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Influence of Tumour Condition on the Macrophage Activity in *Candida albicans* Infection

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Abstract

To better understand the interactions between opportunistic fungi and their hosts, we investigated hydrogen peroxide (H_2O_2), nitric oxide and TNF-α production by peritoneal macrophages from Ehrlich tumour-bearing mice (TBM) during microbial infections. For this purpose, TBM at days 7, 14 and 21 of tumour progression were inoculated intraperitoneally with *C. albicans* and evaluated after 24 and 72 h. We observed that TBM showed significant increases in H_2O_2, TNF-α levels and fungal clearance at day 7 after *C. albicans* infection. However, as the tumour advanced, there was a progressive decline in the release of H_2O_2 and TNF-α that was paired with the dissemination of *C. albicans*. These results demonstrate that protective macrophage activities against *Candida albicans* are limited to the initial stages of tumour growth; continued solid tumour growth weakened the macrophage response and as a consequence, weakened the host's susceptibility to opportunistic infections.

Introduction

Candida albicans systemic infections have an important impact on the clinical course and outcomes of cancer patients [1, 2], as they are responsible for prolonged hospital stays, high healthcare costs and significant mortality [3–6]. Several authors have disagreed with the widely held belief that compromised immune responses are a cause of the susceptibility to infection in tumour patients, and they have reported that the tumoral condition can generate a protective response against this pathogen [7]. Considering that this phenomenon could be associated with the activation state of macrophages, we decided to study this premise using peritoneal macrophages.

Macrophages have been recognized as a critical component of the innate immune system and can regulate both the immune response against micro-organisms and the development of a solid tumour [8]. We chose to use peritoneal macrophages because macrophages have been shown to traffic between the peritoneum and neoplastic tissues [9, 10]. According to those authors, the peritoneal cavity may act as a training ground where macrophages undergo developmental changes in response to several stimuli. To our knowledge, there have been no previous reports with a simultaneous examination of candidiasis on Ehrlich solid tumour mice and the role of macrophage activity in this process. The Ehrlich solid tumour model was chosen because the majority of knowledge concerning *Candida* infections in cancer patients has been obtained from patients with haematological malignancies and candidaemia has been an important complication in patients with solid tumour [6].

Materials and methods

Animals. All protocols are in accordance with the ethical principles for animal research adopted by the Brazilian College of Animal Experimentation (COBEA). Two-month-old male Swiss mice were obtained from the Animal House of Sao Paulo State University, Botucatu, SP, Brazil. Mice were housed in groups of three to five animals and were provided with food and water *ad libitum*.

Experimental design. Mice were divided into four groups: tumour-bearing mice that were inoculated with *C. albicans* (Ca-TBM), uninoculated tumour-bearing mice (TBM), *C. albicans*-inoculated control mice (Ca-CTL), and uninoculated naive mice (CTL). Mice from the TBM group were killed on days 7, 14 and 21 to analyse certain parameters. The Ca-TBM group was inoculated

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intraperitoneally (i.p.) with *C. albicans* (5×10^6 fungi) on days 7, 14 and 21 following tumour implantation and was killed after 24 and 72 h of *C. albicans* inoculation. Finally, the Ca-CTL group was killed 24 and 72 h following *C. albicans* inoculation.

Solid Ehrlich tumour. This neoplasia, which is a spontaneous mammary tumour in mice, does not resolve spontaneously. It may evolve to the ascitic or solid forms depending on the route of inoculation [i.p. or subcutaneous (s.c.) respectively] [11]. For our studies, the tumour was maintained in the ascitic form in Swiss mice and tumour cells were collected according to the methods of Silva *et al.* [12]. 1×10^7 tumour cells/100 μl were inoculated s.c. in both TBM and Ca-TBM mice.

Candida albicans *inoculum. Candida albicans* strain FCF-14 was originally obtained from the fungal collection of the School of Odontology, Sao Paulo State University, Sao Jose dos Campos, SP, Brazil. The fungi were maintained on Sabouraud medium (Difco Lab., Detroit, MI, USA). To prepare the inoculum, fungi were cultured in Sabouraud medium for 24 h at 37 °C and the colonies were resuspended and washed twice. The fungal viability was determined by cotton blue staining, and the final concentration was adjusted to 5×10^7 viable *C. albicans*/ml [13].

Collection of the biological material and macrophage culture. Mice were killed using CO_2 euthanasia, and the peritoneal cells were collected by washing the peritoneal cavity with 10 ml sterile ice-cold phosphate-buffered saline, pH 7.4. The suspension was then centrifuged and the cells were resuspended in 1 ml RPMI-1640 medium (Nutricell, Campinas, SP, Brazil) containing 10% heat inactivated foetal calf serum (Gibco BRL, Grand Island, NY, USA). The cell concentration was adjusted to 2×10^6 macrophages/ml, as judged by 0.02% neutral red uptake. The peritoneal cells were placed in 96-well flat-bottom microtitre plates (Costar, Cambridge, MA, USA) and incubated for 2 h at 37 °C and 5% CO_2, in a humidified chamber to allow macrophages to adhere and spread. Non-adherent cells were removed by washing the wells three times with RPMI and the remaining adherent cells (>97% macrophages as assessed by morphological examination) were used to perform the experiments. The macrophages were cultured in RPMI-1640 with or without 10 μg/ml LPS (Sigma, St Louis, MO, USA), as an internal control of macrophage activity (data not shown), at 37 °C and 5% CO_2. After 24 h, the cell-free supernatants were harvested and stored at −70 °C to posterior NO and TNF-α analysis. Finally, the peritoneal fluid and liver, spleen and kidney fragments were submitted to microbiological evaluation.

Direct organ culture. For organ cultures, 10 fragments (2 × 2 mm) of liver, spleen and kidney were placed on 15 × 90 mm Sabouraud agar plates at 37 °C for 5 days, as described previously [14]. The yeast colonies present on the organ fragments were counted and the results were expressed as the frequency of *Candida*-positive organ fragments.

Fungal loads in peritoneal lavage (PL). Peritoneal lavage fluid samples (30 μl) were placed on 15 × 90 mm Sabouraud agar plates at 37 °C. The yeast colonies were counted after 5 days and the fungal loads were expressed as colony-forming units (CFU).

Nitric oxide production. To determine NO levels, the production of nitrite (a stable end product of NO) was measured in the cell-free supernatants of cultured macrophages according to the methods of Green *et al.* [15]. Briefly, 100 μl of the cell-free supernatant was incubated with an equal volume of Griess reagent containing 1% sulfanilamide (Synth, Diadema, SP, Brazil), 0.1% naphthylene diamine dihydrochloride (Sigma) and 2.5% H_3PO_4, at room temperature for 10 min and the nitrite accumulation was quantified using an ELISA microreader (ELx 800; Bio-tek Instruments Inc., Winooski, VE, USA). The concentration of nitrite was determined using sodium nitrite (Sigma) diluted in RPMI-1640 medium as a standard.

Hydrogen peroxide release (H_2O_2). Macrophages (2×10^6 cells/ml) were obtained as described before and were maintained in RPMI-1640 culture medium at 37 °C and 5% CO_2 for 24 h. At the end of the cell culture period, the supernatant was removed and macrophages were incubated with phenol red solution [dextrose (Sigma), phenol red (Sigma), horseradish peroxidase type II (Sigma)] and plated at 37 °C in 5% CO_2 for 1 h according to the methods of Russo *et al.* [16]. The reaction was stopped with the addition of 1 N NaOH and the H_2O_2 concentration was determined using an ELISA microreader.

TNF-α analysis. TNF-α levels in cell-free supernatant were quantified using the Cytokine Duo-Set Kit (R&D Systems, Minneapolis, MI, USA) according to the manufacturer's instructions.

Statistical analysis. The peritoneal microbiological data were analysed with the Mann–Whitney test. The frequency of *Candida*-positive organ fragments was determined using contingency table analysis and Fisher's exact test. Linear regression analysis was used to correlate the tumoral progression with fungal installation and dissemination. All other data were analysed using one-way ANOVA with a Tukey–Kramer post-test [17]. All statistical analysis was performed in GraphPad InStat version 3.0 for Windows (GraphPad Software, San Diego, CA, USA) and the statistical significance level established was $P < 0.05$.

Results

Tumour implantation

One week after the introduction of Ehrlich ascite tumour cells into the subcutaneous tissue of mice, a firm palpable whitish mass that was relatively mobile with little

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adherence to the adjacent tissues developed at the site of inoculation. This tumour showed continuous and progressive growth that resulted in animal death approximately 28 days after implantation.

Fungal installation and dissemination

CTL and TBM groups

Fungi were not present in samples from uninoculated animals (CTL and TBM).

Ca-CTL group

Ca-CTL mice had acute systemic candidiasis that compromised the site of inoculation and the following organs: spleen, liver and kidneys; the spleen was the most affected organ. After 72 h, the number of fungi in the peritoneal cavity (Fig. 1) and viscera significantly decreased ($P < 0.05$), which suggests that these mice could resolve the infection (Fig. 2A).

Ca-TBM group

In contrast to Ca-CTL mice, Ca-TBM mice had a lower number of fungi in the peritoneum on day 7 of tumour progression (Fig. 1A) and a lower frequency of *Candida*-positive organs 24 h after fungal inoculation (Fig. 2A). At 72 h, fungi were no longer detected (Figs. 1A and 2A). Compared to Ca-CTL mice, it is evident that the neoplastic condition significantly altered the host's response to *Candida* infection.

On day 14 of tumour progression, the Ca-TBM mice exhibited a similar pattern of infection in the peritoneum and organs to Ca-CTL mice at 24 h post-infection (Figs. 1B and 2B). Although the burden of fungi in the peritoneum was similar to that of Ca-CTL mice at 72 h (Fig. 1B), the spleen of those animals was more affected (Fig. 2B).

There was a significant exacerbation of *Candida* infection 72 h after fungi inoculation in Ca-TBM mice on day 21 of tumour progression; the mice had increased numbers of CFUs in the peritoneal cavity fluid (Fig. 1C) and a larger number of *Candida*-positive organ samples than the Ca-CTL group (Fig. 2C). Thus, at the end of the experiment, *C. albicans* dissemination was more extensive in tumour-bearing animals than in non-TBM.

Evaluation of macrophage activity

Ca-CTL group

Peritoneal macrophages of Ca-CTL mice always exhibited higher production of H_2O_2, NO and TNF-α than animals free of fungi and tumours (CTL) (Fig. 3).

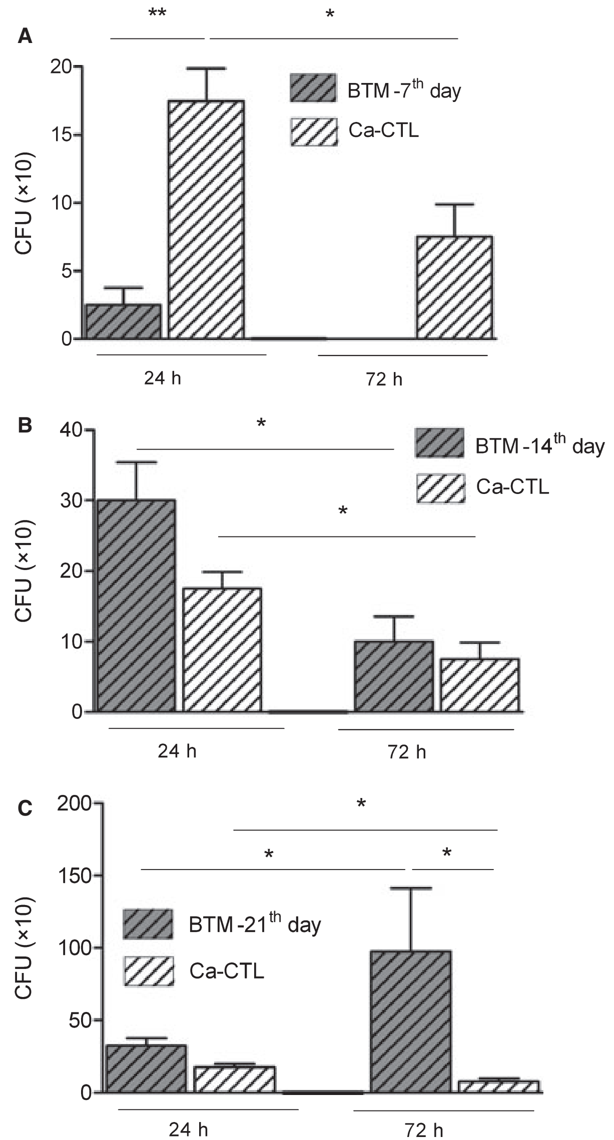

Figure 1 Microbiological evaluation of PL fluid. Tumour-bearing mice were inoculated i.p. with *Candida albicans* on days 7, 14 and 21 of tumour progression (Ca-TBM group, grey striped box) and evaluated at 24 and 72 h after fungal inoculation. Tumour-free mice inoculated with *C. albicans* were used as control group (Ca-CTL, white striped box) and evaluated at 24 and 72 h after *Candida* inoculation. (A) Ca-BTM on day 7 of tumour progression. (B) Ca-BTM on day 14 of tumoral progress. (C) Ca-BTM on day 21 of tumour progression. Data are expressed as the mean ± SEM of colony-forming units (CFU). $*P < 0.05$, $**P < 0.01$, $n = 4$ (each group evaluated). Mann–Whitney test.

TBM group

Macrophages from TBM had similar NO production compared to the CTL group throughout the experiment (Fig. 4B). Similarly, the Ca-TBM mice also exhibited basal NO production (data not shown).

In contrast, macrophages from day 7 TBM had significantly increased H_2O_2 production (Fig. 4A) and TNF-α release (Fig. 4C). After this period, H_2O_2 and TNF-α

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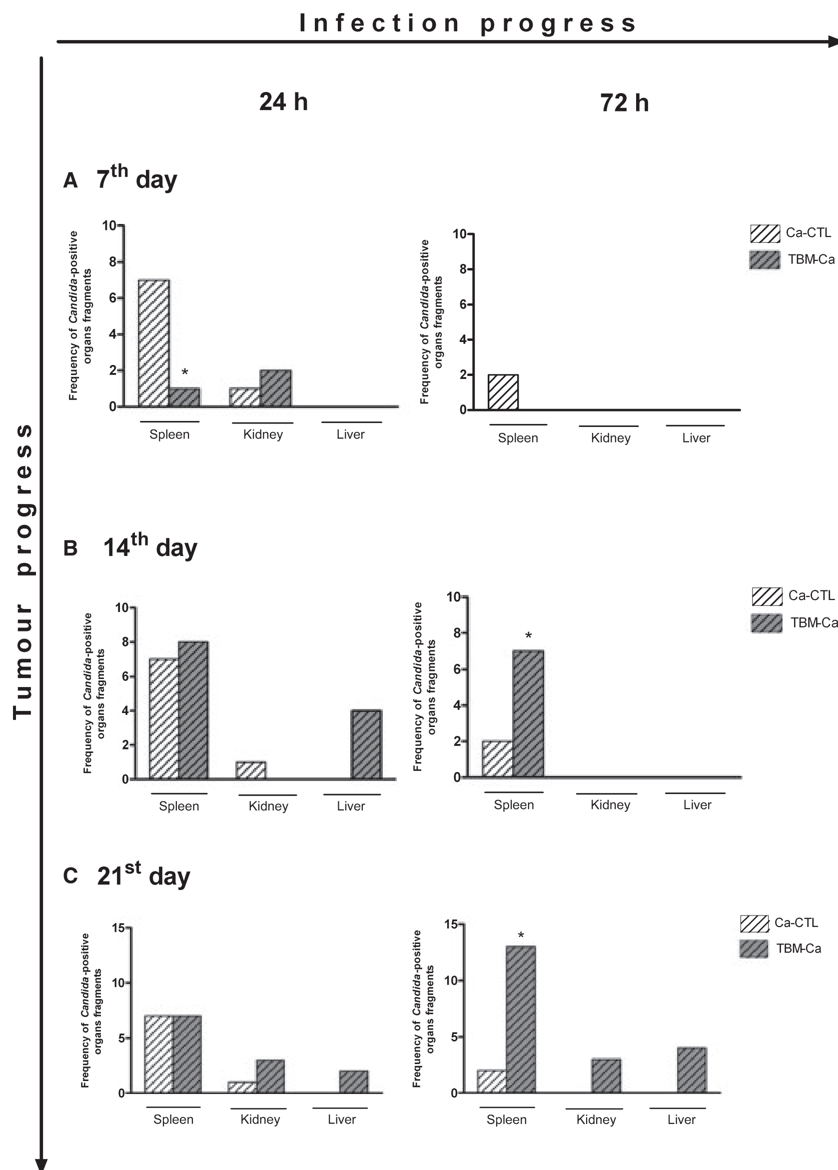

Figure 2 Evaluation of fungal dissemination to the spleen, kidneys and liver. Tumour-bearing mice were inoculated i.p. with *Candida albicans* on days 7, 14 and 21 of tumour progression (Ca-TBM group, grey striped box) and evaluated at 24 and 72 h after fungal inoculation. Tumour-free mice inoculated with *C. albicans* were used as control group (Ca-CTL, white striped box) and evaluated at 24 and 72 h of *Candida* inoculation. (A) Ca-BTM on day 7 of tumour progression. (B) Ca-BTM on day 14 of tumour progression. (C) Ca-BTM on day 21 of tumour progression. Values are expressed as the frequency of *Candida*-positive fragments. $*P < 0.05$, $n = 4$ (each group evaluated). Fisher's exact test.

levels decreased. Interestingly, H_2O_2 production in peritoneal macrophages from day 21 TBM rebounded to significantly higher levels (Fig. 4A).

Ca-TBM group

Macrophages exhibited significantly higher levels of H_2O_2 and TNF-α during *Candida* infection in the presence of tumours (7 days) when compared with the Ca-CTL and TBM groups (Figs. 5A and 6A); the ability of the macrophages to respond to the fungal infection and tumour remained unchanged.

At 24 h after *Candida* inoculation, Ca-TBM (14 days) macrophages produced more H_2O_2 and TNF-α when compared with the TBM group in mice with 14-day-old tumours (Figs. 5B and 6B), which suggests that the Ca-TBM group was still able to respond to fungal infection. At 72 h after *Candida* infection, macrophages from Ca-TBM produced less H_2O_2 than the Ca-CTL group, although the TNF-α levels were unchanged compared to the Ca-CTL and TBM groups (Figs. 5B and 6B). This demonstrates that Ca-TBM mice had a decreased capacity to respond to *Candida* infection by 72 h.

The production of H_2O_2 and TNF-α was unchanged in Ca-TBM mice with 21-day-old tumours (Figs. 5C and 6C). This suggests that progression of the infection, and not the tumoral mass itself, impairs the ability of macrophages to respond to infection.

Discussion

Although it has been reported that neoplastic tissues are capable of causing immunological degeneration [18], the

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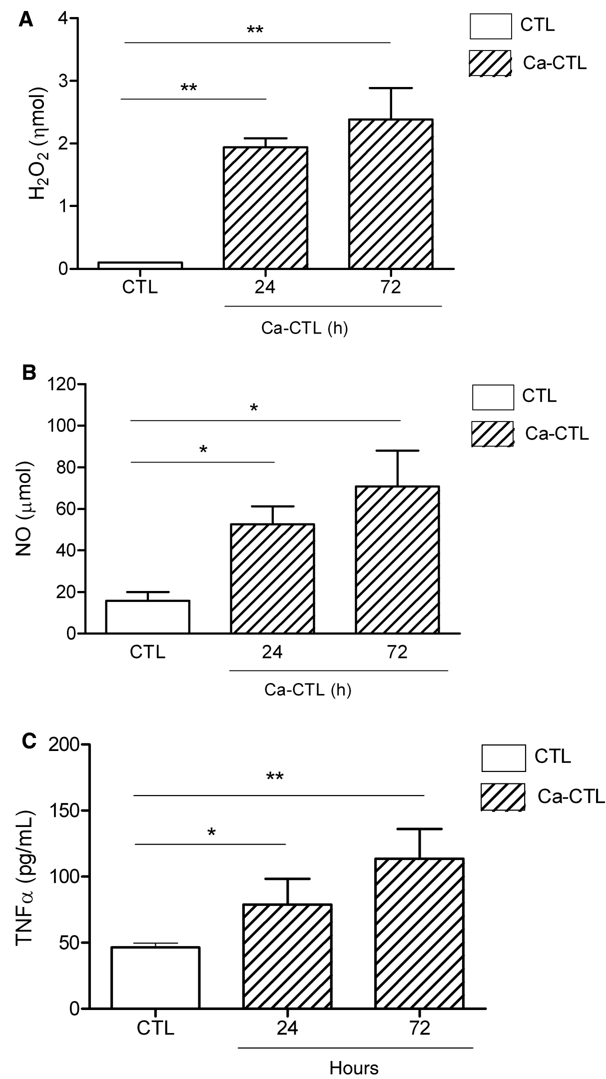

Figure 3 Evaluation of macrophage activity in *Candida albicans*-infected mice (Ca-CTL group). Tumour-free mice were inoculated i.p. with *C. albicans* and the macrophage activity was evaluated at 24 and 72 h after fungal inoculation (Ca-CTL, white striped box). Naïve mice were used as control group (CTL, white box). (A) Production of H_2O_2 by peritoneal macrophages. (B) Release of nitric oxide (NO) by peritoneal macrophages. (C) Release of TNF-α by peritoneal macrophages. Data are expressed as the mean ± SEM. *$P < 0.05$, **$P < 0.01$, $n = 4$ (each group evaluated). ANOVA Tukey's post-test.

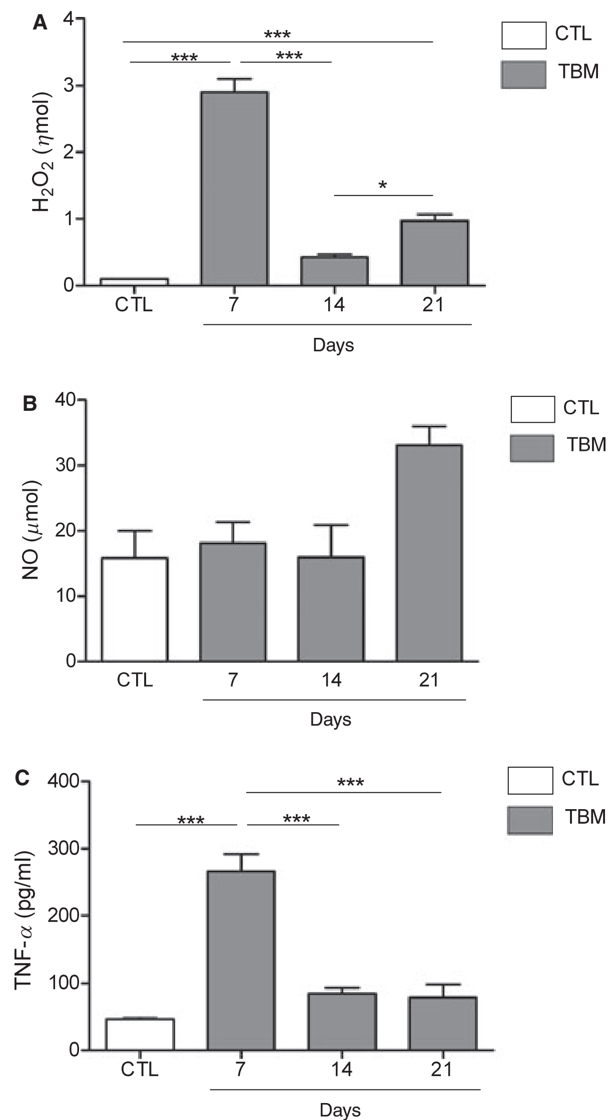

Figure 4 Evaluation of macrophage activity in *Candida*-free tumour-bearing mice (BTM-CTL group). Tumour-bearing mice were evaluated on days 7, 14 and 21 days of tumour progression (BTM-CTL, grey box). Naïve mice were used as control group (CTL, white box). (A) Production of H_2O_2 by peritoneal macrophages. (B) Release of nitric oxide (NO) by peritoneal macrophages. (C) Release of TNF-α by peritoneal macrophages. Data are expressed as the mean ± SEM. *$P < 0.05$, ***$P < 0.001$, $n = 4$ (each group evaluated). ANOVA Tukey's post-test.

mechanisms involved in this process have not been fully clarified. It has been suggested that both the fungus [19] and the tumour factors [20, 21] may depress the ability of macrophages to carry out phagocytosis and lysis, which could facilitate the development of infections, as a consequence.

Similar to other published studies using other experimental models [22–25], we observed that i.p. inoculation of *C. albicans* into tumour-free Swiss mice (Ca-CTL) resulted in acute systemic infection that tended towards resolution by 72 h p.i. The same infection pattern was observed in Ca-TBM until day 7 of tumour progression. After this period, the TBM were more susceptible to infection.

Similar to previously published studies [26, 27], we observed that peritoneal macrophages from *C. albicans*-infected mice produced increased levels of NO. However, the tumour state did not affect NO production by itself, as the TBM and Ca-TBM groups produced a similar quantity of NO as CTL mice. Naama *et al.* [28] demonstrated that upon fungal challenge, TBM produced a similar quantity of NO as healthy control mice, which

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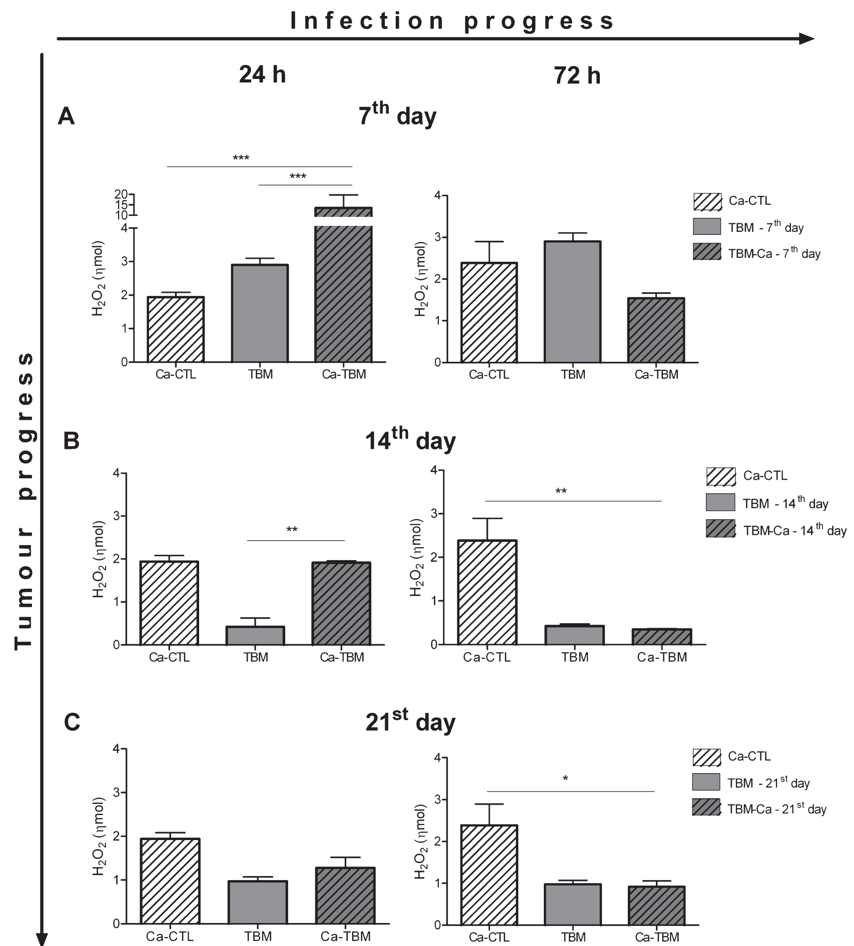

Figure 5 Production of H_2O_2 by peritoneal macrophages. Tumour-bearing mice were inoculated i.p. with *Candida albicans* on days 7, 14 and 21 of tumour progression (Ca-TBM group, grey striped box) and evaluated at 24 and 72 h after fungal inoculation. Free-tumour mice inoculated with *C. albicans* (Ca-CTL, white striped box) and *Candida*-free tumour-bearing mice (TBM-CTL, grey box) were used as control group. (A) Ca-BTM on day 7 of tumour progression. (B) Ca-BTM on day 14 of tumour progression. (C) Ca-BTM on day 21 of tumour progression. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $n = 4$ (each group evaluated). ANOVA Tukey's post-test.

suggests that tumour presence negatively influences the production of NO. In our study, macrophages from infected mice with 7-day-old tumours produced basal levels of NO and were still able to eliminate fungi, which suggests that NO does not significantly contribute to the clearance of *Candida.* Balish *et al.* [29] demonstrated that NO was not essential for killing *C. albicans* using NO-deficient animals.

In addition to NO, phagocytic cells of the host can also use reactive oxygen species such as H_2O_2, as a part of their arsenal for neutralizing *Candida* [30]. Therefore, there are other mechanisms available for clearing *Candida* infection, even if macrophages from tumour-bearing hosts are impaired in their ability to produce NO [29]. This is consistent with our findings concerning H_2O_2. In this study, we demonstrated that peritoneal macrophages from *Candida*-infected animals always exhibited higher levels of this metabolite than macrophages from control animals. We observed that this phenomenon also occurred in peritoneal macrophages from TBM, regardless of the presence or absence of *Candida.* Increased H_2O_2 production was sustained through day seven of tumour growth and coincided with the fast clearance of the fungal burden in infected mice. These data demonstrate that there was an increase in H_2O_2 release at the beginning of the neoplastic progression, but after this period, H_2O_2 production decreased and the infection spread. Our findings are consistent with those described by Okawa *et al.* [7], who verified that the *C. albicans* load in sarcoma-bearing mice was related to the levels of oxygen metabolites released by polymorphonuclear cells.

The dual behaviour of the peritoneal macrophages from the Ca-TBM group against *Candida* suggested that the tumour and/or its products may have modulated the ability of the macrophages to clear *Candida* from a distance. According to Elgert *et al.* [18], circulating factors derived from tumours, such as cytokines and/or tumour cell-membrane debris, were able to activate distal macrophages to produce cytotoxic and suppressor molecules. Confirming these data, we observed this picture was associated with TNF-α levels. For instance, at the beginning of tumour progression, mice showed large quantities of TNF-α and the fungal clearance index was high; at the end of the process, this was inverted (i.e. decrease in H_2O_2 and TNF-α levels and a high fungal load). Therefore, tumour environment could affect the production of macrophage-derived cytokines and consequently the host's resistance to opportunistic infections [7]. Studies

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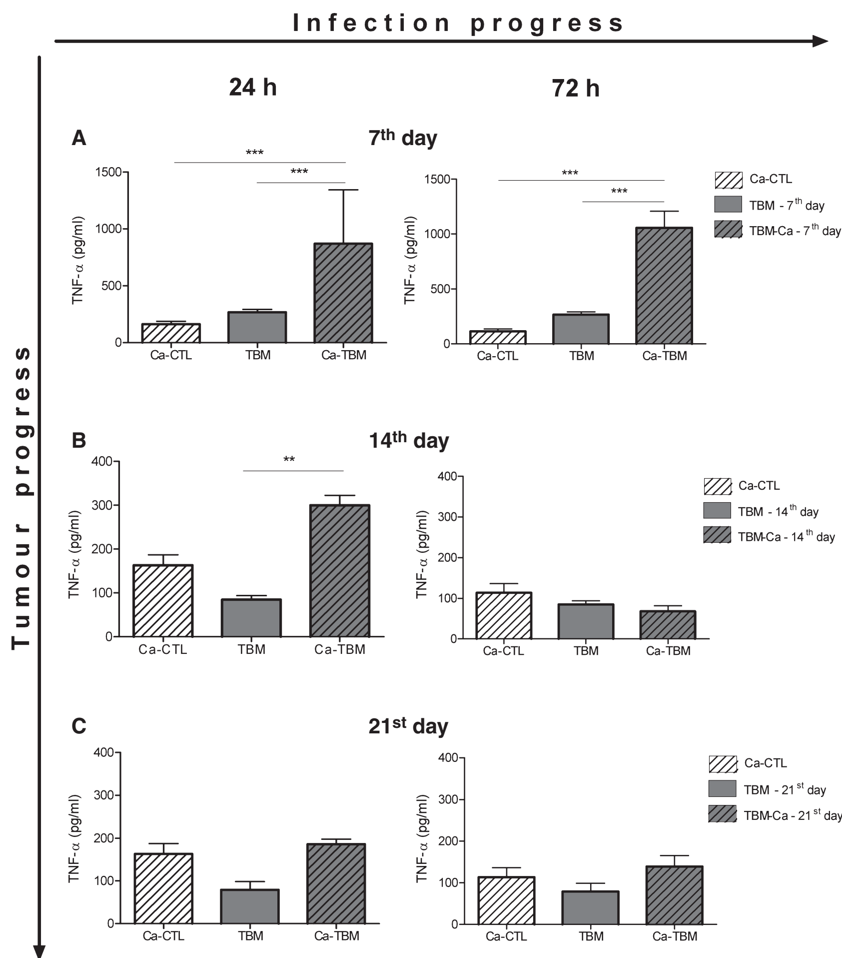

Figure 6 Release of TNF-α by peritoneal macrophages. Tumour-bearing mice were inoculated i.p. with *Candida albicans* at days 7, 14 and 21 of tumoral progression (Ca-TBM group, grey striped box) and evaluated at 24 and 72 h after fungal inoculation. Free-tumour mice inoculated with *C. albicans* (Ca-CTL, white striped box) and *Candida*-free tumour-bearing mice (TBM-CTL, grey box) were used as control group. (A) Ca-BTM at day 7 of tumoral progress. (B) Ca-BTM at day 14 of tumoral progress. (C) Ca-BTM at day 21 of tumoral progress. $^{**}P < 0.01$, $^{***}P < 0.001$, $n = 4$ (each group evaluated). ANOVA Tukey's post-test.

are underway in our laboratory to evaluate the role of other cytokines in this process.

It is possible that we have worked with macrophages from the tumour site (TAMs), as Bhaumik and Khar [9] have reported that macrophages traffic between the peritoneum and neoplastic tissues. Mitra *et al*. [31] demonstrated that macrophages migrated to regional lymph nodes and the peritoneum after ingesting antigens from regressive tumour tissue. In the peritoneal cavity, these cells become activated. This event occurs between days 2 and 5 following tumoral implantation and is coincident with tumour regression.

However, the Ehrlich tumour progressed in the present study, even though there was elevated H_2O_2 and TNF-α production at the beginning of tumour growth (7 days). This progression was associated with a drastic increase in the production of H_2O_2 and TNF-α. Working with several human tumour cell lines, Kuang *et al.* [21] described that factors derived from tumours (including hyaluronan fragments) could induce the transitory activation of monocytes and their subsequent development with suppressor activities. Our results are consistent with those findings, including the Ehrlich solid tumour that was capable of promoting the development of immunosuppressive macrophages after triggering a transient early protective response.

Therefore, our results provide strong evidence that solid tumour development can interfere in the host's response to infection with *C. albicans.* This can be attributed to tumour interference in the production of reactive toxic metabolites by peritoneal macrophages that are located away from the tumour development site, thus creating conditions that favour the progression of both the solid tumour and the fungal infection.

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References

1 Elting LS, Rubenstein EB, Rolston KV, Bodey GP. Outcomes of bacteremia in patients with cancer and neutropenia: observations from two decades of epidemiological and clinical trials. *Clin Infect Dis* 1997;25:247–59.

2 Wisplinghoff H, Cornely OA, Moser S *et al.* Outcomes of nosocomial bloodstream infections in adult neutropenic patients: a

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prospective cohort and matched case-control study. *Infect Control Hosp Epidemiol* 2003;24:905–11.
3 Jugo J, Kennedy R, Crowe MJ *et al.* Trends in bacteraemia on the haematology and oncology units of a UK tertiary referral hospital. *J Hosp Infect* 2002;50:48–55.
4 Velasco E, Byington R, Martins CA, Schirmer M, Dias LM, Gonçalves VM. Prospective evaluation of the epidemiology, microbiology, and outcome of bloodstream infections in hematologic patients in a single cancer center. *Eur J Clin Microbiol Infect Dis* 2003;22:137–43.
5 Wisplinghoff H, Seifert H, Wenzel RP, Edmond MB. Current trends in the epidemiology of nosocomial bloodstream infections in patients with hematological malignancies and solid neoplasms in hospitals in the United States. *Clin Infect Dis* 2003;36:1103–10.
6 Pasqualotto AC, Rosa DD, Medeiros LR, Severo LC. Candidaemia and cancer: patients are not all the same. *BMC Infect Dis* 2006;6:50.
7 Okawa Y, Kobayashi M, Sakai K, Suzuki M. Role of polymorphonuclear leukocytes in the resistance of tumor-bearing mice against *Candida albicans* infection. *Biol Pharm Bull* 2004;27:674–8.
8 Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 2005;5:953–64.
9 Bhaumik S, Khar A. Cytokine-induced production of no by macrophages induces apoptosis and immunological rejection of ak-5 histiocytic tumor. *Apoptosis* 1998;3:361–8.
10 Bhaumik S, Mitra R, Varalakshmi C, Khar A. Activated macrophages migrate to the subcutaneous tumor site via the peritoneum: a novel route of cell trafficking. *Exp Cell Res* 2001;266:44–52.
11 Dagli MLZ, Guerra JL, Saldiva PHN. An experimental study on the lymphatic dissemination of the solid Ehrlich tumor in mice. *Braz J Vet Res Anim Sci* 1992;29:97–103.
12 Silva RJ, da Silva MG, Vilela LC, Fecchio D. Cytokine profile of Ehrlich ascites tumor treated with *Bothrops jararaca* venom. *Mediators Inflamm* 2002;11:197–201.
13 Vilani-Moreno FR, Arruda MSP, Escudero H. Candidíase Experimental: estudo comparativo de dois Métodos de coloração vital na determinação da viabilidade dos fungos em suspensão. *Salusvita* 1999;18:139–42.
14 Bagagli E, Sano A, Coelho KI *et al.* Isolation of *Paracoccidioides brasiliensis* from armadillos (*Dasypus noveminctus*) captured in an endemic area of paracoccidioidomycosis. *Am J Trop Med Hyg* 1998;58:505–12.
15 Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Anal Biochem* 1982;126:131–8.
16 Russo M, Teixeira HC, Marcondes MC, Barbuto JA. Superoxide-independent hydrogen peroxide release by activated macrophages. *Braz J Med Biol Res* 1989;22:1271–3.
17 Norman GR, Streiner DL. *Biostatistics – The Base Essentials*. St. Louis, MO: Mosby Year Book, 1994.
18 Elgert KD, Alleva DG, Mullins DW. Tumor-induced immune dysfunction: the macrophage connection. *J Leukoc Biol* 1998;64: 275–90.
19 Tavares D, Ferreira P, Arala-Chaves M. Increased resistance to systemic candidiasis in athymic or interleukin-10-depleted mice. *J Infect Dis* 2000;182:266–73.
20 Monti P, Leone BE, Zerbi A *et al.* Tumor-derived MUC1 mucins interact with differentiating monocytes and induce IL-10highIL-12low regulatory dendritic cell. *J Immunol* 2004;172:7341–9.
21 Kuang DM, Wu Y, Chen N, Cheng J, Zhuang SM, Zheng L. Tumor-derived hyaluronan induces formation of immunosuppressive macrophages through transient early activation of monocytes. *Blood* 2007;110:587–95.
22 Pitarch A, Díez-Orejas R, Molero G *et al.* Analysis of the serologic response to systemic *Candida albicans* infection in a murine model. *Proteomics* 2001;1:550–9.
23 Elahi S, Pang G, Clancy R, Ashman RB. Cellular and cytokine correlates of mucosal protection in murine model of oral candidiasis. *Infect Immun* 2000;68:5771–7.
24 Ashman RB. Murine candidiasis: susceptibility is associated with the induction of T cell-mediated, strain-specific autoreactivity. *Immunol Cell Biol* 1990;68:179–85.
25 Elahi S, Pang G, Ashman Rb, Clancy R. Nitric oxide-enhanced resistance to oral candidiasis. *Immunology* 2001;104:447–54.
26 Blasi E, Pitzurra L, Puliti M *et al.* Differential susceptibility of yeast and hyphal forms of *Candida albicans* to macrophage-derived nitrogen-containing compounds. *Infect Immun* 1995;63:1806–9.
27 Schröppel K, Kryk M, Herrmann M, Leberer E, Röllinghoff M, Bogdan C. Suppression of type 2 NO-synthase activity in macrophages by *Candida albicans*. *Int J Med Microbiol* 2001;290:659–68.
28 Naama HA, McCarter MD, Mack VE *et al.* Suppression of macrophage nitric oxide production by melanoma: mediation by a melanoma-derived product. *Melanoma Res* 2001;11:229–38.
29 Balish E, Warner TF, Nicholas PJ, Paulling EE, Westwater C, Schofield DA. Susceptibility of germfree phagocyte oxidase and nitric oxide synthase 2-deficient mice, defective in the production of reactive metabolites of both oxygen and nitrogen, to mucosal and systemic candidiasis of endogenous origin. *Infect Immun* 2005; 73:1313–20.
30 Enjalbert B, MacCallum DM, Odds FC, Brown AJ. Niche-specific activation of the oxidative stress response by the pathogenic fungus *Candida albicans*. *Infect Immun* 2007;75:2143–51.
31 Mitra R, Dharajiya N, Kumari L, Varalakshmi C, Khar A. Migration of antigen presenting cells from periphery to the peritoneum during an inflammatory response: role of chemokines and cytokines. *FASEB J* 2004;18:1764–6.

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