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Flying under the radar: *Histoplasma capsulatum* avoidance of innate immune recognition

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Abstract

The dimorphic fungal pathogen *Histoplasma capsulatum* takes advantage of the innate immune system, utilizing host macrophages as a proliferative niche while largely avoiding stimulation of signaling host receptors. As a result, innate immune cells are unable to control *H. capsulatum* on their own. Not all host phagocytes respond to *H. capsulatum* in the same way, with neutrophils and dendritic cells playing important roles in impeding fungal growth and initiating a protective T_H1 response, respectively. Dendritic cells prime T-cell differentiation after internalization of yeasts via VLA-5 receptors and subsequent degradation of the yeasts. Dendritic cell-expressed TLR7 and TLR9 promote a type I interferon response for T_H1 polarization. In contrast to dendritic cells, macrophages provide a hospitable intracellular environment. *H. capsulatum* yeasts enter macrophages via binding to phagocytic receptors. Simultaneously, α -glucan masks immunostimulatory cell wall β -glucans and a secreted endoglucanase removes exposed β -glucans to minimize recognition of yeasts by Dectin-1. This review highlights how phagocytes interact with *H. capsulatum* yeasts and the mechanisms *H. capsulatum* uses to limit the innate immune response.

Keywords

fungal pathogenesis; dimorphism; cell wall; β -glucan; Dectin-1; Complement Receptor

1. Introduction

The fungal pathogen *Histoplasma capsulatum* infects both immunocompromised and immunocompetent individuals. Exposure to *H. capsulatum* is common in endemic regions, namely: the Ohio and Mississippi River Valleys in North America, Central America, scattered regions throughout South America, and parts of Africa [1–4]. In these endemic regions, infection occurs via inhalation of airborne conidia. The severity of the disease correlates with the amount of infecting fungal cells inhaled and the effectiveness of the host immune response. Acquisition of small inocula often results in a subclinical, influenza-like

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illness. Inhalation of larger inocula, often resulting from major soil disturbances, building renovations, farming, or spelunking, can lead to acute pulmonary histoplasmosis marked by fever, fatigue, and pneumonia. Severe pulmonary disease may require antifungal treatment to hasten recovery. In the absence of T-cell-mediated immunity, *H. capsulatum* can spread beyond the lungs, causing life-threatening disseminated histoplasmosis [2–4].

H. capsulatum is a dimorphic fungus with temperature serving as the primary cue for differentiation either as a filamentous mycelia or pathogenic yeasts. In the environment, *H. capsulatum* saprotrophic mycelia absorb nutrients from organic matter in soil often contaminated with bird or bat guano. As the mycelia grow, they produce conidia for dispersal [3, 4]. Upon disturbance of the mycelia and release of conidia into the air, the conidia can be inhaled by a mammalian host, and the elevated body temperature triggers differentiation of the conidia into the pathogenic yeasts. The life cycle of *H. capsulatum* does not inherently require infection of a mammalian host; instead, human infections are thought to be accidental, with the resulting mycoses an unfortunate consequence of *H. capsulatum*'s ability to infect and survive as an intracellular pathogen.

Within the lungs, *H. capsulatum* carefully balances infection of host phagocytes while minimizing immune activation. Resident alveolar macrophages recognize and phagocytose conidia and yeasts [5, 6]. Unlike mycelial cells, *H. capsulatum* yeasts exhibit phase-specific virulence strategies to stimulate their phagocytosis by macrophages, combat phagocyte responses and defense mechanisms, and survive within these immune cells [7–10]. These yeast-phase-specific adaptations allow *H. capsulatum* to thwart the innate immune system, and without T-cell-activation of phagocytes, *H. capsulatum* yeasts proliferate unabated.

As a primary pathogen, *H. capsulatum* infects host cells without eliciting the same antifungal responses as opportunistic fungal pathogens, rendering innate immune cells insufficient to control *H. capsulatum* infections. *H. capsulatum* interacts with a variety of phagocytes in the mammalian host including macrophages, neutrophils, and dendritic cells (Figure 1), and the population of immune cells interacting with *H. capsulatum* evolves over the course of infection [5, 11]. This review examines the interactions between *H. capsulatum* and cells of the innate immune system, focusing on how *H. capsulatum* avoids stimulating immune responses and how the host eventually succeeds in recognizing *H. capsulatum* yeasts.

2. *H. capsulatum* yeasts promote phagocytic uptake by host cells

Many pathogens can proliferate intracellularly to reduce exposure to elements of the host's immune system, but few fungal pathogens take full advantage of this lifestyle. For example, phagocytosis of *Candida albicans* yeasts by macrophages in culture leads to transition of yeasts to a filamentous form which lyses the macrophage, releasing the *Candida* cells via pyroptosis, although whether this occurs *in vivo* is unclear [12–14]. *Cryptococcus neoformans*, like *H. capsulatum*, is capable of growth and division within host macrophages. However, *C. neoformans* also expresses multiple mechanisms to prevent phagocytosis, including production of a polysaccharide capsule that deters non-opsonic phagocytosis [15, 16], secretion of the antiphagocytic protein App1p [17], and formation of titan cells which

resist phagocytosis through their large size [18]. In addition, *C. neoformans* induces non-lytic exocytosis to escape from host cells, though the mechanisms by which this is achieved remain unclear [19]. Together, these mechanisms suggest that the intracellular niche is possible but only temporary for *C. neoformans*. On the other hand, *H. capsulatum* yeasts prefer existence within phagocytes [4, 11], which provide them with a secluded environment for growth as well as a vehicle for extrapulmonary dissemination. Alveolar macrophages ingest *H. capsulatum* conidia and unopsonized yeast cells within 10 minutes of exposure, averaging 120 yeasts or 70 microconidia ingested per 100 macrophages within an hour [6]. Consequently over the course of infection, yeasts are primarily intracellular and only rarely observed outside of host cells *in vivo* [4, 11]. To achieve this predominantly intracellular lifestyle, *H. capsulatum* exploits host cell phagocytosis mechanisms.

2.1. Macrophage uptake: Complement Receptors (CR)

H. capsulatum cells utilize complement receptor-mediated phagocytosis to gain entry into macrophages and neutrophils. Lung infection initially involves uptake of *H. capsulatum* by alveolar and inflammatory macrophages [11]. Both yeasts and conidia adhere to macrophages primarily via CD11/CD18 family leukocyte integrins (LFA-1, and the complement receptors CR3 and CR4; Figure 1), as blocking the integrins' common β -chain CD18 using antibodies substantially decreases both attachment to macrophages and subsequent phagocytosis of *H. capsulatum* cells [6, 20]. Obstruction of the individual α -chain glycoproteins (i.e., CD11a, CD11b, and CD11c) partially inhibits yeast adherence, indicative of redundancy in complement receptor function for *H. capsulatum* uptake [6, 20]. Macrophage phagocytosis of *H. capsulatum* requires actin microfilaments, but not polymerization of microtubules, suggesting that *H. capsulatum* stimulates phagocytosis in a manner separate from that of particles opsonized with iC3b, which require microtubule polymerization for uptake [6]. Consistent with this, phagocytosis of *H. capsulatum* yeasts by macrophages does not require opsonization by C3b, iC3b, or antibodies [20]. Blocking CD18-family receptors, but not Fc γ R or mannose-type receptors, prevents the majority of *H. capsulatum* uptake, suggesting that the CD18-family receptors are the primary factor mediating *H. capsulatum* internalization into macrophages [20]. Expression of CR3 in Chinese hamster ovary cells, a cell line lacking CD18-family integrins, enables binding of *H. capsulatum*, demonstrating that CR3 alone is sufficient for attachment of *H. capsulatum* yeasts [21]. The individual affinities of the other CD18-family integrins to *H. capsulatum* have not yet been determined.

To facilitate phagocytosis, *H. capsulatum* expresses at least one ligand that can bind to CD18 family receptors. Of the CD11/CD18 integrins, only CR3 (CD11b/CD18, also called Mac-1) has been studied at length in its relationship to *H. capsulatum*. Screening of *H. capsulatum* cell wall/membrane protein preparations for CR3 binding identified a single 60 kDa protein, heat shock protein 60 (Hsp60) [21]. Although Hsp60 is primarily a cytoplasmic chaperonin protein, a small amount localizes to the cell surface [21]. Recombinant Hsp60p as well as certain regions of the human Hsp60 protein competitively block attachment of *H. capsulatum* to macrophages [21, 22], showing Hsp60 is necessary for binding to macrophages. In addition, vaccination with Hsp60 protects mice against *H. capsulatum* challenge [23], and antibodies to *H. capsulatum* Hsp60 opsonize *H. capsulatum* yeasts [23],

confirming surface localization of at least some Hsp60. Polystyrene beads coated with *H. capsulatum* Hsp60 bind to macrophages in a CR3-dependent manner, indicating Hsp60 is sufficient to mediate attachment to macrophages [21]. While Hsp60 promotes binding of *H. capsulatum* to macrophages (Figure 1), it does not rule out the participation of other *H. capsulatum* surface proteins that may also contribute to adhesion to macrophage cells.

Binding to CR3 not only facilitates adhesion to macrophages, but as CR3 is a phagocytic receptor, it also serves as an attractive portal of entry for *H. capsulatum*. Complement-receptor-mediated phagocytosis is typically non-inflammatory when not accompanied by other stimulating signals [24]. This may result from lack of cytokine stimulation or active inhibition of cytokine production. In support of the latter, monocytes exposed to heat-killed *H. capsulatum* yeasts have reduced IL-12 production when subsequently stimulated with lipopolysaccharide [25]. Suppression of IL-12 likely contributes to *H. capsulatum* virulence since IL-12 promotes cell-mediated immunity and IFN- γ production, which are crucial for limiting *H. capsulatum* growth in the host [26]. It is worth noting that *H. capsulatum* does not primarily interact with monocytes during an initial infection, and it is unknown whether this dampening effect occurs in other phagocyte types or *in vivo*. Different populations of macrophages vary in their expression levels of LFA-1, CR3, and CR4 component integrins [27–29], which could potentially affect the binding and internalization of *H. capsulatum* cells, especially if these receptors have differing affinities, but this is yet to be determined. Nonetheless, interaction of *H. capsulatum* Hsp60 with CR3 provides *H. capsulatum* with an efficient means to trigger phagocytosis by macrophages.

2.2. Dendritic cell phagocytosis: Very Late Antigen-5 (VLA-5)

In addition to macrophages, *H. capsulatum* also encounters dendritic cells (DCs) in the lung [11]. However, DCs prove less hospitable to *H. capsulatum* yeasts than macrophages. In contrast to yeasts taken up by unactivated macrophages, yeasts ingested by cultured DCs do not proliferate well, and visible degradation of yeasts within DCs has been reported within 2 hours of internalization [30]. DCs are responsible for presenting antigens recovered from the degraded yeasts to lymphocytes of the adaptive immune system and initiating a protective T_H1 immune response. Supporting this, DCs which have phagocytosed *H. capsulatum* yeasts stimulate proliferation of T cells [30]. Interestingly, DCs which phagocytose viable yeasts stimulate more robust T-cell proliferation than that of DCs with heat-killed yeast, suggesting more potent antigen presentation [30].

How are DCs able to kill *H. capsulatum* yeast, whereas macrophages are permissive for yeast replication? One possible reason is that DCs recognize and phagocytose *H. capsulatum* cells primarily through a different pathway. Although DCs also express CD18 complex glycoproteins (CD11c/CD18 in particular), antibodies against CD18 do not block *H. capsulatum* attachment to DCs, nor does recombinant Hsp60 inhibit *H. capsulatum* adherence to DCs as it does with macrophages [21, 30]. Instead, DCs bind *H. capsulatum* via the fibronectin receptor known as very late antigen-5 (VLA-5) [30]. The corresponding ligand on *H. capsulatum* yeasts appears to be the 20 kDa protein cyclophilin A (CypA), which was identified by screening *H. capsulatum* freeze-thaw extract with purified VLA-5 [31]. Excess recombinant CypA inhibits binding of *H. capsulatum* to DCs but not to

macrophages, suggesting a specific ligand-receptor interaction [31]. However, since both macrophages and DCs express VLA-5, CypA-coated polystyrene beads also bind to macrophages [31]. Thus, macrophages and DCs appear to recognize *H. capsulatum* yeasts via separate pathways, despite both phagocytic cell types expressing CD18-family integrins and VLA-5 (Figure 1). The reasons for this differential interaction of *H. capsulatum* between macrophages and DCs as well as the divergent intracellular outcomes for phagocytosed yeasts remain unknown.

2.3. Role of neutrophils

Neutrophils (PMNs) also interact with *H. capsulatum* as part of the innate immune response, but these phagocytes can only achieve fungistatic control. *In vivo*, PMNs localize to *H. capsulatum*-infected lungs early during *H. capsulatum* infection [5, 32, 33] and *H. capsulatum* yeasts can be found within PMNs [11]. While the extent of PMN non-opsonic ligand-receptor interactions with *H. capsulatum* has not been fully defined, PMNs can bind opsonized *H. capsulatum* via a variety of opsonin-related receptors, including CR1 (complement receptor 1, CD35), CR3, and Fc γ RIII (CD16) in an apparently additive fashion [34]. Opsonization of *H. capsulatum* yeasts by serum is necessary for ingestion of yeasts by PMNs. However, uptake of yeasts is not required for the fungistatic effects of PMNs since inhibition of yeasts occurs even when phagocytosis is inhibited by treatment with cytochalasin D [34]. This evidence suggests that the fungistatic effector can be exported out of the PMN cell (Figure 1). Although interaction of PMNs with *H. capsulatum* yeasts can stimulate the PMN respiratory burst [35], reactive oxygen species (ROS) are not required for the fungistatic effect; PMNs from patients with chronic granulomatous disease that lack superoxide production control *H. capsulatum* yeasts as well as PMNs from healthy patients [34]. It is unsurprising that ROS are dispensable for fungistatic effect, as *H. capsulatum* yeasts express efficient ROS-neutralization factors such as catalase and superoxide dismutase to eliminate ROS produced by both PMNs and macrophages [35, 36]. PMN-dependent fungistasis is instead mediated through azurophilic granule contents. These PMN factors include cathepsin G, bactericidal-permeability-increasing protein (BPI), and the human neutrophil defensins HNP-1, HNP-2, and HNP-3, all of which can restrict *H. capsulatum* growth *in vitro* in a dose-dependent manner [37]. Cathepsin G is a protease with specificities similar to chymotrypsin [38] and additionally contains short polypeptide sequences which have demonstrated antimicrobial activity against bacteria via a mechanism independent of proteolytic activity, though the enzyme's antifungal mechanism is uncharacterized [39]. BPI contains two biochemically distinct halves joined by a proline-rich linker region [40]. The N-terminal half, which is Lysine-rich and highly cationic, is known to associate strongly with LPS in gram-negative bacteria and cause membrane disruption [41]. However, as fungal cells lack LPS, further work is needed to elucidate the antifungal activity of BPI. The human neutrophil defensins are thought to inhibit microbial growth via non-lytic membrane disruption, which has been demonstrated in *Candida albicans* with HNP-1 [42]. While PMNs cannot eliminate *H. capsulatum* yeasts, their fungistatic activity and uptake of opsonized yeasts may play an important role in delaying yeast proliferation until adaptive immunity can be activated.

3. Recognition of *H. capsulatum* yeasts by signaling PRRs

Although *H. capsulatum* cells engage host phagocytic receptors to initiate phagocytosis, they must do so without being recognized by signaling pattern recognition receptors (PRRs) which would stimulate immune responses. Mammalian cells carry a variety of PRRs capable of recognizing fungal antigens, especially polysaccharides of the fungal cell wall. Both Toll-like receptors (TLRs) and C-type lectin receptors have been connected to *H. capsulatum* detection. However, it is worth noting that the pathogen-associated molecular patterns (PAMPs) of *H. capsulatum* are recognized to a lesser degree than those of opportunistic fungal pathogens. This partially explains the ineffectiveness of innate cells alone in controlling Histoplasma despite their efficient control of opportunistic fungal pathogens.

3.1. Yeast recognition by TLR2, TLR7, and TLR9

TLRs provide a powerful means for host cells to discriminate between classes of pathogens [43]. While several TLRs have been found to recognize fungal PAMPs [44], few have been shown to detect *H. capsulatum* cells. TLR2 was the first TLR documented to bind and trigger host responses to *H. capsulatum*. The ligand recognized by TLR2 appears to be a yeast-phase-specific surface protein, Yps3 [45], which is only produced by some strains of *H. capsulatum* [46]. Purified Yps3 induces expression of an NF- κ B-driven luciferase reporter in both TLR2-expressing cell lines and primary murine microglial cells, but not in microglial cells extracted from TLR2 knockout mice [45]. These results suggest that *H. capsulatum* Yps3p is recognized by TLR2. However, these tests have not been replicated with lung phagocytic cells or with *in vivo* models of respiratory histoplasmosis. TLR2 can also cooperate with CD18 and Dectin-1 in the formation of lipid bodies, which are correlated with generation of proinflammatory leukotriene B₄ [33]. Higher levels of leukotrienes are correlated with better host control of Histoplasma [47]. However, these responses were triggered by β -glucan-containing cell wall fractions, and the true level of PAMP exposure on intact cells is likely greatly reduced compared to extracted wall constituents (see below). Together these data suggest some potential recognition of *H. capsulatum* yeasts by TLR2 with Yps3 as one ligand (Figure 1), but the precise contribution of TLR2 to host recognition and control of *H. capsulatum in vivo* will require infection studies in TLR2 knockout mice. Interestingly, global loss of the adaptor protein MyD88, through which most TLRs (including TLR2) signal, impairs host control of *H. capsulatum*, suggesting some TLR recognition of yeasts. Without MyD88, proinflammatory cytokine production by lung macrophages and DCs is reduced [48]. Importantly, the loss of MyD88-dependent signaling in lung macrophages and DCs alone does not significantly curtail *H. capsulatum* proliferation in lungs, suggesting the involvement of TLR signaling in other cell types [48].

Full DC responses to *H. capsulatum* involve TLR7 and TLR9. These two Toll-like receptors are canonical PRRs for viral and bacterial nucleic acids (recognizing ssRNA and CpG DNA or RNA:DNA hybrids, respectively). Recently, it was shown that conventional DCs require TLR7 and TLR9 in order to mount an effective type I interferon (IFN-I) response to *H. capsulatum* yeasts [49]. TLR7/9 knockout mice begin to succumb to a normally sublethal infection with *H. capsulatum* around 11 days post infection [49]. Loss of both TLR7 and

TLR9 causes a mild increase in the number of *H. capsulatum* yeasts in the lungs and spleens, but only at two weeks post-infection [49]. This suggests that DC production of type I interferons contributes little during the innate immune response (e.g., fewer than 10 days-post-infection with *H. capsulatum* in mice) but instead influences the host's ability to mount a protective T_H1 response to *H. capsulatum* infection. Given the established ligands of TLR7 and TLR9, destruction of *H. capsulatum* yeasts by DCs and the resultant release of yeast nucleic acids may serve to further direct DCs to prime host protective adaptive responses. However, whether DC killing of *H. capsulatum* is necessary to stimulate TLR7 and TLR9 is unknown.

3.2. C-type lectins

The C-type lectins Dectin-1, Dectin-2, and Mincle are key receptors for immune recognition of fungal polysaccharides and activation of phagocyte responses. Dectin-1 recognizes β -glucans, which are a near-universal constituent of fungal cell walls, while Dectin-2 and Mincle recognize mannans, especially those found on N- and O-linked glycans of surface proteins [50–52]. Of these three receptors, only Dectin-1 and Dectin-2 recognize and respond to *H. capsulatum* [53], but their contribution to host defenses against *Histoplasma* differs by phagocyte type.

DCs express both Dectin-1 and Dectin-2. In DCs, Dectin-2 recognition of *H. capsulatum* leads to increased production of proinflammatory IL-1 family cytokines (Figure 1). Dectin-2 interaction with *H. capsulatum* yeasts activates caspase-1 and inflammasome activation, leading to release of IL-1 [54]. Upon exposure to *H. capsulatum* yeast, Dectin-2 appears to provide signals for both pro-IL-1 β synthesis and stimulation of the NLRP3-inflammasome for release of activated IL-1 β [54]. Dectin-1 is dispensable for inflammasome activation but can provide some signaling in the absence of Dectin-2 [54]. These data confirm that DC-expressed Dectin-2 can recognize *H. capsulatum* yeasts to enhance pro-inflammatory responses. However, *in vivo*, the loss of Dectin-2 or Mincle has no effect on the progression of fungal infection [53], arguing there is relatively minimal contribution by Dectin-2 for control of *H. capsulatum* infections.

In macrophages, which are more permissive for *H. capsulatum* yeasts, C-type lectins play a different role. Macrophages stimulated with heat-killed or live *H. capsulatum* yeasts produce IL-6 and TNF- α [55–57]. Both Dectin-1 and CR3 co-localize in lipid rafts, and each can contribute to stimulation of cytokine production [56], although CR3 stimulation is thought to produce lower cytokine responses [24]. Unlike CR3, however, Dectin-1 plays no role in phagocytosis of yeast, as neither antibody blocking nor competitive inhibition by exogenous β -glucans affects macrophages' ability to engulf *H. capsulatum* [55]. Despite some recognition of *H. capsulatum* by Dectin-1 *in vitro*, the loss of Dectin-1 in mice does not cause a dramatic change in *H. capsulatum* proliferation *in vivo*. Dectin-1 knockout mice show only a mild increase in pulmonary fungal burdens, if at all [53, 56–58] suggesting that Dectin-1 does not play a large role in the immune response to *H. capsulatum*.

4. *H. capsulatum* yeasts minimize detection by Dectin-1

The lack of significant changes to fungal proliferation in Dectin-1 and Dectin-2 knockout mice, in contrast to the data with cultured DCs and macrophages, suggests there is minimal recognition of *H. capsulatum* cells by C-type lectin receptors *in vivo*. In fact, *H. capsulatum* has multiple ways of minimizing detection by host β -glucan receptors, and these strategies involve manipulation of the fungal cell wall.

4.1. The *H. capsulatum* yeast cell wall

The composition and organization of the cell wall of *H. capsulatum* yeasts markedly differs from the avirulent mycelial cell wall. Yeast cell walls are thicker, richer in chitin, and contain less mannose and amino acids than their mycelial counterparts [59]. Early studies found two distinct yeast chemotypes among *H. capsulatum* strains [60] which corresponded to yeast serotypes found in histoplasmosis patients [61] and specific phylogeographic clades [62]. Chemotype I yeast (corresponding to North American type 2 strains; Nam2) contain a higher percentage of chitin than chemotype II cell walls [61]. In contrast to chemotype I yeasts, the cell walls of chemotype II yeasts contain an additional polysaccharide, α -(1,3)-glucan [61]. These two chemotypes also exhibit different infection kinetics in a high-dose mouse model of histoplasmosis [63]. Chemotype II strains, which contain α -(1,3)-glucan, kill macrophages faster and result in a shorter course of infection [63], whereas chemotype I strains that lack α -(1,3)-glucan, although slower at killing macrophages, reach higher fungal burdens overall [63]. Interestingly, both chemotypes have mechanisms to evade detection by Dectin-1.

4.2. α -(1,3)-glucan masks cell wall β -(1,3)-glucans

In chemotype II strains, synthesis of α -(1,3)-glucan is essential for masking β -glucans from Dectin-1 (Figure 1). The first indicator that α -(1,3)-glucan could obscure cell wall features came from assays in which yeast exposed to α -glucanase reacted more strongly with goat antiserum than untreated cells [64]. Spontaneous conversion of yeasts during laboratory culture to chemotype I, which lacks α -(1,3)-glucan, results in strains that are unable to kill macrophages as efficiently [65–67]. *H. capsulatum* yeasts genetically engineered to lack α -(1,3)-glucan due to depletion or deletion of *Ags1* (α -glucan synthase) are severely attenuated in their ability to establish pulmonary infections in the lungs *in vivo* [68]. Immunofluorescent localization of α - and β -glucans revealed that α -(1,3)-glucan forms a polysaccharide layer on top of the β -glucans, which spatially covers the underlying β -glucans [69]. Consistent with this model, yeasts deficient in α -glucan synthesis are recognized to a greater degree by Dectin-1 and stimulate greater cytokine production from macrophages than yeasts with α -(1,3)-glucan [69]. Additionally, phagocytes depleted of Dectin-1 are impaired in their ability to produce TNF- α when exposed to α -glucan-deficient yeasts, confirming Dectin-1 mediates recognition of yeast β -glucans—but only when yeasts lack the α -glucan mask. Intriguingly, depletion of *Ags1* in chemotype I yeasts that naturally lack α -glucan results in no virulence defect, indicating chemotype I yeasts do not rely on α -glucan to obscure β -glucans [70].

4.3. The secreted Eng1 endoglucanase trims exposed β -(1,3)-glucans

In addition to masking of β -glucans, *H. capsulatum* yeasts further reduce recognition of β -glucans by enzymatically removing exposed polysaccharides. Examination of the secreted proteome of pathogenic-phase *H. capsulatum* yeasts identified the Eng1 endoglucanase [71]. Eng1 is a yeast-phase extracellular glucanase with specificity for β -(1,3)-glycosidic linkages [72], implying it could modify β -glucan constituents of the cell wall. Rapidly dividing yeasts have some native β -glucan exposure, but depletion of Eng1 causes increased amounts of surface-exposed β -glucans, which in turn leads to greater Dectin-1-dependent recognition of yeasts [57]. Thus, Eng1 prunes β -glucans from the yeast surface, resulting in reduced Dectin-1 recognition of yeasts and reduced cytokine production by phagocytes (Figure 1). Accordingly, loss of Eng1 decreases fungal burdens in the lung, which are restored in mice lacking the Dectin-1 receptor [57].

Both α -glucan and Eng1 effectively obscure β -glucans from recognition by Dectin-1. Chemotype II strains, which have α -(1,3)-glucan, show a minor increase in Dectin-1 binding when Eng1 is depleted, and this becomes more pronounced when both Eng1 and α -glucan are lost [57]. This indicates that in chemotype II strains, α -glucan and Eng1 have additive effects on reducing β -glucan exposure, with the α -glucan mask providing the major contribution. In both chemotypes, Eng1 facilitates Dectin-1 avoidance by minimizing any exposed β -glucan chains. These data highlight the significant efforts expended by *H. capsulatum* yeasts to minimize their recognition by Dectin-1 receptors.

Concealment versus immune detection does not exist as an all-or-none state in the host, but rather as a spectrum from low to high. As such, the degree of immune response to *H. capsulatum* yeasts reflects the sum of both recognition of yeasts through host PRRs and the degree to which yeast PAMPs remain hidden. Masking of β -glucan by α -glucan is not perfect; thus, the pruning activity of the Eng1p endoglucanase provides additional protection against Dectin-1 recognition. As such, when considering immune detection of fungi, it is helpful to keep the magnitude of the response in context. For example, mutant *H. capsulatum* yeasts with increased β -glucan exposure (either through loss of α -glucan or loss of Eng1) stimulate significant cytokine responses from macrophages. The magnitude of these responses to mutant *H. capsulatum* yeasts is similar to that of the response triggered by the interaction of macrophages with wild-type *Candida albicans* [57, 69]. Thus, although there can be some detection of *H. capsulatum* yeasts through Dectin-1 and other PRRs, it is substantially less than the degree of immune recognition of opportunistic fungal pathogens. Similarly, although interactions between *H. capsulatum* and cultured phagocytes might indicate PRR recognition of yeasts, the lack of substantially increased virulence in *in vivo* studies with PRR-deficient mice underscores the relatively minor degree of Dectin-1 and Dectin-2 recognition of *H. capsulatum* yeasts [53, 56–58, 69].

5. Conclusions

H. capsulatum is remarkable among fungal pathogens in its ability to utilize innate immune cells for its own proliferation while simultaneously minimizing the innate immune response from these host cells. To achieve the first task of internalization by phagocytes, conidia and yeasts exploit phagocytic receptors such as the CD18 integrin family of receptors.

Simultaneously, *H. capsulatum* obscures itself from immune signaling receptors like Dectin-1 by masking its immunostimulatory β -glucans with a non-stimulatory α -(1,3)-glucan layer and by trimming extraneous β -glucan with secreted endonucleases. Once it has achieved internalization, other virulence factors of *H. capsulatum* transform the macrophage phagolysosome into a safe environment for proliferation, thereby isolating the yeast from detection by other immune cells [73].

Fortunately for the host, an infection of *H. capsulatum* does not go completely unnoticed. Before the onset of cell-mediated immunity, most *H. capsulatum* yeasts are minimally recognized and proliferate readily within unactivated macrophages. Some yeasts, however, are encountered by other, less permissive immune cells with their own recognition pathways (Figure 1): neutrophils can inhibit *H. capsulatum* growth through release of azurophilic granule contents, and DCs phagocytize and kill *H. capsulatum* yeast, thereby recovering presentable antigens. Through the combination of antigen presentation and release of critical cytokines such as IL-12 and type I interferons, DCs stimulate T-cell differentiation and a protective T_H1 immune response. Only then, through subsequent activation of phagocytes, can the host finally control infections by *H. capsulatum*. Thus, stealthy yeasts dominate during the initial stages of infection, but DC recognition of *H. capsulatum*—and the ensuing activation of T-cells—converts the macrophage host from a permissive niche into one that controls *H. capsulatum* yeasts.

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References

1. Edwards LB, Acquaviva FA, Livesay VT, Cross FW, Palmer CE. An atlas of sensitivity to tuberculin, PPD-B, and histoplasmin in the United States. American Review of Respiratory Disease. 99(4 Pt 2)1969; :132.
2. Ajello L. The medical mycological iceberg. HSMHA Health Rep. 86(5)1971; :437–48. [PubMed: 5089309]
3. Kauffman CA. Histoplasmosis: a clinical and laboratory update. Clin Microbiol Rev. 20(1)2007; : 115–32. [PubMed: 17223625]
4. Goodwin RA, Loyd JE, Des Prez RM. Histoplasmosis in normal hosts. Medicine (Baltimore). 60(4)1981; :231–66. [PubMed: 7017339]
5. Baughman RP, Kim CK, Vinegar A, Hendricks DE, Schmidt DJ, Bullock WE. The pathogenesis of experimental pulmonary histoplasmosis. Correlative studies of histopathology, bronchoalveolar lavage, and respiratory function. Am Rev Respir Dis. 134(4)1986; :771–6. [PubMed: 3767131]
6. Newman SL, Bucher C, Rhodes J, Bullock WE. Phagocytosis of Histoplasma capsulatum yeasts and microconidia by human cultured macrophages and alveolar macrophages. Cellular cytoskeleton requirement for attachment and ingestion. The Journal of clinical investigation. 85(1)1990; :223–30. [PubMed: 2104879]
7. Nguyen VQ, Sil A. Temperature-induced switch to the pathogenic yeast form of Histoplasma capsulatum requires Ryp1, a conserved transcriptional regulator. Proc Natl Acad Sci U S A. 105(12)2008; :4880–5. [PubMed: 18339808]
8. Webster RH, Sil A. Conserved factors Ryp2 and Ryp3 control cell morphology and infectious spore formation in the fungal pathogen Histoplasma capsulatum. Proc Natl Acad Sci U S A. 105(38)2008; :14573–8. [PubMed: 18791067]

9. Garfoot AL, Rappleye CA. Histoplasma capsulatum surmounts obstacles to intracellular pathogenesis. *FEBS Journal*. 283(4)2016; :619–633. [PubMed: 26235362]
10. Holbrook ED, Rappleye CA. Histoplasma capsulatum pathogenesis: making a lifestyle switch. *Curr Opin Microbiol*. 11(4)2008; :318–24. [PubMed: 18573684]
11. Deepe GS Jr, Gibbons RS, Smulian AG. Histoplasma capsulatum manifests preferential invasion of phagocytic subpopulations in murine lungs. *Journal of leukocyte biology*. 84(3)2008; :669–78. [PubMed: 18577715]
12. Brothers KM, Newman ZR, Wheeler RT. Live imaging of disseminated candidiasis in zebrafish reveals role of phagocyte oxidase in limiting filamentous growth. *Eukaryot Cell*. 10(7)2011; :932–44. [PubMed: 21551247]
13. Brothers KM, Gratacap RL, Barker SE, Newman ZR, Norum A, Wheeler RT. NADPH oxidase-driven phagocyte recruitment controls *Candida albicans* filamentous growth and prevents mortality. *PLoS Pathog*. 9(10)2013; :e1003634. [PubMed: 24098114]
14. Wellington M, Koselny K, Sutterwala FS, Krysan DJ. *Candida albicans* triggers NLRP3-mediated pyroptosis in macrophages. *Eukaryot Cell*. 13(2)2014; :329–40. [PubMed: 24376002]
15. Bulmer GS, Sans MD. *Cryptococcus neoformans*. 3. Inhibition of phagocytosis. *Journal of bacteriology*. 95(1)1968; :5–8. [PubMed: 4866104]
16. Kozel TR, Mastroianni RP. Inhibition of phagocytosis by cryptococcal polysaccharide: dissociation of the attachment and ingestion phases of phagocytosis. *Infect Immun*. 14(1)1976; :62–7. [PubMed: 780279]
17. Luberto C, Martinez-Marino B, Taraskiewicz D, Bolanos B, Chitano P, Toffaletti DL, Cox GM, Perfect JR, Hannun YA, Balish E, Del Poeta M. Identification of App1 as a regulator of phagocytosis and virulence of *Cryptococcus neoformans*. *The Journal of clinical investigation*. 112(7)2003; :1080–94. [PubMed: 14523045]
18. Okagaki LH, Nielsen K. Titan cells confer protection from phagocytosis in *Cryptococcus neoformans* infections. *Eukaryot Cell*. 11(6)2012; :820–6. [PubMed: 22544904]
19. Johnston SA, May RC. The human fungal pathogen *Cryptococcus neoformans* escapes macrophages by a phagosome emptying mechanism that is inhibited by Arp2/3 complex-mediated actin polymerisation. *PLoS Pathog*. 6(8)2010; :e1001041. [PubMed: 20714349]
20. Bullock WE, Wright SD. Role of the adherence-promoting receptors, CR3, LFA-1, and p150,95, in binding of *Histoplasma capsulatum* by human macrophages. *The Journal of experimental medicine*. 165(1)1987; :195–210. [PubMed: 3025331]
21. Long KH, Gomez FJ, Morris RE, Newman SL. Identification of heat shock protein 60 as the ligand on *Histoplasma capsulatum* that mediates binding to CD18 receptors on human macrophages. *Journal of immunology (Baltimore, Md: 1950)*. 170(1)2003; :487–94.
22. Habich C, Kempe K, Gomez FJ, Lillicrap M, Gaston H, van der Zee R, Kolb H, Burkart V. Heat shock protein 60: identification of specific epitopes for binding to primary macrophages. *FEBS Lett*. 580(1)2006; :115–20. [PubMed: 16343489]
23. Guimaraes AJ, Frases S, Gomez FJ, Zancoppe-Oliveira RM, Nosanchuk JD. Monoclonal antibodies to heat shock protein 60 alter the pathogenesis of *Histoplasma capsulatum*. *Infect Immun*. 77(4)2009; :1357–67. [PubMed: 19179416]
24. Aderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. *Annu Rev Immunol*. 171999; :593–623. [PubMed: 10358769]
25. Marth T, Kelsall BL. Regulation of interleukin-12 by complement receptor 3 signaling. *The Journal of experimental medicine*. 185(11)1997; :1987–95. [PubMed: 9166428]
26. Allendoerfer R, Deepe GS Jr. Intrapulmonary response to *Histoplasma capsulatum* in gamma interferon knockout mice. *Infect Immun*. 65(7)1997; :2564–9. [PubMed: 9199420]
27. Misharin AV, Morales-Nebreda L, Mutlu GM, Budinger GR, Perlman H. Flow cytometric analysis of macrophages and dendritic cell subsets in the mouse lung. *Am J Respir Cell Mol Biol*. 49(4)2013; :503–10. [PubMed: 23672262]
28. Hashimoto D, Miller J, Merad M. Dendritic cell and macrophage heterogeneity in vivo. *Immunity*. 35(3)2011; :323–35. [PubMed: 21943488]

29. Becher B, Schlitzer A, Chen J, Mair F, Sumatoh HR, Teng KW, Low D, Ruedl C, Riccardi-Castagnoli P, Poidinger M, Greter M, Ginhoux F, Newell EW. High-dimensional analysis of the murine myeloid cell system. *Nat Immunol.* 15(12)2014; :1181–9. [PubMed: 25306126]
30. Gildea LA, Morris RE, Newman SL. Histoplasma capsulatum yeasts are phagocytosed via very late antigen-5, killed, and processed for antigen presentation by human dendritic cells. *Journal of immunology (Baltimore, Md: 1950).* 166(2)2001; :1049–56.
31. Gomez FJ, Pilcher-Roberts R, Alborzi A, Newman SL. Histoplasma capsulatum cyclophilin A mediates attachment to dendritic cell VLA-5. *Journal of immunology (Baltimore, Md: 1950).* 181(10)2008; :7106–14.
32. Procknow JJ, Page MI, Loosli CG. Early pathogenesis of experimental histoplasmosis. *Arch Pathol.* 69(1960); :413–26. [PubMed: 14434993]
33. Sorgi CA, Secatto A, Fontanari C, Turato WM, Belanger C, de Medeiros AI, Kashima S, Marleau S, Covas DT, Bozza PT, Faccioli LH. Histoplasma capsulatum cell wall {beta}-glucan induces lipid body formation through CD18, TLR2, and dectin-1 receptors: correlation with leukotriene B4 generation and role in HIV-1 infection. *Journal of immunology (Baltimore, Md: 1950).* 182(7)2009; :4025–35.
34. Newman SL, Gootee L, Gabay JE. Human neutrophil-mediated fungistasis against Histoplasma capsulatum. Localization of fungistatic activity to the azurophil granules. *The Journal of clinical investigation.* 92(2)1993; :624–31. [PubMed: 8349801]
35. Youseff BH, Holbrook ED, Smolnycki KA, Rappleye CA. Extracellular superoxide dismutase protects Histoplasma yeast cells from host-derived oxidative stress. *PLoS Pathog.* 8(5)2012; :e1002713. [PubMed: 22615571]
36. Holbrook ED, Smolnycki KA, Youseff BH, Rappleye CA. Redundant catalases detoxify phagocyte reactive oxygen and facilitate Histoplasma capsulatum pathogenesis. *Infection and Immunity.* 81(7)2013; :2116–24.
37. Newman SL, Gootee L, Gabay JE, Selsted ME. Identification of constituents of human neutrophil azurophil granules that mediate fungistasis against Histoplasma capsulatum. *Infect Immun.* 68(10)2000; :5668–72. [PubMed: 10992469]
38. Tanaka T, Minematsu Y, Reilly CF, Travis J, Powers JC. Human leukocyte cathepsin G. Subsite mapping with 4-nitroanilides, chemical modification, and effect of possible cofactors. *Biochemistry.* 24(8)1985; :2040–7. [PubMed: 4016099]
39. Bangalore N, Travis J, Onunka VC, Pohl J, Shafer WM. Identification of the primary antimicrobial domains in human neutrophil cathepsin G. *J Biol Chem.* 265(23)1990; :13584–8. [PubMed: 2116408]
40. Beamer LJ, Carroll SF, Eisenberg D. Crystal structure of human BPI and two bound phospholipids at 2.4 angstrom resolution. *Science.* 276(5320)1997; :1861–4. [PubMed: 9188532]
41. Ooi CE, Weiss J, Elsbach P, Frangione B, Mannion B. A 25-kDa NH2-terminal fragment carries all the antibacterial activities of the human neutrophil 60-kDa bactericidal/permeability-increasing protein. *J Biol Chem.* 262(31)1987; :14891–4. [PubMed: 3667613]
42. Edgerton M, Koshlukova SE, Araujo MW, Patel RC, Dong J, Bruenn JA. Salivary histatin 5 and human neutrophil defensin 1 kill Candida albicans via shared pathways. *Antimicrob Agents Chemother.* 44(12)2000; :3310–6. [PubMed: 11083633]
43. Aderem A. Phagocytosis and the inflammatory response. *J Infect Dis.* 187(Suppl 2)2003; :S340–5. [PubMed: 12792849]
44. Netea MG, Van der Graaf C, Van der Meer JW, Kullberg BJ. Recognition of fungal pathogens by Toll-like receptors. *Eur J Clin Microbiol Infect Dis.* 23(9)2004; :672–6. [PubMed: 15322932]
45. Aravalli RN, Hu S, Woods JP, Lokensgard JR. Histoplasma capsulatum yeast phase-specific protein Yps3p induces Toll-like receptor 2 signaling. *Journal of neuroinflammation.* 52008; :30. [PubMed: 18606009]
46. Bohse ML, Woods JP. Expression and interstrain variability of the YPS3 gene of Histoplasma capsulatum. *Eukaryot Cell.* 6(4)2007; :609–15. [PubMed: 17337639]
47. Medeiros AI, Sa-Nunes A, Soares EG, Peres CM, Silva CL, Faccioli LH. Blockade of endogenous leukotrienes exacerbates pulmonary histoplasmosis. *Infect Immun.* 72(3)2004; :1637–44. [PubMed: 14977971]

48. Coady A, Sil A. MyD88-dependent signaling drives host survival and early cytokine production during *Histoplasma capsulatum* infection. *Infect Immun*. 83(4)2015; :1265–75. [PubMed: 25583527]
49. Van Prooyen N, Henderson CA, Hocking Murray D, Sil A. CD103+ Conventional Dendritic Cells Are Critical for TLR7/9-Dependent Host Defense against *Histoplasma capsulatum*, an Endemic Fungal Pathogen of Humans. *PLoS Pathog*. 12(7)2016; :e1005749. [PubMed: 27459510]
50. Herre J, Gordon S, Brown GD. Dectin-1 and its role in the recognition of beta-glucans by macrophages. *Mol Immunol*. 40(12)2004; :869–76. [PubMed: 14698225]
51. McGreal EP, Rosas M, Brown GD, Zamze S, Wong SY, Gordon S, Martinez-Pomares L, Taylor PR. The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. *Glycobiology*. 16(5)2006; :422–30. [PubMed: 16423983]
52. Yamasaki S, Matsumoto M, Takeuchi O, Matsuzawa T, Ishikawa E, Sakuma M, Tateno H, Uno J, Hirabayashi J, Mikami Y, Takeda K, Akira S, Saito T. C-type lectin Mincle is an activating receptor for pathogenic fungus, *Malassezia*. *Proc Natl Acad Sci U S A*. 106(6)2009; :1897–902. [PubMed: 19171887]
53. Wang H, LeBert V, Hung CY, Galles K, Saijo S, Lin X, Cole GT, Klein BS, Wuthrich M. C-type lectin receptors differentially induce th17 cells and vaccine immunity to the endemic mycosis of North America. *Journal of immunology (Baltimore, Md: 1950)*. 192(3)2014; :1107–1119.
54. Chang TH, Huang JH, Lin HC, Chen WY, Lee YH, Hsu LC, Netea MG, Ting JP, Wu-Hsieh BA. Dectin-2 is a primary receptor for NLRP3 inflammasome activation in dendritic cell response to *Histoplasma capsulatum*. *PLoS Pathog*. 13(7)2017; :e1006485. [PubMed: 28671985]
55. Lin JS, Huang JH, Hung LY, Wu SY, Wu-Hsieh BA. Distinct roles of complement receptor 3, Dectin-1, and sialic acids in murine macrophage interaction with *Histoplasma* yeast. *Journal of leukocyte biology*. 88(1)2010; :95–106. [PubMed: 20360401]
56. Huang JH, Lin CY, Wu SY, Chen WY, Chu CL, Brown GD, Chuu CP, Wu-Hsieh BA. CR3 and Dectin-1 Collaborate in Macrophage Cytokine Response through Association on Lipid Rafts and Activation of Syk-JNK-AP-1 Pathway. *PLoS Pathog*. 11(7)2015; :e1004985. [PubMed: 26132276]
57. Garfoot AL, Shen Q, Wuthrich M, Klein BS, Rappleye CA. The Eng1 beta-Glucanase Enhances *Histoplasma* Virulence by Reducing beta-Glucan Exposure. *MBio*. 7(2)2016; :e01388-15. [PubMed: 27094334]
58. Wuthrich M, Gern B, Hung CY, Ersland K, Rocco N, Pick-Jacobs J, Galles K, Filutowicz H, Warner T, Evans M, Cole G, Klein B. Vaccine-induced protection against 3 systemic mycoses endemic to North America requires Th17 cells in mice. *The Journal of clinical investigation*. 121(2)2011; :554–68. [PubMed: 21206087]
59. Domer JE, Hamilton JG, Harkin JC. Comparative study of the cell walls of the yeastlike and mycelial phases of *Histoplasma capsulatum*. *Journal of bacteriology*. 94(2)1967; :466–74. [PubMed: 6039363]
60. Domer JE. Monosaccharide and chitin content of cell walls of *Histoplasma capsulatum* and *Blastomyces dermatitidis*. *Journal of bacteriology*. 107(3)1971; :870–7. [PubMed: 5095293]
61. Reiss E. Serial enzymatic hydrolysis of cell walls of two serotypes of yeast-form *Histoplasma capsulatum* with alpha(1 leads to 3)-glucanase, beta(1 leads to 3)-glucanase, pronase, and chitinase. *Infect Immun*. 16(1)1977; :181–8. [PubMed: 873605]
62. Kasuga T, White TJ, Koenig G, McEwen J, Restrepo A, Castañeda E, Da Silva Lacaz CDA, Heins-Vaccari EM, De Freitas RS, Zancopé-Oliveira RM, Qin Z, Negroni R, Carter DA, Mikami Y, Tamura M, Taylor ML, Miller GF, Poonwan N, Taylor JW. Phylogeography of the fungal pathogen *Histoplasma capsulatum*. *Molecular Ecology*. 2003
63. Sepúlveda VE, Williams CL, Goldman WE. Comparison of phylogenetically distinct *Histoplasma* strains reveals evolutionarily divergent virulence strategies. *mBio*. 5(4)2014;
64. Reiss E, Miller SE, Kaplan W, Kaufman L. Antigenic, chemical, and structural properties of cell walls of *Histoplasma capsulatum* yeast-form chemotypes 1 and 2 after serial enzymatic hydrolysis. *Infect Immun*. 16(2)1977; :690–700. [PubMed: 405328]
65. Klimpel KR, Goldman WE. Isolation and characterization of spontaneous avirulent variants of *Histoplasma capsulatum*. *Infect Immun*. 55(3)1987; :528–33. [PubMed: 3818083]

66. Klimpel KR, Goldman WE. Cell walls from avirulent variants of *Histoplasma capsulatum* lack alpha-(1,3)-glucan. *Infect Immun*. 56(11)1988; :2997–3000. [PubMed: 3169995]
67. Eissenberg LG, West JL, Woods JP, Goldman WE. Infection of P388D1 macrophages and respiratory epithelial cells by *Histoplasma capsulatum*: selection of avirulent variants and their potential role in persistent histoplasmosis. *Infect Immun*. 59(5)1991; :1639–46. [PubMed: 2019435]
68. Rappleye CA, Engle JT, Goldman WE. RNA interference in *Histoplasma capsulatum* demonstrates a role for alpha-(1,3)-glucan in virulence. *Mol Microbiol*. 53(1)2004; :153–65. [PubMed: 15225311]
69. Rappleye CA, Eissenberg LG, Goldman WE. *Histoplasma capsulatum* alpha-(1,3)-glucan blocks innate immune recognition by the beta-glucan receptor. *Proc Natl Acad Sci U S A*. 104(4)2007; :1366–70. [PubMed: 17227865]
70. Edwards JA, Alore EA, Rappleye CA. The yeast-phase virulence requirement for alpha-glucan synthase differs among *Histoplasma capsulatum* chemotypes. *Eukaryot Cell*. 10(1)2011; :87–97. [PubMed: 21037179]
71. Holbrook ED, Edwards JA, Youseff BH, Rappleye CA. Definition of the extracellular proteome of pathogenic-phase *Histoplasma capsulatum*. *J Proteome Res*. 10(4)2011; :1929–43. [PubMed: 21291285]
72. Garfoot AL, Dearing KL, VanSchoiack AD, Wysocki VH, Rappleye CA. Eng1 and Exg8 are the major β -glucanases secreted by the fungal pathogen *Histoplasma capsulatum*. *Journal of Biological Chemistry*. 292(12)2017; :4801–4810. [PubMed: 28154008]
73. Shen QR, CA. Differentiation of the fungus *Histoplasma capsulatum* into a pathogen of phagocytes. *Curr Opin Microbiol*. 2017

Figure 1. Potential interactions of *H. capsulatum* with host phagocytes

Host phagocytes express a variety of receptors which are capable of recognizing *H. capsulatum* yeasts. Macrophages phagocytose yeasts through CR3 interaction with yeast Hsp60, while DCs engulf yeasts through VLA-5 interaction with yeast CypA. PMNs respond to opsonized yeasts by producing azurophilic granules, which contain fungistatic compounds such as Cathepsin G, BPI, and HNP-1, 2, and 3. The signaling receptors TLR2 and Dectin-1 potentially recognize Yps3 and β -glucans, respectively. However, *H. capsulatum* minimizes many PAMP-PRR interactions. Yeast cells reduce detection of immunostimulatory cell wall β -glucans by masking cell wall PAMPs with α -(1,3)-glucan as well as by trimming exposed β -glucans with the secreted endoglucanase Eng1. TLR7, TLR9, and Dectin-2 are also capable of recognizing *H. capsulatum* yeasts, but the associated *H. capsulatum* ligands have not been confirmed.