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Candida species in cystic fibrosis: A road less travelled.

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CANDIDA SPECIES IN CYSTIC FIBROSIS: A ROAD LESS TRAVELLED

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Abstract

Candida species are isolated with high frequency in cystic fibrosis yet their definitive role in disease remains unclear. Previously considered to have minimal inherent virulence owing to their commensal ability, the last decade has heralded an increasing recognition of *Candida* infection among patients with cystic fibrosis. What has been more recently hypothesized is that the organism possesses virulence factors that play diverse roles at different body sites during varied stages of an infection. Currently, limited data is accessible in the area of cystic fibrosis. This review aims to provide an overview of the role of *Candida* species in cystic fibrosis as is currently understood including the common local and systemic infections observed in clinical practice. The uncertain role of airway colonization and insight into emerging fields such as *Candida*-bacterial interactions are also addressed. Finally, we outline the current understanding of the innate, cellular and humoral immune responses associated with this genus which has been the major focus of work performed to date.

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1. Introduction

Major advances in the care of cystic fibrosis (CF) patients have positively influenced prognosis over the last decade. Significant inroads into understanding the basic defect have accelerated the development of targeted therapies. The disease however continues to present new challenges to clinicians and researchers, for example fungal airway colonization. The consequences of bacterial infection, colonization and need for segregation in outpatient clinics have all been confronted and curbed to the point that fungal colonizers with an undetermined role on disease course and progression are becoming increasingly prevalent. Both yeasts and filamentous fungi have been identified as microbial pathogens in CF particularly in the context of invasive disease in the transplanted population and allergic responses, for instance allergic bronchopulmonary aspergillosis (ABPA). One particular fungal genus isolated at high frequencies from sputum culture is *Candida* and limited literature is available addressing the issues of *Candida* colonization and infection in CF. This is possibly because its manifestations are still considered relatively minor in comparison to other infectious agents. As a consequence it has received little attention in terms of clinical and scientific research. This review aims to provide an overview and our current understanding of the *Candida* species in CF, its associated local and systemic infections and an insight into emerging data on its role in airway colonization and associated immune response.

2. The *Candida* genus

Oral thrush was the first infection of the *Candida* species described in humans and following identification of its reproductive potential by budding, the fungus was originally named *Oidium albicans*. *Candida albicans* became the adopted name used and since then many other species have been identified to play a role in human infection. The most common of

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these are *Candida albicans*, *Candida glabrata, Candida krusei, Candida parapsilosis* and *Candida tropicalis* (1, 2).

The genus has exponentially grown with hundreds of newer member species identified and unlike dimorphic fungi the morphology of a given *Candida* species remains fundamentally comparable *in vitro* or *vivo*. *Candida* species are capable of causing chronic, localized or systemic infection collectively termed 'candidiasis' although most commonly act as commensals within the oropharynx, skin folds, gastrointestinal tract and vagina. On occasion it can cause opportunistic infection (3). Once infection ensues, significant morbidity and mortality results and consequently, systemic candidiasis has high death rates (>75%) (4).

Identification in the microbiology laboratory is achieved on Sabouraud dextrose media and *Candida albicans* can be identified through germ tube testing or colorimetric detection of L-proline aminopeptidase and beta-galactosaminidase. Since recent isolation of *Candida dubliniensis* which produces false positive results in the above tests, a chromogenic agar culture method that allows isolation and identification of *Candida albicans, Candida tropicalis* and *Candida krusei* has been widely adopted. A modified version of this media is available to more clearly distinguish *Candida dubliniensis* (5). Alternative older methods to distinguish between the two organisms include an assessment of growth ability at higher temperatures (45˚C for *Candida albicans*) or specific DNA sequencing.

Patients with CF are at an increased risk of acquiring *Candida* due to use of inhaled steroids, diabetes mellitus and lifelong antibiotic treatment however despite its frequent isolation from sputum, oral and vaginal swabs, it remains unclear what such culture actually means in practical terms for CF clinicians. We believe that a spectrum of "commensal-colonizer-

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pathogen" most likely exists for the organism and where specifically the organism is on this spectrum at a particular time point may be dictated by the clinical state of the CF patient and whether bacterial co-colonizers are concurrently present in the airway.

Prior studies have addressed the notion that although frequently identified in CF, the clinical role of *Candida* species has yet to be definitively determined (6-8). *Bakare* et al identified *Candida* as the second most frequent fungal growth to *Aspergillus* in the CF airway and such growth has been associated with more severe CF where patients receive prolonged treatment with antibiotics, glucocorticoids and probiotics (9-12). In terms of infection, *Cimon* et al performed a five-year epidemiological study assessing the frequency of bronchopulmonary mycoses in a CF population and examined the aetiological role of individual fungal species in disease. The filamentous fungi *Aspergillus* and *Scedosporium apiospermum* together with *Candida* contributed the largest burden. Despite high isolation of *Candida* in CF, a single case of candidiasis was observed and this low rate was attributed to the anti-fungal ability of various bacterial colonizers in CF and whilst invasive airway infection is a rare event, extent of airway damage from hypersensitivity phenomena remain unknown (13). We will now address the localized and systemic infections associated with *Candida* species in CF and subsequently tackle the complex issues of airway colonization, cross kingdom interaction and the immune response.

3. Localized *Candida* infection in CF

3.1 Oral candidiasis

Candida species are isolated from the oral mucosa in up to 40% of healthy adults and therefore considered commensal (14). A cut-off point to distinguish between commensalism and colonization remains undetermined. Common risk factors associated with oral recovery

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include poor dentition, older age, diabetes mellitus, use of inhaled or systemic steroids, smoking, malignancy and frequent antibiotic use. Oral thrush usually presents as discomfort associated with a dry mouth and associated dysphagia. In some cases, altered taste is experienced. The diagnosis is usually straightforward and by direct observation of white membraneous plaques on the buccal mucosa or soft palate. This may be confirmed microbiologically by staining a swab or culturing a rinse from the associated area. Atypically, foci of oral erythematous inflammation or angular cheilitis may present. There are clearly significant risk factors in the CF state that predispose to oral colonization and subsequent infection including impaired salivary secretion, steroid use, CF-related diabetes and recurrent courses of antibiotics for exacerbations. Antibiotics alter the homeostasis of oral flora and as such have a permissive action on *Candida* growth. In a study from Manchester, on direct questioning of major symptoms in the CF population, 40% (n=17) complained of a sore mouth, 24% (n=10) of thrush five times annually and 38% (n=16) of a hoarse voice every three months (15). In our own institution's experience, we encounter regular instances of oral candidiasis annually following courses of antibiotics but which resolve after a short burst of anti-fungal treatment (Fluconazole). We recommend microbiological confirmation by scrapings in all cases unless white plaques are directly observed on oral examination. This is because some of the symptoms described are not specific to oral thrush but can be found in associated vitamin deficiencies (B_6, B_{12}) or by simple blistering. We recommend that CF patients attending routine clinic be screened for risk factors and questioned at three-monthly intervals with regard to the symptoms of oral thrush including frequency of sore or dry mouth, crusting lips, dysphagia, dysphonia or hoarseness and difficulties with taste. Any relationship to antibiotic treatment should be teased out. Clearly oral candidiasis is recognized in CF but the dearth of available literature suggests it is probably misdiagnosed, ignored or missed in several cases. A simple risk factor and symptom screen would see

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improvements both in quality of life and probably compliance with other CF treatments. With the advent of newer and increasingly earlier administration of anti-bacterial therapies, oral candidiasis is likely to become a more significant issue in the future care of CF patients.

3.2 Genital candidiasis

Genital candidiasis is a common occurrence in the normal population with rates of up to 75% having single and 50% recurrent episodes (2). It may be asymptomatic or present with balanitis in males and pruritis with vaginal discharge in females. Most infections are caused by *Candida albicans* (95%) however *Candida glabrata* (5%) infection is described (2). There is limited but important literature available addressing these infections in CF with the majority focused on female manifestations. It has been more than a decade since *Sawyer et al* first reviewed the subject with a self-administered questionnaire in young women with CF (n=55) (16). Vulvovaginal candidiasis was more common in CF (35%) versus controls (13%) and additionally more persistent and difficult to treat. Antibiotic use was a significant association and the work concluded that "health professionals generally trivialize illnesses and diseases that are common, easily treated and not life-threatening". More recent work has included male patients and addressed symptomatic partners. *Lyon et al* evaluated 40 adults with CF (19 male, 21 female) and similarly found large proportions (62.5%, n=25) experiencing symptoms of infection however few (15%, n=6) had been directly questioned about it at CF clinic (17). Patients refused to discuss if their partners were symptomatic. This highlighted to CF clinicians a major deficiency of clinical practice and questions about candidiasis should feature during annual review clinic consultations. It is also important to consider that vaginal discharge in young CF women can be caused by other pathogens such as *Chylamidia, Gonococcus* or *Trichomonas* species and that there is an observed unexplained high incidence of genital *Chylamidia* in CF.

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A third study addressing the same subject was performed by interview in 101 CF patients and addressed symptom frequency and medical risks associated with *Candida* (18). Patients were asked to report on personal risk factors for *Candida* infection and their desire to be questioned about 'thrush' in CF clinic. Many had two or more risk factors (92.1%, n=93) however the only significant factor associated with genital *Candida* was long-term antibiotics (87.1%, n=88, p=0.001). Over seventy percent of patients, both males and females had symptoms of either oral or genital *Candida* or both simultaneously. Forty percent (18/45) of those with oral and two-thirds (33/50) of those reporting genital candidiasis described 'distress' however it did not affect desire for treatment. Most cases of oral infection were diagnosed by a CF physician whilst genital infection was mainly self-diagnosed. It is noteworthy that general practitioners diagnosed more cases of genital infection when compared to CF physicians. This is potentially explained by differing doctor-patient relationships in different settings or alternatively because the focus of the CF unit remains on respiratory or gastrointestinal symptoms leading patients to believe that this forum is inappropriate for discussing other complaints. Most patients in the study did however want to discuss such issues and were unconcerned who their discussant was although some females predictably preferred discussion with female staff. This Manchester based study is the largest to date and detected similar patterns to previous work. The most concerning new discovery was the high incidence of symptoms among CF patients but only on direct questioning placing a future onus on CF clinics. A major criticism of all these studies was that symptom recording was not supported by microbiological confirmation of infection, an important point for future work. Additionally, although several publications have assessed the benefits and efficacy of anti-fungal treatment in vulvovaginal candidiasis in the 'normal' population, importantly none have been performed in CF (19). Despite this clear lack of available literature, we strongly recommend screening questions for infection at all CF clinic visits and

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depending on clinical findings, anti-fungal treatment prescribed either empirically or following microbiological confirmation.

4. Systemic *Candida* infection in CF

4.1 Post-transplant Candidiasis

CF is the 3rd most common indication for lung transplantation and the opportunistic nature of *Candida* species suggest that the post-transplant period is ripe for such infection (20). Despite this, candidiasis post-transplant remains rare and *Aspergillus* species are in fact more commonly encountered in this setting (21). The main *Candida* infection following transplantation is surprisingly tracheobronchitis which includes anastomotic site infections. Bloodstream and other invasive infections secondary to *Candida* are rare and will not be addressed in any detail within this review except to state that when present occur within the first month following transplantation (22, 23). This is primarily a consequence of the major surgical intervention and intensive care unit stay experienced by patients.

4.2 Totally implantable venous access device (TIVAD) infection

A more commonly encountered systemic infection associated with *Candida* involves the presence of a TIVAD commonly referred to as a "port". *Candida* species in this setting are recognized as the most common infecting organism associated with a TIVAD resulting in septicaemia (24-26). Important risk factors for infection remain the same as that for other *Candida* infections. Diagnosed by the presence of swinging pyrexia, systemic septicaemia and positive blood cultures for *Candida* species taken from both the port site and peripherally, the first intervention remains to remove the offending device whose tip should also be sent for microbiological evaluation. Device removal results in significant clinical improvement however aggressive anti-fungal therapy is concurrently administered during

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which time patients on the active transplant list have to be removed temporarily. Recently, we encountered a case series at our centre which presented a different setting to the traditional *Candida* port infection. We experienced three cases of TIVAD thrombosis and superior vena cava obstruction that required use of thrombolytic therapy. In two of the three patients, their post-thrombolysis course was complicated by systemic candidiasis secondary to TIVAD infection. In these cases, we achieved a successful outcome following removal of the device coupled with aggressive anti-fungal treatment. Another more traditional case series described earlier this decade over a six year period was that from a CF centre in Manchester where fifteen adults with CF were diagnosed via positive blood cultures with a *Candida* port infection (15). Here, a variety of *Candida* isolates were identified including *albicans, parapsilosis* and *glabrata* and excellent clinical outcomes again achieved via device removal and systemic anti-fungal treatment dictated through sensitivity testing. Our own practice continues to evolve with regard to optimal treatment and we routinely look for at least two negative blood cultures following completion of the prescribed treatment course. Replacing ports depends on need but we try not to replace before 8 weeks following the last negative blood culture. To date, there remain no clinical trials or evidence based guidelines to support these treatment practices. Another important point is that many *Candida* infections involve biofilm formation particularly with indwelling vascular catheters in the context of CF. These biofilms are microbiologically complex containing matrix enclosed microcolonies containing yeasts and hyphae in a bilayer structure (27). Such *Candida* biofilms can be resistant to conventional anti-fungals through a multitude of mechanisms and as such future research needs to be conducted to determine the best and optimally standardized treatment of *Candida* port infections.

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5. Airway *Candida* colonization in CF

It remains controversial as to whether *Candida* species are transient or persistent colonizers of the respiratory tract in CF. A study by *Muthig* et al showed that the mean persistence of *Candida* species was at least nine months and that the species identified were genetically related and transmissible but susceptible to all anti-fungals tested. Although concerns of transmissibility persist, it is unsure whether this species conclusively contributes to chronic infection and the inflammatory milieu in CF (28). We have assessed colonization rates at our own centre over prolonged time periods and found persistence rates in excess to that previously described. We have established that the main factors predicting colonization by *Candida albicans* in CF are pancreatic insufficiency, osteopenia and co-colonization with *Pseudomonas*. At first glance, this suggests that the more advanced a patient's disease, the likelier their sputum contained *Candida albicans*, a view of many clinicians and it may be that the organism acts as nothing more than a microbiological marker of disease severity in CF. To challenge this paradigm, we are currently prospectively evaluating whether airway colonization by *Candida albicans* may act pathogenically by affecting clinical outcomes in CF including FEV1, BMI, hospitalizations for infective exacerbations and sputum colonization with *Pseudomonas* or *Aspergillus* species. Notably, a previous cross sectional analysis of a European CF registry did show that *Candida albicans* colonization was associated with 5-10% predicted decrease in pulmonary function (29).

Newer airway *Candida* species have also emerged over the last decade and one pertinent example is the high recovery rates (10-25%) of *C. dubliniensis* from the oral cavity of HIV patients (30). This new organism was subsequently described in the non-HIV population particularly in individuals receiving high antibiotic burdens (31). Therefore, its detection in CF came as no surprise but did involve a complex isolation procedure involving Staib agar

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(32). What was surprising was that its prevalence rate in CF was higher than that found in HIV however virtually nothing about its potential for virulence is known. There is no clear clinical or experimental evidence of differences in terms of pathogenic potential when compared to *Candida albicans* (33) however *Candida dubliniensis* is reported to exhibit cell surface hydrophobicity not observed in the *albicans* species (34). Cell surface hydrophobicity is known to play a role in the adhesion of microorganisms and by displaying this feature, *Candida dubliniensis* takes advantage of the dehydrated respiratory secretions in CF and consequently proliferates. These observations may also explain why patients who are older (>30 years) and have more advanced disease are colonized by this yeast. *Peltroch* et al (32) followed six CF patients with *Candida dubliniensis* and whilst all patients remained stable with no invasive infection detected its effect on lung function could not be conclusively established because of the small numbers. A larger epidemiological study of this *Candida* species in CF is warranted.

6. *Candida*-bacterial interactions

According to Costerton (35), biofilms are 'a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface'. Traditionally biofilms have been thought to comprise a single bacterial species however it is now increasingly recognised that mixed biofilms exist involving interactions between both prokaryotes and eukaryotes. Bacteria and fungi are found together in a variety of environments but particularly in biofilms, where adherent species interact through diverse signaling mechanisms. In the host *C. albicans* can often be found growing with bacteria in polymicrobial biofilms and interspecies interactions occur that can impact on the transition of *C. albicans* between virulent and nonvirulent states (27). Under conditions of immune dysfunction, such as in the CF lung, colonising *C. albicans* can become an opportunistic

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pathogen causing mucosal and disseminated infections potentially impacting on mortality. In the biofilm environment, microbial species use 'quorum-sensing' (QS) molecules for cell-to-cell communication to promote collective behaviour within the population, enhance access to nutrients and niches, and provide a combined defense against competitor organisms (36, 37). The process of QS can cross the prokaryote–eukaryote boundary (36-39).

6.1 Interactions with *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is the most prevalent opportunistic pathogen in individuals with CF and is the principal organism associated with biofilm formation in the CF lung; *S. aureus* and *Burkholderia* spp. are also considered important pulmonary pathogens in CF. The dimorphic yeast *C. albicans* is the most common eukaryotic microbe isolated from CF patient sputum (9, 10, 40). *C. albicans* can exist in a mixed biofilm where the prokaryotic and eukaryotic communities exhibit either synergistic or antagonistic interactions. Several studies suggest that *P. aeruginosa* and *C. albicans* interact with each other *in vivo,* and they are commonly found together in mixed infections (41). In their seminal paper Hogan and Kolter (38) first reported a pathogenic relationship between *P. aeruginosa* and *C. albicans.* They demonstrated how *P. aeruginosa* can form a dense biofilm on *C. albicans* filaments and kill the fungus. Interestingly this only occurred when *C. albicans* was growing in its filamentous (or hyphal) form – an essential feature associated with its virulence (42). *P. aeruginosa* neither bound to nor killed the yeast form of *C. albicans* and the ability of *P. aeruginosa* to kill filamentous *C. albicans* was dependent on a number of physiological factors including growth phase, nutrient availability, surface structures including flagellae and type IV pili, secreted QS factors and regulatory molecules such as *rpoN* (43). By forming a biofilm on fungal filaments *P. aeruginosa* may be able to obtain nutrients from *C. albicans* in a nutritionally scarce environment.

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Prior to killing of *C. albicans* by *P. aeruginosa,* signalling can occur between both organisms. The QS molecules of both species are responsible for this communication. For example the bacterial molecule 3-oxo-C12 homoserine lactone can affect *Candida* morphology, whilst the fungal 12-carbon sesquiterpene metabolite, farnesol can interfere with *Pseudomonas* quinolone and pyocyanin production and swarming motility (37, 39, 41, 44-49). Thus eukaryotes and prokaryotes possess diverse signaling mechanisms to detect and respond to each other through QS signal molecules.

6.2 Interactions with *Staphylococcus aureus*

In oral biofilms a mutually beneficial interaction called coaggregation can occur where the adhesion of *C. albicans* to oral bacteria facilitates its colonization of the oral cavity (50-52). In contrast, the interaction between *C. albicans* and *Pseudomonas aeruginosa* as described above is competitive and antagonistic in nature. A third mechanism of interaction that can occur is that evident between staphylococci and *C. albicans*, which appears to be initially synergistic (53-55). Carlson *et al.* (56, 57) described a synergistic effect between *C. albicans* and *S. aureus* in a mouse infection model leading to enhanced mortality following dual infection suggesting that *C. albicans* can either enhance the virulence of *S. aureus* or impair the host's immune defences. Extensive physical interactions are known to occur between *S. aureus* and both the yeast and hyphal forms of *C. albicans* in a mixed biofilm (58) and it has been suggested that farnesol has a role in orchestrating these interactions. After the initial synergy during *C. albicans*-*S. aureus* biofilm formation farnesol then negatively affects staphylococcal biofilm formation, compromises cell membrane integrity, viability and susceptibility of *S. aureus* to a variety of clinically important antibiotics (58). Thus farnesol may represent a therapeutic target for inhibiting the development of a mixed biofilm in the CF lung however once the biofilm has been established farnesol may actually behave as an

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anti-bacterial factor. It remains to be seen which has the more detrimental effect in the CF lung, *C. albicans* growing alone or in combination with *S. aureus* in a mixed biofilm.

6.3 Interactions with other microbes

Notwithstanding the ability of *C. albicans* to modulate bacterial growth, reciprocal evidence indicates that other bacteria may also play an important role in the pathogenesis of *C. albicans* infections. For example in the urinary tract *Escherichia coli* can enhance adhesion of *C. albicans* to bladder mucosa (59) whereas in the gut indigenous microbes can inhibit mucosal adhesion of *C. albicans* (60). Consequently alterations in the normal bacterial flora following treatment with broad-spectrum antibiotics may allow *C. albicans* to proliferate and invade tissues, greatly affecting its pathogenicity (60). This is an important consideration for individuals with CF who are frequently prescribed antibiotics.

This may be most clearly studied in the oral cavity where adhesion of *C. albicans* to saliva-coated surfaces and proline-rich proteins is an important early step in colonization (50, 61-63). Many species of oral bacteria may compete with *C. albicans* for primary adhesion receptor sites (50, 61, 64, 65) however once resident in the mouth *C. albicans* can adhere to the major microbial constituents of early dental plaque.

Similar to *P. aeruginosa,* another opportunistic pathogen *Acinetobacter baumannii* exhibits a predilection for *C. albicans* filaments and can inhibit *C. albicans* filamentation, resulting in attenuated virulence of *C. albicans* in the nematode. Interestingly, similar to its effect on *S. aureus*, *C. albicans* can also inhibit *A. baumannii* growth via farnesol production (66). As mixed bacterial–fungal biofilms have been shown to be associated with a multitude of infections including those affecting endotracheal tubes, biliary stents, silicone voice and orthopedic prostheses and acrylic dentures (35, 67) determining the exact sequence of events

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involved in the development of these mixed biofilms may determine how and when to target the individual microbial or fungal constituents.

7. *Candida* and the immune response

The outer strata of *Candida* species contain elements with antigenic potential. These include mannans and mannoproteins which upon human exposure induce an immunogenic response (68-71). Where mannan-deficient, *Candida* strains are clinically less virulent and during the course of a *Candida* infection, cellular, humoral and innate immune responses all play a role (69, 70, 72-74).

7.1 Innate immunity

Recognition of microbes by the innate immune system depends on activation of specific pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). For fungi the first PAMPs encountered by the immune system are those present in the fungal cell wall. The cell wall of *Candida albicans* is composed of a core structure of β-(1,3)-glucan polysaccharide fibrils covalently linked to chitin (a β-(1,4)-linked polymer of *N*-acetyl glucosamine) and β-(1,6)-glucan. The outer layer consists of *N*-linked (75) or *O*-linked mannosylated proteins called mannans (76). Two classes of PRRs in particular play an important role in antifungal immunity - the C-type lectin receptors (CLRs) and Toll-like receptors (TLRs). Neutrophils, monocytes, macrophages and airway epithelial cells are all involved in defense against fungal pathogens. Dendritic cells (DCs) also respond to fungal PAMPs leading to activation of T-cell-mediated specific immunity. These various cell populations differ in their expression of CLRs and TLRs on the cell membrane, and are therefore capable of initiating different responses. CLRs and TLRs recognize the major

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polysaccharide cell wall components, *N*- and *O*-linked mannans, β-mannosides, β-(1,6)-glucan and phospholipomannan. The mannan structures are detected by the mannose receptor (MR), the dendritic cell-specific ICAM3-grabbing nonintegrin (DC-SIGN), dectin-2, galectin-3 and TLR4 whereas complement receptor 3 (CR3), dectin-1 and TLR2 detect the β-glucans.

7.1.1 CLRs

CLRs comprise a large family of receptors that share at least one carbohydrate recognition domain originally identified in mannose binding lectin (MBL). CLRs are evolutionary conserved and have been shown to be involved in the modulation of the innate immune response and fungal recognition. Although MBL can bind to *C. albicans* (77) and has the ability to opsonize fungal yeasts by activating the complement system (78), MBL-deficient mice do not show decreased survival to infection with *C. albicans* (79). However dectins-1 and -2, galectin-3, DC-SIGN and MR do play important roles in the innate immune response to *C. albicans*.

Dectin-1 recognizes β-(1,3)-glucans, mediates ligand uptake and phagocytosis, and triggers cytokine production (80). Alone it is sufficient in inducing responses to fungi however synergistic proinflammatory responses occur in cooperation with TLRs. For example in collaboration with TLR2, dectin-1 triggers proinflammatory responses by *C. albicans* or zymosan (81, 82). Dectin-2 is mainly expressed on myeloid cells and maturing monocytes. It recognizes high-mannose structures (83) and interacts with the FcγR to induce TNF in response to filamentous *C. albicans* (84). On macrophages the galectin-3 receptor mediates the recognition β-mannosides expressed on *C. albicans*. This PAMP-PRR interaction is also enhanced by TLR2 (85). DC-SIGN, expressed on mature DCs, recognizes

high-mannose structures in *C. albicans* and mediates phagocytosis of fungal particles (86). Finally, MR recognizes chitin, fucose, and mannose and has been implicated in the recognition of several fungi, including *C. neoformans*, *C. albicans*, and *Pneumocystis*. Branched *N*-bound mannans in *C. albicans* are recognised by MR (87) and mice defective in MR display partial impairment in their host defense against *Candida* infections (88).

Modulation of CLR expression and activity thus represent important therapeutic targets in CF that remain, as yet, underexplored. A newly identified CLR, Mincle, has been shown to participate in macrophage recognition of *C. albicans*. Although it remains to be shown which PAMP expressed by *C. albicans* directly activates Mincle, the role of this receptor in fungal innate immunity has been clearly demonstrated. Inhibition studies have shown decreased TNF production by macrophages following stimulation by *Candida* yeast cells and Mincle knockout mice display hypersusceptibility to *Candida* infection (89). It will be interesting to determine the expression and function of Mincle by immune cells and airway epithelium in individuals with CF.

7.1.2 TLRs

Following the initial observation by Lemaitre *et al.* (90) that *Drosophila melanogaster* flies deficient in the Toll receptor succumb readily to infection with *Aspergillus fumigatus* due to defective synthesis of the drosomycin defensin, the role for TLRs in antifungal defense has been extensively studied and recently a human homolog of drosomycin called drosomycin-like defensin has been described. This peptide is expressed mainly in the skin and has activity against a variety of filamentous fungi (91), however its potential as an antifungal or anti-*C. albicans* therapeutic has not yet been exploited. Early studies on TLRs revealed that TLR2 and TLR6 co-operate in recognition of the fungal structure zymosan

derived from *Saccharomyces cerevisiae* (92). With respect to *C. albicans,* blocking of TLR2 has been shown to lead to decreased monocyte production of TNF and IL-1β after stimulation with *C. albicans* (93). Furthermore TLR2$^{-/-}$ mice have decreased TNF and MIP-2 production and reduced neutrophil recruitment after challenge with *Candida* (94). TLR1 and TLR6, two receptors capable of forming heterodimers with TLR2, may also have a minor role in *C. albicans* recognition (95). Further evidence for an anti-fungal role for TLR2 comes from studies showing that TLR2-deficient macrophages have an increased ability to contain *C. albicans* (96), and that TLR2 signalling can promote Th2-type or T-reg-type responses in response to *C. albicans* (97, 98).

TLR4 participates in antifungal host defense by recognizing *O*-linked mannan structures and mediating proinflammatory responses. TLR9 has the potential to recognize fungal DNA and blocking TLR9 either pharmacologically in human monocytes or genetically in TLR9-deficient mouse macrophages leads to a reduced production of cytokines, mainly IL-10, in response to stimulation with *C. albicans* (99). However the contribution of TLR9 to fungal recognition is not believed to be significant. Much is known regarding the expression and function of TLRs in the CF lung and is beyond the scope of this article. Readers are directly elsewhere for comprehensive reviews of the role of TLRs in CF (100, 101).

Recognition of *C. albicans* by the innate immune system therefore occurs through MR and DC-SIGN recognizing branched *N*-linked mannans, and TLR4 recognizing linear *O*-linked mannans. CR3 responds to β-(1,6)-glucan and dectin-1 and galectin-3 in combination with TLR2 each recognize β-glucan/phospholipomannan and β-mannosides, respectively. It is likely that these recognition receptors can operate in combination and that stimulation via multiple PAMP–PRR combinations might increase both the sensitivity and the specificity of the immune recognition process. Notwithstanding these elegant recognition systems, *C.*

albicans frequently colonizes individuals with CF. Exactly why *C. albicans* is so commonly found in CF individuals remains to be determined but may be associated with impairment in a particular component of the innate immune system.

7.2 Cell mediated immunity

Individuals whose cell mediated immunity is compromised are at an increased susceptibility of infection with *Candida* species illustrating the important role that this arm of the immune system plays in the host defence against the organism. The reasons underlying this observation remain ambiguous and little work has been performed in CF to examine this potential link. In non-CF animal models, interleukin (IL)-12 promotes a Th1 response to *Candida* exposure and IL-10 systemic infection (102, 103). In contrast interferon (IFN)-gamma appears to be protective (104). *Allard* et al have shown that CF oropharyngeal exposure to *Candida* lysates evoked a Th2 type immune response similar to that observed in non-CF models. However when exposed to both *Pseudomonas* and *Candida* lysates together a deviation in the adaptive immune response from a Th2 to Th1 type associated with neutrophilia is noted (105). Neutrophils also damage pseudohyphae in association with IFN-gamma to provide another host resistance strategy against *Candida* species. This is mediated through granulocyte colony stimulating factor (G-CSF) (106, 107). Additionally, Dectin-1 by distinguishing cell wall β-glucan can trigger cell mediated defences (81). While the cytokine mileu and neutrophil response in CF continue to be an area of intense investigation among researchers, minimal data to date remains accessible with regard solely to *Candida* species.

7.3 Humoral immunity

The role of the humoral immune response during *Candida* exposure and infection remain controversial. Despite this, the majority of literature with regards to *Candida* in CF exists in

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this area. In the late 1980s, *Przyklenk et al* first assessed serum IgG antibodies to both *Aspergillus funigatus* and *Candida albicans* in CF versus control patients and found that antibody levels were higher in CF irrespective of sputum isolation (108). In contrast to *Aspergillus fumigatus*, antibodies to *Candida albicans* were observed to increase significantly with increased isolation from sputum culture. Minimal work followed until *Maiz et al* assessed the prevalence of *Aspergillus* and *Candida* species in CF sputa and the serologic IgE responses to these fungi. For the first time, they additionally investigated whether the immune response had direct effects on clinical status in CF (109). *Candida* species were isolated in nearly half of all sputum samples analyzed (47.5%) however 87.9% of patients had at least one growth of *Candida albicans* during the study course. One-quarter (26.7%) were sensitized to *Candida albicans* and only patients who grew *Candida albicans* at least once during the study developed an IgE response to the fungi. The clinical parameters assessed (FEV1 and CT scores) were not worse in those sensitized versus the non-sensitized. Interestingly, half of the sensitized group had confirmed ABPA whilst the remaining patients some immunologic characteristics of ABPA. In conclusion, the group found a high prevalence of both colonization and sensitization to *Candida albicans* in CF but could not relate this to disease severity or clinical status. Although serum IgE to *Candida albicans* appeared to represent an immunological marker of ABPA in CF, it is important to note that the studied group was small (n=20) and only FEV1 and CT scores assessed as clinical measures. The same group extended this work recently to assess serum IgG, IgA and IgM against *Aspergillus fumigatus* and *Candida albicans* and found that although no correlation was detected between the presence of *Aspergillus fumigatus* in sputum and an immune response, the converse was true of *Candida albicans*. Increasing sputum isolation heralded an elevated serum response however again this could not be related to respiratory impairment (110).

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8. Conclusion and future directions

In this review, we have highlighted the current knowledge base and infections caused by the *Candida* species in CF. The dearth of CF-specific literature available illustrates that it evidently is a 'road less travelled'. Despite this lack of literature and audit, what remains undoubted is that the species is isolated frequently and has importance in contributing to morbidity and in some cases mortality in CF. There is a high rate of undetected symptomatic oral and genital infection in the adult CF population and the problem should not be ignored with newer anti-bacterial agents on the horizon that will likely select out these fungal pathogens. Long-term use of antibiotics has recurrently emerged as a contributing factor to *Candida* infection and an alteration of flora post therapy lends survival advantages to the pathogenicity of this species. In doing so, *Candida* probably contributes to the inflammatory mileu observed in CF. Although post-transplant candidiasis is a rare occurrence, port infections do occur frequently. When a port is infected, it should be removed promptly and combined with anti-fungal therapy results in excellent clinical outcomes. The *Candida* species interestingly elicits innate, cellular and humoral immune responses that we have yet to fully understand in the context of CF. Clearly an increasing amount of work remains left to be done to address the many unanswered questions. Future avenues for focus in this field lie within clinical care, isolation techniques and biomedical research. Healthcare professionals should maintain a positive approach in looking for manifestations of *Candida* infection during annual review at CF clinics and subsequently pursue microbiology in symptomatic cases. In terms of isolation techniques, selective media needs to be developed to suppress the growth of gram negative pathogens such as *Pseudomonas* and *Burkholderia* species and enhance fungal identification and isolation. Standardization of detection protocols needs to be pursued for fungi in CF as currently lab and international variation persists. Finally, basic science and clinical research avenues with regard to *Candida* species in CF need to be

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actively pursued so as to enable an improved understanding of its role in the CF airway, *Candida*-bacterial interaction and its potential use as a microbiological marker of CF disease severity and progression.

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