Pathogenicity mechanisms and host response during oral Candida albicans infections

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EXPERT REVIEWS

Pathogenicity mechanisms and host response during oral *Candida albicans* infections

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Oral candidiasis remains one of the most common forms of Candida infections and occurs if the balance between host, Candida and microbiota is disturbed, e.g., by broad spectrum antibiotics or immunosuppression. In recent years, identification of fungal factors contributing to host cell damage and new insights into host defense mechanisms have significantly extended our understanding of the pathogenesis of oral candidiasis. In this review, we will provide an overview of the pathogenicity mechanisms during oral Candida infections and discuss some approaches by which this knowledge could be transferred into therapeutic approaches.

Keywords: adhesion • damage • epithelial cells • immunosuppression • infection models • innate immunity • invasion

Epidemiology, risk factors & clinical presentation

Candida albicans is a frequent commensal on human mucosal surfaces of the oral, gastrointestinal and vaginal tracts. It has been estimated that approximately 50% of the healthy human population carry *Candida* species within the oral cavity, whereby *C. albicans* comprises 70–80% of the isolates [1,2]. *Candida glabrata*, *Candida dubliniensis*, *Candida tropicalis* and *Candida parapsilosis* are the frequently isolated non-albicans *Candida* species from the oral cavity. As long as the balance between the fungus, the host defense mechanisms and other members of the oral microbiota is maintained, oral colonization with *C. albicans* can be considered as benign. However, disruption of this balance can lead to fungal overgrowth and oral candidiasis.

Oral candidiasis is the most common fungal infection of the oral mucosa and is commonly associated with predisposing factors. These include disturbance of the oral bacterial community by the prolonged use of broad-spectrum antibiotics [3] and any disorder that directly or indirectly leads to an impaired immune response (reviewed in [4]). The most striking example of the role of host defense mechanisms in the prevention of oral candidiasis are HIV infections: Oral candidiasis is one of the initial clinical manifestations of AIDS [5] and occurs in up to 90% of HIV-infected patients before the introduction of highly active antiretroviral therapy [6]. Aggressive treatment of hematological malignancies by cytotoxic chemotherapy leads not only to severe immunosuppression but also promotes mucosal damage. This is likewise the case in patients receiving radiotherapy for the treatment of solid tumors in the head and neck regions. It is therefore not surprising that these patients constitute another high-risk group for the development of oral candidiasis, with a prevalence of clinical oral mycoses of approximately 30% (reviewed in [7]). Hereditary immune disorders, topical use of corticosteroids, dietary deficits and extremes of age (<1 year and old age) are further predisposing factors for oral candidiasis [8–10]. It should be further noted that reduced saliva flow is associated with an increased risk of oral candidiasis [11]. In addition to mechanical flushing of the oral cavity by saliva, antifungal saliva components such as histatins directly limit fungal growth [12]. Therefore, various conditions and

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treatments that reduce the salivary flow or antifungal salivary proteins and peptides might predispose for oral candidiasis ([13] and is reviewed in [4]).

Clinical manifestations of oral *Candida* infections vary in localization, depth of epithelial invasion and host response. Commonly used classifications differentiate four forms, which are associated to different degrees with specific predisposing factors [14]: pseudomembranous candidiasis, acute erythematous candidiasis, denture-associated erythematous candidiasis and chronic hyperplastic candidiasis. Pseudomembranous candidiasis is also known as thrush, a condition that mainly affects infants and immunocompromised patients. Histologically, the infection is characterized by hyphae invading into the stratum spinosum; in immunocompromised patients invasion might extend into deeper layers. The most prominent features of thrush are white to yellowish plaques, consisting of necrotic tissue, fungal elements and fibrin, which cover the erythematous mucosa and can be easily scraped off. Lesions commonly affect the tongue, palatum, oropharynx and buccal mucosa [2]. Oral candidiasis as a complication of treatment with broad-spectrum antibiotics can present as thrush, but more commonly causes acute erythematous candidiasis characterized by localized mucosal erythema without plaque formation [3]. Similar to thrush, invading fungal hyphae are found in the stratum spinosum. Lesions commonly occur on the tongue and the palate but may also affect the buccal mucosa [8]. Erythematous lesions can also occur in association with oral prosthetic devices, termed prosthetic stomatitis or, if associated with *Candida*, as denture-associated erythematous candidiasis. In this case, lesions are usually restricted to the mucosa which is in direct contact with the denture and *Candida* infections probably occur as a complication of poor hygiene and/or mucosal trauma due to poor fitting of dentures (reviewed in [15]). Finally, *Candida* can also be found in association with hyperplastic alterations, occurring most frequently on the buccal mucosa. Similar to thrush, these lesions appear as white plaques but in contrast to thrush they cannot be scraped off. This form is called chronic hyperplastic candidiasis and is characterized by deep invasion of tissues and associated with a higher risk of dysplasia and malignancy [14].

Infection models to study oral candidiasis

Infection models are essential to study the pathogenesis of candidiasis and have contributed significantly to our understanding of the disease. While biopsy samples from patients have been used to identify fungal genes upregulated during infection [16], the majority of studies employed cell and tissue culture or animal models. Monolayers of oral epithelial cells, for example, the cell lines TR146 and FaDu, present a convenient model to dissect the infection process and to identify factors involved in host–pathogen interaction [17–19]. However, to study invasion into deeper tissue layers, 3D models such as reconstituted human oral epithelium have to be used [20]. These models can be supplemented with immune cells, thereby facilitating the analysis of the cross talk between epithelial and immune cells during *Candida* infections [21]. As these *in vitro* models cannot fully represent the complexity of host–pathogen interactions *in vivo*, rodent models are needed to address specific aspects of pathogenesis (recently reviewed by Costa *et al* [22].). Although *C. albicans* is not a commensal of laboratory rats and mice, both species can be orally infected with *C. albicans* if predisposing factors are present: Treatment with broad-spectrum antibiotics and reduction of salivary flow facilitates the development of erythematous candidiasis in mice and rats, closely resembling the type of oral *Candida* infections in humans [22]. In immunocompromised animals, infection manifests as pseudomembranous candidiasis [23]. In rats, the sufficient size of the animals even allows placement of prosthetic devices to model chronic denture stomatitis [24,25].

Both clinical data and results obtained in the different infection models outlined above clearly demonstrate that the pathogenesis of oral candidiasis depends on both activities of the fungus leading to tissue invasion and the host response. Therefore, we will discuss the current state of knowledge concerning these two aspects in the next part of this review.

Fungal factors contributing to pathogenesis

In order to identify fungal factors involved in infection, transcriptional profiling studies were performed in the past years [16,26,27] based on the hypothesis that genes upregulated during infection might contribute to pathogenesis. These studies used *in vitro*, cell culture-based infection models and, importantly, clinical samples mainly from HIV-positive patients suffering from oral candidiasis. Despite the artificiality of the *in vitro* models, it appears that these models at least partially mimic the clinical setting as the majority of genes constitutively expressed in a reconstituted oral epithelia model were also upregulated by *C. albicans* infecting the oral cavity of HIV-positive patients. Among these were a number of genes known to encode hypha-associated genes and adhesins such as *ALS3*, *HWP1*, *RBT1*, *ECE1* and *SOD5*. However, the global expression patterns indicated a stress response to several environmental stresses due to contact with epithelial cells or oral tissues, as indicated by the upregulation of *YHB1* (nitrosative stress), *ICL1* (carbon starvation) and *GNP1* (amino acid sensing) in two of the studies and a decrease in ribonucleoprotein complex biogenesis and protein catabolic processes in one study (Table 1). Finally, expression analyses of certain genes such as *SAPs*, *ALSs* and *HWP1* using RT-PCR support the view that specific virulence genes contribute to different stages (adhesion, invasion and damage) of oral candidiasis.

Invasion of the host tissue by *C. albicans* hyphae is seen in the different clinical manifestations of oral candidiasis and is thus a hallmark of pathogenesis of these infections. Furthermore, adhesion is an essential step toward invasion, which commonly leads to cell damage. We will therefore discuss adhesion, invasion, cell damage and the fungal genes involved in these processes in the following sections.

Adhesion

The initial step during both commensal and pathogenic relationships of *C. albicans* with the host is the direct or indirect

Table 1. *Candida albicans* genes differentially expressed in oral infection models.

Infection model	Biological process/activity	Transcriptionally regulated genes	Ref.
Reconstituted human epithelium (RHE)/Human patient samples	Hypha-associated genes	[*ALS3, HWP1, SAP5, PHR1, PRA1, FKH1, ATP2, TEF1, SOD5, RBT1, ECE1, HYR1*]†	[16]
	Adhesion	[*ALS3, HWP1*]†	
	Invasion	*ALS3*†	
	Alternative carbon utilization	[*ICL1, MLS1, PCK1, MAL31, FOX3, PXA1, FOX2*]†	
	Nitrogen acquisition	[*GNP1, CAR1, CAR2*]†	
	Nitrosative stress	[*YHB5, SSU1, YHB1*]†	
	Phosphate limitation	*PHO84*†	
Human patient samples	Iron acquisition	[*CFL2, FRE4*]†	
Oral epithelial cell line TR146	Hypha-associated genes	[*ALS3, HWP1, PHR1, PRA1, SOD5, ECE1, RBT1*]†	[30]
	Adhesion	[*ALS3, HWP1*]†	
	Invasion	*ALS3*†	
	Alternative carbon utilization	[*ICL1, MLS1, PCK1, FBP1*]†	
	Nitrogen acquisition	[*GAP1, OPT8*]‡	
	Nitrosative stress	*YHB1*†	
	Phosphate, zinc, copper limitation	[*PHO87, CRP1, ZRT2*]†	
Oral epithelial cell line FaDu	Stress response		[68]
	Ribonucleoprotein complex biogenesis	[*DIP2, HCR1, MPP10, NIP7, RIA1, RLP24, RPB4, RPL3, RPP0, RPS15, RPS21, RPS5, RPS6A, RPS7A, RPS8A, TIF5, UTP20, YST1, orf19.1466, orf19.1833, orf19.2384, orf19.3481, orf19.4479, orf19.501, orf19.6197, orf19.7422*]‡	
	Protein catabolic process	[*CDC48, DOG1, RAD16, RAD7, RPN4, SAP1, SAP6, orf19.6672*]‡	

Note that some genes are involved in more than one process or activity.
†Upregulated genes.
‡Downregulated genes.

attachment of the fungus to mucosal surfaces or the surface of medical devices (Figure 1). Adhesion is mediated by adhesion factors (adhesins) on the surface of the fungal cell and physical properties such as cell surface hydrophobicity. Possible interaction partners include soluble host ligands, such as extracellular matrix (ECM) proteins and serum proteins, and factors on the surface of host cells, such as integrins and cadherins. Binding of *C. albicans* to ECM proteins was shown for laminin, fibronectin, collagen, entactin, vitronectin and tenascin [28]. In addition to direct binding to host structures, interaction of fungal adhesins with each other and the surface of other microorganisms can indirectly mediate attachment to mucosal surfaces [29]. Within the oral cavity, *C. albicans* is often found in mixed biofilms with various bacterial pathogens such as *Streptococcus* spp., *Actinomyces naeslundii* or *Fusobacterium nucleatum*. These biofilms are beneficial for the fungus in several aspects, including drug resistance, colonization and persistence within the complex microbial flora of the oral cavity and enhanced resilience to physical disruption [30].

Although yeast cells can adhere to surfaces, hyphae are generally considered to be the more adhesive *C. albicans* morphology [28,31]. Indeed, *C. albicans* strains unable to produce true hyphae show a reduced ability to adhere to epithelial cells [27]. During interaction with oral epithelial cells, adhesion and hypha formation are intricately linked: adhesion stimulates hypha formation and hypha formation enhances adhesion [16,27,32]. This link is likely mediated by the expression of hypha-associated factors that function as adhesins on filaments [33].

The majority of the *C. albicans* adhesins are GPI-anchored glycoproteins, including the agglutinin-like sequence (Als) family, hyphal wall protein 1 (Hwp1), Eap1 and Iff4 [32], and most of these adhesins are expressed in samples of patients suffering from oral candidiasis (Table 2) [16,32,34]. The Als proteins are characterized by a Thr-rich conserved β-sheet amyloid-forming 'T region' and a tandem repeat domain [35]. The amyloid sequences can form amyloid adhesion nanodomains which strengthen adhesion [36]. The different Als proteins have distinct yet overlapping functions and can cooperatively interact with

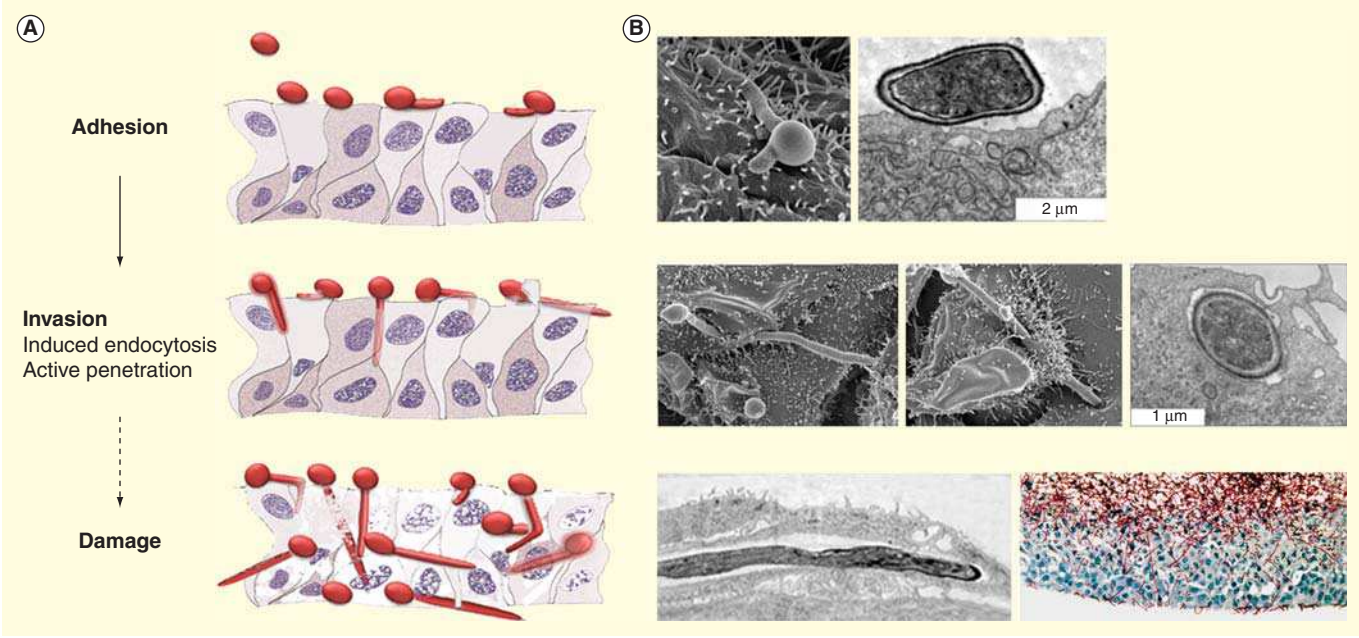

Figure 1. The three major stages of interaction of *Candida albicans* with oral epithelial cells based on *in vitro* data. (A) First, fungal cells adhere to epithelial cells by the interaction of cell wall factors (adhesins) with host ligands, which triggers hyphal formation. Next, the attached organisms can invade both into and between epithelial cells via two different invasion mechanisms: induced endocytosis and active penetration. Fungal invasins, such as Als3, bind to target proteins on the epithelial cell surface, such as E-cadherin, leading to endocytosis of *C. albicans* through the formation of pseudopod-like structures on the epithelial cell. Finally, epithelial damage and tissue destruction is induced by the active penetration of long hyphal elements into and between host cells at later time points. **(B)** Representative scanning and transmission electron micrographs and histology of epithelial tissues for the different stages of oral epithelial infection.

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each other. Als3, in particular, a protein specifically expressed on hyphae of wild-type *C. albicans*, is not only involved in all stages of *C. albicans* interaction with epithelial or endothelial cells and biofilm formation, but also in interaction with bacteria and iron acquisition (reviewed in [37]). In the interaction with epithelial cells, Als3 directly promotes adhesion and triggers clathrin-dependent endocytosis, thereby acting as an invasin [19]. The role of Als3 as a major adhesin of *C. albicans* is further supported by the observation that antibodies directed against the N-terminal region of Als3 reduce both *C. albicans* adhesion to epithelial cells *in vitro* and tongue fungal burden in mice with oropharyngeal candidiasis (OPC) [38,39]. Orthologs of the *ALS* genes of *C. albicans* exist in related species except *C. glabrata*, which does not contain *ALS* genes. However, *ALS3* appears to be specific for *C. albicans* as no ortholog has been identified in any other *Candida* species, including the most closely related species, *C. dubliniensis* [40]. Interestingly, Als3 not only mediates adhesion to host cells but also involved in interactions between *C. albicans* and *Staphylococcus aureus*: *S. aureus* adheres via Als3 to *C. albicans* hyphae. This association appears to mediate invasion of the oral mucosa by *S. aureus* in an *ex vivo* model using murine tongue explants [41].

Another key adhesin is the GPI-anchored cell wall protein Hwp1, which mediates adherence to oral epithelial cells by functionally mimicking host cell proteins. The N-terminal effector domain is recognized as a substrate of mammalian transglutaminases, leading to covalent binding to host surface proteins [42]. Hwp1 appears to have a specific function for interactions with mucosal surfaces as it is required for OPC in mice [34,43,44], but is dispensable for adhesion to endothelial cells and disseminated disease [33,44]. Eap1 is a morphology-independent GPI-anchored cell wall protein that is expressed in both yeast and hyphal cells. It contributes to epithelial cell and polystyrene adhesion, biofilm formation and cell–cell binding in response to α-pheromone [45,46]. Similar to Als3 and Hwp1, Eap1 can facilitate binding to *Streptococcus gordonii*, a colonizing bacterium of the oral cavity [47]. Within the *IFF/HYR* gene family, *IFF4* has been implicated in adhesion of *C. albicans*. Overexpression of the *IFF4* gene promotes adhesion to human epithelial cells but not endothelial cells. Suppression of *IFF4* expression results in decreased adherence to oral epithelial cells and in a murine vaginal candidiasis model, the overexpressing strain showed a slight increase in tissue fungal burden [48]. Although all members of the *IFF/HYR* family have a high degree of sequence similarity in their N-terminal effector domains, no common function for all 12 members of the *IFF/HYR* gene family has been discovered so far. However, the adherence function of *IFF4* seems not to be localized in the conserved domain, thus it is unlikely that the other gene family members are associated with adhesion to epithelial cells [48].

In addition to GPI-linked proteins, other classes of proteins have been implicated in adhesion of *C. albicans* to epithelial

Table 2. *Candida albicans* factors involved during interaction with epithelial cells.

Process	Factor	Description	Ref.
Adhesion	Hwp1	GPI-anchored hyphal wall protein 1 Substrate of mammalian transglutaminases	[42]
	Eap1	Enhanced adherence to polystyrene GPI-anchored cell wall protein	[47]
	Iff4	IPF family F Adhesin-like cell surface protein	[48]
	Mp65	Cell surface mannoprotein	[49]
	ALS gene family	Agglutinin-like sequence Cell surface adhesins	[34]
Invasion	Als3	Adhesin and invasin; interaction with E- and N-cadherin to induce endocytosis	[19,40,41]
	Ssa1	Member of heat shock protein 70 family Induction of induced endocytosis via interaction with host cadherins	[62]
	Saps	Secreted aspartic proteases	[30,59]
Damage	Sap5	E-cadherin degradation	[60]
	Rim101	Transcription factor E-cadherin degradation via Sap4, 5 and 6	[60]
	Eed1	Epithelial escape and dissemination hyphal maintenance	[16]

Some factors are specific for one interaction process (adhesion, invasion, damage), others are involved in more than one process.

cells. For example, the putative β-glucanase mannoprotein Mp65 is required for hyphal morphogenesis and virulence in mucosal *C. albicans* infection models [49]. An *mp65*Δ/Δ deletion mutant displayed a marked reduction in adhesion to buccal epithelial cells, enterocytes and plastic and has severe defects in biofilm formation. Mp65 is involved in maintaining cell wall integrity and hypha formation and thus might indirectly affect adhesion via cell surface expression of other *C. albicans* adhesins [49]. However, the observation that specific antibodies directed against Mp65 were able to block the adherence of wild-type *C. albicans* yeast cells to vaginal epithelial cells in an *ex vivo* assay supports direct contribution of Mp65 to adhesion [50].

Other factors that have been linked with adherence to epithelial cells and virulence in mucosal *C. albicans* infection models include an integrin-like protein [51], glycosidases (Utr2, Sun41) [52,53], the endoplasmic reticulum protein Big1 [54], a protein required for vacuole formation Vps11, the Ras-GTPase Rsr1/Bud1 [27], the yeast cell wall protein 1 [55] and members of the secreted aspartyl proteases, particularly Sap1-3 [56]. Deletion of these genes resulted in reduced epithelial adhesion; however, their role is probably indirect, mediated, for example, through alterations in the fungal cell wall, reduced filamentation or processing of host ligands.

Invasion

Intraepithelial *C. albicans* hyphae are typical in oral candidiasis, and *C. albicans* mutants lacking key regulators of morphology, associated with reduced capacity to invade epithelial cells *in vitro*, usually also have reduced virulence in animal models of mucosal candidiasis [57]. Thus, hyphae-mediated invasion appears to be a critical step in oral *Candida* infections. Invasion of epithelial cells by *C. albicans* occurs via two distinct mechanisms: induced endocytosis and active penetration [17]. Induced endocytosis is a host-cell driven process characterized by the accumulation of epithelial cell actin around the internalized microorganism (Figure 1). It is independent of fungal viability but can be disrupted using the actin microfilament inhibitor cytochalasin D [57]. *In vitro* studies suggest that induced endocytosis occurs early during epithelial cell–*C. albicans* interaction *in vitro* usually within 4 h [58]. Invasion at later stages and invasion into multilayered (stratified) epithelia where the uppermost layer consists of nonproliferating, functionally inactive cells require a second invasion mechanism which is fungal-driven: active penetration. During this process, *C. albicans* hyphae penetrate either directly into host cells or at intercellular junctions between cells (Figure 1) [59,60]. Although induced endocytosis and active penetration can be considered to be mechanistically distinct mechanisms, the two processes likely play complementary roles during the invasion process, which might depend on the epithelial cell type. For example, invasion into oral epithelial cells *in vitro* is driven by both induced endocytosis and active penetration, whereas cell entry into a gastrointestinal epithelial cell line is solely dependent on active penetration [59,60].

This cell type specificity could be explained by the variable presence of host cell receptors mediating induced endocytosis. The first identified defined molecular mechanism of induced endocytosis is the interaction of *C. albicans* Als3 with E-cadherin on host cells [61], which activates a clathrin-dependent

endocytosis pathway (Table 2). In addition, *C. albicans* Ssa1 also interacts with E-cadherin [62]. Knockdown of components of this pathway by RNAi led to a 60% reduction in endocytosis [63]. Albeit significant, this result strongly suggests that additional factors further contribute to induced endocytosis [63]. Recently, Zhu *et al.* identified the EGF receptor and a related protein, HEGF receptor 2 (HER2) as additional epithelial cell receptors for the *C. albicans* Als3 invasin [18]. As host–fungal interactions are affected by the cell type-specific expression of surface receptors, cell lines used for *in vitro* experiments should be chosen with care and ideally closely resemble surface protein expression of primary epithelial cells from the same anatomical location.

Although the exact mechanisms underlying active penetration are incompletely understood, several results indicate that secreted aspartic proteases (Saps) of *C. albicans* in combination with mechanical pressure (likely mediated by hyphae) contribute to active penetration: Mutants lacking either *SAP1-3* or *SAP4-6* displayed moderate, but significant reductions in invasion into oral and intestinal cells; the aspartic protease inhibitor pepstatin A caused a significant and dose-dependent reduction in invasion of oral epithelial cells and enterocytes and SEM pictures showed invasion of pepstatin A-treated hyphal cells without the formation of depressions on host cell surfaces [59]. Other hydrolytic enzymes such as lipases and phospholipases appear to play minor roles during active penetration. Surprisingly, induced endocytosis of killed *sap1-3*Δ/Δ and *sap4-6*Δ/Δ mutant hyphae was also significantly reduced compared to wild-type hyphae. Moreover, simultaneous treatment of *C. albicans* wild-type cells with pepstatin A and inhibitors of induced endocytosis further reduced invasion compared to cells treated with pepstatin A alone. In sharp contrast, no reduced invasion was monitored when enterocytes were treated with the same inhibitors [59]. These data indicate that Sap1–6 are directly or indirectly involved in active penetration and induced endocytosis. As a possible explanation, the activities of these proteases might be involved in the processing of host or fungal cell surface structures necessary for both invasion mechanisms.

Damage

Later phases of infection are characterized by destruction and loss of the superficial oral epithelium due to invasion of long *C. albicans* hyphae [8], which is believed to be responsible for the symptoms observed in patients (Figure 1). *In vitro* studies using epithelial cells and a 3D model of oral mucosa have shown that proteolytic degradation of E-cadherin, mediated at least in part by Saps, results in loss of integrity of the epithelium (Table 2) [60,64]. Similarly, a transcription factor mutant (*rim101*Δ/Δ), which showed reduced expression of *SAP4*, *SAP5* and *SAP6* compared to a wild type, has reduced capacity to degrade E-cadherin and disrupt epithelium in an oral tissue model [60]. In addition, filamentation and active penetration appear to be essential for the induction of epithelial damage. *C. albicans* strains deficient in hyphal formation showing reduced invasion also have reduced damage potential [19,27,65]. However, normal filamentation and invasion capacity is not sufficient for normal damage: For example, the *als3*Δ/Δ knockout strain forms normal hyphae on oral epithelial cells but is reduced in damaging these cells [27]. Furthermore, maintenance of hyphal elongation after initiation of invasion is also crucial for host cell damage, demonstrated by a mutant strain (*eed1*Δ/Δ) which reverts to pseudohyphal and yeast growth after initial hyphal formation. The initial formation of germ tubes by *eed1*Δ/Δ mutant cells is sufficient for normal invasion into epithelial cells. However, once inside the epithelial cells, the *C. albicans* mutant cells are trapped, unable to laterally penetrate the epithelial cells and cause damage [16]. Finally, killed hyphae taken up via induced endocytosis do not cause damage. Thus, damage is not a direct consequence of invasion *per se*.

The mechanisms by which invading *C. albicans* hyphae mediate cell damage are not fully understood yet. While it appears obvious that mechanical disruption of host cell integrity by piercing hyphae might cause necrosis of epithelial cells, recent studies provide evidence that *C. albicans* also activates specific epithelial cell-death pathways in response to infection. *In vitro* cell cycle arrest and early apoptosis events are induced 4 h after infection of epithelial cells, although no LDH release (as a marker of epithelial damage) is detectable at this time point [66]. Isolated *C. albicans* cell walls from both yeast and hyphae are sufficient to diminish epithelial cell proliferation and induce apoptosis. Thus, cell wall glycans, especially N-glycans, are likely to be the molecules inducing epithelial cell apoptosis and proliferation arrest. However, at later phases of *C. albicans* infections antiapoptotic signaling pathways are activated [67]. Consequently, final epithelial cell death is not due to apoptosis but rather due to necrosis at late time points of infection [58].

The host immune response during oral C. albicans infections

Epithelial cells (or keratinocytes) are the primary cell type comprising the oral mucosal surface and are the first line of defense against *C. albicans*. Apart from maintaining barrier function and providing an anchorage point for *C. albicans* colonization, epithelial cells also play a fundamental role in host defense by initiating innate and adaptive immune responses. Recent research has significantly expanded our knowledge of the interactions between *C. albicans* and the host immune system, not only with regard to classical immune cells but also regarding the role of epithelial cells. Therefore, direct epithelial responses and the innate and adaptive responses triggered by epithelial cells will be discussed below.

Epithelial cell recognition & activation by Candida

In conventional immune cells, several innate pattern recognition receptors (PRRs) for *C. albicans* have been identified, including toll-like receptors (TLRs), C-type lectin receptors (CLRs, e.g., dectin-1) and nucleotide binding domain leucine-rich receptors [68,69]. These PRRs recognize pathogen-associated

molecular patterns in the fungal cell wall, which include β-glucans, *N*- and *O*-linked mannans and phospholipomannans [69]. However, the importance of these PRRs in epithelial recognition and immune activation by *Candida* is debatable. With respect to TLRs, the majority of studies have been performed in intestinal, respiratory or uterine epithelial cells – less is known in oral epithelial cells. The predominant TLRs expressed in oral epithelial cells *in vivo* appear to be TLR1, TLR2, TLR4 and TLR8 [21]. TLR expression is unaffected after the addition of heat-killed *C. albicans* [21], and the application of fungal agonists (e.g., β-glucans, mannan, chitin) individually or in combination has no effect on cytokine production [70]. In contrast, viable *C. albicans* cells appear to downregulate all TLRs (except TLR2) and dectin-1 (Naglik JR, UNPUBLISHED DATA) and also induce cytokine production [21,68,71,72], suggesting that activities of viable fungal cells are essential for full epithelial cell stimulation.

Notably, cytokine induction correlates with hypha formation, since those *Candida* species or *C. albicans* strains unable to produce or maintain hyphae do not induce immune responses [16,70,73–75]. Furthermore, cytokine induction appears to occur via mechanisms independent of conventional pathogen associated molecular pattern recognition (or at least TLR2, TLR4 or dectin-1) [70]. With this in mind, the recently identified Als3–Her2 interaction suggests Her2 to be a potential non-PRR involved in *C. albicans* recognition [18]. However, Als3 does not induce cytokine secretion in epithelial cells [75], indicating that *C. albicans* recognition (leading to induced endocytosis) and immunological activation may be separate, autonomous processes.

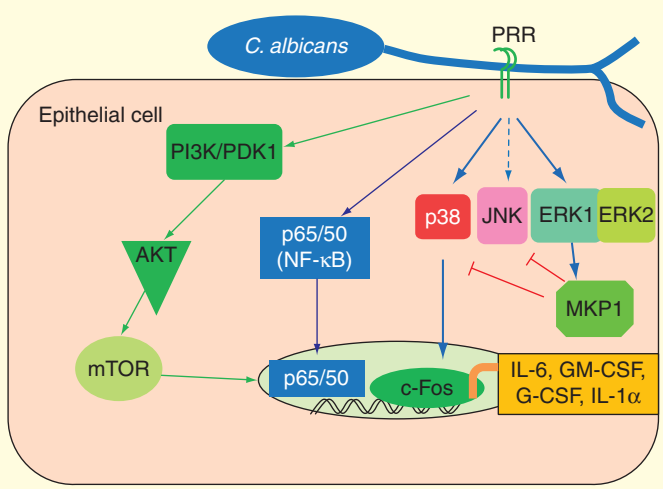

Figure 2. Epithelial immune signaling pathways activated by *Candida albicans* hyphae. *C. albicans* hyphal cells, when in sufficient quantities, are recognized by an unknown PRR mechanism that results in the activation of NF-κB, MAPK and PI3K pathways. MAPK signaling via p38 and ERK1/2 appears to discriminate between yeast and hyphal cells. Activation of p38 by hyphae leads to activation of the c-Fos transcription factor, which, in conjunction with the p65/p50 NF-κB heterodimers and PI3K/AKT, results in upregulation of proinflammatory cytokines. Concurrently, activation of ERK1/2 signaling results in stabilization of the MKP1 phosphatase, which deactivates p38 and JNK, hence acting as part of a negative feedback loop and preventing a potentially deleterious overreaction of the immune system.
PRR: Pattern recognition receptor.
Adapted with permission from [68].
Data taken from [70,73,75,76,107–109].

Epithelial identification of 'pathogenic' Candida

The ability to discriminate between 'commensal' and 'pathogenic' states of an endogenous, mucosal microbe is a fundamental function of epithelial cells. Oral epithelial cells are able to detect different *Candida* species and *C. albicans* in its yeast or hyphal form [70,73]. Notably, initial detection is independent of fungal viability, suggesting that activation of epithelial signaling is the result of specific recognition of the fungus and not necessarily a feature of invasion or damage induction. In *C. albicans*, yeast cells activate NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and PI3K signaling along with weak, transient activation of all three MAPK (mitogen-activated protein kinase) pathways: p38, JNK and ERK1/2 [70,76]. This drives the early and brief activation of the transcription factor c-Jun via JNK and ERK1/2. However, *C. albicans* hyphae induce sustained NF-κB and PI3K signaling along with strong activation of MAPK signaling, resulting in the activation of the transcription factor c-Fos via the p38 pathway. *C. albicans* hyphae also activate the MAPK phosphatase, MKP1 [70], via the ERK1/2 pathway, which stabilizes and regulates MAPK-induced immune responses. This combination of c-Fos activation and MKP1 regulation is specifically associated with hypha formation and correlates with proinflammatory cytokine responses (FIGURE 2) [70,73]. Accordingly, this MAPK-p38/c-Fos pathway is only activated by true hypha-forming *Candida* species (*C. albicans* and *C. dubliniensis*), but not by non-true hypha forming *Candida* species (*C. tropicalis*, *C. glabrata*, *C. parapsilosis* and *C. krusei*) [73]. Furthermore, this hyphal response is highly dependent on fungal burdens, indicating that a threshold level needs to be reached prior to full activation [70]. Thus, this mechanism may represent a 'danger response' mechanism allowing epithelial surfaces to remain quiescent in the presence of colonizing *C. albicans* (low burdens of yeast and/or hyphae), but permitting a specific and strong response to potentially dangerous levels of invasive hyphae common in disease pathologies. If so, this mechanism may be critical to the host's ability to identify when this normally benign fungus has become pathogenic. Similarly, the induction of IL-1β in macrophages differs between yeasts and hyphae. Differences in cell wall architecture between these two morphologies lead to different inflammasome activation, partially mediated by the dectin-1/Syk pathway [77].

Protective innate immunity initiated by epithelial cells

Ultimately, epithelial activation by *C. albicans* results in the induction of effector immune molecules such as cytokines and chemokines, including G-CSF, GM-CSF, IL-1α, IL-1β, IL-6, RANTES and IL-8 [21,70,72,74]. This results in the recruitment

and activation of a variety of immune cells, particularly neutrophils. Neutrophils possess a dual role in antifungal responses. First, neutrophils phagocytose *C. albicans* via TLRs and CTLs (non-opsonized) or via complement receptor 3 and Fc receptor (opsonized) [78]. Phagocytosis leads to intra- and extracellular killing of *Candida* via oxidative and nitrosative mechanisms, although fungicidal activity varies between different *Candida* spp. [79,80]. Second, neutrophils can indirectly mediate protection against oral *C. albicans* infections via cross talk with epithelial cells [21]. Interestingly, protection appears to be independent of phagocytosis, neutrophil transmigration or neutrophil–epithelial cell contact. Furthermore, epithelial TLR4 appears essential for protection since both *C. albicans* invasion and cell damage could be restored by TLR4 blockade (antibody) or 'knockdown' of TLR4 using siRNA, even in the presence of PMNs [21]. TNF-α production by neutrophils was required to induce this protective TLR4-mediated response [21], which confirms the important role of TNF-α in host defense against fungal infections. A similar neutrophil–epithelial protective cross talk mechanism was found using *in vitro* models of esophageal candidiasis [81]. Therefore, neutrophil-induced stimulation of epithelial immune responses could represent an important protective mechanism in the control of oral candidiasis. Other key epithelial responses include the production of S100A8/9 alarmins, which act as key chemotactic mediators and appear critical in recruiting neutrophils to the vagina during *C. albicans* infections [82]. Oral epithelial cells also possess direct innate antifungal activity via an annexin-A1 dependent mechanism [83]. This fungistatic activity does not require live epithelial cells, suggesting that surface epithelial layers can naturally inhibit *C. albicans* growth and proliferation at mucosal surfaces [84], which may help to maintain *C. albicans* in the commensal state.

Protective adaptive immunity initiated by epithelial cells

During *C. albicans* infection, epithelial cells produce proinflammatory molecules, including CCL20 and β-defensin 2, which act as chemoattractants to recruit mucosal-homing dendritic cells that express CCR6. Recruited dendritic cells recognize *C. albicans* through conventional PRRs, including TLRs and CTLs [69], which result in fungal ingestion, dendritic cell activation and trafficking to the local lymph nodes. In the lymph nodes, dendritic cells present process *C. albicans* antigens to naïve and memory T cells, initiating adaptive immunity. The nature of the T-cell response is determined by the cytokine milieu the T cells encounter during activation: IL-12/IFN-γ for Th1 cells, IL-4 for Th2 cells, IL-1β/IL-6/IL-23 for Th17 cells and IL-2/TGF-β for Treg cells.

The key T-cell subsets most strongly associated with protection against oral *Candida* infections are Th1 and Th17. Murine and human clinical studies indicate a central role for cell-mediated immunity and specifically the Th1 phenotype in combating oral and gastrointestinal *C. albicans* infections [85,86]. Indeed, a high proportion of AIDS patients who have low $CD4^+$ T-cell levels develop OPC [5], indicating the importance of $CD4^+$ T cells in host defense against oral infections [87]. Therefore, for many years, Th1 responses were regarded as the main protective T cell against *Candida* infections [86]. This viewpoint was supported by studies demonstrating that T-cell deficient mice, although susceptible to OPC, could be protected using adoptive transfer of $CD4^+$ T cells [88].

More recently, a new T-cell phenotype, Th17, has been identified and shown to contribute substantially to immunity to mucosal *Candida* infections (reviewed in [89]). Th17 cells are induced by a combination of IL-6, IL-1β and TGF-β and further matured (or reactivated) upon stimulation with IL-23. Th17 cells secrete IL-17A, IL-17F and IL-22 and are playing a major role in preventing extracellular infections and autoimmunity [90]. Furthermore, IL-17A and IL-17F are known to activate a variety of cells (e.g., epithelial cells and fibroblasts) to produce antimicrobial peptides, metalloproteases and chemokines that promote neutrophil recruitment and activation, ultimately resulting in clearance of fungal infections [89]. Concurrently, IL-22 limits fungal growth and promotes epithelial barrier integrity [91].

IL-17 production appears to be a key event in the protection against oral *C. albicans* infections [89]. For example, mice lacking the Th17-driving IL-23 cytokine show increased susceptibility to oral infection, while those lacking Th1 cytokines (e.g., IFN-γ) resist oral infections [92]. In addition, $IL\text{-}17RA^{-/-}$ and $IL\text{-}23p19^{-/-}$ deficient mice have increased susceptibility to OPC [93]. Patients with impaired IL-17 production also suffer from mucosal *C. albicans* infections in hyper-IgE syndrome and chronic mucocutaneous candidiasis. Recent studies investigating patients with autoimmune conditions (e.g., chronic mucocutaneous candidiasis) have also highlighted the importance of Th17 responses in protection against *C. albicans* (recently reviewed in [94]).

Therapeutic strategies

In many cases, oral candidiasis can be controlled by topical or systemic treatment with antifungals [4,6]. However, relapses may occur if the underlying predisposing condition cannot be corrected. This is, for example, the case in patients with persistent immunosuppression due to hereditary immune defects and some HIV patients. Prolonged treatment with azoles in these patients can result in the development of azole-resistant *C. albicans* strains [95]. In addition to novel antifungal approaches, such as the use of photodynamic therapy [96] and essential oils [97,98], better understanding of the pathogenesis of oral candidiasis might facilitate the development of effective adjunctive therapies. Imbalance of the oral microbiota by antibiotic treatment promotes the development of oral candidiasis, thus restoration of balance by benign bacteria interacting antagonistically with *C. albicans* might alleviate clinical disease. Indeed, studies using murine models or oral candidiasis demonstrated that the severity of disease can be significantly reduced by the application of specific probiotic bacteria [99,100]. Some effects were also observed in oral *Candida* colonization in the elderly [101], but further trials are needed to determine

the suitability of probiotics as adjunctive therapy to treat oral candidiasis.

Immune modulation by topical application of cytokines or PRR agonists might allow effective management of oral candidiasis in chronically infected patients (reviewed in [102]). However, implementation of this strategy will require appropriate delivery systems and clinical trials. Another strategy is the development of effective vaccines for high-risk patients. In fact, an Als3-based vaccine is currently under investigation in a Phase I clinical trial against candidiasis in high-risk patients [10] and has shown promising results against mucosal candidiasis in animal models [103]. Interestingly, mice immunized with the recombinant N-terminus of Als3p were not only protected against *C. albicans*, but even protected against systemic *S. aureus* infections via induction of a Th1/Th17 response, suggesting potential cross protection [104]. Thus, it appears likely that the vaccine could also mediate cross protection against other *Candida* species [105].

The identification of host receptors and pathways that mediate epithelial invasion by *C. albicans* might also be exploited for the development of alternative therapeutic strategies: Experiments using a murine model of OPC showed that treatment with inhibitors targeting the EGF receptor and HER2 kinases reduces the severity of infection [18]. Finally, strategies targeting hyphae formation as an essential step in invasion and damage (reviewed in [106]) could be used to alleviate clinical symptoms of oral candidiasis.

Conclusion

Oral candidiasis develops if the balance between *Candida*, the mucosal microbiota and the host defense system is disturbed. Specific fungal factors mediate adhesion to oral epithelial cells, invasion into oral mucosa and damage, thereby contributing significantly to pathogenesis. A protective host response relies on intact Th1 and Th17 responses. Additionally, epithelial cells are essential for an appropriate response toward *Candida* and are able to differentiate between the commensal and the invasive stage by responding differentially to yeast and invading hyphae. These recent insights provide avenues for new therapeutic strategies, which are especially important for the treatment of patients in whom the predisposing factors cannot be corrected, and who are therefore at risk for relapsing infections due to the development of strains resistant to antifungals.

Expert commentary & five-year view

Oral candidiasis mainly occurs in immunocompromised patients. As this patient group will likely continue to increase due to the advances in modern medicine, oral candidiasis will continue to be a common problem in patients at risk. Although standard therapy is effective in most patients, relapsing infections in long-term immunocompromised patients provide a therapeutic challenge as resistant *Candida* strains develop under long-term treatment. Recent insights into pathogenesis and host-defense mechanisms provide the basis for new approaches to target oral candidiasis.

The identification of molecular interactions between *C. albicans* and oral epithelial cells has already provided possible targets for the development of new antifungal strategies and vaccination. Additional factors and fungal activities will likely be identified in the following years – the challenge will be to identify suitable inhibitors of adhesion, invasion and damage. However, the localized nature of oral *Candida* infections also provides opportunities for novel local treatment approaches such as photodynamic therapy and immunomodulation. While management of systemic infections by immunomodulation is difficult because of side effects, local immune enhancement within the oral cavity appears feasible if suitable topical application methods are developed.

Despite recent advances in our understanding of the pathogenesis of oral candidiasis, many questions still remain to be answered. For example, it is not yet understood why specific risk factors lead to specific clinical forms of oral *Candida* infections. Furthermore, although the occurrence of oral candidiasis under long-term antibiotic treatment underlines the role of the oral microbiota in preventing fungal infection, the players and mechanisms behind this antagonism are not clear. With the advance of techniques and tools to study the microbiome, we will likely gain novel insights into the interplay between *Candida* and microbiota within the following years and might be able to exploit the antagonistic potential of other microbes to combat *C. albicans* infections in the oral cavity.

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Key issues

- *Candida albicans* occurs as a commensal in the oral cavity of healthy humans.
- Oral candidiasis is a frequent fungal infection in patients undergoing prolonged broad-spectrum antibiotic therapy or suffering from immunosuppression.
- Adhesion to epithelial cells, invasion of hyphae into the mucosa and epithelial damage are distinct steps during the pathogenesis of oral candidiasis.
- Distinct fungal factors contribute to these steps.
- The immune response of epithelial cells appears targeted to *C. albicans* hyphae, which provides a mechanism that may allow mucosal tissues to discriminate between commensal/colonizing and invasive/pathogenic *C. albicans*.
- Cross talk between epithelial cells and innate immune cells is likely an important protective mechanism in the control of oral candidiasis.
- Th1 and Th17 are key T-cell subsets strongly associated with protection against oral *Candida* infections.
- The elucidation of pathogenesis of oral candidiasis provides leads for novel therapeutic strategies such immunomodulation.

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