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Production of pro-inflammatory cytokines during the early stages of experimental *Paracoccidioides brasiliensis* infection

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Pro-inflammatory cytokines play an important role in both recruitment and activation of leukocytes migrating into tissues in response to invading pathogens. In this study the production of pro-inflammatory cytokines, determined by ELISA assays, and the recruitment of leukocytes into the lungs of BALB/c mice infected with *Paracoccidioides brasiliensis* conidia were evaluated during the early stages of infection. The results showed that infected mice had a significant increase in leukocytes in the lung during the first 4 days with a peak at day 2 post-challenge; infiltrates were composed mainly of polymorphonuclear neutrophils (PMN). Pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin (IL) 6, IL-1β and macrophage inflammatory protein (MIP) 2 were produced at elevated levels during the first 4 days post-challenge, but only in pulmonary samples and not in sera. Additionally, during the early stages of infection, overall weight loss was recorded in infected mice. These results suggest that pro-inflammatory cytokines could be responsible for the recruitment of leukocytes into the lung during the early stages of *P. brasiliensis* infection. In addition, both pro-inflammatory cytokine production and leukocyte recruitment may participate in the control of infection by influencing the organization of the immune response in the host exposed to *P. brasiliensis* conidia.

Keywords interleukin-1, interleukin-6, MIP-2, *Paracoccidioides brasiliensis*, TNF alpha

Introduction

Paracoccidioidomycosis (PCM), a systemic mycosis restricted to Latin America and produced by the dimorphic fungus *Paracoccidioides brasiliensis*, is thought to be acquired by inhalation of conidia produced by the fungus's mycelial form [1]. Rarely, the initial pulmonary infection overwhelms the host's immune defences, causing juvenile type PCM, an acute to subacute disease predominantly involving the reticuloendothelial system [2]. The most common and most extensively studied clinical presentation of the disease, however, is the less severe adult type disease, also known as the chronic form [1].

Monocyte-derived macrophages (MΦs) play an important role in host defence. They are implicated in innate resistance mechanisms against fungi via their direct microbicidal capacity and via their synthesis of cytokines such as tumour necrosis factor alpha (TNF-α). TNF-α has a beneficial effect contributing to the prevention of fungal infections [3–7]. MΦs are the main

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source of TNF-α and interleukin-1 (IL-1) – cytokines that mediate both local and systemic inflammatory responses [3,6,7].

It has been shown that *in-vitro* interferon gamma (IFN-γ)-activated MΦs are able to kill both *P. brasiliensis* conidia [8] and yeast cells [9]. In hamsters infected with strain Pb 18, Paris-Fortes *et al.* [10] found that in the early period after infection, MΦs had a spreading ability that was increased compared to that seen in the absence of infection. This ability was associated with increased production of TNF-α and enhanced fungicidal activity. TNF-α levels remained elevated throughout the experimental period while IL-1β was produced at low levels. These findings suggest that during the early post-challenge stages of *P. brasiliensis* infection, MΦs activated by TNF-α limit fungal dissemination. In addition, Kurita *et al.* [11], observed that during this period polymorphonuclear neutrophils (PMN) activated by IFN-γ, granulocyte–macrophage colony-stimulating factor (GM-CSF) and/or IL-1β appear to play an important role in host defence due to their enhanced antifungal activity.

The initial stages of the host–parasite interaction in PCM are unknown due to our inability to detect the precise moment when infection occurs [12]. For this reason, our group has developed a mouse model that reproduces human infection from its inception. The naturally infecting propagules, conidia, are given intranasally (i.n.) to the animals, mimicking naturally acquired infection. The early inflammatory stages of PCM in the model system are characterized by few MΦs and abundant PMN. Even if this response is non-specific, such a stage is important because it is at just this moment that resident pulmonary MΦs interact with the fungus for the first time and become activated. This process stimulates the recruitment of the other inflammatory cells such as PMN [13,14]. It has also been shown that TNF-α is one of the cytokines produced during the first week of the experimental infection [15]. This pro-inflammatory cytokine is capable of inducing the production of other cytokines mediating leukocyte migration to inflammation sites [6].

The role of pro-inflammatory cytokines has also been studied in other experimental models. In mice infected with *Coccidioides immitis,* TNF-α, IL-6 and IL-1β were induced during the early stage of pulmonary challenge [16], just as was seen in PCM.

Recent studies have shown that TNF-α, IL-6 and G-CSF play an important role in PMN recruitment at the site of invasive *Candida albicans* infection. It was shown that in the absence of either TNF-α or IL-6, the course of experimental disseminated candidiasis was relatively severe due to defective PMN recruitment [17].

In addition, human PMN from normal subjects produced the pro-inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α in response to stimulation with *Cryptococcus neoformans* yeast cells [18].

Vassallo *et al.* [19], utilizing a *Pneumocystis carinii* cell wall fraction, found that this component induced production of TNF-α and macrophage inflammatory protein-2 (MIP-2) resulting in infiltration of neutrophils into the lungs.

Previous studies using the experimental model in which *P. brasiliensis* conidia are used to initiate infection have revealed an important early up-regulation of the inflammatory reaction; this process is characterized by abundant recruitment of PMN [13,14]. We have shown that in the early interaction of the host with conidia, at least one pro-inflammatory cytokine, TNF-α, was present at elevated levels [15]. We hypothesized that a study on the array of pro-inflammatory cytokines seen at different stages following infection in our mouse model could reveal details of any connections in cellular signalling processes linking the observed recruitment of leukocytes into the lungs and the subsequent PMN influx.

Materials and methods

Animals

Isogenic 6-week-old BALB/c male mice obtained from the breeding colony of the Corporación para Investigaciones Biológicas (CIB), in Medellín, Colombia, were used in all experiments and were kept and fed under the conditions previously indicated [13]. Mice were supplied with sterilized commercial food pellets, sterilized bedding and fresh acidified water. Once infected, they were kept under restricted conditions in isolator chamber.

Fungus cultures and conidia production

P. brasiliensis isolate ATCC 60855 (American Type Culture Collection, Manassas, VA, USA), which is known to sporulate freely on special media, was used [20]. All steps involved in this procedure and in the experimental infection were carried out with sterile material and under a protective class II isolator hood. The techniques used to grow the mycelial form, and collect and dislodge conidia have been reported previously [20]. Briefly, the stock mycelial culture was grown in a liquid synthetic medium, the modified McVeigh–Morton broth [21], at $18 \pm 4^{\circ}C$ with shaking. Growth was homogenized and portions were used to

inoculate agar plates; the latter were incubated at 18 ± 4°C for 2 months. After this time, sterile physiological saline containing 0.01% Tween 20, plus 100 U penicillin and 100 μg streptomycin per ml was used to flood the culture surface. Growth was removed with a bacteriological loop and the resulting suspension pipetted into an Erlenmeyer flask containing glass beads. This was then shaken in a reciprocating shaker at 250 r.p.m. for 45 min. The homogeneous suspension was filtered through a syringe packed with sterile glass wool (Pyrex fibre glass, 8 μm; Corning Glasswork, Corning, NY, USA). The filtrate was collected in a polycarbonate centrifuge tube and centrifuged for 30 min at 1300 *g*; the pelleted conidia were washed, counted with a haemocytometer, and assessed for viability by the ethidium bromide-fluorescein diacetate technique [22]. For the experiments, only inocula with a conidial viability of > 90% were used.

Experimental infection

Animals were anaesthetized by intramuscular injection with 50 μl of solution containing a mixture of ketamine hydrochloride (100 mg) (Park-Davies, Bogotá, Colombia) and xylazine (20 mg) (Bayer, Brazil). When deep anaesthesia was obtained, 4×10^6 conidia suspended in a 60-μl inoculum divided in two portions were instilled intra-nasally over a 10 min period. The abdomen was compressed, and droplets were deposited on the nares. When the pressure was released, the mouse inhaled deeply. Control mice received an intranasal inoculum of 60 μl phosphate-buffered saline (PBS). To observe changes in body weight, each mouse was weighed both before inoculation and after killing.

To control possible changes in cytokines and cellularity of the bronchoalveolar lavage fluids (BAL) during experiments, each group of infected mice was studied alongside age-matched controls housed in the same conditions as infected mice.

The experiments required a total of 196 animals, inoculated as described below and killed at the following time intervals 0 (2 h post-inoculation), 1, 2, 3, 4, 7 and 14 days. At each period, eight mice from each experimental group, as well as six non-infected control animals, were killed by an intraperitoneal injection of 1.0 ml 2.5% sodium pentothal (Abbott Laboratories, Chicago, IL, USA). Different mouse groups were used for studies of either BAL or lung homogenates.

BAL cellularity

After killing, the trachea was exposed and a blunt-tipped needle was inserted; 10 ml PBS was then flushed into the lungs, with 1.0-ml volumes being injected each time and withdrawn serially to obtain samples of BAL fluids. This suspension was centrifuged at 4°C, and the pellet was suspended in 2.0 ml PBS. After harvesting, the cells were counted with a haemocytometer and cytospin slides were prepared and stained with toluidine blue or Wright's stain [23].

Cytokine assays

Samples

BAL

After the mice were killed, the trachea was exposed as described below. 1 ml PBS was then flushed into the lungs once and aspirated. This suspension was centrifuged at 4°C, and the supernatants were frozen at −70°C until assayed [15].

Lung homogenates

Another group of mice was killed, their lungs removed and homogenized in 2.0 ml PBS with tissue grinders (Tissue Tearor, model 985-370; Biospec Products, Racine, WI, USA). Liberation of proteases during homogenization was controlled by adding a cocktail of inhibitors (pepstatin 0.1 μmol/l, leupeptin 0.1 μmol/l, phenylmethyl sulfonide fluoride 1 mmol/l, N-tosyl-L-phenylalanine chloromethyl ketone 0.2 mmol/l, (α) p-methyl L lysine chloromethyl ketone 0.1 mmol/l) (Sigma Chemical, St Louis, USA) plus ethylenediamine tetra-acetic acid (EDTA) 1 mmol/l (Merck, Darmstadt, Germany). The homogenates were kept in ice and then centrifuged at 4°C; the supernatants were kept at −70°C until their use.

Sera

Animals were anaesthetized and bled from the axillary plexus. Approximately 1 ml of blood was obtained and then centrifuged. Serum was kept at −70°C until assayed.

In subsequent studies, samples of BAL, lung homogenates and sera were thawed only once immediately prior to performance of commercial ELISA assays for the murine cytokines TNF-α (OptEIA set; PharMingen, San Diego, CA, USA), IL-6 (OptEIA set), and IL-1β (Duo Set, R&D Systems, Minneapolis, MN, USA). For MIP-2 determination we used anti-mMIP-2 purified rat monoclonal IgG2b (capture antibody), anti-MIP-2 biotinylated MIP-2 affinity purified goat IgG (detection antibody) and recombinant mouse macrophage inflammatory protein 2 (rmMIP-2) (all from R&D Systems). The methodology used was as described by each manufacturer.

Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM). Comparisons between groups (inoculated and non-inoculated) were done using Student's *t*-test and, in all groups, time changes were analysed by one-way analyses of variance (ANOVA). The program used was STATISTIC for Windows, version 5.0, with the significance level taken to be $P < 0.05$.

Results

Recruitment of pro-inflammatory leukocytes into the lungs

As shown in Fig. 1, intranasal challenge with *P. brasiliensis* conidia induced an increase in the cellularity of BAL. Over the whole time-course of the study, with measurement points 2 h and 1, 2, 3, 4, 7 and 14 days post-challenge, recruitment of pro-inflammatory leukocytes could be seen to have caused the total number of cells in BAL to increase significantly. Strong differences were found as early as day 1 ($1.11 \pm 0.06 \times 10^6$ cells in infected mice compared to $0.13 \pm 0.01 \times 10^6$ in control mice) and continued up to 4 days post-challenge ($2.07 \pm 0.06 \times 10^6$ cells in infected mice vs. $0.11 \pm 0.009 \times 10^6$ cells in controls). The maximal value was observed at day 2 post-challenge ($4.81 \pm 0.30 \times 10^6$ cells vs. $0.29 \pm 0.03 \times 10^6$ cells in controls; $P < 0.0001$). BAL cellularity decreased markedly in infected mice from days 7 to 14, dropping from $0.40 \pm 0.03 \times 10^6$ to $0.27 \pm 0.02 \times 10^6$ cells.

To assess the type of cells recruited into the lung, the absolute number of leukocytes was enumerated and the proportion of PMN, MΦs and lymphocytes was calculated on the basis of morphology.

As shown in Fig. 2, the majority of the pro-inflammatory leukocytes in the BAL of infected mice were PMN. These cells were present during the first 4 days post-inoculation with the maximal value ($4.37 \pm 0.30 \times 10^6$ cells) being observed at day 2. This number was significantly higher ($P < 0.0001$) than that obtained with control animals ($0.02 \pm 0.04 \times 10^6$ cells).

However, at 0 and 1 days post-challenge MΦs were present in equal proportions in both infected and control mice. They increased significantly in infected animals at day 2 and reached a maximum of $0.94 \pm 0.04 \times 10^6$ cells at day 4, a significant differences ($P < 0.0001$) from control levels ($0.11 \pm 0.009 \times 10^6$ cells).

Lymphocytes were observed only in infected mice. The maximal value was seen at day 4 ($0.21 \pm 0.03 \times 10^6$ cells; $P < 0.0001$ in comparison with control levels).

Determination of pro-inflammatory cytokines in the different compartments

TNF-α

As shown in Fig. 3A, during the first and second days post-challenge there was a significant increase ($P < 0.0001$) in TNF-α levels in the BAL of infected mice

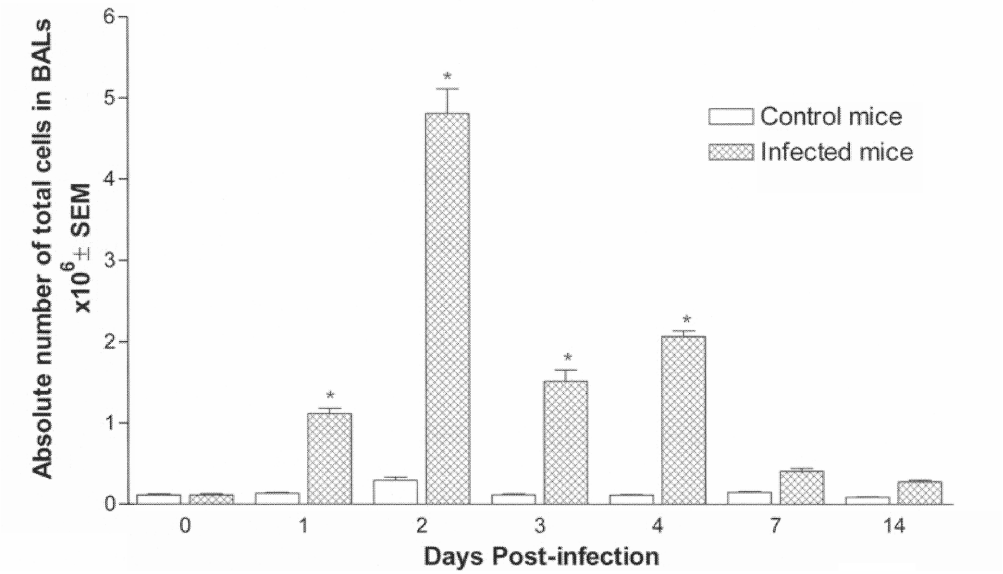

Fig. 1 Cellularity in bronchoalveolar lavage (BAL) fluid from mice inoculated with phosphate-buffered saline (PBS, open bars, control) or 4×10^6 *Paracoccidioides brasiliensis* conidia (hatched bars). The mice were killed on day 0 (at 2 h post-inoculation) and on days 1, 2, 3, 4, 7 and 14 post-inoculation. Each bar represents the mean ± standard error of the observations from eight mice per group in the case of infected mice and from six animals in the PBS-inoculated group. *Significant values ($P < 0.0001$) between control and infected mice.

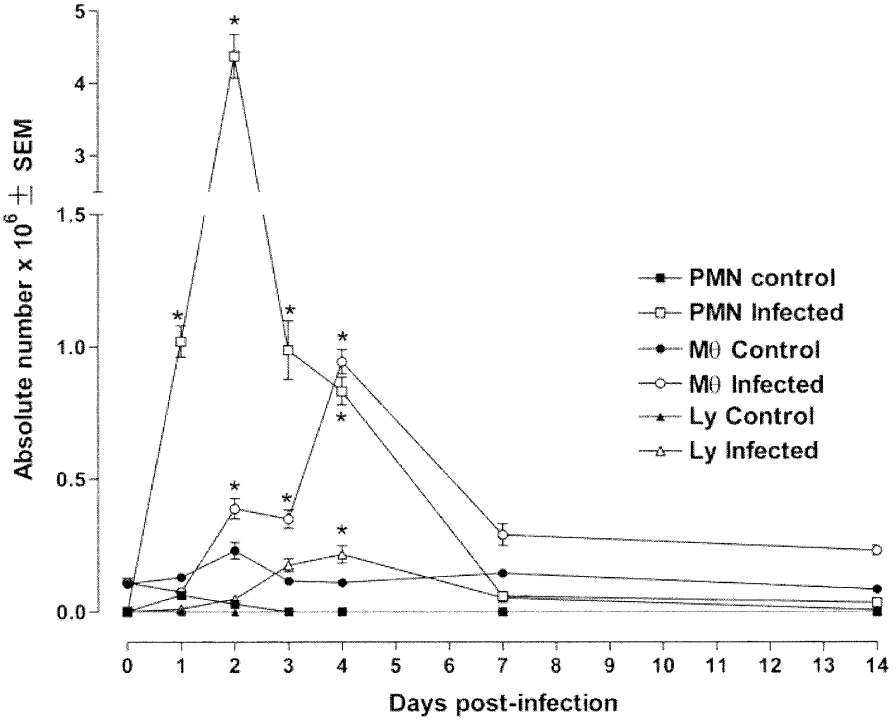

Fig. 2 Numbers of polymorphonuclear neutrophils (PMN), macrophages (MΦ) and lymphocytes (Ly) in BAL from BALB/c mice inoculated with PBS (control) or with 4×10^6 *P. brasiliensis* conidia. Measurement times, numbers of animals and replication procedures are as in Fig. 1. *Significant values ($P < 0.0001$) between control and infected mice.

(660 ± 4.5 pg/ml vs. a control value 52.3 ± 8.7 pg/ml at day 2; 383.8 ± 57.6 pg/ml vs. a control value of 52.0 ± 6.3 pg/ml at day 3). At other measurement times, TNF-α levels were similar in inoculated and control animals.

In contrast, no difference was seen in TNF-α levels in lung homogenates of infected and control mice throughout the observation period, except at day 2 post-infection when infected animals showed a moderate increase in comparison with controls (108.5 ± 20.5 vs. 55.8 ± 4.3 pg/ml; $P < 0.03$). TNF-α concentrations were low in sera of both infected and control mice, with serum levels always under 10 pg/ml.

IL-6

A significant ($P < 0.0001$) increase in IL-6 was observed in both BAL and lung homogenates from mice infected with *P. brasiliensis* conidia between days 1 and 3 post-inoculation (Fig. 3B). Maximal values were observed at day 1 post-infection with values of 1230.3 ± 9.1 and 737.3 ± 72.9 pg/ml in BAL and lung homogenates, respectively. In addition, the IL-6 serum levels were significantly increased at day 1 (43.6 ± 5.6 pg/ml vs. control level 2.3 ± 0.8 pg/ml; $P < 0.01$).

IL-1β

As shown in Fig. 3C, lung homogenates from infected mice, when compared with homogenates from controls, exhibited a significant increase in IL-1β. The maximal concentration was observed at day 2 post-infection, 1723.2 ± 33.0 pg/ml, in contrast to control value 215.1 ± 13.9 pg/ml ($P < 0.0001$). Also, BAL from infected mice had higher levels of IL-1β than control BAL had. The peak concentration was observed at day 2, with 351.3 ± 35.2 pg/ml in test animals vs. 13.7 ± 1.1 pg/ml in controls ($P < 0.0001$). IL-1β serum levels from control and experimental animals showed no differences.

MIP-2

Levels of MIP-2 in both BAL and lung homogenates from infected mice exhibited significant increases ($P < 0.0001$) over control levels at days 1 to 3 post-inoculation. As showed in Fig. 3D, the maximal values were observed at day 1 and were 1148.5 ± 36.9 and 1305.8 ± 8.2 pg/ml for BAL and lung homogenates, respectively. There were no differences in serum MIP-2 levels of control and test animals.

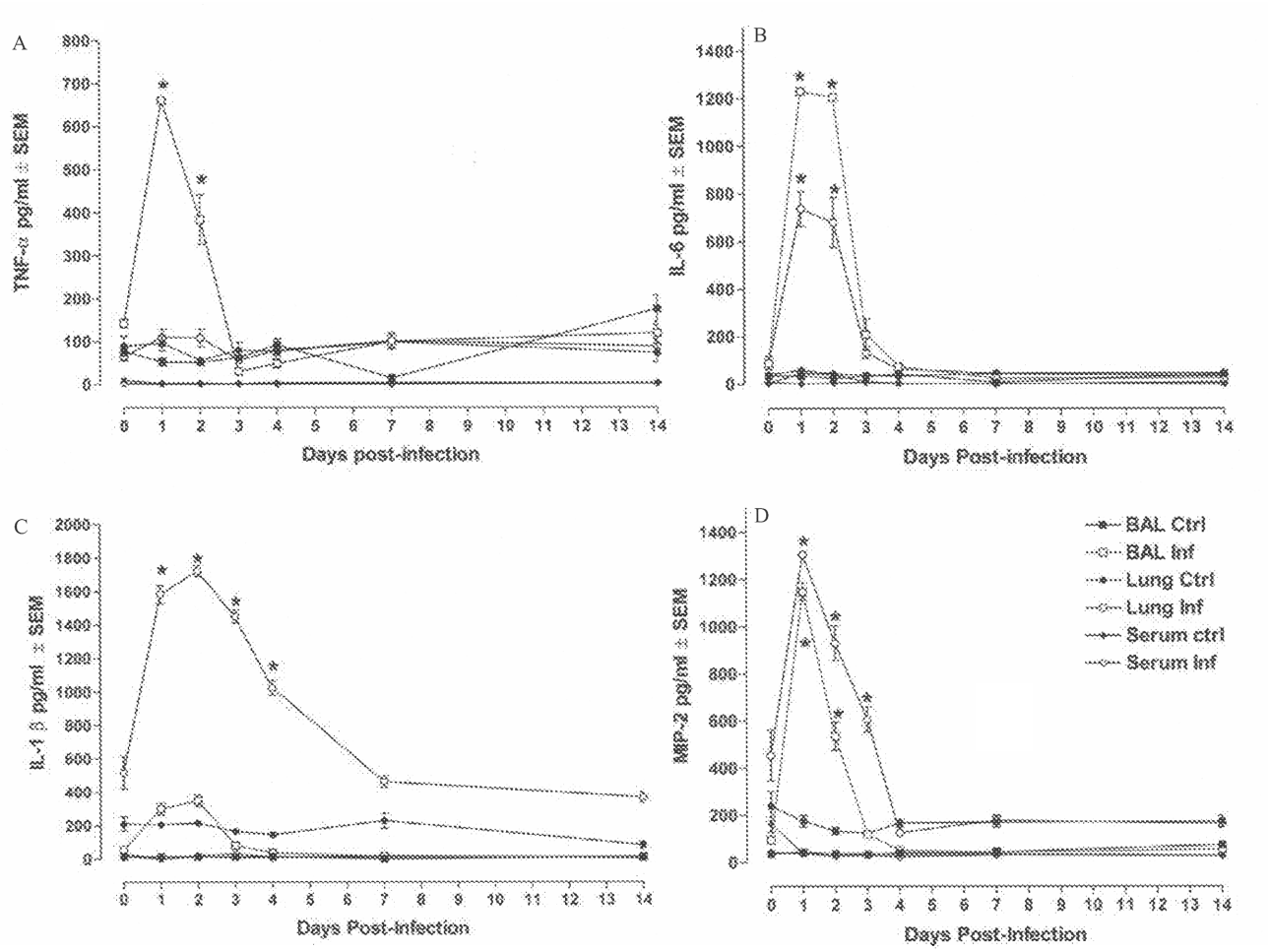

Fig. 3 Levels of pro-inflammatory cytokines in mice infected with 4×10^6 *P. brasiliensis* conidia. A) TNF-α, B) IL-6, C) IL-1β and D) MIP-2, were measured in BAL fluids, lung homogenates and sera. Measurement times, numbers of animals and replication procedures are as in Fig. 1. $^*P < 0.0001$ when comparing infected animals with PBS-inoculated mice.

Body weight loss during the early post-infection period

The animals were weighed before inoculation with *P. brasiliensis* or PBS and after killing. The difference in weight of each animal was calculated. During the first 4 days post-challenge the infected animals showed weight loss contrasting with the normal weights seen in controls (Fig. 4). The maximal loss, approximately 12% of total body weight, was observed between the second and the third days, and was highly significant ($P < 0.0002$).

Discussion

This study shows that during the early stages of infection brought about by intranasal introduction of *P. brasiliensis* conidia, BALB/c mice respond with leukocyte recruitment into the lung and with significant elevation of levels of the pro-inflammatory cytokines TNF-α, IL-6 and IL-1β and MIP-2. The highest levels of these cytokines were found in BAL fluids and lung homogenates but not in sera, indicating that these molecules predominate in pulmonary compartments. Perenboom *et al.* [24], in a *P. carinii* pneumonia rat model, similarly found increased levels of IL-1β in lung homogenates and IL-6 in BAL fluids, whereas in plasma cytokines remained within normal limits. In contrast, Futenma *et al.* [25], using a pulmonary candidiasis model, found a considerable level of TNF-α in the serum of infected mice, with peak levels at 24–48 h. Although this observation is not consistent with our results, both the Perenboom *et al.* [24] and Futenma *et al.* [25] studies did concur with our own in demonstrating an enhanced level of TNF-α in BAL fluid during the same experimental periods [24,25].

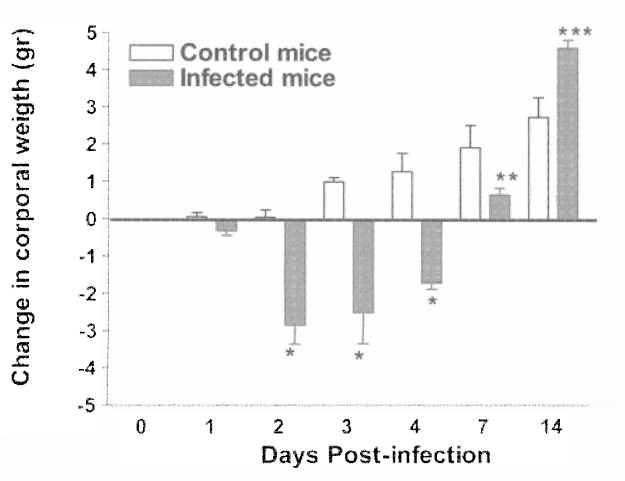

Fig. 4 Loss of body weight in mice infected with 4×10^6 *P. brasiliensis* conidia. The mice were weighed at the beginning of the trial and upon killing. Measurement times are as in Fig. 1. Each bar represents the mean ± standard error of the observations for eight infected mice per group (dotted bars) and for six PBS-inoculated controls (open bars). Significant values $*P < 0.0002$; $**P < 0.03$; $***P < 0.002$ for differences between infected and control mice.

It is well established that the expression of pro-inflammatory cytokines by macrophages or endothelial cells results in recruitment of leukocytes; subsequently, the latter adhere to infected tissues by means of adhesion molecules [6]. Experimental models with other fungi such as *C. albicans* [17,26], *Aspergillus fumigatus* [27] and *P. carinii* [28] have demonstrated that the recruitment of PMN is indeed mediated by pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β and granulocyte-macrophage colony stimulating factor (GM-CSF). These early events have been observed in other models also. Baughman *et al.* [29], utilizing an experimental model of pulmonary histoplasmosis, found that during the first week the histopathology revealed sub-acute inflammation with components consisting of PMN, lymphocytes and MΦs, while in BAL fluids there was a large influx of PMN during the same period. Our results agree in that we also found an important recruitment of leukocytes, mainly PMN, into the lung during the first 4 days post-inoculation, especially at day 2. However, in contrast to other investigators, Williams *et al.* [30], in studies on pulmonary infection in a model using mice inoculated with *Blastomyces dermatitidis* conidia, observed mostly MΦ infiltration on day 1 with just occasional accompanying PMN. In BAL measured at day 28, PMN levels rose 17% over control levels, and attained a 40% increase in comparison with control levels on day 42.

Granulocytes, mainly PMN, are the first cells to migrate into tissues in response to invading pathogens. It has long been recognized that their main role in the inflammatory and immune responses is accomplished through phagocytosis and killing of microorganisms, *via* the generation of reactive oxygen intermediates (ROI) and the release of the lytic enzymes stored in their granules [31]. Consequently, human PMN, either constitutively or in response to appropriate stimulation, have the capacity to express mRNA for a variety of proteins involved in defence functions. Among these molecules, cytokines, with their broad spectrum of biological activity, are the most important components.

PMN are able to synthesize IL-1β, IL-8, TNF-α, TGF-β, MIP-1α, GM-CSF and IFN-α [31]. Some of these pro-inflammatory cytokines have the capacity to induce fungicidal activity in PMN directed against fungi such as *C. albicans* [32] and *C. neoformans* [18]. Kurita *et al.* [11,33,34] observed that in *P. brasiliensis* infection, both murine and human PMN are fungicidal to yeast phase cells if they have previously been activated by cytokines such as IFN-γ, GM-CSF or IL-1β. The efficacy of this response varied considerably among fungal isolates [11,33,34]. Such differences proved to be unrelated to any differences in the ability of PMN to phagocytose yeast cells of the various isolates, as most yeasts from all strains were too large to be phagocytosed by PMN [34]. On the other hand, live PMN were necessary for accomplishing the degree of control seen. PMN lysates appeared to have no fungistatic or fungicidal effects [34].

McEwen *et al.* [35] were able to demonstrate that killing of *P. brasiliensis* yeasts by PMN obtained from infected mice was correlated with the capacity of these PMN to produce reactive oxygen intermediates. In studies conducted using non-activated PMN against *B. dermatitidis*, resistance to killing was found to result from inefficient generation of inhibitory materials by PMN myeloperoxidases [36]. The *in-vivo* and *in-vitro* effects of cytokines on the fungicidal activity of murine peripheral blood PMN were studied by Morrison *et al.* [37–39], who found that these cells, when activated with IFN-γ, had enhanced fungicidal activity against *B. dermatitidis*. This activity involved one or more oxidative mechanisms, with a prominent role being played by the respiratory burst. Newman *et al.* [40,41] demonstrated that human PMN mediate potent and long-lasting fungistasis against *Histoplasma capsulatum* yeasts. Fungistatic activity resides in the azurophil granules, defensins, capthesin G, and the bactericidal-permeability-increasing protein, all of which act as major anti-*H. capsulatum* effector molecules.

In the present study, the high TNF-α levels detected in the BAL of infected mice during the first two days post-infection may have been responsible for the MΦ activation seen. Such a relationship has been suggested

by Paris-Fortes *et al*. [10], who found that, during the early post-infection period, MΦs from hamsters infected experimentally with *P. brasiliensis* yeasts had a higher spreading ability than those from control animals. This spreading ability was associated with increased TNF-α production and enhanced fungicidal activity, and resulted in restriction of fungal dissemination.

Calich and Kashino [42] studied responses to *P. brasiliensis* yeast cells in mice from a resistant strain, A/Sn, and a susceptible strain, B10.A. They found that peritoneal MΦs from the susceptible strain secreted low levels of TNF-α, while MΦs from resistant animals released high levels of this cytokine. The high level of TNF-α secretion seen in resistant animals coincided with IFN-γ production by lymph node cells and was followed by sustained secretion of IL-12. The authors suggested that production of these cytokines plays a crucial role in resistance to *P. brasiliensis* infection [42]. A similar conclusion was reached by Souto *et al*. [43], who also investigated the role of these cytokines in resistance to PCM by using GKO strain knock-out mice lacking IFN-γ and p55KO mice lacking the TNF-α receptor. Such animals were unable to control the proliferation of yeast cells and succumbed to infection shortly after inoculation, while wild-type mice controlled the infection. These results reveal that both IFN-γ and TNF-α mediate resistance to *P. brasiliensis*.

In the present study we observed that during the first 4 days post-infection, the infected mice appeared to have diminished vitality as reflected in decreased body weight. The weight loss observed in our model could be attributed to the high TNF-α and IL-1 levels, as these pro-inflammatory cytokines have been associated with symptoms such as weight loss in connection with various inflammatory processes [7,44–47].

In our model of pulmonary PCM, the production of pro-inflammatory cytokines during the early stages of infection appears be responsible for leukocyte recruitment into the lung. The higher levels of these cytokines in the lung combined with the increase in the PMN and MΦ numbers suggest that these factors play an important role in innate resistance to *P. brasiliensis*. There is some evidence that during the initial days after infection with *P. brasiliensis* yeast cells in the mouse model, there is a decrease in colony forming units (c.f.u.) [48]. Experiments based on use of *P. brasiliensis* conidia also show a reduction in both c.f.u. and directly estimated propagule numbers in the lungs during the early stages of infection, suggesting the presence of a functional fungicidal mechanism [14]. These data lead us to suggest that phagocytic cells, including PMN and macrophages, participate in controlling *P. brasiliensis* infection both directly and by prompting, at the same time, the transition from a Th2- to a Th1-type response pattern mediated by an up-regulated expression of TNF-α.

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