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Review Article

Adherence mechanisms in human pathogenic fungi

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Interactions of human pathogenic fungi with the host tissues are key factors in the pathogenesis of mycoses. Based on the concept that adherence of microorganisms is a prerequisite for initiation of the disease, numerous studies have been conducted to identify the fungal adhesins and their respective receptors. Several adhesins recognizing different host ligands, sometimes with multifunctional properties, have been described. Some of them have been extensively characterized, and their expression analyzed according to morphological changes or culture conditions. For some ligands, the amino acid or carbohydrate motifs participating in these interactions have been identified. Various host proteins or glycoproteins have been suggested as ligands, including components of biological fluids, or extracellular matrix and basement membrane proteins; equally adherence to several cell types, mainly epithelial and endothelial cells, or to biomaterials has been considered. This review synthesizes available information regarding adherence of the most important human fungal pathogens. It is divided into three sections corresponding to the three main groups of pathogenic fungi: *Candida* yeasts, opportunistic moulds and other filamentous fungal pathogens, and dimorphic fungi.

Keywords Adherence, extracellular matrix, *Candida* yeasts, Pathogenic aspergilli, dimorphic fungi

Introduction

Due to the markedly increased incidence of fungal infections during the past decades, a particular attention has been paid to elucidating host-pathogen relationships. Initial interactions of pathogenic fungi with the host cells, which are essential for colonization of the host tissues and the initiation of the disease, have been the topic of much scientific investigations [1–9].

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Fungal pathogens display a large variety of adhesins that are expressed at their surface. When introduced into the host, they are therefore capable of adhering to a large variety of cell types, and of interacting with numerous ligands present in various host sites like biological fluids, extracellular matrix or basement membranes.

As an interface between the pathogen and its host, the fungal cell wall plays a pivotal role in these interactions. This macromolecular structure is characterized by a highly complex organization and biochemical composition [10–16]. Each fungal species has unique glycan polymers and proteins, interconnected to each other within the cell wall. Cell wall proteins are highly glycosylated and have negatively charged phosphate groups in their carbohydrate side chains, which

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strongly affect the electrostatic charge. They may also contribute to the cell surface hydrophobicity, which is important for adherence to biomaterials. In addition, growth of the fungus into the host implies continuous biosynthesis and remodeling of their cell wall, and many fungal pathogens may invade the host at different morphological states, which may differ greatly in the organization and biochemical composition of the cell wall.

The increasing knowledge of the composition and structure of the cell wall, together with the recent availability of fungal genomes, has led to the identification and characterization of adhesins in many pathogenic species and of their host ligands. The present knowledge of the adherence mechanisms in the major pathogenic fungi is presented in this review.

Adherence of *Candida* species

Interactions of *Candida* yeasts and/or hyphae with the host cells are crucial for the initial colonization of the host, as well as for eliciting the immune reponse and invasion of the tissues. *Candida albicans*, which remains the major cause of candidiasis, is an opportunistic pathogen responsible for a wide variety of infections, particularly in immunocompromised hosts. This pleomorphic fungus can grow either in a yeast form (commensal) or in a hyphal form (invasive) which is initiated by germ tubes arising from yeast cells, these two forms differing in cell surface components. Initial interactions of the fungus with the host are mediated by a particular compartment of the fungal cell, the cell wall, which comprises various components involved in adherence and designated as adhesins. Adherence of *C. albicans* to inert surfaces or biological substrates is considered as a major attribute of its virulence [1–4,6,7,9]. Elucidation of its molecular mechanisms has been the subject of many studies which have highlighted the large repertoire of adhesins displayed by *C. albicans*, in relation to the high complexity of the cell wall.

The cell wall of C. albicans

The cell wall of *C. albicans* is organized in several layers mainly composed of polysaccharides comprising three types of carbohydrates: D-glucose, N-acetyl-D-glucosamine and D-mannose [12,14]. Any layer may be considered as a zone of enrichment, the cell wall being thought as a highly dynamic structure capable to modulate its composition and organization according to environmental conditions [11].

Together with chitin, glucans form the rigid skeleton of the cell wall. Glucans consist in highly branched polymers whith β-1,3 and β-1,6 linkages. Some mannoproteins are linked to β-1,6-glucans through a glycosyl-phosphatidyl-inositol (GPI) anchor. Chitin is a linear polymer of N-acetyl D-glucosamine residues linked by β-1,4 bonds forming the core of the septum between bud and mother cell. However, some chitin is found attached to β-1,3 glucans, particularly in hyphae, helping to determine the shape and rigidity of the cell wall.

Mannose polymers (mannans) are found only in covalent association with proteins (mannoproteins). These polysaccharides consist in short chains of mannose residues with α-1,2 or α-1,3 bonds, linked to the hydroxyl group of seryl or threonyl residues of the protein core, or in a linear chain of α-1,6 linked mannose residues with branched side chains of α-1,2 or α-1,3 mannose. Additional β-1,2-oligomannosides are linked through phosphodiester bridges to the carbohydrate chains. Sialic acids are also constituents of the *Candida* cell wall, presumably as terminal residues of the carbohydrate side chains of mannoproteins. Mannoproteins are the major components of the outer cell wall layer (Fig. 1) where they appear as a dense network of fibrils arranged perpendicularly to the cell surface and called fimbriae.

Most of the cell wall proteins (CWPs) are mannoproteins [15,17,18], representing 30–50% of the cell wall

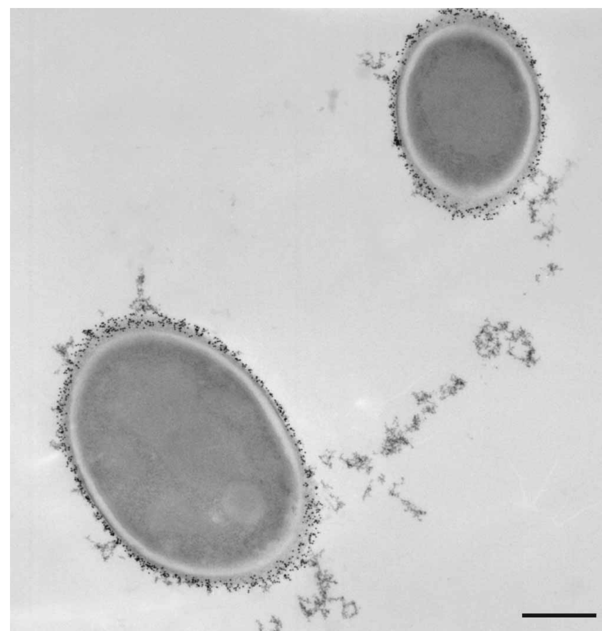

Fig. 1 Mannoproteins of the outer cell wall layer of *Candida albicans* germ tube as revealed by treatment with ConA-gold. Bar corresponds to 1 μm.

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dry weight. Two main classes of CWPs have been described in the cell wall: GPI proteins (GPI-CWPs) such as the adhesins Als1p and Als3p, which are localized in the outer cell wall layer and linked to other cell wall components (mainly β-glucans) by their GPI anchor, and proteins encoded by members of the *PIR* (proteins with internal repeats) gene family (Pir-CWPs) which are uniformly distributed throughout the inner layer of the cell wall and covalently linked to β-1,3-glucans. These last proteins present an N-terminal signal peptide, an internal repeat region, and a highly conserved C-terminal region.

Adhesins and other binding proteins

Glycans. The outer cell wall layer is rich in highly glycosylated mannoproteins. Oligosaccharidic part of phosphomannoprotein complex (PMPC) may contribute to host-fungus interactions. A β-1,2-linked mannotetraose appeared to mediate adherence of yeasts to spleen and lymph nodes in an *ex vivo* experiment [19]. Complete digestion of the acid-stable moiety of PMPC with α-1-2-mannosidase or its hydrolysis with 0.6 N sulfuric acid abolished the adhesive porperties [20]. In an adherence model using differentiated Caco-2 cells, Dalle et al. [21] found that MAbs specific for α-1,2 or β-1,2 mannan epitopes inhibited adherence in a dose-dependent manner. Among all carbohydrates tested in competitive assays, α-1,2 and β-1,2 tetramannosides were the most potent inhibitors of adherence. Likewise, administration of β-1,2 oligomannosides in a mouse model prior to inoculation with C. albicans was shown to prevent colonization by a virulent strain, whereas synthetic α-mannosides had no effect [22].

The glycosylation process in mannoproteins is highly complex, involving several mannosyltransferases (Mnt) or protein mannosyltransferases (Pmt) for O-glycosylation, Pmt-proteins in the endoplasmic reticulum and Mnt-proteins in the Golgi. The lack of Pmt6p results in a significant decrease in adherence to endothelial cells and, compared to their parent strain, both *mnt1Δ* and *mnt2Δ* mutants exhibit a significant reduction in their capability to adhere to human buccal epithelial cells (BECs) and to surfaces coated with a commercial cell-free basement membrane preparation (Matrigel™) [23,24].

Other cell wall carbohydrates are also involved in adherence. For instance, sialic acids which contribute to the electronegative charge of fungal cells, partly mediate the adherence to a cationic solid phase substrate (poly-L-lysine), since the number of adherent yeast cells was significantly reduced after treatment with bacterial sialidase [25].

Adherence to biomaterials and biofilm formation. *C. albicans* adheres to various biomaterials, such as catheters, prostheses or medical implants, where it forms biofilms which contribute to tissue colonization [26]. Cell surface hydrophobicity (CSH) plays a crucial role in this adherence process and hyphae are more hydrophobic than yeast cells [27,28]. Using an adherence assay to polystyrene Petri dishes, we demonstrated that *C. albicans* germ tubes developed an outer fibrillar layer which promotes adherence to plastic (Fig. 2) [29]. After removal of the adherent germ tubes, four components of 60, 68, 200 and >200 kDa were solubilized from the plastic surface, suggesting that these proteins are hydrophobic. Interestingly, these major components of the fibrillar cell wall layer of germ tubes

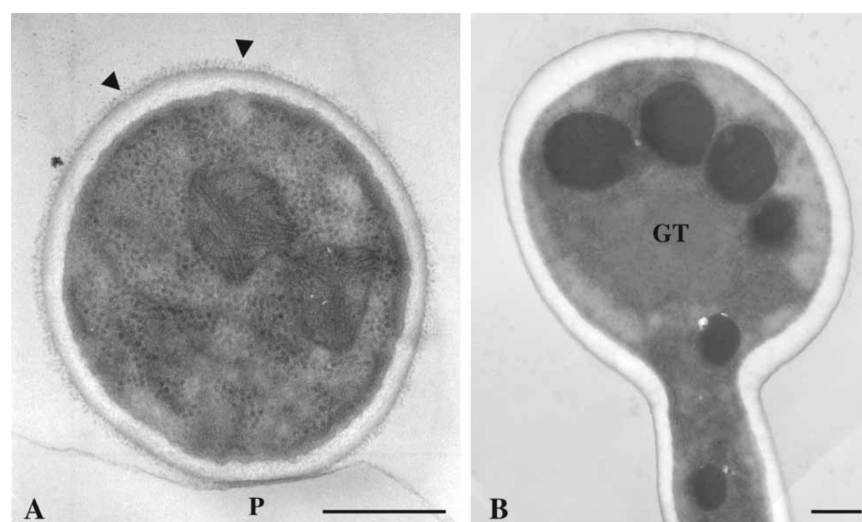

Fig. 2 Transverse sections of *Candida albicans* germ tubes (GT) adherent to a polystyrene (P) Petri dish (A) or produced in glass tube (B). Note the fibrillar layer (arrowheads) surrounding the cell wall in germ tubes adherent to the plastic substrate. Bars correspond to 0.5 μm.

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also act as receptors for some host proteins including laminin, fibrinogen and complement [30]. These results are in agreement with the fact that hydrophobic *C. albicans* strains bound in greater numbers than hydrophilic strains to immobilized fibrinogen, fibronectin, collagen type IV or laminin [31]. Likewise, a significant increase in CSH and in adherence to BECs was noted during progression of HIV disease [32]. Additionally, *C. glabrata* demonstrated a four-fold greater CSH and a two-fold greater tendency to adhere to denture acrylic surfaces compared with *C. albicans* [33], and adherence of *C. parapsilosis* to epithelial and acrylic surfaces correlated with CSH [34]. However, further studies are needed to specify the role of hydrophobic interactions in adherence.

Biofilm formation is considered as one of the most critical event in virulence of *C. albicans* and *C. dubliniensis* [35]. *C. albicans* biofilms consist in a multilayer of cells adhering to materials like latex, silicone elastomer or polyvinyl chloride, and embedded within a matrix of extracellular polymeric material [36]. Recent studies showed that the transcription factor Bcr1p regulates biofilm formation in an *in vitro* catheter model [37,38]. Expression of the gene *ALS3* which encodes an important adhesin at the surface of *Candida* cells, is also regulated by this transcription factor. Likewise, other known adhesins such as Hwp1p, a hyphae-specific adhesin, also contribute to biofilm formation [39].

Adherence and enzymes. Many cell wall-associated proteins are enzymes involved in cell wall synthesis and remodeling. A putative glycosidase Csf4p has been described as an important factor for cell wall integrity. Interestingly, deletion of CSF4 which leads to a defect in filamentation, also resulted in a reduced adherence to mammalian cells *in vitro* and in a diminished virulence in an immunocompetent mouse model of disseminated candidiasis [40].

CAMP65 of *C. albicans* encodes a putative β-glucanase of 65 kDa which has been characterized as the main target of the host immune response [41]. Disruption of *CaCAMP65* severely affected hyphal morphogenesis both *in vitro* and *in vivo* and resulted in the loss of the capabilities to adhere to plastic. Likewise, adherence to plastic was markedly inhibited in the presence of anti-Camp65p antibodies. The null mutant was also significantly less virulent than its parent strain in both systemic and mucosal infection models.

Secreted aspartate proteinases (Saps) of *C. albicans* may also contribute to adherence. It was found that pepstatin A reduced adherence of *C. albicans* to vaginal epithelial cells, suggesting that these enzymes may have an auxiliary role in adherence [42]. More, the adherence capabilities of strains harbouring specific disruptions in various members of the *SAP* gene family have been compared to those of their parent strain [43]. The adhesiveness of Δ*SAP1*, Δ*SAP2*, Δ*SAP3* null mutants and of a triple Δ*SAP4-6* disruptant was examined on glass surfaces coated with poly-L-lysine or with Matrigel™, as well as on human BECs. For each single *SAP* null mutants, adherence was either reduced or not affected significantly compared to the parent strain. The adherence of the Δ*SAP4-6* mutant was reduced on poly-L-lysine and Matrigel™, but increased on buccal cells [43]. In addition, inhibition of Saps by HIV protease inhibitors reduced adherence to epithelial cancer cells [44,45].

Agglutinin-like sequence proteins. Als (agglutinin-like sequence) proteins are the most widely expressed adhesins in *C. albicans* [6,46]. The *ALS* gene family encodes a group of GPI-anchored proteins that function as adhesins. There are at least eight distinct *ALS* genes in *C. albicans* genome. Mature Als proteins which are highly homologous to each other, consist in a 300-residue N-terminal region predicted to have an immunoglobulin-like fold (Ig), a 104-residue conserved threonine-rich region (T), a central domain comprising a variable number of tandem repeats (TR) of a 36-residue threonine-rich sequence, and a heavily glycosylated C-terminal serine/threonine-rich stalk region, also of variable length [47]. Motifs mediating substrate-specific adherence are localized in the N-terminal domain [48].

Among all Als proteins, Als1p, Als3p and Als5p have been extensively characterized, and recent works suggest that they play the major role in adherence of *C. albicans* to the host cells. Als1p and Als3p were reported to bind to endothelial and epithelial cells [49], whereas Als5p binds to extracellular matrix (ECM) proteins [47]. *ALS* gene expression in clinical specimens and experimental candidiasis was dependent of the infection site [50]. Als1p is important for adherence to the oral mucosa during the early stage of the infection [51]. Likewise, it was shown that *ALS2* and *ALS5* genes were up-regulated following incubation with Hep2 cells [52]. Soluble Als5p Ig-T and Ig-T-TR(6) fragments bound to fibronectin *in vitro*, but the inclusion of the TR region increased affinity [53]. *C. albicans* and *Saccharomyces cerevisiae* expressing the adhesin Als1p and Als5p adhere to accessible threonine, serine or alanine patches [54], and to a variety of protein and peptide ligands [55], indicating a degenerate adherence recognition system. Additionally, it

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was shown that the als1/als1mutant formed significantly fewer germ tubes and showed decreased adherence to human umbilical vein endothelial cells (HUVECs), but adherence to BECs and fibronectin was unchanged [56]. Conversely, the *ALS3* null mutant was defective in adherence to both HUVECs and BECs, but not to fibronectin-coated plastic plates [56]. Both *ALS1* and *ALS3*, as genes encoding other adhesins such as *HWP1*, are expressed mainly by hyphae. Expression of these genes is governed by the transcription factor Bcr1p (biofilm and cell wall regulator). A *BCR1* null mutant had diminished adherence to plastic, but *ALS3* overexpression restored biofilm formation in this genetic background [38]. The contribution of a single protein to *C. albicans* adherence seems difficult to determine by mutant analysis because of compensatory upregulation mechanisms affecting genes encoding other adhesins. For example, there was about 40% reduction in adherence of a strain with low expression of Als2p and 30% reduction in adherence of strain that lacked Als4p. Interestingly, there was a 2.8-fold increase in *ALS2* expression in the *ALS4* null mutant and a 3.2-fold increase in *ALS4* expression in a strain that had low expression of *ALS2* [57].

Hwp1p (hyphal wall protein 1), which is found exclusively at the germ tube surface, is another important adhesin of *C. albicans*, mediating tight binding to BECs. Hwp1 is the first cell surface protein of *C. albicans* known to be required for biofilm formation *in vivo*. In an *in vivo* model using venous catheters, the *HWP1* null mutant was defective in biofilm formation, producing only yeast microcolonies in the catheter lumen [39]. In addition, as a substrate for mammalian transglutaminase, Hwp1p mediates the attachment of germ tubes to transglutaminase-expressing epithelial cells in the superficial layers of stratified epithelia [58].

Integrins. Because *C. albicans* bind and adhere to numerous ECM proteins, it has been hypothesized the presence of integrin-like receptors at its surface. In mammalian cells, integrins are heterodimeric or heterotrimeric transmembrane proteins which recognize various ECM components by their peptide sequence, usually the tripepide RGD, and are therefore involved in various physiological or pathological processes such as diapedesis, cohesion inside tissues, and tumour metastasis. Numerous antibodies to human integrins, recognizing αM, αX, β1, β3 or β5 subunits, bound to *Candida* cells, and RGD peptides inhibited adherence of *Candida* cells to various ligands [59]. Research for integrin-like proteins led to the isolation of *INT1* gene in *C. albicans*. *INT1* expression in *S. cerevisiae* allowed this normally non-adherent yeast to adhere to human epithelial cells. Furthermore, disruption of *INT1* in *C. albicans* suppressed adherence to epithelial cells and virulence in mice [60]. Other integrin-like proteins have been suggested in *C. albicans*. For example, using polyclonal antibodies to mammalian α5β1 and αvβ3 integrins, as well as MAb directed towards the human vitronectin receptor, a 37-kDa protein was detected in cell wall fractions of *C. albicans* [61].

Lectin-like proteins. In *C. glabrata*, a major group of adhesins is encoded by the *EPA* (epithelial adhesin) gene family [62]. The overall structure of Epa proteins is similar to that of the Als proteins of *C. albicans*. Epa1p is a Ca^{2+}-dependent lectin that binds to N-acetyllactosamine-containing glycoconjugates [63]. Despite the large number of *EPA* genes, deletion of just *EPA1* reduces adherence *in vitro* to background levels because the other *EPA* genes are expressed at low levels [62]. However, although *EPA6* is not expressed *in vitro*, its expression increases during experimental urinary infection, suggesting that *C. glabrata* is capable to adapt to a specific host niche [64].

Lectin-like proteins have also been investigated as putative adhesins in *C. albicans*. Fucose, glucosamine and GlcNAc were the most potent inhibitors of adherence to human BECs for different *C. albicans* strains [65]. Moreover, as it will be discussed later, recognition of collagen IV is inhibited by several sugars constitutive of its N-linked oligosaccharide chains [66]. N-acetylglucosamine, L-fucose and methylmannoside caused a similar inhibition, but N-acetyllactosamine was a more effective inhibitor, whereas glucose, galactose or lactose did not affect the binding. However, if lectin-like adhesins have been suggested in *C. albicans*, they have not been characterized as yet.

Other putative adhesions. Although it is still a matter of debate, numerous data suggest that transition of *C. albicans* from the yeast to the hyphal phase is associated with virulence. Filamentation which occurs in response to environmental conditions (including temperature, pH), implies important changes in the structure and organization of the cell wall. Multiple genes that regulate the yeast to hyphae transition or which affect the cell wall biosynthesis and integrity also indirectly affect adherence [67–70].

Fimbriae are long filamentous appendages of the cell surface which can mediate adherence to the host cells. Their major structural subunit is a glycoprotein of about 66 kDa [71]. It has been reported that fimbriae bind to glycosphingolipid lactosylceramide [72] and to

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asialo-GM1 (gangliotetraosylceramide) [73]. So, fimbrial protein could represent sites for attachment of fungal cells to glycosphingolipids displayed on the surface of human BECs [73].

Host ligands

During the 1980s, considerable attention has been directed to elucidating the complex interactions between *C. albicans* and the host components and to the identification of the corresponding fungal receptors. Binding of the ligands and the distribution of their receptors at the cell surface have been studied by indirect immunofluorescence, flow cytometry, confocal fluorescence microscopy and immunoelectron microscopy. Moreover, the role of these ligands in adherence of fungal cells has been evaluated in *in vitro* models using cultured cells or proteins immobilized on microtiter plates, or in *ex vivo* models on tissue sections.

Plasma proteins. *C. albicans* yeast cells or hyphae can interact with a large variety of plasma proteins. Among them, fibrinogen, which plays a key role in inflammatory reaction, has been particularly studied. *In vitro*, human fibrinogen binds intensely to germ tubes and hyphae, but not to yeast cells grown on Sabouraud medium [74,75]. Interaction takes place through the recognition of the D domains of the fibrinogen molecule by cell surface mannoproteins of 68 and 60–62 kDa [76,77]. Using other strains and culture conditions (Lee medium), Casanova *et al*. [78] reported the binding of fibrinogen to both yeasts and mycelia and identified a mannoprotein of 58 kDa (Mp58) in β-mercaptoethanol (βME) extracts as the fungal receptor. This mannoprotein was shown to be encoded by the gene *FBP1* (for fibrinogen binding protein) whose expression is dependent on the environment. Interaction with fibrinogen seems to be mediated by the O-linked carbohydrate chains of Mp58. Moreover, Mp58 comprises both collagenous domains and ubiquitin-like epitopes [79,80] and is expressed *in vivo*, but the relevance of Mp58 ubiquitin-like epitopes to adherence should be clarified. The binding of *C. albicans* to the ECM protein fibronectin or to blood platelets, however, seems to be mediated by fibrinogen [81,82].

Other works also suggested the ability of *C. albicans* to interact with the iC3b and C3d products of the C3 component of complement [83]. Major C3d binding activity was supported by two components of 50 and 60-kDa from a hyphal extract [84], the second component being associated with the hyphal wall. However, additional components exhibiting antigenic cross-reactivity with the human C3d receptor were identified in βME extracts from both blastoconidia and hyphae [85]. The candidal C3d receptor which is called CR2-like because of its functional similarities with the mammalian C3d receptor CR2 [86], is expressed *in vivo* as demonstrated by immunoelectron microscopy [86,87], but its role in adherence remains to be defined.

Identification of the candidal receptors for iC3b was performed using monoclonal antibodies (MAbs) directed towards the α-subunit of the human complement receptor 3 (CR3). Several polypeptides were detected, with molecular masses of 130, 100 and 50 kDa [88], 165 kDa [89] or 70, 66, 55, and 42 kDa [90]. According to antigenic and structural similarities between some of these proteins and human CR3 and CR4, the *C. albicans* iC3b receptor is usually considered as an integrin analog [91,92]. Additionally, several observations suggest that it may be involved in pathogenesis [91–94].

Extracellular matrix (ECM) proteins. ECM components form a complex network which provides multiple binding sites for attachment of microorganisms. In mammals, molecules involved in adherence to ECM components are highly conserved receptors, usually members of the integrin family that mediate cell-matrix and cell-cell interactions. Among ECM proteins, laminin, fibronectin, collagen type IV, entactin and vitronectin could be involved in adherence in *C. albicans.*

Laminin, the major non-collagenous component of basement membranes, binds to germ tubes, but not to yeasts cells of *C. albicans* [30]. Indirect immunofluorescence and immunoelectron microscopy revealed that binding sites are localized on the outermost fibrillar layer. The binding was shown to be saturable and specific, with about 8000 binding sites per cell and a *Kd* of 1.3×10^{-9} M. Ligand blotting performed with a cell wall extract of *C. albicans* germ tubes revealed two components of 68 and 60–62 kDa as the laminin receptors [30]. Moreover, laminin receptors were shown to interact also with fibrinogen and C3d and to enhance adherence to plastic surfaces, suggesting their multifunctional nature [76]. However, in other studies, yeasts cells were also shown to adhere to immobilized laminin [95,96], and attachment seemed to be mediated by two polypeptides of 37 (p37) and 67 kDa, the former cross-reacting with the carboxy-terminal laminin-binding domain present in the human 67-kDa high-affinity laminin receptor [97].

Fibronectin is a high molecular weight multifunctional dimeric glycoprotein that can be found under a soluble form in plasma and an insoluble form as part of ECM. Adherence of *C. albicans* yeast cells to immobilized fibronectin was first demonstrated [98].

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Fibronectin can mediate adherence of yeasts to vaginal epithelial cells [99], but CSH and composition of the growth medium largely affect the binding capabilities [100,101]. Several fibronectin binding components with molecular masses of 60, 62, 72 and 105 kDa were isolated from a detergent extract of yeast cells [102]. Moroever, the fibronectin receptors which were detected in the cell membrane and the cell wall of both yeasts and germ tubes, are antigenically and functionally related to the human α5β1 integrin receptor for fibronectin [59]. MAbs directed towards the α5 and the β1 integrin subunits stained germ tubes in flow cytometry and inhibited adherence to immobilized fibronectin [59]. Likewise, fibronectin binding was partially inhibited by RGD-containing peptides. Less pathogenic *Candida* species, including *C. glabrata* and *C. tropicalis*, also exhibit reactivity with anti-α5 antibodies [103]. Moreover, cell adherence assays with *S. cerevisiae* showed that Als5p mediates adherence to fibronectin and that binding is significantly enhanced by the TR region [53].

Interections with immobilized type I and type IV collagen, respectively, found in connective tissue and basement membrane, have also been investigated. Recognition of the proteins seems to be mediated by distinct fungal receptors. Among fragments of gelatin (denatured type I collagen), a 47 amino-acid fragment of the α1 chain (residues 40–86) which contains three RGX sequences, completely inhibited adherence to immobilized type I collagen [104]. Two gelatin binding components with molecular mass similar to that of some fibronectin binding proteins, 60 and 105 kDa, have been isolated from yeast cell extracts [102]. In contrast, several sugars known to be part of the N-linked oligosaccharide chains of collagen IV, particularly N-acetyllactosamine inhibited adherence to the immobilized 7S domain [66]. Thus, it seems that at least one of the candidal adhesins interacting with type IV collagen is a lectin which recognizes the 7S(IV) oligosaccharide residues.

Other ECM components may also interact with *C. albicans*. Yeast and hyphal forms bind to entactin and an RGD peptide inhibited binding by approximately 50% [105]. The interaction occurs through components of 25, 44 and 65 kDa present in βME extracts, possibly by collagenous domains interacting with the collagen binding sites of entactin. *C. albicans* yeasts also bind to vitronectin, a constituent of vascular walls and dermis [106]. This interaction is mediated by fungal receptors antigenically related to the vertebrate α5β3 integrins [107]. Biochemical analysis of yeast lysates with anti-human αv, β3 or β5 antibodies revealed molecular species of 130, 110, 100, and 84 kDa [108]. The 130-kDa band was identified as α5, whereas the doublet of 110/100 kDa and the 84-kDa band corresponded to β3 and β5 subunits, respectively. However, other studies suggested recognition of the glycosaminoglycan-binding domain of vitronectin (50–85% inhibition of the binding with heparin) [107,109], or binding to β-glucans as described for *Pneumocystis carinii* [110]. Since vitronectin is highly adsorbed to biomaterials, attachment to this glycoprotein may represent a major cause of infections [106]. As revealed by immunofluorescence, *C. albicans* germ-tubes, but not blastoconidia, also bind to soluble human tenascin-C, a large multimeric ECM protein involved in tissue and organ morphogenesis, as well as in adherence and cell migration. Fibronectin, but not fibrinogen, inhibited the binding, thus indicating a role of the fibronectin type III repeats in this interaction [111].

Clearly, the role of adherence to ECM in the initiation of candidiasis has not been established as yet. Considering the multiple fungal receptors described, it seems that several distinct mechanisms are involved in the binding of *C. albicans* to ECM components, and it is not sure that a genetic approach using mutant strains deficient in binding to the ECM proteins will be helpful to define the contribution of these interactions to pathogenesis.

Salivary proteins. Attachment of *C. albicans* to dental prostheses or to salivary macromolecules adsorbed on their surface is believed to be a critical event in the development of denture stomatitis. Whole saliva enhances the adherence of *C. albicans* yeasts cells to hydroxyapatite beads [112] and saliva proteins selectively adsorbed to silicone promotes adherence of *C. albicans* [113]. Among salivary components which may be involved in the interaction, are basic proline-rich proteins, particularly four components of 17, 20, 24 and 27 kDa, statherin, and a low-molecular-weight mucin (MG2) in human saliva or the rat submandibular gland mucin (RSMG) in rat saliva [112,114]. However, the presence of whole saliva was also shown to decrease adherence of germinated cells to polystyrene [115] or to herculite [116] and this inhibition seemed to be due to the blockage of germ tubes surface adhesins by salivary IgA rather than to an inhibition of germination. Recently, Jeng *et al*. [117] purified two fungal polypeptides of 97.4 and 35 kDa that bind immobilized salivary components, the last one corresponding to the 1,3-β-glucosyltransferase. Adherence of *C. albicans* to BECs was also quantitatively inhibited by graded concentrations of intestinal mucin. However, this inhibition was reversed by pretreatment of mucin with pronase or by *C. albicans* secretory aspartyl

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proteinase Sap2p, suggesting that *C. albicans* may both adhere to and degrade mucins [118]. The reactive component of intestinal mucin was identified as a 66-kDa cleavage product of the 118-kDa C-terminal glycopeptide.

Cell surface components. *C. albicans* adheres to a large variety of mammalian host cells *in vitro* and *in vivo*, including epithelial and endothelial cells. Besides the ECM proteins, numerous components of the host cell surface were suggested to mediate the attachment. For example, the intercellular adhesion molecule 1 (ICAM-1) has been demonstrated to play a role in adherence of *C. albicans* to pulmonary vascular endothelial cells, and by extension in invasion of the lung tissue [119]. Likewise, *C. albicans* hyphae bind to N-cadherin on the surface of vascular endothelial cells *in vitro*, and this interaction stimulates endocytosis [120].

Adherence mechanisms in pathogenic filamentous fungi

Because of the dramatic increase in the frequency of fungal infections due to opportunistic moulds during the past decades, much work has focused on elucidating the pathogenesis of these infections. These fungi are primarily responsible for respiratory infections by inhalation of their airborne conidia. Therefore, adherence of conidia to the epithelial cells or to the underlying basement membrane was thought to play a crucial role in the establishment of the fungus and the initiation of the disease in a receptive host. Particular attention has been paid to *Aspergillus fumigatus*, which is the major causative agent of human and animal aspergillosis [121], and several components have been suggested as virulence factors, as proteolytic enzymes and phospholipases, or catalases and superoxide dismutases, as well as non ribosomal peptide synthases involved in the synthesis of hydroxamate siderophores necessary for iron uptake [121,122]. Other work has focused on adherence of the fungus to the host tissues. The interaction with plasma or ECM proteins has been extensively investigated [123], but non-specific interactions also seem to contribute to adherence. These mechanisms will be reviewed here and compared to those reported for other moulds and some non opportunistic human pathogenic filamentous fungi.

Adherence mechanisms in Aspergillus fumigatus

Adherence of *A. fumigatus* conidia to the host tissues has been the subject of many investigations, mainly using A549 cells which are a pulmonary epithelial cell line with characteristics of human type II pneumocytes. These studies showed that conidia attached rapidly to confluent monolayers, reaching a plateau within 40 min; this is followed by germination, and finally hyphal invasion of the epithelial cell layer [124]. However, conidia may also be internalized by A549 cells [124–126]. By confocal microscopy, conidia expressing the green fluorescent protein were found to co-localize with the lysosomal proteins LAMP-1 and CD63 [127]. Moreover, it was shown that some of the internalized conidia may survive within the epithelial cells [127]. In another study, 30% of conidia bound to A549 cells were internalized in three hours, and a large number of them survived and germinated in phagolysosomes [128], suggesting adherence to and internalization within alveolar epithelial cells as a route of entry of the fungus within the host tissues. As an angioinvasive fungus, *A. fumigatus* was shown to interact with HUVECs. Recently, it was demonstrated that not only conidia, but also hyphae can adhere to and be internalized by HUVECs. Upon interaction, hyphae can injury and activate endothelial cells to a prothrombotic phenotype [129].

However, epithelial tissue damage often accompanies the predisposing factors for aspergillosis. They are a common side effect of the neutropenic chemotherapy used in preparation to bone marrow transplantation, which is the main risk factor for invasive pulmonary aspergillosis. Likewise, previous chronic broncho-pulmonary infections due to *Staphylococcus aureus* or to *Pseudomonas aeruginosa* usually precede the colonisation of the airways by *A. fumigatus* in patients with cystic fibrosis (CF) [130]. It has therefore been suggested that components of the exposed subepithelial basement membrane and connective tissue, such as laminin, fibronectin and type IV collagen, or the fibrinogen/fibrin deposits resulting from the inflammatory reaction, would allow the attachment of the inhaled conidia [123].

Specific recognition of the ECM proteins. As revealed by immunofluorescence performed on different morphological stages of the fungus, from resting conidia to germ tubes, the binding of fibrinogen and laminin is associated mainly with the conidia [131,132]. Binding assays using radiolabelled ligands and flow cytometry experiments revealed characteristics of receptors. Binding was saturable and specific [132–134]. Moreover, these experiments highlighted the high affinity of the binding sites and assigned the adhesintopes to the outer D domains of the fibrinogen molecule.

The possible involvement of these interactions in adherence was first suggested by Microtitre plate

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adherence assays [133]. Conidia adhered avidly to wells coated with laminin or fibrinogen, but also to collagen and, to a lesser extent, to fibronectin substrates, whereas no adherence was observed for wells coated with other plasma proteins or with glycosaminoglycans (GAG). Involvement of these interactions in adherence was further supported by the ability of fibrinogen [135] or laminin [136] to inhibit the adherence of conidia to A549 cells.

Biomolecules which can diffuse from the conidial surface may influence the attachment of spores to immobilized laminin and A549 cells [137]. Preincubation of laminin substrates or A549 cells with a spore diffusate enhanced the attachment of conidia to laminin by 137% and to A549 cells by 250%. The laminin receptor was identified by ligand-blotting experiments using a cell wall extract from resting conidia [138]. A unique band of 72 kDa was observed, which displayed laminin-binding activity. Conflicting results were reported by Gil *et al*. [139] who identified by ligand blotting experiments, the laminin receptor as a 37-kDa protein. However, these experiments were conducted with a cytoplasmic extract from resting conidia, and not with a cell wall extract, so that this 37-kDa protein may be a cytoplasmic precursor of the 72-kDa receptor, as it occurs for the human 67-kDa high affinity laminin receptor with its 32-kDa cytoplasmic precursor [140]. Supporting this hypothesis are the results obtained by the same group for the characterization of laminin receptors in *C. albicans* [97], since two proteins of 67 and 37 kDa were identified with cross immunogenicity. Moreover, the *ASPF2* gene which encodes one of the major allergens of *A. fumigatus* has been cloned and sequenced by Banerjee *et al*. [141]. A recombinant protein of 37 kDa, rAspf2p, was produced which was shown to bind to laminin, and a database search for protein sequence homologies revealed important similarities with *C. albicans FBP1* gene.

Fibrinogen and fibronectin also interact with *A. fumigatus*. The fungal receptors for fibrinogen and laminin seem to be the same. Binding sites for these two ligands have a similar distribution at the conidial surface, since they are located mainly on the protrusions of the outer cell wall layer of resting conidia. Moreover, there is a cross-reactivity between the two ligands, and a similar evolution of receptor expression during the maturation of the conidia [131–133,138]. Together these data suggest the presence of a multifunctional adhesin in *A. fumigatus* as it occurs for numerous pathogens, and for instance, it has already been mentioned the identification of a multifunctional adhesin of 68 kDa with laminin, fibrinogen and C3d binding activities from a cell wall extract of *C. albicans* germ tubes [142].

Distinct receptors seem to be involved in fibronectin binding. Binding is mediated by two fungal polypeptides of 23 and 30 kDa, and it is inhibited by RGD peptides [143]. Conversely, all the synthetic peptides tested which mimic adhesive recognition sequences common to laminin and fibrinogen such as RGD peptides, failed to inhibit the binding of fluorescent ligands to *A. fumigatus* conidia, as well as the binding of conidia to immobilized ligands [133,134]. Likewise, no inhibition was seen in the presence of peptides mimicking adhesive motifs specific for fibrinogen or laminin, including the motifs GPRP and YIGSR, involved in the interaction of fibrinogen with platelets or in attachment of laminin to various substrates, respectively.

Given the high carbohydrate content of laminin and fibrinogen, the possibility of a lectin interaction was investigated [134]. Laminin is a heavily glycosylated protein containing about 15% of carbohydrates and, interestingly, *N*-linked oligosaccharide chains of both ligands share a common structure, with bi-, tri- or tetra-antennary carbohydrate side chains composed of sialyllactosaminyl residues branched by mannose residues on the protein core [144]. Among the different carbohydrates tested, only *N*-acetylneuraminic acid (NANA) and sialyl-lactose inhibited the binding of laminin and fibrinogen, suggesting the involvement of a sialic-acid specific lectin [134]. Sialic-acid dependent lectins have also been reported in numerous microorganisms including fungal pathogens such as the dimorphic fungus *Penicillium marneffei* [145].

Hemagglutination assays and inhibition of the hemagglutination by carbohydrates are widely used for lectin characterization. Therefore, cytoplasmic and cell wall extracts were prepared from resting or swollen conidia, germ tubes and mycelium. A lectin activity was detected in all fungal extracts, but the highest specific activity was seen for a cell wall extract from resting conidia, suggesting an increased synthesis of the lectin during conidiation and its accumulation within the cell wall [146]. SDS-PAGE analysis of the hemagglutinin purified from a cytosolic hyphal extract revealed a single polypeptide of 32 kDa, which is similar to that of a fucose-specific lectin described by Ishimaru *et al.* [147] in *A. fumigatus*. Nevertheless, inhibition experiments of the hemagglutination demonstrated the specificity for sialic acids. The relationship between this 32-kDa sialic acid-specific lectin and the 72-kDa laminin receptor remains to be defined.

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Long range non-specific interaction. As an interface between the fungus and the host tissues, the outer cell wall layer of the conidia plays an important role in the early steps of the infectious process and a considerable attention has been paid on elucidation of its biochemical characterization. Thus, at the surface of *A. fumigatus* resting conidia, it has been reported that there are proteins belonging to the hydrophobin family [148,149]. Hydrophobins are small proteins of about 100 amino acids, so that their molecular mass is comprised between 12 and 18 kDa [150]. These proteins, which are thought to play a role in adherence, have been detected in all filamentous fungi that have been studied. They exhibit a poor amino acid homology, but they have all a conserved spacing of eight cysteinyl residues. Moreover, these moderately hydrophobic proteins present a signal peptide necessary for their secretion during growth in liquid medium. However, when confronted with a hydrophilic-hydrophobic interface such as between water and air, monomers self-assemble into an amphipathic protein film at the surface of aerial structures, and particularly on resting conidia. This process leads to the formation of a hydrophobic rodlet layer which can be visualized using the freeze-etching technique [151] or by atomic force microscopy [152]. Two genes encoding hydrophobins, *RODA* and *RODB*, have been identified in *A. fumigatus*, and single or double disruptants have been prepared [149,153]. However, no changes were seen in the capabilities of mutant strains to attach to immobilized matrix components (except for collagen) or to adhere to epithelial cells.

Long range non-specific interactions, i.e., electrostatic or hydrophobic interactions, are involved in adherence for numerous microorganisms. Microelectrophoresis and two phase-partitioning for determination of the net charge of the conidia and CSH, respectively, revealed that *A. fumigatus* resting conidia present a high hydrophobicity and an electronegative charge [123]. Moreover, the variations of CSH according to culture conditions superimposed exactly with those of the capabilities of the conidia to bind the ECM proteins [123]. In addition, these experiments highlighted the importance of the outer layer of the cell wall in the adhesive properties of the conidia. This was particularly obvious when studying the influence of the maturation of the conidia. Indeed, CSH as well as binding of the conidia to ECM proteins, increased with the age of the cells to reach a maximum for cells from 3- to 5-day-old cultures [133,134]. These results are in agreement with observations from electron microscopy, since the binding sites for fibrinogen and laminin were seen to be localized on the echinulations of the cell wall which appear progressively at the conidial surface along with their maturation [132,138].

The importance of the convoluted outer cell wall layer on *A. fumigatus* conidia is also illustrated by the reduction in the adhesive properties during the early steps of germination. Indeed, swelling which leads to the shedding of the outer cell wall layer, results in the loss of laminin/fibrinogen receptors, as demonstrated by flow cytometry and Microtitre plate adherence assays [123]. Interestingly, swelling also resulted in a marked increase in CSH [123], as well as in the agglutination of the conidia [154], a phenomenon which also reflects the high hydrophobicity of the cells.

In light of these data, it has been proposed the presence at the conidial surface of two distinct adherence systems with: (i) recognition of fibronectin by two fungal polypeptides of 23 and 30 kDa localized on the protrusions of the conidial wall, which bind to the ligand through its tripeptide RGD sequence; and (ii) binding of laminin and fibrinogen through a fungal lectin, also localized on the protrusions of the conidia, which recognizes the terminal sialic acid residues of the carbohydrate side chains of these glycoproteins. However, hydrophobic interactions may also contribute to adherence of resting conidia by mediating dehydratation and apposition of interacting surfaces, thus allowing specific ligand-receptor interaction to occur. Moreover, these hydrophobic interactions could play a major role in the adhesive properties of swollen conidia.

Considering the negative charge of the conidial surface, Wasylnka *et al.* [155] proposed another model for the adherence to fibronectin. They found by microtitre plate attachment assays that *A. fumigatus* conidia bound equally well to glycosylated and deglycosylated fibronectin and that the different RGD-containing peptides tested did not significantly inhibit the adherence. It was therefore suggested that adherence to fibronectin could occur via negatively charged carbohydrates of the conidial surface, mainly sialic acids, and the positively charged GAG-binding domain on fibronectin. Differences in pathogenicity between *Aspergillus* species could therefore be related to differences in the density of sialic acids at the conidial surface [156]. Structure of sialic acids in *A. fumigatus* was recently determined and it was found that unsubstituted NANA was the predominant sialic acid [157]. Besides, it was shown that desialylation of the conidia decreased their binding to fibronectin and that sialylated molecules on *A. fumigatus* conidia were ligands for both professional and non-professional phagocytes.

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Other Aspergillus species

Very little information is available regarding the adherence mechanisms in non-*fumigatus Aspergillus* species. Hydrophobins have also been detected at the surface of *Aspergillus nidulans* conidia, and two genes designated *RODA* and *DEWA* were identified for this species. Disruption of the gene *RODA* in *A. fumigatus* and *A. nidulans* which leads to the absence of the hydrophobic rodlet layer on the conidial wall, had a slight effect on the hydrophobicity of conidia for *A. fumigatus*, whereas a marked decrease in CSH and in the electronegative charge was observed for *A. nidulans* [158]. These differences may be related to variation in the composition of the outer cell wall of the conidia between the two species.

Regarding specific interactions, binding assays using radiolabeled human fibrinogen demonstrated that, among 33 filamentous fungal species belonging to different groups, including some *Aspergillus* species, dermatophytes or related keratinolytic fungi, and some phytopathogenic or strictly saprophytic fungi, only the pathogenic aspergilli significantly bound fibrinogen (in decreasing order *Aspergillus niger* > *A. fumigatus* > *Aspergillus flavus* > *Aspergillus terreus*) [159]. In addition, as mentioned above, Wasylnka *et al*. [155] investigated the attachment of conidia from different *Aspergillus* species to immobilized fibronectin and intact basal lamina. They showed that *A. fumigatus* conidia bound to fibronectin more efficiently than those of the three other *Aspergillus* species tested, i.e., *A. flavus* (10- to 50-fold lower), *A. wentii* and *A. ornatus* (up to 90-fold lower).

Studies on fungal respiratory infections in CF patients revealed *A. terreus* as the second *Aspergillus* species colonizing the airways of these patients [130]. Interactions between *A. terreus* conidia and the ECM proteins laminin and fibronectin were therefore investigated [Pihet, unpublished data]. An intense labelling was seen by immunofluorescence at the conidial surface for both FITC-conjugated ligands. However, contrary to *A. fumigatus*, the binding interested resting conidia as well as germ tubes or hyphae (Fig. 3A to 3D). Electron microscopy confirmed this binding pattern, showing the uniform distribution of laminin binding sites at the surface of all fungal elements (Fig. 3E, 3F). Nevertheless, many similarities were observed between the two *Aspergillus* species. Flow cytometry experiments demonstrated the saturability, specificity and reversibility of laminin binding which also seemed to be mediated by a sialic acid-specific lectin, whereas fibronectin binding was inhibited by RGD-peptides. Microtiter plate adherence assays suggested a role for these interactions in adherence of the conidia and confirmed the involvement of a sialic acid-specific lectin in attachment to laminin substrates. Additionally, due to the aberrant glycosylation of mucins in patients with CF, and their increased content in sialic acid residues [160], the presence of sialic-acid specific lectins in *A. fumigatus* and *A. terreus* as well could facilitate the entrapment of their inhaled airborne spores in the viscous bronchial mucus.

The influence of culture conditions (culture medium and incubation time) was also investigated (Pihet, unpublished data). The expression of laminin receptors at the conidial surface increased with their maturation, reaching a maximum for conidia from 6- or 7-day-old cultures (Fig. 3G). Moreover, the binding capabilities of *A. terreus* conidia were affected by the presence of Zn^{2+} cations in the culture medium, which induced a marked reduction in the expression of laminin receptors (Fig. 3H). Recent sequencing of the whole genome of *A. terreus* should allow the identification of the laminin receptors by 2-D electrophoresis and MALDI-TOF analysis of the proteins differentially expressed in cultures grown with or without Zn^{2+}.

Non-Aspergillus opportunistic moulds

Despite the medical importance of Mucorales, little is known about their mechanisms of adherence to the host tissues. As with the aspergilli, Zygomycetes are opportunistic fungi which may cause invasive pulmonary or disseminated infections in patients with haematological malignancies.

Germination and adherence to plastic were investigated for *Rhizopus oryzae*, which is the main causative agent of mucormycosis. Attachment occurred prior to germination and decreased dramatically with germ tube formation [161]. Moreover, attachment assays to immobilized ECM components showed that *R. oryzae* conidia adhered readily to immobilized laminin or type IV collagen, but not to fibronectin or GAG (Fig. 4A). As for *A. fumigatus*, attachment to laminin and collagen substrates was dose-dependent and specific, and immunofluorescence revealed that laminin and type IV collagen interacted exclusively with conidia and mother cells of germ tubes (Fig. 4B, 4C). However, adherence of *R. oryzae* spores to laminin substrates was not inhibited by the different carbohydrates tested, and further experiments should be conducted in order to identify the fungal receptors.

Dong *et al*. [162] induced fungal keratitis in rabbits by inoculating yeast cells of *C. albicans* or conidia of *A. fumigatus*, *Fusarium solani* or *Penicillium citreo-viride* in order to investigate the role of adherence and matrix

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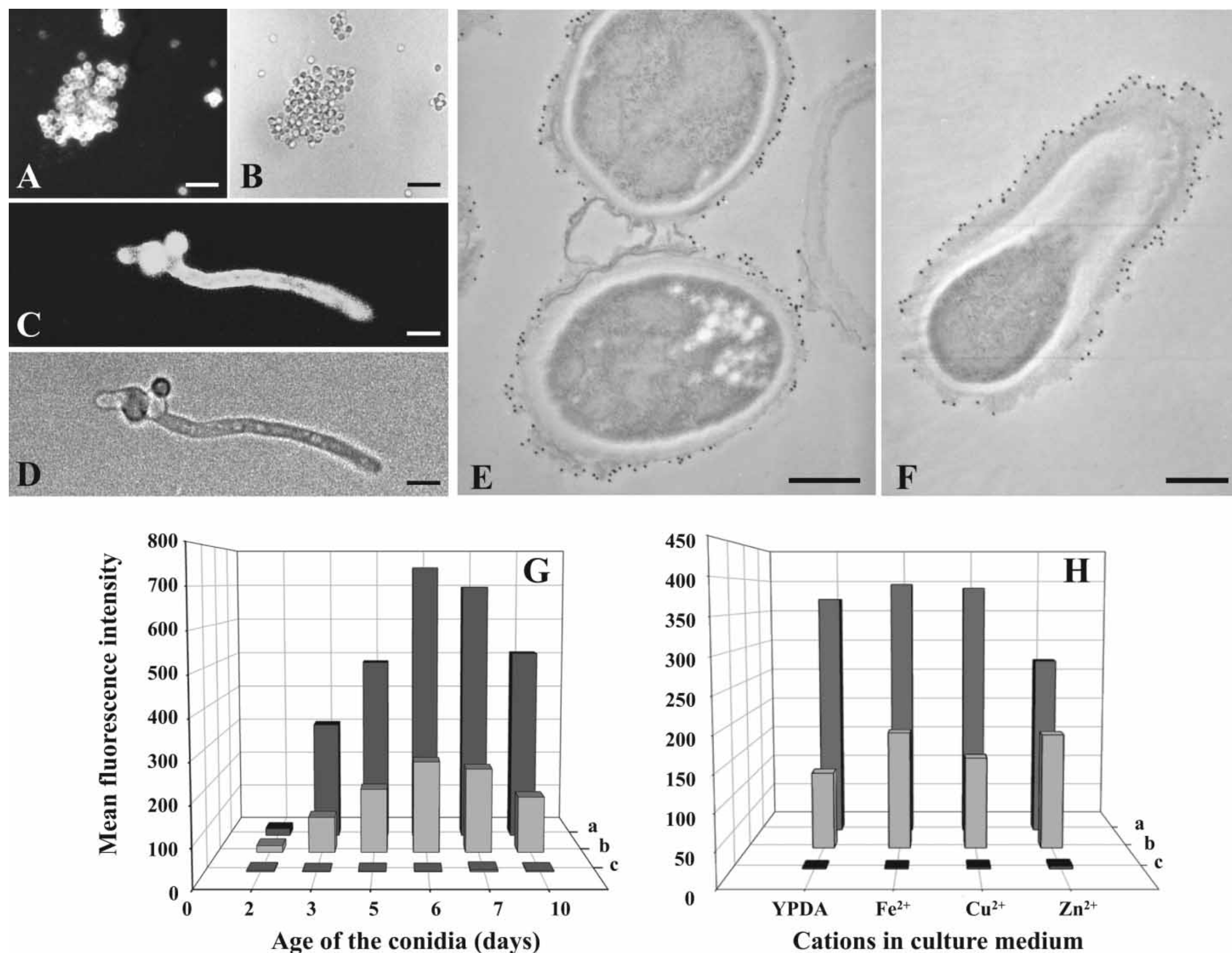

Fig. 3 Adherence mechanisms in *Aspergillus terreus*. (A–D), indirect immunofluorescence detection of the binding of laminin to *A. terreus* conidia (A) or germ tubes (C). As revealed by examination of the same fields by phase-contrast microscopy (B and D), all fungal elements incubated in the presence of laminin exhibited a bright and uniform fluorescence at their surface. Bars correspond to 10 μm for A and B, to 5 μm for C and D. E and F, electron microscopy detection (protein A-gold labelling) of laminin binding sites at the surface of *A. terreus* conidia (E) and germ tube (F). Fungal elements were successively treated with laminin, rabbit anti-laminin antibodies and protein A-gold. The labelling was uniformly distributed at their surface. Bars correspond to 0.5 μm. (G and H) influence of the maturation of the conidia (G) and of the composition of the culture medium (H) on binding of laminin to *A. terreus* conidia as revealed by flow cytometry. Conidia from 2 to 10-day old cultures on yeast extract-peptone-dextrose-agar medium (YPDA; G) or from cultures grown in YPDA medium supplemented with Fe^{2+}, Cu^{2+} or Zn^{2+} (H) were incubated with FITC-conjugated laminin alone (a) or in the presence of a 10-fold excess of unlabeled laminin (b), or without any ligand (c) as control. Results are expressed as mean fluorescence intensity of the cells calculated from the analysis of 10,000 cells.

metalloproteinases (MMPs) in growth patterns of the major fungal pathogens in cornea. Using electron microscopy and histopathological examination, they showed that binding to corneal epithelial basement membrane was initiated earlier with *A. fumigatus* and *C. albicans* than with *F. solani* and *P. citreo-viride*. Moreover, there were positive correlations between the number of bound conidia, the degree of inflammation, and the level of MMP-9 produced by the fungi. The adherence ability, chemotaxis to neutrophils, and MMP-9 expression level differed in eyes with the different fungal pathogens tested. These differences may contribute to the different growth patterns of these fungi in cornea.

Pseudallescheria boydii is usually responsible for chronic colonization of the airways in patients with CF [130], but it may also cause severe invasive pulmonary infections in immunocompromised patients such as lung transplant recipients. Virulence mechanisms of this fungus are largely unknown, but it was demonstrated recently that its conidia attached to, and were internalized by, HEp 2 cells through a lectin-mediated process involving a peptido-rhamnomannan of the fungal wall [163]. In contrast, carbohydrate-specific

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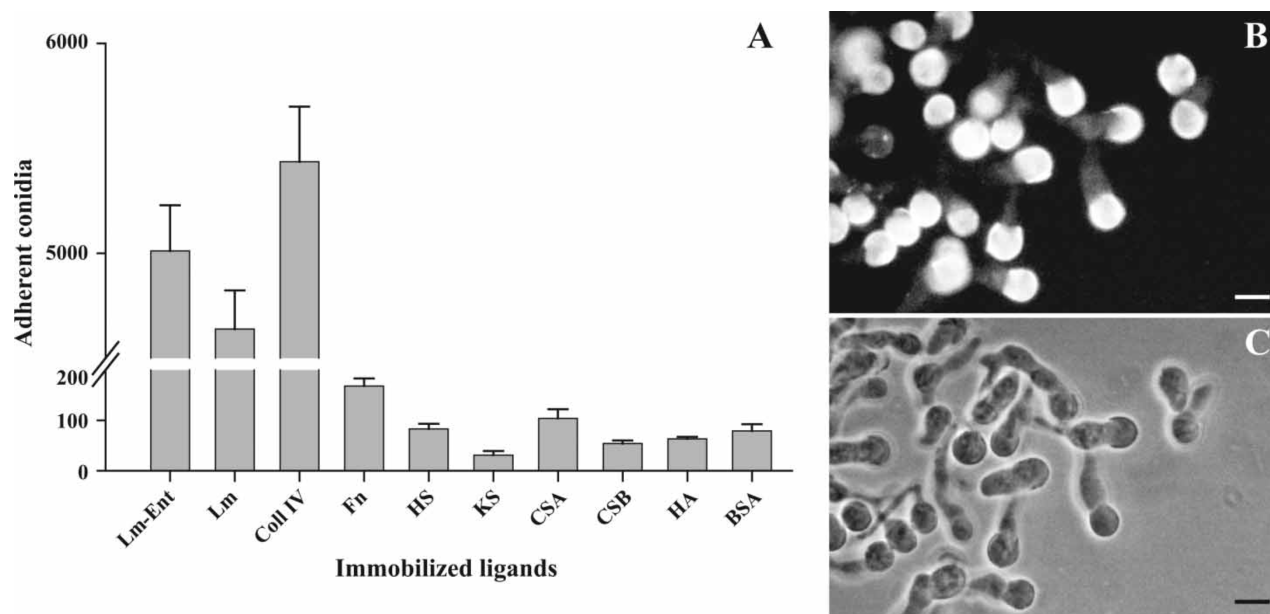

Fig. 4 Adherence mechanisms in *Rhizopus oryzae*. (A) attachment of conidia to immobilized ECM components (Lm-Ent, laminin-entactin complex; Lm, entactin-free laminin; Coll IV, type IV collagen; Fn, fibronectin; HS, heparan sulphate; KS, keratan sulphate; CSA, chondroitin sulphate A; CSB, chondroitin sulphate B; HA, hyaluronic acid; and BSA, bovine serum albumin). (B and C) indirect immunofluorescence detection of the binding of laminin to *R. oryzae* germ tubes. Binding was restricted to the mother cells of germ tubes, as revealed by examination of the same field by phase-contrast microscopy (C). Bars correspond to 10 μm.

adhesins seem to mediate adherence in some non-opportunistic human pathogenic filamentous fungi, including *Trichophyton mentagrophytes* and *T. rubrum* [164,165].

Mannose- and galactose-specific lectins have been identified, at the surface of microconidia of the dermatophytes *T. mentagrophytes* [164] and *T. rubrum* [165], by adherence assays to chinese hamster ovary (CHO) epithelial cells and their glycosylation-deficient mutants (Lec cells). For both species, Lec1 cells which express terminal mannose residues at the end of their N-linked carbohydrate chains, gave the higher adherence index (Fig. 5). Addition of this carbohydrate to the medium, as well as pretreatment of Lec cells with concanavalin A (a mannose-specific lectin) or with sodium periodate or trypsin treatment of microconidia, led to a decrease in the adherence index.

Dimorphic fungi and adherence

These fungi, which undergo dimorphic transition within the host tissues, from a mycelial saprophytic phase to a yeast or spherule parasitic phase, are endemic in several parts of the world, including Africa, South Asia, South America, and parts of North and Central America. Due to the high incidence of the corresponding diseases, much scientific works have been focused during these past few years on elucidation of the pathogenic mechanisms of these fungi [8]. Current knowledge on their adherence mechanisms to the host tissues will be reviewed here.

Blastomyces dermatitidis

Blastomyces dermatitidis is a primary fungal pathogen of man and other mammals, but like many other human fungal pathogens, relatively little is known about the factors that account for its virulence and pathogenicity [166]. This fungus which exists in nature in mycelial phase and converts to yeast phase at body temperature is responsible for blastomycosis, an endemic systemic infection acquired by inhalation of conidia from soil [167].

BAD-1 (*Blastomyces* adhesin-1), formely known as WI-1, is an essential virulence factor of *B. dermatitidis* which functions as an adhesin and immunomodulator [168–174]. This 120-kDa protein is expressed on the yeast phase but not in mycelium [173] and is present both on the cell surface and extracellularly [170]. The secreted adhesin is incorporated back onto the yeast cell surface by non-covalent and also, covalent links through cysteine residues [175]. Structurally, BAD-1 is composed of a short N-terminal region, a core of 30 amino acids tandem repeats critical for virulence and a C-terminal epidermal growth factor (EGF)-like domain that binds the protein to the yeast cell surface

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CHO cell lines	Carbohydrate chains		Adherence index*		
	N-linked	O-linked	*T. mentagrophytes* [164]	*T. rubrum* [165]	*F. pedrosoi* [214]
Parental line			40 ± 2	29 ± 7.7	28 ± 2.6
Lec1			78 ± 3	70 ± 5.8	49 ± 1.3
ldlLec1			/	/	51 ± 1.8
Lec2			71 ± 2.5	34 ± 5.8	31 ± 2.7
Lec8			/	40 ± 5.8	26 ± 5.3

*Adherence index is calculated by multiplying the mean number of attached conidia per CHO cell by the percentage of infected cells observed by microscopic examination.

▲, Sialic acid; ●, Gal; ■, GlcNAc; ○, Man; □, GalNAc; R, arginine; S/T, serine or threonine.

Fig. 5 Structure of the N- and O-linked oligosaccharide chains of membrane glycoproteins found in CHO cells or their glycosylation mutants and adherence indexes for *Trichophyton mentagrophytes*, *T. rubrum* and *Fonsecaea pedrosoi*.

chitin [171,172]. Binding of calcium ions changes the conformation of the protein and reflects on its biological function. Interestingly, the calcium binding domain exhibits similarity with that of the extracellular matrix protein, thrombospondin [176].

The adhesive properties of BAD-1 were demonstrated with both native and recombinant protein. BAD-1 was shown to mediate binding of yeast cells to human macrophages through their CD14 and CR3 (CD11b/CD18) receptors [170,171]. Binding sites were located into the lipopolysaccharide binding site on CR3, and the tandem repeats within the fungal adhesin. *Blastomyces dermatitidis* strains showing differences in the expression level of BAD-1 exhibited different capabilities to interact with human macrophages [177]. Further evidence that adherence parallels with virulence was achieved by the construction of isogenic strains of *B. dermatitidis* differing in BAD-1 expression. The lack of this protein on the yeast cell surface (knock-out strain) significantly diminished the adherence to macrophages and lung tissue, and increased the survival of infected mice [172].

Although BAD-1 protein exhibits a mosaic architecture with tandem interaction domains containing regions of great similarity with invasin, a 103 kDa adhesin of *Yersinia* spp., adjacent to the EGF-like domain, a motif exhibited by numerous molecular species that mediate adhesive interactions with ECM proteins [178], there is no experimental evidence for the interaction of BAD-1 with ECM components.

Histoplasma capsulatum

Histoplasma capsulatum is a dimorphic intracellular fungal pathogen of worldwide importance. Infection of the host occurs upon inhalation of microconidia and small mycelial fragments that convert to pathogenic yeast form responsible for the clinical manifestations of histoplasmosis. Although most infections involving *H. capsulatum* are unapparent, this organism may cause a broad range of clinical manifestations including progressively disseminated infections [179].

It was demonstrated that the yeast phase of this fungal pathogen bind to laminin and also, to matrigel™ [180]. In the presence of the synthetic peptide IKVAV which mimicks a recognition motif localized in the long arm of laminin α chain, the binding of ^{125}I-laminin to the yeast phase of *H. capsulatum* significantly decreased while no inhibition was observed with the peptide YIGRS, which mimicks another recognition motif also localized in laminin α chain, but in the short arm of the cross. A putative adhesin of 50 kDa, localized at the surface of yeast cells, was identified by Western blot analysis and mediated binding of ^{125}I-laminin to *H. capsulatum* [180].

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Another protein expressed on the surface of *H. capsulatum* mediates binding to CR3 receptors on human macrophages. This protein which migrates as a single spot in 2-D gels, showing an isolectric point of 5.9 and an apparent molecular mass of 60 kDa, was identified as a heat shock protein (hsp). The cell surface hsp60 mediates binding and internalization of *H. capsulatum* yeasts and conidia by macrophages, but not dendritic cells, via the CD18 family integrin receptor [181]. Previously, it is was shown that binding of *H. capsulatum* to dendritic cells was Ca^{2+} dependent and mediated by the fibronectin receptor VLA-5 [182]. More recently, competition experiments with *H. capsulatum* hsp60 have shown that it is able to inhibit by 69% the binding of human hsp60 to primary macrophages in a dose-dependent manner [183]. Moreover, several recombinant *H. capsulatum* hsp60-derived fragments, corresponding to distinct regions of the full-length hsp60 molecule, i.e. fragment 1 (aa 40–328), fragment 2 (aa 131–394) and fragment 3 (aa 214–484), could inhibit in some extent (34–73% inhibition) the interaction of hsp60 with bone marrow macrophages. By contrast, only fragment 3 competed with hsp60-binding to J7774.1 macrophages [183].

The *YPS3* gene of *H. capsulatum* encodes a protein that is both resident on the cell wall and also released into the culture medium [184]. This protein is produced only during the pathogenic yeast phase of infection and is also expressed differently in *H. capsulatum* strains that differ in virulence. The product of this gene, Yps3p, shows homology (2e-23) with the *B. dermatitidis* adhesin and virulence factor BAD-1. Alignments of the predicted protein products of these genes revealed homology in both an N-terminal signal sequence region and a cysteine-rich EGF domain similar to the EGF-like domain that characterizes the C terminus of BAD-1 [184]. However, no experimental data is available indicating a biological function of Yps3p as an adhesin.

Paracoccidioides brasiliensis

Paracoccidiomycosis, also called American blastomycosis, is a chronic, progressive and insidious systemic mycosis caused by the thermally dimorphic fungus, *Paracoccidioides brasiliensis.* Inhalation of microconidia causes a primary lung infection and is followed by the morphological transition into the pathogenic yeast form [185].

The major immunodiagnostic antigen of *P. brasiliensis*, gp43, found as an exocellular compound of either the yeast-phase or the mycelial-phase of the fungus [186] is also present on the cell wall [187]. Binding of the glycoprotein gp43 to laminin was first reported by Vicentini *et al*. [188]. The ECM protein laminin was shown to bind specifically to yeast forms of *P. brasiliensis* and also, to enhance adherence of the fungus to the surface of epithelial Madin-Dardy canine kidney cells *in vitro* [188]. Gp43 was also reported to mediate adherence to other host cells, including macrophages and epithelial cells (Vero cells) [189,190]. In competitive assays with a synthetic peptide that represented a specific sequence of gp43, NLGRDAKRHL, an inhibition of 57% was observed in the interaction between gp43 and Vero cells [191]. Additionally, gp43 cross-reacted with the laminin-binding protein from *Staphylococcus aureus*, suggesting the possibility of evolutionary conservation of the reactive epitope in phylogenetically very distant organisms [192].

Beyond laminin, *P. brasiliensis* yeast cells also interact with several other ECM proteins, fibronectin, fibrinogen and type I and type IV collagens [191,193]. Interaction with collagen I was mediated by two components of 47 and 80 kDa, whereas gp43 bound both laminin and fibronectin [191]. However, according to Gonzalez *et al*. [193], two adhesins of 19 and 32 kDa present in yeast cell extracts (total homogenates, β-mercaptoethanol and SDS extracts) were also suggested to mediate binding to laminin, fibrinogen and fibronectin. These two polypeptides were detected at the surface of either yeast cells, hyphae and conidia of *P. brasiliensis*, and both the purified 32-kDa protein and a MAb directed towards this adhesin inhibited the adherence of conidia to the three ECM-proteins in a dose-dependent manner [194]. Furthermore, the binding of conidia to fibronectin and fibrinogen enhances its interaction with human lung adenocarcinoma cell line (A549 cells) which in turn is inhibited by sialic acid (NANA) and a MAb anti-32-kDa protein. These data brings new insights by showing that adherence of *P. brasiliensis* conidia to A549 cells is probably mediated by adhesin-type molecules or a sialic acid based recognition system [195]. As mentioned in a previous section (*Specific recognition of the ECM proteins*), a 32 kDa lectin-like molecule present on the surface of *A. fumigatus* conidia mediates the interaction with ECM proteins by a sialic acid recognition system (146). Therefore, it is tempting to speculate the existence of similar adhesion mechanisms in different fungal species.

Besides the adhesin gp43, *P. brasiliensis* yeasts release and express on their surface a GlcNAc-binding lectin with an apparent molecular weight of 70 kDa, which interacts with laminin by a sugar-dependent manner. This lectin which is called paracoccin, was evidenced on the surface of yeast cells, especially in the budding

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regions [196]. Also, a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of *P. brasiliensis* was recently characterized as an adhesin [197]. GAPDH was detected in the cytoplasm and on the cell wall of the yeast cells. The recombinant GAPDH was shown to mediate adherence of yeast cells to the ECM proteins fibronectin, laminin, and type I collagen, and also to type II pneumocytes [197]. Pretreatment of pneumocytes with the recombinant protein inhibited adherence, and internalization of *P. brasiliensis* yeast cells. Interestingly, the participation of a cell surface GAPDH in adherence process has also been described for several other microorganisms, including *C. albicans* [198–200]. Moreover, recent analysis of *P. brasiliensis* triosephosphate isomerase, a protein predominantly expressed in the yeast phase and localized in the cell wall and cytoplasm, suggested a potential for adhesin function as revealed by adherence assays to cultured epithelial cells and attachment to laminin substrates [201].

Sporothrix schenckii

Sporothrix schenckii is widely distributed in nature and can be found in water and in soil associated with decaying organic matter (particularly thorns, dry leaves and wood, among others). This pathogen causes a deep mycosis, with diverse clinical manifestations, through the traumatic inoculation of elements of the mycelial saprophytic phase. Within the host, occurs a morphological transition to the yeast parasitic phase [202]. Although known as a benign subcutaneous disease, disseminated sporotrichosis is a serious fungal infection with a substantial number of recent cases involving patients with the acquired immunodeficiency syndrome (AIDS). However, individuals with other conditions that affect the immune system also are at increased risk [203].

The yeast-phase of *S. schenckii*, as well as conidia, is capable to interact with immobilized fibronectin, laminin and type II collagen while no adhesive capacity was observed for either thrombospondin or fibrinogen [204]. Among the ECM proteins, fibronectin seems to be the most important substrate for adherence of the parasitic phase of this fungus, followed by laminin [204,205]. The adherence to fibronectin is positively modulated by Ca^{++}, but not by Mg^{++} [206].

Changes in the conformation of matrix proteins can influence adherence of microorganisms [207]. Soluble ECM proteins conjugated to FITC, FITC-fibronectin and FITC-laminin, were demonstrated to bind to yeast cells of *S. schenckii* more intensely than to the conidia [205]. In this model, the tripeptide RGD could inhibit by 50% the interaction with fibronectin and YIGSR peptides caused a 30% inhibition of laminin binding. However, neither the RGDS peptide nor heparin inhibited the interaction of *S. schenckii* yeast cells with immobilized fibronectin, but a significant decrease in adherence was seen in competition assays with both a crude cell wall extract and a peptido-rhamnomannan fraction of the cell wall extract [206].

The yeast cells of *S. schenckii* adhere to and are internalized by either phagocytic and non phagocytic host cells, macrophages and HUVECs monolayers [208,209]. The ingestion of yeast forms of *S. schenckii* by thioglycolate-elicited mouse peritoneal macrophages was inhibited by partially purified cell wall polysaccharides, galactomannan and rhamnomannan (62% and 53% inhibition, respectively) [208]. Differences in the phagocytic index were reported according to the cell morphotype [210].

The interaction of *S. schenckii* with HUVECs was mediated by two major receptors of the endothelial cells with molecular masses of 90 kDa and 135 kDa [210]. Although yeast cells can be internalized by endothelial cells, no injury occurs after endocytosis. Furthermore, upon interaction with endothelial monolayers, a higher number of yeast cells were located at the intercellular junctions what could suggest a higher affinity to exposed components of the subendothelial matrix or the involvement of junction proteins such as cadherin. This last hypothesis was discharged as an anti-cadherin commercial antibody did not inhibit the adherence of *S. schenckii* to endothelial monolayers (unpublished data). In contrast, the interaction of *S. schenckii* with HUVECs monolayers was markedly affected in the presence of anti-laminin and anti-fibronectin antibodies, as well as the transmigration through endothelial cell cultivated onto membrane inserts [211]. None of the fungal adhesins mediating the interaction of *S. schenckii* with either ECM proteins or host cells has been identified as yet. However, we have new evidence for the presence of a 70-kDa protein expressed on the surface of yeast cells of several clinical isolates which is recognized by fibronectin in an affinity blotting assay (unpublished data).

Coccidioides immitis

A *Coccidioides immitis* gene, *SOWgp*, encodes an immunodominant glycoprotein of spherule outer wall [212]. This glycoprotein, which is presented as parasitic phase-specific, is a component of the membranous layer at the spherule surface. The *SOWgp* gene encodes a protein with a predicted molecular size of 46.4 kDa and a pI of 4.2 composed by a signal peptide and a propeptide, a variable number (four to six according to

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the isolates studied) of tandemly repeated proline- and aspartic acid-rich motifs, and a GPI anchor signal consensus sequence. The recombinant protein (rSowgp58) was shown to bind to laminin and significantly less to fibronectin and collagen type IV. Additionally, deletion of *SOWgp* gene resulted in partial loss of the ability of spherules to bind the ECM proteins and a significant reduction in virulence. This protein does not seem to be involved in the interaction of *C. immitis* to host phagocytes [212].

Fonsecaea pedrosoi

Fonsecaea pedrosoi, which is the major causative agent of chromoblastomycosis, a chronic subcutaneous mycotic infection, can be isolated from plant debris under a saprophytic conidial phase. Usually, infection occurs following traumatic inoculation through splinter wounds and, in the host tissues, the fungus converts into small clusters of cells, known as muriform bodies [213,214]. Due to the endemic feature of the disease in several regions, and to the poor effectiveness and serious side effects of the treatment, several studies have been conducted in order to improve knowledge on the biology of the fungus and on its pathogenic mechanisms. For instance, adherence mechanisms to the host cells have been investigated by Limongi *et al.* [215]. Using adherence assays to CHO cells and Lec mutants, they suggested the involvement of a mannose-specific lectin in the adherence of *F. pedrosoi* to cell surface glycoforms (Fig. 5), whereas the subsequent ingestion of the fungus seemed to be mediated by N-acetyl glucosamine residues. Flow cytometry analysis of the binding of FITC-conjugated neoglycoproteins (mannose- and GlcNAc-BSA) confirmed these results [216] and affinity chromatography on immobilized mannose or GlcNAc allowed the recovery of a single 50-kDa protein from a conidial extract, suggesting that both carbohydrates are recognized by the same lectin-like protein [216].

Conclusion

Apart from *C. albicans*, knowledge of the adherence mechanisms in human pathogenic fungi is still limited. However, a significant improvement in this research field was achieved in the last few years. As an interface between the host and the pathogen, the cell wall plays a crucial role for colonization and infection. Elucidation of its structure, composition and biogenesis therefore would lead to a better understanding of the pathogenesis of fungal infections, but also to an improvement of their treatment since some cell wall components or enzymes involved in their synthesis or assembly, may constitute valuable targets for antifungal drugs.

Several proteins with adhesive function have been identified in *C. albicans* (for a schematic view, see Fig. 6), but the ability of the microorganism to adhere to the host cells seems to be mediated mainly by the Als protein family. Other fungal pathogens express at their surface lectin-like adhesins which mediate binding to sugar residues present on biotic surfaces. Such adhesins have been particularly characterized in *C. glabrata* and their role in adherence of this yeast species is well established. In other pathogenic fungi, multifunctional adhesive surface components, known in bacteria as

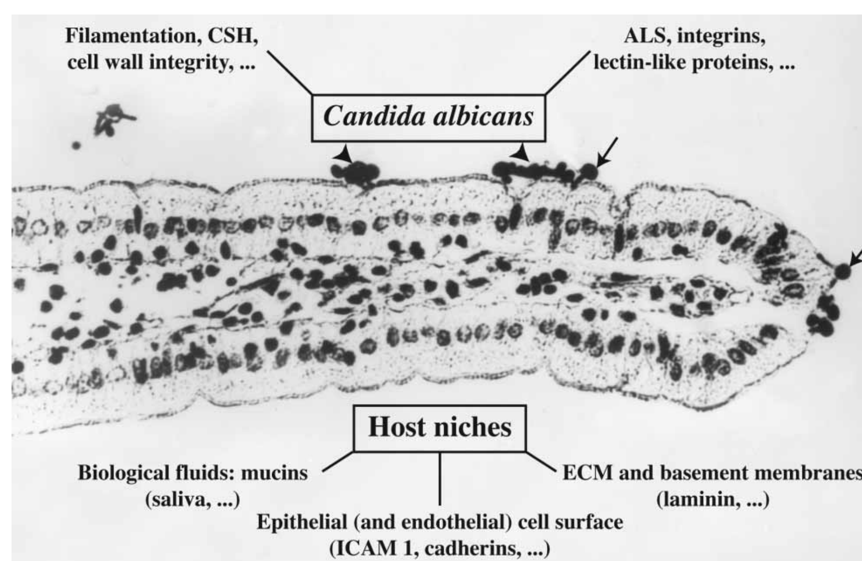

Fig. 6 Main fungal components or properties and putative host ligands involved in adherence of *Candida albicans*. The background view shows *C. albicans* yeasts (arrowheads) and germ tubes (arrows) adhering to an intestinal villus.

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MSCRAMM (microbial surface components recognizing adhesive matrix molecules), mediate not only the adherence to several ECM proteins such as laminin or fibronectin, but also internalization of the microorganisms by epithelial or endothelial cells. The development of proteomic tools and the availability of genomic data will be of great importance to elucidate the mechanisms of the host-fungus interplay, and particularly of its adherence to the host tissues. Additionally, almost all studies in this field have been conducted *in vitro*, and the potential interest of adhesins in preventing fungal infections remains to be investigated in animal models.

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