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Candida albicans morphogenesis and host defence: discriminating invasion from colonization

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Abstract

Candida albicans is a common fungal pathogen of humans that colonizes the skin and mucosal surfaces of most healthy individuals. Until recently, little was known about the mechanisms by which mucosal antifungal defences tolerate colonizing *C. albicans* yet react strongly when hyphae of the same microorganism attempt to invade tissue. In this Review, we describe the properties of yeast cells and hyphae that are relevant to the interaction with the host, and the immunological mechanisms that differentially recognize colonizing versus invading *C. albicans*.

Many fungal pathogens of humans such as *Candida albicans*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Penicillium marneffei and Blastomyces dermatitidis* are capable of growing as unicellular budding yeast cells or as filamentous hyphae or pseudohyphae — a trait termed fungal dimorphism or, more precisely, fungal polymorphism. Those fungi with a life cycle that includes a saprophytic environmental phase, such as *H. capsulatum*, *P. brasiliensis*, *P. marneffei* and *B. dermatitidis*, normally grow in filamentous forms outside the human body, but convert to yeast forms in human tissues[1]. By contrast, there is no known terrestrial life cycle for *C. albicans*, the most common fungal pathogen of humans, and this microorganism can grow as both yeast and filamentous forms in the host[2] (Figure 1). The relative attributes of the yeast and filamentous forms of *C. albicans* during the colonization of skin and mucosae, and later in the invasion of the bloodstream and deep tissues, have long been debated[3-6].

One intriguing aspect of the interaction between the human host and the microbial flora that colonizes the skin and mucosal surfaces is the immunological tolerance of colonizing microorganisms by the mucosal host defences. This contrasts with the induction of potent defence mechanisms following tissue invasion. *C. albicans* colonizes the skin, genital and/or intestinal mucosae of 30-70% of healthy individuals at any given moment, and it is therefore noteworthy that under normal circumstances the fungus does not cause significant disease[7]. However, in the absence of proper immune recognition, the inability to control colonizing *C. albicans* on mucosal surfaces can lead to severe infection (e.g. in chronic mucocutaneous candidiasis (CMC) patients)[8]. Perturbations in the composition of the competitive commensal bacteria can also predispose individuals to *C. albicans* infections, and distorted immunological responses against *C. albicans* have been proposed to contribute to the pathological autoinflammation that occurs in patients with Crohn's disease[9].

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In this Review, we describe the properties of *C. albicans* yeast cells and hyphae that are relevant to the interaction with the host, and the immunological consequences of the shifting nature of the cell surface of these different cellular forms of the fungus. We discuss the molecular mechanisms that drive the morphogenetic changes of *C. albicans* from yeast cells to hyphae (a crucial step for invasion), the changes that lead to differential interaction with the host, and the immunological mechanisms that discriminate between tissue colonization and invasion. Although many aspects discussed in this review could be extrapolated to other microorganisms, we will focus on *C. albicans* as a prototypic fungal pathogen, primarily in relation to the immunological balance between tolerance of colonization and host immunity to invasion.

Morphogenetic changes in the cell wall

The cell wall of *C. albicans* contains carbohydrates and cell wall proteins (CWPs) that are not present in the human body. The cell wall therefore represents an ideal immunological target to discriminate self from non-self and consequently the majority of fungal PAMPs (pathogen-associated molecular patterns) that activate and modulate immune responses are cell wall components[10, 11].

In well-preserved images of the *C. albicans* cell wall, two main layers can be distinguished: an outer layer that is composed of glycoproteins and an inner layer that contains skeletal polysaccharides (Figure 2). The *C. albicans* cell wall contains 80-90% carbohydrate, with the outer layer predominantly consisting of *O*- and *N*-linked mannose polymers (mannans) that are covalently associated with proteins to form glycoproteins**.** The expression of CWPs is highly regulated during the yeast-to-hypha transition, and genes encoding hypha-specific proteins such as Hwp1, Hyr1 and Als3 are amongst the most highly upregulated genes during this switch[12-1415, 16]. Cytoplasmic immunodominant antigens that are not normally associated with the cell wall have also been recognised [16, 17]. Nevertheless, the hypha-specific CWPs are major antigens and in addition they function as adhesins and invasins that can modulate immune responses[18, 19]. The extent to which the structure of the mannans decorating these CWPs differs in yeast cells and in hyphae is not certain, although it is known that CWPs in hyphae contain less phosphodiester-linked, β-1,2 manno-oligosaccharides than do CWPs in yeast cells [20]. Other properties of the mannan might also differ between the yeast and hyphal forms.

The inner layer of the cell wall contains the skeletal polysaccharides chitin and β-1,3 glucan, which confer strength and cell shape (Figure 2). The CWPs from the outer layer are attached to this framework, predominantly by glycosylphosphatidylinositol (GPI) remnants that are linked to the skeletal polysaccharides through a more flexible β-1,6 glucan. In *C. albicans* yeast cells, chitin normally comprises ~2% of the cell wall dry weight, whereas β-1,3 glucan and β-1,6 glucan account for 40% and 20%, respectively. However, the chitin content of yeast cells can be increased 3- to 4-fold by conditions that affect the cell wall, such as inhibition of β-1,3 glucan synthesis by caspofungin [21]. Hyphal cells contain approximately 3-5 times more chitin than yeast cells [22]. In addition, most chitin in yeast cells resides in the bud scars [23]. Because hyphae have no bud scars, it is likely that the actual chitin content of the lateral cell walls of yeast and hyphae could differ by as much as an order of magnitude. This could be relevant for the interaction with the immune system, as chitin purified from *C. albicans* blocks recognition of *C. albicans* yeast cells by monocytes [24]. By contrast, the β-1,3 glucan content of yeast and hyphal cells is similar. However, β-1,3 glucan might be less exposed at the hyphal cell surface than on yeast cells, as it also normally only occurs to a significant extent at the sites of bud scars[25]. How the structural and chemical properties of the cell wall differ in pseudohyphal cells compared with yeast and hyphal cells, and how

pseudohyphae differ from yeast cells and hyphae in terms of immune recognition, is presently unknown.

Therefore, although the basic components of the *C. albicans* cell wall are similar in the yeast and filamentous forms, the surface proteome and the amounts of individual PAMPs presented to immune cells differ substantially. In addition, the conditions under which cells are grown can cause substantial changes in the cell wall, even if the morphology of the cell is unaltered [26]. Owing to its highly regulated and responsive nature, the cell wall thus represents a moving target that presents a significant challenge for the host immune system.

Regulation of morphogenesis

As described above, cellular morphogenesis in *C. albicans* is associated with changes in cell wall composition and architecture, and this influences detection by the host immune system and the subsequent response. In general, these observations have been made using standard experimental conditions for inducing hypha formation *in vitro*, including the addition of serum or the use of mutants that are defective in genes encoding regulators of hypha formation such as the *cph1/cph1 efg1/efg1* double mutant [27] or the *hgc1*/*hgc1* mutant[28]. However, morphogenesis in *C. albicans* can be induced by several mechanisms (Figure 3) that are activated by various environmental triggers that might have different effects on the fungal cell surface. Therefore, *C. albicans* probably generates subtly different forms of hyphae in different host niches, thereby affecting the host immune response in a niche-specific manner.

Protein kinase A signalling

Signalling through cAMP–protein kinase A (PKA) is the major pathway by which hyphal growth is induced in *C. albicans* (Figure 3A). A range of environmental signals regulate cAMP–PKA signalling, with most inputs being coordinated through the adenylate cyclase Cyr1 (also called Cdc35). Some signals appear to act directly on the adenylate cyclase itself, such as serum muramyl dipeptide (MDP) and CO_2 [29, 30]. Other signals, such as glucose and amino acids [31-33], act indirectly on the adenylate cyclase by upstream signalling through the small GTPase Ras1 or the G-protein coupled receptor Gpr1 and its Gα protein Gpa2, respectively. Additional environmental signals influence cAMP–PKA signalling, although their exact mode of action has not yet been clarified. For example, hyphal development is inhibited at temperatures below 35°C through the action of the molecular chaperone heat shock protein 90 (Hsp90)[34]. Also, the quorum sensing molecule farnesol, which accumulates at high *C. albicans* cell densities, inhibits hyphal formation by downregulating cAMP-PKA signalling [35], indicating that cell density probably affects morphogenesis at the site of infection.

Adenylate cyclase activation leads to elevated levels of cAMP, which causes dissociation of the PKA regulatory subunit (Bcy1) from the catalytic subunits (Tpk1 or Tpk2), thereby activating the kinase. PKA is thereafter thought to phosphorylate and activate the transcription factor Efg1 [36], which is essential for the activation of hypha-specific genes in *C. albicans* [37]. These genes include those encoding the cell surface adhesins, invasins and immune modulators Als3, Hwp1 and Hyr1, as well as the hypha-specific cyclin Hgc1. Hgc1 interacts with the cyclin-dependent kinase (CDK) Cdc28 to mediate phosphorylation of the septins Cdc11 and Sep7, thereby promoting polarized growth and cell separation [38] (Figure 3A). These inputs promote the maintenance of hyphal development once polarized growth has been triggered by the Cdc28-Ccn1 complex, which binds to septin complexes and phosphorylates the septin Cdc11 [39].

Alternative signalling mechanisms

Additional environmental signals stimulate hyphal development through alternative signalling mechanisms (Figure 3B). Low levels of nitrogen due to starvation activate hyphal development through a mitogen-activated protein (MAP) kinase cascade as well as the cAMP–PKA pathway. This MAP kinase cascade, which includes proteins with structural similarity to MAP kinase components in other organisms, namely Hst7 and Cek1, subsequently activates the transcription factor Cph1 (Figure 3B) [33]. Cph1 also plays a role in pheromone signalling during mating in *C. albicans* [40]. Whether responding to starvation or mating pheromone, the activation of this MAPK pathway plays an important role in the formation of chlamydospores, which are thought to promote *C. albicans* survival in unfavourable conditions[41]. Ambient pH also influences morphogenesis and CWP synthesis through the Rim101 pathway. Rim101 is a zinc finger-containing transcription factor that is inactive under acidic conditions but activated at neutral to alkaline conditions and alters gene expression [42, 43], Hypha development can also be stimulated by hypoxia and by embedding *C. albicans* cells in a matrix. These inputs activate morphogenesis through additional alternative pathways that involve the transcription factors Efg1 and Efh1 during hypoxia-induced hypha formation and the morphogenetic regulator Czf1 during matrix-induced hypha formation (Figure 3B) [44, 45].

Genotoxic stress

C. albicans morphogenesis can also be driven by other mechanisms. Genotoxic stresses that disturb cell cycle progression can stimulate filamentous growth in *C. albicans* [46] (Figure 3C). For example, mutations that interfere with DNA damage repair, or the pharmacological inhibition of DNA replication (for example with hydroxyurea), can trigger hyphal growth [47, 48]. These genotoxic insults can act through the DNA damage or DNA replication checkpoints to promote hyphal development through the Rad53 checkpoint kinase [46]. Reactive oxygen species generated by phagocytic cells also induce genotoxic stress. For example, hydrogen peroxide activates hyphal development through Rad53 signalling [49]. Therefore, the *C. albicans* hyphae that escape from macrophages might not be equivalent to the hyphae that invade mucosal surfaces, further complicating the host-pathogen interaction.

Clearly, *C. albicans* morphogenesis is regulated by a complex regulatory network that coordinates an array of environmental inputs. As a result, not all *C. albicans* hyphae are identical and the fungal cell surface will differ depending on the nature of the microenvironment that the cell occupies. This will influence the correspondingly complex network of immune detection mechanisms and responses. It is interesting to note that the presence of pseudohyphae has yet to be considered in terms of their relevance to pathogenesis and the immune response.

Morphogenesis and pathogen recognition

How the immune system interacts with the various morphogenetic forms of fungi is relatively poorly understood. In the case of *C. albicans*, one intriguing possibility is that differential recognition of yeast and hypha could be the key to understanding the mechanisms through which different immune responses are elicited during colonization and invasion.

During an infection with *C. albicans*, the initial response of the innate immune system is determined by the recognition of fungal cell wall components by pattern recognition receptors (PRRs) on the surface of innate immune cells [50, 51]. Many excellent reviews have been published on this subject [10, 11, 52], and therefore we will only briefly summarize the most important aspects (Figure 4). Immune responses can be stimulated not only by components from the outer layer of the cell wall but also by components from the inner

layer, as these can be exposed at the cell surface at bud scars or by the action of antifungal drugs and host enzymes [53]. Several classes of PRRs have been implicated in recognition of *C. albicans* PAMPs and the induction of innate host responses: the Toll-like receptors (TLRs), the C-type lectin receptors (CLRs) and the NOD-like receptors (NLRs).

TLRs

Mammalian TLRs have an extracellular domain containing leucine-rich repeats that is responsible for the recognition of microbial structures and a cytoplasmic Toll/IL-1 receptor (TIR) domain that is responsible for inducing intracellular responses. Shortly after the discovery of TLRs, TLR2 and TLR6 were shown to be involved in the recognition of the fungal cell wall preparation zymosan[54]. Moreover, MyD88, which is an adaptor molecule that is shared by most TLRs, is crucial for antifungal defence *in vivo*[55], strongly suggesting that TLRs have a key role in host defence against fungi. TLR2, TLR4 and TLR9 are involved in recognition of *C. albicans* and the induction of proinflammatory cytokine production *in vitro* [56-58], and knock-out mice deficient in TLR2 or TLR4 have an altered susceptibility to disseminated candidiasis [57, 59]. By contrast, TLR1 and TLR6 have redundant roles in host defence against *C. albicans* [60].

CLRs

CLRs mostly recognize polysaccharide structures of microorganisms and are probably the most important PRR family in the recognition of fungi. *C. albicans* is recognized by several CLRs including dectin-1, dectin-2, the macrophage mannose receptor (MMR), the dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN), the macrophage-inducible C-type lectin (Mincle), and the circulating mannose-binding lectin (MBL).

Dectin-1 recognizes β-1,3-glucans in a calcium-independent manner, and is involved in both ligand uptake and phagocytosis, as well as proinflammatory cytokine production [61]. Dectin-1 intracellular signalling is mediated either through the adaptor molecule CARD9 [62] or the non-canonical nuclear factor (NF)-κB pathway through Raf1[63]. In addition, dectin-1 cooperates with TLRs, leading to synergistic proinflammatory responses [64, 65]. *In vivo* studies [66-68] together with the discovery of primary immunodeficiencies associated with fungal infections due to host mutations in dectin-1 or CARD9 [69, 70], have confirmed the important role of dectin-1 and CARD9 in antifungal host defence.

MMR is involved in recognition of *C. albicans* and other fungi [71] . In particular, it recognizes branched *N*-bound mannans from *C. albicans* [72]. It is unclear whether MMR has a role in phagocytosis [73], similar to that of dectin-1, but it might mediate the intracellular signals leading to cytokine production [74, 75].

Dectin-2 is mainly present on myeloid cells and maturing inflammatory monocytes [76]. It recognizes mannose-rich structures [77] and interacts with the Fc gamma receptor (FcγR) to induce TNF production in response to *C. albicans* hyphae [78]. Dectin-2 has important T-helper 17 (T_H17) cell-inducing activities that include the recruitment and activation of neutrophils and the induction of defensins during *C. albicans* infection [79]. Another CLR, DC-SIGN, is primarily expressed on mature dendritic cells (DCs) and exhibits calcium-dependent recognition of mannose-rich structures [80]. DC-SIGN mediates uptake of *C. albicans* yeast cells and recognizes *N*-linked mannans from *C. albicans* [81, 82]. Although DC-SIGN can induce immunosuppression by stimulating IL-10 production[83], this effect remains to be demonstrated for fungal infections. Several other CLRs, such as Mincle [84] and the CD36/Scarf scavenger receptors [85], might also contribute to host defence against *C. albicans*. Another CLR, galectin-3, which is mainly expressed by macrophages, is involved

in the recognition of *C. albicans* β– mannosides , in close collaboration with TLR2, especially at the intestinal mucosa [86, 87].

NOD-like receptors (NLRs)—In addition to TLRs and CLRs, which are usually associated with the cell membrane, mammals have evolved a second line of recognition receptors located inside the cytoplasm, that can trigger an innate immune response to intracellular pathogens. Two main classes of cytoplasmic PRRs have been described to date: RigI helicases, which are mainly receptors for viruses, and NLRs. NLRs have two important functions for host defence against intracellular pathogens: recognition of bacterial peptidoglycans (by NOD1 and NOD2) and activation of the inflammasome. Inflammasomes are protein platforms that, upon recognition of a microbial PAMP or an endogenous danger signal (e.g. uric acid or ATP released from damaged epithelial cells), induce activation of caspase-1, which in turn processes pro-IL-1β and pro-IL-18 into active cytokines [88]. Although NOD1 and NOD2 are not involved in the recognition of *C. albicans* [89], inflammasomes containing another NLR, NLRP3, have an important role in *C. albicans*-induced inflammation. Mice that are deficient in either IL-1α or IL-1β show increased mortality when infected with *C. albicans*, and endogenous IL-1α and IL-1β are required for the induction of T-helper 1 (T_H1) responses that are important for host defence against disseminated candidiasis [90]. Nlrp3-deficient and apoptosis-associated speck-like protein containing a caspase recruitment domain (Asc)-deficient mice are more susceptible to both systemic [91-93] and mucosal [94] *C. albicans* infections, suggesting an important role for NLRP3-inflammasome-mediated production of IL-1 during host defence against *C. albicans*. In addition, the inflammasome-mediated production of IL-1β and the subsequent T_H17 response could be a major mechanism of mucosal antifungal host defence, as detailed below.

Differential recognition of yeast and hyphae

Although much progress has been made on the recognition of *C. albicans* PAMPs, much less is known regarding the differential recognition of the two main morphogenetic forms of this fungus, yeast and hyphae. In contrast to yeast cells**,** hyphae do not induce IL-12 production from DCs, but instead induce IL-4, resulting in a more anti-inflammatory immune response [95]. Hyphae also fail to induce the T_H1-typical cytokine IFNγ, which is attributed to the lack of hyphae recognition by TLR4 [96]. The structure that is recognized by TLR4 on yeast cells, and that is absent or hidden on hyphae, is unknown but it could be a mannan structure, as TLR4 can recognize mannans [72] and mannans can induce IFNγ [75]. In addition, the differential exposure of β–glucans on the surface of yeasts and hyphae has been proposed to account for differences in cytokine stimulation, with β–glucans being exposed on the bud scars of yeasts, but not on hyphae [97]. However, hyphal β–glucans can be recognized by the immune system through a process mediated by dectin-1, suggesting that β–glucan might be accessible on the hyphal surface, probably because mannan fibrils are shorter and less abundant in hyphae than in yeast cells [53, 98]. In addition, differential recognition of mannans from hyphae and yeasts by dectin-2 has been proposed [99], although the differences in mannan structure that are responsible for these effects are unclear [20, 100].

Discriminating mucosal colonization and tissue invasion

The capacity to undergo a reversible yeast-hypha transformation is linked to the virulence of *C. albicans* [27]. When infecting humans and animals, *C. albicans* hyphae predominate at the primary site of infiltration of epithelial cell layers and tissues, whereas yeast cells are generally found either on the epithelial cell surface or emerging from penetrating hyphae that are infiltrating tissues [101, 102]. The molecular responses of *C. albicans* are modulated by population density through quorum sensing, and this could modulate fungal growth *in vivo* [31, 103]. Nevertheless, it is thought that there is a threshold for the amount of *C. albicans*

that is tolerated by the host**.** The host must keep the fungal burden below this threshold and distinguish non-pathogenic *C. albicans* cells from invasive and potentially life-threatening cells of the same fungus to maintain homeostasis [104].

Sensing invasion by epithelial cells

Mucosal epithelial cells not only provide a physical barrier but can also recognize fungi and respond by producing cytokines[105, 106]. *C. albicans* interacts closely with these non-phagocytic cells both as a commensal (Figure 5A) and during the active phases of invasion (Figure 5B). Mucosal surfaces in healthy individuals are frequently colonized with *C. albicans*. The relatively small number of yeast cells present does not induce epithelial cell damage, and thus does not trigger a cytokine response in epithelial cells or in mucosal macrophages. Invasive disease occurs when *C. albicans* crosses tissue surfaces. One mechanism by which *C. albicans* cells cross the epithelial surface is endocytosis, whereby the fungus is internalized by epithelial cells [107-109]. Although both yeast and hyphae can induce endocytosis, *C. albicans* hyphae are thought to be more efficient at stimulating this process. This view is supported by the observation that the *efg1/efg1* mutant, which is not able to form hyphae, is much less capable of inducing endocytosis than wild-type cells [110]. Hyphae might thus have molecules on their cell wall that can bind to epithelial cell receptors leading to the induction of endocytosis. One such molecule could be Als3, an hyphal CWP that binds to E-cadherin and N-cadherin and induces endocytosis [19].

C. albicans can also directly penetrate tissues, which can lead to damage of the epithelial surface. Hyphae are more potent in causing epithelial damage than yeast cells [109, 111], and the damage occurs by the production of lytic enzymes, such as secreted aspartyl proteinases (SAPs) [112, 113], although it also seems to require normal hyphal growth [111, 114].

Epithelial cells can respond to *C. albicans* in a two-phase MAP kinase (MAPK) pathway that enables them to discriminate between the commensal yeast and the invasive hyphal form of the fungus [104]. *In vitro* and *in vivo* experiments indicate that a first phase of MAPK activation involves the activation of the MAPK p38 and subsequent activation of the transcription factors c-Jun and c-Fos. This is independent of the morphological status of *C. albicans*. However, a second phase of activation of the MAP kinases p38 and ERK1/ERK2 results in activation of the transcription factor c-Fos and the MAPK phosphatase MKP1, which is necessary to induce a cytokine response by oral epithelial cells. Importantly, this second phase can be induced by *C. albicans* hyphae but not yeast. Although previously it has been shown that the upregulation of TLR4 expression in *C. albicans*-infected human oral epithelium is directly associated with protection against fungal invasion of this tissue [106], TLR4 was not identified as an important receptor for the induction of these dual MAPK pathways. In addition, there was no evidence supporting a role for TLR2, dectin-1 or MMR. The addition of neutrophils to *C. albicans*-infected oral human epithelium not only strongly upregulated epithelial TLR4 expression, but also resulted in the release of LL-37, a cathelicidin-derived antimicrobial peptide that correlates with protection to infection [115]. It remains to be elucidated which PRRs mediate recognition of hyphae, allowing the epithelial cell to discriminate between colonization and invasion with *C. albicans*.

Mucosal antifungal immune mechanisms

In addition to epithelial cells, tissue macrophages and DCs sample the contents of the microbial flora on the mucosa. The mechanisms that allow these immune cells to discriminate between yeasts and hyphae were unknown until recently, but the description of T_H17 cells and the role of inflammasomes in host defence has shed new light on these processes (Figure 5).

CMC is a disease that is associated with defects in controlling *C. albicans* at the skin and mucosal surfaces. CMC can present itself as an isolated disease (autosomal dominant CMC (AD-CMC)), or it can be part of a primary immunodeficiency disorder, such as hyper-IgE syndrome (HIES) or autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy (APECED). The common defect in these diseases that leads to the inability to control *C. albicans* at the mucosa seems to be an insufficient *C. albicans*-induced T_H17 response. Patients with AD-CMC show a defect in the production of the T_H17 cytokines IL-17A, IL-17F and IL-22 [116, 117]. Most AD-CMC patients have mutations in the coiled-coil domain of signal transducer and activator of transcription 1 (STAT1), which encodes one of the main adaptor molecules of the IL-23R and IL-12R pathways. As IL-23 stimulation of IL-23R is crucial for the induction of T_H17 responses, and the IL-12–IL-12R interaction is central for the induction of T_H1 cytokines, defects in STAT1 result in defective T_H17 and T_H1 responses[118, 119]. Moreover, a recent report identified genetic defects in the IL-17 receptor (IL-17R) and in IL-17F in some patients with CMC [120]. On the other hand, patients with APECED were found to have neutralizing autoantibodies against T_H17 cytokines [121, 122]. Furthermore, patients with HIES have a defect in STAT3, which has been shown to be crucial for the development of T_H17 cells [123-125]. These data strongly argue that the T_H17 subset and its cytokines IL-17A, IL-17F and IL-22 are pivotal for maintaining the balance between colonization and mucosal disease caused by *C. albicans*. Indeed, it has been shown that patients with defects in the dectin-1–CARD9 pathway, which is important for an optimal *C. albicans*-induced T_H17 response[69,70], have mucosal candidiasis. Furthermore, heterozygous carriers of an early-stop-codon mutation (Tyr238X) in the β-glucan receptor dectin-1, which leads to defective β-glucan binding, are more likely to be colonized with *C. albicans* when undergoing stem-cell transplantation 126. Thus, T_H17 responses and the pathways that induce these responses play an essential part in controlling commensal *C. albicans* at the site of the mucosa.

Interestingly, patients with T_H17 immunodeficiencies suffer from oral and skin candidiasis, but not from vulvovaginal candidiasis (VVC). It should also be noted that two decades of research from animal models and clinical studies have revealed that adaptive immunity does not protect against VVC[127]. A live challenge model in humans revealed that symptomatic infection correlated with a neutrophil infiltrate in the vaginal lumen and elevated fungal burden. Similar to the human setting, a robust vaginal polymorphonuclear neutrophil (PMN) migration occurs in a subset of mice without affecting vaginal fungal burden. Furthermore, the neutrophil chemotactic factors S100A8 and S100A9 can be produced by vaginal epithelial cells following interaction with *C. albicans*, demonstrating that the vaginal epithelium can contribute to a strong neutrophilic influx in VVC[128]. The pathophysiology of VVC seems to differ from other mucosal candidiasis, and therefore it has been proposed that VVC occurs when overwhelming, rather than deficient, immune responses are present [127, 128].

Sensing invasion: the caspase-1–T_H17 axis

Important differences between stimulation of host defence mechanisms by yeast and hyphae have been shown, and certain immune responses such as the T_H17 response are known to be crucial for mucosal defence. Nevertheless, the question remains why are these mechanisms induced by invading, but not by colonizing, fungi? To answer this question, we need to decipher the steps necessary for mounting an effective T_H17 response.

The induction of T_H17 responses requires a specific cocktail of cytokines that are produced by antigen-presenting cells: IL-23, IL-1, IL-6 and (at least in mice) TGFβ [129-132]. Whereas no differences in the induction of IL-23 and IL-6 production are found between yeast and hyphae, only hyphae can induce production of IL-1β by macrophages [133]. This is followed

by the induction of an antifungal T_H17 response [98]. Additionally, the products of tryptophan metabolism released by *C. albicans* hyphae can downmodulate T_H17 responses[140] (Figure 5).

As mentioned earlier, the induction of IL-1β is mediated by the NLRP3 inflammasome. Importantly, *C. albicans* mutants that cannot form hyphae are unable to activate the inflammasome and induce IL-1β secretion by macrophages [133], which suggests that hyphae, but not yeasts, can induce the activation of the NLRP3 inflammasome. In addition, the dectin-1 pathway is important for pro-IL-1β production, which can be processed into IL-1β [134].

These observations allow us to propose a model to explain the mechanism by which tissue antigen-presenting cells discriminate between *C. albicans* colonization and invasion (Figure 5). According to this model, once *C. albicans* yeast start to germinate into hyphae, they trigger activation of the NLRP3 inflammasome in macrophages and DCs, which leads to the activation of caspase-1. Activation of PRRs, such as dectin-1, TLR2 and MR, by the recognition of fungal components induces transcription of pro-IL-1β[94, 134]. Under normal circumstances of mere colonization, no significant activation of caspase-1 takes place and therefore very little pro-IL-1β is processed into its active form. It should be noted however that there is low-grade activation of the cellular immune response during colonization, as suggested by the presence of memory T_H17 responses in healthy individuals[75].

When macrophages sense the formation of *C. albicans* hyphae, caspase-1 is activated and active IL-1β is produced. IL-1β subsequently induces a T_H17 response which includes production of cytokines IL-17 and IL-22, and which is essential for the control of *C. albicans* at mucosal surfaces. IL-17 recruits neutrophils [135] that phagocytose and kill the invading hyphae, whereas IL-22 induces the production of defensins from epithelial cells [136], which also contribute to the killing of invading *C. albicans* cells. An additional activator of the inflammasome is the ATP [137] that is released from damaged epithelial cells during mucosal invasion. Interestingly, ATP released from epithelial cells can also directly drive T_H17 cell differentiation (Figure 5B) [138]. In conclusion, there is a two-way interaction between epithelial and immune cells during invasion: epithelial cells release cytokines and ATP to activatethe inflammasome in immune cells and IL-22 released by T_H17 cells induces defensin production from epithelial cells.

Conclusions and future perspectives

Much has recently been learned about the mechanisms through which the immune system discriminates between *C. albicans* colonization and invasion of the mucosa. These discoveries not only have important consequences for our understanding of the immune response to fungi, but they also open new avenues for future research. Many details regarding the precise fungal PAMPs and the host PRRs that recognize them remain to be discovered. 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? What are the consequences of the deregulation of antifungal mucosal immunity for diseases such as Crohn's disease and ulcerative colitis? What is the relevance of circulating *C. albicans*-induced memory T_H17 cells in the pathogenesis of autoimmune disorders related to T_H17 such as rheumatoid arthritis, psoriasis and multiple sclerosis? Do T_H17 cells in the mucosa that have been induced by *C. albicans* have a similar protective effect against intestinal Gram-negative pathogens as do the intestinal T_H17 cells that are induced by segmented filamentous bacteria [139]?

Finally, although this review focused on *C. albicans*, it is likely that similar mechanisms may contribute to the discrimination between colonization and invasion of other fungal or bacterial pathogens. The gastrointestinal tract of mammals is inhabited by hundreds of distinct species of commensal microorganisms that exist in a mutualistic relationship with the host. The commensal microbiota throughout the whole gastrointestinal tract might protect the body against invading mucosal pathogens, but when the mechanisms that control the growth of these commensals are disturbed we might pay a high price: autoimmunity. Understanding how *C. albicans* influences local immune responses at mucosal surfaces may lead to the development of new and exciting ways to modulate mucosal immunity for therapeutic purposes.

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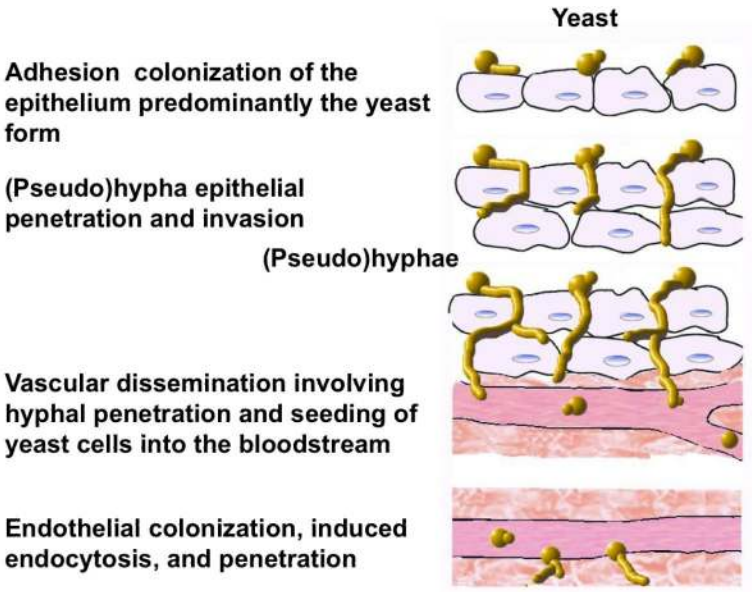

Figure 1. ***Candida albicans*** **tissue invasion**

The figure shows several steps in tissue invasion by *C. albicans* of a stylised epithelial cell surface: adhesion to the epithelium; epithelial penetration and invasion by hyphae; vascular dissemination, which involves hyphal penetration and seeding of yeast cells into the bloodstream; and finally endothelial colonization and penetration during disseminated disease.

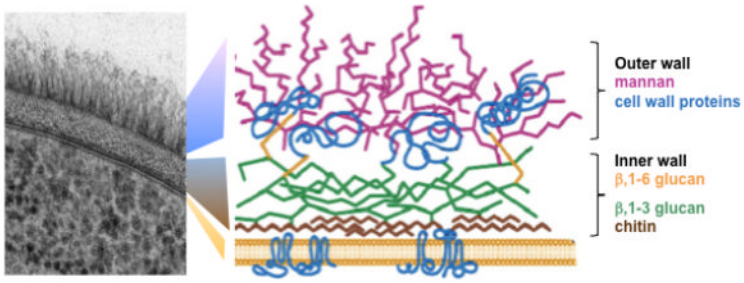

Figure 2. Structure of the *Candida albicans* cell wall

Two layers can be distinguished in the *C. albicans* cell wall. The outer layer is highly enriched with *O*- and *N*-linked glycoproteins whereas the inner layer contains the skeletal polysaccharides chitin and β-1,3 glucan that confer strength and cell shape. The outer cell wall proteins are attached to this framework predominantly by glycosylphosphatidylinositol (GPI)-remnants that are linked to the skeleton through a more flexible β-1,6 glucan.

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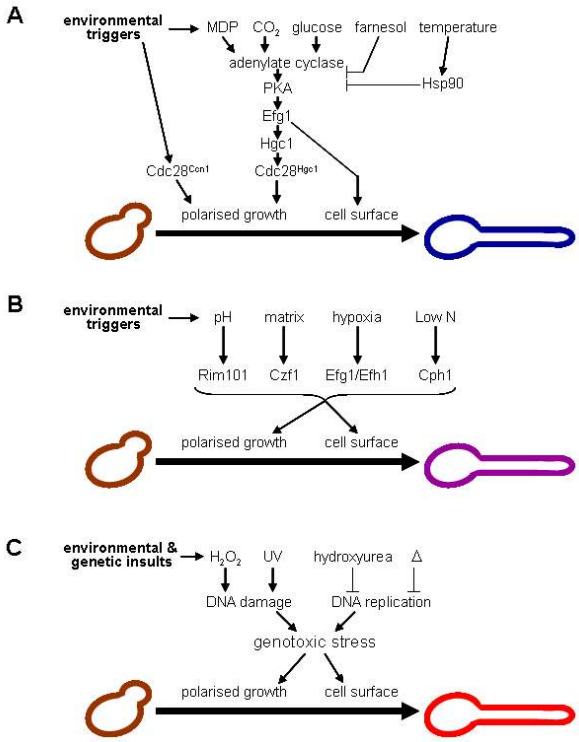

Figure 3. Different stimuli promote the formation of subtly different *Candida albicans* hyphae

(A) A variety of environmental triggers modulate hypha formation through the cyclic AMP (cAMP)–protein kinase A (PKA) signalling pathway**.** Muramyl dipeptide and CO_2 act directly on the adenylate cyclase Cyr1 and other signals, including amino acids and glucose, act on Cyr1 indirectly. Many signals act through the transcription factor Efg1 to activate the hypha-specific cyclin Hgc1. The cyclins Ccn1 and Hgc1 form complexes with the cyclin-dependent kinase (CDK) Cdc28. These complexes promote persistent actin polarization for hyphal growth. Farnesol is a quorum-sensing molecule that accumulates at high *C. albicans* cell densities and inhibits hyphal formation. Low temperatures inhibit hyphal formation through the inhibitory activity of the molecular chaperone heat shock protein 90 (Hsp90) on the PKA signalling pathway, which involves Ras1. The environmental triggers that induce signalling through the Ccn1 pathway are thought to be similar to those that induce signalling through the PKA pathway but the precise details of Ccn1 activation are unclear. (B) Additional environmental factors stimulate hypha formation through alternative signalling pathways. These factors include ambient pH (through the Rim101 pathway), physical embedding of *C. albicans* cells within a matrix (through the transcription factor Czf1), hypoxia (which involves the transcription factors Efg1 and Efh1) and low nitrogen due to starvation (which activates hyphal development by the transcription factor Cph1). (C) In addition, genotoxic stress by hydrogen peroxide (H_2O_2), UV radiation, hydroxyurea or gene deletions leading to DNA damage or interference with DNA replication cell-cycle checkpoints also cause cell filamentation**.** The alternative signalling mechanisms illustrated in parts A, B and C will yield different signalling outputs, and as a result the cell surface and immunological properties of these hyphal forms are likely to differ (denoted by the different colours of hyphae).

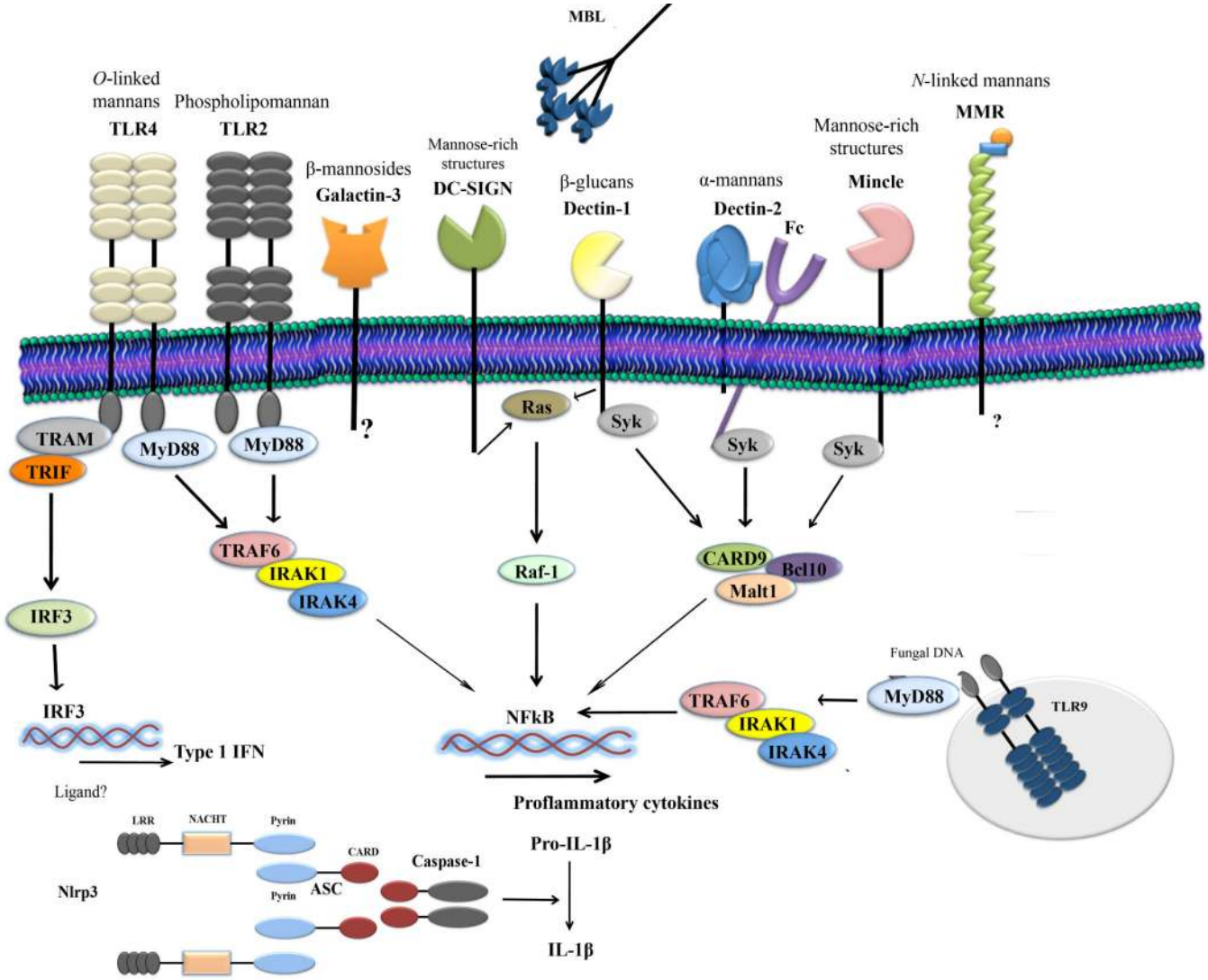

Figure 4. The main pattern recognition receptors involved in recognizing *Candida albicans*

The soluble lectin receptor mannose-binding lectin (MBL) can bind mannose-rich structures. In addition, the membrane-bound C-type lectin receptors macrophage mannose receptor (MMR), dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN) and the macrophage-inducible C-type lectin (Mincle) also recognize mannose-rich structures. Dectin-1 can bind β-glucans and dectin-2, together with the Fc gamma receptor (FcγR), recognizes α-mannans. TLR4 recognizes *O*-linked mannans and TLR2 can recognize phospholipomannans or, together with galectin-3, recognizes β-mannosides. TLR9 is located in the cytosol and recognises fungal DNA. Furthermore, the NOD-like receptor NLRP3 forms an inflammasome complex with apoptosis-associated speck-like protein containing a caspase recruitment domain (Asc) and the enzyme caspase-1. The ligand that triggers the Nlrp3 inflammasome is currently unknown.

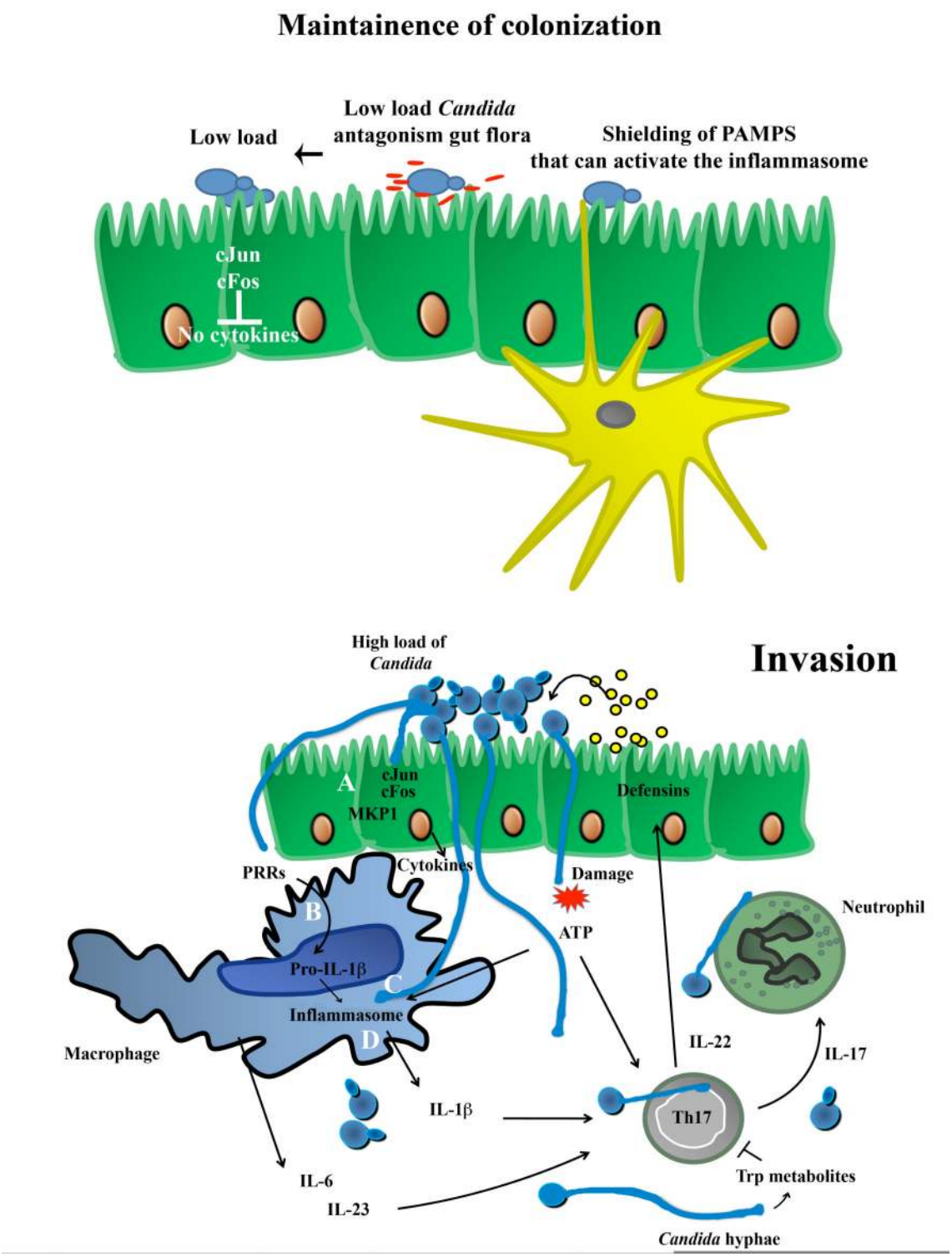

Figure 5. Colonization versus invasion

(A) *Candida albicans* colonization of the skin and mucosal surfaces. In healthy individuals, mucosal surfaces are often colonized with *C. albicans*. The relatively small numbers of *C. albicans* do not induce epithelial cell damage, and as a result no cytokine response is induced from the epithelial cells or mucosal macrophages and dendritic cells. In *C. albicans*

yeast cells, the pathogen-associated molecular patterns (PAMPs) responsible for inflammasome activation are hidden, and no interleukin (IL)-1β or T helper 17 (T_H17) responses are induced**.** *C. albicans* yeast cells can trigger a mitogen-activated protein kinase (MAPK) pathway leading to the activation of the transcription factors c-Jun and c-Fos, but this is not sufficient to trigger a cytokine response in epithelial cells. The normal microbial flora acts as a natural antagonist against abundant fungal growth. (B) Invasion of mucosal surfaces by *C. albicans* [109]. The switch between yeast and hyphae has an important role in the invasiveness of *C. albicans*. On one hand, the hyphae induce cytokine production from epithelial cells by inducing not only the MAPK pathway described in (A) but also a second MAPK pathway that leads to the activation of MKP1. This triggers the production of IL-1α and IL6. On the other hand, hyphae activate the inflammasome and induce IL-1β production in immune cells such as macrophages, and stimulate T_H17 cells to produce cytokines. T_H17-produced cytokines such as IL-17 activate neutrophils, while IL-22 induces the release of defensins from epithelial cells, both crucial components of mucosal antifungal defense. Products of tryptophan metabolism released by *C. albicans* hyphae can downmodulate T_H17 responses [140].