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# Leukotrienes are involved in leukocyte recruitment induced by live *Histoplasma capsulatum* or by the β-glucan present in their cell wall

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**1** The inflammatory cell influx towards the peritoneal cavity in mice inoculated i.p. with live or dead *Histoplasma capsulatum* or with its subcellular preparations was studied. We also evaluated the effects of dexamethasone (Dexa) or MK886, an inhibitor of leukotriene (LT) biosynthesis, on the recruitment of leukocytes.

**2** Live yeast form of fungus (LYH) induced an increase in neutrophils (NE) which was highest 4 to 24 h after inoculation. Mononuclear cell (MN) migration beginning at 24 h with a gradual increase over 48 and 168 h, and an eosinophil (EO) recruitment occurs between 24 and 48 h.

**3** NE and EO recruitment induced by dead mycelial form of fungus (DMH) was greater than that observed for dead yeast form of fungus (DYH). A similar leukocyte migration pattern was seen after i.p. injection of the alkali-insoluble fraction (F1) from DYH (F1Y) and F1 from DMH (F1M) this being more active than former. The difference in concentration of β-glucan in DYH and DMH could explain the different inflammatory capacity exhibited by the two forms of *H. capsulatum*.

**4** LT seems to be the principal mediator of leukocyte migration in response to LYH, DYH or DMH or to β-glucan. However, other mediators appear to contribute to NE and EO migration since the treatment with Dexa was more effective in inhibiting cell migration than MK886. Complement dependent leukocyte migration may participate in this recruitment. Treatment with MK886 completely abolished MN cell migration, indicating its dependence on the presence of LT.

**Keywords:** Eosinophils; neutrophils; mononuclear cells; leukotrienes; dexamethasone; MK886; *Histoplasma capsulatum*; histoplasmosis; β-glucan; inflammation

**Abbreviations:** βM, β-glucan isolated from F1M; βY, β-glucan isolated from F1Y; BHI, brain heart infusion; CM, chitin isolated from F1M; CY, chitin isolated from F1Y; Dexa, dexamethasone; DMH, dead mycelial form of fungus; DYH, dead yeast form of fungus; EO, eosinophil; F1M, alkali-insoluble fraction F1 from DMH; F2M, alkali-soluble fraction F2 from DMH; F3M, alkali-soluble fraction F3 from DMH; F1Y, alkali-insoluble fraction F1 from DYH; F2Y, alkali-soluble fraction F2 from DYH; F3Y, alkali-soluble fraction F3 from DYH; ILs, interleukins; $LTB_4$ (C4, D4, E4), leukotriene $B_4$ (C4, D4, E4); LYH, live yeast form of fungus; M, mycelial or mycelium form of fungus; MCP-1, monocyte chemoattractant protein; MN, mononuclear cell; NE, neutrophil; PAF, platelet-activating factor; PMN, polymorphonuclear leukocytes; TNF, tumour necrosis factor; Y, yeast form of fungus

## Introduction

*Histoplasma capsulatum* is a dimorphic pathogenic fungus that causes a wide spectrum of disease when condidiospores or mycelial fragments are inhaled (Schwartz, 1981). By far the most common clinical manifestation of acute *H. capsulatum* infection is pulmonary disease that usually is self-limiting over a period of several weeks and is characterized by chronic granulomatous and suppurative inflammatory reaction. During the last few decades the incidence of histoplasmosis infection has dramatically increased worldwide, mainly as the result of alterations in immune status associated with the acquired immunodeficiency syndrome (AIDS) epidemic, cancer chemotherapy, and organ and bone marrow transplantation (Georgopapakadou & Walsh, 1996). Many factors are associated with the disease, some of them involved within the fungus, such as fungal strain and virulence (Goodwin & Des Prez, 1978) and others related to host competence (Goodwin *et al*., 1981). Few attempts have been made to determine what cellular factor(s) might be responsible for the inflammatory reaction caused by this fungus and how it could be controlled. The causative organism, *H. capsulatum*, is a dimorphic fungus with a yeastlike (Y) morphology in tissue or in artificial media at 37°C. However, in soil or artificial media at room temperature it forms a filamentous mycelium (M) that carry the reproductive conidia. In the body, the Y form is found intracellularly in macrophages, where it survives and multiplies. Chemical and ultrastructural studies of the cell walls of the Y and M forms of the fungus revealed that the Y form contained high amounts of α-glucan and low level of β-glucan, galactomannan and chitin, whereas the cell wall of the M form contained high amounts of β-glucan and low level of α-glucan, galactomannan, and chitin (Kanetsuma *et al*., 1974). We have previously demonstrated such differences in another dimorphic fungus, *Paracoccidioides brasiliensis*. Only β-glucan from *P. brasiliensis* was able to trigger attraction of neutrophils (NE) and mononuclear (MN) cells at the inflammatory foci, and the difference in the concentration of this compound in the cell walls of Y and M forms could explain the different inflammatory capacity exhibited by the two forms of the fungus (Cararetto-Alves *et al*., 1987; Silva *et al*., 1994).

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Previous studies of the inflammatory response in lungs of humans or experimental animals to pulmonary histoplasmosis have described the cell infiltration in lung tissues (Procknow *et al*., 1960; Baughman *et al*., 1986). Although these studies have detailed the serial changes in the inflammatory response to *H. capsulatum*, no information is available regarding the dynamics of inflammatory cells within lungs and other organs during the course of infection. We believe that the early inflammatory response to live fungus or to its cell wall components is of critical importance to both host and parasite relationship and to the establishment of infection.

Inflammatory cells release a wide variety of mediators, including local preformed mediators, newly synthesized metabolites of arachidonic acid, and soluble pro-inflammatory proteins including kinins and cytokines (Stewart, 1995; Lee & Arm, 1991; Xing *et al*., 1999). Glucocorticoids are potent inhibitors of virtually every type of inflammatory reaction and exert a strong effect on leukocyte recruitment (Flower, 1988; Perretti & Flower, 1993; Barnes, 1998). Dexamethasone (Dexa) is routinely used to modulate negatively cell migration into sites of inflammation and this action is accomplished, in part, by its potent effect on the synthesis of pro-inflammatory cytokines and chemokines coupled to a reduced degree of leukocyte responsiveness (van der Velden, 1998). On the other hand, leukotrienes (LT) are products of arachidonic acid metabolism derived through an oxygenation of the fatty acid and a subsequent dehydrase step by 5-lipoxygenase (Miller *et al*., 1990). Leukotriene B4 ($LTB_4$) is a potent stimulant of leukocyte functions including the chemotaxis, chemokinesis, and aggregation of polymorphonuclear leukocytes (PMN) (O'Byrne, 1988). Moreover, $LTB_4$, $LTD_4$, $LTC_4$ and $LTE_4$ are chemoatractant for eosinophils (EO) (Faccioli *et al*., 1991; Woodward *et al*., 1991; Bureau *et al*., 1997; O'Byrne, 1997). The compound MK886 (Gillard *et al*., 1989; Ford-Hutchinson *et al*., 1991) is a potent inhibitor of LT biosynthesis in NE, both *in vivo* and *in vitro*, thus indicating that the compound is suitable for studying the role of LT in a variety of inflammatory situations (Puustinen *et al*., 1988). The objective of the present investigation was: to characterize the kinetics and type of cells recruited to the peritoneal cavity of mice induced by i.p. injection of live or dead fungus, or by fungal cell wall fractions; to explore the nature of the cell wall components of *H. capsulatum* involved in inflammatory cell recruitment leading to the development of an intense inflammatory reaction; and to characterize the possible mediators involved in cell migration.

## Methods

### *Animals*

Outbred female Swiss mice were obtained from the animal house of the University Campus of Ribeirão Preto, University of São Paulo, and were maintained under standard laboratory conditions. Infected animals were kept in biohazard facilities, housed in cages within a laminar flow safety enclosure.

### *Fungal strain and culture conditions*

The strain of *H. capsulatum* used in this work was isolated from a patient of the Clinical Hospital, School of Medicine of Ribeirão Preto, University of São Paulo. The live mycelial phase was obtained by the culture of fungus at 25°C in BHI (Difco) media. The live yeast form of fungus (LYH) was obtained and subcultured at 37°C on glucose-cysteine-blood BHI (Howard *et al*., 1971). Yeast cells were used when the viability was over 95% by fluorescein diacetate and ethidium bromide staining (Corrêa *et al*., 1990).

### *Preparation and fractionation of cell walls*

The Y and M forms of the fungus, cultured as described above, were harvested, formalin-killed and washed several times with distilled water. These preparations were used as dead yeast form of fungus (DYH) and dead mycelial form of fungus (DMH), or used to obtain the cell wall fractions. Briefly, the washed DYH or DMH cells were disrupted by ultrasonic vibration at 200 W for 3 min; this process was repeated six times. Extensive disruption was confirmed by microscopy. The cell walls were collected and washed three times with distilled water by centrifugation at $5000 \times g$ for 5 min. Lipids were extracted by repeated soaking of the walls in chloroform/methanol (2 : 1, v $v^{-1}$) with stirring at room temperature for 2 h. The extracts were separated by centrifugation at $5000 \times g$ for 5 min and the insoluble residue was re-extracted three more times as described above. The resulting insoluble cell wall residue was fractionated as previously described (Silva & Fazioli, 1985; Carareto-Alves *et al*., 1987). Briefly, cell wall was suspended in 1N NaOH and gently stirred at room temperature for 1 h. After centrifugation at $5000 \times g$ for 10 min, the supernatants were collected and the procedure was repeated four times. The alkali-insoluble sediment was washed with water until it reached pH 7.0 and then washed with ethanol, followed by acetone and diethyl ether. After drying, the resulting white powder was named the F1 fraction from DYH (F1Y) and from DMH (F1M). The pooled supernatants obtained after treatment of cell walls with NaOH, were neutralized with acetic acid and left to stand overnight at 4°C, after which a precipitate formed. The suspension was then centrifuged as before. The precipitate and the supernatant were collected, dialyzed separately against distilled water and freeze-dried, yielding fraction F2 (alkali-soluble and precipitable with acid from DYH, F2Y, and from DMH, F2M) and F3 (alkali-soluble and non-precipitable with acid, from DYH, F3Y and DMH, F3M), respectively.

### *Preparation of β-glucan by enzymatic treatment of F1 fractions*

Samples (10 mg) of F1Y or F1M fractions were incubated at 37°C for 24 h with 1 ml 0.6 mg chitinase $ml^{-1}$ in 0.05 M sodium acetate buffer (pH 5.0) and 5 $\mu$l toluene. After incubation, the supernatant was removed and the residue was further treated with chitinase. The procedure was repeated five times and the resulting sediment was washed with water and with ethyl alcohol and finally dried in an oven at 37°C. The chitinase-treated F1 fractions were also submitted to Pronase E treatment (1 mg or 4 units $ml^{-1}$) for varying times at 37°C in a 0.05 M Tris/HCl buffer, pH 7.5, containing ($ml^{-1}$): CaCl2, 555 $\mu$g; cycloheximide, 50 $\mu$g; and chloramphenicol, 25 $\mu$g. After several washes the resulting precipitates were called $\beta$-glucan isoled from F1Y ($\beta$Y) and $\beta$-glucan isolated from F1M ($\beta$M). To obtain chitin, both F1 fractions were incubated with cellulase (EC 3.2.1.4), $\beta$-glucosidase (EC 3.2.1.21) or $\alpha$-glucosidase (EC 3.2.1.20), obtained from Sigma (1 mg or 10 units in 1 ml 0.05 M acetate buffer, pH 5.0) and the mixture was incubated at 37°C. After 24 h incubation, the reaction mixture was placed in a boiling-water bath for 5 min to inactivate the enzymes. Qualitative analyses for monosaccharides were performed as previously described (Silva *et al*., 1994)

and infrared spectra were recorded on KBr discs using a Shimadzu IR-27G spectrophotometer.

### *Evaluation of leukocyte influx into the peritoneal cavity*

Groups of five mice were injected i.p. with $6 \times 10^5$ forms of LYH, 400 $\mu$g of DYH or DMH, or with 100 $\mu$g of the following preparations in 1 ml phosphate-buffered saline (PBS): F1Y, F1M, F2Y, F2M, F3Y and F3M. Control groups received only PBS. At 4, 24, 48 or 168 h after the injection of stimulants the animals were killed with anaesthetic and the cells from the peritoneal cavities were harvested by injection of 3 ml PBS containing 5 u $ml^{-1}$ of heparin. The abdomens were gently massaged and blood-free cell suspension was carefully withdrawn with a syringe. Abdominal washings were placed in plastic tubes and total cell counts were performed immediately in a Neubauer Chamber. Differential counting were obtained using Rosenfeld-stained cytospin preparations (Faccioli *et al*., 1990).

### *Treatment with Dexa and MK886*

The effects of systemic treatment of mice with Dexa or with the compound MK886, an inhibitor of LT biosynthesis, on the recruitment of NE, EO or MN cells to the peritoneal cavity were analysed according to the following protocol. All groups received one dose of Dexa (1 mg $kg^{-1}$) s.c. or MK886 (1 mg $kg^{-1}$), p.o., 1 h before the injection of stimulants and another dose 1 h before the sacrifice. Moreover, groups that were sacrificed 48 or 168 h after stimulus received extra doses of inhibitors 24 or 24 and 48 h, respectively.

### *Materials*

L-663,536 (3-[1*p*-chlorobenzyl)-5-isopropyl-3-tert-butylthioindo-2-yl]-2,2-dimethylpropanoic acid) or MK886 (Gillard *et al*., 1989) was a generous gift from Merck Frosst Canada Inc. Dexa (sodium phosphate salt) was from Merck Co. Inc.

### *Statistical analysis*

All results are shown as the mean $\pm$ s.e.mean. Statistical differences were analysed by non-parametric Mann-Whitney. For comparison between DMH and DYH data we also used ANOVA test. A $P<0.05$ value was considered to be statistically significant.

## Results

### *Comparison of the inflammatory cell recruitment activity induced in mice by live (LYH) or dead fungus (DYH and DMH)*

The response of mice to i.p. administration of live or dead fungus was studied by evaluating the influx of leukocytes into the peritoneal cavity. The number of NE, EO and MN cells in the peritoneal fluid measured 4, 24, 48 and 168 h after injection of $6 \times 10^5$ LYH or 400 $\mu$g of DYH or DMH into the peritoneal cavity of mice is shown in Figure 1. All of the stimulus significantly increased the number of inflammatory cells in the peritoneal cavity when compared with the control group receiving only PBS. The kinetics of cell recruitment induced by LYH or by both forms of dead fungus revealed a gradual increase in NE which was highest 4–48 h after inoculation and which declined slowly over 48 h. In contrast, the MN cell phase beginning at 24 h and with a gradual increase over 48 and 168 h after injection was observed for all stimulus. Interestingly, an intermediate phase of cell recruitment was observed for EO, which peaked between 24 and 48 h. Comparisons of EO and MN cells recruitment between LYH and DYH showed that the live fungus was more effective in recruiting both types of cells than the dead fungus. These results suggest that the somatic structure of the fungus *H. capsulatum* could be involved in the inflammatory cell recruitment. Moreover, DMH was more active in inducing NE and EO recruitment than DYH (NE 4 h $P=0.002$; EO 24 h $P=0.02$; EO 48 h $P=0.04$).

### *Comparison of the inflammatory cell recruitment activity induced in mice by cell wall fractions*

In an attempt to determine which cell wall components are involved in the inflammatory cell recruitment, the DYH and DMH were fractionated according to their different solubilities in alkali. Three main fractions were obtained from each mass of dead fungus: F1 (containing $\beta$-glucan) F2 (containing $\alpha$-glucan), and F3 (containing galactomannan). The results shown in Figure 2 are for experiments where the NE, EO and MN cell counts in the peritoneal fluid were measured at 4, 24, 48 and 168 h after injection of F1Y, F1M, F2Y, F2M, F3Y, F3M or PBS. With both F1 fractions, F1Y and F1M, the number of NE was highest after 4–24 h, and declined slowly after 48 h with F1M being more active than F1Y. An intermediate phase of cell recruitment was observed for EO which peaked between 24 and 48 h. On the other hand, a late MN cell phase beginning at 48–96 h after F1 injection was observed. F2 and F3 had no effect on NE and EO influx under the experimental conditions used. However, F2 and F3 were able to induce MN cell migration after 168 h of injection but to a lower extent than that observed for the F1 fraction. When equal amounts of both F1 fractions were injected separately into the mouse peritoneal cavity they induced different numbers of leukocyte recruitment as shown in Figure 2. F1M was able to induce more cell recruitment than the F1Y fraction, suggesting that the active components present in both cell wall forms may be different or that they may be the same but found in different concentrations.

Quantitative analyses (data not shown) showed that both F1 fractions contained the same amount of glucosamine. However, glucose was 2 fold less abundant in the F1Y than in F1M chitinase treatment of both F1 fractions, which were previously treated with trypsin and pronase, yielded a product characterized as $\beta$-glucan by infrared spectroscopy (data not shown). Moreover, cellulase and $\beta$-glucosidase treatment of F1Y and F1M yielded a product characterized as chitin by the same spectroscopic technique. These results suggest that F1Y and F1M contain chitin and $\beta$-glucan, with chitin found in the same amount in both fractions and $\beta$-glucans, at a higher concentration in F1M than in F1Y. To test whether chitin or $\beta$-glucan was responsible for the leukocyte cell migration activity of both F1 fractions, we isolated chitin (CY and CM, obtained from F1Y and F1M, respectively) and $\beta$-glucan ($\beta$Y and $\beta$M, obtained from F1Y and F1M, respectively) by enzymatic treatment as described above and looked at total leukocyte influx into the peritoneal cavity of mice, 24 h after i.p. injection of the preparations. Injections of 100 $\mu$g of $\beta$Y or $\beta$M into the peritoneal cavity of mice produced a significant increase in total leukocytes 24 h after inoculation ($7.4 \pm 0.8 \times 10^6$ ml and $7.2 \pm 0.7 \times 10^6$ ml, respectively) when compared to the inoculation of CY or CM ($1.7 \pm 0.2 \times 10^6$ ml and $1.9 \pm 0.2 \times 10^6$ ml, respectively) or compared to the

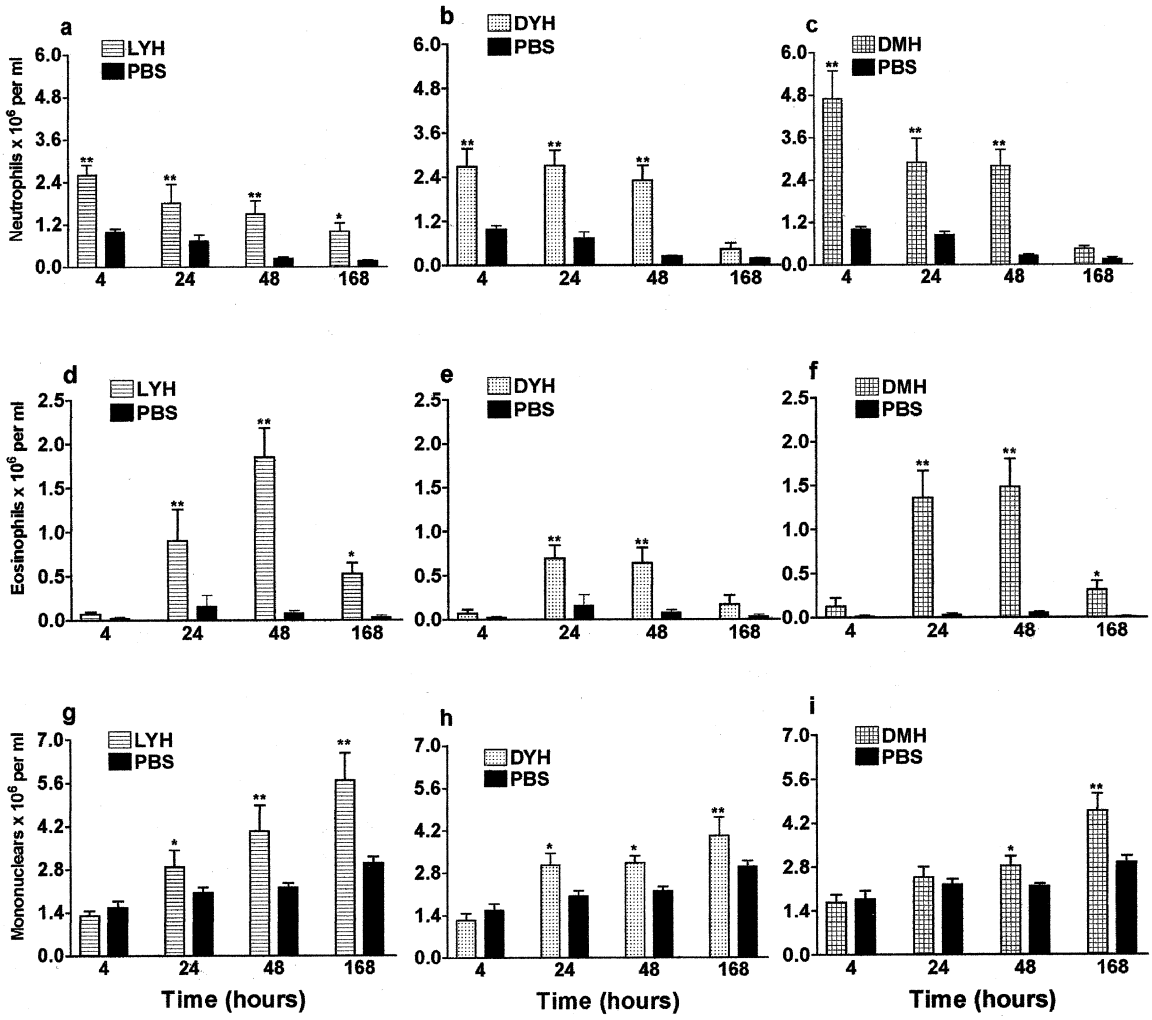


**Figure 1** NE, EO and MN cell recruitment induced by i.p. inoculation of mice with $6 \times 10^5$ yeast-like forms of LYH, or with 400 $\mu$g of DYH or DMH. Cell numbers are expressed as the mean of 12–15 mice per group $\pm$ s.e.mean. $^*P<0.05$ and $^{**}P<0.01$ when compared to PBS group.

inoculation of PBS ($1.6 \pm 0.3 \times 10^6$ ml). The results demonstrated that $\beta$-glucan is the active component of the F1 fractions of both forms of the fungus.

### *Effect of MK886 and Dexa on leukocyte recruitment*

As glucocorticoids are potent inhibitors of virtually every type of inflammatory reaction and the compound MK886 is a leukotriene biosynthesis inhibitor, we studied the effect of Dexa or MK886 on leukocyte recruitment induced by live or dead fungus or by its F1 fraction. The effects of MK886 and Dexa on fungal (fractions)-induced inflammatory cell recruitment are shown in Figures 3–5. Treatment of animals with MK886 (1 mg/kg/day) was effective in reducing: the number of NE in the peritoneal cavity at 4, 24 and 48 h after inoculation of LYH, DYH, DMH, F1Y or F1M (Figure 3); the number of EO at 24 and 48 h after inoculation of the same stimulants (Figure 4); or the number of MN cells at 48 and 168 h after inoculation of the fungus or its F1 fractions (Figure 5). The same pattern of leukocyte recruitment inhibition as described above for MK886 was also observed by the treatment of animals with Dexa (1 mg/kg/day). However, Dexa was more efficient in inhibiting inflammatory cell recruitment than MK886 in the presence of the same stimulants (Figures 3–5).

## Discussion

The experiments carried out in the present study clearly demonstrate that *H. capsulatum* induces migration of leukocytes into the peritoneal cavity of mice injected i.p. with live or dead forms of fungus or with the cell wall fraction F1, which contains mainly $\beta$-glucan. The number of leukocytes observed after F1 treatment was greater than in mice inoculated with the other cell wall fractions or with PBS. The kinetics of the cell migration induced by the active preparations permitted us to characterize the gradual increase in NE, which was reduced at later times, simultaneously with an increase in MN cells. An intermediate phase of EO migration was also observed that peaked between 24 and 48 h after inoculation of stimulants. These data are in agreement with that described by Baughman *et al.* (1986), showing that in bronchoalveolar lavage fluid obtained from a patient with a yeast pulmonary infection, an influx of NE occurs at the beginning of infection followed by MN cell infiltration after 2 weeks. Both types of cells are present during the chronic infection. On the other hand, NE migration induced by LYH and DYH were of similar magnitude, but EO migration under the same conditions of stimulus was reduced. It appears that the live fungus secretes other components that take part in EO and MN cell recruitment. When cell migration

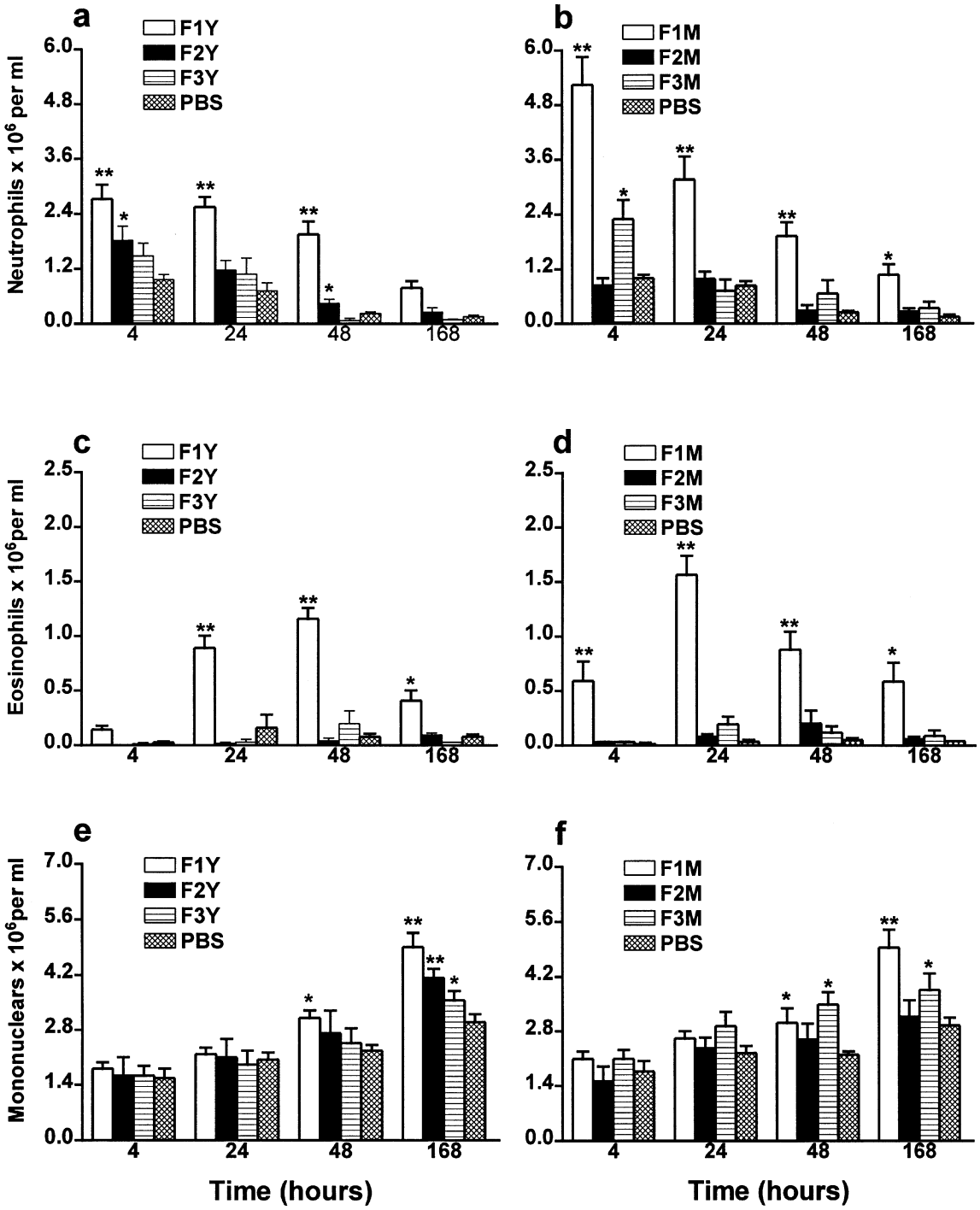


**Figure 2** NE, EO and MN cell recruitment induced by i.p. inoculation of mice with 100 $\mu$g of the polysaccharide fractions F1, F2 and F3, isolated from the cell walls of either DYH or DMH. Cell numbers are expressed as the mean of 12–15 mice per group $\pm$ s.e.mean. $^{*}P<0.05$ and $^{**}P<0.01$ when compared to PBS group.

was compared at 48 and 168 h post-injection, DYH was less potent than LYH in inducing EO recruitment. However, DMH had similar activity to LYH, which can be explained by the high amount of $\beta$-glucan found in DMH.

Eosinophilia is not usually recognized as a manifestation of histoplasmosis. Nonetheless, eosinophilia exceeding 15–20% of the leukocyte count is not unusual in the disseminated form of the disease (Bullock *et al*., 1979). The onset of eosinophilia in our experimental model is earlier than that found in a well established parasitic model of eosinophilia, where EO migration began at 6 days and peaked at 18 days after infestation with *Toxocara canis* (Faccioli *et al*., 1996).

Polysaccharides are one of the main component of the cell wall of *H. capsulatum*. Mice injected i.p. with the polysaccharide F1 fraction showed a greater influx of NE, EO and MN cells when compared with animals treated with other polysaccharides present in fractions F2 and F3 and with PBS. These results, taken as a whole, suggest that the active F1 fraction stimulates the chemotactic systems of the host. Chemical and spectroscopic analysis showed that the chemical constituents present in both F1 fractions, F1Y and F1M, are similar, so that the constituent responsible for the induction of cell migration to the mouse peritoneum may have been present in different concentrations in the two fungal forms studied. In

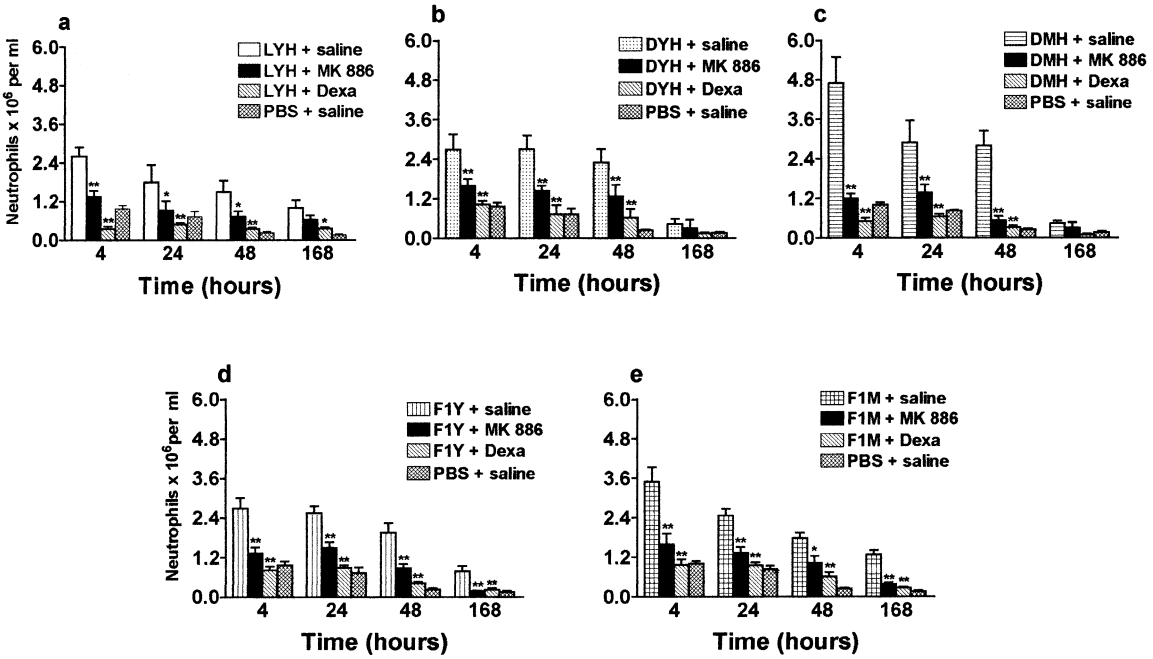


**Figure 3** Effect of MK886 and Dexa on NE recruitment induced by i.p. inoculation of mice with $6 \times 10^5$ yeast-like forms of LYH, 400 $\mu$g of DMH or DYH, or with 100 $\mu$g of a polysaccharide fraction F1 isolated from cell walls of either DYH or DMH. Cell numbers are expressed as the mean of 12–15 mice per group $\pm$ s.e.mean. $^*P<0.05$ and $^{**}P<0.01$ when compared to stimulated group without drug treatment.

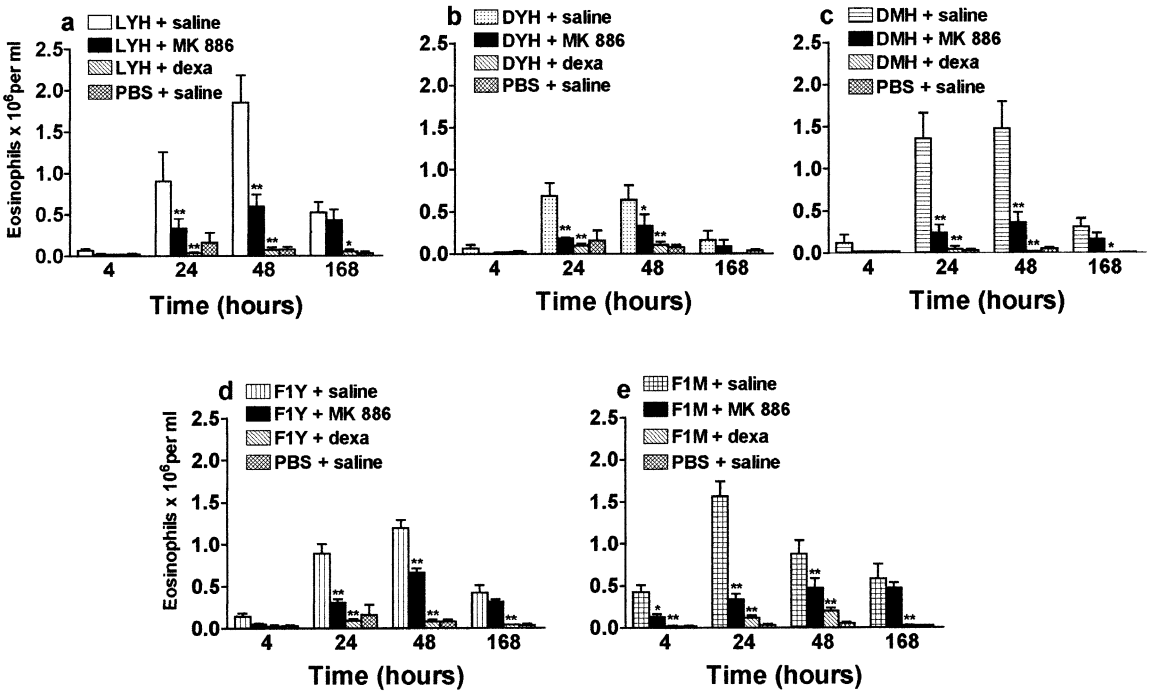


**Figure 4** Effect of MK886 and Dexa on EO recruitment induced by i.p. inoculation of mice with $6 \times 10^5$ yeast-like forms of LYH, 400 $\mu$g of DMH or DYH, or with 100 $\mu$g of a polysaccharide fraction F1 isolated from cell walls of either DYH or DMH. Cell numbers are expressed as the mean of 12–15 mice per group $\pm$ s.e.mean.. $^*P<0.05$ and $^{**}P<0.01$ when compared to stimulated group without drug treatment.

fact, we found higher levels of $\beta$-glucan in M form than in Y form of the fungus. In contrast, chitin levels were similar in the two F1 fractions. Moreover, the results also demonstrated that $\beta$-glucan was the main active component in both F1 fractions. The results obtained here suggest that the ability of DMH and F1M to recruit more leukocytes was related to the higher proportion of $\beta$-glucan in the cell wall of the mycelial form of the fungus. When the transition of the mycelial form to the

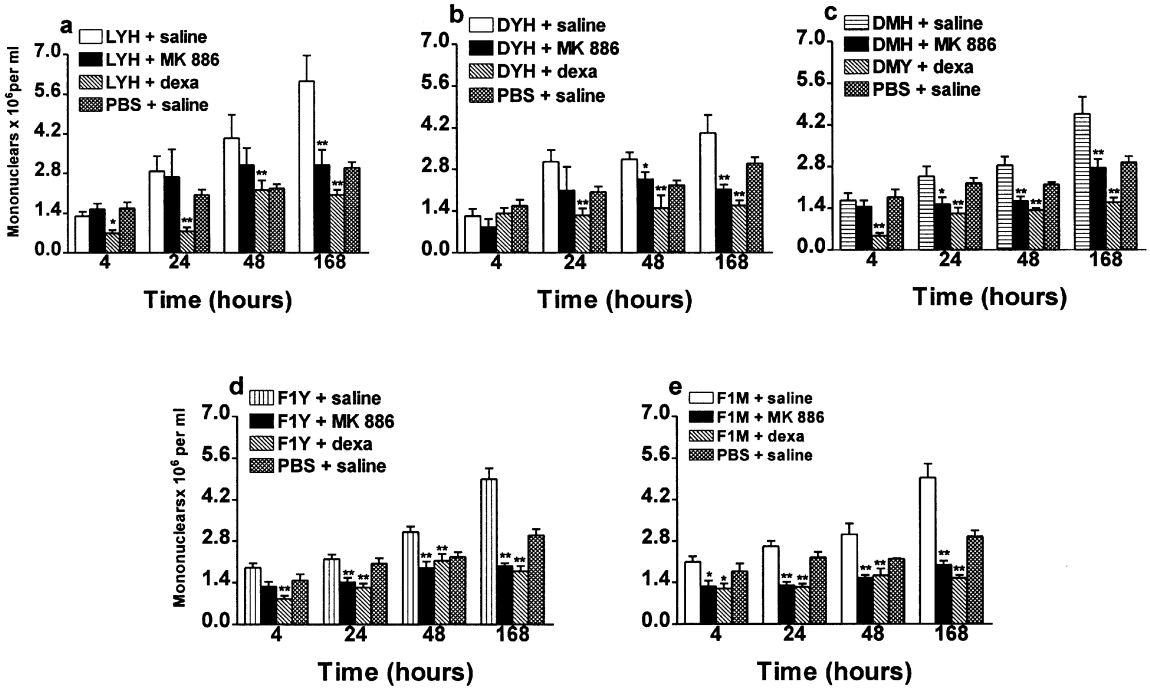


**Figure 5** Effect of MK886 and Dexa on MN cell recruitment induced by i.p. inoculation of mice with $6 \times 10^5$ yeast-like forms of LYH, 400 $\mu$g of DYH or DMH, or with 100 $\mu$g of a polysaccharide fraction F1 isolated from cell walls of either DYH or DMH. Cell numbers are expressed as the mean of 12–15 mice per group $\pm$ s.e.mean.. $^*P<0.05$ and $^{**}P<0.01$ when compared to stimulated group without drug treatment.

yeast form of *H. capsulatum* occurs at 37°C, there is activation of $\beta$-glucanases enzymes responsible for $\beta$-glucan lyses (Maresca & Kobayashi, 1989). Consequently the yeast form has less $\beta$-glucan than the mycelial form. Moreover, as demonstrated by Kanetsuma *et al.* (1974) and Hallak *et al.* (1982) in the transition process from the mycelial to the yeast form, the enzyme $\beta$-glucan synthetase induces a change in the quaternary structure of this molecule. Thus, the preservation of $\beta$-glucan structure in the mycelial form may be responsible for the more efficient binding of this compound to the surface of host cells where it could induce the release of NE and EO chemoattractants.

Several investigators have shown that the integrins CD11b/CD18 (Mac-1), present on NE and MN cell surface, mediate the binding and phagocytosis of bacteria and yeast-like fungus by these cells through the presence of $\beta$-glucan in the external layer of the bacterial and fungal cell walls (Petersen *et al.*, 1994; Steadman *et al.*, 1996). Moreover, the binding of $\beta$-glucan to Mac-1 integrin on NE surface induces the activation of the enzyme 5-lipoxygenase and the concomitant release of $LTB_4$ contributing to the amplification of the inflammatory response. This process was inhibited by an antibody to Mac-1 (Steadman *et al.*, 1991, 1996; Petersen *et al.*, 1994).

Cell wall components also have been associated with the dimorphism and virulence of *H. capsulatum* (Kobayashi & Guilliacci, 1967), $\alpha$-glucan, present in the F2 fractions, has been strongly implicated in the virulence of the fungus (Maresca & Kobayashi, 1989). This polysaccharide exists in high concentration only in the parasitic Y form of the fungus. The reduced amount of $\alpha$-glucan in the cell walls mutant fungi is directly correlated with a decrease in virulence. The M form of the fungus used here contains 50 fold less $\alpha$-glucan than the Y form of the fungus (unpublished data). In the present study, fraction F2, which is rich in $\alpha$-glucan and located externally on the fungal cell wall (Kanetsuma *et al.*, 1974) did not induce a significant leukocyte migration towards the peritoneal cavity when compared with the controls. The relatively greater proportion of $\beta$-glucan and lesser proportion of $\alpha$-glucan in the cell wall of the M form of the fungus can explain its diminished virulence through elicitation of a more vigorous inflammatory response and presumably more efficient clearance by the host. In contrast, the high proportion of $\alpha$-glucan, which is located more externally on the cell wall of the Y forms of the fungus, protects $\beta$-glucan from metabolism by the host. Therefore, the $\beta$-glucan is degraded more slowly, resulting in a continuous stimulus for a long period of time at the site of inoculation.

Chemical signals generated at sites of inflammation initiate the process of leukocyte accumulation *in vivo*. These may be generated in tissue fluid (e.g., the complement-derived protein fragment C5a), or released from the host cells (e.g., cytokines, PAF, and LTB4). The complement system in tissue fluid plays an important role in certain immune and nonimmune inflammatory reactions. Probably the most important product of complement activation as a trigger for NE and EO accumulation is C5a (Faccioli *et al*, 1991; Shuster *et al.*, 1997). In previous work we demonstrated that Y forms of *P. brasiliensis*, a dimorphic fungus closely related to *H. capsulatum*, can activate the complement system (Crott *et al.*, 1993) and the products of the complement cascade are involved in leukocyte migration and activation (Crott *et al.*, 1997). In *P. brasiliensis* also the F1 fraction seems to be one of the fungal components responsible for this activation. The F1 fraction from less virulent strains is more effective in complement activation, therefore this differential action on complement may account for the more rapid elimination of less virulent fungal strains. This fact could also explain why the host does not permit the establishment of infections through the mycelial forms of the fungus once their cell wall composition becomes high in $\beta$-glucan. However, these considerations require further investigation specifically directed at the study of elimination of *H. capsulatum* by the host.

The lipid mediators PAF and LTB4, released on stimulation of cells at inflammatory sites, can induce NE and EO activation *in vivo* and *in vitro* and are potent chemoattractants for these cells (Faccioli *et al*., 1991; Bureau *et al*., 1997; O'Byrne, 1997). Moreover these mediators play a role in pulmonary inflammations such as asthma and respiratory allergy (Lee & Arm, 1991). In order to verify the contribution of LTs on leukocyte recruitment induced by LYH, DYH, DMH, F1Y and F1M, mice were treated daily with the compound MK 886. The treatment significantly inhibited NE, EO and MN cell migration, suggesting the involvement of LTs. Because the lipoxygenase inhibitor MK886 inhibited EO migration induced by F1M and F1Y, it appears that the main EO-chemoattractants are LT. It is known that LTB4, LTD4, LTC4, and LTE4 are chemoattractant for EO (Faccioli *et al*., 1991; Woodward *et al*., 1991; O'Byrne, 1997). On the other hand, among the LTs, only LTB4 is a chemotactic factor for NE and macrophages (Lee & Arm, 1991; Henderson, 1994). Moreover, the lipoxygenase products of arachidonic acid, mainly LTB4, LTC4 and LTE4, are able to enhance the expression of complement (C3b) receptors and to evoke chemotaxis of NE and EO (Nagy *et al*., 1982).

MK886 was more effective in inhibiting NE migration induced by DMH (62%) and F1M (75%) than that induced by DYH (45%) and F1Y (53%). We can speculate that $\beta$-glucan, present in the M forms of the fungus is more efficient in interacting with receptors in resident inflammatory cells than $\beta$-glucans found in Y forms of the fungus. The efficient interaction between $\beta$-glucans and receptors in phagocytic cells results in highest liberation of LTs and concomitantly the greatest cell migration. Also, LTs, probably LTB4 (Henderson, 1994), are the mediators involved in MN cell recruitment to the peritoneal cavity of mice injected with F1Y and F1M, since both MK886 and Dexa equally inhibited MN cell recruitment. However, Dexa was more efficient than MK886 in abrogating MN cell accumulation between 24–48 h after LYH inoculation, suggesting the release of other mediators. Conversely, MN cell migration appears to be totally dependent of LTs at 168 h after the injection of stimulants.

Although LTs contribute to leukocyte recruitment at the beginning of the inflammatory response induced by *H. capsulatum* or its cell wall fraction F1, our results suggest that other mediators may be involved in cell migration between 4–48 h, since Dexa was more efficient than MK-886 in inhibiting recruitment (Figures 3–5). Dexa is an anti-inflammatory drug that inhibit several chemoattractants such as leukotriene (Flower, 1988; Hirata *et al*., 1986) and inflammatory cytokines, such as IL-1, TNF, IL-8 and MCP-1 (Perretti & Flower, 1993; Oliveira *et al*., 1997; Joyce *et al*., 1997; van der Poll & Lowry, 1997; Miyamasu *et al*., 1998).

We have demonstrated for the first time that $\beta$-glucan of *H. capsulatum* is the main component involved in leukocyte recruitment to the site of inoculation of stimulants and that LTs are the main chemoattractant involved in the NE, EO and MN cell migration. Our results will be useful in understanding the mechanism of the inflammatory response in deep mycosis, and provides clues for the treatment of inflammation during histoplasmosis.

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