ABSTRACT

Glycoproteins, glycosphingolipids and polysaccharides exposed at the most external layers of the wall are involved in several types of interactions of fungal cells with the exocellular environment. These molecules are fundamental building blocks of organisms, contributing to the structure, integrity, cell growth, differentiation and signaling. Several of them are immunologically active compounds with potential as regulators of pathogenesis and the immune response of the host. Some of these structures can be specifically recognized by antibodies from patients’ sera, suggesting that they can be also useful in the diagnosis of fungal infections.

Key-words: Glycoproteins, polysaccharides, glycosphingolipids, immune system, fungi

INTRODUCTION

The cell wall is a vital structure for all fungi, controlling shape and protecting the organism from the environment. It contains molecules involved in morphogenesis, reproduction, cell-cell and cell-matrix interactions. Although the fungal cell wall is a rigid structure, it must be dynamic in order to allow budding, growth and adaptation to environmental stress (43). This structure is composed of a number of unique interconnected polysaccharides, including chitin and a variety of glucans that are not found in mammalian cells, therefore it defines a prime target for drug development. However, the composition of the cell wall can differ substantially among fungal species. In general, they present a very similar polysaccharide structure but differ significantly in their protein composition which underscores the importance of cell wall proteins for pathogenesis.

In Saccharomyces cerevisiae, chitin is found at the cell budding sites representing 1-2% of the cell mass. β 1-6 glucan chains are directly attached to β 1-3 glucan and both glucan (50%) can be linked to chitin. In Aspergillus fumigatus branching of β 1-3 glucan results in an increase of acceptor sites for chitin, galactomannan and a linear β 1-3/1-4 – glucan which substitutes the β 1-6 glucan commonly expressed in other fungi. Glucans can also covalently bind to cell wall proteins (CWP). There are two major types of glycosyl modifications of proteins. N-linked glycans are attached to specific asparagine residues within a protein sequence and O-linked glycans to either serine or threonine residues. Many of these CWPs are attached to a glycosylphosphatidylinositol (GPI) anchor during their transport to the cell wall. At the cell surface, GPI-containing CWP are found linked to the other components through a remnant of their GPI anchor (see review 77).

Polysaccharides and peptidopolysaccharides are fundamental building blocks of organisms, contributing to the structure, integrity, and function of prokaryotic and eukaryotic cells. These molecules are especially relevant for the architecture of the cell wall, but several of them are immunologically active compounds with great potential as regulators of pathogenesis and the immune response of the host. In addition, some of these molecules can be specifically recognized by antibodies from patients’ sera, suggesting that they can be also useful in the diagnosis of fungal infections.

Proteins and glycoproteins exposed at the most external layers of the wall structure are involved in several types of
interactions of fungal cells with the exocellular environment. Thus, coating of fungal cells with host antibodies has the potential to strongly influence the host-parasite interaction by affecting antibody-mediated functions such as opsonin-enhanced phagocytosis and blocking the binding activity of fungal adhesins to host ligands (57).

It is known that host defence mechanisms influence the manifestation and severity of fungal infections, such that the clinical forms of the disease depend on a patient’s immune response.

Glycoproteins have long been known to influence T cell immune responses to a wide variety of antigens. Carbohydrate-binding receptors (CBR) are part of the larger pattern-recognition receptor (PRR) family and are highly expressed on front-line immune cells, particularly macrophages and dendritic cells. For fungi, the polysaccharide-rich cell wall is a major source of pathogen-associated molecular patterns (PAMPs), and it comprises the initial structure recognized by cells of the immune system. Identified PRRs for the detection of fungal surface components include Toll-like receptors TLR2 and TLR4, collectins (81), lectins (95,28,109,72). Recent work on the nonclassical C-type lectin (DC)-specific ICAM-3-grabbing nonintegrin (DC-SIGN). Similar to other members in their class, MMR and DC-SIGN exist as single transmembrane chains and require Ca2+ for their carbohydrate-binding properties, and thus are termed C-type lectins (95,28,109,72). Recent work on the nonclassical C-type lectin, dectin-1, has defined its substrate to be oligomers of β-(1,3)-glucan (81) a constituent of the cell wall of all fungi and a potent immunostimulatory molecule that induces TNF-α production by macrophages.

CBR affinity for sugars is diverse, but mannose is the most common monosaccharide recognized by this receptor (reviewed in Refs 109,23). Another important glycoconjugate class are glycosphingolipids (GSLs), which are the glycosides of either ceramide or myo-inositol-(1-O)-phosphoryl-(O-1)-ceramide. It is a structurally and functionally diverse sphingolipid subclass; GSLs are ubiquitously distributed among all eukaryotic species and are found in some bacteria (52). These molecules have been implicated in many fundamentals cellular processes including growth, differentiation, morphogenesis and contribute to host immune response. GSLs may also modulate cell signaling by controlling the assembly and specific activities of plasma membrane proteins (33,38). Phosphorylinoisitol-containing sphingolipids, which are absent in animals, have been reported in many plants, fungi, and protozoan (50). GSLs are present in fungi of the most primitive class of Phycomycetes (132) as well as in the most complex Basidiomycetes (6). Neutral and acidic GSLs have been characterized from fungal cells.

Polysaccharides and glycoproteins

Opportunistic yeasts: Cryptococcus neoformans

The incidence of infections caused by Cryptococcus neoformans greatly increased in individuals with compromised T-cell-mediated immune systems and cryptococcosis has emerged as the second most common cause of death in persons with AIDS. The cryptococcal infection follows the inhalation of poorly encapsulated yeasts, which are deposited into the alveolar space and then reach the lung interstitium. The infection is normally limited to the lung, but can disseminate to other tissues (55).

Earlier studies demonstrated that protective T cell responses to the pathogenic yeast Cryptococcus neoformans are dependent on heavily mannosylated antigens termed mannoproteins. Extensive O-mannosylation, which occurs at the serine/threonine region, facilitates recognition by mannose receptors on antigen-presenting cells, particularly dendritic cells. This results in an efficient antigen uptake, processing and presentation to T cells. Inhibition of mannose receptors or deglycosylation of mannoproteins profoundly inhibits T-cell responses, demonstrating the crucial contribution of mannosylation to immunogenicity (55,61). Human and murine dendritic cells (DC) are able to capture fluorescent-labeled mannoprotein by a mannose receptor-mediated process. By confocal microscopy, intracellular mannoprotein trafficked to an endo-lysosomal compartment in DC, and at later time points extended into tubules in a similar fashion to the degradation marker DQ-OVA. Mannoprotein colocalized intracellularly with CD206 and CD209. These data suggest that DC provide the crucial link between innate and adaptive immune responses to C. neoformans via a process that is dependent upon the efficient uptake of mannoprotein by mannose receptors (60).

In addition, incubation of human peripheral blood mononuclear cells with cryptococcal mannoprotein leads to the secretion of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), IL-1β, IL-6, IL-8 and IL-10 (131).

Other studies have demonstrated that secreted cryptococcal antigens were separated by concanavalin A affinity chromatography into adherent (mannoprotein [MP]) and nonadherent (flowthrough [FT]) fractions, and the fractions were tested in murine models of disseminated cryptococcosis. Mice that received two inoculations of MP and FT exhibited prolonged survival and reduced brain and kidney fungal loads following intravenous challenge with C. neoformans and MP-immunized animals had increased brain levels of tumor necrosis...
factor alpha, gamma interferon, and interleukin-2. In this context, FT and MP immunization protected B-cell-deficient, but not T-cell-deficient mice, suggesting that protection was T-cell mediated (62).

During C. neoformans infection, mannoprotein reinforced IL-12 and IFN-γ secretion that coincided with enhanced antifungal activity of natural effector cells, early resolution of the inflammatory process, and clearance of fungal load from the brain. These studies show that MP is a key inflammatory mediator that induces a protective immune response against C. neoformans infection (85).

Glucuronoxylomannan (GXM), the major polysaccharide component of Cryptococcus neoformans, is found bound to the fungal cell in the form of a capsule or shed in soluble form as an exopolysaccharide during growth in vivo and in culture. GXM is a (1→3)-linked, linear α-D-mannopyranan with β-D-xylopyranosyl (Xylp), β-D-glucopyranosyluronic acid (GlcUA), and 6-O-acetyl constituents (14) Variation in the structure of GXM results in antigenic differences that permit classification of C. neoformans strains into five serotypes known as A, B, C, D and AD. This molecule is associated with a variety of immunomodulatory effects. It inhibits the production of proinflammatory cytokines (125) induces inhibitory factors such as IL-10 (97), inhibits activation and maturation of dendritic cells (124), suppresses T cell proliferation in the presence of APC (98,111), dampens Th1 response and delayed-type hypersensitivity response (96), limits MHC class II expression (98,111), dampens Th1 response and delayed-type hypersensitivity response (96), limits MHC class II expression (98,111), and induces apoptosis in splenic mononuclear cells from normal rats (68). These effects are believed to contribute to the pathogenesis of C. neoformans infections. Glucuronoxylomannan induces expression of Fas ligand in monocytes/macrophages resulting in apoptosis of T cells expressing Fas. The induction of FasL occurs in part through GXM-TLR4 interaction (30). IgM, IgG1, and IgA mAbs to the capsule of C. neoformans are protective in murine models of cryptococcosis. Taborda and coworkers (2002) (112) reported that IgM and IgA to the Cryptococcus neoformans capsular glucuronoxylomannan (GXM) promote complement-independent phagocytosis by macrophages with efficiency comparable to that of IgG1. IgM- and IgA-mediated phagocytosis of C. neoformans was proportional to CR3 expression, inhibited by Abs to CR3 (CD11b/CD18) and CR4 (CD11c/CD18), and dramatically reduced with macrophages of CD18-deficient mice.

Pericollini and coworkers (2006) (82) investigated the effect of purified soluble GalXM on human T lymphocytes. Results indicate that, GalXM (i) can affect selected immune responses; (ii) causes significant impairment of T cell proliferation and increases interferon-γ and interleukin-10 production; and (iii) induces apoptosis of T lymphocytes through activation of caspase-8 that terminates with fragmentation of DNA.

Candida albicans

Fungal opportunistic infections, in particular, those caused by Candida species, have gained considerable significance as a cause of morbidity and mortality. Commensalism can easily turn into mucosal candidiasis in immunocompromised subjects, such as AIDS patients or those affected by idiopathic CD4+ T lymphocytopenia (17); moreover, deep-seated candidiasis predominantly occurs in neutropenic bone-marrow transplant patients. Finally, a large incidence of vaginal infection by Candida is recorded in otherwise healthy women of premenopausal age (25,83,79).

Candida albicans has a multilayered cell wall composed of an outer layer of proteins glycosylated with N- or O-linked mannosyl residues and an inner skeletal layer of α-glucans and chitin. The glucans are in the form of phosphomannoprotein complexes and are important in fungal-host interactions, as they make first contact with the immune system. The beta-oligomannosides, which make up the acid-labile part of the phosphomannan complex, and alpha-oligomannosides, which make up the acid-stable part of the complex, serve as adhesins in the attachment of C. albicans yeast cells to host splenic and lymph node macrophages. The β-oligomannosides can induce release of tumour necrosis factor (TNF)-alpha, and antibodies specific to certain beta-oligomannosides enhance host resistance to various forms of candidiasis (18). Several Mabs generated against C. albicans have been shown to react to β-1,2-oligomannosides (35,36,57) In addition, Han and collaborators (37) demonstrated that a mannan-based vaccine formulation elicited antibodies that protected against disseminated systemic and vaginal candidiasis and that a monoclonal agglutinating IgM (MAb 6.1) specific for a C. albicans cell surface β-1,2-mannosiose was also protective in both types of infection (36).

The cytokine production by human mononuclear cells or murine macrophages is markedly reduced when stimulated by C. albicans mutants defective in mannosylation. The recognition of mannosyl residues is mediated by mannose receptor binding to N-linked mannosyl residues and by TLR4 binding to O-linked mannosyl residues. Residual cytokine production is mediated by recognition of β-glucan by the dectin-1/ TLR2 receptor complex. C. albicans mutants with a cell wall defective in mannosyl residues were less virulent in experimental disseminated candidiasis and elicited reduced cytokine production in vivo (75). A 65-kDa mannoprotein (MP65) has long been studied as a major, immunodominant antigen of the human opportunistic pathogen Candida albicans. Pietrella and coworkers (2006) (84) demonstrated that MP65 stimulates dendritic cells (DC) and induces the release of TNF-alpha, IL-6 and the activation of IL-12 gene. MP65 induces DC maturation by increasing costimulatory molecules and decreasing CD14 and Fc gammaR molecule expression. The latter effect is partly mediated by Toll-like receptor 2 (TLR2) and TLR4, and the
MyD88-dependent pathway is involved in the process. MP65 enables DC to activate T cell response, its protein core is essential for induction of T cell activation, while its glycosylated portion primarily promotes cytokine production. (84) MAbC7, a monoclonal antibody directed against a Candida albicans cell wall mannanprotein exerts three anti-C. albicans activities, i.e., inhibition of adherence to HEp2, inhibition of germination, and direct candidicidal activity. (71). The candidicidal activity of macrophage was strongly enhanced when C. albicans was opsonized by C7 (108).

The 58-kDa surface mannanprotein of Candida albicans (mp58) elicits strong antibody responses during infection. A monoclonal antibody directed towards the C-terminal epitope conferred protection in serum therapy experiments in a murine model of hematogenously disseminated candidiasis (130).

Lectins play a critical role in host protection against infection. The galectin family of lectins recognizes saccharide ligands on a variety of microbial pathogens. Galectin-3, a galectin expressed by macrophages, dendritic cells, and epithelial cells, bind to Candida albicans species that bear beta-1,2-linked oligomannans on the cell surface, but did not bind to Saccharomyces cerevisiae that lacks beta-1,2-linked oligomannans. This fact, induced death of Candida species containing specific beta-1,2-linked oligomannosides. Unlike other lectins of the innate immune system that promote opsonization and phagocytosis, galectin-3 has direct fungicidal activity against opportunistic fungal pathogens (45).

Filamentous fungi:
Aspergillus sp

Species of the genus Aspergillus are among the most common causal agents of deep mycoses in the developed world. They have been implicated as etiological agents of several lung diseases including allergic asthma, allergic bronchopulmonary aspergillosis (ABPA), aspergillosis, and invasive aspergillosis (IA). Over 90% of cases of Aspergillus-related diseases are caused by A. fumigatus (47,41). Cell-wall polysaccharides and glycoproteins have been characterized in Aspergillus spp. and galactomannans are an important structural component of the Aspergillus cell-wall, being widely distributed among most Aspergillus species (49, 31). A galactomannan was isolated from a culture filtrate (48) or extracted from the fungal cell wall and it consisted of a main chain of (1→6)-linked alpha-D-mannopyranosyl residues substituted at O-2 by 1 to 3 consecutive alpha-D-mannopyranosyl units that were (1→2)-linked, and beta-D-Galactofuranosyl-containing side-chains, with (1→5)-links. Such beta-D-Gal-bearing chains are regarded as immunodominant epitopes, especially when they are (1→5)-linked. Antibodies directed against this type of polysaccharide have been detected in patients with aspergillosis and in experimentally infected animals, or in rabbits or mice hyperimmunized with total extracts of A. fumigatus (106). Galp-containing molecules have been described to be important antigens among several human fungal pathogens, such as Paracoccidioides brasiliensis (1) and are not present in the human host and could help it recognize the fungus as non-self and induce cytokine synthesis to activate cellular immunity.

Monoclonal antibodies have been raised against these structures and are used with some success for detecting circulating antigens (110). Periodate treatment, partial acid hydrolysis, and alkaline, reductive beta-elimination of peptidogalactomannans (pGM) removed most of the antibody-binding capacity (31).

An immunodominant 35 kDa antigen containing 70% carbohydrate and 30% protein, isolated from a strain of Aspergillus flavus, involved in invasive mold sinusitis, was fractionated by ConA-Sepharose chromatography. The role of the carbohydrate moiety in sera recognition has been demonstrated (3).

Leitão et al. (2003) (49) have demonstrated that oligosaccharides terminate oligosaccharide may account for a significant part of the A. fumigatus peptidogalactomannan antigenicity, because de-O-glycosylation decreased by 50% its activity. The immunodominant epitopes were present in the tetra- and hexasaccharides, which contain beta-Galp-(1→5)-beta-Gal terminal groups. These hapten are potent inhibitors (90%) of the recognition between the sera of patients and pGM.

Mature A. fumigatus conidia and germ tubes stimulate NF-kappaB, secretion of proinflammatory cytokines and production of reactive oxygen by human monocyte-derived macrophages and murine macrophages from multiple anatomical sites. These responses are in part mediated by dectin-1, which binds cell wall beta-glucan that is not present on the surface of dormant conidia, but is present after cellular swelling and loss of the hydrophobic proteinaceous cell wall (29).

Binding and internalization of A. fumigatus conidia correlates with DC-SIGN cell surface expression levels and is abolished in the presence of A. fumigatus-derived cell wall galactomannans. The clinical relevance of this interaction is emphasized by the presence of DC-SIGN in lung DC 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. These dates suggest the involvement of DC-SIGN in the initial stages of pulmonary infection as well as in fungal spreading during invasive aspergillosis. (107).

C-type lectins represent a family of receptors, which recognize pathogen-specific carbohydrates. One of them is beta-1,3 glucan, a major component of the fungal cell wall. Luther and coworkers (2006) (58) provide evidence that beta-1,3 glucan plays an important role for the elimination of A. fumigatus conidia. Laminarin, a soluble beta-1,3 glucan and antibodies to dectin-1, a well known beta-1,3 glucan receptor, significantly inhibited conidial phagocytosis. Additionally, TLR2 and the adaptor protein MyD88 are required for efficient conidial phagocytosis,
suggesting a link between the TLR2-mediated recognition of \( A. \) \( fumigatus \) and the phagocytic response. TLRs as well as the TLR-associated adaptor molecule MyD88 have been implicated in the recognition of the fungal pathogens \( C. \) \( albicans \), \( A. \) \( fumigatus \), \( C. \) \( neoformans \), and \( P. \) \( boydii \). \( PRM \) is a homopolymer of glucose with \( \alpha \)-D-\( \text{Glc} \) residues substituted at position 6 with \( \alpha \)-D-\( \text{Glc} \) branches. Soluble \( \alpha \)-glucan, but not \( \beta \)-glucan, led to a dose-dependent inhibition of conidia phagocytosis. Furthermore, a significant decrease in the phagocytic index occurred when \( \alpha \)-glucan from conidial surface was removed by enzymatic treatment with alpha-amyloglucosidase, thus indicating an essential role of \( \alpha \)-glucan in \( P. \) \( boydii \) internalization by macrophages. \( \alpha \)-glucan stimulates the secretion of inflammatory cytokines by macrophages and dendritic cells; again this effect is abolished by treatment with alpha-amyloglucosidase. Finally, alpha-glucan induces cytokine secretion by cells of the innate immune system in a mechanism involving toll-like receptor 2, CD14, and MyD88. These results might have relevance in the context of infections with \( P. \) \( boydii \) and other fungi, and \( \alpha \)-glucan could be a target for intervention during fungal infections (9).

**Emerging filamentous fungus: \( P. \) \( boydii \)**

Several types of pathogenicity have been associated with \( P. \) \( boydii \). It is known to cause human white-grain mycetoma (99) and it has recently emerged as an agent of systemic and disseminated mycoses. \( P. \) \( boydii \) resembles other fungal species in tissue specimens and is not easily distinguished from \( A. \) \( fumigatus \) species (7).

In the search for structures that could be helpful in the diagnosis of pseudallescheriasis, much attention has been paid to the study of \( P. \) \( boydii \) cell-wall antigens. Polysaccharides and peptidopolysaccharides have been isolated from its mycelial form and characterized by our group using chemical and immunological methods. The chemical structure of a peptidoglycan of \( P. \) \( boydii \) was investigated. Chemical analysis showed it to contain \( \alpha \)-Rhap-(1→3)-\( \alpha \)-Rhap- side-chain epitopes linked (1→3) to \( \alpha \)-(1→6)-linked \( \alpha \)-Manp core. (88) PRM reacted poorly with an antiserum raised against whole cells of \( S. \) \( schenckii \) and strongly with one against \( P. \) \( boydii \) hyphae. These characteristics and immunological differences suggest that this major rhamnose-containing antigen of \( P. \) \( boydii \) may be useful for diagnostic purposes, mainly in mixed allergic bronchopulmonary fungal disease due to \( P. \) \( boydii \) and \( A. \) \( fumigatus \). Nonreducing, \( O \)-linked oligosaccharides were obtained from PRM by alkaline \( \beta \)-elimination under reducing conditions. Three oligosaccharide fractions were obtained and the major oligosaccharide (oligo 1) was characterized. Oligo 1 was a branched structure, with a main chain of \( \alpha \)-Rhap-(1→3)-\( \alpha \)-Rhap-(1→3)-\( \alpha \)-Manp-(1→2)-Man-ol substituted at O-6 of mannitol with an \( \alpha \)-GlcP-(1→4)-\( \beta \)-Galp group.Oligo 2, was a substructure of Oligo 1, lacking a hexose from Glc-Gal branch. Both oligo 1 and 2 blocked the reaction between PRM and rabbit anti-\( P. \) \( boydii \) mycelium hyperimmune serum by 75% (87).

Bittencourt et al (2006) (9) isolated and characterized the structure of an alpha-glucan from \( P. \) \( boydii \) cell wall and evaluated its role in the induction of innate immune response. These analyses indicated that \( \alpha \)-glucan of \( P. \) \( boydii \) is a glycogen-like polysaccharide consisting of linear 4-linked \( \alpha \)-D-GlcP residues substituted at position 6 with \( \alpha \)-D-GlcP branches. Soluble \( \alpha \)-glucan, but not \( \beta \)-glucan, led to a dose-dependent inhibition of conidia phagocytosis. Furthermore, a significant decrease in the phagocytic index occurred when \( \alpha \)-glucan from conidial surface was removed by enzymatic treatment with alpha-amyloglucosidase, thus indicating an essential role of \( \alpha \)-glucan in \( P. \) \( boydii \) internalization by macrophages. \( \alpha \)-glucan stimulates the secretion of inflammatory cytokines by macrophages and dendritic cells; again this effect is abolished by treatment with alpha-amyloglucosidase. Finally, alpha-glucan induces cytokine secretion by cells of the innate immune system in a mechanism involving toll-like receptor 2, CD14, and MyD88. These results might have relevance in the context of infections with \( P. \) \( boydii \) and other fungi, and \( \alpha \)-glucan could be a target for intervention during fungal infections (9).

**Dimorphic fungi**

**Histoplasma capsulatum**

There are two varieties of \( H. \) \( capsulatum \) that are pathogenic to humans, \( H. \) \( capsulatum \) var. \( capsulatum \) and \( H. \) \( capsulatum \) var. \( duboisi \), and a third variety that is an equine pathogen, \( H. \) \( capsulatum \) var. \( farciminosum \). \( H. \) \( capsulatum \), a dimorphic fungus, exists as a mold in the environment and two types of conidia (macroconidia or tuberculate and microconidia) are formed. At 37°C in vitro and in tissues, the organism converts into the yeast phase that is composed of tiny oval budding yeasts found both inside and outside macrophages. Infection with \( H. \) \( capsulatum \) var. \( capsulatum \) occurs commonly in areas in the Midwestern United States and Central America, but symptomatic disease requiring medical care is manifest in very few patients. The extent of disease depends on the number of conidia inhaled and the function of the host’s cellular immune system (40).

The major diagnostic antigens of \( H. \) \( capsulatum \) var. \( capsulatum \) are the \( H \) and \( M \) antigens, pluripotent glycoproteins that elicit both humoral and T-cell-mediated immune responses. The \( H \) antigen from \( H. \) \( capsulatum \) var. \( capsulatum \), isolated from histoplasmin, have carbohydrate-to-protein ratios of 0.78 and contains galactose, glucose, mannose, and hexosamine, with higher concentrations of galactose, mannose, glycine, and alanine. The amino acid sequence of this glycoprotein showed homology with \( \beta \)-glucosidases (20,21) Recombinant \( H \) antigen is able to stimulate splenocytes from mice immunized with viable yeast cells or with antigen suspended in adjuvant. Mice inoculated with \( H \) antigen were not protected against either a sublethal or a lethal inoculum of yeast cells. Thus, \( H \) antigen stimulates a cell-mediated immune response in BALB/c mice but does not induce a protective response to \( H. \) \( capsulatum \) (20).

One such yeast phase-specific component is \( \alpha \)-(1,3)-glucan, a homopolymer of glucose with \( \alpha \)-glycosidic linkages, which has been linked to fungal virulence. The cell walls of most
medically important fungi contain α-(1,3)-glucan. Reduction in α-(1,3)-glucan, in Histoplasma (94) has no effect on in vitro growth but severely attenuates virulence in murine respiratory infection models. Rappleye and collaborators (2007) (93) present evidence showing that Histoplasma cell wall α-(1,3)-glucan blocks host PRR recognition of the fungal pathogen-associated molecular patterns (PAMP) β-glucan, enabling Histoplasma yeast to avoid detection as a fungal invader. However, the role of this polysaccharide during infection, its organization within the cell wall, and its synthesis and regulation remain poorly understood. Organisms that present α-(1,3)-glucan as the most external cell wall layer may thus effectively mask their β-glucan signature and avoid alerting the host immune system and the production of proinflammatory TNFα by phagocytes. The generation of these cytokines necessary for a protective immune response to both primary and secondary histoplasmosis, was 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. Consistent with this hypothesis, the parasitic forms of the dimorphic fungal pathogens each possess α-(1,3)-glucan and can cause disease even in the face of normal host immune function.

Paracoccidioides brasiliensis

Paracoccidioidomycosis (PCM) is a human systemic granulomatous disease, prevalent in South America, which is caused by a dimorphic fungus, Paracoccidioides brasiliensis (Pb). This fungus grows in the mycelial phase at room temperature and in the yeast phase at 37°C as well as in infected tissues. This disease is one of the most prevalent human systemic mycoses in Latin America (11). Inhalation is probably the common route of introduction of air-borne conidia into the human host, with the lung being the primary organ infected. Transformation of conidia into yeast forms starts the infectious process (67,105). Once these cells are installed, characteristic granulomatous lesions are formed in the lungs and, if not contained, yeast cells invade the lymphatic system (acute form). Granulomas are rich in viable fungi which, in immunologically compromized hosts, can disseminate to virtually all organs and tissues (74) It is suggested that cell-mediated immunity is the most important host defense mechanism against this fungus, although specific antibodies may also have a protective role (66). The gp43, first described by Puccia et al. (1986) (91), is the major diagnostic antigen of P. brasiliensis in a variety of serological tests (12,121). The 43 kDa glycoprotein (gp) was the predominant IgG reactive antigen, recognized by 100% of the patient’s sera (13). Monoclonal antibodies to gp43 modulated the laminin-mediated fungal adhesion to epithelial cells and moderated pathogenesis of yeast cells in a hamster testicle infection model (126). The gp43, a high-mannose glycoprotein, was also immunodominant in crude antigenic preparation for eliciting delayed hypersensitivity reactions in guinea pigs (100).

The gp43 gene was cloned and completely sequenced by Cisalpino et al. (1996) (16). It encodes a polypeptide of 416 amino acids with a leader peptide of 35 residues: the mature protein has a single high mannose N-glycosylation site. It contains a neutral high-mannose core (Man7GlcNAc2) to which a (1→6)-linked alpha-D-Manp chain of variable length, substituted at the 2-O positions by single alpha-D-Manp residues, is attached. A terminal unit of beta-D-Glactofuranose is (1→6)-linked to one of the (1→2)-linked mannosyl residues, either in the C or in the A arm of the oligosaccharide. The heterogeneity of the oligosaccharide is determined by the different sizes of the A arm and the sites of insertion of the beta-Glactofuranosyl unit. (Almeida et al. 1996) (1). It bears peptide sequences homologous to those of beta-1,3-glucanases from Candida albicans and Saccharomyces cerevisiae. The gp43 is, however, devoid of hydrolytic activity and does not cross-react immunologically with fungal glucanases.

Specific conformational peptide epitopes are recognized by the human antibodies as determined by antigen deglycosylation (120).

Apart from eliciting high antibody titers, gp43 is also immunodominant in delayed-type hypersensitivity reactions in infected animals and humans. The cellular immune response in mice to gp43 administered in complete Freund’s adjuvant involves CD4+ Th-1 lymphocytes, secreting gamma interferon (IFN-gamma) and interleukin 2 (IL-2) but not IL-4 and IL-10. The T-cell epitope of this antigen was mapped to a 15-amino-acid peptide (P10) based on lymphoproliferations with primed cells from three different haplotypes. The HTLAIR inner core of P10 is the essential domain of the epitope, with various flanking regions possible. Immunization of mice with both gp43 and P10 led to vigorous protection against intratracheal challenge by virulent P. brasiliensis, with a >200-fold decrease in lung CFU and halting of dissemination to the spleen and liver. The protective effect of P10 is mainly attributed to an IFN-gamma-mediated cellular immune response. Unlike gp43, which induces an antibody response (IgG1, IgG2a, IgE and IgG2b subclasses) compatible with both Th-1 and Th-2 activation in infected BALB/c mice, P10 does not induce a humoral response. Protection by gp43 and P10 was characterized by a well-demarcated lung granulomas with numerous nonviable yeast forms or resolved lesions with no detectable fungal cells (113,119). The treatment combined with peptide P10 and chemotherapy showed an additive protective effect when administered at 48 h or 30 days after intratracheal challenge in BALB/c mice (63).

In resistant mice (A/Sn), purified gp43 seems to have been preferentially presented by macrophages and stimulated Th1 lymphokine production. On the other hand, in susceptible animals (B10) gp43 was distinguishably presented by B cells, which led to stronger activation of Th2 subsets. Moreover, T cells from resistant mice responded as those from susceptible animals when stimulated by gp43 presented by APCs from...
susceptible mice and vice versa, indicating that there are no significant differences in the T cell repertoires from resistant and susceptible mice (2).

Addition of different concentrations of gp43 to the culture medium inhibited, in a dose-dependent pattern, phagocytosis of live or heat-killed Pb by peritoneal macrophages from both B10.A and A/Sn mice. Gp43 also inhibits phagocytosis of zymosan particles but did not interfere with the uptake of opsonized sheep red blood cells. It was also shown that both gp43 and heat-killed Pb have an inhibitory effect on the release of NO by zymosan - stimulated macrophages. Moreover, gp43 inhibits the fungicidal ability of macrophages from both lineages (90).

Antigen presentation is an essential stage in the development of immune response to a specific antigen. This response can lead to the production of antibodies and/or effector T lymphocyte activation. Macrophages, dendritic cells and B-lymphocytes, among others, act as antigen presenting cells. B-1a and B1-b cells represent a small population in the adult spleen and are abundant in the peritoneal and pleural cavities. Previous studies demonstrated that B1-b cells express constitutively high levels of class II MHC and costimulatory molecules inducing an efficient proliferation of gp43 sensitized T lymphocytes. (128) Granulomas were observed either when total adherent peritoneal cells or when isolated B-1 cells were added to macrophage cultures. The data strongly suggest that an interaction of B-1 cells and macrophages plays an important role in granuloma-like formation in this experimental model and that the presence of gp43 strongly stimulates this response (127).

Purified gp43 lead to down-regulation of MHC-II and adhesion properties of immature DCs and in LPS-induced DCs maturation. It was also shown that purified gp43 from P. brasiliensis has the same inhibitory effect on IL-12 release. Mice infected with P. brasiliensis that received DCs plus gp43 plus LPS had a significant increase of the lung colony forming units when compared with control (not immunized) or those that received only DCs plus LPS. These data suggest that gp43 affects many functions of the host cells, indicating that these alterations might be used by P. brasiliensis to reduce the effectiveness of the immune response thus facilitating the establishment and fate of primary infection in susceptible host (24).

Another expressed glycoprotein, gp70, is recognized by 96% of sera from PCM patients and is able to induce lymphoproliferation. Using anti-gp70 MAbs, it was observed by confocal microscopy that gp70 is located mainly in the intracellular compartment of the fungus, although it was also detected in the culture supernatant. Purified gp70 was able to inhibit the activity of macrophages through the mannose receptors and also through the Fc receptors and inhibits NO and H(2)O(2) liberation by peritoneal macrophages in vitro. Passive immunization of mice during intratracheal infection with P. brasiliensis using anti-gp70 MAbs almost completely abolished granuloma formation in the lungs, suggesting that this glycoprotein may facilitate fungal establishment and progression of lesions in the primary fungal infection (65).

**Glycosphingolipids**

Glycosphingolipids (GSLs) are amphipathic molecules consisting of a ceramide (N-acylsphingosine) lipid moiety to a glycan chain of variable length and structure. Glycoconjugates, fusoceramides, and sphingolipids are glycosphingolipids. They are structurally different from their mammalian counterparts in that the latter contain saturated, non-hydroxylated fatty acids bound to a sphingobase lacking branches, consequently, fungal CMHs can be selectively recognized by antifungal agents. Several studies revealed that fungal CMHs are the cellular targets for the action of human, rabbit and mouse antibodies with antimicrobial activity. The side effects and drug resistance are commonly observed during treatment of deep mycoses, which motivates the search for new antifungal drugs.

**Structural aspects**

Glycosphingolipids are composed of a sugar unit, usually glucose or galactose, bound to a hydrophobic ceramide, containing the conserved C19 sphingoid base with a C-9 methyl group and two unsaturated linkages (Δ9, Δ8) in amidic linkage to 2-hydroxoctadecanoic or 2-hydroxyhexadecanoic acids. These molecules are conserved structures, in which modifications include different sites of unsaturation as well as the varying length of fatty acids residues in the ceramide moiety. In plants the monosaccharide is normally glucose and the sphingoid usually phytosphingosine. Galactose, sphingosine or dihydrophosphingosine are the main components of animal glycolipids. The gangliosides contain at least one sialic acid residue. CMHs differ from globosides in that these glycolipids contain multiple sugar moieties and also from gangliosides that contain at least one sialic acid residue. CMHs have been widely detected in fungal cells and the current literature indicates that cerabiosides seem to be present in almost all the fungal species studied so far, with *Saccharomyces cerevisiae*, *Torulaspora delbrueckii*, *Candida glabrata*, *Kluyveromyces polysporus*, and *K. yarrowii* representing exceptions (103) Cerebrosides from *Saccharomyces kluveri* have a rare trihydroxy sphingoid base as a unique feature (115).

The major CMH species from *F. pedrosai* produced by conidial and mycelial forms display the same structure, an N-2'-hydroxyhexadecanoyl-1-beta-d-glucopyranosyl-9-methyl-I-4,8-sphingadienine. However, the major cerebroside species purified
from sclerotic cells carries an additional hydroxyl group, bound to its long-chain base (78).

Using several chromatographic approaches, mass spectrometry, and nuclear magnetic resonance, Maciel and coworkers (2002) (59) identified ceramide mono- and dihexosides (CDH) in purified lipid extracts from Magnaporthe grisea cells. As described by other authors, CMH consists of a ceramide moiety containing 9-methyl-1,8,8-sphingadienine in amide linkage to 2-hydroxyoctadecenoic or 2-hydroxyhexadecenoic acids and a carbohydrate segment consisting of one residue of glucose. CDHs, however, contain beta-galactose (1→4)-linked to beta-glucose as sugar units and phytosphingosine as the long-chain base, bound to a C24-alpha-hydroxylated fatty acid. This is the first report on the occurrence of CDH in a fungal species and illustrates the existence of an alternative path of ceramide glycosylation in fungal cells.

The long chain base 9-methyl-1,8,8-sphingadienine was first described in monohexosylceramides from Aspergillus oryzae (27) and was subsequently isolated from Schizosaccharomyces pombe (19), Cryptococcus neoformans (101), Fonsecaea pedrosoi (78), Fusarium sp (22), Hansenula anomala (76), Histoplasma capsulatum (118), Kluyveromyces waltii (115), Magnaporthe grisea (44,122), Paracoccidioides brasiliensis (114), Pichia pastoris (104), Pseudallescheria boydii (89), Saccharomyces kluverieri (115), Sporothrix schenckii (117), Termitomyces albuminosus (92).

Several studies (reviewed in reference Lester and Dickson 1993) (50) allowed the identification of three classes of GIPCs in S. cerevisiae. These classes consist of inositolphosphoceramides (IPCs), mannosylinositolphosphorylceramides (MIPCs) and the major sphingolipid, M(IP)2C which contains two IPCs, mannosylinositol phosphoceramides (MIPCs) and the long-chain base, bound to a C24-alpha-hydroxylated fatty acid. This is the first report on the occurrence of CDH in a fungal species and illustrates the existence of an alternative path of ceramide glycosylation in fungal cells.

Only the GSL Pb-1 antigen, which presents the carbohydrate structure Galβ1-6(Manch1-3) Manß1, is reactive with the PCM patient sera. The PCM patient sera did not react with Pb-2, which lacks the Galβ residue and which is considered the biosynthetic precursor of Pb-1, indicating that the Galβ residue is essential for antibody reactivity. The Pb-1 glycolipid from nontreated patients elicited a primary immune response with immunoglobulin M (IgM) production and subsequent switching to IgG1 production. The IgG1 titers increase after the start of antifungal treatment, and general decreases in the anti-Pb-1 antibody titers are observed after 5 months of treatment. Probably, the Pb-1 antigen, an acidic GSL with terminal Galβ residue, has potential application as an elicitor of the host immune response in patients with PCM (8). The PCM patient sera recognized mainly CMH and one fraction of acidic glycolipid. Anti-CMH and anti-acidic glycolipid Abs were used as opsonizing agents in phagocytosis assays increasing the internalization of the yeast cells and production of NO by macrophages. A protective role of these Abs is thus suggested.

A monoclonal antibody to the glucosylceramide synthesized by P. brasiliensis was produced and demonstrated to react with its long-chain base (78).
with the reproductive structures (conidiophore) of *A. fumigatus* (120). This evidence supported the idea that CMHs are preferentially accumulated in surface sites related to fungal growth, but also suggested that they are involved in the differentiation process. Accordingly, the same research group reported the involvement of GlcCer in fungal development on *A. fumigatus* and *A. nidulans* using a family of compounds known to inhibit GlcCer synthase in mammals. Two analogs inhibited germination and hyphal growth. Neutral lipids from *A. fumigatus* cultured in the presence of these inhibitors displayed a significantly reduced GlcCer/GalCer ratio. These results suggest that synthesis of GlcCer is essential for normal development of these species (53).

*Pseudallescheria boydii* is a fungal pathogen that causes disease in immunocompromised patients. Ceramide monohexosides were isolated and purified from mycelia of *P. boydii* and their structures were determined by chemical, spectrometric, and spectroscopic methods. This fungus appears to synthesize only glucosylceramides containing 9-methyl-4,8-sphingadienine as long chain base. The different molecular species can thus be attributed to CMH molecules differing only in the chain length of hydroxylated fatty acids (16:0 and 18:0). This molecule was recognized by serum of an infected rabbit, confirming that antibodies to cerebrosides are producing by *P. boydii*. (89) Hydroxylation at position 2 of the fatty acid is apparently important for antigenicity of the CMH (73, 134), and possible epitopes involve both glucose and the hydroxylated fatty acid, with modulation by the sphingosine-derivated base. Conformer 4 of glucosylceramide – as studied by Nyholm and Pascher (1993) (80) which is allowed in a membrane layer, and further stabilized by a hydrogen bond between the 2-OH group on the fatty acid and the 6-OH group on the glucose residue, in addition to the hydrogen bond between glucose O5 and the amide hydroxide – is a candidate for epitopes reactive with anti-CMH antibodies. CMH accumulated on the surface of mycelia was recognized by antibodies from rabbits immunized with *P. boydii* whole cells. Interestingly, conidial cells did not react with antibodies to CMH, suggesting that CMHs are differentially expressed in *P. boydii* according with morphological phase. These antibodies were able to inhibit the formation of germ tube–like structures in *P. boydii*, although they did not influence mycelial growth. We have shown that germ tubes are induced after the contact of *P. boydii* conidia with animal cells, a step preceding efficient fungal invasion. (86). Germ tube formation is also recognized as a crucial event in tissue invasion by *C. albicans* (30), a fungus that synthesizes CMHs (64) structurally similar to those previously described in other fungi and to that characterized from *P. boydii*. In this context, the influence of antibodies to CMH on *C. albicans* differentiation was also evaluated. As with *P. boydii*, anti-CMH antibodies inhibited germ tube formation in *C. albicans* (89). Our most recent results demonstrate that polyclonal and monoclonal antibodies to CMH strongly inhibit the differentiation of the plant pathogen *Colletotrichum gloeosporioides* (19). The mechanism by which anti-CMH antibodies inhibit fungal growth and/or differentiation remain to be established, but there is a possibility that CMHs are associated with enzymes involved in the hydrolysis and synthesis of the cell wall and/or with GPI-anchored precursors during cell differentiation and division. In this context, binding of antibodies to CMHs could impair the action of CMH-associated functional proteins inhibiting cell wall synthesis.

By using various mass spectrometric techniques, a cryptococcal cerebroside was characterized by Rodrigues and coworkers (2000) (102) as a β-glucosylceramide, with the carbohydrate residue attached to 9-methyl-4,8-sphingadienine via an amidos linkage to 2-hydroxyoctadecanoic acid. This molecule was recognized by sera from patients with cryptococcosis and a few other mycoses, indicating that CMHs are immunogenic glycolipids that induce the production of human antibodies during fungal infections.

The presence of CMHs as structural components of the cell wall of *C. neoformans* was demonstrated by electron microscopy of yeast cells labeled with immunogold-antibodies (102). An abundant deposition of gold particles was observed on the cryptococcal wall rather than on the plasma membrane, indicating that the antibody-reactive epitopes of CMH may be sterically accessible only after transfer of the glycosphingolipids to the cell wall. Labeling was also observed on membrane formations, putatively vesicles, across the periplasmic space, linking the plasma membrane to the inner face of the cell wall (5, 78, 102) suggesting that cerebrosides can be hydrophobic components involved in the vesicular traffic of surface molecules.

Confocal analysis demonstrated that human anti-CMH antibodies mainly reacted at the cell budding sites of *C. neoformans*, suggesting a relationship between CMH distribution and cell growth (102). To confirm this hypothesis, human antibodies to cerebrosides were added to cultures of *C. neoformans* and yeast growth determined at 12 h intervals. In their presence, Rodrigues and coworkers (2000) (102) observed an immediate arrest of cell growth and budding. Although fungal cultures were also supplemented with human serum, this effect was independent of the action of the complement system. Both acapsular and encapsulated strains of *C. neoformans* had budding and cell growth inhibited by the antibodies. Analysis of antibody-treated cells by transmission electron microscopy revealed intense cellular damage, with organelle destruction, membrane retraction, and increased vacuolization (78).

Infection with *F. pedrosoi*, etiological agent of chromoblastomycosis, begins with traumatic inoculation of conidia or mycelial fragments from the soil, but in vivo these
cells differentiate into sclerotic bodies. CMHs from conidial forms of *F. pedrosoi* were purified and characterized as N-2’-hydroxyhexadecanoyl-1-β-D-glucopyranosyl-9-methyl-4,8-sphingadienine. However, the major cerebroside species purified from sclerotic cells carries an additional hydroxyl group, bound to its long-chain base (78). The structural difference between cerebrosides from mycelial and sclerotic cells was apparently not relevant for their antigenicity, since they were both recognized at similar levels by sera from individuals with chromoblastomycosis and a monoclonal antibody to a conserved cerebroside structure. *F. pedrosoi* conidia were treated with the antibody to CMH and the growth of antibody-treated cells was analyzed by counting the number of colony-forming units. Treatment with anti-CMH antibody killed at least 60% of the conidial population. The addition of the anti-CMH antibody to conidial cell cultures of *F. pedrosoi* also resulted in inhibition of fungal growth. Fungal cells treated with a monoclonal anti-cerebroside antibody were more efficiently internalized and killed by phagocytes, showing for the first time that, besides their immediate antifungal action, CMH-binding antibodies can help host cells to eliminate internalized fungi. Sclerotic cells display a unique shape, along with a muriform arrangement within the tissue, which impairs an efficient host cell attack and access of anti-fungal drugs (34). Pre-incubation of fungal cells with the antibody had no effect on the interaction of *F. pedrosoi* sclerotic cells with murine macrophages. In addition, sclerotic bodies were completely resistant to the antifungal action of anti-CMH antibodies. Immunofluorescence analysis showed that recognition of sclerotic cells by these antibodies only occurs at cell wall regions in which melanization is not evident. Accordingly, melanin removal with alkalis results in an increased reaction of fungal cells with anti-CMH antibodies. These results indicate that cerebroside expression in *F. pedrosoi* cells is associated with dimorphism and melanin assembly on the fungal cell wall (78).

To understand how cerebrosides influence the biology of fungal cells, a profound knowledge of structural and biosynthetic aspects of these molecules is still required. The development of chemical or immunological agents with unquestionable selectivity to inhibit CMH synthesis and expression is also necessary to evaluate if cerebrosides are in fact good targets for the treatment of fungal infections.

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RESUMO

Glicocojugados e polissacarídeos de parede celular de fungos e ativação do sistema imune

Glicoproteínas, glicoessingolipídios e polissacarídeos, expostos nas camadas mais externas da parede celular dos fungos, estão envolvidos em diferentes tipos de interações com o ambiente extracellular. Essas moléculas são componentes essenciais desses organismos, contribuindo para a estrutura, integridade, crescimento celular, diferenciação e sinalização. Alguns são compostos imunologicamente ativos com potencial para regular a patogênese e a resposta imune do hospedeiro. Algumas dessas estruturas podem ser especificamente reconhecidas por anticorpos presentes no soro de pacientes, sugerindo uma possível utilização como ferramenta no diagnóstico das infecções fúngicas.

Palavras-chave: Glicoproteínas, polissacarídeos, glicoessingolipídios, sistema imune, fungos

REFERENCES

1. Almeida, I.C.; Neville, D.C.; Treumann, A.; Ferguson, M.A.; Previanto, J.O.; Travassos, L.R. (1996). Structure of the N-linked oligosaccharide of the main diagnostic antigen of the pathogenic fungus *Paracoccidioides brasiliensis*. Glycobiology, 6, 507-515.
2. Almeida, S.R.; Moraes, J.Z.; Camargo, Z.P.; Gesztesi, J.L.; Mariano, M.; Lopes, J.D. (1998). Pattern of immune response to GP43 from *Paracoccidioides brasiliensis* in susceptible and resistant mice is influenced by antigen-presenting cells. Cell. Immunol., 190 (1), 68-76.
3. Bahia, M.C.F.S.; Haido, R.M.T.; Figueiredo, M.H.G.; dos Santos, G.P.L.; Lopes-Bezerra, L.M.L.; Heurn, V.M.; Barreto-Bergter, E. (2003). *Current Microbiol.*, 47, 163-168.
4. Ballio, A.; Casinovi, C.G.; Framondino, M.; Marino, G.; Nota, G.; Santurbano, (1979). A new cerebroside from *Fusicoccum anygdali Del. Biochim Biophys Acta.*, 573 (1), 51-60.
5. Barreto-Bergter, E.; Pinto, M.R.; Rodrigues, M.L. (2004). Structure and biological functions of fungal cerebrosides. *An. Acad. Bras. Cienc.*, 76 (1), 67-84.
6. Barreto-Bergter, E.; Villas Boas, M.H.S. (1996). In: Studies in Natural Products Chemistry (Rachman, A.-u., Ed.) Vol 18, pp. 785-817, Stereoselective Synthesis (part k), Structural Chemistry of Glycolipids from Fungi and Protozoa.
7. Berenguer, J.; Díaz-Mediavilla, J.; Urra, D.; Munoz, P. (1989) Central nervous system infection caused by *Pseudallescheria boydii*: case report and review. *Rev. Infect. Dis.*, 11, 890-896.
8. Bertini, S.; Colombo, A.L.; Takahashi, H.K.; Straus, A.H. (2007). Expression of antibodies directed to *Paracoccidioides brasiliensis* glycosphingolipids during the course of paracoccidioidomycosis treatment. *Clin. Vaccine Immunol.*, 14 (2), 150-156.
9. Bittencourt, V.C.; Figueiredo, R.T.; da Silva, R.B.; Mourao-Sa, D.S.; Fernandez, P.L.; Sassaki, G.L.; Mulloy, B.; Bozza, M.T.; Barreto-Bergter, E. (2006). An alpha-glucan of *Pseudallescheria boydii* is involved in fungal phagocytosis and Toll-like receptor activation. *J. Biol. Chem.*, 281 (32), 22614-23
10. Brown, G.D. (2006). Dectin-1: a signalling non-TLR pattern-recognition receptor. *Nat. Rev. Immunol.*, 6 (1), 33-43.
Hakomori, S. (1990). Bifunctional role of glycosphingolipids. In Cryptococcus neoformans. ASM Press, 71-114.

Chiapello, L.S.; Aoki, M.P.; Rabinstein, H.R.; Masih, D.T. (2003). Apoptosis induction by glucuronoxystromannan of Cryptococcus neoformans. Med. Mycol., 41 (4), 347-352.

Cisalpino, P.S.; Pucci, R.; Yamauchi, L.M.; Cano, M.I.; da Silveira, J.F.; Travassos, L.R. (1996). Cloning, characterization, and epitope expression of the major diagnostic antigen of Paracoccidioides brasiliensis. J. Biol. Chem., 271, 4553-4560.

Coleman, D.C.; Bennett, D.E.; Sullivan, D.J.; Gallagher, P.J.; Henman, M.C.; Stanley, D.B.; Russell, R.J. (1993). Oral Candida in HIV infection and AIDS: new perspectives/new approaches. Crit. Rev. Microbiol., 19 (2), 19-82.

Cutler, J.E. (2001). N-glycosylation of yeast with emphasis on Candida albicans. Med. Mycol., 39, Suppl 1, 75-86.

da Silva, A.F.; Rodrigues, M.L.; Farias, S.E.; Almeida, I.C.; Pinto, M.R.; Barreto-Bergter, E. (2004). Glucosyleramides in Colletotrichum gloeosporioides are involved in the differentiation of conidia into mycelial cells. FEMS Lett., 561 (1-3), 137-143.

Depe, G.S.Jr.; Ureche, G. (1995). Immunobiological activity of recombinant H antigen from Histoplasma capsulatum. Infect. Immun., 63, 3151-3157.

Depe, G.S.Jr.; Woods, J.P. (1999). Histoplasma capsulatum strain variation in both H antigen production and beta-glucosidase activity and occurrence of HAG1 from a telomeric linear plasmid. Infect. Immun., 67, 3312-3316.

Duarte, R.S.; Polycarpo, C.R.; Wait, R.; Hartmann, R.; Bergter, E.B. (1998). Structural characterization of neutral glycosphingolipids from Fusarium species. Biochim Biophys Acta, 1390 (2), 186-196.

East, L.; Isacke, C.M. (2002). The mannose receptor family. Biochim. Biophys. Acta, 1572, 364-386.

Ferreira, K.S.; Almeida, S.R. (2006). Immunization of susceptible mice with gp43-pulsed dendritic cells induce an increase of pulmonary Paracoccidioidomycosis. Immunol. Lett., 103 (2), 121-126.

Fidel, P.L.R.; Sobel, J.D. (1994). The role of cell-mediated immunity in candidiasis. Trends Microbiol., 2, 202-206.

Fodegal, M.; Mickos, H.; Norberg, T. (1986). Isolation of N2' hydroxydecanoyl-1-0-β-D-glucopyranosyl-9-methyl-4,8-D-erythro-sphingadienine from fruiting bodies of two Basidiomycetes fungi. Glycoconjugate J., 3, 233-237.

Fujino, Y.; Ohnishi, M. (1976). Structure of cerebroside in Aspergillus oryzae. Biochim Biophys Acta, 486 (1), 161-171.

Geijtenbeek, T.B.; Toornstra, R.; van Vliet, S.J.; van Duijnhooven, G.C.; Adema, G.J.; van Kooiy, Y.; Figdor, C.G. (2000). Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell, 100, 575-585.

Gersuk, G.M.; Underhill, D.M.; Zhu L.; Mar K.A. (2000). Dectin-1 and TLRs permit macrophages to distinguish between different Aspergillus fumigatus cellular states. J. Immunol., 176, 3717-3724.

Gow, N.A. (1997). Germ tube growth of Candida albicans. Curr. Top. Med. Mycol., 8, 43-55.

Haido, R.M.; Silva, M.H.; Ejzemburk, R.; Leitão, E.A.; Hearn, V.M.; Evans, E.G.; Barreto Bergter, E. (1998). Analysis of peptidogalactomannans from the mycelial surface of Aspergillus fumigatus. Med Mycol., 36 (5), 313-321.

Hakomori, S. (1990). Bifunctional role of glycosphingolipids. Modulators for transmembrane signaling and mediators for cellular interactions. J. Biol. Chem., 265 (31), 18713-18716.

Hakomori, S. (1993). Structure and function of sphingoglycolipids in transmembrane signalling and cell-cell interactions. Biochem. Soc. Trans., 21 (3), 583-595.

Hamza, S.H.; Mercado, P.J.; Skelton, H.G.; Smith, K.J. (2003). An unusual dematiaceous fungal infection of the skin caused by Fonsecaea pedrosoi: a case report and review of the literature. J. Cutan. Pathol., 30, 340-343.

Han, Y.; Kane, B.; Cherniak, R.; Cutler, J.E. (1997). Biochemical characterization of Candida albicans epitopes that can elicit protective and non-protective antibodies. Infect. Immunol., 65, 4100-4107.

Han, Y.; Ulrich, M.A.; Cutler, J.E. (1999). Candida albicans mannann extract-protein conjugates induce a protective immune response against experimental candidiasis. J Infect Dis., 179 (6), 1477-1484.

Han, Y.; Morrison, R.P.; Cutler, J.E. (1998). A vaccine and monoclonal antibodies that enhance mouse resistance to Candida albicans vaginal infection. Infect. Immunol., 66, 5771-5776.

Kasahara, K.; Sanai, Y. (2000). Functional roles of glycosphingolipids in signal transduction via lipid rafts. Glycoconj. J., 17 (3-4), 153-162.

Kauffman, C.A. (2003). Histoplasmosis. In Dismukes W. E.; Pappas, P. G.; Sobel J.D. (ed), Clinical mycology. Oxford University Press, New York, NY. p. 285-298.

Kauffman, C.A. (2007). Histoplasmosis: a Clinical and Laboratory Update. Clin. Microbiol. Rev., 20, 115-132.

Kauffmann, C.A. (2006). Fungal Infections. Proc. Am. Thorac. Soc., 3 (1), 35-40.

Kawai, G.; Ikeda, Y. (1982). Fruiting inducing activity of cerebrosides observed with Schizopyllum commune. Biochim Biophys Acta, 719, 612-618.

Kluts, J.S.; Yoneda, A.; Reilly, M.C.; Bose, I.; Doering, T.L. (2006). Glycocoly transferases and their products: cryptococcal variations on fungal themes. FEBS Yeast Research, 6 (4), 499-512.

Koga, J.; Yamauchi, T.; Shimura, M.; Ogawa, N.; Oshima, K.; Umemura, K.; Kikuchi, M.; Ogasawara, N. (1998). Cerebrosides A and C, sphingolipid elicitors of hypersensitive cell death and phytoalexin accumulation in rice plants. J. Biol. Chem., 273 (48), 31985-31991.

Kohatsu, L.; Hsu, D.K.; Jegalain, A.G.; Liu, F.T.; Baum, L.G. (2006). Galectin-3 induces death of Candida species expressing specific beta-1,2-linked mannans. J. Immunol., 177 (7), 4718-4726.

Koecieklak, J. (1986). A possible biological function of carbohydrate structures which are typical of erythrocytes. Med. Biol., 64 (6), 311-334.

Lagé, J.P. (1999). Aspergillus fumigatus and aspergillosis. Clin Microbiol Rev., 12 (2), 310-350.

Lagé, J.P.; Kobayashi; H.; Debeauvais, J.P.; Diaquin, M.; Sarafati, J.; Wieruszeski, J.M.; Parra, E.; Bouchard, J.P.; Fournet, B. (1994). Chemical and immunological characterization of the extracellular galactomannan of Aspergillus fumigatus. Infect. Immun., 62, 5424-5433.

Leitao, E.A.; Bittencourt, V.C.; Haido, R.M.; Valente, A.P.; Peter-Katalinic, J.; Letzel, M.; de Souza, L.M.; Barreto-Bergter, E. (2003). Beta-galactofuranose-containing O-linked oligosaccharides present in the cell wall peptidogalactomannan of Aspergillus fumigatus contain immunodominant epitopes. Glycobiology, 13 (10), 681-692.

Lester, R.L.; Dickson, R.C. (1993). Sphingolipids with inositolphosphate-containing head groups. Adv. Lipid. Res., 26, 253-274.

Lester, R.L.; Smith, S.W.; Wells, G.B.; Rees, D.C.; Angus, W. (1974). The isolation and partial characterization of two novel sphingolipids from Neurospora crassa: diinositolphosphoryl ceramide and (gal3)glucosyryamide. J. Biol. Chem., 249 (11), 3385-3394.

Levery, S.B. (2005). Glycosphingolipid structural analysis and glycosphingolipidomics. Methods Enzymol., 405, 300-369.
53. Levery, S.B.; Momany, M.; Lindsey, R.; Toledo, M.S.; Shayman, J.A.; Fuller, M.; Brooks, K.; Doong, R.L.; Strauss, A.H.; Takahashi, H.K. (2002). Disruption of the glycosylceramide biosynthetic pathway in Aspergillus nidulans and Aspergillus fumigatus by inhibitors of UDP-GLC:glycosyltransferase strongly affects spore germination, cell cycle, and hyphal growth. FEBS Lett., 525 (1-3), 59-64.

54. Levery, S.B.; Toledo, M.S.; Doong, R.L.; Strauss, A.H.; Takahashi, H.K. (2000). Comparative analysis of ceramide structural modification found in fungal cerebrosides by electrospray tandem mass spectrometry with low energy collision-induced dissociation of Li+ adduct ions. Rapid Commun Mass Spectrom., 14 (7): 511-563.

55. Levitz, S.M.; Specht, C.A. (2006). The molecular basis for the immunogenicity of Cryptococcus neoformans mannoproteins. FEMS Yeast Res., 6, 513-524.

56. Lhomme, O.; Bruneteau, M.; Costello, C.E.; Mas, P.; Molot, P.M.; Dell, A.; Tillier, P.R.; Michel, G. (1990). Structural investigations and biological activity of inositol sphinchoospholipids from Phytophthora capsici. Eur. J. Biochem., 191 (1), 203-209.

57. López-Ribot, J.L.; Casanova, M.; Murgui, A.; Martínez, J.P. (2004). Antibody response to Candida albicans cell wall antigens. FEMS Immunol Med Microbiol., 41 (3), 187-196.

58. Luther, K.; Torosantucci, A.; Brakhage, A.A.; Heesemann, J.; Ebel, F. (2007). Phagocytosis of Aspergillus fumigatus conidia by murine macrophages involves recognition by the dectin-1 beta-glucan receptor and Toll-like receptor 2. Cell. Microbiol., 9 (2): 368-381.

59. Maciel, D.M.; Rodrigues, M.L.; Wait, R.; Villas Boas, M.H.; Tischer, C.A.; Barreto-Berger, E. (2002). Glycosphingolipids from Magnaporthe grisea cells: expression of a ceramide dihexoside presenting phytosphingosine as the long-chain base. Arch. Biochem. Biophys., 405 (2), 205-213.

60. Mansour, M.K.; Latz, E.; Levitz, S.M. (2006). Cryptococcus neoformans glycoconjugates are captured by multiple lectin receptors and presented by dendritic cells. J. Immunol., 168 (6), 2872-2879.

61. Mansour, M.K.; Vach, L.E.; Rottman, J.B.; Stuart M.; Levitz, S.M. (2004). Protective efficacy of antigenic fractions in mouse models of cryptococcosis. Infect. Immun., 72 (3), 1746-1754.

62. Marques, A.F.; da Silva, M.B.; Juliano, M.A.P.; Travassos, L.R.; Taborda, C.P. (2006). Peptide immunization as an adjuvant to immunogenicity of C. albicans. J. Immunol. Med Microbiol., 41 (3), 187-196.

63. Matsubara, T.; Hayashi, A.; Banno, Y.; Morita, T.; Nozawa, Y. (1993). Comparative analysis of the glucose-ceramide and the glucose-glyceride linkages chains of glycolipids at the membrane surface: conformational structural difference in the ceramide moiety. FEBS Lett., 525, 2814-2819.

64. Mattos Grosso, D.; Almeida, S.R.; Mariano M.; Lopes, J.D. (2003). Comparative analysis of 65-kilodalton mannoprotein, a major antigenicity, and biological functions of Cryptococcus neoformans capsular polysaccharide component galactoxylomannan induces expression of Fas ligand in Macrophages. J. Immunol., 174, 3461-3468.

65. Monari, C.; Netzi, D.; Bistoni, F.; Kozel, T.R.; Vecchiarelli, A. (2003). Differences in outcome of the interaction between Cryptococcus neoformans glucuronoxylomannan and human monocytes and neutrophils. Eur. J. Immunol., 33 (4), 1041-1051.

66. Moragues, M.D.; Omae, S.R.; Elguezabal, N.; Sevilla, M.J.; Conti, S.; Polonelli, L.; Ponton, J. (2003). A monoclonal antibody directed against a Candida albicans cell wall mannoprotein exerts three anti-C. albicans activities. Infect Immun., 71 (9), 5273-5279.

67. Mullin, N.P.; Hitchen, P.G.; Taylor, M.E. (1997). Mechanism of Ca2+ and monosaccharide binding to a C-type carbohydrate-recognition domain of the macrophage mannose receptor. J. Biol. Chem., 272, 5668-5671.

68. Nakakuma, H.; Arau, M.; Kawai, T.; Hida, M.; Sakamoto, K.; Iwamori, M.; Sakai, Y.; Takatsuki, K. (1996). Monoclonal antibody to galactosylceramide: discrimination of structural difference in the ceramide moiety. FEBS Lett., 258, 230-232.

69. Negroni, R. (1993). Paracoccidioidiomycosis (South American blastomycosis, Lutz’s mycosis). Int. J. Dermatol., 32, 847-859.

70. Nisini, R.; Romagnoli, G.; Gomez, M.J.; La Valle, R.; T.R.; Vecchiarelli. A. (2006). The molecular basis for the immune sensing of Cryptococcus neoformans glucuronoxylomannan. J. Biol. Chem., 281, 1740-1748.

71. Nisini, R.; Romagnoli, G.; Gomez, M.J.; La Valle, R.; T.R.; Vecchiarelli. A. (2006). Role of mannoprotein in function of dendritic cells. Microbes Infect., 7 (4), 789-798.

72. Nisini, R.; Romagnoli, G.; Gomez, M.J.; La Valle, R.; T.R.; Vecchiarelli. A. (2006). The multitude of targets for the immune system and drug therapy in the fungal cell wall. Microbes Infect., 7 (4), 789-798.

73. Nyholm, P.G.; Pascher, I. (1993). Antibody response to Candida albicans cell wall antigens. FEMS Immunol Med Microbiol., 41 (3), 187-196.

74. Nyholm, P.G.; Pascher, I. (1993). Orientation of the saccharide recognition domain of the macrophage mannose receptor. J. Cell. Biol., 174, 3769-3774.

75. Palma, A.S.; Feizi, T.; Zhang, Y.; Stoll, M.S.; Lawson, A.M.; Diaz-Rodriguez, E.; Campanero-Rhodes, M.A.; Costa, J.; Gordon, S.; Brown, G.D. (2006). J. Biol. Chem., 281, 5771-5779.

76. Pericolini, E.; Cenci, E.; Monari, C.; De Jesus, M.; Bistoni, F.; Casadevall, A.; Vecchiarelli, A. (2006). Cryptococcus neoformans capsular polysaccharide component galactoxylomannan induces apoptosis of human T-cells through activation of caspase-8. Cell. Microbiol., 8 (2), 267-275.

77. Pietrella, D.; Bistoni, G.; Corbucci, B.; Perito, S.; Vecchiarelli, A. (2006). Candida albicans mannoprotein influences the biological function of dendritic cells. Cell. Microbiol., 8 (4), 602-612.

78. Pietrella, D.; Bistoni, G.; Corbucci, B.; Perito, S.; Vecchiarelli, A. (2006). Candida albicans mannoprotein influences the biological function of dendritic cells. Cell. Microbiol., 8 (4), 602-612.

79. Pinto, M.R.; Momany, M.; Lindsey, R.; Toledo, M.S.; Shayman, J.A.; Fuller, M.; Brooks, K.; Doong, R.L.; Strauss, A.H.; Takahashi, H.K. (2002). Disruption of the glycosylceramide biosynthetic pathway in Aspergillus nidulans and Aspergillus fumigatus by inhibitors of UDP-GLC:glycosyltransferase strongly affects spore germination, cell cycle, and hyphal growth. FEBS Lett., 525 (1-3), 59-64.

80. Pinto, M.R. (2006). Paracoccidioides brasiliensis Characterization of gp70 and anti-gp70 monoclonal antibodies in Candida albicans. Chem. Phys. Lipids, 139 (1), 1-12.

81. Pinto, M.R. (2006). Paracoccidioides brasiliensis Characterization of gp70 and anti-gp70 monoclonal antibodies in Candida albicans. Chem. Phys. Lipids, 139 (1), 1-12.

82. Pinto, M.R. (2006). Paracoccidioides brasiliensis Characterization of gp70 and anti-gp70 monoclonal antibodies in Candida albicans. Chem. Phys. Lipids, 139 (1), 1-12.
97. Retini, C.; Kozel, T.R.; Pietrella, D.; Monari, C.; Bistoni, F.; Barreto-Bergter, E. (2004). Involvement of peptidorrhpanomannan in the interaction of Pseudallescheria boydii and HEp2 cells. Microbes Infect., 6 (4), 1259-1267.

98. Pinto, M.R.; Mulloy, B.; Haido, R.M.T.; Travassos, L.R.; Bergter, E.B. (2004). RNA interference function of monocytes in response to stimulation with interleukin-12 in regulation of T-cell differentiation and effector Vecchiareli. A. (2001). Interdependency of interleukin-10 and Vecchiarelli, A. (1999). Specific activated T cells regulate IL-12 production by human monocytes stimulated with Cryptococcus neoformans. J. Immunol., 162 (3), 1618-1623.

99. Qi, J.; Ojika, M.; Sakagami, Y. (2001). Neuritogenic cerebrosides from an edible Chinese mushroom. Part 2: Structures of two additional termitomycesphins and activity enhancement of an inactive cerebroside by hydroxylation. Bioorg. Med. Chem., 9 (8), 2171-2177.

100. Rappleye, C.A.; Eisenberg, L.G.; Goldman W.E. (2007). Histoplasma capsulatum α-(1,3)-glucan blocks innate immune recognition by the β-glucan receptor. PNAS, 104, 1366-1370.

101. Rodrigues, M.L.; Travassos, L.R.; Miranda, K.R.; Franzen, A.J.; Barreto-Bergter, E. (2002). Characterization of gluco cerebrosides in Pseudallescheria boydii and their involvement in fungal differentiation. Glycobiology, 12 (4), 251-260.

102. Popi, A.F.; Lopes J.D.; Mariano, M. (2002). GP43 from Pseudallescheria boydii inhibits the antigen-presenting capacity of monocytes. J. Immunol., 66 (2), 786-793.

103. Puccia, R.; Schenkman, S.; Gorin, P.A.; Travassos, L.R. (1986). Mapping of the T-cell epitope in the major 43-kDa antigen of Pseudallescheria boydii in yeast and its relation to alkali tolerance of P. boydii. Med. Mycol., 3rd edn. W. B. Saunders, Philadelphia, pp 651-680.

104. Sakaki, T.; Zähringer, U.; Warnecke, D.C.; Fahl, A.; Knogge, W.; Heinz, E. (2001). Sterol glycosides and cerebrosides accumulate in Pichia pastoris, Rhynchosporium secalis and other fungi under normal conditions or under heat shock and ethanol stress. Yeast, 18 (8), 679-695.

105. San-Blas, G.; Calderone, A. (2004). Morphogenesis of agents of endemic mycoses. In: Pathogenic Fungi: Structural Biology and Taxonomy., eds. Caister Academic Press. Wymondham, Norfolk, UK, p. 167-220.

106. Sakati, J.; Boucias, D.G.; Latgé, J.P. (1995). Antigens of Aspergillus fumigatus produced in vivo. J. Med. Vet. Mycol., 33 (1), 9-14.

107. Serrano-Gomez, D.; Dominguez-Soto, A.; Ancococha, J.; Jimenez-Hefferman, J.A.; Leal, J.A.; Corbi, A.L. (2004). Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin mediates binding and internalization of Aspergillus fumigatus conidia by dendritic cells and macrophages. J. Immunol., 173 (9), 5653-5643.

108. Sevilla, M.J.; Robledo, B.; Rementeria, A.; Moragues, M.D.; Ponton, J. (2006). A fungalicidal monoclonal antibody protects against murine invasive candidiasis. Infect. Immun., 74 (5), 3042-3045.

109. Stahl, P.D.; Ezekowitz, R.A. (1998). The mannose receptor is a pattern recognition receptor involved in host defense. Curr. Opin. Immunol., 10, 50-55.

110. Styven, D.; Sarafit, J.; Goris, A.; Prévost, M.C.; Lesourd, M.; Kamphuis, H.; Darras., V., Latgé, J.P. (1992). Rat monoclonal antibodies against Aspergillus galactomannan. Infect. Immun., 60, 2237-2245.

111. Syme, R.M.; Bruto, T.F.; Kozel, T.R.; Mody, C.H. (1999). The capsule of Cryptococcus neoformans reduces T-lymphocyte proliferation by reducing phagocytosis, which can be restored with anticapsular antibody. Infect. Immun., 67 (9), 4620-4627.

112. Taborda, C.P.; Casadevall, A. (2002). CR3 (CD11c/CD18) and HEp2 cells. Pseudallescheria boydii are involved in complement-independent antibody-mediated phagocytosis of Cryptococcus neoformans. Immunity, 16 (6), 791-802.

113. Taborda, C.P.; Juliano, M.A.; Puccia, R.; Franco, M.; Travassos, L.R. (1998). Mapping of the T-cell epitope in the major 43-kilodalton glycoprotein of Paracoccidioides brasiliensis which induces a Th-1 response protective against fungal infection in BALB/c mice. Infect. Immun., 66 (2), 786-793.

114. Takahashi, H.K., Levery, S.B.; Toledo, M.S.; Suzuki, E.; Salay, M.E.; Hakomori, S.; Straus, A.H. (1996). Isolation and possible composition of glucosylceramides from Paracoccidioides brasiliensis. Braz. J. Med. Biol. Res., 29 (11), 1441-1444.

115. Takakuwa, N.; Kinoshita, M.; Oda, Y.; Ohnishi, M. (2002). Existence of 2-hydroxy fatty acyl (E)-Δ3-unsaturation in the interaction of Paracoccidioides brasiliensis with Cryptococcus neoformans. J. Immunol., 69, 6064-6073.

116. Takakuwa, N.; Kinoshita, M.; Oda, Y.; Ohnishi, M. (2002). Existence of cerebrosides in Saccharomyces kluverii and its related species. FEMS Yeast Res., 2 (4), 533-538.

117. Toledo, M.S.; Levery, S.B.; Strauss, A.H.; Suzuki, E.; Momany, M.; Glushka, J.; Moulton, J.M.; Takahashi, H.K. (1999). Characterization of sphingolipids from mycopathogens: factors correlating with expression of 2-hydroxy fatty acyl (E)-Δ3-unsaturation in cerebrosides of Paracoccidioides brasiliensis and Aspergillus fumigatus. Biochemistry, 38 (22), 7294-7306.

118. Toledo, M.S.; Levery, S.B.; Strauss, A.H.; Takahashi, H.K. (2000). Dimorphic expression of cerebrosides in the mycopathogen Sporothrix schenckii. J. Lipid Res., 41 (5), 797-806.

119. Toledo, M.S.; Levery, S.B.; Strauss, A.H.; Suzuki, E.; Momany, M.; Glushka, J.; Moulton, J.M.; Takahashi, H.K. (2001). Characterization of cerebrosides from the thermally dimorphic mycopathogen Histoplasma capsulatum: expression of 2-hydroxy fatty N-acyl (E)-Δ3-unsaturation correlates with the yeast-mycelium phase transition. Glycobiology, 11 (2), 113-124.

120. Travassos, L.R.; Casadevall, A.; Taborda, C.P. (2004). Immunomodulation and immunoprotection in fungal infections: humoral and cellular immune responses. In: Pathogenic fungi: Host interactions and emerging strategies for control. San-Blas, G. and Calderone, R.A. pp 241-283.

Fungal cell wall and activation of immune system
120. Travassos, L.R.; Puccia, R.; Cisalpino, P.; Taborda, C.; Rodrigues, E.G.; Rodrigues, M.; Silveira, J.F.; Almeida, I.C. (1995). Biochemistry and molecular biology of the main diagnostic antigen of *Paracoccidioides brasiliensis*. *Arch Med Res.* Autumn., 26 (3), 297-304.

121. Travassos, L.R.; Taborda, C.P.; Iwai, L.K.; Cunha Neto, E.; Puccia, R. (2004). The gp43 from *Paracoccidioides brasiliensis*: A major diagnostic antigen and vaccine candidate. In: The Mycota XII, Human Fungal Pathogens. J.E. Domer and G.S. Kobayashi, eds. Springer-Verlag Berlin Heidelberg. P. 279-296.

122. Umemura, K.; Ogawa, N.; Yamauchi, T.; Iwata, M.; Shimura, M.; Koga, J. (2000). Cerebroside elicitors found in diverse phytopathogens activate defense responses in rice plants. *Plant Cell Physiol.*, 41 (6), 676-683.

123. Vecchiarelli, A. 2000. Immunoregulation by capsular components of *Cryptococcus neoformans*. *Med. Mycol.*, 38: 407-417.

124. Vecchiarelli, A.; Pietrella D.; Lupo P.; Bistoni, F.; McFadden D.C.; Casadevall, A. (2003). The polysaccharide capsule of *Cryptococcus neoformans* interferes with human dendritic cell maturation and activation. *J. Leukocyte Biol.*, 74, 370-378.

125. Vecchiarelli, A.; Retini, C.; Pietrella, D.; Monari, C.; Tascini, C.; Beccari, T.; Kozel. T. R. (1995). Down-regulation by cryptococcal polysaccharide of tumor necrosis factor a and interleukin-1 b secretion from human monocytes is recognized by a monoclonal antibody raised against *Staphylococcus aureus* laminin receptor. *J. Med. Vet. Mycol.*, 35, 37-43

127. Vigna, A.F.; Almeida, S.R.; Xander, P.; Freymüller, E.; Mariano, M.; Lopes, J.D. (2002). Characterization of B-1b cells as antigen presenting cells in the immune response to gp43 from *Paracoccidioides brasiliensis* in vitro. *Immunol Lett.*, 83 (1), 61-66.

128. Vigna, A.F.; Godoy, L.C.; Almeida, S.R.; Mariano, M.; Lopes; J.D. (2003). Characterization of B-1b cells as antigen presenting cells in the immune response to gp43 from *Paracoccidioides brasiliensis* in vitro. *Immunol Lett.*, 232 (2), 133-138.

129. Viudes, A.; Lazzell, A.; Perea, S.; Kirkpatrick, W.R.; Peman, J.; Patterson, T.F.; Martinez, J.P.; Lopez-Ribot, J.L. (2004). The C-terminal antibody binding domain of *Candida albicans* mp58 represents a protective epitope during candidiasis. *FEMS Microbiol. Lett.*, 26 (3-4), 309-318.

130. Weiss, B.; Stiller, R.L.; Jack, R.C. (1973). Sphingolipids of the fungi *Phycomycetes blakesleeanus* and *Fusarium lini*. *Lipids.*, 8 (1), 25-30.

131. Wells, G.B.; Lester, R.L. (1983). The isolation and characterization of a mutant strain of *Saccharomyces cerevisiae* that requires a long chain base for growth and for synthesis of phosphosphingolipids. *J. Biol. Chem.*, 258 (17), 10200-10203.

132. Young, W.W.; Durdik, J.M.; Urdal, D.; Hakomori, S.; Henney, C.S. (1981) Glycolipid expression in lymphoma cell variants: chemical quantity, immunologic reactivity, and correlations with susceptibility to NK cells. *J. Immunol.*, 126, 1-6.