

Polysaccharide-rich fraction of *Agaricus brasiliensis* enhances the candidacidal activity of murine macrophages

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A polysaccharide-rich fraction (ATF) of medicinal mushroom Agaricus brasiliensis was evaluated on the candidacidal activity, H₂O₂ and nitric oxide (NO) production, and expression of mannose receptors by murine peritoneal macrophages. Mice received three intraperitoneal (i.p.) injections of ATF and after 48 h their peritoneal resident macrophages were assayed against Candida albicans yeast forms. The treatment increased fungicidal activity and it was associated with higher levels of H₂O₂, whereas NO production was not affected. We also found that the treatment enhances mannose receptor expression by peritoneal macrophages, which are involved in the attachment and phagocytosis of non-opsonized microorganisms. Treatment of animals with ATF was able to enhance the clearance of C. albicans during the first 6 h after the experimental i.p. infection. Our results suggest that this extract can increase host resistance against some infectious agents through the stimulation of microbicidal activity of macrophages.

Key words: *Candida albicans* - microbicidal activity - mushroom

Candida albicans is a very common dimorphic fungus that can cause opportunistic infection and, although most people are resistant to this agent, candidiasis is the most frequent fungal infection among immune depressed individuals such as diabetes (Donders 2002), cancer (Ridola et al. 2004) and HIV patients (Klein et al. 1984). Resistance to *C. albicans* depends on a coordinated action of innate and adaptive immune defenses, a process to which phagocytosis by granulocytes and macrophages is crucial. The destruction of microorganisms by phagocytic cells involves both the recognition/attachment to cell surfaces and phagocytosis/endocytosis, followed by the killing processes. The binding of pathogens to macrophage surfaces initiates the secretion of a wide array of inflammatory mediators including arachidonic acid metabolites, neutral proteases and reactive oxygen intermediates (H₂O₂, O₂⁻, OH⁻) and nitric oxide (NO), all of which are highly toxic to most of microorganisms (Goldsby et al. 2000). Administration of β -glucan obtained from different sources has been demonstrated to stimulate the microbicidal activity of macrophages (Suzuki et al. 1990, Sakurai et al. 1991). These cells show a variety of surface molecules that enhance the phagocytic function, such as receptors for complement (CR1 and CR3), IgG Fc portion, and the pattern-recognition receptors (PRR), like the mannose-receptors (MR) and toll-like recep-

tors, which are involved in the recognition and phagocytosis of non-opsonized microorganisms. MR was first identified on Küpffer cells of rats as a specific uptake system for mannosylated/N-acetylglucosamine-terminal and fucosylated glycoproteins (Schlesinger et al. 1978). Further studies have demonstrated their presence on alveolar (Stahl et al. 1978) and peritoneal macrophages (Stahl et al. 1982) as well as on human mononuclear phagocytes (Shepherd et al. 1982). Many studies have suggested that the main role of MR is the endocytic clearance of host-derived glycoproteins (Smedsrod et al. 1988) and it is also widely accepted that MR can mediate the phagocytosis of non-opsonized microorganisms through interaction with polysaccharide structures of cell walls, such as yeast mannan, bacterial capsules, lipopolysaccharides, and lipoarabinomannan (Ofek et al. 1995).

Agaricus blazei Murrill - whose Brazilian variety was recently suggested for classification as a new species to be named *Agaricus brasiliensis* sp. nov. (Wasser et al. 2002) - is a medicinal mushroom whose characteristics include a wide range of medicinal properties including antitumoral (Mizuno et al. 1990a, b) and immunostimulatory activities (Ito et al. 1997, Fujimiya et al. 1998). The main bioactive substances of this mushroom are polysaccharides obtained from the fruiting bodies (Mizuno et al. 1990a, b, Ebina & Fujimiya 1998, Fujimiya et al. 1998), and its immunomodulatory activity is attributed mainly to β -glucans (Mizuno et al. 1990a, b, Ito et al. 1997), which are also found in other species of edible mushrooms such as *Lentinus edodes* and *Ganoderma lucidum* (Borchers et al. 1999).

There are few reports on the metabolic pathway of (1 \rightarrow 3)- β -D-glucans in the host. Vertebrate cells do not possess (1 \rightarrow 3)- β -D-glucanases and cannot rapidly metabolize these carbohydrates, but in fact metabolize them

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slowly through oxidation (Nono et al. 1991). In vivo the clearance of (1→3)- β -D-glucans depends on their molecular weight so that low-molecular-weight glucans are excreted through glomerular filtration, whereas large molecules are retained primarily in the liver. These larger β -glucans are metabolized by K upffer cells and this process may take several weeks (Suda et al. 1996).

A polysaccharide-rich fraction obtained by acid treatment of the ammonium oxalate-soluble extract of *A. blazei* (ATF) was shown to be able to cause tumor infiltration by NK cells; and it inhibits in vitro tumor cell growth by inducing apoptosis (Fujimiya et al. 1998, 1999). Sorimachi et al. (2001) have observed that extracts from *A. blazei* are able to activate macrophage functions. Our previous results have shown that the oxalate-soluble polysaccharide fraction was able to inhibit the growth of Ehrlich tumor and partially inhibit the production of IL-10 by spleen cells of tumor-bearing mice (unpublished observations). Although there are only two works reporting the effects of *A. blazei* on human diseases (Ahn et al. 2004, Grinde et al. 2006), people of many countries habitually consume this mushroom prepared as tea or pills (whole dried and powdered fruiting bodies).

Since mushroom polysaccharides are able to activate lymphocytes and macrophages (Ebina & Fujimiya 1998, Fujimiya et al. 1998, Borchers et al. 1999, Sorimachi et al. 2001) in the present work we aimed to explore, for the first time, the usefulness of ATF against opportunistic fungi. Thus we evaluated the effect of ATF inoculation on candidacidal activity, spontaneous H₂O₂ and NO release, and the expression of MR on peritoneal resident macrophages.

MATERIALS AND METHODS

Animals and ATF treatment - Specific pathogen-free male BALB/c mice aged 45 days were handled according to the recommendations of the Brazilian College of Animal Experimentation. All procedures were approved by the Animal Research Ethics Committee of the Institute of Biosciences (CEEA n  039/04). For assaying the ex vivo fungicidal activity, phagocytosis, H₂O₂ and NO release, and MR expression, animals (n = 6) were treated intraperitoneally (i.p.), with 500 μ g of ATF or phosphate-buffered salt (PBS) (0.01M PBS solution, pH 7.2) for three consecutive days, following standardization in a tumor-bearing mice model (unpublished observations).

Forty-eight hours after the last dose, peritoneal cells were collected by washing the cavity with cold PBS. Afterwards cell suspension was plated on 24- or 96-well culture plates, as detailed in a later item.

ATF - The ATF of *A. brasiliensis* was obtained according to Fujimiya et al. (1998). Before use, ATF was rehydrated with PBS and the sample was autoclaved in order to obtain a sterile solution (previous experiments have shown that autoclaved samples were more efficient against tumor growth than filtered ones). The use of *A. brasiliensis* in this study is in accordance with the Brazilian rules for Biodiversity (IBAMA – Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renov veis).

The presence of oxalate was checked by heating ATF samples with 500 μ l of 0.1N potassium permanganate for 1 min at 100 C. The final solution of ATF was compared with a standard curve (4.0; 2.0; 0.5; 0.25 and 0.125%) prepared with 1N HCl (Borchers et al. 1999), showing that the residual concentration of oxalate was lower than 0.125%. Endotoxin was analyzed by a *Lymlulus* amoebocyte lysate test (E-toxate kit - Sigma ET0200), and this extract presented less than 0.06 EU/ml.

***C. albicans* suspension** - Yeast cells of *C. albicans*, sample H-428/03, originally isolated from a patient of Hospital das Cl nicas de Botucatu (SP, Brazil) and maintained at -70 C, were defrosted and grown in Sabouraud-Dextrose-Agar medium (Oxoid, Ltd.), at 35 C for 24 h. Cells were collected and washed with sterile pyrogen-free salt-solution and adjusted to 2 x 10⁶ yeasts ml⁻¹. The viability of yeast cells was evaluated by phase-microscopy (99% of viable cells).

Macrophage monolayer preparation and challenge with *C. albicans* - Peritoneal resident macrophages were collected by washing the cavity with cold PBS. Cell suspensions were washed twice with cold complete tissue culture medium (CTCM = RPMI 1,640 liquid culture medium supplemented with 10% fetal calf serum, 20 mM HEPES, 200 mM L-glutamine and 40 μ l ml⁻¹ gentamicine) and macrophages were counted with neutral red solution (0.02%) set at 4 x 10⁵ cells ml⁻¹. Two hundred microliters of cell suspensions was dispensed into 24-well cell culture plates (Nunc, Life Tech. Inc., Maryland, USA) following incubation for 2 h at 37 C under 5% CO₂. Wells were rinsed twice with CTCM at 37 C and non-adherent cells were removed by aspiration of the supernatant.

Adherent cells were then challenged with 2 x 10⁶ yeast cells of *C. albicans* (ratio yeast:macrophage = 25:1) for 30 min at 37 C under 5% CO₂. *C. albicans* yeast cells diluted with CTCM at the same concentration (2 x 10⁶) were plated into wells of the culture plate without macrophages (*C. albicans* control cultures). After this time, the adherent monolayer was detached and lysed by washing each well with sterile non-pyrogenic distilled water (final volume of 2 ml). Samples of recovered suspensions were diluted 1:300 and 100 μ l of each suspension was plated on Sabouraud medium for 48 h at 35 C, following the counting of colony forming units (CFU). The percentage of *C. albicans* recovery was determined by the formula: %CFU recovery = (mean CFU of experimental culture/mean CFU of control culture) x 100.

Phagocytosis assay - Peritoneal resident macrophages were collected and 3 x 10⁵ cells/chamber were cultured in 8-chamber slides (Nunc). The cells were incubated at 37 C, under 5% CO₂ tension and after 2 h non-adherent cells were removed by washing each chamber with CTCM. Adherent cells were then challenged with 15 x 10⁵ yeast cells of *C. albicans* (yeast:macrophage ratio = 5:1) for 30 min at 37 C under 5% CO₂. After this period the slides were washed with complete culture medium, dyed with May-Gr nwald-Giemsa and analyzed under light microscopy. Results were expressed as phagocytic index

calculated as the average number of attached plus ingested yeasts per phagocytizing cell multiplied by proportion of phagocytizing cells (Muniz-Junqueira et al. 2005).

Assay for spontaneous release of hydrogen peroxide (H_2O_2) and NO - H_2O_2 release by peritoneal macrophages was determined using the horseradish peroxidase-dependent phenol red oxidation microassay according to Pick and Mizel (1981). NO release was determined by assaying culture supernatants for nitrite using Griess reagent according to Green (1981).

Analysis of MR expression - Normal mice were inoculated with ATF sterile solution (500 μ g/day) for three days, according to previous standardization. Normal control mice were inoculated with 0.5 ml of sterile PBS and positive controls received 500 μ g of concanavalin A (Con A) 24 h before the collection of peritoneal cells. Expression of MR by peritoneal adherent cells was evaluated by using FITC-labeled bovine serum albumin (FITC-BSA - Sigma), according to Loyola et al. (2002).

*Clearance kinetics of *C. albicans* in the peritoneal cavity* - Animals were i.p. treated with PBS (control group) or different doses of ATF (100, 500 or 2,000 μ g/animal) for three consecutive days. Forty-eight hours after the last dose, the animals were i.p. inoculated with 5×10^6 *C. albicans*; and 1, 6 or 24 h after infection, the peritoneal cavity was washed with 20 ml of PBS. Twenty-five microliters of this suspension was plated on Sabouraud medium and colonies were enumerated after 48 h of culture at 35°C.

Statistical analysis - All the experiments, except analysis of MR, were performed at least twice. Data from one representative experiment were analyzed by the unpaired Student's *t* test when we had only two groups or transformed to square roots and analyzed by Student-Newman-Keuls for multiple comparisons (INSTAT software - Graph Pad, San Diego, CA). Significant difference between groups was concluded for $p < 0.05$.

RESULTS

Ex vivo fungicidal activity - As can be observed in Fig. 1, the i.p. administration of ATF was able to significantly enhance the capacity of peritoneal resident macrophages to kill *C. albicans* yeast cells in a 30 min in vitro challenge assay (Control vs. ATF; $p < 0.05$). The average number of CFUs observed in the control group was 100.17 ± 13.27 CFUs, whereas ATF-treated animals showed 80.17 ± 6.88 CFUs ($p < 0.01$). Analysis of the phagocytic index of peritoneal cells on *C. albicans* yeasts (Fig. 2) showed a significant effect of the treatment on phagocytotic activity (Control = 3.42 ± 0.91 vs. ATF = 7.93 ± 2.00 ; $p = 0.0005$). Morphological observation of these cells have shown that macrophages of ATF-treated animals showed many more phagocytic vacuoles than cells of normal controls (Fig. 3).

Spontaneous production of H_2O_2 and NO - Production of H_2O_2 by peritoneal macrophages is shown in Fig. 4, where it can be observed that ATF-treated mice produced higher levels (2.00 ± 0.54 nmol/2 $\times 10^5$

cells) of this metabolite than control animals (1.06 ± 0.16 nmol/2 $\times 10^5$ cells; $p < 0.001$). Our data indicate that ATF treatment was unable to modify spontaneous NO production (Fig. 5).

MR expression - The treatment of normal mice with ATF increased the expression of MR on macrophage surfaces. In Fig. 6 it can be observed that i.p. inoculation of 500 μ g of ATF (3 consecutive days) induced a more intense reactivity than did the positive control (Con A).

*Clearance kinetics of *C. albicans* in the peritoneal cavity* - Since ATF caused a significant increase in the in vitro killing activity of peritoneal resident macrophages (Fig. 1) and produced higher levels of H_2O_2 (Fig. 4), we decided to evaluate the in vivo effect of this extract. Thus animals were pretreated with different doses of ATF in order to assess their ability to clear *C. albicans* inoculated in the peritoneal cavity. As can

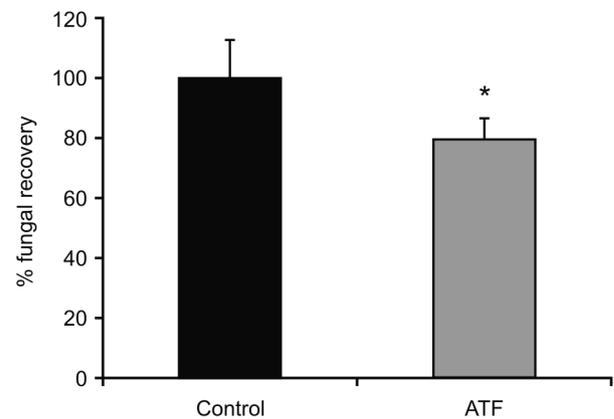


Fig. 1: enhancement of killing activity against *C. albicans* by i.p. inoculation of ATF in normal BALB/c mice. Results represent the mean \pm SD of six mice in each group of a representative experiment (asterisks indicate $t = 3.276$ with 10 degrees of freedom, $p = 0.0083$; Student's *t* test).

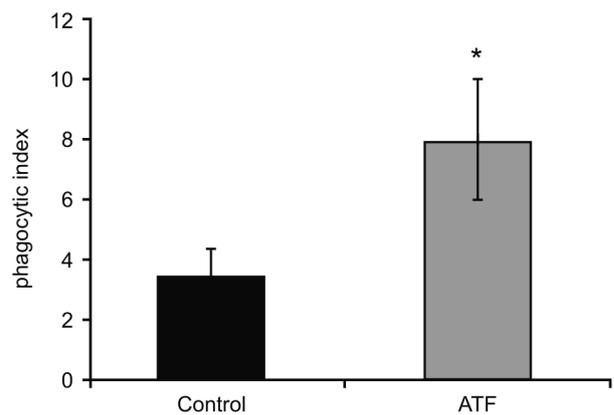


Fig. 2: increased phagocytosis activity by peritoneal macrophages of normal BALB/c mice, after i.p. inoculation of ATF. Animals received 500 μ g/day during three consecutive days before evaluation. Results represent the mean phagocytic index \pm SD of six mice in each group of a representative experiment (asterisks indicate $t = 5.018$ with 10 degrees of freedom, $p = 0.0005$; Student's *t* test).

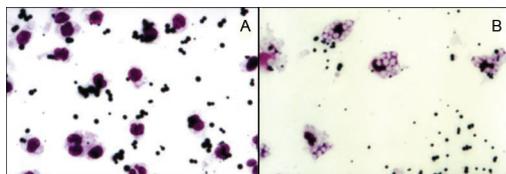


Fig. 3: effect of i.p. inoculation of ATF in normal BALB/c mice on the phagocytic activity of peritoneal macrophages against *C. albicans* (yeast:macrophage ratio = 5:1). A: control group; B: macrophages of ATF group show increased number of digestive vacuoles after in vivo treatment. May-Grünwald-Giemsa, 400X.

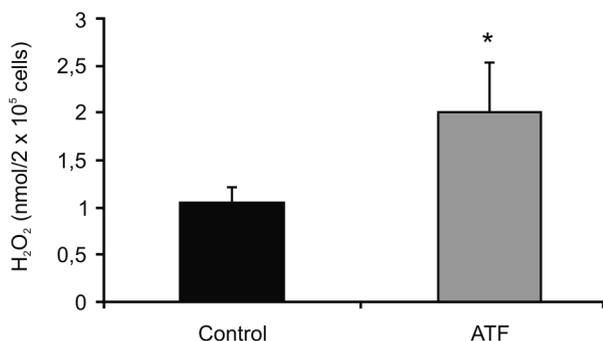


Fig. 4: increased spontaneous production of H₂O₂ by peritoneal macrophages of normal BALB/c mice, after i.p. inoculation of ATF. Animals have received 500 µg/day during three consecutive days before evaluation. Results represent the mean ± SD of six mice in each group of a representative experiment (asterisks indicate $t = 4.025$ with 10 degrees of freedom, $p = 0.0024$; Student's t test).

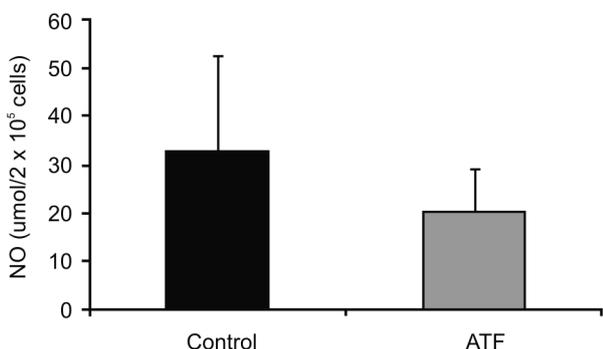


Fig. 5: spontaneous production of NO by peritoneal macrophages of normal BALB/c mice, after i.p. inoculation of ATF. Animals have received 500 µg/day during three consecutive days before evaluation. Results represent the mean ± SD of six mice in each group of a representative experiment ($t = 1.543$ with 12 degrees of freedom, $p = 0.1489$; Student's t test).

be observed in Fig. 7, i.p., administration of ATF for three consecutive days enhanced the killing of *C. albicans* in the first 6 h after infection. The best results were observed by using 500 µg/day (control vs. ATF 500; $p < 0.05$). The number of CFUs found in the control group 1 h after infection was 25.13 ± 3.11 CFUs, whereas animals treated with 500 µg ATF showed 19.35 ± 5.70 CFUs. The number of CFUs observed in the control group 6 h after infection diminished to 8.42 ± 4.97 CFUs, whereas animals treated with 500 µg ATF presented only 4.02 ± 2.06 CFUs.

DISCUSSION

The antitumoral activity of *A. brasiliensis* (formerly *A. blazei*) has been documented through several experimental studies and it is mainly attributed to β-glucan (Mizuno et al. 1990a, b, Fujimiya et al. 1998, Sorimachi et al. 2001); however, there is only one report of its effect against infectious diseases in which the authors analyzed patients with hepatitis C (Grinde et al. 2006). In the present study we analyzed, for the first time, the antifungal effect of a polysaccharide fraction of *A. brasiliensis* ATF and observed that the i.p. inoculation of ATF for just three consecutive days was able to enhance the ability of peritoneal macrophages to kill *C. albicans* yeast both ex vivo (Fig. 1) and in the peritoneal cavity (Fig. 7).

Fujimiya et al. (1998) demonstrated that ATF inhibits the development of Meth A fibrosarcoma by inoculating 500 µg/day. This same dose was chosen in our study because it was also effective against the development of Ehrlich tumor cells and during the standardization phase; it gave us the best results on the in vitro *C. albicans* phagocytosis assay. Extraction of ATF was previously standardized in our laboratory (unpublished observations), according to Fujimiya et al. (1998); and analysis by nuclear magnetic resonance demonstrated that our extract is similar to that presented earlier by the first group to show the antitumoral property of *A. blazei* (Kawagishi et al. 1989, 1990). The protein (13.4%) and carbohydrate (86.6%) concentrations indicate that this extract is rich in carbohydrates as also demonstrated by Ebina et al. (1998). Fujimiya et al. (1998) demonstrated that the antitumoral and immunostimulatory effects of ATF were due to (1→4)-α-D-glucan with (1→6)-β-D-glucan branches, in a 4:1 ratio. Analysis by HMQC showed that the main compound of our extract has a (1→6)-β-glucan structure (data not shown).

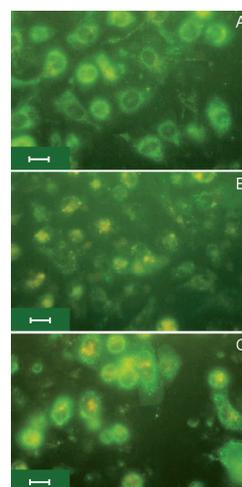


Fig. 6: enhanced expression of mannose receptors (MR) by peritoneal macrophages of normal BALB/c mice, after i.p. inoculation of ATF. Samples were analyzed through fluorescent microscopy after labeling with FITC-labeled α-D-mannosylated BSA for 60 min. A: control group, inoculated with PBS; B: positive control inoculated with 500 µg of Con-A 24 h before evaluation; C: ATF group treated with 500 mg/day during three consecutive days before evaluation. 400X.

Although patients habitually consume the whole dried mushroom or tea orally, we decided to administer ATF through the i.p. route because: a) we were interested in its effect on macrophages; b) we would like to avoid loss of activity due to digestive enzymes; c) vertebrate hosts do not have β -glucanases to metabolize ATF, an absence which could drastically reduce its biological effects, and d) this route was previously used by other authors (Sakurai et al. 1991). Of course the oral route should also be analyzed in further studies to assess the feasibility of using it for human diseases.

Higher ex vivo candidacidal activity of macrophages in ATF-treated mice was associated with higher phagocytic ability, as compared to normal mice (Figs 2, 3). It was also associated with higher spontaneous production of H_2O_2 (Fig. 4) suggesting that the generation of reactive oxygen intermediates (ROIs) could be responsible for the higher fungicidal activity of those cells. Therefore the increased clearance of *C. albicans* from the peritoneal cavity after pretreatment with ATF can probably be attributed to a combination of these two factors. Our results are supported by the findings of Stevenhan and Furth (1993) who showed that the candidacidal activity of human granulocytes is associated with enhanced H_2O_2 production after stimulation with rIFN- γ .

Phagocytosis is essential for the destruction of some pathogens and during this process macrophages enhance the production of ROIs, including H_2O_2 , O_2^- and OH \cdot (Nathan & Root 1977, Johnston et al. 1978), which are highly toxic to microorganisms (Sasada & Johnston 1980). Our observations that ATF enhances the phagocytic and fungicidal activity of peritoneal macrophages are in agreement with this concept. In fact, the attachment of pathogens to macrophages can trigger the secretion of ROIs and NO, thus leading to their destruction (Goldsby et al. 2000). The linking of fungi to polymorphonuclear cells also induces the production of cytotoxic products such as H_2O_2 and O_2^- , which exert fungicidal activity (Calderone et al. 1994).

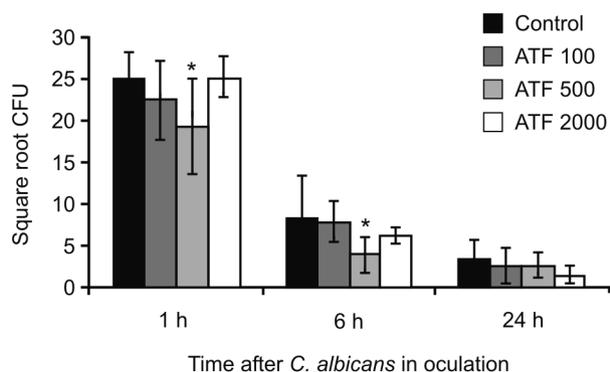


Fig. 7: effect of i.p. treatment with ATF on the in vivo clearance of *C. albicans*. Animals were treated with different doses of ATF and further infected i.p. with *C. albicans*. Clearance of yeast cells was checked by culturing peritoneal exsudate on Sabouraud culture medium plates. Results represent the mean \pm SD of eight mice in each group of a representative experiment (asterisks indicate $p < 0,05$ compared to the respective controls; Student-Newman-Keuls Multiple Comparisons test applied on square roots of data).

Suzuki et al. (1990) demonstrated that oral administration of the β -1,3-glucan fraction, obtained from the *Sclerotinia sclerotiorum* culture supernatant, increases the candidacidal activity of murine peritoneal macrophages, in addition to H_2O_2 and IL-1 production. Sakurai et al. (1991) demonstrated that i.p. administration of this product also increases IL-1 production, which enhances the production of colony-stimulating factor, promoting the proliferation of alveolar macrophages and increasing their candidacidal activity. These data suggest that polysaccharides from this mushroom also could be useful against infectious diseases.

Administration of (1 \rightarrow 3)- β -D-glucan from *Grifola frondosa* also increases the production of NO by murine peritoneal macrophages and induces the expression of IFN- γ mRNA in the liver and peritoneal cells, suggesting that the synthesis of NO is mediated by IFN- γ (Ohno et al. 1996). In fact, Ding et al. (1988) stimulated peritoneal macrophages in vitro with 12 different cytokines and observed that only IFN- γ was able to induce the production of NO. However, in the present study the increased candidacidal activity does not appear to involve NO production since we did not find any significant differences between the ATF-treated and control animals.

The enhancement of phagocytosis could be due to the higher expression of MR, a type of PRR associated with the phagocytosis of non-opsonized microorganisms like *C. albicans* (Asterie-Dequeker et al. 1998), *Escherichia coli* (Ofek et al. 1995) and even *Mycobacterium tuberculosis* (Shepherd & Hoidal 1990). Actually, we observed that the increased MR expression induced by ATF was more intense than the effect of Con A, used as positive control, indicating the high activity of our product (Fig. 6). Mannosylated glycoproteins are recognized by these receptors, promoting endocytosis by macrophages and dendritic cells (Sallusto et al. 1995); and the attachment of microorganisms to MR causes intracellular signaling that is associated with several functional changes, including O_2^- delivery (Berton & Gordon 1983), and the synthesis of IL-1, IL-6, and GM-CSF (Yamamoto et al. 1997).

MR also appears to improve the integration of peptides to MHC molecules, thus enhancing the antigen presentation process (Sallusto et al. 1995, Yamamoto et al. 1997), indicating that this structure could have an important role in the development of specific immune response. However, MR is considered a marker of non-stimulated macrophages, since IFN- γ induces it decreased expression on cell surface due to a lower level of mRNA transcription (Harris et al. 1992). Marodi et al. (1993) observed that the high *C. albicans* killing activity of IFN- γ -activated macrophages is associated with low MR expression, although the remaining MR showed increased phagocytic function.

In agreement with these reports, in the present study we observed that macrophages of ATF-treated animals showed increased MR but did not enhance the production of NO, suggesting that they were not activated in an IFN- γ -dependent way. Since ATF failed to induce NO production in our experimental conditions, it is possible to consider that the expression of MR and increasing

of candidacidal activity were due to the stimulation of TH2 lymphocytes. This idea is supported by previous observation by our group that subcutaneous, inoculation of ATF was not able to induce the in vitro production of IFN- γ and TNF- α , but stimulated the production of IL-10 by spleen cells of normal mice (unpublished observations). In addition, Longoni et al. (1998) observed that IL-10 enhances MR expression by human monocyte-derived dendritic cells, and Stein et al. (1992) demonstrated that IL-4, a TH2 cytokine, increases both MR expression and MR-mediated endocytosis. In the present study we did not evaluate the effect of ATF on the production of IFN- γ but, in a parallel investigation employing experimental infection with *Paracoccidioides brasiliensis*, we observed that the number of IFN- γ -producing cells (ELISpot) was diminished under treatment with ATF on the 14th day.

Although no toxicity had been observed, even by using the higher dose of 2,000 μg , our data showed that 500 μg had a better effect on fungicidal activity of macrophages than 2,000 μg , both 1 and 6 h after *C. albicans* inoculation. We do not have an explanation for this, but 500 μg appears to be the optimum concentration for treatment, since in the tumor model we also observed that this concentration had a better effect than higher ones (data not shown). Besides, we previously observed that β -glucan from *Saccharomyces cerevisiae* also presented a better effect on fungicidal activity of macrophages against *P. brasiliensis* when animals were treated with a lower dose (20 μg vs. 100 μg) (Pelizon et al. 2005).

In summary, our data suggest that the polysaccharide-rich fraction of *A. brasiliensis* could enhance host resistance against infectious diseases by stimulating the microbicidal activity of macrophages. Considering that the increase of H₂O₂ was not followed by a similar increase in NO production, we suggest that H₂O₂ helped by MR-promoted phagocytosis may be sufficient to promote this phenomenon.

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