The host response to fungi is in part dependent on activation of evolutionarily conserved receptors including toll-like receptors (TLR) and phagocytic receptors. However, the molecular nature of fungal ligands responsible for this activation is largely unknown. Herein, we describe the isolation and structural characterization of an α-glucan from *Pseudallescheria boydii* cell wall, and evaluate its role in the induction of innate immune response. These analyses indicate that α-glucan of *P. boydii* is a glycogen-like polysaccharide consisting of linear 4-linked α-D-Glcp residues substituted at position 6 with α-D-Glcp branches. Soluble α-glucan, but not a β-glucan, led to a dose dependent inhibition of conidia phagocytosis. Furthermore, a significant decrease in the phagocytic index occurred when α-glucan from conidial surface was removed by enzymatic treatment with α-amylglucosidase, thus indicating an essential role of α-glucan in *P. boydii* internalization by macrophages. α-Glucan stimulates the secretion of inflammatory cytokines by macrophages and dendritic cells; again this effect is abolished by treatment with α-amylglucosidase. Finally, α-glucan induces cytokine secretion by cells of the innate immune system in a mechanism involving TLR2, CD14 and MyD88. These results might have relevance in the context of infections with *P. boydii* and other fungi, and α-glucan could be a target for intervention during fungal infections.

*Pseudallescheria boydii* is a saprophytic fungus widespread in soil and polluted water and has recently emerged as an agent of localized as well as disseminated infections in both immunocompromised and immunocompetent hosts. Clinical observations and data obtained from experimental models indicate the essential role of innate immunity in resistance against infections caused by pathogenic fungi. Macrophages provide a major line of defense against fungal cells by ingesting and killing conidia by oxidative and non-oxidative processes (1-4). Innate immunity recognition causes the release of proinflammatory mediators that induce recruitment of polymorphonuclear leukocytes (5). Failure of fungicidal activity of macrophages and neutrophils permits germination of conidia in hyphae with subsequent tissue invasion (6).

Innate immunity performs pathogen surveillance by detection of pathogen associated components through germline encoded receptors that are expressed in resident leukocytes. Mammalian toll-like receptors (TLRs) are a family of closely related transmembrane proteins, first
α-glucan of fungi activates the innate immune system

identified as homologues of the Toll receptor in *Drosophila* (7,8). TLRs mediate the recognition of a large array of molecules present in pathogens, triggering the production of proinflammatory cytokines, activation of microbicidal mechanisms and the induction of adaptive immunity (9-11). TLR activation initiates a signaling cascade through a conserved pathway shared by IL-R1 and IL-18R that requires adaptor proteins such as MyD88 leading to NFκB activation and the induction of different proinflammatory genes (12). A great variety of pathogen molecules have been described to signal through TLRs, especially in case of TLR2 and TLR4, the best-characterized TLRs. TLR2 recognizes lipoteichoic acid, bacterial lipopeptides, mycobacterial lipoarabinomannans and GPI anchors of protozoan parasites (13-16). TLR4 recognizes bacterial LPS and cytolysins of Gram-positive bacteria (17-19).

Recent studies have demonstrated the involvement of TLRs in the recognition of fungal pathogens such as *Aspergillus fumigatus*, and *Candida albicans*. *A. fumigatus* induces cytokine release as well NFκB activation through TLR2 and TLR4 activation (20,21). Genetic deficiency of TLR4 makes mice more susceptible to an experimental *A. fumigatus* infection following immunosuppression, and TLR2 and TLR4 deficient mice present an increased fungal load in the lungs upon *A. fumigatus* intranasal challenge (22,23). However, the nature of the pathogen-associated molecular patterns expressed by *A. fumigatus* that trigger the synthesis of TNF-α, IL-12 and MIP-2 remains to be established. Resistance to experimental infection with *C. albicans* requires TLR2 and TLR4 as observed by the increased fungal load on the kidneys of TLR4 mutant mice, C3H/HeJ, and the higher susceptibility of TLR2/- mice to *C. albicans* infection (24,25). In addition, *C. albicans* induces cytokine release through TLR2 and TLR4 (24,25). Although the results demonstrate that TLR2 and TLR4 participate in the recognition of pathogenic fungi, the molecules that trigger the activation of the TLR-associated signaling pathway leading to the induction of the innate immune response are largely unknown.

In the present study we describe the structural characterization of an α-glucan, a glycogen-like polysaccharide extracted from *P. boydii* cell wall, and evaluate its role in the induction of innate immune response. The α-glucan from *P. boydii* is essential to conidial phagocytosis by macrophages and induces cytokine secretion by cells of the innate immune system in a mechanism involving TLR2, CD14 and MyD88.

**Experimental Procedures**

*Mice* - C57/BL6, Balb/c and C57BL/10 mice were obtained from the Fundaçao Oswaldo Cruz Breeding Unit (Rio de Janeiro, Brazil). C57BL/10ScN mice were obtained from the Universidade Federal do Rio de Janeiro and C57BL/10ScCr mice were obtained from the Universidade Federal Fluminense (Rio de Janeiro, Brazil). MyD88, TLR2 and CD14 knockout mice (on a C57BL/6 background) were kindly provided by Dr. Akira from the Research Institute for Microbial Diseases, Osaka University, Osaka, Japan, and Dr. Douglas Golenbock from the University of Massachusetts, Worcester, MA, USA.

*Reagents* - LPS (O111:B4), zymosan and thioglycolate were purchased from Sigma (St Louis, MO), Pam3Cys-Ser-(Lys)4 (Pam3Cys) was obtained from EMC Microcollections (Tübingen, Germany). Laminarin from *Laminaria digitata* was kindly supplied by Prof. Michael Noseda from the Departamento de Bioquímica, Universidade Federal do Paraná, Brazil. Polymixin B was obtained from Bedford Laboratories (Bedford, OH). Detoxifying polymixin column was purchased from Cambrex. RPMI medium for macrophage culture was obtained from Sigma and was supplemented with Penicillin and Streptomycin (100 UI/ml and 100 µg/ml, respectively) obtained from Gibco.

*Microorganism and growth conditions* - *P. boydii*, isolated from eumycotic mycetoma, was kindly supplied by Bodo Wanke from Evandro Chagas Hospital, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil. Cells were grown on Sabouraud solid slants, inoculated in liquid culture medium, and incubated for 7 days at 25 °C with shaking. Cultures were then transferred to the same medium and incubated for 7 days at the same temperature with shaking; the mycelium was filtered, washed with distilled water, and stored at -20 °C. For phagocytic assays, conidia were grown on Petri plates containing modified Sabouraud medium at 25 °C. After 7 days in culture, conidia cells were obtained by washing the plate surface with
phosphate-buffered saline (PBS) and filtered through gauze to remove hyphae fragments and debris. Conidial suspensions were heat-killed by autoclaving the preparation at 120 °C, for 15 minutes, washed and counted.

**Extraction and fractionation of P. boydii glucan -** Hyphae of *P. boydii* were extracted with 2% KOH for 2h at 100 °C. The alkali extract was neutralized with glacial acetic acid and precipitated with 3 volumes of ethanol. The resulting precipitate was recovered by centrifugation, dialyzed against distilled water and lyophilized. The crude polysaccharide was fractionated by gel filtration chromatography on a Superdex S-200 column (30 x 1.0 cm) coupled to an AKTA Purifier liquid chromatography (Pharmacia-Amersham, Uppsala, Sweden) using phosphate buffer 0.01M, 0.15M NaCl, pH 7.0 as eluent pumped at 0.5ml/min for 60 min. The elution profiles of the gel filtration chromatography were monitored by refractive index detection and the collected fractions were assessed for their carbohydrate content.

**Sugar analysis** - Total carbohydrate was determined by the phenol-sulphuric acid method (26) and protein by the Folin phenol reagent method (27). Glucan (1 mg) was hydrolyzed with 2M Trifluoracetic acid (TFA) at 100 °C for 3 h, the solution evaporated to dryness. The resulting monosaccharides were characterized by HPTLC and quantified by GC as alditol acetate derivatives (28) using a capillary column of OV-225 (30 m x 0.25 mm i.d.), with temperatures programmed from 50 to 220 °C at 50 °C/min.

**Methylation analysis** - Methylation analysis was carried out by the method of Tischer et al., (29) using a modification of the method of Ciucanu & Kerek (30). Glucan (1 mg) was dissolved in a drop of H2O, which was diluted with Me2SO (~ 1 ml) and then MeI (1 ml). Powdered NaOH (0.3 g) was added and the mixture agitated vigorously with a vortex for 30 min and then left overnight. After neutralization with HOAc the product was then extracted with CHCl3 and washed three times with H2O. On evaporation, the resulting per-O-methylated product was converted into partially O-methylated alditol acetates by successive treatments with 3% MeOH-HCl for 2 h at 70 °C, 0.5 M H2SO4 for 14 h at 100 °C, reduction with NaBD4, and acetylation with Ac2O-pyridine. The products were analyzed by GC-MS on a capillary column of DB-225 (31), programmed from 50 °C (1 min) at 40 °C/min to 210 °C (constant temperature).

**Treatment with yeast alpha-amylloglucosidase** - Glucan (1 mg) was incubated with 1.5 mg of yeast α-amylloglucosidase in 0.05 ml of 0.05M sodium acetate buffer (pH 4.8) at 37 °C for 22 h. At the end of the incubation period, the mixture was heated at 100 °C for 5 min to inactivate the enzyme, centrifuge and the supernatant concentrated and applied to a TLC plate. This was eluted with n-BuOH- MeC2O- H2O (4:5:1 v/v) and developed with 0.05 % (w/v) orcinol in 10% H2SO4 at 100 °C for 10 min, using 10μg of glucose as standard.

**Endotoxin removal** - Glucan (2 mg) was dissolved in 1 ml of pyrogenic saline and then applied to an immobilized polymixin B gel column (Detoxi-Gel™ Endotoxin removing gel, Pierce), according to the manufacture’s instructions.

**NMR spectroscopy** - NMR spectra at 500 MHz (°H) and 125 MHz (°C) were recorded, using a Varian INOVA spectrometer, for a sample (10 mg) of the glucan in D2O, at 60 °C. Chemical shifts were relative to internal trimethylsilylpropionic acid-d4 sodium salt (TSP) at 0 ppm (°H) and external trimethylsilane (TMS) at 0 ppm (°C). Two-dimensional spectra (COSY, TOCSY, HSQC) were performed using the pulse sequences supplied by the instrument manufacturer.

**Phagocytic Assay** - Elicited peritoneal macrophages were obtained by the i.p. instillation of 2 ml of 3% sterile thioglycollate. After 4 days, mice were sacrificed and the peritoneal macrophages were harvested and washed with chilled HBSS and plated. Elicited macrophages (2 x 10⁵cells/ml) were cultured over round glass cover slips (13 mm) in 24-well flat bottom microtest plates. Adherent monolayers were challenged with 500μl of suspensions of heat-killed conidia containing 10⁶ cells/ml. After incubation at 37 °C in 5% of CO₂ for 60 minutes in RPMI-1640 medium, the cells were rinsed with HBSS for removal of non-internalized conidia. The preparations were fixed in Bouins fixative and stained with Giemsa. The influence of α-glucan on conidia phagocytosis was evaluated by adding different concentrations of the polysaccharide (25, 50 and 100μg/ml) and of the polysaccharide (100μg/ml) after digestion with α-amylloglucosidase to the cultures simultaneously.
with the addition of conidia. The influence of a different purified glucans in the phagocytosis of conidia was also tested by adding 100μg/ml of selected glucans to the cultures. To determine the phagocytic indexes (PI), 200 cells were counted and the percent of cells that ingested at least one particle was multiplied by the mean number of internalized particles (32).

**Phagocytosis of zymosan particles** - Macrophage monolayers were challenged with 500μl of suspensions of zymosan particles (10⁶ particles/ml). After incubation at 37 °C in 5% of CO₂ for 60 minutes in RPMI-1640 medium, the cells were rinsed with HBSS for removal of non-internalized particles. The preparations were fixed in Bouins fixative and stained with Giemsa. The influence of α-glucan and laminarin on the phagocytosis of zymosan was carried out by adding 100μg/ml of each glucan, simultaneously to zymosan particles.

**Macrophage culture and stimulation** - Elicited peritoneal macrophages were obtained by the i.p. instillation of 2 ml of 3% sterile thioglycollate. After 4 days, mice were sacrificed and the peritoneal macrophages were harvested and washed with chilled HBSS and plated at a density of 2x10⁵ cells/well, in a 96-well plate. The plate was incubated for 2h at 37 °C in 5% of CO₂. Non-adherent cells were removed by washing with HBSS. Adherent cells were stimulated for 4h, in RPMI medium, with the α-glucan, LPS or Pam3Cys, at concentrations indicated in the Figure legends. After this period the supernatant was recovered for TNF determination by ELISA according to the manufacturer’s instructions. Polymixin B (1 or 10 μg/ml) was added 5 minutes before the stimulation with α-glucan, to rule out the possibility that the stimulating activity was due to contaminating lipopolysaccharides.

**Generation and stimulation of murine bone marrow-derived dendritic cell** - Dendritic cells were generated as previously described (33), with some modifications. Briefly, bone marrow was harvested from the tibia and femur of C57/B6 or TLR2 -/- mice. The cells were resuspended at 10⁶ per milliliter in RPMI 1640 (Sigma) supplemented with vitamins, amino acids, 50μM of 2-mercaptoethanol, rmGM-CSF and rmIL-4 at 10ng/mL. After 5 days, fresh medium was added to culture and with 7 days of culture, the cells were collected and BMDCs were separated by Optiprep gradient (Sigma) by centrifugation at 600g for 30min at 24 °C. This protocol generated more than 75% of CD11c+ cells. BMDCs were plated in 96-well at density of 2x10⁵/well and incubated for 16h with the stimuli, after which the supernatant was recovered for the determination of cytokines by ELISA.

**Statistical analysis** - Statistical analysis was performed using the statistical software SPSS for Windows (Version 10.0.1, SPSS Inc., 1989-1999, USA). Statistical differences among the experimental groups were evaluated by analysis of variance with Newman-Keuls correction or with the t-test. Values are expressed as the mean ± s.e.m. The level of significance was set at p<0.05.

**RESULTS**

**Isolation and characterization of the glucan from P. boydii** - Mycelia of P. boydii were extracted with hot 2% aqueous potassium hydroxide at 100 °C followed by neutralization with acetic acid and precipitation with ethanol. The crude precipitate was applied to a Superdex 200 column, which was eluted with a discontinuous gradient of aqueous NaCl. Carbohydrate-containing fractions F-1, F-2 and F-3 were obtained. Hydrolysis of F-1 followed by TLC examination of the products showed only glucose, confirmed by GC-MS analysis of derived alditol acetates. These results indicate that the F1 fraction contains a glucan. Methylation-GC-MS analysis of the glucan gave rise to partially O-methylated alditol acetates, which corresponded to non-reducing end units of Glc (13%), 4-O-(64%), and 4,6-di-O-substituted Glc units (24%) (Table 1). The P. boydii glucan yields 2,3-di-O-methyl glucose, which is characteristic of branch points with glucose units in (1→6) linkage, and a high proportion of 2,3,6-tri-O-methylglucose, indicating the presence of linear portions of (1→4)-linked glucopyranosyl units.

The ¹H NMR spectrum of the purified glucan (Fig. 1A) was very similar to that of glycogen from A. fumigatus, Mycobacterium bovis, and rabbit liver (34-37). An envelope of two α-anomeric signals was seen at 5.39 ppm with a smaller α-anomeric signal at 4.98 ppm. The signals between 3.9 and 3.4 ppm were assigned to ring proton resonances (H-2 to H-6) by means of two-dimensional COSY (not shown) and TOCSY spectra (Fig. 1B). The three residue types to which
signals were assigned were designated A (\(\rightarrow\)4-\(\alpha\)Glcp-(1\(\rightarrow\)4)-), B (terminal \(\alpha\) Glcp-(1\(\rightarrow\)4)-) and C (\(\rightarrow\)4-\(\alpha\)Glcp-(1\(\rightarrow\)6)-) by comparison with earlier studies (36,37). Table 2A lists \(^1\)H chemical shifts in comparison with published values.

The \(^{13}\)C NMR spectrum of the \(\alpha\)-glucan of \(P.\) boydii was partially assigned by means of a heteronuclear correlated spectrum (not shown) and is compared with corresponding published values in Table 2B. A DEPT-135 spectrum confirmed the assignment of the resonance at 61.3 ppm in the \(^{13}\)C spectrum to C-6, as this methylene carbon signal was inverted with respect to the methine ring carbon signals (Fig. 2A). A small, broad \(^{13}\)C resonance at about 69 ppm was also inverted, and may be assigned to C-6 of 6-linked glucose, though no other signals from the branch point, 4,6-linked residues could be identified. Assignments of resonances from 4)-\(\alpha\)Glc-(1\(\rightarrow\)4)- (residue type A) and terminal \(\alpha\)Glc-(1\(\rightarrow\)4)- (residue type B) were in good agreement with published values (Table 2B); limited assignments could also be made for 4)-\(\alpha\)Glc-(1\(\rightarrow\)6)- (residue type C). The ratio C-4(A + C): C-4 (B) = 89:11 by integration of signals in the \(^{13}\)C NMR spectrum indicates that about 11% of residues are not glycosylated at the 4-position. These results indicate that the \(\alpha\)-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.

\(\alpha\)-glucan has an important role in the phagocytic process of \(P.\) boydii by macrophages - To investigate whether the \(\alpha\)-glucan is involved in the phagocytic process of \(P.\) boydii, macrophages were incubated with heat-killed conidia at a ratio of 5:1 for 1h in the presence or absence of \(\alpha\)-glucan. Macrophages endocytosed heat killed conidia \(P.\) boydii avidly and similar phagocytic indexes were obtained by challenging macrophages with live conidia (Fig. 3A; B). The addition of increasing concentrations of \(\alpha\)-glucan led to a dose dependent inhibition of \(P.\) boydii phagocytosis (Fig. 3C). The concentration of 100\(\mu\)g/ml of \(\alpha\)-glucan consistently caused a 50% inhibition of conidia phagocytosis. To exclude the possibility of contaminants being responsible for the inhibition of conidial phagocytosis, an \(\alpha\)-amyloglucosidase-treated \(\alpha\)-glucan (100\(\mu\)g/ml) was added to the culture at time zero of interaction. The phagocytic index of conidia returned to the control level when macrophages were allowed to interact with the digested \(\alpha\)-glucan (Fig. 3D). To further characterize the role of \(\alpha\)-glucan in the phagocytosis of \(P.\) boydii, conidia were submitted to treatment with \(\alpha\)-amyloglucosidase for 22h at 37 \(^\circ\)C and their phagocytic index was compared to that from untreated conidia (Fig. 3E). A significant decrease in the phagocytic index occurred when \(\alpha\)-glucan from conidial surface was removed by enzymatic treatment. These results suggest that \(\alpha\)-glucan present in \(P.\) boydii surface plays an essential role in the internalization of conidia by macrophages.

\(\alpha\)-Glucan induces TNF release by macrophages through TLR2 and CD14 - To study the role of \(\alpha\)-glucan in cytokine production, peritoneal macrophages were stimulated with increasing concentrations of \(\alpha\)-glucan in the presence of purified \(\alpha\) or \(\beta\)-glucans (Fig. 4A). A significant decrease of \(P.\) boydii phagocytosis occurred for the cultures treated with different ramified \(\alpha\)-glucans, but not with pullulan, a linear \(\alpha\)-glucan from the lichen \(Teloschistes\) flavicans, or laminarin, a \(\beta\)-glucan isolated from \(L.\) digitata. Recent studies have shown that \(\beta\)-glucan plays a central role in the phagocytosis of zymosan and this effect is dependent on the phagocytic receptor Dectin-1 (38). As expected, soluble laminarin strongly reduced the ingestion of zymosan, while purified \(\alpha\)-glucan had only a minor effect on zymosan phagocytosis (Fig. 4B).
α-glucan of fungi activates the innate immune system

... indicate that highly purified α-glucan from P. boydii is recognized by macrophages triggering fungal phagocytosis and TNF release. To investigate the involvement of TLR on α-glucan induction of macrophage activation, we stimulated wild-type and MyD88 deficient macrophages with α-glucan and evaluated TNF release. The secretion of TNF induced by α-glucan was abolished from MyD88-/- macrophages (Fig. 6A). As controls we stimulated wild-type and MyD88 deficient macrophages with LPS and Pam3Cys, TLR4 and TLR2 ligands, respectively. As previously reported, TNF release triggered by these ligands was completely dependent on MyD88 (39,40). The requirement for MyD88 on macrophage activation induced by α-glucan indicates that a TLR is involved in the recognition of α-glucan. TLR2 and TLR4 are the best studied TLRs and a great variety of molecules are potential ligands for these receptors. We investigated the role of these TLRs in the recognition of α-glucan, by stimulating macrophages from mice lacking TLR4 (C57BL/10ScN) or TLR2 (TLR2-/-) and their respective counterparts. TLR4 deficient macrophages showed a partial but significant reduction in TNF secretion induced by α-glucan, while the response was completely abrogated in the absence of TLR2-/- (Fig. 6B; C). As controls LPS and Pam3Cys were included in the experimental protocol. Similar results were obtained with the TLR4 mutant strains C57BL/10ScCr and C3H/HeJ (data not shown).

Previous studies have shown the involvement of CD14 in the recognition of fungi by macrophages (20,41). To investigate the contribution of CD14 to the macrophage activation induced by α-glucan, wild-type and CD14 deficient macrophages were stimulated by α-glucan and TNF release was evaluated. Wild-type macrophages responded to α-glucan by the secretion of TNF, while CD14-/- macrophages were unable to release TNF in response to α-glucan (Fig. 6D). LPS, a well known ligand of CD14, did not induce optimal TNF production at low concentrations in the absence of CD14. These results indicate that TLR2 and CD14 are essential to the recognition of α-glucan by the innate immune system.

**DISCUSSION**

In this work, we described the structure of a highly purified α-glucan, obtained from P. boydii, that mediates P. boydii conidial phagocytosis and triggers macrophage activation in a mechanism involving CD14, TLR2 and MyD88. The immune response to the infections caused by fungi like A. fumigatus and P. boydii requires phagocytosis and killing of conidia with induction of a strong inflammatory response, preventing the development of hyphae and tissue colonization.

P. boydii glucan structure was determined based on a combination of several techniques including gas chromatography, 1H TOCSY, 1H and 13C - NMR spectroscopy and methylation analysis, to be a glycogen-like polysaccharide consisting of linear 4-linked α-D-Glcp residues substituted at position 6 with -α-D-Glcp branches. Like oyster and and A. fumigatus glucan (34,43), the P. boydii glucan yields 2,3-di-O-methyl glucose, which is characteristic of branch points with glucose units in (1→6) linkage, and a high proportion of 2,3,6-tri-O-methylglucose, indicating the presence of linear portions of (1→4)-linked glucopyranosyl units. The 1H NMR spectrum of the purified glucan confirmed the similarity of the glucan of P. boydii with glycogen from other species including A. fumigatus, Mycobacterium bovis, and rabbit liver (34-37).

We have demonstrated that conidial phagocytosis depends to a significant extent on α-glucan recognition. The inhibition of conidial phagocytosis by soluble α-glucan or by amyloglucosidase treatment indicates that α-glucan is accessible on the conidial surface and...

The activation of dendritic cells by α-glucan is dependent on TLR-2 - Dendritic cells play a central role in naïve T cell activation and Th1 versus Th2 differentiation (42). The ability of dendritic cells to exert these functions is largely dependent on its previous activation by TLR ligands (11). Treatment of bone marrow derived dendritic cells with α-glucan caused the release of IL-12 and TNF (Fig 7A; B). Similar to macrophages, the effect of α-glucan on cytokine secretion by dendritic cells was abolished in the absence of TLR2.
mediates its interaction with macrophages. In this way, α-glucan recognition resembles the role of β-glucans in the interaction of other fungi with macrophages. The phagocytosis of yeast forms of C. albicans and S. cerevisiae are critically dependent on the recognition of surface associated β-glucans by Dectin-1 expressed by macrophages (44,45). The phagocytosis of P. boydii by macrophages was not significantly affected by treatment with soluble β-glucan laminarin. This result further supports the concept that α-glucan is an important molecule involved in the recognition and phagocytosis of P. boydii, and also suggests that Dectin-1 is not essential to the recognition of P. boydii α-glucan. However, our results do not formally exclude a possible role of Dectin-1 in α-glucan recognition by cells of the innate immune system. Moreover, the partial effect of α-glucan on phagocytosis clearly indicates that other ligands are involved in the recognition of conidia by macrophages. Future analyses are required to define the phagocytic receptor involved on α-glucan recognition, as well as the nature of other putative phagocytic ligands present on P. boydii conidia.

Several studies have demonstrated that TLR2, TLR4 and CD14 are involved in the recognition of fungi (20,21,41). Although many details of TLR recognition and signaling in response to different developmental forms of fungal pathogens are well known, the molecules expressed by fungal cells that trigger TLR signaling by fungi are largely unknown. We demonstrated that α-glucan induces cytokine secretion by macrophages via CD14, TLR2 and MyD88. Interestingly, in the absence of TLR4 we observed a significant reduction of TNF secretion induced by α-glucan but not by Pam3Cys. A major concern in the identification of new putative TLR ligands is the presence of undesirable contaminants. To rule out contamination as the explanation for these results several strategies were used in the present study. First, a highly purified molecule was used as assessed by the analytical methods; second, the endotoxin was specifically removed by using a polymixin B column; and third, the α-glucan was digested with an α-amyloligosidase that completely abolished the α-glucan stimulation of macrophages but not that induced by Pam3Cys or LPS (data not shown).

Jouault et al. also showed that a phospholipomannan of C. albicans requires TLR2 and to a lesser extent TLR4 and TLR6 to induce TNF release by murine peritoneal macrophages (46). On the other hand, A. fumigatus and zymosan induce macrophage activation, respectively, by TLR2/4 (20,21) and TLR2/Dectin-1 (45,47) but the molecules responsible for TLR triggering are unknown. A. fumigatus presents α-glucans as well β-glucans in its cell wall, so it is possible that α-glucans could be the TLR2 activating molecules representing typical PAMPs of filamentous fungi like P. boydii.

A number of α-glucans from lichens and oyster were ineffective in inducing TNF secretion. Interestingly, pullulan was unable to affect the phagocytosis of conidia or cause TNF secretion. On the other hand, glycogen caused inhibition of phagocytosis of conidia to a similar extent to α-glucan from P. boydii despite not inducing TNF. These results indicate different requirements of putative phagocytic and TLR2 receptors on recognizing α-glucans. Pullulan and nigeran are linear molecules while glycogen, amylopectin and α-glucan from P. boydii present different grades of ramification. Our results suggest that the degree of ramification is important for the recognition by phagocytic receptors. In contrast, the induction of TNF release was triggered only by P. boydii α-glucan, the α-glucan with the higher degree of ramification. These results suggest that extensive ramification is required for TLR2 recognition by α-glucans. Interestingly, curdlan, a linear β-glucan from fungal cell wall shows MyD88-dependent macrophage activation (48). In fact, for β-glucans, increasing the degree of ramification caused a reduction in macrophage activation. The activation of macrophages by mannuronic acid polymers (poly-M) is preferentially performed by TLR4 but also requires TLR2 (49). Interestingly, the potency of high molecular poly-M is reduced by polymeric breakdown, while attaching oligomeric M blocks enhances particles potency (50).

In recent years the central role of dendritic cells as the link between innate and adaptative response has become clear. These cells are responsible for the activation of naïve T lymphocytes, presenting antigen, providing co-stimulation and secreting polarizing cytokines (11,31). The secretion of IL-12 is essential to the
α-glucan of fungi activates the innate immune system

induction of a Th1 phenotype. An effective immune response to fungi such as A. fumigatus, C. albicans and P. brasiliensis requires a strong Th1 response and consequent IFN-γ production (51-53). The ability of dendritic cells to recognize α-glucan through TLR2, secreting IL-12 and TNF, suggests a possible mechanism for the induction of a protective Th1 polarizing response during P. boydii infection. Future studies are necessary to define the involvement of this pathway in the in vivo host response to P. boydii infection.

Here, we described an α-glucan that represents a characteristic PAMP of filamentous fungi. We also demonstrated that this molecule participates in the phagocytosis of conidia and that TLR2 and CD14 are involved in the innate immune activation upon the recognition of α-glucan. These results might have relevance in the context of infections with P. boydii and other fungi. Recognition of α-glucan could be a target for immunomodulation during fungal infections increasing the host resistance through IL-12 secretion and Th1 induction; alternatively α-glucan could also contribute to the pathology by inducing local and systemic TNF release, promoting tissue injury.

REFERENCES

1. Washburn, R. G., Gallin, J. I., Bennett, J. E. (1987) Infect. Immun. 55, 2088-92.
2. Morgenstern, D. E., Gifford, M. A., Li, L. L., Doerschunk, C. M., Dinauer, M. C. (1997) J. Exp. Med. 18, 207-18.
3. Sasada, M., Jonhston, R. B. (1980) J Exp Med. 152, 85-98.
4. Gil-Lamagniere, C., Roilides, E., Lyman, C. A., Simitsopoulo, M., Stergiopoulo, T., Maloukou, A., Walsh, T. J. (2003) Infect. Immun. 71, 6472-8.
5. Ley, K. (2002) Immunol. Rev. 186, 8-18.
6. Romani, L. (2004) Nat. Rev. Immunol. 4, 1-23.
7. Rock, F. L., Hardiman, G., Timans, J. C., Kastelein, R. A., Bazan, J. F. (1998) Proc. Natl. Acad. Sci. U.S.A. 95, 588-593.
8. Medzhitov, R. M., Preston-Hurlburt, P., Janeway, C. A. Jr. (1997) Nature 388, 394-397.
9. Takeda, K., Kaisho, T., Akira, S. (2003) Annu Rev Immunol. 21, 335-76.
10. Thoma-Uszynski, S., Stenger, S., Takeuchi, O., Ochoa, M. T., Engele, M., Sieling, P. A., Barnes, P. F., Rollinghoff, M., Bolcskei, P. L., Wagner, M., Akira, S., Norgard, M. V., Belisle, J. T., Godowsky, P. J., Bloom, B. R., Modlin, R. L. (2001) Science 291, 1544-1547.
11. Iwasaki, A., Medzhitov, R. (2004) Nat Immunol. 5, 987-95.
12. Akira, S., Takeda, K. (2004) Nat. Rev. Immunol. 4, 499-511.
13. Schroder, N. W., Morath, S., Alexander, C., Hamann, L., Hartung, T., Zahringer, U., Gobel, U. B., Weber, J. R., Schumann, R. R. (2003) J. Biol. Chem. 278, 15587-94.
14. Lien, E., Sellati, T. J., Yoshimura, A., Flo, T. H., Rawadi, G., Finberg, R. W., Carroll, J. D., Espevik, T., Ingalls, R. R., Radolf, J. D., Golenbock, D. T. (1999) J. Biol. Chem. 274, 33419-25.
15. Campos, M. A., Almeida, I. C., Takeuchi, O., Akira, S., Valente, E. P., Procopio, D. O., Travassos, L. R., Smith, J. A., Golenbock, D. T., Gazzinelli, R. T. (2001) J. Immunol. 167, 416-23.
16. Means, T. K., Lien, E., Yoshimura, A., Wang, S., Golenbock, D. T., Fenton, M. J. (1999) J. Immunol. 163, 6748-55.
17. Poltorak, A., He, X., Smirnova, I., Liu, M. Y., Van Huffel, C., Du, X., Birdwell, D., Alejos, E., Silva, M., Galanos, C., Freudenberg, M., Ricciardi-Castagnoli, P., Layton, B., Beutler, B. (1998) Science 282, 2085-8.
18. Park, J. M., Ng, V. H., Maeda, S., Rest, R. F., Karin, M. (2004) J. Exp. Med. 200, 1647-55.
19. Malley, R., Henneke, P., Morse, S. C., Cieslewicz, M. J., Lipsitch, M., Thompson, C. M., Kurt-Jones, E., Paton, J. C., Wessels, M. R., Golenbock, D. T. (2003) Proc. Natl. Acad. Sci. U S A. 100, 1966-71.
20. Mambula, S. S., Sau, K., Henneke, P., Golenbock, D., Levitz, S. (2002). J. Biol. Chem. 18, 39320-39326.
α-glucan of fungi activates the innate immune system

21. Meier, A., Kirschning, C. J., Nikolaus, T., Wagner, H., Heesemann, J., Ebel, F. (2003). *Cell. Microbiol.* 5, 561-570.

22. Belloccchio, S., Montagnoli, C., Bozza, S., Gaziano, R., Rossi, G., Mambula, S. S., Vecchi, A., Mantovani, A., Levitz, S. M., Romani, L. (2004). *J. Immunol.* 172, 3059-69.

23. Balloy, V., Si-Tahar, M., Takeuchi, O., Philippe, B., Nahori, M. A., Tanguy, M., Huerre, M., Akira, S., Latge, J. P., Chignard, M. (2005) *Infect Immun.* 73, 5420-5.

24. Netea, M. G., Van Der Graaf, C. A., Vonk, A. G., Verschueren, I., Van Der Meer, J. W., Kullberg, B. J. (2002) *J. Infect. Dis.* 185, 1483-9.

25. Villamon, E., Gozalbo, D., Roig, P., O’Connor, J. E., Fradelizi, D., Gil, M. L. (2004) *Microbes Infect.* 6, 1-7.

26. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956) *Anal. Chem.* 28, 350-6.

27. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) *J. Biol. Chem.* 193, 265-75.

28. Sawardeker, J. S., Sloneker, J. H., Jeanes, A. (1965) *Anal. Biochem.* 37, 1602-4.

29. Tischer, C. A., Gorin, P. A., Gorin, Iacomini, M. (2002) *Carbohydr. Polymers* 47,151-8.

30. Ciucanu, I., Kerek, F. A. (1984) *Carbohydr. Res.* 131, 209-17.

31. Jansson, P. E., Kenne, L., Liedgren, H., Lindberg, B., Lönngren, J. (1976) *Univ. Stockol. Chem. Commun.* 8, 1-76.

32. Popi, A. F. F., Lopes, J. D., Mariano, M. (2002) *Cell. Immunol.* 218, 87-94.

33. Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S., Steinman, R. M. (1992) *J Exp Med.* 176, 1693-702.

34. Bahia, M.C.F.S., Vieira, R. P., Mulloy, B., Hartmann, R., Barreto-Bergter, E. (1997) *Mycopathologia*, 137:17-25.

35. Dinadayala, P., Lemassu, A., Granovski, P., Cérantola, S., Winter, N., Daffé M. (2004) *J. Biol. Chem.* 279, 12369-12378.

36. Zang, L.H., Howseman, A.M., Shulman, R.G. (1991) *Carbohydr. Res.* 220, 1-9.

37. Wang, R., Klegerman, M.E., Marsden, I., Sinnott, M., Groves, M.J., (1995) *Biochem. J.* 311, 867-872.

38. Brown, G. D., Gordon, S. (2003) *Immunity* 19, 311-5.

39. Kawai, T., Adachi, O., Ogawa, T., Takeda, K., Akira, S. (1999) *Immunity* 11, 115-22.

40. Takeuchi, O., Kaufmann, A., Grote, K., Kawai, T., Hoshino, K., Morr, M., Muhlradt, P. F., Akira, S. (2000) *J. Immunol.* 164, 554-7.

41. Newman, S. L, Chaturvedi, S., Klein, B. S. (1995) *J. Immunol.* 154, 753-61.

42. Moser, M., Murphy, K. M. (2000) *Nat. Immunol.* 1, 199-205.

43. Cheetham, N. W., Hansawek, N., Saecou, P. (1991) *Carbohydr. Res.* 215, 59-65.

44. Brown, G. D., Taylor, P. R., Reid, D. M., Willment, J. A., Williams, D. L., Martinez-Pomares, L., Wong, S. Y., Gordon, S. (2002) *J. Exp. Med.* 196, 407-12.

45. Brown, G. D., Herre, J., Williams, D. L., Willment, J. A., Marshall, A. S., Gordon, S. (2003) *J. Exp. Med.* 197, 1119-24.

46. Jouault, T., Ibata-Ombetta, S., Takeuchi, O., Trinel, P. A., Sacchetti, P., Lefebvre, P., Akira, S., Poulinel, D. (2003) *J. Infect. Dis.* 188, 165-72.

47. Gantner, B. N., Simmons, R. M., Canavera, S. J., Akira, S., Underhill, D. M. (2003) *J. Exp. Med.* 197, 1107-17.

48. Kataoka K., Muta T., Yamazaki S., Takeshige K. (2002) *J. Biol. Chem.* 277:36825-31.

49. Flo T. H., Ryan L., Latz E., Takeuchi O., Monks, B. G., Lien E., Halsass O., Akira S., Skjak-Braek G., Golenbock D. T., and Espevik T. (2002) *J. Biol. Chem.* 277:35489-35495.

50. Flo T. H., Ryan L., Kilaas L., Skjak-Braek G., Ingalls R. R., Sundan A., Golenbock D. T., and Espevik T. (2000) *Infect. Immun.* 68, 6770–6776.

51. Cenci, E., Mencacci, A., Del Sero, G., Bacci, A., Montagnoli, C., d'Ostiani, C. F., Mosci, P., Bachmann, M., Bistoni, F., Kopf, M., Romani, L. (1999) *J. Infect. Dis.* 180, 1957-68.
52. Cenci, E., Mencacci, A., Del Sero, G., d'Ostiani, C. F., Mosci, P., Bacci, A., Montagnoli, C., Kopf, M., Romani, L. (1998) *J. Immunol.* 161, 3543-50.
53. Cano, L. E., Kashino, S. S., Arruda, C., Andre, D., Xidieh, C. F., Singer-Vermes, L. M., Vaz, C. A., Burger, E., Calich, V. L. (1998) *Infect. Immun.* 66, 800-6.
α-glucan of fungi activates the innate immune system

Footnote: VCBB and RTF contributed equally to the study. The research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES), Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ), Programa de Núcleos de Excelência (Pronex) and UFRJ.

The abbreviations used are: BMDC, bone-marrow derived cell; COSY, correlated spectroscopy; DEPT, distortionless enhancement by polarization transfer; ECL, electrochemiluminescence; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GC-MS, gas-liquid chromatography-mass spectrometry; GPI, glycosylphosphatidylinositol; HBSS, Hanks balance salt solution; HPTLC, high performance thin layer chromatography; HSQC, heteronuclear single quantum coherence; MyD88, myeloid differentiation protein-88; TFA, trifluoroacetic acid; TLC, thin layer chromatography; TLR, Toll-like receptors; TOCSY, total correlation spectroscopy; NMR, nuclear magnetic resonance; Pam3Cys, (S)-(2,3-bis(palmitoyloxy)-(2RS)-propyl)-N-palmitoyl-(R)-Cys-(S)-Ser(S)-Lys(4)-OH, trihydrochloride; PAMP, pathogen-associated molecular pattern; PMSF, phenylmethanesulfonyl fluoride

Acknowledgements

We are grateful to Shizuo Akira and Douglas Golenbock for providing mutant mice strains (MyD88, TLR2 and CD14), Patricia Bozza, Ricardo Gazzinelli and Maria Belio for providing mice and reagents, Leonardo Travassos for critical reading of the manuscript and Maria de Fátima Ferreira Soares, Universidade Federal do Rio de Janeiro, for technical assistance.
**FIGURE LEGENDS**

**Fig. 1.** A, 500 MHz ¹H-NMR of the glucan from *P. boydii* recorded at 60 °C in D₂O. Chemical shifts are relative to internal TSP at 0 ppm. B, Two-dimensional TOCSY NMR spectrum of the glucan from *P. boydii*. Residue A: linear (→4)-αGlcp-(1→4)-; Residue B: terminal α Glcp-(1→4)-; Residue C: branched (→4)-αGlcp-(1→6)-. H1A, H1B, H1C- anomeric protons of the residues A, B and C; H2 to H5- ring protons; HOD- deuterated water (D₂O).

**Fig. 2.** A, DEPT ¹³C-NMR spectrum of the glucan from *P.boydii* recorded at 60 °C in D₂O. Chemical shifts are relative to external TMS at 0 ppm. B, A simplified structural diagram of the glycogen-like glucan from *P. boydii*, showing the three residue types A (→4-αGlcp-(1→4)-), B (terminal α Glcp-(1→4)-) and C (→4-αGlcp-(1→6)-) identified by NMR and methylation analysis. The lengths of backbone and side-chains are not known from this study, nor is it clear whether the glucan has a single backbone with simple side-chains, or a more complex structure in which the branches themselves bear further branches.

**Fig. 3.** Influence of *P. boydii* α-glucan on the PI of conidia by peritoneal macrophages from BALB/c mice. A, Light micrograph of the interaction between heat killed *P. boydii* conidia and peritoneal macrophages after 1h. Conidia were allowed to interact with macrophages in a E:T ratio of 5:1. Note ingested fungi (arrowhead) within vacuole. B, Comparison of phagocytosis of live and heat killed *P. boydii* conidia. C, Addition of increasing concentrations α-glucan inhibited the phagocytosis of fungal conidia. D, α-glucan (100μg/ml) or α-glucan treated with α-amyloglucosidase were added to the cultures of macrophages interacting with conidia. E, Conidia treated with α-amyloglucosidase were allowed to interact with macrophages in a E:T ratio of 5:1. Cells were stained with Giemsa and counted to determine the PI. Data are shown as the mean ± S.E. of three independent experiments performed in duplicate. Asterisks denote values statistically different from control (p<0,05).

**Fig. 4.** Influence of different glucans on the phagocytosis of conidia and zymosan by peritoneal macrophages from BALB/c mice. A, Phagocytosis of conidia was inhibited by a variety of α-glucans, but not by laminarin. B, Phagocytosis of zymosan was inhibited by laminarin and only marginally inhibited by *P. boydii* α-glucan. Glucans were added to the culture medium at the concentrations of 100 μg/ml, simultaneously with addition of the particles. Cells were stained with Giemsa and counted to determine the PI. Data are shown as the mean ± S.E. of three independent experiments performed in duplicate. Asterisks denote values statistically different from control (p<0,05).

**Fig. 5.** Effect of glucans on TNF secretion by macrophages. A, α-glucan induces TNF release by peritoneal macrophages. Thioglycollate-elicited peritoneal macrophages were plated on 96-well plates and stimulated with the indicated concentrations of α-glucan, α-glucan (100 μg/mL) digested with α-amyloglucosidase, LPS (100 ng/mL) or Pam3Cys (100 ng/mL). B, Macrophages were stimulated with several glucans at 100 μg/ml as indicated. After 4h, the supernatant was recovered and TNF was evaluated by ELISA. Data represent mean ± S.E.M. of three different experiments and the stimulations were performed in duplicates. Asterisks denote values statistically different from control and from α-amyloglucosidase treated α-glucan (p<0,05).

**Fig. 6.** α-glucan is a ligand of TLR2 and CD14 requiring MyD88. A, α-glucan requires MyD88 to induce TNF secretion by macrophages. Thioglycollate-elicited peritoneal macrophages, WT or MyD88−/−, were stimulated with α-glucan (100 μg/ml), LPS (100 ng/mL) or Pam3Cys (100 ng/mL). After 4h, the supernatant was harvested and TNF was measured by ELISA. Results are expressed as
mean ± S.E.M. of three different experiments and stimulation was performed in duplicates. B,C, TNF release induced by α-glucan is dependent of TLR2 but not TLR4. Peritoneal macrophages obtained from WT, TLR2-/-, C57Bl/10 (TLR4+/+) or C57BL/10ScN (TLR4-/-) were stimulated with α-glucan (100 μg/ml), LPS(100 ng/mL) or Pam3Cys (100 ng/mL). After 4h, the supernatant was harvested for TNF determination. Data represent mean ± S.E.M. of two independent experiments and the stimulation was performed in duplicate. D, CD14 recognition is required to TNF release induced by α-glucan in macrophages. Thioglycollate-elicited peritoneal macrophages were obtained from WT or CD14-/- mice. Macrophages were stimulated with α-glucan (100 μg/ml), LPS(100 ng/mL) or Pam3Cys (100 ng/mL). After 4h, the supernatant was harvested for TNF determination. Data represent mean ± S.E.M. of two independent experiments and the stimulation was performed in duplicate.

**Fig. 7.** A, α-glucan induces TNF and B, IL-12 release by dendritic cells cells in mechanism involving TLR2. Dendritic cells differentiated with IL-4 and GM-CSF were plated in 96-well at a density of 2x10⁵ cells/well, and stimulated for 16h in presence of α-glucan (100 μg/mL), LPS (100 ng/mL) or Pam3Cys (100 ng/mL). The supernatant was evaluated for TNF and IL12 by ELISA. Data represent mean ± S.E.M. of stimuli performed in duplicates of one representative experiment from two different experiments with similar results.
Table 1
Molar percentages of $O$-methyl alditol acetates obtained on methylation analysis of the glucan of *P. boydii*.

| $O$-methyl alditol acetate | Molar % $O$-methyl alditol acetate |
|----------------------------|-----------------------------------|
| 2,3,4,6-Me$_4$-Glc          | 13                                |
| 2,3,6-Me$_3$-Glc            | 63                                |
| 2,3-Me$_2$-Glc              | 24                                |

Column: DB- 225
Table 2

$^1$H and $^{13}$C NMR chemical shifts (ppm) of glucan from *P. boydii* (a) compared to rabbit liver glycogen (b) and *Mycobacterium bovis* (c).

| Residue | A (a) | A (b) | A (c) | B (a) | B (b) | B (c) | C (a) | C (b) | C (c) |
|---------|------|------|------|------|------|------|------|------|------|
| 1       | 5.39 | 5.38 | 5.38 | 5.39 | nd   | 5.38 | 4.98 | 4.98 | 4.99 |
| 2       | 3.66 | 3.66 | 3.66 | 3.62 | 3.62 | 3.62 | 3.61 | 3.62 | 3.64 |
| 3       | 3.96 | 3.97 | 3.98 | 3.71 | 3.72 | 3.72 | 4.00 | nd   | 4.03 |
| 4       | 3.66 | 3.64 | 3.66 | 3.44 | 3.44 | 3.44 | 3.65 | nd   | 3.66 |
| 5       | 3.88 | 3.85 | 3.85 | 3.79 | 3.73 | 3.73 | 3.89 | nd   | 3.88 |
| 6, 6'   | 3.87 | 3.90 | nd   | 3.86 | 3.8  | 3.8/3.9 | nd | nd   | nd |

$^1$H chemical shifts in ppm, relative to internal TSP at 0ppm.

| Residue | A (a) | A (b) | A (c) | B (a) | B (b) | B (c) | C (a) | C (b) | C (c) |
|---------|------|------|------|------|------|------|------|------|------|
| 1       | 100.5 | 100.5 | 100.1 | 100.5 | nd   | 100.1 | 99.2 | nd   | 98.5 |
| 2       | 72.2  | 72.3  | 72.0  | 72.5  | 72.0 | nd   | nd   | nd   | nd   |
| 3       | 73.9  | 74.0  | 73.8  | 73.6  | 73.7 | 73.4 | nd   | nd   | nd   |
| 4       | 78.0  | 78.0  | 78.0  | 70.1  | 70.1 | 70.0 | 78.8 | nd   | nd   |
| 5       | 71.9  | 72.0  | 72.0  | 73.4  | 73.4 | nd   | nd   | nd   | nd   |
| 6, 6'   | 61.3  | 61.3  | 61.3  | 61.3  | nd   | 61.3 | nd   | nd   | nd   |

$^{13}$C Chemical shifts in ppm, relative to external TMS at 0 ppm.

- **Residue A** = linear $\rightarrow$4-αGlc-(1→4)-
- **Residue B** = terminal -αGlc-(14)-
- **Residue C** = branched$\rightarrow$4-αGlc-(1→6)-

(a) This work
(b) Reference 36
(c) Reference 35
\( \alpha \)-glucan of fungi activates the innate immune system
α-glucan of fungi activates the innate immune system

FIGURE 2

A

B
α-glucan of fungi activates the innate immune system

FIGURE 3

A

B

C

D

E

live conidia
heat-killed conidia

0
10
20
30
40
50
60
70

Phagocytic index

α-glucan (μg/ml)

0
25
50
100

Phagocytic index

* * *

control-glucan
α-glucan digested
α-glucan

0
25
50
75
100

Phagocytic index

* * *

control
digested conidia
$\alpha$-glucan of fungi activates the innate immune system

**FIGURE 4**

A

B

CTR $\alpha$-glucan glycogen pullulan laminarin

CTR $\alpha$-glucan laminarin
\[\alpha\text{-glucan of fungi activates the innate immune system}\]
FIGURE 6

**A**

![Bar chart showing TNF-α levels in MyD88 +/- and MyD88 -/- mice.]

**B**

![Bar chart showing TNF-α levels in TLR2 +/- and TLR2 -/- mice.]

**C**

![Bar chart showing TNF-α levels in TLR4 +/- and TLR4 -/- mice.]

**D**

![Bar chart showing TNF-α levels in CD14 +/- and CD14 -/- mice.]

α-glucan of fungi activates the innate immune system
α-glucan of fungi activates the innate immune system

FIGURE 7

A

![Bar graph showing TNF-α (pg/ml) response to different treatments.

- Wild-type
- TLR2 -/-

B

![Bar graph showing IL-12 (pg/ml) response to different treatments.

- Wild-type
- TLR2 -/-

Wild-type

CTR α-glucan LPS PamCys
An α-glucan of Pseudallescheria boydii is involved in fungal phagocytosis and TLR activation
Vera Carolina B. Bittencourt, Rodrigo T. da Figueiredo, Rosana B. Silva, Diego S. Mourão-Sá, Patricia L. Fernandez, Guilherme L. Sassaki, Barbara Mulloy, Marcelo T. Bozza and Eliana Barreto-Bergter

J. Biol. Chem. published online June 9, 2006

Access the most updated version of this article at doi: 10.1074/jbc.M511417200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts