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Candida albicans interactions with epithelial cells and mucosal immunity

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Abstract

Candida albicans interactions with epithelial cells are critical for commensal growth, fungal pathogenicity and host defence. This review will outline our current understanding of *C. albicans*-epithelial interactions and will discuss how this may lead to the induction of a protective mucosal immune response.

Keywords

Candida albicans; yeast; hyphae; epithelial cells; oral; vaginal; mucosal; innate immunity; induced endocytosis; active penetration; cytokines; chemokines; commensal; pathogen

1. Introduction

A few specialised *Candida* species reside as harmless commensal organisms within the normal microbial flora in approximately half the world's population. Although normally these fungi cause no pathology, under suitable predisposing conditions, including long-term antibiotic treatment and compromised local immune/barrier defences, these fungi may become 'pathogenic'. As such, *Candida* species are the most common fungal pathogens of humans and are the causative agents of oral and vaginal candidiasis, giving rise to severe morbidity in millions of individuals worldwide.

Candida albicans is the most pathogenic *Candida* species and the host-fungal interaction can be regarded as an encounter between fungal virulence and host defence mechanisms. *C. albicans* possesses many putative virulence attributes that contribute to general survival, fitness and persistence within the host as well as specific factors associated with adhesion, invasion, cell damage and induction/evasion of host responses [1–3]. The host defences

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include mechanical barriers to fungal penetration such as epithelial surfaces, soluble antimicrobial factors, and innate and adaptive cellular immune mechanisms. The observation that only slight alterations in the physiological state of the host can turn this normally harmless commensal yeast into a dangerous pathogen capable of inflicting debilitating illness points both to the importance of host defence in maintaining *C. albicans* in the commensal state and the virulence potential of *C. albicans* when suitable predisposing conditions arise.

Epithelial cells (ECs) at mucosal surfaces are in the unique position of being in constant contact with *C. albicans* and thereby constitute the first line of defence against the fungus. Given that potentially fatal systemic infections can arise from breaches of the mucosal barrier it is of paramount importance to understand how *C. albicans* interacts with ECs and how this fungus is restricted to the mucosal surface in health. Critical to this is an appreciation of how ECs recognise *C. albicans* either in the 'commensal' or 'pathogenic' stage leading to either passive interaction or an active immune response.

2. Initiation of *C. albicans* infection: an overview

A prerequisite for colonisation of and commensal growth on mucosal surfaces is the ability of *C. albicans* to adhere to ECs, while invasion into and damage of ECs are regarded as infection specific activities (Fig. 1). Adhesion requires interaction between the fungal cell wall and the surface of ECs, and ranges from non-specific (e.g. hydrophobicity) to specific (e.g. protein-protein). Given that the composition of the *C. albicans* cell surface is continually changing, especially during the yeast-to-hypha transition, the precise nature of *C. albicans* adhesion to ECs is a complex, multifactorial process that probably involves several types of candidal adhesins on a morphologically changing cell surface [4].

Initial adhesion is invariably mediated by the yeast form as nearly all *in vitro* studies add *C. albicans* in the yeast phase to establish epithelial infections. Whether yeast-mediated adhesion is a true reflection of the initial adhesion process *in vivo* is uncertain as there is no reason to believe *C. albicans* can not be 'transferred' by human-to-human contact when in the hyphal form, especially as individuals asymptomatically colonised with *C. albicans* may harbour the fungus in the hyphal form [5–8]. Irrespective, the physical interaction of the *C. albicans* yeast cell with ECs is a potent stimulator of germ-tube/hypha formation [9;10], thereby intrinsically linking adhesion with filamentation in mucosal infections. This is a two-way intimate relationship as filamentation provides the platform for enhanced binding by utilisation of surface moieties specifically expressed in the hyphal form. Indeed, *C. albicans* hyphae adhere more strongly to ECs than yeast cells [11] and wild-type *C. albicans* strains unable to produce true hyphae have a reduced ability to adhere to ECs [10;12].

Hypha formation allows tight contact between fungal and host cells. This interaction results in a ligand/receptor-mediated reorganisation of the host cytoskeleton, envelopment of hyphae by membrane-derived pseudopod-like structures and uptake of the fungal cell [3;10;13;14] Such a microorganism-triggered epithelial-driven invasion process, known as 'induced endocytosis', is well described for bacteria such as *Salmonella, Shigella* or *Yersinia* [15–17]. This early phase of hypha formation, attachment and induced endocytosis is followed by an invasion phase, which is characterised by extensive epithelial penetration by hyphae in a pathogenic scenario which ultimately leads to tissue damage. Invasion resulting in tissue destruction is mediated predominantly by the process of 'active penetration', distinct from induced endocytosis and not relying on host cellular machinery. Rather, it is dependent exclusively on fungal attributes that include physical (turgor) pressure and penetration by the advancing hyphal tip and the production/secretion of hyphal factors that assist the invasion process. However, hypha formation is not necessarily

sufficient to cause cell damage and is almost certainly not the only factor that contributes to tissue destruction [10;13].

Given the strong relationship between hypha formation, adhesion and invasion and that the transcriptional programmes associated with dimorphism appear critical for virulence, it is difficult to delineate the precise contribution of hypha formation per se and morphology-associated genes/proteins to *C. albicans* pathogenicity at mucosal surfaces. However, recent studies have implicated a number of fungal moieties as being important in the initiation of *C. albicans* infection of ECs. We shall collate the recent literature to identify which fungal factors are required for adhesion, induced endocytosis, active penetration and cell damage of ECs. However, we stress that these processes are not necessarily mutually exclusive and are likely to involve significant overlap in function.

3. *C. albicans* adhesion to ECs

3.1. The Als (agglutinin-like sequence) family and adhesion

In both the 'commensal' and 'pathogenic' state *C. albicans* has to first attach to host cells/ligands through use of surface adhesins (Fig. 1). A key adhesin family identified as having a major role in epithelial attachment is the Als family, particularly Als3 which is a hypha-specific protein. Als3 is member of a family of eight glycosylphosphatidylinositol (GPI)-linked Als proteins that mediate adhesion to numerous cell types and host substrates [18;19]. The N-terminal region of all Als proteins contain the substrate binding region and constitute anti-parallel β-sheets, and the C- terminal region is serine and threonine rich and contains the GPI anchor sequence [3;20]. *ALS3* gene expression is upregulated during infection of oral ECs *in vitro* [9;10] and can been detected *in vivo* during vaginal *C. albicans* infections [21]. Both gene disruption and heterologous protein studies have demonstrated a direct role for Als3 (as an 'adhesin') in adherence to oral ECs [18;22]. In addition, human antibody fragments (single-chain variable) to Als3 reduce *C. albicans* adhesion to human ECs. Similar approaches have also demonstrated a potential role for Als1, Als2, Als4, Als5 and Als6 in EC adherence, although the data are partially contradictory depending on whether gene disruption or heterologous expression was used [3]. In addition to its role as an adhesin, Als3 was also shown to act as one of the ligands (invasin) which can trigger endocytosis [23] (see below) and to act as a receptor for host ferritin, thereby facilitating iron acquisition [24]. An important approach to understand the nature of Als-host substrate interactions will be to determine their structure by X-ray crystallography or NMR (nuclear magnetic resonance). Recently, en route to full structural determination by NMR, the resonance assignments of backbone atoms plus Ile, Leu and Val methyls for residues 18–329 of Als1 (comprising the 33.5 kDa N-terminal binding domain) were determined [25]. Full structural analysis of Als1 and Als9 has now been completed and this should provide valuable insights into Als interactions with both fungal and host substrates (Ernesto Cota, personal communication).

3.2. Hypha-associated genes and adhesion

Another key adhesin that has a major role in epithelial attachment is Hwp1 (hyphal wall protein 1). Hwp1 is also a GPI-linked protein expressed on the hyphal cell surface. The N-terminal domain acts as a substrate for epithelial transglutaminases, which covalently links Hwp1 to other epithelial proteins to form a strong attachment [26]. *HWP1* expression is upregulated during infection of oral ECs *in vitro* [9;10] and is expressed *in vivo* in both asymptomatic colonised individuals and patients with symptomatic oral infection [7]. Furthermore, oral exposure to Hwp1 protein induces salivary and systemic antibody responses [7]. *HWP1* is required for mucosal pathogenicity as a *hwp1*Δ/Δ mutant adhered poorly to oral ECs and had significantly reduced virulence in a murine model of

oropharyngeal candidiasis [27]. *HWP1* may also contribute to biofilm formation at oral surfaces as *HWP1* overexpression in a *bcr1*Δ/Δ mutant (a key fungal transcription factor regulating biofilm formation) partly rescued the *bcr1*Δ/Δ biofilm phenotype *in vivo* [28]. Since the *bcr1*Δ/Δ mutant itself did not form biofilms *in vitro* or on tongues of immunocompromised mice, Bcr1 may also be critical for mucosal biofilm infection, probably via regulation of hypha-associated genes [28].

Two other well known hypha-specific genes include *HYR1* and *ECE1* but their role in epithelial adhesion/interactions are unclear. Whilst one study has shown that *HYR1* overexpression does not alter *C. albicans* adherence to oral ECs [29], others have shown that a *hyr1*Δ/Δ mutant has significantly attenuated virulence in a mouse oral biofilm model [28]. However, the host ligands, if any, mediating interactions with Hyr1 are unknown. No data is currently available on Ece1 function.

3.3. Morphology-independent genes and indirect contributions to *C. albicans* adherence

There have been numerous reports regarding the role of morphology-independent genes and cell wall-associated proteins in adherence to ECs and mucosal pathogenicity. However, many of these genes/proteins have complex functions and the phenotypes observed may be due to indirect effects rather than the identification of a specific adhesin important for epithelial binding and subsequent infection. The cell wall possesses different classes of proteins that could interact with ligands on ECs. The most abundant class of cell wall protein, which are transported via the secretory pathway, are the GPI proteins. Als3 and Hwp1 are two such examples (covered above), which are hypha specific and act as direct adhesins. However, other morphology-independent cell wall GPI proteins potentially mediate adhesion to ECs.

Eap1 is a GPI protein and has structural homology with the Als family. Heterologous expression of *EAP1* in *S. cerevisiae* and autonomous expression in a *C. albicans efg1*Δ/Δ mutant (a key transcription factor controlling the yeast-hypha transition) enhanced attachment to HEK293 kidney ECs [30]. Likewise, a *eap1*Δ/Δ mutant had reduced adherence to the same cell line [31]. However, adhesion studies to oral, vaginal or intestinal ECs and virulence studies in animal mucosal models have not been undertaken, so the role of Eap1 in epithelial binding and mucosal infection is unclear.

Iff4 is another GPI protein and one of 12 Iff family proteins (related to Hyr1). Overexpression of *IFF4* increased *C. albicans* adherence to human ECs, but not endothelial cells, and modestly increased tissue fungal burdens during murine vaginal candidiasis [29]. Limited data is available regarding other Iff proteins, but *IFF2* and *IFF3* overexpression had no affect on *C. albicans* adherence to oral ECs. It is possible that other Iff family members may act as adhesins for non-EC types.

Ecm33 is a cell wall GPI protein that affects adherence as an *ecm33*Δ/Δ mutant had reduced binding to FaDu oral ECs. However, overexpression of *C. albicans ECM33* in *S. cerevisiae* did not alter adherence or invasion capabilities suggesting it does not act as an adhesin or invasin [32;33]. Since Ecm33 plays a critical role in cell wall integrity, this epithelial phenotype most likely reflects the indirect effects of Ecm33 on cell wall function rather than a specific role in mucosal infections. For example, adhesins such as Als3 or Hwp1 may not be properly exposed to the cell surface in the *ecm33*Δ/Δ mutant. Likewise, Utr2 is a GPI-linked glycosidase on the cell surface that is required for adherence to oral ECs but is also required for cell wall integrity, so these observations are likely to be indirect [34;35].

Apart from GPI-linked proteins, another class of cell wall protein that has been linked with *C. albicans* adherence is the non-covalent wall-associated proteins, such as Mp65 and Phr1.

Mp65 is a putative β-glucanase (GH17 family) which cleaves β-glucans, usually β-1,3 glucan, and gene disruption studies indicate that Mp65 is required for hypha formation and virulence in *C. albicans* mucosal infection models [36]. Notably, domain antibodies to Mp65 blocked *C. albicans* adhesion to vaginal ECs [37]. However, as with Ecm33, given its location and general function, it is likely that Mp65 affects cell wall integrity and/or modifies other adhesins required for epithelial binding rather than acting as an adhesin itself. Similar arguments can be made for Phr1, which is a β-1,3 glucanosyl transferase (GH72 family) that also plays a crucial role in cell wall assembly of β-1,3 and -1,6 glucan linkages [38]. Deletion of *PHR1* reduced *C. albicans* adherence and invasion of oral epithelium, however, this correlated with a lack of hypha formation. Although *PHR1* is not required for hyphal induction, it appears important for maintenance of hyphal growth, thereby indirectly affecting epithelial adhesion and invasion. Sap9 and Sap10, which are cell-surface associated proteases with function in cell wall integrity [39], may also indirectly contribute to adhesion by targeting covalently-linked fungal cell wall proteins such as Cht2, Ywp1, Als2, Rhd3, Rbt5, Ecm33, and Pga4 and glucan cross-linking protein Pir1 [40]. Thus, the role of Mp65, Phr1 and Sap9/10 in mucosal infections is likely to be multi-factorial.

Int1 is a *C. albicans* surface protein with similarities to vertebrate integrins and is implicated in filamentation and promoting intestinal colonisation in mice [41;42]. Furthermore, *C. albicans INT1* overexpression in *S. cerevisiae* was sufficient to enable adhesion of this normally non-adherent yeast to human ECs.

Internal proteins also affect *C. albicans* adhesion and virulence through indirect effects on the fungal cell wall. Big1 is an endoplasmic reticulum protein that is involved in β-1,6, glucan biosythesis and deletion of *BIG1* affects filamentation, adhesion to ECs and virulence [43]. Vps11 is involved in protein trafficking and required for a functional vesicle transport system [44]. Therefore, the *vps11*Δ/Δ mutant has multiple defects, including deficiencies in epithelial adhesion [10]. Rsr1/Bud1, encoding a Ras-GTPase, or Bud2, the GTPase activating protein of Rsr1, also indirectly affect epithelial adhesion [10]. Since both proteins are involved in hyphal orientation and bud formation, this potentially demonstrates a role for contact sensing in mucosal infections.

Finally, secreted proteins have also been linked with promoting adherence to ECs, although data is sparse. Gene disruption and inhibitor studies indicate that members of the Sap (secreted aspartyl proteinase) proteinase family, particularly Sap1–3, contribute to epithelial adhesion by *C. albicans* [2]. However, this function is probably indirect as the Sap enzymes may be able to process host ligands, thus facilitating or preventing access for fungal adhesins to attach to the epithelial surface.

4. *C. albicans* invasion into ECs: induced endocytosis vs active penetration

Invasion into and damage of ECs are considered to be ‘pathogenic’ features rather than ‘commensal’ attributes. Unlike bacteria, which have developed several strategies to trigger uptake by induced endocytosis [45], *C. albicans* can utilise two distinct invasion mechanisms for epithelial entry: induced endocytosis and active penetration [3;9;46;47] (Fig. 1). Induced endocytosis is mediated by the EC and active penetration by the fungus. Even killed hyphae are endocytosed and killed ECs can be penetrated by viable hyphae. However, which is the predominant route of EC entry? Recent studies indicate that both induced endocytosis and active penetration mediate epithelial entry, however, the mechanism used is dependent on the epithelial lineage. For example, both mechanisms are utilised during oral EC invasion, whereas intestinal ECs are only invaded via active penetration [23;47].

Since yeast cells do not appear to induce their own uptake into ECs, it is evident that epithelial invasion (passive or active) is triggered by hypha-associated factors. Furthermore, only hyphae adhere strongly to ECs (see above). This close association between hypha formation, adhesion and epithelial invasion is evident in studies demonstrating a lack of adhesion and epithelial invasion (and damage) when screening *C. albicans* hypha-deficient mutants, including *efg1*Δ/Δ, *ras1*Δ/Δ, *tec1*Δ/Δ, *cka2*Δ/Δ, *rim101*Δ/Δ, *tpk1/2*Δ/Δ, *czf1*Δ/Δ [8;10;46;48–50]. These hypha-deficient strains all have defects in the cAMP/PKA pathway, which signal through Efg1. Therefore, it appears that this signalling pathway controls expression of the hypha-associated factors that promote adhesion, epithelial invasion and subsequent damage. The fungal MAPK pathway seems to have little role in promoting epithelial infection as null mutations in the three MAPK proteins (*cek1*Δ/Δ, *hog1*Δ/Δ and *mkc1*Δ/Δ) and associated transcription factors (*cph1*Δ/Δ and *cph2*Δ/Δ) all produce hyphae on epithelial monolayers and invade and induce damage similar to wild-type cells [10].

4.1. *C. albicans* invasion by induced endocytosis

Induced endocytosis is the process by which a fungal ‘invasin’ protein expressed on the hyphal surface interacts with an epithelial surface protein, triggering pseudopod formation and fungal engulfment into the cell. The process is passive as killed hyphae are endocytosed as efficiently as viable hyphae [46], indicating that induced endocytosis is mediated solely by the EC. Induced endocytosis occurs early in the epithelial-*C. albicans* interaction, usually within 4 h [51], and is thought to be mediated in part via epithelial E-cadherin through a mechanism that is actin-dependent and requiring clathrin [14]. Actin dependency was demonstrated by blocking endocytosis with the microfilament inhibitor cytochalasin D, which prevents actin polymerisation [46;47;52;53]. Furthermore, knockdown of proteins associated with the clathrin-mediated pathway (cortactin, dynamin and clathrin) by RNA interference (RNAi) demonstrated a 60% reduction in endocytosis [14]. However, as endocytosis was not completely abolished, it is likely that other clathrin-independent mechanisms may also be involved in inducing endocytosis in ECs, but probably still in an actin-dependent manner. Recent work in rabbit corneal ECs has identified another potential mechanism of induced endocytosis, which requires recruitment of small GTPases (Cdc42, Rac1 and RhoA) and ZO-1 (zonula occludens) to the site of cytoskeletal (actin) reorganization, as inhibition of endocytosis was observed in corneal ECs expressing dominant-negative mutants of Rac1 and RhoA GTPases [54]. However, as actin, GTPases and ZO-1 were co-localized in ECs during uptake of beads coated with spent medium from a *C. albicans* culture, this endocytosis mechanism might be mediated by molecules that are shed or secreted by *C. albicans* rather than being cell-associated.

Currently, two *C. albicans* invasins have been described that induce endocytosis in ECs, Als3 [53] and Ssa1 [55]. Als3 has been addressed above but Ssa1 is a member of the heat shock protein (HSP) 70 family that is expressed on the cell surface. Respective *als3*Δ/Δ and *ssa1*Δ/Δ mutants exhibited defective binding to oral ECs, had reduced capacity to invade ECs and had attenuated virulence in a murine model of oropharyngeal candidiasis [10;53;55]. Furthermore, latex beads coated with the recombinant N-terminal region of Als3 or recombinant Ssa1 were avidly endocytosed. Both Als3 and Ssa1 bind to E-cadherin and probably initiate epithelial entry via clathrin-mediated mechanisms [23], although this has not been officially demonstrated for Ssa1.

Other candidate proteins that may contribute to induced endocytosis are Cht2, Pga7 and Zrt1. Cht2 is a cell surface chitinase, Pga7 is a surface protein and Zrt1 a plasma membrane zinc transporter and all three are regulated by the RIM101 transcription factor (a transcription factor that controls the alkaline pH-mediated fungal response [56]). They were identified by overexpressing them in a *rim101*Δ/Δ mutant, which has reduced capacity to induce its own endocytosis [49]. Overexpression of these genes enhanced endocytosis in

oral ECs, although whether these proteins directly mediate endocytosis or act indirectly through effects on other cell wall functions is currently unknown.

Finally, the Sap proteases may contribute to induced endocytosis as pepstatin A, an aspartyl protease inhibitor, inhibits early events of epithelial [oral and intestinal] invasion and *sap1–3*Δ/Δ and *sap4–6*Δ/Δ triple mutants are poorly invasive [47]. However, the fact that non-viable *sap1–3*Δ/Δ and *sap4–6*Δ/Δ hyphal cells show reduced uptake by ECs even in the absence of endocytosis inhibitors together with the need to pre-incubate *C. albicans* with pepstatin A to see its endocytosis inhibitory effects, would argue that any effects on induced endocytosis of these aspartyl proteases is indirect, being a result of their action on the fungal or host surface and/or proteins.

In oral ECs, once endocytosed, *C. albicans* hyphae gain access to the vacuolar endosomal compartments [52]. The fate of *C. albicans* at this point is less clear, however, *C. albicans*-containing endosomes transiently acquire the early endosomal marker EEA1 but show marked defects in acquisition of late endosomal marker LAMP1 and lysosomal marker cathepsin D. Thus, endocytosed *C. albicans* hyphae appear to prevent endolysosomal maturation, which may promote intracellular fungal growth. This survival strategy may permit *C. albicans* to survive for prolonged periods within the EC up until such time the hypha penetrates out of the cell to infect neighbouring ECs, which will result in necrosis and cell damage (see later sections).

4.2. *C. albicans* invasion by active penetration

Active penetration is a separate mechanism to induced endocytosis and occurs at later time points than endocytosis. Active penetration requires fungal viability and results in hypha invasion in between or through ECs. *C. albicans* hyphae can invade killed oral ECs and viable ECs when endocytosis-mediated mechanisms are blocked, demonstrating that active penetration is a fungal-driven process [10;47].

It is still unclear which fungal proteins contribute to active penetration. Given its role as an adhesin and invasin, Als3 could be considered a prime candidate. However, Als3 is not essential for active penetration as the *als3*Δ/Δ mutant still invades cytochalasin D-treated oral ECs at a rate identical to untreated ECs. Also, since *als3*Δ/Δ hyphae demonstrated only 50% invasion of untreated ECs as compared with the wild type strain, one could conclude that Als3 predominantly contributes to epithelial entry via induced endocytosis [10]. Despite this, Als3 probably contributes to active penetration through indirect mechanisms such as epithelial anchoring.

The Sap enzymes may also contribute to active penetration, although their role is contentious, with some studies using pepstatin A showing a reduced ability of *C. albicans* to invade and damage oral epithelium [8] whereas others show little effect [57]. The main contribution of Saps to epithelial invasion, however, might be through facilitation of hyphal penetration between epithelial layers as opposed to direct penetration into cells. The Sap4–6 subfamily in particular has been implicated in the modulation of expression of keratinocyte differentiation markers [58] whilst Sap5 was responsible for E-cadherin degradation [59], which is present at high concentrations at inter-epithelial junctions. E-cadherin degradation would disrupt the integrity of the mucosal tissue, thereby enabling hyphal penetration between ECs. Other secreted enzymes of *C. albicans* including the lipases and phospholipases appear to play no role in active penetration (or induced endocytosis) [47].

It should be noted that in mucosal tissues of stratified, squamous epithelium like the oral cavity and vaginal lumen, the uppermost surface epithelial layers are non-proliferative and regarded as functionally inactive. Such cells are unlikely to support a mechanism of cell

entry dependent solely upon induced endocytosis as this requires healthy, viable ECs. Therefore, *in vivo*, active penetration is likely to be the main mechanism of initial mucosal invasion, either through direct EC entry or inter-epithelial penetration that allows access to sub-mucosal layers. In the sub-mucosal layers *C. albicans* hyphae will encounter viable, active ECs which will enable cell entry via induced endocytosis. Thus, although induced endocytosis and active penetration may be regarded as distinct mechanisms, *in vivo* both processes are likely to play complementary roles during the invasion of oral and vaginal mucosal tissues. It should also be noted that oral and vaginal ECs possess innate antifungal activity via an annexin-A1 dependent mechanism [60]. This fungistatic effect does not require live ECs and suggests that the uppermost surface epithelial layers are able to naturally inhibit the growth and proliferation of *C. albicans* (and probably other pathogens) at the mucosal surface.

5. *C. albicans* induced epithelial damage

Once *C. albicans* hyphae have gained access to ECs or to sub-mucosal layers, the final feature of the invasion process is induction of tissue damage. This might occur via a combination of two distinct mechanisms: necrosis and apoptosis. Necrosis is characterised by mitochondrial swelling and increased plasma membrane permeability, and is induced by factors external to cells and will be caused directly by *C. albicans* hyphal factors (cell-associated or secreted). This is in contrast to apoptosis, which involves an articulated biochemical breakdown of the cell into membrane-bound apoptotic bodies and is a naturally occurring cause of cellular death. While apoptosis can provide beneficial effects to the host, necrosis is nearly always detrimental.

5.1. Epithelial damage by necrosis

The relationship between epithelial invasion and cell damage is not fully understood. The original consensus was that if an EC is invaded then this must induce damage. However, this is not necessarily the case (see below). In addition, when cell damage does occur, the proportion that can be ascribed to induced endocytosis and active penetration is a contentious issue. Since induced endocytosis is the main mechanism that mediates early-stage entry of oral ECs, one may postulate that endocytosis is first required in order to permit full induction of cell damage. This is supported by data demonstrating reduction in epithelial damage when cells are treated with the microfilament inhibitor cytochalasin D, which prevents endocytosis [46]. However, given that killed hyphae are also readily endocytosed [46] but do not cause epithelial damage [47;61], the process of endocytosis is not necessarily indicative that damage will be induced. More likely, endocytosis is required for initial cell entry, which then enables the fungus to cause damage through the process of active penetration. This is strongly supported by data showing that a *C. albicans* mutant strain (*EED1* deficient) with normal adhesion and endocytosis properties is unable to induce damage [9]. Currently, this is the only mutant known that invades ECs without causing damage [although other mutants with an attenuated ability to induce damage exist, see below] and demonstrates that endocytosis may be unconnected to damage induction.

Active penetration appears essential for induction of EC damage and, unlike endocytosis, is a fungal-mediated process that requires hyphal viability. Damage by active penetration may be achieved after initial cell entry by endocytosis or direct from the epithelial surface. Numerous fungal factors have been suggested as being contributors of cell damage via this mechanism, not least the Sap proteins, although this is a contentious issue (see above). Clearly, other fungal or hyphal factors are required for cell damage that are currently unidentified. To address this, a series of defined *C. albicans* mutant strains was recently screened to delineate which genes or fungal processes were required for adhesion, invasion and damage of ECs [10]. Strains that were deficient in all three processes were also deficient

in hypha formation to some degree (*rim101*Δ/Δ, *hgc1*Δ/Δ, *tup1*Δ/Δ, *tec1*Δ/Δ, *tpk1*Δ/Δ, *tpk2*Δ/Δ, *ras1*Δ/Δ, *efg1*Δ/Δ, *vps11*Δ/Δ and *ecm33*Δ/Δ). The only exception was *als3*Δ/Δ, which had normal hypha formation, thereby confirming its important role as both an adhesin and invasin. Since some of these hypha-deficient strains have defects in genes regulating the cAMP/PKA pathway, the data confirm that this fungal signalling pathway controls the biogenesis of multiple hypha-associated factors that promote epithelial adhesion, invasion and damage.

Of specific interest were strains that exhibited normal invasion but were deficient in adhesion and damage induction (*cka2*Δ/Δ, *bcr1*Δ/Δ, *hwp1*Δ/Δ, *bud2*Δ/Δ, *rsr1*Δ/Δ), and strains that could adhere and invade normally but were deficient in damage (*gpd2*Δ/Δ, *gpp1*Δ/Δ, *eed1*Δ/Δ) [10]. These two groups of strains may identify factors specifically involved in damage induction by active penetration, as cell entry mediated by endocytosis appeared to be unaffected. However, since *cka2*Δ/Δ, *bud2*Δ/Δ and *rsr1*Δ/Δ have deficiencies in hypha formation this complicates the role of these genes in epithelial damage induction. The data for *bcr1*Δ/Δ, *hwp1*Δ/Δ, *gpd2*Δ/Δ, *gpp1*Δ/Δ and *eed1*Δ/Δ are more promising as these strains form normal hyphae. Hwp1 is already known to have an important role in epithelial adhesion and is probably required to establish a firm footing on mucosal surfaces which enables other hyphal factors to invade and cause damage of ECs. As *HWP1* gene expression is under the control of Bcr1 (a transcription factor), the phenotype of the *bcr1*Δ/Δ mutant may be ascribed to the genes Bcr1 regulates, such as *HWP1*. Both Gpd2 and Gpp1 are involved in glycerol metabolism, suggesting glycerol accumulation within the fungal hyphal cell may contribute to cell damage during late-stage invasion, possibly by influencing turgor pressure and morphogenic plasticity. Finally, *EED1* is known to be essential for maintenance of hyphal elongation [9;62]. This explains the damage phenotype of the *eed1*Δ/Δ mutant, as although the mutant is able to adhere and invade (as it initially forms germ tubes) it soon reverts back to the pseudohyphal and yeast form and remains trapped within the EC. Thus, the *eed1*Δ/Δ mutant is unable to laterally penetrate ECs and cause damage. This intriguing phenotype indicates that not only is initial germ tube formation critical in mediating adhesion and invasion but that maintenance of hyphal extension is also crucial for damage induction.

5.2. Epithelial damage by apoptosis

Apoptosis may play an important role in a variety of mucosal diseases as it appears to be an evolutionary conserved response to pathogens across the animal and plant kingdoms [63]. Although there are number of reports of bacterial-induced apoptosis in myeloid cells, very few studies have been performed with *C. albicans*. However, two reports demonstrate that *C. albicans* can inactivate anti-apoptotic proteins Bcl-2 and Bcl-xL in macrophages and neutrophils and can trigger activation of cellular caspases [64;65]. Equivalent apoptosis studies in ECs are sporadic and somewhat controversial and most of the evidence for the induction of epithelial apoptosis again comes from the bacterial field. These data indicate that apoptosis is a delayed event and thus a late stage response to infection [66]. This may indicate that during early epithelial infection anti-apoptotic mechanisms may be activated, which ultimately are overcome by pro-apoptotic mechanisms leading to cell death.

However, this hypothesis may not apply to *C. albicans* as microarray screens of *C. albicans*-infected oral ECs indicate that both anti- and pro-apoptotic genes are activated at 6 h and 24 h post-infection, suggesting both mechanisms are activated simultaneously (Naglik laboratory, unpublished data). Indeed, a recent study demonstrated that *C. albicans* induces early apoptosis events in oral ECs, which is subsequently followed by necrotic death [51]. Early apoptotic events were determined by significantly increased Annexin V positive cells as well as internucleosomal degradation of chromosomal DNA within the first few hours of *C. albicans* infection. This was confirmed by incubation with the irreversible pan-caspase

inhibitor Z-VAD-FMK, which resulted in a significant reduction of EC death during the first 9–12 h post-infection but not after 12 h. Notably, the ability of *C. albicans* to induce early apoptotic events was inhibited cytochalasin D, indicating that epithelial entry via induced endocytosis may be a key process that leads to early apoptosis induction. Epithelial necrosis followed apoptosis, which was determined by incorporation of propidium iodide into cells and the fact that *C. albicans* induced cell death even in the presence of the pan-caspase inhibitor at later time points. These data suggest that *C. albicans* induces both apoptosis and necrosis in oral ECs but the cross-over between these two mechanisms and how they may interact is still unclear.

Little data is available on the fungal factors that induce EC apoptosis. One study suggested that farnesol, a quorum sensing molecule of *C. albicans*, is able to decrease proliferation and induce apoptosis in oral ECs [67]. However, given that physical separation of *C. albicans* and oral ECs completely abolishes endocytosis and apoptosis, the physiological relevance of this finding is unclear since farnesol is a secreted molecule. It is possible that only high concentrations of farnesol induce epithelial apoptosis but it is unlikely such high concentrations are present during oral infections *in vivo* given the diluting action of saliva. Recent work also indicates that the surface *N*- and *O*- mannans of *C. albicans* can directly induce cell cycle arrest and apoptosis in oral ECs (Schaller Laboratory, unpublished data). Since the *C. albicans* hyphal cell wall is highly glycosylated this may provide an important mechanism for the induction of apoptosis *in vivo*, as it can be envisaged that once *C. albicans* hyphae are endocytosed into the EC the glycan moieties might then induce cell cycle arrest and activate apoptotic mechanisms to promote survival and potentially pathogenicity. Irrespective of the fungal moieties that activate apoptosis, once triggered, the production of nitric oxide (via peroxynitrite) by ECs may enhance *C. albicans*-induced mucosal injury [68].

It should be noted that induction of apoptosis by *C. albicans* in oral ECs may not be applicable to other EC lineages or indeed other fungi. For example, endocytosis of *Paracoccidioides brasiliensis* triggers apoptosis in type II pneumocytes whereas endocytosis of *Aspergillus fumigatus* conidia is thought to suppress apoptosis in the same cell type [69–71]. Therefore, there is still a lot to learn about how *C. albicans* and fungi in general induce apoptosis in ECs and the physiological relevance of this during mucosal infections *in vivo*.

6. Host response and mucosal immunity to *C. albicans*

One of the key characteristics of *C. albicans* is that it is an opportunistic pathogen, being part of the normal mucosal microbiome in approximately 50 % of healthy individuals but capable of causing debilitating disease in the immunocompromised. This indicates the importance of immune defence in preventing infection and in restricting *C. albicans* to the superficial epithelial layers in the commensal state. Thus, it is essential to understand the host defence processes involved in initiating immune responses at mucosal surfaces and how ECs discriminate between the “commensal” and “pathogenic” states of this fungus.

Nearly all studies investigating the epithelial response to *C. albicans* utilise cytokine and chemokine production as the sole read-out mechanism. We and others have shown that infected ECs produce cytokines/chemokines with a proinflammatory profile, including IL-1α/β, IL-6, G-CSF, GM-CSF and TNFα as well as the chemokines RANTES, IL-8 and CCL20 [61;72–74]. However, effector responses are usually measured *in vitro* at 24 h, which is a late time point at which *C. albicans* has already adhered, invaded and induced cell damage. Therefore, it is difficult to delineate which of these processes contributes to the immune effector phenotype. What is known, however, is that *C. albicans* hypha formation is crucial for inducing cytokine responses from ECs [61]. This is supported by the fact that

other *C. albicans* species, which do not form hyphae in EC culture systems, or *C. albicans* strains unable to produce hyphae, correspondingly do not induce strong effector responses [50;73;75]. Therefore, the three key questions that need answering are: (i) How do ECs discriminate between the yeast and hyphal form of *C. albicans*, (ii) what are the fungal moieties and epithelial receptors that mediate epithelial recognition and activation and (iii) how does epithelial activation result in protection against fungal infection? Recently, there have been a number of important developments in these areas that we will now summarise.

6.1. How do ECs discriminate between the yeast and hyphal form of *C. albicans*?

Given the apparent importance of hypha formation in pathogenicity and epithelial activation, determining how ECs are activated by the yeast and hyphal form of *C. albicans* is of fundamental importance. To this end, recently a novel mechanism was identified that enables oral ECs to discriminate between *C. albicans* yeast and hyphae [61] (Fig. 2). Oral ECs orchestrate responses to *C. albicans* via NF-κB and a bi-phasic MAPK signaling response. Activation of NF-κB and an initial, transient MAPK response, resulting in activation of the c-Jun transcription factor, is independent of morphology and thus activated by both yeast and hyphae. However, activation of a second, stronger MAPK response, resulting in activation of the c-Fos transcription factor and production and stabilisation of the MAPK phosphatase MKP1, is specifically associated with hypha formation and correlates with proinflammatory responses and cell damage. A key finding was that the hypha-mediated response was strongly dose-dependent, indicating that this MAPK/MKP1/c-Fos activation system may constitute a ‘danger response’ mechanism that permits epithelial tissues to remain quiescent in the presence of low fungal burdens whilst responding specifically and strongly to damage-inducing hyphae when burdens increase. If so, this mechanism may be critical to the host’s ability to identify when this normally benign fungus has become pathogenic [61].

6.2. What are the fungal moieties and epithelial receptors that mediate epithelial recognition and activation?

Since *C. albicans* yeast and hyphae activate ECs differently, it is critically important to identify the fungal moieties and epithelial receptors that mediate recognition and activation. However, in terms of the EC response “recognition” and “activation” are not the same, in that both the yeast and hyphae of *C. albicans* are recognised by ECs but only hyphae induce full activation and effector responses in ECs. As mentioned above, only one specific fungal-epithelial interaction has been demonstrated to date. This is between Als3 on the hyphal surface and E-cadherin on the oral EC, which results in endocytosis of the fungus via clathrin-mediated mechanisms [23]. However, whether E-cadherin engagement results in activation of epithelial immune responses is currently unknown.

Some data can be extrapolated from myeloid/lymphoid cell studies, which indicate that the main inducers of immune responses are polysaccharide components of the fungal cell wall, specifically *N*- and *O*-mannan, phospholipomannan and β-glucan [76]. The other polysaccharide constituent of the fungal cell wall, chitin, is not directly stimulatory to myeloid cells but does have the ability to block the recognition of *C. albicans* by human peripheral blood mononuclear cells and murine macrophages [77]. Together, these fungal agonists activate cells via specific pattern recognition receptors (PRRs), namely mannose receptor, toll-like receptor (TLR)4, TLR2 and dectin-1, respectively [76] (a receptor for chitin has not yet been found). However, the relative importance of these interactions in the detection of *C. albicans* at mucosal surfaces is unclear given that the EC is the first cell that encounters *C. albicans*. Although ECs are known to express a range of PRRs including TLRs and dectin-1 [72;78], they appear to play a minimal role in activating epithelial immunity as blockade or inhibition of these receptors does not affect activation of the

MAPK/MKP1/c-Fos system or cytokine production against *C. albicans* [61]. Likewise, stimulation of oral ECs with purified *N*- and *O*-mannan and β-glucan from *C. albicans* does not activate cytokine production. This lack of activation was also recently demonstrated in skin keratinocytes when using heat-killed *C. albicans* [79] (which might result in exposure of multiple cell surface antigens and/or cell wall structures), suggesting that *C. albicans* cell wall polysaccharides may have a limited role in inducing EC responses. Notably, however, *N*- and *O*-mannan and β-glucan do activate NF-κB and the first phase of the MAPK bi-phasic response, activating c-Jun [61]. This suggests a model of EC-*C. albicans* interaction whereby recognition of *C. albicans* cell wall polysaccharides informs the host of the presence of fungus but is insufficient to induce immune effector responses. Induction of EC immune effector responses are instead possibly driven by a combination of the recognition of a surface moiety specific to the hyphal form of *C. albicans*, whose identity is currently unknown, and detection of cell damage.

Together, these studies suggest that ECs utilise different sensing mechanisms for immune activation than myeloid cells, possibly as they target alternative fungal moieties. It has been demonstrated that myeloid cells respond strongly to the yeast form of *C. albicans*, whilst being less responsive to the hyphal form. In contrast, despite recognising yeast cells, ECs only 'activate' in response to the hyphal form [61]. These differences probably reflect differing needs at the locations that each cell type is likely to encounter the fungus and the different morphological forms that are pathogenic or invasive at these sites.

6.3. How does epithelial activation result in protection against fungal infection?

Despite advances in our knowledge of immune cell- and epithelial-fungal interactions, the relative importance of each interaction in the context of a mucosal infection and protective immunity is unclear. However, one would expect a significant level of immunological crosstalk between ECs and local immune cells in order to either maintain homeostasis (commensal state) or to elicit a protective immune response (pathogenic state). As in most mucosal infections, the secretion of pro-inflammatory cytokines and chemokines by ECs in response to *C. albicans* will result in the recruitment, differentiation and activation of a variety of immune cells including neutrophils, dendritic cells and T cells. These cells are integral in mediating innate and adaptive responses that will result in protective mucosal immunity against *C. albicans*.

The innate arm of the anti-*C. albicans* response involves the release of epithelial cytokines that will recruit and activate neutrophils [80;81] which appear to have a dual role in anti-*C. albicans* mucosal immunity. Neutrophils can directly kill *C. albicans* cells through phagocytosis and degranulation [82] or through the recently discovered Neutrophils Extracellular Traps (NETs) [83]. NETs occur as a specialised form of neutrophil cell death and comprise a web of chromatin 'fibres' coated with serine proteases, antimicrobial proteins and other molecules which capture and kill *C. albicans*. Neutrophils also appear to protect against *C. albicans* infection indirectly via immunological cross-talk with the epithelium. This intriguing mechanism was described by Weindl *et al.* [72] who found that addition of neutrophils to an epithelial model resulted in protection against *C. albicans* infection and cell damage via epithelial TLR4. Notably, *C. albicans*-induced cell damage was abolished irrespective of whether the PMNs were separated by a membrane or applied directly to the epithelium. This demonstrated that PMN-dependent protection against *C. albicans* infection was independent of PMN migration into the epithelial tissue and direct cell-cell contact with the oral epithelium. Furthermore, *C. albicans* invasion and cell injury could be restored with TLR4 blocking antibodies or knockdown of TLR4 using RNAi, even in the presence of PMNs, demonstrating a direct role for epithelial TLR4 in antifungal defence at mucosal surfaces. This data is of specific interest as it demonstrates that although epithelial TLR4 is not required for the initial recognition or activation of ECs [61;72], it

plays a critical role in subsequent epithelial protection induced by neutrophils. This is the first description of such a PMN-dependent TLR4-mediated protective mechanism at epithelial surfaces and may provide significant insights into how fungal infections are controlled at the oral tissues (Fig. 3). It also demonstrates that far from being bystanders during infections, ECs play an active and integral role in innate protection against pathogens. Interestingly, PMNs do not appear to play an obvious protective role in the vaginal lumen and indeed, in humans, PMNs might even exacerbate vaginal disease [84]. The reasons for this are as yet unknown.

The adaptive arm of the anti-*C. albicans* response is initiated through recruitment of dendritic cells (or langerhans cells) to the site of mucosal infection (Fig 4). This will likely be through the actions of epithelial CCL20 and β-defensin 2, which are ligands for CCR6 expressing mucosal-homing dendritic cells. Dendritic cells will recognise *C. albicans* through established PRRs, including TLR2/4, mannose receptor, dectin-1, dectin-2 and DC-SIGN, and traffic to the local lymph node where processed fungal antigen will be presented to T cells to initiate adaptive immunity, which can take the form of Th1/2/17 and T regulatory (Tregs) cell induction. Good evidence exists indicating that cellular immunity, mediated by Th1 CD4+ cells, protects against oral and gastrointestinal *C. albicans* infection as demonstrated experimentally in murine models and clinically in humans, in which various immunocompromised patient populations (e.g. AIDS and transplant patients, and patients undergoing steroid therapy) are more susceptible to oropharyngeal candidiasis [85]. However, in vaginal candidiasis, although a Th1-type cellular immunity can also be generated, it does not appear to be protective against *C. albicans* infection. Rather, local mucosal immunity together with immunoregulation by γδ T cells and dendritic cells might be the predominant immune mechanisms governing protection at the vaginal mucosa [86].

The epithelial cytokine/chemokine profile elicited by *C. albicans* also permits the recruitment of Th17 cells [87] and dendritic cell recognition of fungi through dectin-1 and dectin-2 appears to play an instrumental role in driving Th17 development [88]. Also, recently the dectin-1/inflammasome pathway in macrophages was identified as being responsible for the induction of protective Th17 responses that discriminate between yeasts and hyphae of *C. albicans* [89]. With regard to immune cell interplay, Treg cells may also promote Th17 cells *in vitro* and enhance host resistance in mouse model of oral candidiasis [90]. Th17 cells secrete IL-17A and IL-17F as well as IL-22 and have been associated with mucosal defense against oral candidiasis [91] but the exact role of these cells in anti-*C. albicans* immunity is not fully understood, with evidence to suggest both a positive [91;92] and negative [93] role. Irrespective, it is becoming clear that the Th17 cytokines IL-17A, IL-17F and IL-22 play a significant role in anti-fungal immunity [94;95]. IL-17A and IL-17F act on ECs and neutrophils by inducing antimicrobial peptides, metalloproteases and other inflammatory mediators, thereby functioning as a bridge between the adaptive and innate immune responses. IL-22 has similar effects to IL-17 on ECs but may also control yeast cell growth and epithelial integrity during infection [94].

The importance of the Th17 response in mucosal immunity to *C. albicans* infections is highlighted by several recent studies linking defects in Th17 responses to chronic mucocutaneous candidiasis (CMC) [95;96]. This link is supported by the finding that in cases of autoimmunity with neutralising antibodies to Th17 cytokines (IL-17A, IL-17F and IL-22), there is an increased incidence of CMC [97]. Recently, in a breakthrough study, two genetic etiologies of CMC were identified: autosomal recessive deficiency in the IL-17 cytokine receptor, IL-17RA, and autosomal dominant deficiency of IL-17F [98]. IL-17RA deficiency was complete and abolished cellular responses to IL-17A and IL-17F homo- and heterodimers. By contrast, IL-17F deficiency was partial, with mutant IL-17F-containing homo- and heterodimers displaying impaired, but not abolished, activity. These findings

indicate that IL-17A and IL-17F are essential for mucocutaneous immunity against *C. albicans* [98].

7. Summary and conclusions

It is becoming clear that the *C. albicans*-epithelial cell interaction at mucosal surfaces is critical for commensalism, pathogenicity and host defence. *C. albicans* is able to bind to many EC lineages, which results in adhesion and epithelial invasion via two mechanisms: induced endocytosis or active penetration. Hypha formation is key to both mechanisms but we are only just beginning to identify the fungal and epithelial components associated with these processes. Both induced endocytosis and active penetration may ultimately result in cell damage, which can be induced via necrosis or apoptosis. However, it is still uncertain how either mechanism promotes pathogenicity and it is likely that numerous other fungal and host factors, which are currently unknown, are required for both mechanisms. The task of identifying these factors is clearly warranted but determining their role in *C. albicans* pathogenesis will be challenging and complicated. Concomitantly, fungal-epithelial interactions activate innate immune responses in the EC but the contribution of induced endocytosis or active penetration to this process is also unclear. Irrespective, a key aspect of epithelial activation is the specific recognition of *C. albicans* hyphae via a MAPK-based mechanism that involves c-Fos transcription factor activation. This response is highly dependent upon hyphal burdens and may constitute a ‘danger response’ mechanism informing the host of potentially dangerous levels of fungal hyphae. Epithelial activation results in secretion of proinflammatory cytokines and chemokines that recruit neutrophils, dendritic cells and T cells to the site of infection, ultimately resulting in either clearance or a return to the non-activatory colonization ‘commensal’ state. During an infection episode, fungal proliferation and immune activation may translate into signs and symptoms of infection, and the cycle of immune activation (based on fungal burdens and hypha formation) followed by subsequent clearance may represent what occurs in patient populations that experience acute *C. albicans* infections. In the clinical setting, the successful targeting of specific epithelial signaling networks or novel *C. albicans* processes/proteins resulting in the prevention or control of infection could provide a basis for novel treatment strategies against mucosal fungal infections.

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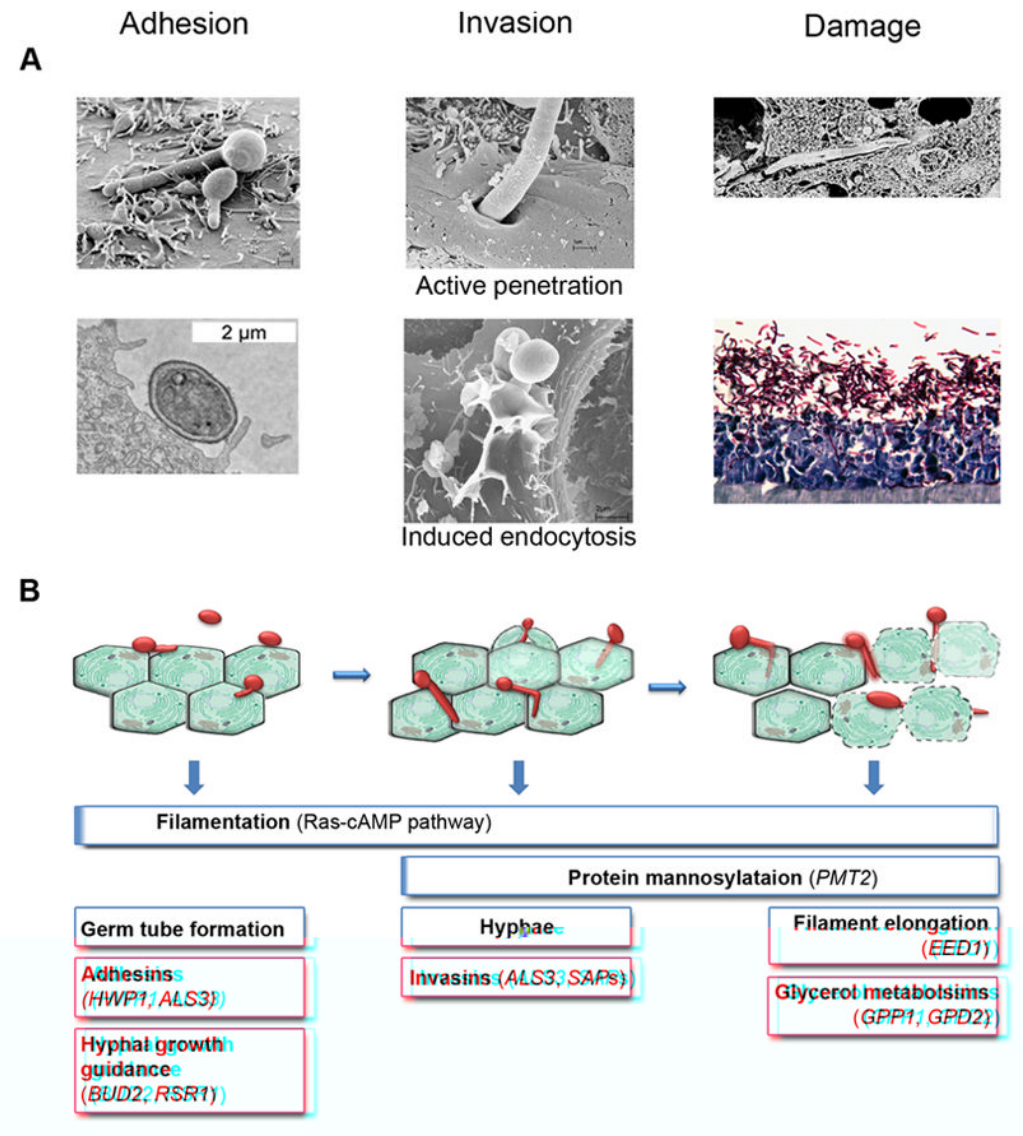

Figure 1. Stages of *C. albicans* oral infection

The progression of *C. albicans* infection of oral epithelial cells is characterised by three distinct stages (from left to right): adhesion, invasion via two different routes (active penetration and induced endocytosis), and tissue damage. (A) Scanning and transmission electron micrographs and histology of epithelial tissue (kindly provided by Holland, Özel, Zakikhany, Schaller, Wächtler, Hube). (B) Schematic drawing of the three distinct stages including factors and activities that are involved. Examples of genes necessary for each activity are in brackets. SAPs - secreted aspartic proteases.

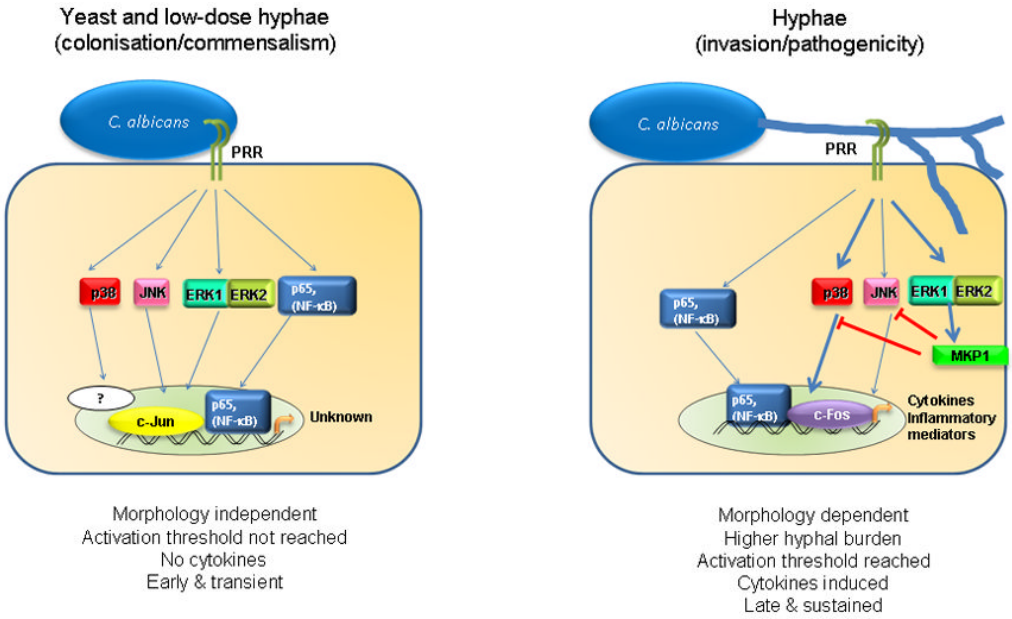

Figure 2. Signalling pathways that discriminate between *Candida albicans* yeast and hyphae

Oral epithelial cells are able to discriminate between the two morphological forms of *C. albicans* via the MAPK pathway using a biphasic response. In the first response, a colonising yeast cell is recognised by an as yet undetermined Pattern Recognition Receptor (PRR) resulting in activation of the NF-κB pathway and weak, early and transient activation of all three MAPK pathways (p38, ERK1/2 and JNK). This results in activation of p65/p50 heterodimer transcription factor activity (NF-κB) and activation of c-Jun DNA binding via the ERK1/2 and JNK pathways. What role, if any, is played by p38 at this point is unknown. Hyphal cells present at low dose also activate this first response. In the second phase, when in sufficient quantities, *C. albicans* hyphae are recognised by an unknown mechanism that results in continued activation of the NF-κB pathway along with further, stronger activation of the MAPK pathways – in particular p38 and ERK1/2. Activation of p38 leads to activation of the c-Fos transcription factor, which, in conjunction with the p65/p50 NF-κB heterodimers, results in upregulation of cytokine and inflammatory mediator expression. At the same time, activation of ERK1/2 signalling results in stabilisation of the MKP1 phosphatase, which targets p38 and JNK to deactivate them, hence acting as part of a negative feedback loop and preventing a potentially deleterious over-reaction of the immune system.

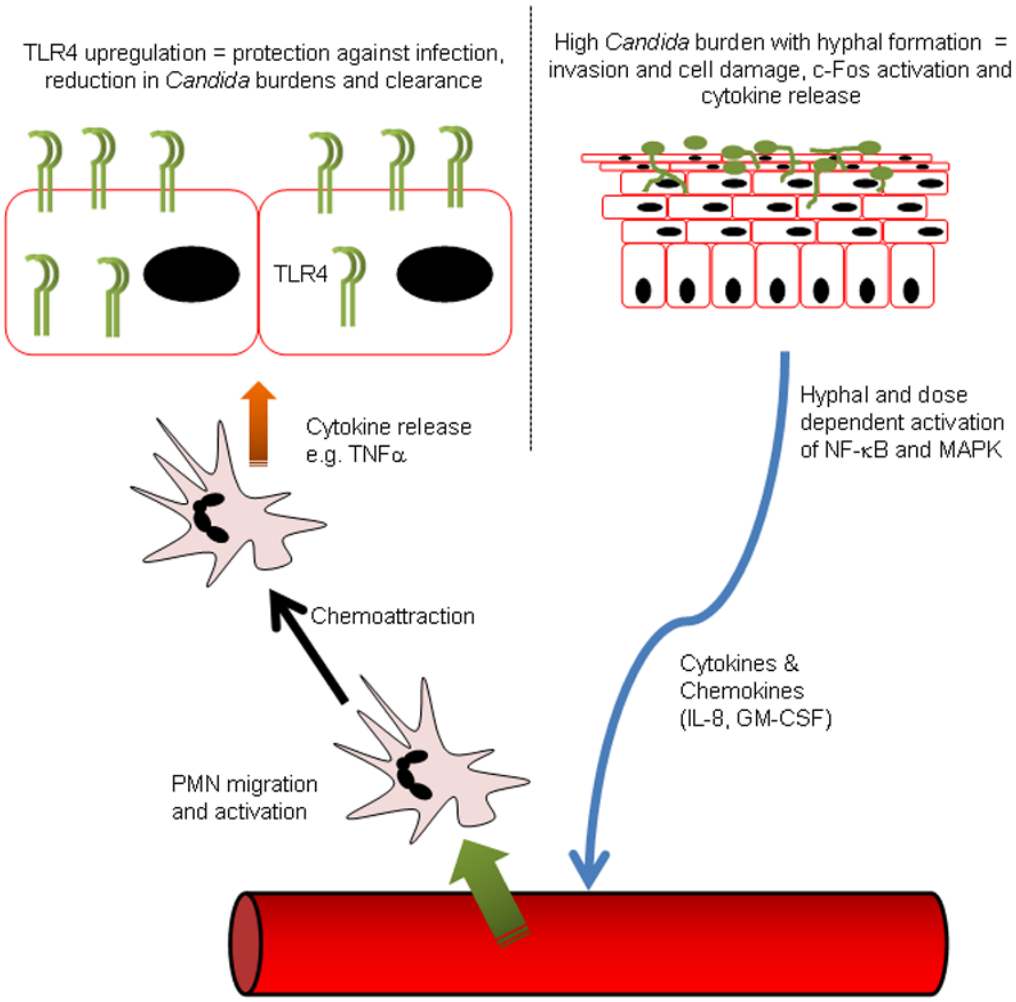

Figure 3. Model of mucosal protection against *Candida albicans* infection

Infection and subsequent invasion of the epithelial surface by *C. albicans* hyphae results in the production of cytokines and chemokines in a dose dependent fashion as described in the text and in the legend to Fig. 2. Secreted chemokines, particularly IL-8, will recruit neutrophils (polymorphonuclear leukocytes (PMN)) from the circulating blood to the infected epithelium. Here, EC-secreted cytokines (e.g. GM-CSF) will further activate the PMNs and induce them to secrete their own cytokines, including TNFα, which then act on the local epithelial cells causing upregulation of TLR4 and potentially other mediators. TLR4 upregulation induces a protective/resistance phenotype and promotes clearance of the invading fungus by an as yet unknown mechanism.

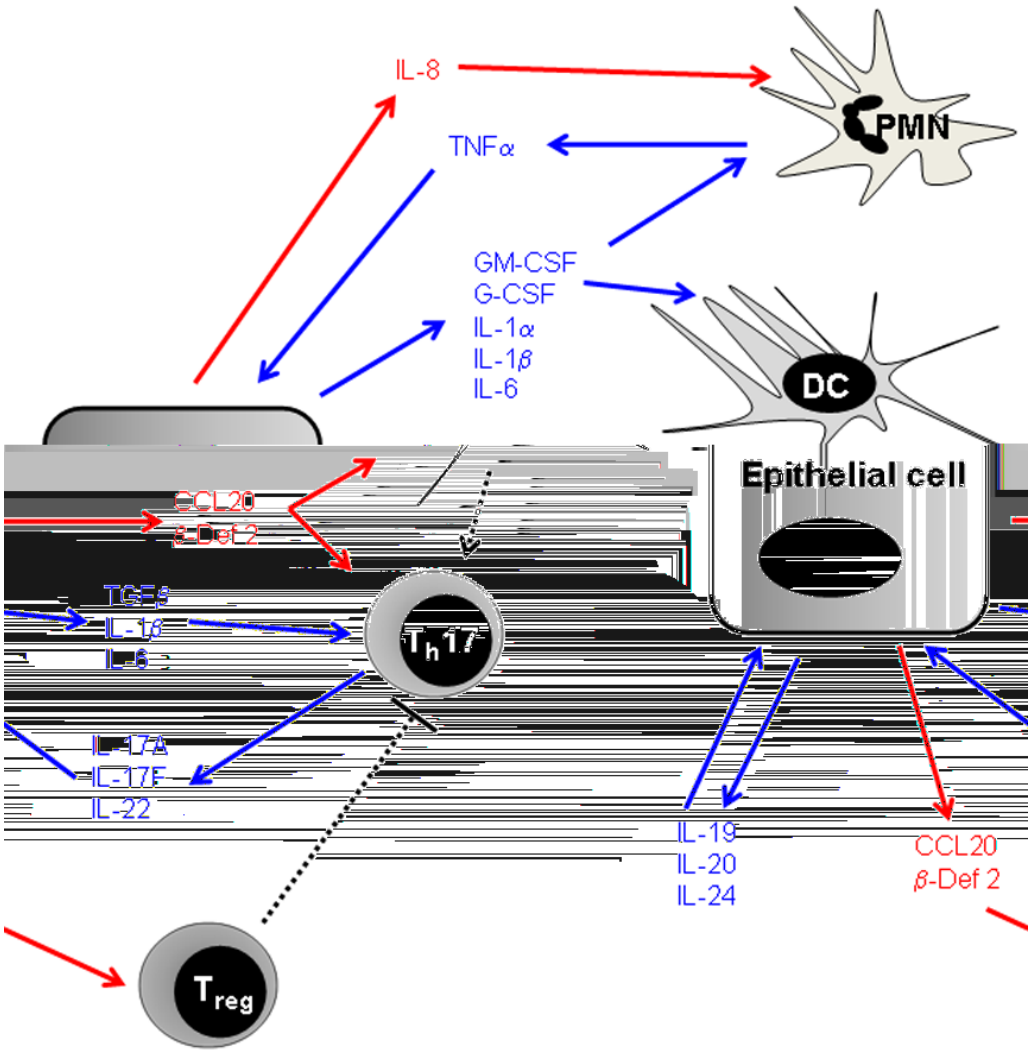

Figure 4. Cytokine/chemokine network induced during *C. albicans* infection of mucosal epithelium

Infection of epithelial cells by *C. albicans* results in the production of cytokines (blue) and chemokines (red) which recruit and activate various other immune cells. The best documented of these networks is initiated by IL-8. IL-8 recruits circulating neutrophils (PMNs) that are then activated by a variety of cytokines including GM-CSF, G-CSF and IL-1 family members. Activated PMNs then produce TNFα which then affect epithelial gene transcription. TGFβ is produced constitutively by epithelial cells and will act with IL-1β and IL-6 to induce T cell differentiation to the Th17 phenotype. Mucosal homing cells including Th17 T cells and activating dendritic cells will also be recruited by the increased expression of CCL20 and β-defensin 2, acting through the CCR6 receptor. This will lead to the presence of active Th17 T cells in the region to combat the fungal infection. CCL20 and β-defensin 2 will also recruit in Treg cells which will act to suppress and control the Th17 response. Finally, infection of epithelial cells leads to the production of IL-20 family cytokines including IL-19, IL-20 and IL-24. These cytokines will function in an autocrine fashion, although their role in fungal immunity is not fully understood.