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Fungal Cell Wall Dynamics and Infection Site Microenvironments: Signal Integration and Infection Outcome

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Abstract

Upon entrance into the host, fungi encounter a myriad of host effector products and microenvironments that they sense and adapt to for survival. Alterations of the structure and composition of the cell wall is a major fungal adaptation mechanism to evade these environments. Here we discuss recent findings of host-microenvironmental induced fungal cell wall changes, including structure, composition, and protein content, and their effects on host immune responses. A take home message from these recent studies is an emerging understanding of how integration of multiple signals, of both fungal and host responses to dynamic infection site microenvironments, determines outcomes of infection. A challenge moving forward is to further understand these mechanisms and harness them for therapeutic benefit.

Introduction

Fungi encounter diverse microenvironments in mammalian hosts, including macro and micro nutrient limitation, pH changes, oxygen levels, antifungal drugs, and host derived proteins/effectors. An important response to these microenvironments is modulation of a major defensive apparatus, the fungal cell wall (CW). CW's of fungi are dynamic structures that fluctuate not only during growth, but in direct response to environmental stimuli and stress [1]. While the underlying mechanisms are not fully understood, they are important because detection of CW components drives immune responses through host pattern recognition receptor (PRRs) sensing of pathogen associated molecular patterns (PAMPs) [2]. Responses between the fungus and host may lead to a protective response or host mediated damage. For example, CW components can lead to host damage, such as in immune reconstitution syndrome (IRIS) [3], or they can suppress protective responses, such as with the recently described *Aspergillus fumigatus* CW component galactosaminogalactan [4]. These interactions have traditionally been depicted as one-dimensional and there is much unknown about how host microenvironments affect this relationship at the initiation of the interaction and throughout the course of infection during antifungal therapy. Here, we discuss recent observations illustrating how host microenvironments affect CW dynamics of fungi associated with human disease (Figure 1).

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Carbon source

Carbon sources utilized by fungi *in vivo* remain to be fully defined and vary with host niche and fungus. Most host environments are sugar limited causing the fungi to rely on alternative energy sources, such as amino and organic acids [5,6]. However, most *in vitro* analyses of the fungal cell wall (and growth) occur in sugar, usually glucose, rich conditions. Growth of *A. fumigatus* on glucose alone was found to influence the effect of antifungal inhibitory properties [7]. The importance of the variety and dynamism of these *in vivo* nutrient sources on the fungal CW is now being understood. Lactate, a non-fermentable carbon source, produced at sites of infection has strong effects on the CW of *C. albicans*. Growth on lactate induced a decrease in the CW mass, increased expression of CW remodeling proteins, and increases in CW synthase gene transcripts [6,8]. Additionally, the lactate grown CW was more resistant to osmotic stress, CW perturbing agents, and antifungal drugs [8]. Lactate induced changes in *C. albicans* affected the yeasts interaction with the immune response, with decreased phagocytosis and more efficient killing and evading of macrophages [9]. There is less known about the effects of carbon source on CW and PAMP changes for other fungi even though nutrient limiting conditions were found *in vivo* in infection transcriptomic data [10]. Utilization of different carbon sources for *A. fumigatus* and *C. neoformans* are involved in virulence, but whether there is contribution from CW changes and immune recognition is unknown [11,12]. With regard to mechanism of carbon source induced CW changes, differences in ubiquitination targets between fungi may lead to metabolic flexibility that is likely critical for fungal survival in mammalian hosts [13].

Immune Effector Products

During infection, fungi encounter immune effector products. An exciting emerging area of fungal pathogenesis research is the observation that immune effector products such as PRRs, antifungal/microbial peptides, and antibody/complement alter fungal CW composition, metabolism, and virulence. Binding of murine soluble PRR Ptx3, causes remodeling of the CW and alterations in the inflammatory response with *C. albicans* and increased phagocytosis of *A. fumigatus* conidia [14]. Remodeling of CWs by antimicrobial peptides, such as LL-37, was prevented in *C. albicans* through reduction of Xogp1 activity [15,16]. For *C. neoformans*, an antimicrobial peptide from a cattle tick causes defects in melanization and capsule formation, leading to decreased survival [17]. One resulting hypothesis is that fungal interactions with immune cells, through PRRs, trigger CW remodeling as an immune evasion strategy.

One immune evasion technique that *C. neoformans* utilizes is the formation of enlarged, multinucleated “titan” yeast cells that cannot be phagocytosed. An unknown effector product in the lung stimulates the formation of these cells by signaling through the G-coupled receptor Grp5 and activating CW and cell structure remodeling [18]. Moreover, antibody binding to *C. neoformans* caused changes, different between serotypes and strains, in the CW diameter, capsule size, and overall rigidity of the CW [19–21]. Additionally, use of antibody alone caused inhibition of capsule formation and growth in *C. neoformans* and *C. albicans*, respectively, but the changes induced in the CW or capsule by antibody binding are unknown. Recently the cytokine IL-17A was observed to bind to fungal cells and induce significant changes in fungal transcriptomes that affected responses to the host [22]. Thus, changes in CW and how they affect the immune response in relation to immune effector products is an exciting area of study, particularly considering their involvement in vaccine and therapeutic strategies.

Internal Cell Environment

Advances in understanding how internal host cell environments, such as the phagosome, are involved in recognizing fungi has highlighted the exposure of CW PAMPs in this mechanism. Fungi have mechanisms for sensing these attacks, which results in CW restructuring and morphological changes. One mechanism utilized by *C. albicans* and *A. fumigatus* involves the morphological switch from yeast/conidia to hyphae. The phagosome contains several factors that can alter the CW including low pH, low nitrogen, reactive oxygen and nitrogen species, and digestive enzymes (upon lysosome fusion). For *C. albicans*, this switch involves sensing lower pH as a trigger to begin remodeling, but also prevention of phagolysosome formation [23]. *A. fumigatus* conidia are protected from the low pH and ROS by melanin, however, how these conditions affect the CW are unknown [24]. In response to acidic conditions, *C. neoformans* forms a “secondary” CW, with increased chitin and β -glucan, underneath the original CW in order to prevent autolysis [25]. For resistance to oxidative stress in *C. neoformans*, capsule formation is required, but the effect on the CW is unknown [26].

Oxygen/Iron Availability

Oxygen and iron are required for maintaining fungal growth and survival. Within the host, these compounds are limited at sites of infection [27,28]. Fungal responses to low oxygen (hypoxia) and low iron have, for the most part, similar outcomes in transcriptional and proteomic alterations [29–31]. The response to hypoxia and iron starvation affects the CW and membrane of fungi, as both oxygen and iron are required for enzymes involved in their synthesis. Recent studies have demonstrated that the transcriptional and translational response of *A. fumigatus* to hypoxia involves the upregulation of a subset of genes and proteins involved in CW synthesis, including glycolysis, β -glucan, and chitin [30,32]. This led to overall changes in the structure of the CW and component distribution causing an increased inflammatory response by host phagocytes [32]. Similar results were demonstrated in *C. albicans* exposed to hypoxic conditions, with increased transcript levels of CW biosynthesis, remodeling, precursor metabolism (glycolysis), and protein genes [33,34]. Intriguingly, in *C. neoformans* there is minimal change for glycolytic transcriptional responses under hypoxic conditions and its effects on CW dynamics has not been studied [35]. In the case of CW remodeling, responses to low iron largely mimic those found with hypoxia, except for *C. neoformans* [29,34]. In *C. neoformans*, low iron, unlike the known hypoxic responses, results in increased capsule and melanin production in the CW [36].

Biofilm/Secondary infection

Considering recent advances in our understanding of mammalian microbiomes, it seems clear that fungi will often encounter other microbes during fungal pathogenesis. There are diseases or disease states that are characterized as being comprised of multiple microbes, such as biofilm formation or cystic fibrosis. Under biofilm conditions *A. fumigatus* induces expression of genes involved in rodlet production, leading to immune evasion [37]. Biofilm conditions for several fungi also result in increased expression and production of extracellular matrix proteins, that provide biofilm CWs with resistance to host and drug effectors [37–40]. Within these co-habiting microbial environments, bacteria exist and the effects of these microbe-microbe interactions, through soluble and non-soluble compounds, on the CW are unknown. For example, *Pseudomonas aeruginosa* produces soluble compounds, phenazines, that cause inhibition of hyphal growth and hyphal death in *C. albicans* [41]. At low concentrations, phenazines also impact colony morphology and block aerobic respiration. The effect of phenazines on the CW of *C. albicans* has yet to be determined. The interaction of CW components with bacteria affect not only the response of

the microbes to each other, but also how the immune system responds [42]. Interactions between *A. fumigatus* and *C. neoformans* with the microbiomes they encounter during infection are understudied, but is an exciting area for future investigation. It will be important to determine whether microbe-microbe interactions in the host induce microbial defense responses that subsequently alter host immune efficacy. It seems clear that these dynamics will be critical for understanding commensalism, microbial pathogenesis, and many human diseases [43].

Other Host Niches

Increased host damage during infection by fungi is often caused by dissemination. Growth of *C. albicans* on media containing blood or serum resulted in alterations in the CW, including increased mannan [44,45]. For *C. neoformans*, serum affects the half-life of the CW component GXM, however, GXM branching also contributes [46]. Extracts from serum, specifically phospholipids, also cause an increase in capsule size [47]. *A. fumigatus* and other molds are known to be angioinvasive. However, effects from growth in blood and serum on the CW are undefined. Within the brain and CNS *C. neoformans* can utilize L-DOPA as a precursor for CW melanin production [48]. L-DOPA also induces genes that are involved in stress responses, but the overall effects of the CNS on CW structure are not known.

Antifungal drugs can change the microenvironment that the fungi/host occupies through modulation of the CW and plasma membrane. Echinocandins cause increased chitin levels, which confer echinocandin resistance [49,50]. Treatment of *A. fumigatus* with echinocandins resulted in increased β -glucan exposure and an increased inflammatory response [51,52]. Additionally, biofilm formation in *C. albicans* caused an increase in the secretion of β -glucan into the extracellular matrix, which acts to sequester antifungal drugs [53]. *C. neoformans* treated with the triazole drug fluconazole resulted in increased transcripts of genes involved in plasma membrane and CW synthesis, CW maintenance, and stress [54].

Conclusions

Recent studies have shown the dynamic nature of the fungal CW in response to microenvironments encountered *in vivo*. However, much remains to be learned regarding the significance of these dynamics for the outcome of infections. Studies to investigate the mechanisms underlying these observed *in vivo* dynamics have great promise to answer this important question. Signal transduction pathways regulating CW dynamics and composition are now being identified and characterized building on previous observations identifying a cell wall integrity (CWI) pathway [55]. For *C. neoformans*, RIM101 is involved in responding to host microenvironments and recently was observed to control proper remodeling of the CW and in turn, capsule attachment [56]. Null mutants were determined to cause severe disease because unshielded PAMPs resulted in increased inflammation. Another signaling cascade, the MAP kinase pathway involved in heat shock, was observed to control long term viability and thermotolerance through CW remodeling in *C. albicans* [57]. *C. albicans* also controls cell wall remodeling through the catalytic protein kinase A subunit Tpk1, which has roles in biofilm formation, CW integrity (CWI), and repression of cell surface proteins [58]. Underlying all these CW changes are mechanisms linked to fungal metabolism. Mitochondrial function has recently been linked to CWI, where a mitochondrial outer membrane protein SAM37 is required for proper CW structure and virulence [59]. One important issue that is now being addressed is the importance of strain differences in host responses, where different wild types of the same species have different responses in the host [20]. Ultimately, the complexity of the *in vivo* microenvironments remains a challenge

in defining *in vivo* relevant mechanisms. While, reductionism is critical to understand mechanism, it is clear for example, that host receptor costimulation contributes to disease outcome [60]. What remains a significant but exciting challenge is understanding how microenvironment “fungal costimulation” (aka multiple signal integration) impacts fungal metabolism and CW dynamics and how these responses ultimately affect the outcome of fungal infections. An intriguing idea is to alter specific signals driving CW and fungal metabolic changes that are detrimental to the host through modulation of *in vivo* microenvironments to change the signal to a host beneficial response.

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Highlights

- Fungal cell wall composition and structure are influenced by microenvironments.
- In vivo microenvironments remain to be fully defined.
- How the complex nature of *in vivo* microenvironments is sensed by fungi to alter the cell wall is an emerging area of investigation.
- Fungal signal transduction cascades integrating host derived signals are being identified.
- Manipulation of *in vivo* microenvironment is a potential avenue to improve fungal infection outcomes

Figure 1.

Potential Responses of the Fungal Cell wall to Signals encountered in infection site microenvironments. The diagram presents the various signals that are known to occur *in vivo* during fungal pathogenesis and the potential responses of the fungal cell wall in *Aspergillus fumigatus*, *Candida albicans*, and *Cryptococcus neoformans*. The diagram highlights the complexity of *in vivo* microenvironments and the number of signals that the invading fungus must integrate into the subsequent response. A challenge moving forward is to understand these *in vivo* microenvironments and how signal integration leads to disease outcome.