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Minireview

The role of the cell wall in fungal pathogenesis

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Summary

Fungal infections are a serious health problem. In recent years, basic research is focusing on the identification of fungal virulence factors as promising targets for the development of novel antifungals. The wall, as the most external cellular component, plays a crucial role in the interaction with host cells mediating processes such as adhesion or phagocytosis that are essential during infection. Specific components of the cell wall (called PAMPs) interact with specific receptors in the immune cell (called PRRs), triggering responses whose molecular mechanisms are being elucidated. We review here the main structural carbohydrate components of the fungal wall (glucan, mannan and chitin), how their biogenesis takes place in fungi and the specific receptors that they interact with. Different model fungal pathogens are chosen to illustrate the functional consequences of this interaction. Finally, the identification of the key components will have important consequences in the future and will allow better approaches to treat fungal infections.

Introduction

Fungal infections represent today a serious and not-yet-solved health problem even in industrialized countries (Sternberg, 1994; Edmond *et al*., 1999; Zaoutis *et al*., 2005; Pfaller and Diekema *et al*., 2007). Not only HIV infection, but life-prolonging technologies – invasive technologies, therapy prior to organ transplantation and anticancer drugs – have provided an opportunity for fungi to colonize and cause disease in humans. In Europe, fungal infections account for 17% of the intensive care unit infections (Rupp, 2007) and in the USA, deaths caused by fungal infections have increased from the 10th most common cause of death among hospitalized individuals to 7th in the last 10 years (Martin *et al*., 2003). And not only the incidence but the diversity of pathogenic fungi encountered as etiological factors of fungal infections has increased in the last years in immunocompromised individuals. In these patients, eradication of the infection relies in chemotherapy. While superficial infections caused by dermatophytes are relatively simple to eradicate, treatment of deep invasive mycosis is more complex as the therapeutic arsenal available is more limited. Polyenes and azoles (and only recently echinocandines) are the frequent choice and pharmacokinetics limits, in some cases, their utility while the development of resistance against azoles, the most common agents, may limit their usefulness in the coming years (Sanglard *et al*., 1995; vanden Bossche *et al*., 1998). The identification of novel therapeutic approaches to fight against disease is therefore of primary importance in basic research.

Different fungal species are found associated to human diseases. *Candida albicans* is still the most frequently fungus encountered in clinical specimens. The main reason for this relies on its common niche, as this fungus inhabits the human gastrointestinal and urogenital tract in a significant part of the population, where it behaves as a harmless commensal organism (Odds, 1988). However, upon alteration of the host defences, *C. albicans* disseminates within the human body gaining access to internal organs and causing severe infections (called candidiasis). *Candida albicans* thus behaves as an opportunistic pathogen. The ability of this fungus to change its morphology from a yeast-like (unicellular) to a filamentous (hyphal or mycelia) form (a property called dimorphism) is environmentally regulated by factors, such as the temperature (Lee *et al*., 1975), the pH (Soll *et al*., 1978; Odds, 1994) or the availability of nutrients (Gow and Gooday, 1982), and plays a major, albeit non-exclusive, role in its ability to produce disease (Ryley and Ryley *et al*., 1990; Lo *et al*., 1997; Kobayashi and Cutler, 1998; Romani, 2004). Other fungal species associated to human disease are *Aspergillus* spp. This microbe cause serious invasive mycoses in patients undergoing organ transplantation. Spores (resting conidia) of *A. fumigatus* are ubiquitous in the environment and are continuously inhaled and deposited in the lungs where they are phagocytosed and eliminated.

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However, in the impaired immune individuals, conidia germinate and destroy alveolar macrophages, mature into germ tubes and hyphae that can in turn invade vessels and disseminate hematogenously (Latge, 1999). Due to the mechanism of dissemination of spores (air), infections are difficult to control even in carefully controlled environments and may lead to death of the patient. *Cryptococcus neoformans* is the most common cause of fungal infections of the central nervous system. *Cryptococcus neoformans* is a basidiomycete yeast-like fungus found in the environment that can infect and cause disease in a wide variety of animal hosts, insects, birds and humans (Perfect and Casadevall, 2002). Infection in humans also occurs by inhalation of basidiospores that enter the lungs, either proliferating immediately or establishing a dormant infection that can be reactivated at a later stage depending on the host. A characteristic feature of this fungus is the presence of a capsule, which is unique in fungal pathogens and impedes phagocytosis in the absence of host antibodies (McFadden *et al*., 2006). The capsule enables dissemination of *C. neoformans* to the central nervous system, where it generates a fatal meningoencephalitis if untreated. Finally, *Histoplasma capsulatum*, is a dimorphic specie frequently encountered in the environment as saprophyte. Mold-produced spores that are inhaled germinate in the lung where they are converted to a yeast form, which is a process absolutely required for pathogenicity (Medoff *et al*., 1986; Nemecek and Klein *et al*., 2006).

While the biology (life cycle, metabolism and morphogenesis) of all these fungal species greatly differ, they also share certain common features that enable a successful colonization of the human host and are able to counteract its defence mechanisms. Such features are frequently called virulence factors and they comprise metabolic, structural and morphological features (Navarro-García *et al*., 2001) among others, although it has been proposed that only those involved in the direct interaction with host cells should be considered as true virulence factors (Odds *et al*., 2003). Their identification is an active area of research as they may provide the basis for the development of novel therapies to treat fungal infections (Alonso-Monge *et al*., 2003). While several virulence traits have been identified in many fungal species, the cell wall is still the most promising target in drug discovery for different reasons. First, it is unique to the fungus, and therefore, fulfils *a priori* the requirement of selectivity for drug discovery. Second, it is an essential structure to the cell, whose inhibition leads to cell death and most frequently lysis. Third, and most importantly, it is the most external structure present in the fungal cell and therefore, mediates the interaction of the fungus with the mammalian host cells. As a consequence, it is involved in adhesion, colonization, signaling and immune recognition, and therefore plays a major role during infection.

We aim to summarize here the structural and functional aspects of the cell wall in fungal pathogens. We highlight recent findings that indicate the relevance of the cell wall in the interaction with the host immune cells and how this process is essential to prime and develop a protective immune response (Romani, 2004) and contributes significantly to the control and pathology of the infection (Casadevall and Pirofski, 1999; Casadevall and Pirofski, 2001).

Structure and biogenesis of the fungal cell wall

The cell wall is an essential structure that maintains the viability of fungal cells, conferring their typical morphology and protecting the cell against external injuries. As the most external cellular structure of pathogenic microorganisms, it also carries important antigenic determinants and mediates adhesion to the host tissues, being therefore crucial to initiate colonization and therefore, cause disease (Calderone and Fonzi, 2001; Sundstrom, 2002). The cell wall is the structure sensed by the host immune cells. As a consequence, it participates in triggering and orchestrating the whole innate and adaptive immune response against the microorganism. Structurally, it accounts for 15–30% of the cell dry weight. It is normally a multilayered structure composed by glucans, D-glucose polymers with β-1,3, β-1,6 and α-1,3 (in the case of *Histoplasma,* see below) linkages that account for 50–60% of the total cell wall. A minor, but essential, component is chitin, a β-1,4 *N*-acetylglucosamine polymer (1–2%) while mannan (also called phosphopeptidomannan) is composed of mannoproteins and represents about 35–40% of it. The β-1,3 glucan and chitin moiety are mainly responsible for providing the cell wall strength and appear as a dense inner layer by transmission electron microscopy. The synthesis of both polymers is coordinated and regulated in response to different environmental conditions. Drugs or stress conditions that alter the amount of one of these components frequently trigger a mechanism that increases the synthesis of the others, thus providing the necessary strength to maintain cell integrity (Ram *et al*., 1998; Lagorce *et al*., 2003). The external outer layer is composed of mannoproteins and appears much lighter, being largely responsible for determining the porosity of the cell wall. The molecular linkages between all the components are not completely understood. β-1,3 glucan is distributed through the cell surface and is covalently linked to some chitin chains, providing a scaffold to which mannoproteins are also covalently attached (Fig. 1).

In *C. albicans*, the most external part of the cell wall is mainly composed of mannoproteins, also called cell wall proteins (CWPs), which are normally highly glycosylated (either O- or N-glycosylated) with mannose-containing polysaccharides; carbohydrates can account for up to 90% of their molecular mass. O-glycosylation occurs

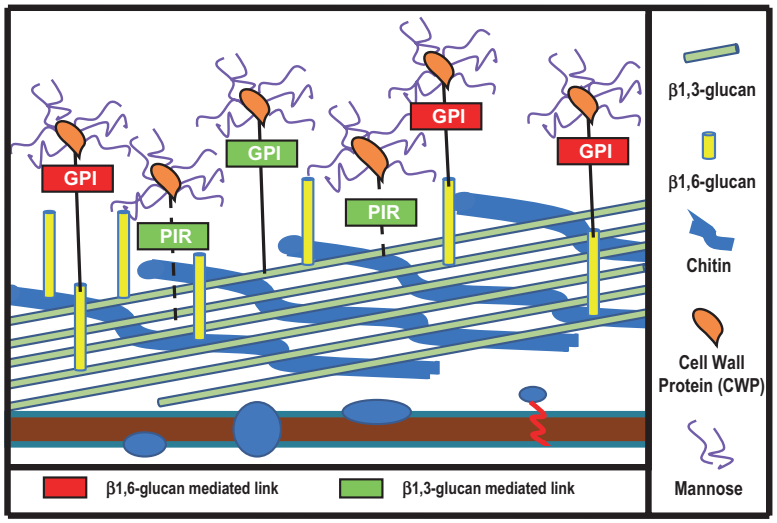

Fig. 1. Schematic organization of the fungal cell wall. The major structural components are shown. β-1,3 glucan and chitin are the main structural components located in the inner layer. The outer layer is composed of cell wall proteins (CWPs) that are attached to the inner layer via glycosylphosphatidylinositol (GPI) or internal repeat domains (PIR) linkages.

among fungi at serine and/or threonine residues and involves the addition of mannoses through α-1,2 or α-1,3 (as occurs in *S. cerevisiae*) type linkages. As the linkage of some cell wall proteins to β-1,3 glucan can be cleaved by treatment with alkalis, it has been hypothesized that O-chains are involved (Kapteyn *et al.*, 1999). N-glycosylation takes place at asparagine residues; the inner core of the added glycan moiety is composed of α-1,6 mannose to which moieties are sequentially incorporated. In *C. albicans*, CWPs frequently contain internal repeats (named Pir-CWPs) that are directly linked to β-1,3 glucan, whereas others contain a glycosylphosphatidylinositol derived-structure (GPI-CWPs) and are attached to the β-1,6 glucan. Interestingly, a different type of linkage (β-1,2 type) does exist (Suzuki *et al.*, 1995; Kobayashi *et al.*, 1998; Shibata *et al.*, 2003), and it has been shown to play a role in protection against disseminated candidiasis (Dromer *et al.*, 2002).

Hyphae of *A. fumigatus* contain four major carbohydrate polymers: chitin, galactomannan, branched β-1,3/β-1,6 glucans and linear β-1,3/β-1,4 glucans (Fontaine *et al.*, 2000). The complex composition of the conidial cell wall is incompletely defined (Bernard and Latge, 2001), but in contrast to hyphae, conidia have other morphologically distinct features: an outermost layer (Paris *et al.*, 2003a,b) and an inner cell wall layer that is exposed during the 'swelling' (Tronchin *et al.*, 1995), a process that can occur within macrophages (Philippe *et al.*, 2003) and participates in infection. The outer conidial cell wall consists largely of hydrophobic proteins and is lost when conidia develop into filaments. The cell wall of mycelia, which has been more extensively characterized, consist of branched β-1,3 glucan covalently bound to chitin, β-1,3 and β-1,4 glucans and galactomannan (Fontaine *et al.*, 2000).

The most distinctive feature of *Cryptococcus neoformans* is the polysaccharide capsule (Janbon, 2004). The presence of a polysaccharide capsule gives to this fungus some pathogenic attributes comparable to those of classical encapsulated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*. The polysaccharide capsule of *C. neoformans* is highly anti-phagocytic, poorly immunogenic and is essential for virulence, as deduced from the avirulence of strains in which key components of its biosynthetic pathway have been deleted (Chang and Kwon-Chung, 1994; Chang *et al.*, 1996). The capsule of *C. neoformans* contains two major polysaccharides, glucuronoxylomannan (GXM) and galactoxylomannan (GalXM). GXM is a linear α-1,3 mannan with a β-1,2 glucuronic acid residue attached at every third mannose (Cherniak *et al.*, 1988). Each trisaccharide of the backbone is also substituted with up to four xyloses, which are either β-1,2 or β-1,4-linked (Cherniak *et al.*, 1988). Xylosylated mannoses tend not to be acetylated (Cherniak *et al.*, 1988; Janbon *et al.*, 2001; Moyrand *et al.*, 2004). GXM forms a complex and large molecular-weight structure that involves the self-association of molecules and the self-entanglement of different fibres (Turner and Cherniak, 1991; McFadden *et al.*, 2006). GXM is also released from the capsule and accumulates in tissues during infection, blocking effective immune responses and contributing to the pathology of the disease (Vecchiarelli, 2000). High concentrations of GXM in tissue were hypothesized to cause dysfunction of cellular processes (Graybill *et al.*, 2000) because of the high viscosity achieved in tissues. However, recent studies suggest that this does not occur at concentrations that are relevant *in vivo* (McFadden *et al.*, 2006). GalXM is an α-1,6 galactan that contains branches of β-1,3 galactose-α-1,4 mannose-α-1,3 mannose. In turn, the branch sugars can be linked to β-1,3 or β-1,2 xylose. Whereas all sugars in GXM and most of the sugars in GalXM are in the pyranose configuration, GalXM does

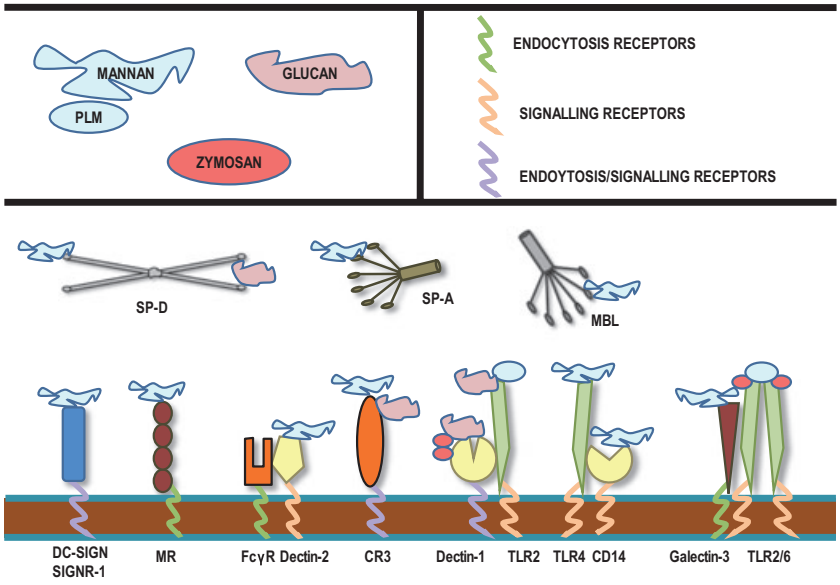

Fig. 2. Receptors involved in the interaction of fungal cell wall components with immune system cells. This figure depicts the multiple receptors that act either alone or simultaneously as sensors for different cell wall components of fungal cells walls. Receptor engagement induces intracellular signals that lead either to endocytosis and phagocytosis signalling or both of them. The receptors are not drawn to scale.

contain a small amount of galactofuranose (Vaishnav *et al.*, 1998). The cell wall of *Histoplasma* yeast contain primarily three polysaccharides: chitin, glucans (β-1,3-, β-1,6-linked and α-1,3-glucan) (Domer, 1971; Reiss, 1977). Immunofluorescence localization in cross-sections of wild-type yeast cells with α-1,3- and β-1,3-glucan-specific antibodies show a non-homogeneous spatial distribution. Although some overlap exists between α-1,3 glucan and β-1,3 glucan, the cell wall is somewhat layered, with α-1,3 glucan being more external.

Recognition of fungi by host cells

Recognition of pathogens by the innate immunity requires the identification of Pathogen-Associated Molecular Patterns (PAMPs). These structures represent surface determinants that are absent in mammalian cells and are sensed by specific structures (called Pattern Recognition Receptors PRRs) present on the surface of the immune cells (Underhill and Ozinsky, 2002; Underhill, 2004). Different PRRs recognize different PAMPs and contribute in this way to the generation of a balanced response against microorganisms (Underhill, 2003b) (Fig. 2).

Toll-Like Receptors (TLRs) are cell membrane associated (TLR1, TLR2, TLR4, TLR5 y TLR6) or intracellular (TLR3, TLR7, TLR8 y TLR9) PRR receptors. Several TLRs have been implicated in the recognition of fungal components: TLR2 was initially described to play a role in zymosan (a glucan enriched particle) recognition (Underhill *et al.*, 1999). Recent studies have shown that TLR2 recognizes phospholipomannan (PLM) (Jouault *et al.*, 2003), while TLR4 recognizes O-linked mannans (Netea *et al.*, 2006). TLR6 is involved in the recognition of zymosan (Underhill, 2003a) while TLR9 detects fungal DNA (Wagner, 2001). In addition to TLRs, C-type Lectin Receptors (CLRs) are mainly membrane-bound receptors that also recognize polysaccharide structures from fungal cells: dectin-1 recognizes β-glucans (Brown and Gordon, 2001), whereas the macrophage mannose receptor (MR) and DC-SIGN (dendritic cell-specific ICAM-3-grabbing non-integrin) recognize N-linked mannans (Stahl and Ezekowitz, 1998; Cambi *et al.*, 2003).

Structurally, PRRs normally have an extracellular pathogen-recognition domain [the Leu-Rich Repeat (LRR) domain in TLRs and the C-type Lectin Domain (CLD) in CLRs] (Fig. 2). The intracellular signalling domain (the TLR-Interleukin 1 Receptor, TIR domain) of TLRs and the Immunoreceptor Tyrosine-based Activation-like Motif (ITAM) from some CLRs, such as dectin-1, are responsible for transducing the intracellular signals that are ultimately responsible for the functional activity of the receptor.

Although ubiquitous, the amount of each individual PRRs differs greatly for each type of immune cell, stage of growth/differentiation and biological specie (Netea *et al.*, 2008). Pattern recognition receptors are differentially expressed in the surface of immune cells (Fig. 3). Phagocytes are essential components of the innate system in the control of the fungal infection as they contribute to eliminate the microbe. They comprise both monocytes and neutrophils in the circulation and macrophages in infected tissues. Monocytes express high levels of TLRs on their cell membranes and moderate levels of CLRs. Macrophages present high expression levels of TLRs and CLRs and DCs, which are essential for antigen processing and presentation to T cells, and also express most of the PRRs that are important for the recognition of fungal pathogens. By contrast, neutrophils show moderate expression of TLRs and a high expression of phagocytic

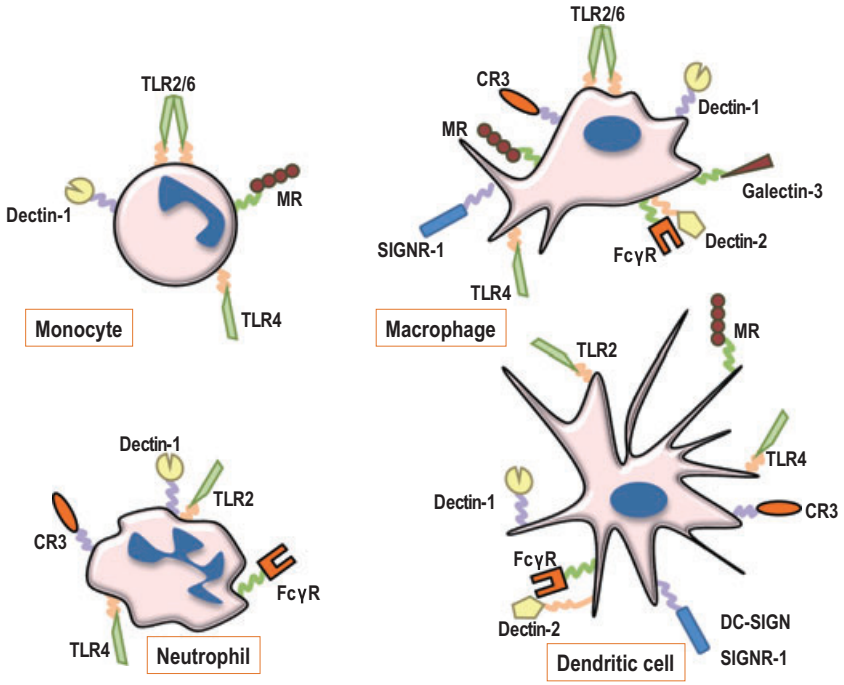

Fig. 3. Cell populations of immune system and pattern-recognition receptors involved in fungal recognition. The main cells of the host innate immune response are monocytes, macrophages, neutrophils and dendritic cells. The receptors expressed by these cell types are shown. TLR, Toll-like receptors; MR, mannose receptor; FcγR, Fcγ receptor; CR3, complement receptor.

receptors. Thus, the mixture of PRRs that is expressed by each of these cell types contributes to generate the initial response following recognition of fungal pathogens.

Due to the localization of mannoproteins and mannans in the outermost part of the fungal cell wall, mannan detection may be expected to be one of the first steps in the recognition mediated by immune system cells. However, other components such as glucan and chitin (at an inner level) also influence the recognition of fungal cells by leucocytes. We will summarize how this process occurs in the different fungal species with special emphasis in the model fungal pathogen *C. albicans*.

Mannans and mannoproteins

Both mannans and mannoproteins from the *C. albicans* cell wall have important immunostimulatory activities (Garner *et al.*, 1994; Gomez *et al.*, 1996; Gomez *et al.*, 2000; Pietrella *et al.*, 2005; 2006). The C-type-lectin MR was among the first described PRRs for fungi (Stahl and Ezekowitz, 1998). It was the first receptor on the surface of macrophages to be described as a mannan receptor (Wileman and Stahl, 1986; Stephenson and Shepherd, 1987). The MR recognizes carbohydrates with terminal mannose, fucose or GlcNAc (Taylor *et al.*, 2005a). Recognition of the PAMP occurs via the carbohydrate-recognition domain (CRDs) present in the extracellular region of the receptor (Linehan *et al.*, 2000). *In vitro* studies have demonstrate that the MR preferentially recognizes α-linked oligomannoses with branched, rather than linear, structures (Kery *et al.*, 1992). A recent study has demonstrated that in monocytes and macrophages, the MR recognizes branched N-bound mannans from *C. albicans* (Netea *et al.*, 2006). The function of the MR in host defence *in vivo* has been addressed. No significant differences were found between normal and MR–/– (knock out) mice using a experimental model of infection with *C. albicans*, although knock-out mice had higher average fungal burdens in some of the organs, they were competent in inflammatory cell recruitment and antibody production, indicating that the MR is not required for the normal host defence during disseminated candidiasis or even for phagocytosis (Lee *et al.*, 2003). By contrast, recognition of the shorter linear structures of O-bound mannan is mediated by TLR4 (Netea *et al.*, 2006) with the resultant production of appropriate cytokines (Tada *et al.*, 2002). Dendritic cells, however, recognize *C. albicans* mannans through the MR and DC-SIGN. DC-SIGN is a receptor that is specifically expressed on the cell membrane of DCs (Cambi *et al.*, 2003) and some subpopulations of tissue macrophages (Soilleux *et al.*, 2002; van Lent *et al.*, 2003). DC-SIGN recognizes carbohydrates such as high mannose structures in a Ca^{2+}-dependent manner with specificity being achieved through unique interactions with its ligands and tetramerization of the receptor (Koppel *et al.*, 2005). It has been shown recently that DC-SIGN is able to bind *C. albicans* in DC-SIGN-transfected cell lines and in human monocyte-derived DCs; this results in the internalization of *C. albicans* in specific DC-SIGN-enriched vesicles, which are distinguishable from those containing the MR, also expressed in DCs (Cambi *et al.*, 2003).

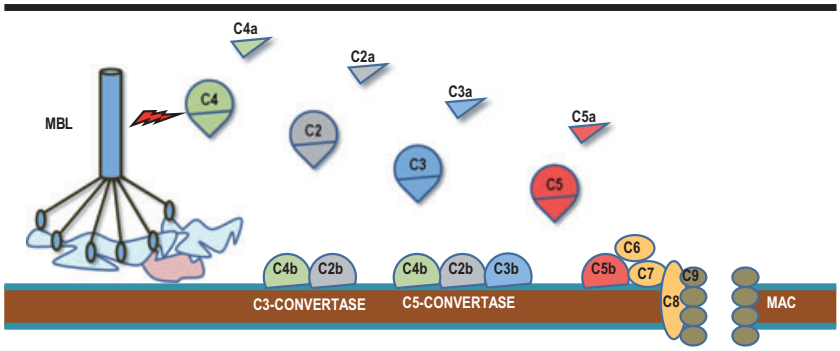

Fig. 4. Activation of the complement system via MBL. The figure shows the activation of the complement cascade in response to fungal mannan recognition mediated by MBL. MBL, mannan-binding lectin; MAC, membrane attack complex.

The α-linked mannose structures on the surface of *C. albicans* are recognized by the MR, TLR4 and DC-SIGN, and β-1,2 mannosides, present in mannoproteins and PLM (Fradin *et al*., 2008; Mille *et al*., 2008), are recognized through TLR2 (Jouault *et al*., 2003). A recent study has shown that galectin-3, a S-lectin involved in the recognition of β-1,2 mannosides on the surface of the cell (Fradin *et al*., 2000), can discriminate between pathogenic *C. albicans* and non-pathogenic *S. cerevisiae*, and that an association between galectin-3 and TLR2 is involved in this process (Jouault *et al*., 2006).

Dendritic cells are able to recognize the different morphologies of *C. albicans* and generate a different response: yeast cells trigger IL-12 production and activate a protective T_H1 response, whereas hyphal forms repress these processes but trigger IL-4 production. Dendritic cells pulsed with the yeast forms were able to generate an antifungal protective immunity, indicating that DCs can sense and differentiate between both morphologies and types of response that can lead to eradication of the microbe or acquisition of a commensalism state (d'Ostiani *et al*., 2000).

Another lectin family member, dectin-2, has also been described to function as a receptor for *C. albicans* mannans (Ariizumi *et al*., 2000). Dectin-2 is present on tissue macrophages, Langerhans cells and DCs (Taylor *et al*., 2005b). Due to its short intracytoplasmic tail, dectin-2 must interact with the FcγR to induce intracellular signals. This receptor seems to be mainly involved in the recognition of *C. albicans* hyphae (Sato *et al*., 2006).

Candida albicans mannan is also recognized by other proteins, called collectins, which are all secreted as large multimeric complexes. At least three collectins, mannose-binding lectin (MBL), surfactant protein A (SP-A) and surfactant protein D (SP-D), have been implicated in antifungal immunity. Mannose-binding lectin is a serum protein that recognizes selected terminal monosaccharides, such as mannose, fucose and *N*-acetylglucosamine (Turner and Hamvas, 2000). Fungal binding by MBL triggers a protease cascade through the MBL-associated serine proteases (MASP-1 and MASP-2) that leads to activation of the complement pathway and deposition (or opsonization) of complement components, such as C3b, on the microbial surface, thereby promoting opsonic fungal recognition (Kilpatrick, 2002; Holmskov *et al*., 2003) (Fig. 4). MBL plays an important role in the first-line defence against *C. albicans* without the need for opsonophagocytosis by DCs, in which a direct interaction of MBL with *C. albicans* results in agglutination and accelerated complement activation via the lectin pathway, leading to inhibition of growth (Ip and Lau, 2004). However, a recent work indicates that the lectin pathway of complement activation in human neutrophils is important for the opsonophagocytosis of yeasts but not of bacteria (Brouwer *et al*., 2008). SP-A and SP-D recognize a broad range of microbes, including *H. capsulatum*, *A. fumigatus*, *C. albicans and C. neoformans* (Kishore *et al*., 2006), but they do not trigger complement activation and their primary role appears to involve microbial agglutination.

Conidia of *A. fumigatus* are recognized specifically by DC-SIGN as demonstrated using stable transfectants and monocyte-derived DCs. Binding and internalization of *A. fumigatus* conidia correlates with DC-SIGN surface expression levels and is abolished in the presence of *A. fumigatus*-derived cell wall galactomannans (Serrano-Gomez *et al*., 2004). The clinical relevance of this interaction is emphasized by the presence of DC-SIGN in lung DCs and alveolar macrophages, and further illustrated by the DC-SIGN-dependent attachment of *A. fumigatus* conidia to the cell membrane of IL-4-treated monocyte-derived macrophages (Serrano-Gomez *et al*., 2004). On the other hand, conidia have been shown to adhere to Langerhans cells in a dose- and time-dependent manner involving in this interaction a receptor with galactomannan structure specificity (Persat *et al*., 2003).

Mannose-binding lectin plays an important role in host defence against aspergillosis. Recent studies suggest a therapeutic role of *ex vivo*-administered MBL, possibly through MBL-mediated complement activation and other protective mechanisms aimed both directly at the pathogen, and indirectly through modulation of the host inflammatory responses (Kaur *et al*., 2007).

Protective T-cell responses to *C. neoformans* are also dependent on mannoproteins. Manoproteins of this fungal pathogen are efficiently recognized by DCs and competitive mannosylated inhibitors and calcium chelators interfere with T-cell stimulation. Human and murine DCs rapidly capture fluorescent-labelled mannoprotein by a MR-mediated process. Furthermore, using transfected cell lines, the type II C-type lectin receptor DC-specific ICAM-3-grabbing non-integrin (CD209) was shown to have affinity for *Cryptococcus* mannoproteins. Dendritic cells are also able to stimulate mannoprotein-specific T cells, suggesting that DCs provide a crucial link between innate and adaptive immune responses to *C. neoformans* via a process that is dependent upon the efficient uptake of mannoprotein by MRs (Mansour *et al*., 2006). However, in the presence of macrophages, binding of encapsulated *C. neoformans* is minimal in absence of opsonins. Following incubation in serum, *C. neoformans* potently activates complement, resulting in surface deposition of the third component of complement. Macrophages bind and phagocytose opsonized *C. neoformans* via three major complement receptors (CR) for C3 fragments, designated CD35 (CR1), CD11b/CD18 (CR3) and CD11c/CD18 (CR4). Antibody in normal human serum generally lacks opsonic activity, although vaccination can elicit anticapsular antibodies that are opsonic. The major component of cryptococcal capsule, GXM, is shed from the fungus and circulates in the blood and cerebrospinal fluid of patients with cryptococcosis. Cellular receptors defined for GXM include CD14, TLR2, TLR4 and CD18. GXM binding to macrophage receptors triggers activation of nuclear factor-kB (NF-κB), but not mitogen-activated protein kinases (MAPK). This results in no pro-inflammatory gene expression or release (Levitz, 2002)

Glucans

As stated before, the skeletal component of the cell wall of the majority of fungal pathogens is based on a core structure of β-1,3 glucan covalently linked to β-1,6 glucan and chitin. These polymers form hydrogen bonds between adjacent polysaccharide chains to form a three-dimensional network of microfibrils. It is generally accepted that these skeletal components of the cell wall are found close to the cell membrane in an inner layer; however, some chitin and glucan can be present throughout the thickness of the whole wall.

Candida albicans cell wall comprises approximately 60% β-glucan (Klis *et al*., 2001). Although initially thought to be hidden under the mannoprotein external layer, recent evidences suggests that β-glucans are indeed exposed on the cell surface although restricted to specific regions, such as bud scars (Gantner *et al*., 2005). β-Glucans can stimulate leucocytes *in vitro*, which induces cytotoxic and antimicrobial activities as well as the production of pro-inflammatory mediators, cytokines and chemokines (Brown and Gordon, 2005). β-Glucans are released into the circulation during systemic fungal infections (Obayashi *et al*., 1995). The recognition of β-glucans is attributed to two receptors, CR3 and dectin-1. CR3 is an integrin that recognizes pathogens opsonized by iC3b (the inactivated form of complement component C3b) and β-glucans. Carbohydrate recognition is mediated by a lectin domain (Diamond *et al*., 1993; Thornton *et al*., 1996), which is distinct from the normal ligand-binding site (the I domain) of CR3 (Diamond *et al*., 1993). The lectin domain mediates recognition of both the yeast and hyphal forms of *C. albicans* (Forsyth *et al*., 1998; Forsyth and Mathews, 2002), as well as several other fungi. Recognition by CR3 does not trigger protective host responses, such as the respiratory burst (Wright and Silverstein, 1983), and can repress pro-inflammatory signals (Wright and Silverstein, 1982; Brandhorst *et al*., 2004). Dectin-1 is a transmembrane receptor that contains a non-classical extracelular C-type lectin domain that specifically recognizes β-1,3 glucans (Brown and Gordon, 2001; Brown, 2006) and which is expressed in the surface of myeloid cells (monocyte/macrophage, DCs and neutrophil lineages) (Taylor *et al*., 2002; Brown, 2006). It was initially demonstrated that this receptor triggers the phagocytosis of β-glucan containing particles when expressed on the surface of non-phagocytic cells (Brown and Gordon, 2001) and that significantly contributes to the immunological response against fungal glucans (Brown *et al*., 2003). Later, it was shown that this molecule, which is expressed at low levels on macrophages but higher levels on dendritic cells, was recruited to phagosomes containing zymosan (Brown *et al*., 2003; Gantner *et al*., 2003). Dectin-1 can recognize several fungi, including *C. albicans* yeast, although it does not appear to recognize *C. albicans* hyphae (Gantner *et al*., 2005). The cytoplasmic tail of dectin-1 contains an ITAM, which can mediate various protective responses through spleen tyrosine kinase and caspase recruitment domain protein 9 (Syk–CARD9)-dependent pathways, such as the stimulation of interleukin 2 (IL-2), IL-10 (Rogers *et al*., 2005), IL-6 and IL-17 production (Leibundgut-Landmann *et al*., 2007). Although Syk-dependent signalling from dectin-1 is sufficient for these responses, stimulation of the MAPK NF-κB pathways, with subsequent production of pro-inflammatory cytokines, such as tumour necrosis factor (TNF), requires collaborative signalling with the TLR2 receptor (Brown *et al*., 2003; Gantner *et al*., 2003). A recent study suggests that phagocytosis of *C. albicans* by neutrophils can be mediated by the recognition of the cell wall component β-1,6 glucan (Rubin-Bejerano *et al*., 2007). Beads coated with β-1,6 glucan are well ingested by neutrophils, and the treatment of yeast cells with β-1,6

glucanase results in a reduced phagocytosis. This recognition appears to be mediated by CR3 following opsonization by C3d fragments that bind β-1,6 glucan.

In *A. fumigatus* dectin-1 is involved in generating inflammatory responses to specific morphological forms of this organism both *in vitro* and *in vivo*. *Aspergillus fumigatus* possesses a cell wall significantly made up of β-glucans (similar to *C. albicans*) (Beauvais and Latge, 2001). Alveolar macrophages are critical for recognizing and reacting to *A. fumigatus* leading to production of pro-inflammatory cytokines such as TNF-α, IL-1β, CCL3/MIP-1α, CXCL2/MIP2, IL-6, GM-CSF and G-CSF, all of which are significantly attenuated by blocking dectin-1 with monoclonal antibodies (Steele *et al*., 2005). In addition, TLR2 plays an accessory role with dectin-1 in mediating the alveolar macrophage inflammatory response to live fungal cells. As stated before, disease is initiated when 'resting conidia' are inhaled into the lung and go through phenotypic changes that lead to 'swollen conidia' followed by germination. Recent evidences suggest that the inflammatory response triggered by alveolar macrophages is different depending on the morphological stage and β-glucan exposure of the fungal cells recognized. In this sense, cytokine and chemokine production mediated by dectin-1 occurs only during the swelling and germination of conidia (Steele *et al*., 2005). This result confirms that no specific morphology is intrinsically associated in fungi with virulence, as it was already known from the clinical experience with fungi. Dectin-1 recognition of germ tubes also stimulates TNF-α production in the absence of TLR2 and MyD88 signalling (Gersuk *et al*., 2006).

An interesting observation is that yeast cells normally mask the β-glucan to immune cells. When *C. albicans* cells are switched to a filamentous mode of growth under host environmental conditions, they mask β-glucan that is exposed in yeast cells and, as a consequence, they are unable to activate dectin-1 mediated defences (Gantner *et al*., 2005). This result suggests the appealing possibility that fungi have evolved to escape immune system recognition through the masking of specific components of the cellular surface that could be able to trigger an effective antifungal response. In fact, a recent screening in *S. cerevisiae* for the enhanced recognition by β-glucan antibodies has led to the identification of several genes involved in this process and, among them, some signalling pathways controlling cell integrity (Wheeler and Fink, 2006). A similar behaviour has been observed in other fungal pathogens. In *A. fumigatus* resting conidia are ingested by macrophages but generate a reduced and controlled immune response without absence of reactive oxygen species. This is consistent with the fact that glucan is also masked in this cellular type. In contrast, maturing conidia and germ tubes are able to bind dectin-1 and generate a productive antifungal response in collaboration with TLRs (Gersuk *et al*., 2006).

In *H. capsulatum,* the less common polysaccharide, α-1,3 glucan, has been correlated with pathogenicity or linked directly to virulence by a yet-unknown mechanism. *Histoplasma capsulatum* exposes α-1,3-glucan in the outermost layer of cell wall and contributes to pathogenesis by concealing immunostimulatory β-glucans from detection by host phagocytic cells. Production of pro-inflammatory TNF-α by phagocytes is suppressed either by the presence of the α-1,3 glucan layer on yeast cells or by RNA interference based depletion of the host β-glucan receptor dectin-1. Thus, it has revealed an important mechanism by which *H. capsulatum* thwarts the host immune system (Rappleye *et al*., 2007). By contrast, little is known regarding glucan recognition in *C. neoformans*. A recent work, however, suggests that dectin-1 is not required for the host defence to C. *neoformans* as the authors did not found significant differences in the clinical course and cytokines production between dectin-1 gene-deficient and control mice, suggesting that dectin-1 is not essential for the development of host protective responses to the fungal pathogen (Nakamura *et al*., 2007).

Other cell wall components

Other structures of fungal pathogens can also be recognized as fungal PAMPs. On one hand, chitin, a less studied polysaccharide component of the *C. albicans* cell wall, induces recruitment of immune cells that principally release IL-4 and IL-13 (Van der Graaf *et al*., 2006). Little is known about the recognition pathways of chitin, its role during *C. albicans* infections and recognition receptors. On the other hand, bacterial and fungal DNA that is poorly methylated, in contrast to mammalian DNA, has been proposed to be instrumental in the recognition of non-self DNA by TLR9 (Wagner, 2001). Although the recognition of fungal DNA has not been properly demonstrated, the involvement of TLR9 in the recognition of *C. albicans* is supported by the observation that cytokine production from CD4+ T cells from TLR9–/– mice is skewed (higher IL-4, lower IFN-γ) compared with cytokine production from CD4+ T cells derived from wild-type mice upon challenge with *C. albicans* yeast (Bellocchio *et al*., 2004). No studies have investigated the possible role of RNA recognition systems (that is, TLR3, TLR7 and TLR8) in the host response to *C. albicans* infection.

Concluding remarks

Although the surface structures being recognized by the host cells are beginning to be determined precisely, much more work is needed to understand the interaction

of fungal PAMPs with their receptors on the host cell membrane. For example, little is known about the recognition of components of the fungal cell wall like chitin, surface proteins or products that are secreted by fungal cells. Recognition of the different morphologies associated with fungi promises to be an attractive area for future research as they are important evasion mechanisms. In this sense, it is important to note the differential exposure of several structures of the cell wall (such as glucan), depending on the fungal morphology with mannans and α-glycans acting as shields that may mask immune responses. Although both mannans and glucans can induce pro-inflamatory signals, the parallel stimulation of the mannan and β-glucan recognition pathways has a synergistic effect on the amplification of the immune response. This synergism may be lost under certain circumstances. Understanding these mechanisms may therefore provide essential information in order to develop novel antifungal therapies and a challenge for basic research.

It is clear that the pathogen–host interaction results in a bidirectional talk, where both the microorganism and the host cells are in a permanent dialogue, influencing each other's behaviour. The identification of the key components of such interplay at the molecular level (signals, mechanisms and responses) will have important consequences in the future and will surely allow better approaches to treat fungal infections.

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