several aspects of the innate immune machinery are involved in the recognition and removal of *Aspergillus* from the CF lung (Fig. 2). Physical barriers, phagocytosis, anti-microbial peptides and pattern recognition receptor (PRR) signalling all play important roles however some are defective based on the pathogenesis of CF lung disease. This promotes fungal persistence and the ability to cause clinical disease in this setting.

The voluntary action of coughing in addition to anatomical barriers including the airway surface liquid (ASL) all provide the first line of defence. All these mechanisms are dysfunctional in CF making the CF patient prone to fungal acquisition. A rodlet protein layer detected on some conidial fungal surfaces additionally aids immune-evasion allowing induction of detrimental responses (Aimanianda et al., 2009).

The two major airway phagocytes, neutrophils and macrophages both attempt to remove the invading fungus. Through both ingestion and cell content release, the neutrophil plays a central role in fungal killing. The alveolar macrophage recognizes *Aspergillus* through surface PRRs and uses oxygen dependent and independent methods for killing (Brakhage et al., 2010; Ibrahim-Granet et al., 2003). Acidification of the phagolysosome (an area of ongoing controversy in CF) is normally followed with conidial degradation by cathepsin D through a non-oxidative mechanism (Barriere et al., 2009; Di et al., 2006). Chitinase (an enzyme possessing the ability to digest chitin, a component of fungal cell walls) is released from macrophages and augments fungal killing (Boot et al., 1995; Hartl et al., 2009; Ibrahim-Granet, Philippe, 2003). Reactive oxygen species (ROS) generation occurs concurrently with the generation of extracellular traps (NETosis) to facilitate further fungal elimination through cationic antimicrobial peptide release (Bruns et al., 2010; Philippe et al., 2003; von Kockritz-Blickwede and Nizet, 2009; Wiesner and Vilcinskis, 2010).

Anti-microbial peptides are important in early contact with *Aspergillus* however may be altered in the CF milieu due to the high protease burden. Defensins (α - from neutrophils and β - from epithelial cells), cathelicidins (LL-37) and secretory leucoprotease inhibitor (SLPI) target the disruption of fungal cell walls lysing them while simultaneously modulating other immune responses (Alekseeva et al., 2009; Baranger et al., 2008; Benincasa et al., 2006; Bjorstad et al., 2009; Wiesner and Vilcinskis, 2010). Lactoferrin inhibits fungal replication through iron-chelation starving conidia and inducing death (Balloy and Chignard, 2009). Nearly all

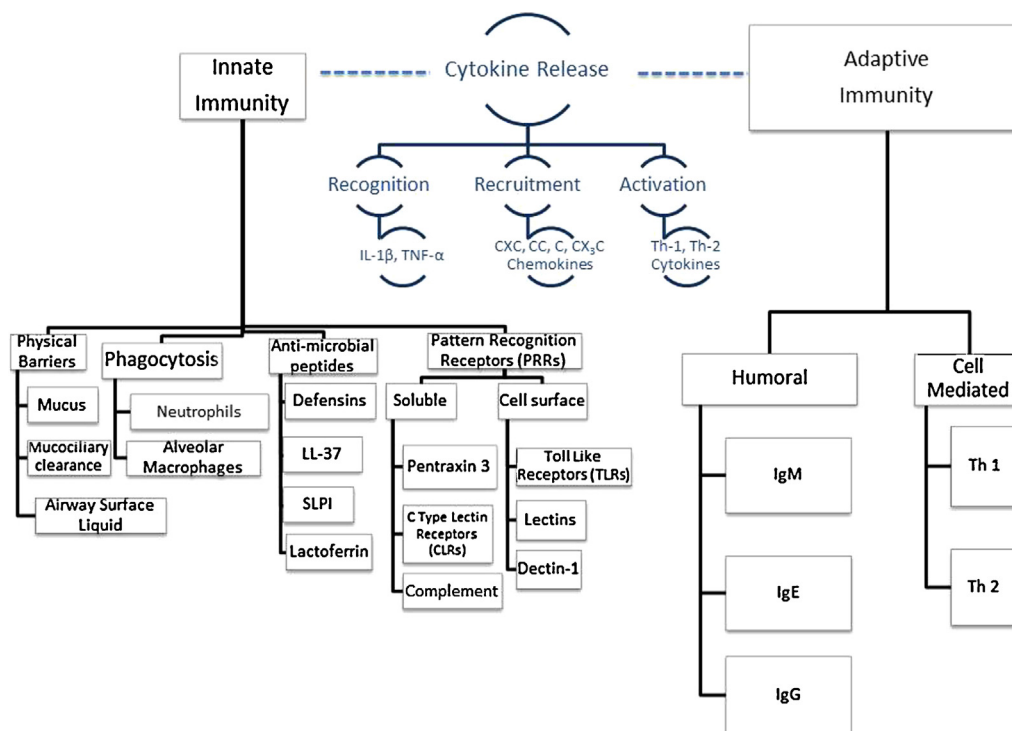


Fig. 2. Immunological responses to *Aspergillus* species exposure in the cystic fibrosis airway.

the anti-microbial peptides are susceptible to proteolytic degradation by cysteinyl cathepsins or serine proteases (Rogan et al., 2004; Taggart et al., 2003; Weldon et al., 2009). In addition to the putative adverse effects of the CF airway surface liquid ionic milieu on anti-microbial activity (Pezzulo et al., 2012) there are now emerging data that the CF neutrophil is less effective at secreting many of these anti-microbial peptides due to defective degranulation (Pohl et al., 2013).

Recognition of *Aspergillus* cell wall components including β -glucan, mannan, chitin and galactomannan by PRRs induces protective immunological responses. Secreted PRRs such as Pentraxin-3, C-type lectins, and complement all have roles. Pentraxin-3 is a galactomannan opsonin while C-type lectin family members surfactant proteins (SP) A and D cause conidial agglutination and enhance phagocytosis associated killing (Allen et al., 1999; Garlanda et al., 2002; Madan et al., 1997). Mannose binding lectin (MBL) activates lectin complement pathways while C3 and C5 have independent roles in anti-*Aspergillus* defences (Hector et al., 1990; Kaur et al., 2007; Neth et al., 2000). Cell surface associated PRRs such as toll-like receptors (TLRs), lectins and dectin-1 all possess important functions in the defence against *Aspergillus*. Key roles for TLRs 2, 4 and 9 are described with the latter activated by hypomethylated fungal DNA and TLR2 recognizing chitin (Da Silva et al., 2008; Mambula et al., 2002; Meier et al., 2003; Ramirez-Ortiz et al., 2008; Wang et al., 2001). Lectins such as DC-SIGN and the mannose receptor (MR) facilitate fungal binding and ingestion by phagocytes. Dectin-1 identifies β -1,3-glucans but also co-operates with TLR2 to influence the response to *Aspergillus* (Gersuk et al., 2006; Persat et al., 2003; Serrano-Gomez et al., 2004; Steele et al., 2005; Taylor et al., 2007). Importantly, *A. fumigatus* induces macrophages independent of dectin-1 through Th2 and transcription protein 6 mediated signalling which are both valuable mechanisms in overall fungal clearance (Bhatia et al., 2011).

Cytokines initiate, maintain and resolve host defences to *Aspergillus* and importantly bridge messages between the innate and adaptive immune response to the fungus (Fig. 2). Their role and precise function in *Aspergillus*-related lung disease is

reviewed elsewhere (Chotirmall et al., 2013). In summary, the initial response and recruitment of immune cells to the site of invading *Aspergillus* is mediated by 'recognition cytokines' including IL-1 β and TNF- α . 'Recruitment cytokines' next assume control. The superfamily encompasses four groups based on the position of their cysteine residue relative to the N-terminus i.e. CXC, CC, C and CX₃C chemokines. Released in response to *Aspergillus* exposure, CC chemokines MCP-1 and MIP-1 α drive mononuclear recruitment and clearance (Chotirmall et al., 2013; Strieter et al., 2002). 'Activation cytokines' are linked to the adaptive T-helper cell response and subdivided into Th1 and Th2 subtypes. Th1 driven responses including IFN- γ , IL-2, IL-12 and TNF- α promote *Aspergillus* resistance whereas Th2 piloted inflammation via IL-4 and IL-10 associates with progressive disease (Cenci et al., 1999; Cenci et al., 1997; Mosmann et al., 2005). Our group has previously shown that high levels of BAL associated Th2 cytokines IL-5 and IL-13 are associated with *Aspergillus* colonization (Coughlan et al., 2012). Further recent work has shown that CFTR-deficient lymphocytes are prone to develop into Th2 cells upon *in-vivo* challenges with *Aspergillus* (Mueller et al., 2011).

4. Yeasts: *Candida* species in cystic fibrosis

There is increasing recognition of the diversity of membership of this particular fungal genus. Unlike dimorphic fungi, morphology remains comparable *in-vitro* or *-vivo* and they are second only to *Aspergillus* as the commonest isolated fungi in CF. The most common *Candida* species are *C. albicans*. Capable in the CF setting of causing localized and systemic infections, these fungi induce oral and genital thrush, vascular access device related infections and post-transplant complications (Chotirmall et al., 2010a). *C. albicans* is also isolated at high frequency from CF sputum owing to risk factors unique to the condition including inhaled steroid use, CF-related diabetes and recurrent exposure to antibiotics. Despite their increasing recognition in CF airways, precise roles in disease remain uncertain. Limited genotyping data is available for this yeast. In a single study of 442 isolates, long-term persistence (~16 months)

of *C. albicans* was detected with microevolution. Similar genotypes were found in only cases of siblings suggestive of transmissibility (Muthig et al., 2010).

4.1. Mucosal infection

Oral candidiasis remains relevant in CF. Significant risks associated with CF include impaired salivation, steroid use, diabetes and antibiotic exposure which allow *Candida* proliferation. Genital candidiasis should also be considered and may be more common, persistent and difficult to treat in CF compared to controls (Sawyer et al., 1994). Studies show that these forms of *Candida* infection are infrequently evaluated in CF clinics (Lyon et al., 2004; Woolnough et al., 2004). Topical or systemic anti-fungal treatment with fluconazole is recommended with good outcomes.

4.2. Device-related infection

Infections of implantable vascular devices are a primary source of CF *Candidaemia* (Bonacorsi et al., 1996; Horn and Conway, 1993; Munck et al., 2004). Diagnosed in the presence of risk factors, pyrexia and positive blood culture, first line treatment is aimed at removal of the offending device and aggressive intravenous anti-fungal therapy. The device is replaced based on need however two negative blood cultures are necessary along with significant clinical improvement prior to re-implantation. A major issue in treating such infections is biofilms associated with such devices causing anti-fungal resistance.

4.3. Post-transplant infection

Candida infection in this setting is relatively uncommon. Tracheobronchitis is surprisingly the major manifestation in addition to anastomotic site infections (Orens et al., 2006). Invasive infection is rare and predominantly occurs in the first month post-transplant.

4.4. Airway colonization and infection

Long-term persistence of *Candida* species in CF airways is recognized and while transmission between siblings is one possible means of infection, it is unclear if long-term colonization contributes to chronic infection and the inflammatory state (Muthig et al., 2010). We prospectively assessed the frequency of *C. albicans* in CF sputum over a prolonged time period in the largest clinical study to date (Chotirmall et al., 2010b). We found frequent colonization by *C. albicans* that is best predicted by pancreatic insufficiency, osteopenia and co-colonization with *P. aeruginosa*. Colonization independently predicted increases in hospitalized exacerbations and longitudinally illustrated greater rates of decline for both FEV₁ and BMI (Chotirmall et al., 2010b). What our work could not determine was whether the sputum presence of the yeast represented a marker for advancing disease rather than possess individual pathogenic potential. What drives the increases in hospitalizations observed in our work remains unclear however the fungus itself, a virulent co-colonizer or cross-kingdom interaction are all possibilities. Interestingly, European registry data similarly associated the presence of *C. albicans* with reductions in pulmonary function however similarly no clear cause and effect was established (Navarro et al., 2001).

4.5. Innate and adaptive immunity against *Candida* species

Mannans and mannoproteins form a structural basis for *Candida* and all induce host responses. Similar to *Aspergillus*, C-lectin type receptors (CLRs) and TLRs respond to antigens within the yeasts structure. Both groups of PRRs recognize major polysaccharide

cell wall components including N- and O-linked mannans, β -mannosides, β -1,6-glucan and phospholipomannan. The key PRRs involved in *Candida* recognition include MR, DC-SIGN, galectin-3 (CLRs), dectin-1 and TLRs 2 and 4 (Ferwerda et al., 2008). Adaptive immunity plays a more prominent role in the context of yeast infection as individuals with compromised cell-mediated immunity are at increased risks of systemic spread. Despite this, little work has been performed to date from a CF context. In non-CF animal models, IL-12 induces Th1 responses while IFN- γ is protective (Lavigne et al., 1998; Mencacci et al., 1998; Vazquez-Torres et al., 1999). CF upper airway exposure to *Candida* lysates induced Th2 responses however when *Pseudomonas* lysates were added, a digression to a Th1 response with neutrophilia occurs (Allard et al., 2009). The humoral response to *Candida* exposure in CF however has been assessed but results are difficult to interpret. In early studies, IgG antibodies to *Candida* were found elevated in CF irrespective of sputum isolation however greater concentrations were seen with enhanced sputum detection techniques (Przyklenk et al., 1987). Other work similarly found antibody elevations but IgE responses to the fungi could not be related to any worsening of clinical status (Maiz et al., 2002). Most recently, it was confirmed that increases in IgG, IgA and IgM concentrations correlated with isolation of *C. albicans* from sputum but again no relation to respiratory status was detectable (Maiz et al., 2008).

5. Fungal-bacterial interactions in cystic fibrosis

Bacteria and fungi co-exist in biofilms within the CF lung. These adherent species interact in this context through diverse signalling pathways that may contribute to clinical disease.

5.1. Fungal–*Pseudomonas* interactions

Gram negative bacteria such as *Pseudomonas* influence the airway environment and subsequently exert inhibitory effects on fungal growth. For instance, through secretion of small carbon chain molecules and pyocyanin a direct effect on *Aspergillus* biofilm formation may occur (Kerr et al., 1999; Mowat et al., 2010). What effect, not clear whether positive or negative. We found at our own centre inverse relationships between *Aspergillus* isolation in sputum and *Pseudomonas* colonization (unpublished work). Recent work however illustrates a reduced *Aspergillus* burden following intravenous antibiotic treatment with anti-pseudomonals (Baxter et al., 2013c). While this goes against prior published work, post antibiotic therapy induced changes in bacterial community profiles likely impact upon *Aspergillus* as other bacteria also possess inherent abilities to inhibit fungal growth. It may be that the inhibitory effects of *P. aeruginosa* on *A. fumigatus* observed in early work only becomes significant prior to biofilm formation (Kerr, 1994; Mowat, Rajendran, 2010). Unlike short-term antibiotic bursts, long-term therapy reduces bacterial diversity that select out more favourable growth conditions and host immunological responses for *Aspergillus* colonization (Tunney et al., 2011). *P. aeruginosa* biofilms provide some protection against antibiotics as do *A. fumigatus* biofilms against anti-fungals but biofilms can be disrupted by macrolides. This disruption may permit a re-exertion of the inhibitory effect on *A. fumigatus* growth (Kobayashi, 2005; Lutz et al., 2012; Seidler et al., 2008). In general, the interdependence of such organisms in CF remains poorly understood.

Interactions between *P. aeruginosa* and *C. albicans* have also been described. The former forms biofilms on the latter and kills the fungus. This only occurs when *C. albicans* grows in filamentous and not yeast forms (Lo et al., 1997). Preceding such killing, signalling occurs between the two organisms involving quorum sensing (Williams, 2007; Williams and Camara, 2009).

5.2. Fungal–*Staphylococcus* interactions

Minimal data addresses the interactions between *Aspergillus* and *Staphylococcal* interactions however co-aggregation, competitive and antagonistic relationships may occur with *C. albicans* (El-Azizi et al., 2004; Rickard et al., 2003). Initial synergism leads to enhancement of virulence for *Staphylococcus* in addition to an impairment of host defences against the bacteria (Carlson, 1983a,b). Extensive intra-biofilm interaction facilitated by farnesol has been described with overall anti-staphylococcal effects (Jabra-Rizk et al., 2006).

6. Other fungi in cystic fibrosis

In addition to *Aspergillus* and *Candida* species, the fungal microbiome is expanding rapidly and we are only beginning to appreciate the diversity of fungi within the CF airway. Whether such rarer organisms represent colonizers or pathogens remain in most cases an issue of uncertainty. We here outline the major other fungi described in the context of CF.

6.1. Rarer *Aspergillus* species

Some uncommon *Aspergillus* species isolated from CF specimens include *A. lentulus* and *Neosartorya pseudofischeri*. The former has been detected intermittently over a decade in a CF patient while the latter has been isolated in three different European countries all cases co-colonized with *A. fumigatus*. Both fungi show low susceptibility to anti-fungals (Symoens et al., 2010).

6.2. *Scedosporium apiospermum*

This saprophyte is the second most detected filamentous fungi in CF with prevalence ranging from 6.5% to 10% (Cimon, Carrere, 2000; Williamson et al., 2001). Commonly isolated in association with other fungi, it is best detected by selective media such as YPDA + cycloheximide or SceSel+ (Rainer et al., 2008). Importantly, potted plants have been described as an exposure risk and while this fungi is rarely airborne, it may be detectable in environmental sources such as water and soil (Summerbell et al., 1989; Tinteln et al., 2009). Colonization is not associated with any clinical parameters including FEV₁, steroid or anti-fungal therapy. Patients with *S. apiospermum* are less likely to be colonized with *Pseudomonas* while prior therapy with anti-staphylococcal penicillin is a documented risk factor for colonization. Bacterial colonization is less likely to influence *S. apiospermum* colonization (Blyth et al., 2010). It is also critical to distinguish between *S. apiospermum* and other emerging species such as *S. aurantiacum* (Delhaes et al., 2008). The discrepancy between high detection frequency and paucity in the environment begs the question as to how CF patients acquire the fungus. Adherence to the CF airway through a lectin-mediated process is described and two putative virulent factors have been identified, an extracellular protease similar to that of *Aspergillus*-associated alkaline protease and a copper-zinc cytosolic superoxide dismutase (Larcher et al., 1996; Lima et al., 2007; Pinto et al., 2004). TLRs and phagocytes respond to *S. apiospermum* however less efficiently than that observed with *Aspergillus*. Colonization by this fungus is chronic and genotype analysis illustrates that each patient is colonized by a unique phenotype conserved over time (Defontaine et al., 2002). In addition to allergic responses, a major feared clinical consequence is disseminated infection in the immunocompromised (Guarro et al., 2006). Voriconazole therapy is recommended however eradication is challenging to achieve.

6.3. *Exophiala Dermatitidis*

This fungus has unique morphology growing as black yeast at 37 degrees and a filamentous fungus at room temperature. Highest prevalence is reported in Germany and Sweden with isolation difficult unless a prolonged incubation period is permitted (Blaschke-Hellmessen et al., 1994; Haase et al., 1990, 1991; Horre et al., 2004; Kondori et al., 2011; Kusenbach et al., 1992). Use of selective media is helpful and while usually asymptomatic in most cases, it can also cause true infection (Haase et al., 1990; Kusenbach et al., 1992). Conflicting results have been reported for genotyping. While some studies found that all isolates belonged to the same genotype although obtained from multiple patients at multiple sites other work found patient-specific clusters, and in a single case, two distinct genotypes within the same sputum sample (Packer et al., 2012; Rath et al., 1997). Pancreatic insufficiency remains a risk factor for colonization with this fungus (Kondori et al., 2011; Lebecque et al., 2010). Considered a biomarker of CF-related dysfunction, its isolation should prompt a search for atypical forms of CF in cases labelled as bronchiectasis (Pihet et al., 2009). Whether the organism generates an immune response is an ongoing matter of research as is whether it impacts upon clinical outcomes and lung function in CF.

6.4. Other fungal species

Other fungi detected in CF specimens include *Alternaria*, *Cladosporium* and *Penicillium* species, as well as *Paecilomyces variotii*. *Rasamsonia argillacea* (ex *Geosmithia argillacea*) initially identified as *Penicillium emersonii*, and *Acrophialophora fusispora* are isolated almost exclusively in the CF context. None are usually associated with clinical consequences in CF. Because of their rare nature they are usually misidentified even in the most experienced of mycology laboratories. *Rasamsonia argillacea* complex for instance has been previously misidentified as *Penicillium* or *Paecilomyces* species hence its true incidence under-reported. Role in the CF airway is unclear. Thermo-tolerant and resistant to azole therapy, chronic colonization of this fungus in CF may in fact pose problems (Giraud et al., 2013).

7. Conclusion and future directions

Significant strides have been made in terms of both fungal detection and evaluation of the effects of fungi on the CF airways. *Aspergillus*, *Candida* and *Scedosporium* species have all been investigated. Insight into the pathogenesis and signalling pathways for such fungi remain an active area of ongoing research. One major difficulty within the field is the cross-sectional nature of many of the clinical studies making it difficult to determine cause and effect. Prospective well controlled studies with treatment arms are now a viable future option to overcome this. The biggest question remains what is the clinical significance of the fungi detected in CF samples and when and if treatment is indicated. The answers will differ depending on the fungus involved, the clinical status of the PWCF, and the individual's response to infection.

7.1. Ongoing controversies

While we have learned a significant amount in recent times, several controversies persist within the field of fungal isolation in CF. Are fungi pathogenic and what are the major mechanisms? When are fungi pathogenic? Is it from the beginning of infection or only after colonization and sensitization? Does the clinical setting or particular fungus matter? Should attempts be made to eradicate fungi? If so what therapies and for how long? These are all questions difficult to answer based on available data but significant

strides have been made in providing answers to these questions. Differences in both isolation and identification techniques exist across laboratories causing a misidentification of certain fungi that affect our recording of 'real' incidence in CF populations. This perhaps causes an underestimation of both the importance and significance of fungi in the CF airway. Use of molecular based detection techniques and genotyping for fungi represent great hope for the future but require standardization before being accepted into routine clinical care while the diagnosis of ABPA and the use of biomarkers particularly in early disease have potential but require further refinement. Future work elucidating specific consequences of fungal colonization in CF are warranted and the recent acknowledgement of *Aspergillus* bronchitis as a significant clinical entity in the non-immunocompromised state aids our understanding of the complex clinical phenotypes involved (Chrdle et al., 2012). Emerging immunological classifications continue to identify sub-groups of fungal 'colonizers' previously unrecognized that may be at risk of clinical deterioration. Classifications including serological markers, RT-PCR and galactomannan data have been described and may direct future therapy by permitting improved clinical phenotyping, optimizing pathogenesis studies, and facilitating management decisions (Baxter et al., 2013a; Liu et al., 2013).

7.2. Bystanders or pathogens?

This remains the pivotal question into the role of fungi in CF. We are closer to answering this question than at any time in the past. We now recognise that host derived factors including the underlying state of the immune system, severity of CF disease, effect of inflammation and the burden of treatment all play an important role in determining susceptibility, while the type of fungus, its chronicity, virulence, genotype and ability to evade immune recognition also have an important role. It is this combination of host and pathogen-related factors which determine if a particular fungus at a given time-point will be a bystander or pathogen in the CF lung and armed with this knowledge we can now anticipate the development of strategies to detect and treat as required.

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