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The interplay between *Candida albicans* and the mammalian innate host defense

Running title: Host defense against *Candida albicans*

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Abstract

Candida albicans is both the most common fungal commensal microorganism in healthy individuals, as well as the major fungal pathogen causing high mortality in at-risk populations, especially of immunocompromised patients. In this review, we summarize the interplay between the host innate system and *C. albicans*, ranging from how the host recognizes, responds and clears *C. albicans* infection, to how *C. albicans* evades, dampens and escapes from host innate immunity.

Introduction

Candida species, the most common human fungal pathogen, ranks as the fourth cause of nosocomial bloodstream infections, with up to 40% mortality in epidemiological studies (119). *Candida* species colonize asymptomatically around 30 to 50% of individuals in a population at any given time, but during conditions when the host defense of the individuals is weakened, they can cause both mucosal and systemic infections (14). Risk factors such as neutropenia, systemic antibiotics exposure, central venous catheter, and prolonged ICU (intensive care unit) stay, predispose individuals to invasive and even life-threatening systemic candidiasis (119).

In the past decades, a sustained effort has been done to unravel the interplay between the host immune system and *Candida*. On the one hand, ample knowledge has been gained regarding the host defense mechanisms against *Candida* species, starting from recognition, to signal transduction and fungal clearance/killing. On the other hand, the mechanisms through which *Candida* evades the host defense armory were also investigated extensively. In this review, we aim to bring these two fields together and present a comprehensive view of the interplay between *Candida* and host innate defense, with a specific focus on how yeast-to-hyphae morphological transition contributes to recognition by the host and to the triggering of a protective immune response against *Candida* infection. While the incidence of non-*albicans* *Candida* species as etiologic agents of invasive candidiasis increased in the last decades (42), *Candida albicans* remains the most prevalent species in both mucosal and systemic infections. Most of the *Candida*-host interaction studies have investigated the interaction of *C. albicans* with the immune system, and therefore this review will focus on this pathogen.

Recognizing the intruder

I. Pattern Recognition Receptors

The first fundamental aim of host innate immunity is to distinguish self from non-self. Since Janeway proposed the concept of pattern recognition (66), a plethora of pattern recognition receptors (PRRs) have been identified that recognize so-called pathogen-associated molecular patterns (PAMPs). Several excellent reviews have extensively discussed how innate immune system recognizes *Candida* species (32,78,80). In this review we will therefore only point out the key receptors and their specific fungal ligands (Figure 1).

Candida cell wall structure is composed of chitin, β-glucans and mannoproteins. The polysaccharide structures of the cell wall of *C. albicans* are recognized by two classes of membrane-bound PRRs: the Toll-like receptors (TLRs) and the C-type lectin receptors (CLRs). The first PRRs discovered to recognize *C. albicans* were the TLRs, with TLR2 recognizing phospholipomannan (48), while the O-linked mannan has been shown to be recognized by TLR4 (79,101). In contrast, other TLRs such as TLR1 and TLR6 play a secondary role, and they do not seem to be essential for antifungal defense in candidiasis (81). The second major PRR family that recognizes *Candida* PAMPs is the CLRs. While β-glucans are recognized by dectin-1 (12), the N-linked mannan is recognized by the macrophage mannose receptor (79). Dectin-2 was initially reported to recognize the high-mannose structure in hyphae (63,95), but recently α-mannan on both yeast and hyphae was shown to be recognized by dectin-2 as well (93). DC-SIGN is another important receptor on the dendritic cells that recognizes *Candida* mannan (16). Galectin-3 has been shown to play a role in recognizing the β-mannosides of *C. albicans* (47). Besides, several additional C-type lectin receptors (CLR), such as Mincle (13) and SCARF1/CD36 (65) were reported to be involved in *Candida* recognition, but the specific ligands are yet to be identified. Last but not least,

MBL (mannose-binding lectin), a soluble CLR, mediates *Candida* opsonization and uptake via binding to *Candida* mannan and to the surface C1q receptor on the phagocyte (11).

In addition to the recognition of fungal PAMPs by membrane-bound receptors, several PRRs were shown to recognize *Candida* intracellularly. TLR9 has been demonstrated to recognize *C. albicans* DNA and induce cytokine production in dendritic cells (70). However, there was no difference reported of susceptibility between wild type and *Tlr9*$^{-/-}$ mice in a model of disseminated candidiasis, suggesting a redundant role of TLR9 for systemic anti-*Candida* defense (107). Although TLR9 is recruited to *C. albicans* containing phagosomes, one study showed that the macrophages from *Tlr9*$^{-/-}$ mice produce higher TNF-α, suggesting a modulatory role of TLR9 in host anti-*Candida* innate immune response (50). Receptors of the nucleotide-binding domain leucine-rich repeat-containing receptors (NLRs) are PRRs recognizing intracellular PAMPs, and one of their main function is to activate caspase-1 within a protein complex called the inflammasome, leading to processing and activation of cytokines of the IL-1 family (10). Among the NLRs, NLRP3 (NLR family pyrin domain-containing 3) has been suggested to play an important role for anti-*Candida* host defense. It has been reported that *Nlrp3* and *ASC* knockout mice were more susceptible to both systemic (41,52) and mucosal (43) *Candida* infections, suggesting a role of NLRP3 inflammasome for anti-*Candida* defense. Intriguingly, caspase-1 knockout mice are not more susceptible to disseminated candidiasis (67), arguing for the presence of alternative inflammasome-independent mechanisms for the production of bioactive IL-1β. Therefore, further investigations of the role of NLRP3 and ASC in inflammasome-independent function are warranted.

II. Danger Recognition Receptors

In addition to PRRs, danger recognition receptors have been proposed to activate host defense by recognizing endogenous danger signals. The protease-activated receptors (PARs) are G-protein coupled receptors that are activated upon proteolytic cleavage of their N-terminal tail. Instead of directly sensing the PAMPs, PARs function as danger-sensing receptors that are activated by either a protease from host, e.g. elastase and cathepsin G from neutrophils, or by proteases from *Candida*, e.g., secreted aspartic proteases. It has been shown that PAR1 expression was upregulated in mice infected with *Candida* and the cross talk between PAR1 and TLR2 could promote *Candida*-induced inflammation (71). However, in an attempt to translate these finding from mice to humans, we were not able to find direct evidence of the involvement of PAR1/PAR2 in *C. albicans*-induced pro-inflammatory cytokine in human peripheral mononuclear cells (17). Nevertheless, this does not yet exclude an *in-vivo* role of PARs in *Candida* infections. Therefore, future studies of the role of PAR during *Candida* infection in different niches are needed.

Cell types involved in host innate defense against *Candida* infection

I. Epithelial cells

The mucosal epithelium is the first line of defense against *Candida* species. It has been long acknowledged that the epithelium has a function as a passive physical barrier to restrain *Candida* from invasion of the underlying tissue. However, recent studies have broadened our knowledge about the active role played by epithelial cells in triggering immune responses. Oral epithelial cells express most of the TLRs, with the exception of TLR5 and TLR7 (114), to recognize invading microorganisms. Upon recognition of the invading *Candida*, epithelial cells secrete antimicrobial peptides, such as β-defensins (2) and LL-37 (53), to clear/control fungal infection directly. For example, in response to *C. parapsilosis*, human gingival epithelial cells upregulate TLRs and anti-microbial pepetides, such as hBD-1 (human β-

defensin 1) and hBD-2, to inhibit fungal growth (5). Similar results were also observed when *C. famata* was used to stimulate oral epithelial cells (6).

In addition, both oral (100) and vaginal (8) epithelial cells could inhibit *Candida* growth in a contact-dependent manner. Although proinflammatory cytokines produced by epithelial cells have no direct anti-fungal effects (55), they serve as signals to mucosal inflammatory cells to boost their anti-fungal function. Weindl and colleagues have shown in a reconstituted human epithelial model that epithelial cells were protected from *Candida* infection when neutrophils were present (114). By addition with anti-TNF-α antibody, the protective effect was partially inhibited. Therefore, epithelial cells may "sound the alarm" by inducing the production of cytokines and chemokines to recruit/activate other immune cells.

Cytokines produced from immune cells also play an important role in epithelial immunity against *Candida* infection. It has been shown that IL-22, the key cytokine produced by T-helper 22 subset of lymphocytes (Th22), synergistically induce the production of hBD2, S100A7 and CXCL-10 together with TNF-α in keratinocytes (26). IL-22 and TNF-α combination also render a protective effect of increasing epidermal integrity against *C. albicans* infection (26). This highlights the cross-talk between epithelial and immune cells in anti-*Candida* infection.

Site-specific differences of anti-*Candida* immunity also need to be taken into account. Oral and vaginal candidiasis are two most commonly found *Candida* infections in humans. It is generally considered that innate and cell-mediated immunity are important for mucosal anti-fungal defense, as exemplified by the high prevalence of oropharyngeal candidiasis (OPC) in the AIDS patients due to the loss of CD4 T cells (30). The role of cell mediated immunity for

host defense at the level of vaginal mucosa is less clear, and no solid evidence for the protective role of the innate immunity against vaginal infection was found (29). Moreover, vaginal epithelia was shown to express S100A8 and S100A9 upon *Candida* infection, which recruit PMNs to the infected vagina (121). However, unlike the protective role of PMNs in the oral candidiasis (96), the infiltrated PMNs in the vagina are associated with symptomatic vaginal infection (31).

II. Phagocytic cells

A. Polymorphonuclear neutrophils - PMNs

Phagocytes are believed to be the most effective cell type for controlling and clearing *Candida* infection. Among the phagocytes, PMNs play a critical role in host defense against both mucosal and disseminated candidiasis (105). Several proinflammatory cytokines have been reported to be responsible for the recruitment of PMNs to the site of infection, such as IL-6 (92,109), IL-8 (7) and TNF-α (82). Recently, IL-17 has been shown to be crucial to stimulate granulopoiesis (97) and recruitment of neutrophils to the site of infection (122). Several studies, though not all, have shown that mice deficient in IL-17 or IL-17 receptor are more susceptible to systemic (45) or mucosal *Candida* infection (22). In contrast, others have suggested deleterious role of IL-17 through overwhelming inflammatory reactions (24). In humans, Th17 responses are severely defective in patients with chronic mucocutaneous candidiasis (108). Similarly, patients with hyper-Ig E syndrome also suffer from oral and mucocutaneous candidiasis due to the defective Th17 response (21). Another line of evidence on the role of Th17 for antifungal defense comes from the dectin-1/CARD9/Th17 pathway, as well as for the occurrence of chronic mucocutaneous candidiasis in patients with IL-17F or IL-17 receptor deficiencies (89). Patients with defective dectin-1 (28) and /or downstream

adaptor CARD9 (38) suffer from mucocutaneous candidiasis. Therefore, Th17 response is less likely to be deleterious, but rather protective in human mucosal anti-fungal response.

In addition to proinflammatory cytokines, the hematopoietic growth factors granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are also critical for recruitment and activation of PMNs (51,54). In addition to direct killing of *C. albicans*, it was demonstrated that PMNs are the only cell type in the blood which could inhibit *C. albicans* germtube formation (33).

Phagocytes, and especially PMNs, kill *Candida* both intracellularly and extracellularly. Once *Candida* is phagocytosed by phagocytes, the engulfed microorganisms are processed through fusion with lysosomes into phagolysosomes. The engulfed *Candida* is killed within the phagolysosome by hydrolytic enzymes, antimicrobial peptides and the reactive oxygen species (ROS) (3). The formation of the candidacidal radical peroxynitrite ($ONOO^-$) due to superoxide anion (O_2^-) and nitric oxide release is another mechanism of intracellular killing (111). Recently, a novel extracellular mechanism of killing *Candida* was shown to be exerted by neutrophils. Upon encountering *Candida*, in addition to direct killing through phagocytosis, neutrophils inhibit *Candida* growth by releasing neutrophil extracellular traps (NETs) which contain the antifungal peptide calprotectin (104).

B. Mononuclear phagocytes – monocytes/macrophages

The role of mononuclear phagocytes in disseminated candidiasis is less well established. In a mouse model of macrophage depletion, a slower clearance of *Candida* from the bloodstream was observed (90), suggesting the involvement of macrophages in host defense against systemic *Candida* infections. However, one study using depletion of monocytes has suggested

that mice with monocytopenia are equally susceptible to *Candida* as control mice, reinforcing the dominant role played by PMNs in terms of anti-*Candida* infection by the host (105). It was proposed that the low candidacidal activity of macrophages is due to the reduced myeloperoxidase activity and decreased superoxide generation during the macrophage differentiation (94). In addition to the oxidative candidacidal mechanism, macrophages adherent to type 1 collagen matrices were more capable of killing ingested *Candida* by enhancing the fusion of yeast-containing phagosomes with the lysosomes (83). This implies that macrophages in contact with the extracellular matrix might be more efficient to kill *Candida*, compared to macrophage in an in vitro experimental setup.

C. Dendritic cells

As a professional antigen-presenting cell, DCs reside and patrol in the skin and mucosal surface, and they ingest *Candida* once tissues are invaded. *Candida* is internalized by DCs via MR and DC-SIGN (15,16), leading to processing and presentation of *Candida* specific antigen via MHC class II. DCs discriminate between yeast and hyphae forms of *C. albicans*, and induce T helper cell differentiation. Ingestion of yeasts primes T helper type 1 cells (Th1), whereas ingestion of hyphae inhibits IL-12 and Th1 differentiation, favoring Th2 differentiation. Thus, DCs bridge the innate and adaptive antifungal response by recognizing different morphologies of *Candida* (23).

Soluble factors

In addition to aforementioned cell-mediated anti-fungal responses, several blood soluble factors, such as complement and antibodies, also contribute to host anti-*Candida* immunity. The complement system can be activated through three pathways: the classical pathway (CP), the alternative pathway (AP) and the lectin pathway (LP). All three pathways can be activated

by *Candida* (98,124,125). The opsonized *Candida* can be more efficiently ingested by phagocytes through the interaction between the CR3 and C3b, which is deposited on the *Candida* surface (62), or Fc receptor and the anti-*Candida* antibody (4). In contrast, the thick fungal cell wall prevents the killing mechanisms mediated by the membrane attack complex.

Apart from the role of mediating phagocytosis through surface opsonization, we have identified a crucial role of anaphylatoxin C5a in augmenting *C. albicans*-induced IL-6 and IL-1β production in PBMCs (18). By using the specific blocking antibody against C5a or the C5a receptor antagonist, a clear reduction of cytokine production induced by *C. albicans* in the presence of serum was observed. Moreover, using serum isolated from patients with various complement deficiencies, we demonstrated a crucial role of C5, but not the membrane attack complex, for *C. albicans*-induced IL-6 and IL-1β. These findings reveal a central role of anaphylatoxin C5a in augmenting host proinflammatory cytokine production upon contact with *C. albicans*. It was also demonstrated that C5-deficient mice are more susceptible to systemic *C. albicans* infection, resulting in a higher fungal burden in the organs (73). A recent study using computational analysis proposed that different combinations of *C5* and *C1r/s* alleles could predict the survival of different mouse strains in the systemic *Candida* infection model (86). This implies that reduced C1 deposition in the susceptible mice resulted in reduced C5 binding and activation.

Evasion of *Candida* from the host defense mechanisms

As a commensal microorganism surviving in various host niches, *Candida* encounters a continuously hostile environment, in terms of host immune system, pH, nutrition acquisition and competition with the other microorganisms in the microflora. Here we will specifically focus on the strategies employed by *Candida* to escape/evade host innate defense (Figure 2).

I. Yeast-to-hyphae transition

C. albicans is a dimorphic fungus. The morphological switch between yeast and hyphae is considered to be the main virulence factor of *C. albicans*. Through the dissection of the molecular mechanisms responsible for the yeast-to-hyphal transition, several transcriptional factors have been identified to be responsible for the morphological transition. These transcriptional factors are activated by different environmental stimuli and have been reviewed previously (118). Nonfilamentous *C. albicans* strains with defective transcriptional factors such as *efg1* and *cph1* has been shown to be avirulent or less virulent in mice infection models (56). This highlights the fact that morphological transition is an important virulence factor for *C. albicans*. In the systemic infection model in mice, *C. albicans* was readily recognized and phagocytosed in the blood stream. Once the yeast form of *C. albicans* is phagocytosed, the production of carbon dioxide within the macrophages induces the adenylcyclase and cAMP-dependent protein kinase A pathway, thereby activating *Efg1p*, which is the major transcription factor responsible for yeast-to-hyphal transition. Formation of hyphae will eventually lead to piercing and killing of macrophages by *C. albicans* hyphae (37,61). In the oral experimental candidiasis model, hyphae formation was also shown to inhibit human-defensins expression, as another example of how yeast-to-hyphae transition subverts host innate immunity (57).

Intriguingly, hyphae-locked mutants as well as yeast-locked mutant both have been demonstrated to be less virulent than wild type strains (9,74). This implies that the morphological switch from yeast to hyphae, and vice versa, accounts for the full virulence of *C. albicans*. While hyphae might be regarded as an invasive form required for piercing

266 through phagocytes and invading epithelium barrier, the yeast form is also needed for the free
267 dissemination in the systemic infection.
268
269 *II. Epithelium invasion*
270 *C. albicans* invades the epithelial barrier via two different routes: active tissue invasion and
271 passively-induced endocytosis. Recently, Wachtler and colleagues have performed an
272 extensive study to elucidate the genes involved in the active penetration of epithelium by *C.*
273 *albicans* at different stages: from epithelial attachment, tissue invasion and, eventually, tissue
274 damage (112). Many hyphal-associated genes, including *ALS3, HWP1, ECE1, SOD5, PHR1*
275 and *PRA1* are upregulated in *C. albicans* in contact with epithelial cells. Hyphae is the
276 invasive form of *C. albicans* found within epithelial cells in the invaded tissue (91).
277 Therefore, upregulation of hyphal-associated genes upon contact with epithelial cells might be
278 crucial for *C. albicans* active penetration of epithelial cells. In addition to active penetration,
279 *C. albicans* can also cause transepithelial infection through induced endocytosis. It is
280 demonstrated that ALS-3 mimics host cadherins and induces endocytosis through binding to
281 E-cadherin on oral epithelial cells (87). This endocytosis process is passive and does not
282 require cell viability, because even the killed *C. albicans* could be endocytosed by the
283 epithelial cells. Once *C. albicans* is inside the epithelial cells, it forms hyphae leading to
284 piercing of the cells through the function of *EED1* (Epithelial Escape and Dissemination 1).
285 An *eed1Δ* deficient strain failed to maintain hyphae formation and was trapped within the
286 cells (123). In addition to invasion of epithelial cells, *C. albicans* is also able to downregulate
287 epithelial TLR4 expression, which in turn increased the vulnerability of epithelial cells to *C.*
288 *albicans* infection (114).
289
290 *III. Escape from Phagocytosis*

A. Shielding of the surface PAMPs

To phagocytose *Candida*, the host cells first need to "sense" the microorganism, which is achieved through recognizing the PAMPs of *Candida*. One mechanism through which this step is prevented is shielding of important PAMPs from recognition by PRRs. It has been shown that β-glucan is shielded by the outer cell-wall components, thus preventing the recognition of dectin-1 (35). In line with this, live *C. albicans* induced low amounts of cytokines in human peripheral blood mononuclear cells, yet heat-killed *C. albicans* in which the architecture of the cell wall is disrupted induced significant amounts of cytokines through the recognition of the now-exposed β-glucan by dectin-1 (39). Mckenzie and colleagues have also demonstrated that mutants deficient in O-linked and N-linked mannans were more readily phagocytosed by macrophages (64). However, during a live infection model, β-glucans are exposed in the damaged *Candida* cells by the action of host factors, demonstrating the continuous "arm race" between the host and the pathogen (117).

B. Complement inhibition and degradation

C. albicans possesses several strategies to interfere with complement activation in order to avoid phagocytosis or to reduce production of proinflammatory cytokines. It has been shown that secreted aspartic protease degrades C3b, thus inhibiting the opsonization of *Candida* by human serum *in vitro* (40). Furthermore, *C. albicans* could also bind on the cell surface the complement regulatory proteins, such as complement regulator C4b-binding protein, factor H, FHL-1 and plasminogen-binding surface protein, in order to inhibit the activation of the complement system (68,69,88). A recently identified *C. albicans* surface protein, Pra1, has been shown to bind factor H and C4b-binding protein to regulate complement activation (58,60), and subsequently blocks the activation and conversion of C3 (59). On the other hand, strikingly, Pra1 also serves as the primary ligand recognized by CR3 and facilitates

316 phagocytosis (99). This demonstrates once more the complex interplay between *Candida* and
317 host innate immune system.
318
319 *C. Inhibition of phagolysosome formation*
320 An important step in the process of killing of a pathogen is the fusion of the phagosome
321 containing the microorganism with the lysosomes. It has been recently reported that *C.*
322 *albicans* can modulate intracellular membrane trafficking by inhibiting the formation of
323 phagolysosome. Only live *C. albicans* was able to inhibit phagolysosome formation, but not
324 heat-killed *C. albicans*, implying that this is an active inhibition dependent on the viability of
325 the fungi. Interestingly, wild-type *C. albicans* is more capable of controlling phagosomal
326 composition than the non-filamentous mutants (27). This is also in line with the fact that
327 morphological transition is one of the critical virulence factors of *C. albicans*. However, the
328 genetic background of *C. albicans* strains also plays an important role in the ability to survive
329 within the phagosome. Tavanti and colleagues have reported that *C. albicans* isolates with c-
330 karyotype are more resistant to intracellular killing and able to replicate and escape from
331 THP-1 cells as compared to the b-karyotype (103). It is to be expected that a further dissection
332 of the underlying mechanisms through which *C. albicans* prevents the phagolysosome fusion
333 may be translated into potential novel anti-fungal intervention strategies.
334
335 *D. ROS inhibition*
336 ROS production is a major antifungal mechanism in phagocytes. To counteract the oxidative
337 stress, *Candida* species possess several defensive armories. *C. albicans* catalase has been
338 suggested to counteract the respiratory burst, and a *cat1*Δ *C. albicans* mutant is less virulent
339 and was cleared faster than a wild-type strain in an experimental model (76). Similarly, the *C.*
340 *albicans* surface superoxide dismutase has also been implicated for counteracting the ROS

production from the phagocytes (34). In line with this, Wellington and colleagues have demonstrated that *C. albicans* and *C. glabrata,* but not *S. cerevisiae,* could actively suppress ROS production in a murine macrophage cell line. Interestingly, although the recognition of fungal cell wall is needed for the ROS production, as demonstrated by stimulating macrophages with heat-killed *Candida* or caspofungin-treated *Candida*, the *Candida* viability is needed for the suppression effect, implying an active role for live *Candida* in suppressing the ROS production (115). *Candida* vacuole formation was also suggested to play a role in resistance against stress and for hyphal growth (84). *vps11Δ* strain is defective in vacuole biogenesis, and as a consequence, more sensitive to oxidative stress and severely retarded in filamentous growth. However, although the partially functional *vps11hr* strain also bears similar defect in hyphae formation, *vps11hr* strain shows similar survival pattern as wild type strain in the macrophage J774A.1 cell line (85).

E. Farnesol

Farnesol was first identified as a quorum-sensing molecule (QSM) that repressed the yeast-to-hyphae transition of *C. albicans* in an autoregulatory manner (44). Recently, farnesol has also been suggested to be a virulence factor of *C. albicans*. It has been demonstrated that farnesol might decrease macrophage viability through induction of ROS (1). Furthermore, farnesol has been suggested to protect *C. albicans* from oxidative stress via upregulating CAT1, SOD1, SOD2 and SOD4 (116). In an in-vivo infection model, the pretreatment with exogenous farnesol led to inhibition of Th1 cytokine IFN-γ and IL-12, and enhanced Th2 cytokine (77).

On the other hand, farnesol also seems to function as a danger signal that activates anti-fungal defense. Exogenous farnesol upregulates TLR2 expression in epithelial cells, which results in more IL-6 and β-defensin 2 expression upon *C. albicans* stimulation (25). It has also been

366 demonstrated that murine macrophages produced more IL-6 when stimulated with wild-type
367 *C. albicans*, than with a farnesol-deficient strain (36). In addition, the conditioned medium of
368 *C. albicans* cultures has also been demonstrated to potentiate IL-6 and IL-8 production in
369 human PBMCs (17), and it has been suggested that this may be attributed to the presence of
370 farnesol.
371
372 *IV. Modulating cytokine production by soluble factors*
373 A lot has been learned in the past decades about the mechanisms through which *Candida*
374 induces production of cytokines in the host, yet little is known about the active role of *C.*
375 *albicans* in exploiting host cytokine production for its own benefit.
376
377 Live *C. albicans,* but not *C. krusei,* has been demonstrated to inhibit IL-12 and IFN-γ
378 production from human PBMCs (120). This IL-12 inhibitory effect was dependent on the
379 viability of *C. albicans*, because both heat-killed *C. albicans* and *C. krusei* induced similar
380 amounts of IL-12. Further studies showed that IL-12 inhibitory activity is due to the secretion
381 of a glycoprotein (113) and signaling through selective activation of ERK mitogen-activated
382 protein kinase (102). However, the identity of this soluble glycoprotein and the receptor
383 responsible for the IL-12 inhibition signaling is unknown.
384
385 Recently, we have also reported the active role played by soluble factors released by *C.*
386 *albicans*. We have demonstrated that although conditioned medium from *C. albicans* culture
387 by itself did not induce host cytokine production, it could amplify host IL-6 and IL-8
388 production (17). On the other hand, the conditioned medium downregulated host IFN-γ
389 synthesis, yet upregulated IL-10 production, thus shifting the T helper cell response from a

beneficial Th1 to a detrimental Th2 response (17). Further investigations about how and which soluble factor(s) are responsible are warranted.

V. Inhibition of IL-17 production

IL-17 has been suggested to be an important component of host defense against *Candida* infection (22,45). *Candida* cell wall components, especially mannans and β-glucans, are recognized by CLRs such as MR, dectin-1, and dectin-2, leading to inflammasome activation, IL-1β production, and subsequent induction of IL-17 (106,110). Recently, it was demonstrated that *C. albicans* could actively inhibit host IL-17 production by altering host tryptophan metabolism. Tryptophan metabolism is regulated by two distinct enzymes: Indoleamine 2,3-dioxygenase (IDO) and tryptophan hydroxylase. By inhibiting IDO expression, *C. albicans* could shift tryptophan metabolism and this leads to less kynurenines and more 5-hydroxytrptophan metabolites. The increased 5-hydroxytryptophan subsequently inhibits host IL-17 production (20).

Recognition of *Candida* colonization versus invasion-the Achilles' heel of *C. albicans*

C. albicans is a commensal microorganism in healthy individuals, but it is capable of causing serious infections if the protective mucosal barrier is breached. Therefore, immune discrimination between *Candida* colonization and invasion is of particular significance.

A biphasic MAPK response has been proposed to be responsible to discriminate between *C. albicans* yeasts and hyphae by the epithelial cells (72). Moyes and colleagues have demonstrated that during the commensal stage of *C. albicans*, c-Jun was activated in the epithelial cells upon recognition of fungal cell wall components. The activation of c-Jun is independent of fungal morphology and leads to NF-κB activation, but not to production of

proinflammatory cytokines. However, activating of the second MAPK phase, consisting of MKP1 and c-Fos activation, is dependent on hyphae germination and an increased fungal burden, and thus induces a potent inflammatory response. A subsequent study further demonstrates that *C. albicans* cell wall glycosylation was indirectly required for induction of proinflammatory cytokines production, but not the activator of MAPK/MKP1/c-Fos pathway, in epithelial cells (75). This reveals a possible mechanism of epithelial discrimination between fungal colonization and invasion.

In addition, hyphae formation was also identified to be the key event for triggering inflammasome activation and IL-1β secretion in murine macrophage (46). Since IL-1β is indispensible for Th17 differentiation, the recognition of invasive hyphae might be the crucial step for macrophages to discriminate between *Candida* colonization and invasion. We have demonstrated that *Candida* hyphae could specifically activate the inflammasome through the exposure of fungal PAMP such as β-glucans that are originally shielded in yeast (19), because β-glucan was demonstrated to induce both IL-1β mRNA transcription and inflammasome activation (49,52). Subsequently, the inflammasome activation and IL-1β production is crucial for Th17 differentiation and IL-17 production, and yeast-locked *C. albicans* strains defective in hyphae formation fail to induce IL-17 production. Therefore, macrophages serve as a gatekeeper to induce protective Th17 responses against *C. albicans* invasion by recognizing invading hyphae.

Yeast-to-hyphae transition has been demonstrated to be the crucial virulence factor for *C. albicans*, and is important for tissue invasion and for escaping from phagocytes. This, however, also puts *C. albicans* at risk to be more efficiently recognized by the host and induces an additional array of host defense mechanisms (Figure 3).

Concluding remarks and future directions

In the past decades, much has been learned about the mechanisms through which host innate immunity recognizes, responds to, and defends against *Candida* species. In addition, many of the fungal virulence factors that contribute to pathogenesis have been identified, and sustained efforts have been made to study the interplay between *Candida* and the host defense. However, one can envisage that the interaction between *Candida* and the host in real life will be more complicated, and important questions remain to be answered. One such topic is represented by the mechanisms through which the sensing of invading *Candida* by the epithelial cells prepare and educate the innate cells in the fight against invasion. It is to be expected that the cross-talk between epithelial cells and immune cells will draw more attention in the years to come. Similarly, much remains to be investigated on the pathways through which the morphology of *Candida* facilitates its pathogenicity. Moreover, several crucial questions related to mucosal antifungal immunity remain unanswered. For example, what are the differences between the host immune responses at the oral mucosa and the vaginal mucosa, and what are the consequences of the deregulation of antifungal mucosal immunity for autoinflammatory diseases such as Crohn's disease and ulcerative colitis? These are only a few of the questions that need to be answered in the future in order to get an overall view about the interplay between *Candida* and host innate immune defense.

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Reference List

1. **Abe, S., R. Tsunashima, R. Iijima, T. Yamada, N. Maruyama, T. Hisajima, Y. Abe, H. Oshima, and M. Yamazaki**. 2009. Suppression of anti-Candida activity of macrophages by a quorum-sensing molecule, farnesol, through induction of oxidative stress. Microbiol. Immunol. **53**:323-330.

2. **Abiko, Y., Y. Jinbu, T. Noguchi, M. Nishimura, K. Kusano, P. Amaratunga, T. Shibata, and T. Kaku**. 2002. Upregulation of human beta-defensin 2 peptide expression in oral lichen planus, leukoplakia and candidiasis. an immunohistochemical study. Pathol. Res. Pract. **198**:537-542.

3. **Aratani, Y., H. Koyama, S. Nyui, K. Suzuki, F. Kura, and N. Maeda**. 1999. Severe impairment in early host defense against Candida albicans in mice deficient in myeloperoxidase. Infect. Immun. **67**:1828-1836.

4. **Bagasra, O., A. Howeedy, and A. Kajdacsy-Balla**. 1988. Macrophage function in chronic experimental alcoholism. I. Modulation of surface receptors and phagocytosis. Immunology **65**:405-409.

5. **Bahri, R., S. Curt, D. Saidane-Mosbahi, and M. Rouabhia**. 2010. Normal human gingival epithelial cells sense C. parapsilosis by toll-like receptors and module its pathogenesis through antimicrobial peptides and proinflammatory cytokines. Mediators. Inflamm. **2010**:940383.

6. **Bahri, R., D. Saidane-Mosbahi, and M. Rouabhia**. 2010. Candida famata modulates toll-like receptor, beta-defensin, and proinflammatory cytokine expression by normal human epithelial cells. J. Cell Physiol **222**:209-218.

7. **Balish, E., R. D. Wagner, A. Vazquez-Torres, J. Jones-Carson, C. Pierson, and T. Warner**. 1999. Mucosal and systemic candidiasis in IL-8Rh-/- BALB/c mice. J. Leukoc. Biol. **66**:144-150.

8. **Barousse, M. M., C. Steele, K. Dunlap, T. Espinosa, D. Boikov, J. D. Sobel, and P. L. Fidel, Jr.** 2001. Growth inhibition of Candida albicans by human vaginal epithelial cells. J. Infect. Dis. **184**:1489-1493.

9. **Braun, B. R., D. Kadosh, and A. D. Johnson**. 2001. NRG1, a repressor of filamentous growth in C.albicans, is down-regulated during filament induction. EMBO J. **20**:4753-4761.

10. **Brodsky, I. E. and D. Monack**. 2009. NLR-mediated control of inflammasome assembly in the host response against bacterial pathogens. Semin. Immunol. **21**:199-207.

11. **Brouwer, N., K. M. Dolman, H. M. van, M. Sta, D. Roos, and T. W. Kuijpers**. 2008. Mannose-binding lectin (MBL) facilitates opsonophagocytosis of yeasts but not of bacteria despite MBL binding. J. Immunol. **180**:4124-4132.

12. **Brown, G. D., P. R. Taylor, D. M. Reid, J. A. Willment, D. L. Williams, L. Martinez-Pomares, S. Y. Wong, and S. Gordon**. 2002. Dectin-1 is a major beta-glucan receptor on macrophages. J. Exp. Med. **196**:407-412.

13. **Bugarcic, A., K. Hitchens, A. G. Beckhouse, C. A. Wells, R. B. Ashman, and H. Blanchard**. 2008. Human and mouse macrophage-inducible C-type lectin (Mincle) bind Candida albicans. Glycobiology **18**:679-685.

14. **Calderone, R. A. and W. A. Fonzi**. 2001. Virulence factors of Candida albicans. Trends Microbiol. **9**:327-335.

15. **Cambi, A., K. Gijzen, J. M. de Vries, R. Torensma, B. Joosten, G. J. Adema, M. G. Netea, B. J. Kullberg, L. Romani, and C. G. Figdor**. 2003. The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for Candida albicans on dendritic cells. Eur. J. Immunol. **33**:532-538.

16. **Cambi, A., M. G. Netea, H. M. Mora-Montes, N. A. Gow, S. V. Hato, D. W. Lowman, B. J. Kullberg, R. Torensma, D. L. Williams, and C. G. Figdor**. 2008. Dendritic cell interaction with Candida albicans critically depends on N-linked mannan. J. Biol. Chem. **283**:20590-20599.

17. **Cheng, S. C., L. Y. Chai, L. A. Joosten, A. Vecchiarelli, B. Hube, J. W. Van der Meer, B. J. Kullberg, and M. G. Netea**. 2010. Candida albicans releases soluble factors that potentiate cytokine production by human cells through a protease-activated receptor 1- and 2-independent pathway. Infect. Immun. **78**:393-399.

18. **Cheng, S. C., T. Sprong, L. A. Joosten, J. W. Van der Meer, B. J. Kullberg, B. Hube, L. Schejbel, P. Garred, J. T. van Dissel, and M. G. Netea**. 2011. Complement plays a central role in Candida albicans-induced cytokine production by human PBMCs. Eur. J. Immunol. DOI: 10.1002/eji.201142057

19. **Cheng, S. C., F. van de Veerdonk, M. Lenardon, M. Stoffels, T. Plantinga, S. Smeekens, L. Rizzetto, L. Mukaremera, K. Preechasuth, D. Cavalieri, T. D. Kanneganti, J. W. Van der Meer, B. J. Kullberg, L. A. Joosten, N. A. Gow, and M. G. Netea**. 2011. The dectin-1/inflammasome pathway is responsible for the induction of protective T-helper 17 responses that discriminate between yeasts and hyphae of Candida albicans. J. Leukoc. Biol. **90(2)**:357-366

20. **Cheng, S. C., F. van de Veerdonk, S. Smeekens, L. A. Joosten, J. W. Van der Meer, B. J. Kullberg, and M. G. Netea**. 2010. Candida albicans dampens host defense by downregulating IL-17 production. J. Immunol. **185**:2450-2457.

21. **Conti, H. R., O. Baker, A. F. Freeman, W. S. Jang, S. M. Holland, R. A. Li, M. Edgerton, and S. L. Gaffen**. 2011. New mechanism of oral immunity to mucosal candidiasis in hyper-IgE syndrome. Mucosal. Immunol. **4**:448-455.

22. **Conti, H. R., F. Shen, N. Nayyar, E. Stocum, J. N. Sun, M. J. Lindemann, A. W. Ho, J. H. Hai, J. J. Yu, J. W. Jung, S. G. Filler, P. Masso-Welch, M. Edgerton, and S. L. Gaffen**. 2009. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. J. Exp. Med. **206**:299-311.

23. **d'Ostiani, C. F., S. G. Del, A. Bacci, C. Montagnoli, A. Spreca, A. Mencacci, P. Ricciardi-Castagnoli, and L. Romani**. 2000. Dendritic cells discriminate between yeasts and hyphae of the fungus Candida albicans. Implications for initiation of T helper cell immunity in vitro and in vivo. J. Exp. Med. **191**:1661-1674.

24. **De, L. A., T. Zelante, C. D'Angelo, S. Zagarella, F. Fallarino, A. Spreca, R. G. Iannitti, P. Bonifazi, J. C. Renauld, F. Bistoni, P. Puccetti, and L. Romani**. 2010. IL-22 defines a novel immune pathway of antifungal resistance. Mucosal. Immunol. **3**:361-373.

25. **Decanis, N., K. Savignac, and M. Rouabhia**. 2009. Farnesol promotes epithelial cell defense against Candida albicans through Toll-like receptor 2 expression, interleukin-6 and human beta-defensin 2 production. Cytokine **45**:132-140.

26. **Eyerich, S., J. Wagener, V. Wenzel, C. Scarponi, D. Pennino, C. Albanesi, M. Schaller, H. Behrendt, J. Ring, C. B. Schmidt-Weber, A. Cavani, M. Mempel, C. Traidl-Hoffmann, and K. Eyerich**. 2011. IL-22 and TNF-alpha represent a key cytokine combination for epidermal integrity during infection with Candida albicans. Eur. J. Immunol.

27. **Fernandez-Arenas, E., C. K. Bleck, C. Nombela, C. Gil, G. Griffiths, and R. ez-Orejas**. 2009. Candida albicans actively modulates intracellular membrane trafficking in mouse macrophage phagosomes. Cell Microbiol. **11**:560-589.

28. **Ferwerda, B., G. Ferwerda, T. S. Plantinga, J. A. Willment, A. B. van Spriel, H. Venselaar, C. C. Elbers, M. D. Johnson, A. Cambi, C. Huysamen, L. Jacobs, T. Jansen, K. Verheijen, L. Masthoff, S. A. Morre, G. Vriend, D. L. Williams, J. R. Perfect, L. A. Joosten, C. Wijmenga, J. W. Van der Meer, G. J. Adema, B. J. Kullberg, G. D. Brown, and M. G. Netea**. 2009. Human dectin-1 deficiency and mucocutaneous fungal infections. N. Engl. J. Med. **361**:1760-1767.

29. **Fidel, P. L., Jr.** 2002. Distinct protective host defenses against oral and vaginal candidiasis. Med. Mycol. **40**:359-375.

30. **Fidel, P. L., Jr.** 2011. Candida-host interactions in HIV disease: implications for oropharyngeal candidiasis. Adv. Dent. Res. **23**:45-49.

31. **Fidel, P. L., Jr., M. Barousse, T. Espinosa, M. Ficarra, J. Sturtevant, D. H. Martin, A. J. Quayle, and K. Dunlap**. 2004. An intravaginal live Candida challenge in humans leads to new hypotheses for the immunopathogenesis of vulvovaginal candidiasis. Infect. Immun. **72**:2939-2946.

32. **Filler, S. G.** 2006. Candida-host cell receptor-ligand interactions. Curr. Opin. Microbiol. **9**:333-339.

33. **Fradin, C., G. P. De, D. MacCallum, M. Schaller, F. Klis, F. C. Odds, and B. Hube**. 2005. Granulocytes govern the transcriptional response, morphology and proliferation of Candida albicans in human blood. Mol. Microbiol. **56**:397-415.

34. **Frohner, I. E., C. Bourgeois, K. Yatsyk, O. Majer, and K. Kuchler**. 2009. Candida albicans cell surface superoxide dismutases degrade host-derived reactive oxygen species to escape innate immune surveillance. Mol. Microbiol. **71**:240-252.

35. **Gantner, B. N., R. M. Simmons, and D. M. Underhill**. 2005. Dectin-1 mediates macrophage recognition of Candida albicans yeast but not filaments. EMBO J. **24**:1277-1286.

36. **Ghosh, S., N. Howe, K. Volk, S. Tati, K. W. Nickerson, and T. M. Petro**. 2010. Candida albicans cell wall components and farnesol stimulate the expression of both inflammatory and regulatory cytokines in the murine RAW264.7 macrophage cell line. FEMS Immunol. Med. Microbiol. **60**:63-73.

37. **Ghosh, S., D. H. Navarathna, D. D. Roberts, J. T. Cooper, A. L. Atkin, T. M. Petro, and K. W. Nickerson**. 2009. Arginine-induced germ tube formation in Candida albicans is essential for escape from murine macrophage line RAW 264.7. Infect. Immun. **77**:1596-1605.

38. **Glocker, E. O., A. Hennigs, M. Nabavi, A. A. Schaffer, C. Woellner, U. Salzer, D. Pfeifer, H. Veelken, K. Warnatz, F. Tahami, S. Jamal, A. Manguiat, N. Rezaei, A. A. Amirzargar, A. Plebani, N. Hannesschlager, O. Gross, J. Ruland, and B. Grimbacher**. 2009. A homozygous CARD9 mutation in a family with susceptibility to fungal infections. N. Engl. J. Med. **361**:1727-1735.

39. **Gow, N. A., M. G. Netea, C. A. Munro, G. Ferwerda, S. Bates, H. M. Mora-Montes, L. Walker, T. Jansen, L. Jacobs, V. Tsoni, G. D. Brown, F. C. Odds, J. W. Van der Meer, A. J. Brown, and B. J. Kullberg**. 2007. Immune recognition of Candida albicans beta-glucan by dectin-1. J. Infect. Dis. **196**:1565-1571.

40. **Gropp, K., L. Schild, S. Schindler, B. Hube, P. F. Zipfel, and C. Skerka**. 2009. The yeast Candida albicans evades human complement attack by secretion of aspartic proteases. Mol. Immunol. **47**:465-475.

41. **Gross, O., H. Poeck, M. Bscheider, C. Dostert, N. Hannesschlager, S. Endres, G. Hartmann, A. Tardivel, E. Schweighoffer, V. Tybulewicz, A. Mocsai, J. Tschopp, and J. Ruland**. 2009. Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defense. Nature **459**:433-436.

42. **Hajjeh, R. A., A. N. Sofair, L. H. Harrison, G. M. Lyon, B. A. rthington-Skaggs, S. A. Mirza, M. Phelan, J. Morgan, W. Lee-Yang, M. A. Ciblak, L. E. Benjamin, L. T. Sanza, S. Huie, S. F. Yeo, M. E. Brandt, and D. W. Warnock**. 2004. Incidence of bloodstream infections due to Candida species and in vitro susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. J. Clin. Microbiol. **42**:1519-1527.

43. **Hise, A. G., J. Tomalka, S. Ganesan, K. Patel, B. A. Hall, G. D. Brown, and K. A. Fitzgerald**. 2009. An essential role for the NLRP3 inflammasome in host defense against the human fungal pathogen Candida albicans. Cell Host. Microbe **5**:487-497.

44. **Hornby, J. M., E. C. Jensen, A. D. Lisec, J. J. Tasto, B. Jahnke, R. Shoemaker, P. Dussault, and K. W. Nickerson**. 2001. Quorum sensing in the dimorphic fungus Candida albicans is mediated by farnesol. Appl. Environ. Microbiol. **67**:2982-2992.

45. **Huang, W., L. Na, P. L. Fidel, and P. Schwarzenberger**. 2004. Requirement of interleukin-17A for systemic anti-Candida albicans host defense in mice. J. Infect. Dis. **190**:624-631.

46. **Joly, S., N. Ma, J. J. Sadler, D. R. Soll, S. L. Cassel, and F. S. Sutterwala**. 2009. Cutting edge: Candida albicans hyphae formation triggers activation of the Nlrp3 inflammasome. J. Immunol. **183**:3578-3581.

47. **Jouault, T., B. M. El Abed-El, M. Martinez-Esparza, L. Breuilh, P. A. Trinel, M. Chamaillard, F. Trottein, and D. Poulain**. 2006. Specific recognition of Candida albicans by macrophages requires galectin-3 to discriminate Saccharomyces cerevisiae and needs association with TLR2 for signaling. J. Immunol. **177**:4679-4687.

48. **Jouault, T., S. Ibata-Ombetta, O. Takeuchi, P. A. Trinel, P. Sacchetti, P. Lefebvre, S. Akira, and D. Poulain**. 2003. Candida albicans phospholipomannan is sensed through toll-like receptors. J. Infect. Dis. **188**:165-172.

49. **Kankkunen, P., L. Teirila, J. Rintahaka, H. Alenius, H. Wolff, and S. Matikainen**. 2010. (1,3)-beta-glucans activate both dectin-1 and NLRP3 inflammasome in human macrophages. J. Immunol. **184**:6335-6342.

50. **Kasperkovitz, P. V., N. S. Khan, J. M. Tam, M. K. Mansour, P. J. Davids, and J. M. Vyas**. 2011. Toll-Like Receptor 9 Modulates Macrophage Antifungal Effector Function during Innate Recognition of Candida albicans and Saccharomyces cerevisiae. Infect. Immun. **79**:4858-4867.

51. **Kullberg, B. J., M. G. Netea, A. G. Vonk, and J. W. Van der Meer**. 1999. Modulation of neutrophil function in host defense against disseminated Candida albicans infection in mice. FEMS Immunol. Med. Microbiol. **26**:299-307.

52. **Kumar, H., Y. Kumagai, T. Tsuchida, P. A. Koenig, T. Satoh, Z. Guo, M. H. Jang, T. Saitoh, S. Akira, and T. Kawai**. 2009. Involvement of the NLRP3 inflammasome in innate and humoral adaptive immune responses to fungal beta-glucan. J. Immunol. **183**:8061-8067.

53. **Li, M., Q. Chen, R. Tang, Y. Shen, and W. D. Liu**. 2011. The expression of beta-defensin-2, 3 and LL-37 induced by Candida albicans phospholipomannan in human keratinocytes. J. Dermatol. Sci. **61**:72-75.

54. **Lieschke, G. J. and A. W. Burgess**. 1992. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor (2). N. Engl. J. Med. **327**:99-106.

55. **Lilly, E. A., J. E. Leigh, S. H. Joseph, and P. L. Fidel, Jr.** 2006. Candida-induced oral epithelial cell responses. Mycopathologia **162**:25-32.

56. **Lo, H. J., J. R. Kohler, B. DiDomenico, D. Loebenberg, A. Cacciapuoti, and G. R. Fink**. 1997. Nonfilamentous C. albicans mutants are avirulent. Cell **90**:939-949.

57. **Lu, Q., J. A. Jayatilake, L. P. Samaranayake, and L. Jin**. 2006. Hyphal invasion of Candida albicans inhibits the expression of human beta-defensins in experimental oral candidiasis. J. Invest Dermatol. **126**:2049-2056.

58. **Luo, S., A. M. Blom, S. Rupp, U. C. Hipler, B. Hube, C. Skerka, and P. F. Zipfel**. 2011. The pH-regulated Antigen 1 of Candida albicans Binds the Human Complement Inhibitor C4b-binding Protein and Mediates Fungal Complement Evasion. J. Biol. Chem. **286**:8021-8029.

59. **Luo, S., A. Hartmann, H. M. Dahse, C. Skerka, and P. F. Zipfel**. 2010. Secreted pH-regulated antigen 1 of Candida albicans blocks activation and conversion of complement C3. J. Immunol. **185**:2164-2173.

60. **Luo, S., S. Poltermann, A. Kunert, S. Rupp, and P. F. Zipfel**. 2009. Immune evasion of the human pathogenic yeast Candida albicans: Pra1 is a Factor H, FHL-1 and plasminogen binding surface protein. Mol. Immunol. **47**:541-550.

61. **Marcil, A., D. Harcus, D. Y. Thomas, and M. Whiteway**. 2002. Candida albicans killing by RAW 264.7 mouse macrophage cells: effects of Candida genotype, infection ratios, and gamma interferon treatment. Infect. Immun. **70**:6319-6329.

62. **Marodi, L., H. M. Korchak, and R. B. Johnston, Jr.** 1991. Mechanisms of host defense against Candida species. I. Phagocytosis by monocytes and monocyte-derived macrophages. J. Immunol. **146**:2783-2789.

63. **McGreal, E. P., M. Rosas, G. D. Brown, S. Zamze, S. Y. Wong, S. Gordon, L. Martinez-Pomares, and P. R. Taylor**. 2006. The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose. Glycobiology **16**:422-430.

64. **McKenzie, C. G., U. Koser, L. E. Lewis, J. M. Bain, H. M. Mora-Montes, R. N. Barker, N. A. Gow, and L. P. Erwig**. 2010. Contribution of Candida albicans cell wall components to recognition by and escape from murine macrophages. Infect. Immun. **78**:1650-1658.

65. **Means, T. K., E. Mylonakis, E. Tampakakis, R. A. Colvin, E. Seung, L. Puckett, M. F. Tai, C. R. Stewart, R. Pukkila-Worley, S. E. Hickman, K. J. Moore, S. B. Calderwood, N. Hacohen, A. D. Luster, and K. J. El**. 2009. Evolutionarily conserved recognition and innate immunity to fungal pathogens by the scavenger receptors SCARF1 and CD36. J. Exp. Med. **206**:637-653.

66. **Medzhitov, R. and C. A. Janeway, Jr.** 1997. Innate immunity: the virtues of a nonclonal system of recognition. Cell **91**:295-298.

67. **Mencacci, A., A. Bacci, E. Cenci, C. Montagnoli, S. Fiorucci, A. Casagrande, R. A. Flavell, F. Bistoni, and L. Romani**. 2000. Interleukin 18 restores defective Th1 immunity to Candida albicans in caspase 1-deficient mice. Infect. Immun. **68**:5126-5131.

68. **Meri, T., A. M. Blom, A. Hartmann, D. Lenk, S. Meri, and P. F. Zipfel**. 2004. The hyphal and yeast forms of Candida albicans bind the complement regulator C4b-binding protein. Infect. Immun. **72**:6633-6641.

69. **Meri, T., A. Hartmann, D. Lenk, R. Eck, R. Wurzner, J. Hellwage, S. Meri, and P. F. Zipfel**. 2002. The yeast Candida albicans binds complement regulators factor H and FHL-1. Infect. Immun. **70**:5185-5192.

70. **Miyazato, A., K. Nakamura, N. Yamamoto, H. M. Mora-Montes, M. Tanaka, Y. Abe, D. Tanno, K. Inden, X. Gang, K. Ishii, K. Takeda, S. Akira, S. Saijo, Y. Iwakura, Y. Adachi, N. Ohno, K. Mitsutake, N. A. Gow, M. Kaku, and K.**

Kawakami. 2009. Toll-like receptor 9-dependent activation of myeloid dendritic cells by Deoxynucleic acids from Candida albicans. Infect. Immun. **77**:3056-3064.

71. **Moretti, S., S. Bellocchio, P. Bonifazi, S. Bozza, T. Zelante, F. Bistoni, and L. Romani**. 2008. The contribution of PARs to inflammation and immunity to fungi. Mucosal. Immunol. **1**:156-168.

72. **Moyes, D. L., M. Runglall, C. Murciano, C. Shen, D. Nayar, S. Thavaraj, A. Kohli, A. Islam, H. Mora-Montes, S. J. Challacombe, and J. R. Naglik**. 2010. A biphasic innate immune MAPK response discriminates between the yeast and hyphal forms of Candida albicans in epithelial cells. Cell Host. Microbe **8**:225-235.

73. **Mullick, A., M. Elias, S. Picard, L. Bourget, O. Jovcevski, S. Gauthier, A. Tuite, P. Harakidas, C. Bihun, B. Massie, and P. Gros**. 2004. Dysregulated inflammatory response to Candida albicans in a C5-deficient mouse strain. Infect. Immun. **72**:5868-5876.

74. **Murad, A. M., P. Leng, M. Straffon, J. Wishart, S. Macaskill, D. MacCallum, N. Schnell, D. Talibi, D. Marechal, F. Tekaia, C. d'Enfert, C. Gaillardin, F. C. Odds, and A. J. Brown**. 2001. NRG1 represses yeast-hypha morphogenesis and hypha-specific gene expression in Candida albicans. EMBO J. **20**:4742-4752.

75. **Murciano, C., D. L. Moyes, M. Runglall, A. Islam, C. Mille, C. Fradin, D. Poulain, N. A. Gow, and J. R. Naglik**. 2011. Candida albicans cell wall glycosylation may be indirectly required for activation of epithelial cell proinflammatory responses. Infect. Immun.

76. **Nakagawa, Y., T. Kanbe, and I. Mizuguchi**. 2003. Disruption of the human pathogenic yeast Candida albicans catalase gene decreases survival in mouse-model infection and elevates susceptibility to higher temperature and to detergents. Microbiol. Immunol. **47**:395-403.

77. **Navarathna, D. H., K. W. Nickerson, G. E. Duhamel, T. R. Jerrels, and T. M. Petro**. 2007. Exogenous farnesol interferes with the normal progression of cytokine expression during candidiasis in a mouse model. Infect. Immun. **75**:4006-4011.

78. **Netea, M. G., G. D. Brown, B. J. Kullberg, and N. A. Gow**. 2008. An integrated model of the recognition of Candida albicans by the innate immune system. Nat. Rev. Microbiol. **6**:67-78.

79. **Netea, M. G., N. A. Gow, C. A. Munro, S. Bates, C. Collins, G. Ferwerda, R. P. Hobson, G. Bertram, H. B. Hughes, T. Jansen, L. Jacobs, E. T. Buurman, K. Gijzen, D. L. Williams, R. Torensma, A. McKinnon, D. M. MacCallum, F. C. Odds, J. W. Van der Meer, A. J. Brown, and B. J. Kullberg**. 2006. Immune sensing of Candida albicans requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. J. Clin. Invest **116**:1642-1650.

80. **Netea, M. G. and L. Marodi**. 2010. Innate immune mechanisms for recognition and uptake of Candida species. Trends Immunol. **31**:346-353.

81. **Netea, M. G., F. van de Veerdonk, I. Verschueren, J. W. Van der Meer, and B. J. Kullberg**. 2008. Role of TLR1 and TLR6 in the host defense against disseminated candidiasis. FEMS Immunol. Med. Microbiol. **52**:118-123.

82. **Netea, M. G., L. J. van Tits, J. H. Curfs, F. Amiot, J. F. Meis, J. W. Van der Meer, and B. J. Kullberg**. 1999. Increased susceptibility of TNF-alpha lymphotoxin-alpha double knockout mice to systemic candidiasis through impaired recruitment of neutrophils and phagocytosis of Candida albicans. J. Immunol. **163**:1498-1505.

83. **Newman, S. L., B. Bhugra, A. Holly, and R. E. Morris**. 2005. Enhanced killing of Candida albicans by human macrophages adherent to type 1 collagen matrices via induction of phagolysosomal fusion. Infect. Immun. **73**:770-777.

84. **Palmer, G. E.** 2011. Vacuolar trafficking and Candida albicans pathogenesis. Commun. Integr. Biol. **4**:240-242.

85. **Palmer, G. E., M. N. Kelly, and J. E. Sturtevant**. 2005. The Candida albicans vacuole is required for differentiation and efficient macrophage killing. Eukaryot. Cell **4**:1677-1686.

86. **Peltz, G., A. K. Zaas, M. Zheng, N. V. Solis, M. X. Zhang, H. H. Liu, Y. Hu, G. M. Boxx, Q. T. Phan, D. Dill, and S. G. Filler**. 2011. Next-generation computational genetic analysis: multiple complement alleles control survival after Candida albicans infection. Infect. Immun. **79**:4472-4479.

87. **Phan, Q. T., C. L. Myers, Y. Fu, D. C. Sheppard, M. R. Yeaman, W. H. Welch, A. S. Ibrahim, J. E. Edwards, Jr., and S. G. Filler**. 2007. Als3 is a Candida albicans invasin that binds to cadherins and induces endocytosis by host cells. PLoS. Biol. **5**:e64.

88. **Poltermann, S., A. Kunert, H. M. von der, R. Eck, A. Hartmann, and P. F. Zipfel**. 2007. Gpm1p is a factor H-, FHL-1-, and plasminogen-binding surface protein of Candida albicans. J. Biol. Chem. **282**:37537-37544.

89. **Puel, A., S. Cypowyj, J. Bustamante, J. F. Wright, L. Liu, H. K. Lim, M. Migaud, L. Israel, M. Chrabieh, M. Audry, M. Gumbleton, A. Toulon, C. Bodemer, J. El-Baghdadi, M. Whitters, T. Paradis, J. Brooks, M. Collins, N. M. Wolfman, S. Al-Muhsen, M. Galicchio, L. Abel, C. Picard, and J. L. Casanova**. 2011. Chronic mucocutaneous candidiasis in humans with inborn errors of interleukin-17 immunity. Science **332**:65-68.

90. **Qian, Q., M. A. Jutila, R. N. Van, and J. E. Cutler**. 1994. Elimination of mouse splenic macrophages correlates with increased susceptibility to experimental disseminated candidiasis. J. Immunol. **152**:5000-5008.

91. **Ray, T. L. and C. D. Payne**. 1988. Scanning electron microscopy of epidermal adherence and cavitation in murine candidiasis: a role for Candida acid proteinase. Infect. Immun. **56**:1942-1949.

92. **Romani, L., A. Mencacci, E. Cenci, R. Spaccapelo, C. Toniatti, P. Puccetti, F. Bistoni, and V. Poli**. 1996. Impaired neutrophil response and CD4+ T helper cell 1

development in interleukin 6-deficient mice infected with Candida albicans. J. Exp. Med. **183**:1345-1355.

93. **Saijo, S., S. Ikeda, K. Yamabe, S. Kakuta, H. Ishigame, A. Akitsu, N. Fujikado, T. Kusaka, S. Kubo, S. H. Chung, R. Komatsu, N. Miura, Y. Adachi, N. Ohno, K. Shibuya, N. Yamamoto, K. Kawakami, S. Yamasaki, T. Saito, S. Akira, and Y. Iwakura**. 2010. Dectin-2 recognition of alpha-mannans and induction of Th17 cell differentiation is essential for host defense against Candida albicans. Immunity. **32**:681-691.

94. **Sasada, M., A. Kubo, T. Nishimura, T. Kakita, T. Moriguchi, K. Yamamoto, and H. Uchino**. 1987. Candidacidal activity of monocyte-derived human macrophages: relationship between Candida killing and oxygen radical generation by human macrophages. J. Leukoc. Biol. **41**:289-294.

95. **Sato, K., X. L. Yang, T. Yudate, J. S. Chung, J. Wu, K. Luby-Phelps, R. P. Kimberly, D. Underhill, P. D. Cruz, Jr., and K. Ariizumi**. 2006. Dectin-2 is a pattern recognition receptor for fungi that couples with the Fc receptor gamma chain to induce innate immune responses. J. Biol. Chem. **281**:38854-38866.

96. **Schaller, M., U. Boeld, S. Oberbauer, G. Hamm, B. Hube, and H. C. Korting**. 2004. Polymorphonuclear leukocytes (PMNs) induce protective Th1-type cytokine epithelial responses in an in vitro model of oral candidosis. Microbiology **150**:2807-2813.

97. **Schwarzenberger, P., R. La, V, A. Miller, P. Ye, W. Huang, A. Zieske, S. Nelson, G. J. Bagby, D. Stoltz, R. L. Mynatt, M. Spriggs, and J. K. Kolls**. 1998. IL-17 stimulates granulopoiesis in mice: use of an alternate, novel gene therapy-derived method for in vivo evaluation of cytokines. J. Immunol. **161**:6383-6389.

98. **Sealy, P. I., B. Garner, E. Swiatlo, S. W. Chapman, and J. D. Cleary**. 2008. The interaction of mannose binding lectin (MBL) with mannose containing glycopeptides and the resultant potential impact on invasive fungal infection. Med. Mycol. 1-9.

99. **Soloviev, D. A., S. Jawhara, and W. A. Fonzi**. 2011. Regulation of innate immune response to Candida albicans infections by alphaMbeta2-Pra1p interaction. Infect. Immun. **79**:1546-1558.

100. **Steele, C., J. Leigh, R. Swoboda, and P. L. Fidel, Jr.** 2000. Growth inhibition of Candida by human oral epithelial cells. J. Infect. Dis. **182**:1479-1485.

101. **Tada, H., E. Nemoto, H. Shimauchi, T. Watanabe, T. Mikami, T. Matsumoto, N. Ohno, H. Tamura, K. Shibata, S. Akashi, K. Miyake, S. Sugawara, and H. Takada**. 2002. Saccharomyces cerevisiae- and Candida albicans-derived mannan induced production of tumor necrosis factor alpha by human monocytes in a CD14- and Toll-like receptor 4-dependent manner. Microbiol. Immunol. **46**:503-512.

102. **Tang, N., L. Liu, K. Kang, P. K. Mukherjee, M. Takahara, G. Chen, T. S. McCormick, K. D. Cooper, and M. Ghannoum**. 2004. Inhibition of monocytic interleukin-12 production by Candida albicans via selective activation of ERK mitogen-activated protein kinase. Infect. Immun. **72**:2513-2520.

103. **Tavanti, A., D. Campa, A. Bertozzi, G. Pardini, J. R. Naglik, R. Barale, and S. Senesi**. 2006. Candida albicans isolates with different genomic backgrounds display a differential response to macrophage infection. Microbes. Infect. **8**:791-800.

104. **Urban, C. F., D. Ermert, M. Schmid, U. bu-Abed, C. Goosmann, W. Nacken, V. Brinkmann, P. R. Jungblut, and A. Zychlinsky**. 2009. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. PLoS. Pathog. **5**:e1000639.

105. **van 't Wout, J. W., I. Linde, P. C. Leijh, and F. R. van**. 1988. Contribution of granulocytes and monocytes to resistance against experimental disseminated Candida albicans infection. Eur. J. Clin. Microbiol. Infect. Dis. **7**:736-741.

106. **van de Veerdonk, F., R. J. Marijnissen, B. J. Kullberg, H. J. Koenen, S. C. Cheng, I. Joosten, W. B. van den Berg, D. L. Williams, J. W. Van der Meer, L. A. Joosten, and M. G. Netea**. 2009. The macrophage mannose receptor induces IL-17 in response to Candida albicans. Cell Host. Microbe **5**:329-340.

107. **van de Veerdonk, F., M. G. Netea, T. J. Jansen, L. Jacobs, I. Verschueren, J. W. Van der Meer, and B. J. Kullberg**. 2008. Redundant role of TLR9 for anti-Candida host defense. Immunobiology **213**:613-620.

108. **van de Veerdonk, F., T. S. Plantinga, A. Hoischen, S. P. Smeekens, L. A. Joosten, C. Gilissen, P. Arts, D. C. Rosentul, A. J. Carmichael, Smits-van der Graaf CA, B. J. Kullberg, J. W. Van der Meer, D. Lilic, J. A. Veltman, and M. G. Netea**. 2011. STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. N. Engl. J. Med. **365**:54-61.

109. **van Enckevort, F. H., M. G. Netea, A. R. Hermus, C. G. Sweep, J. F. Meis, J. W. Van der Meer, and B. J. Kullberg**. 1999. Increased susceptibility to systemic candidiasis in interleukin-6 deficient mice. Med. Mycol. **37**:419-426.

110. **van, d., V, R. J. Marijnissen, B. J. Kullberg, H. J. Koenen, S. C. Cheng, I. Joosten, W. B. van den Berg, D. L. Williams, J. W. Van der Meer, L. A. Joosten, and M. G. Netea**. 2009. The macrophage mannose receptor induces IL-17 in response to Candida albicans. Cell Host. Microbe **5**:329-340.

111. **Vazquez-Torres, A., J. Jones-Carson, and E. Balish**. 1996. Peroxynitrite contributes to the candidacidal activity of nitric oxide-producing macrophages. Infect. Immun. **64**:3127-3133.

112. **Wachtler, B., D. Wilson, K. Haedicke, F. Dalle, and B. Hube**. 2011. From attachment to damage: defined genes of Candida albicans mediate adhesion, invasion and damage during interaction with oral epithelial cells. PLoS. One. **6**:e17046.

113. **Wang, M., P. K. Mukherjee, J. Chandra, A. A. Lattif, T. S. McCormick, and M. A. Ghannoum**. 2008. Characterization and partial purification of Candida albicans Secretory IL-12 Inhibitory Factor. BMC. Microbiol. **8**:31.

114. **Weindl, G., J. R. Naglik, S. Kaesler, T. Biedermann, B. Hube, H. C. Korting, and M. Schaller**. 2007. Human epithelial cells establish direct antifungal defense through TLR4-mediated signaling. J. Clin. Invest **117**:3664-3672.

115. **Wellington, M., K. Dolan, and D. J. Krysan**. 2009. Live Candida albicans suppresses production of reactive oxygen species in phagocytes. Infect. Immun. **77**:405-413.

116. **Westwater, C., E. Balish, and D. A. Schofield**. 2005. Candida albicans-conditioned medium protects yeast cells from oxidative stress: a possible link between quorum sensing and oxidative stress resistance. Eukaryot. Cell **4**:1654-1661.

117. **Wheeler, R. T., D. Kombe, S. D. Agarwala, and G. R. Fink**. 2008. Dynamic, morphotype-specific Candida albicans beta-glucan exposure during infection and drug treatment. PLoS. Pathog. **4**:e1000227.

118. **Whiteway, M. and C. Bachewich**. 2007. Morphogenesis in Candida albicans. Annu. Rev. Microbiol. **61**:529-553.

119. **Wisplinghoff, H., T. Bischoff, S. M. Tallent, H. Seifert, R. P. Wenzel, and M. B. Edmond**. 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect. Dis. **39**:309-317.

120. **Xiong, J., K. Kang, L. Liu, Y. Yoshida, K. D. Cooper, and M. A. Ghannoum**. 2000. Candida albicans and Candida krusei differentially induce human blood mononuclear cell interleukin-12 and gamma interferon production. Infect. Immun. **68**:2464-2469.

121. **Yano, J., E. Lilly, M. Barousse, and P. L. Fidel, Jr.** 2010. Epithelial cell-derived S100 calcium-binding proteins as key mediators in the hallmark acute neutrophil response during Candida vaginitis. Infect. Immun. **78**:5126-5137.

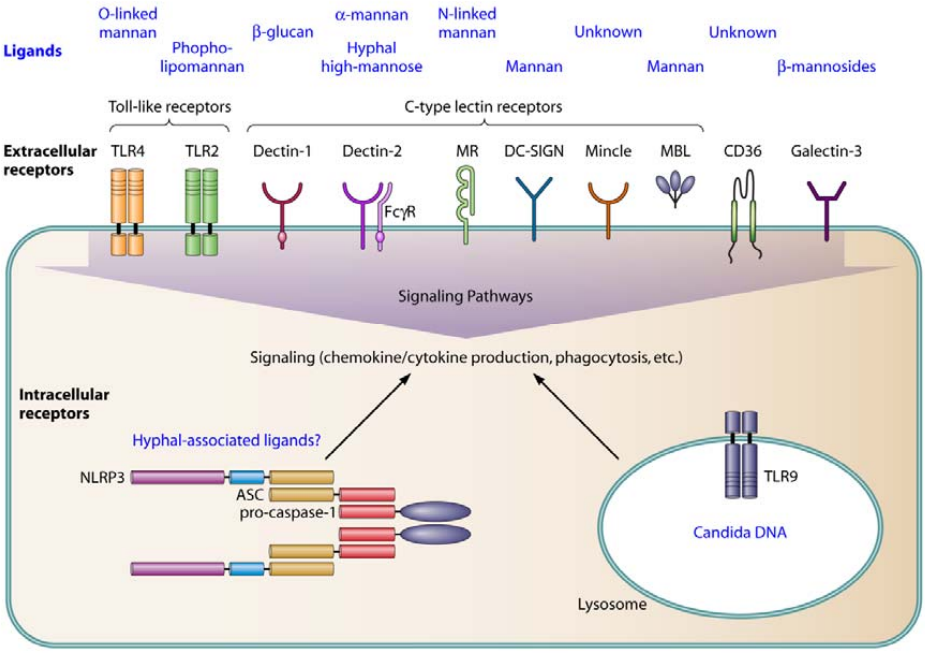
122. **Ye, P., F. H. Rodriguez, S. Kanaly, K. L. Stocking, J. Schurr, P. Schwarzenberger, P. Oliver, W. Huang, P. Zhang, J. Zhang, J. E. Shellito, G. J. Bagby, S. Nelson, K. Charrier, J. J. Peschon, and J. K. Kolls**. 2001. Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. J. Exp. Med. **194**:519-527.

123. **Zakikhany, K., J. R. Naglik, A. Schmidt-Westhausen, G. Holland, M. Schaller, and B. Hube**. 2007. In vivo transcript profiling of Candida albicans identifies a gene essential for interepithelial dissemination. Cell Microbiol. **9**:2938-2954.

124. **Zhang, M. X. and T. R. Kozel**. 1998. Mannan-specific immunoglobulin G antibodies in normal human serum accelerate binding of C3 to Candida albicans via the alternative complement pathway. Infect. Immun. **66**:4845-4850.

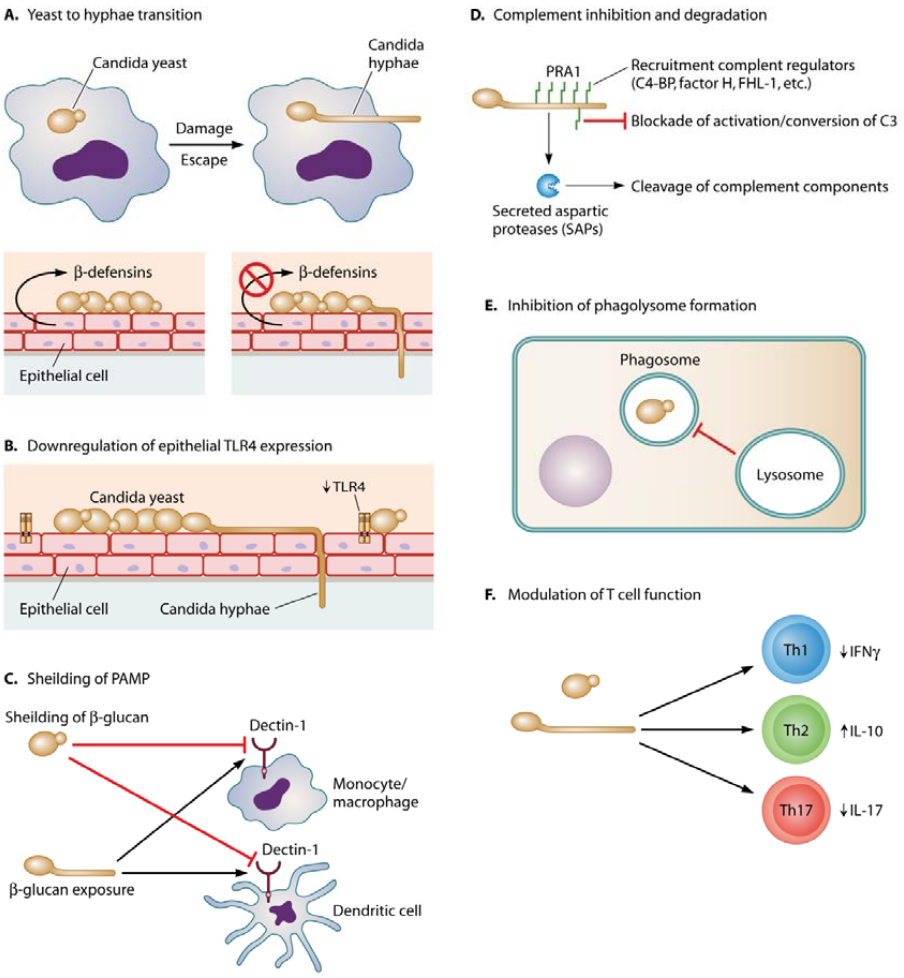
125. **Zhang, M. X., D. M. Lupan, and T. R. Kozel**. 1997. Mannan-specific immunoglobulin G antibodies in normal human serum mediate classical pathway initiation of C3 binding to Candida albicans. Infect. Immun. **65**:3822-3827.

Figure Legends
Figure 1



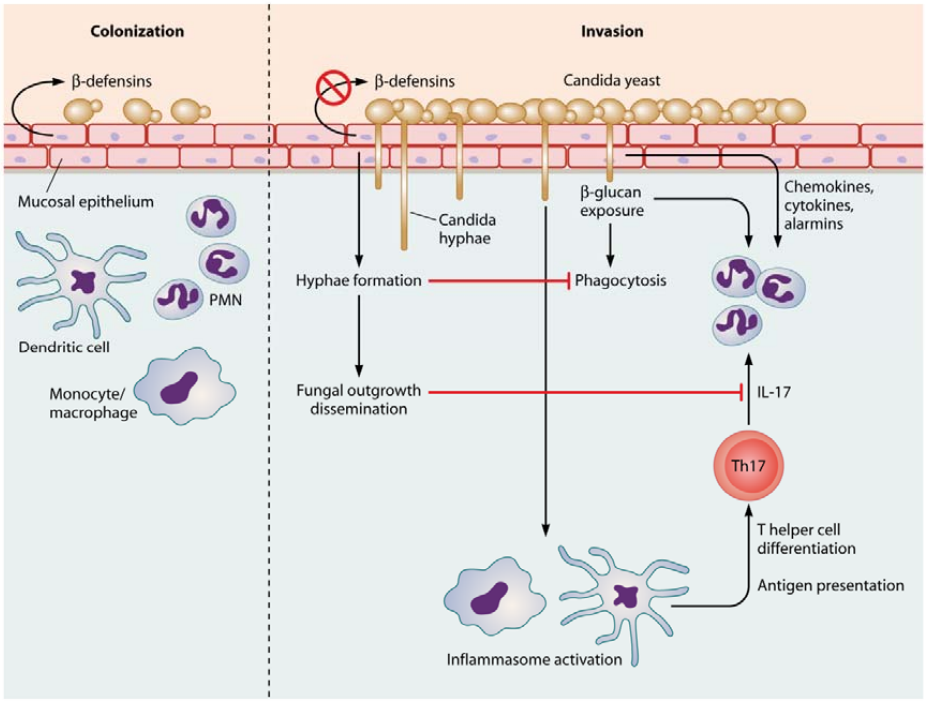
The major pattern recognition receptors (PRRs) and their corresponding *Candida* PAMPs. Candida cell wall components are mainly recognized extracellularly by Toll-like receptors and C-type lectin receptors on the host cell surface and leads to different downstream signaling such as chemokine/cytokine production and phagocytosis. Once *Candida* is internalized/phagocytosesd, the fungal PAMPs could further activate TLR9 or NLRP3 inflammasome activation.

Figure 2

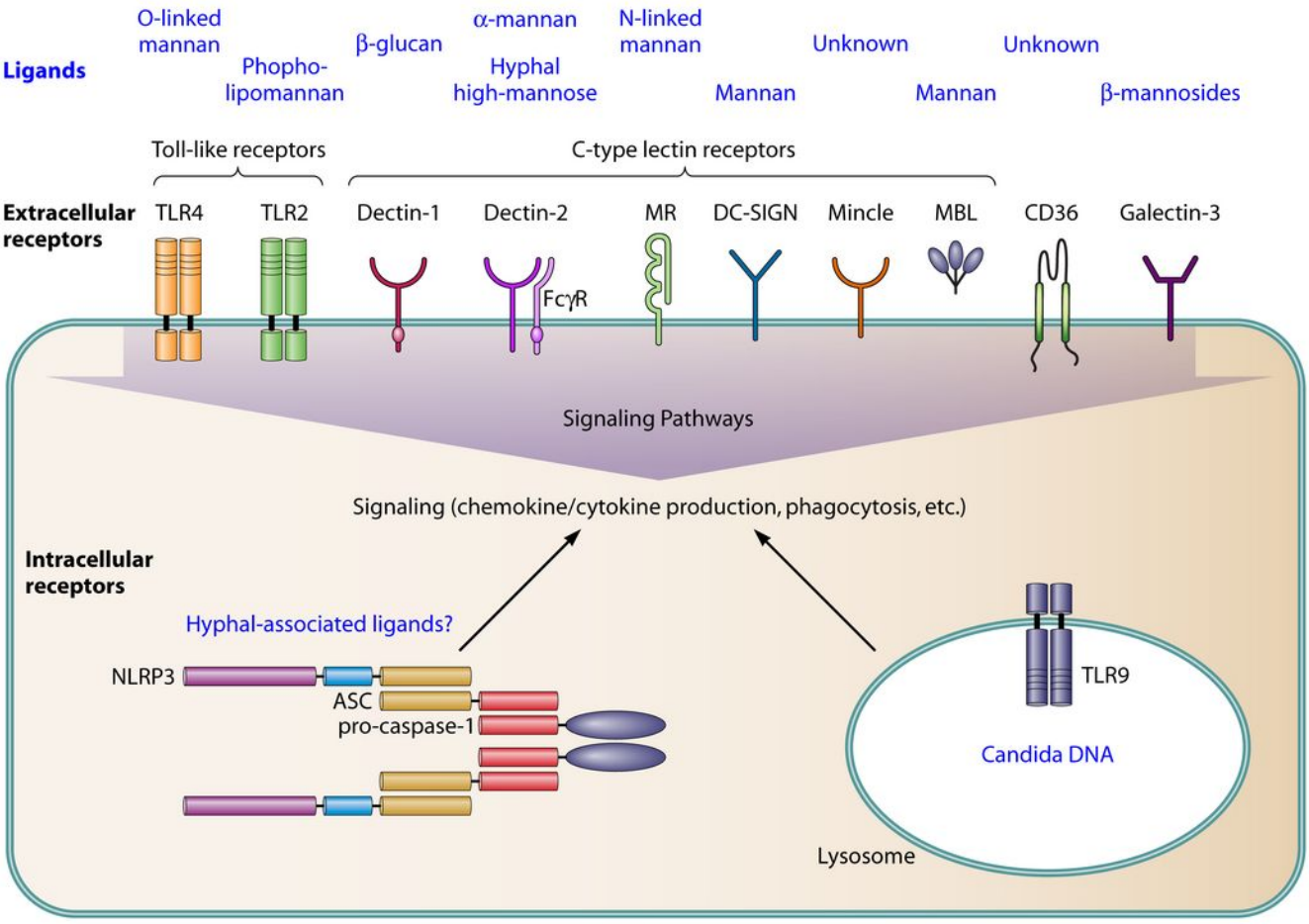


Candida albicans host innate system evasion strategies. **A.** Yeast to hyphae transition. **B.** Downregulation of epithelial TLR4 expression. **C.** Shielding of PAMP from PRR recognition. **D.** Inhibition or degradation of complement system. **E.** Inhibition of phagolysome formation. **F.** Modulation of T cell function.

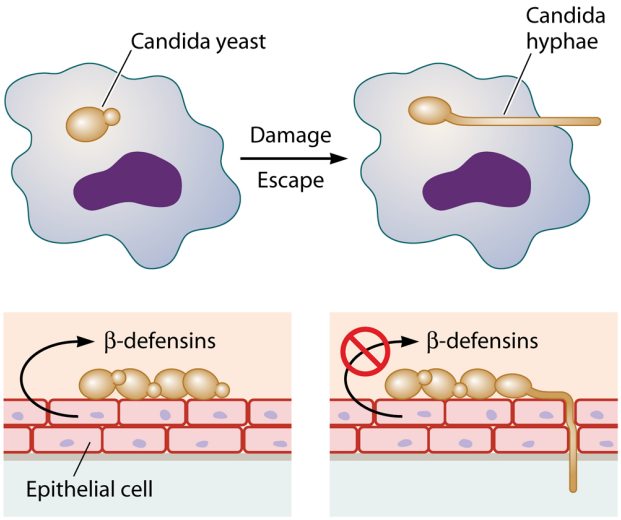
Figure 3



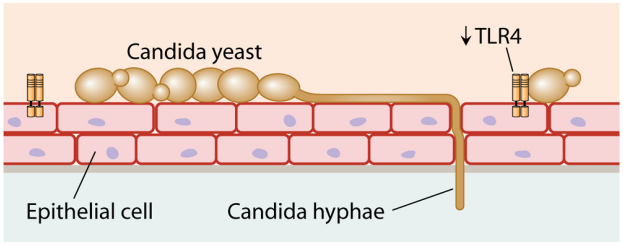
The schematic diagram of the interplay between *Candida albicans* and host innate immune system at the mucosal surface. Black lines denote host defense mechanisms. Red lines denote *Candida* invasion/escape mechanisms



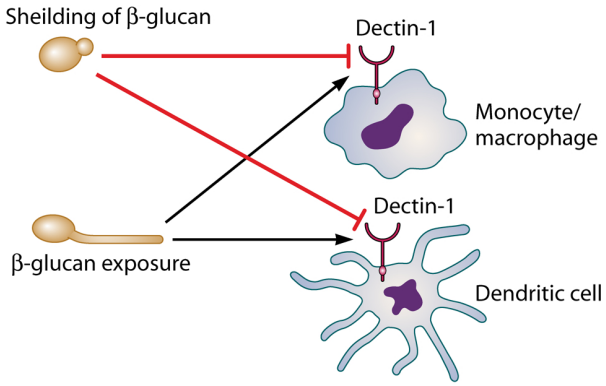
A. Yeast to hyphae transition



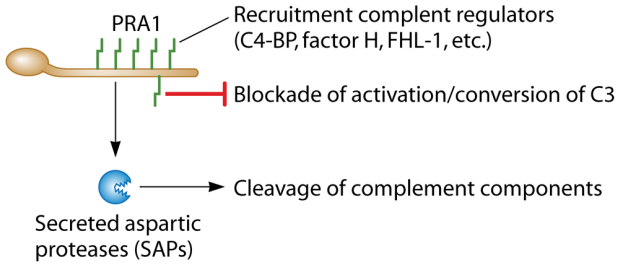
B. Downregulation of epithelial TLR4 expression



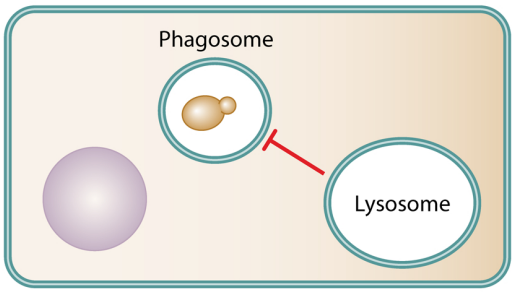
C. Sheilding of PAMP



D. Complement inhibition and degradation



E. Inhibition of phagolysome formation



F. Modulation of T cell function