Available online at www.sciencedirect.com
SCIENCE DIRECT
Acta Tropica 98 (2006) 130–136
ACTA TROPICA
www.elsevier.com/locate/actatropica

Hyperbaric oxygen therapy reduces the size of *Leishmania amazonensis*-induced soft tissue lesions in mice

Wagner Welber Arrais-Silva [a], Eduardo Fonseca Pinto [b], Bartira Rossi-Bergmann [b], Selma Giorgio [a,*]

[a] *Departamento de Parasitologia, Instituto de Biologia, Universidade Estadual de Campinas, Caixa Postal 6109, CEP 13083-970, Campinas, São Paulo, Brazil*
[b] *Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, 21.949-900 Rio de Janeiro, Brazil*

Received 15 June 2005; received in revised form 11 February 2006; accepted 3 March 2006
Available online 25 April 2006

Abstract

In this study we determined whether exposing mice to hyperbaric oxygen (HBO) would alter various disease parameters of a susceptible mouse strain infected with *Leishmania amazonensis*. BALB/c mice exposed to HBO (100% O_2 at a pressure of 2.5 ATA, 1 h before parasite inoculation and subsequently for 20 days) showed significant delay in lesion development and reduction in lesion parasite burdens compared with HBO-unexposed mice. Circulating levels of interferon gamma (IFN-γ) and tumor necrosis factor (TNF-α) were significantly elevated in HBO-exposed as compared to HBO-unexposed mice. Concanavalin A-stimulated lymph nodes cultures from HBO-exposed mice released significantly more IFN-γ and less interleukin 10 (IL-10) than cultures from HBO-unexposed mice, consistent with a skewed Th1 response. These results demonstrate, for the first time, that HBO can play a pathogen control role during leishmaniasis. Further studies are needed to elucidate whether hyperoxia alone or increased atmospheric pressure alone can exert a similar effect.

Keywords: Leishmaniasis; *Leishmania amazonensis*; Hyperbaric oxygen

1. Introduction

Leishmaniasis is a parasitosis caused by a protozoan of the genus *Leishmania* affecting more than 12 million people worldwide (Herwaldt, 1999; Desjeux, 2004). The severity of the disease produced by several *Leishmania* species varies enormously, ranging from cutaneous or mucosal to visceral or diffuse cutaneous infection. The former is generally caused by *Leishmania amazonensis*, a species transmitted mainly in the Amazon region, which is associated with localized cutaneous lesions (Grimaldi and Tesh, 1993; Herwaldt, 1999). Chemotherapy remains the mainstay for the control of leishmaniasis, as effective vaccines have yet to be developed (Handman, 2001). The first line of therapy for all forms of the disease requires potentially toxic and painful multiple injections of pentavalent antimonials (Berman, 1997). The problem is further aggravated by the appearance of resistance to these drugs in some endemic areas (Berman, 2003). Amphotericin B and pentamidine are second-line drugs and remain of limited value because of their toxicity and difficulty in administration (Berman, 1997). Many studies have been conducted to find an

* Corresponding author. Tel.: +55 19 37886287; fax: +55 19 37886282.
E-mail address: sgiorgio@unicamp.br (S. Giorgio).

0001-706X/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2006.03.001

effective therapy for leishmaniasis that avoids exposure to potentially toxic drugs (Taha and Tabbara, 1997; Khaskhely et al., 2001, 2002; Macharia et al., 2004; Gonçalves et al., 2005).

Several characteristics of leishmanial lesions in humans and animals such as microcirculation impairment, parasite proliferation and secondary bacterial infection (El-On et al., 1992; Grimaldi and Tesh, 1993; Giorgio et al., 1998) and the demonstration that the hypoxia-inducible transcriptional factor (HIF-1α) is expressed in the cutaneous lesions of BALB/c mice infected with *L. amazonensis* (Arrais-Silva et al., 2005b) are strong indications of a hypoxic microenvironment in the lesions. Numerous clinical observations strongly supported by experimental evidence, have led to the conclusion that hypoxia retards isquemic injured tissue healing and repair (Sitkovsky. et al., 2004; Tandara and Mustoe, 2004). Hyperbaric oxygen (HBO) therapy has been shown to increase both systemic and tissue oxygen levels and to assist as an adjuvant treatment for some soft tissue infections (Clark and Monn, 1999). Exposure to HBO can relieve tissue hypoxia, restore oxygen necessary for normal oxidative metabolism and stimulate repair and angiogenesis, as well as cytokines and growth factor synthesis (La Van and Hunt, 1990; Tandara and Mustoe, 2004). Exposure to elevated oxygen tensions affects the viability and proliferation of some bacteria and pathogenic fungi (Park et al., 1992). Recently, we demonstrated that HBO is toxic for *L. amazonensis* promastigotes and amastigotes and can reduce macrophage susceptibility to leishmanial infection in vitro (Arrais-Silva et al., 2005a). In the present study we evaluated the effect of HBO therapy on various disease parameters of mice infected with *L. amazonensis*.

2. Materials and methods

2.1. Parasite and infection of mice

L. amazonensis (MHOM/BR/73/M2269) amastigotes were obtained from footpad lesions of susceptible mice as previously described (Barbiéri et al., 1993). Female BALB/c mice (6 weeks old) were subcutaneously infected in the right hind footpad with 10^5 amastigotes.

2.2. Treatment of mice using hyperbaric oxygen

Mice caged in groups of seven were exposed to 100% O_2 at a pressure of 2.5 ATA for 1 h per day in a small animal hyperbaric chamber (Research Chamber, model HB 1300B, Sechrist, Anaheim, CA, USA). The chamber was pressurized and decompressed at the rate of 0.5 ATA/min (Arrais-Silva et al., 2005a). Animals were exposed to HBO 1 h before *L. amazonensis* inoculation and subsequently for 20 days. Glucantime (*N*-methyl glucamine antimonite; Rhodia, Santo Amaro, SP, Brazil) at 100 mg/kg/day injected via i.p. for 20 days after *L. amazonensis* inoculation was used as the standard anti-leishmanial agent.

2.3. Evaluation of infection

The course of the infection was monitored by measuring the increase in the footpad thickness with a dial caliper compared with the contralateral uninfected footpad. All measurements were made by an observer who was blinded to treatments. To estimate the parasite burden in the lesions, mice were sacrificed at designated periods, the entire infected footpads were weighed and the total number of amastigotes was estimated as previously described (Arrais-Silva et al., 2005b). The spleen and liver were removed to determine their weight. The mice were also regularly examined to detect cutaneous ulcers, secondary lesions and secondary infection with bacteria.

2.4. Histological studies

Mice foot tissues were fixed by immersion in 4% paraformaldehyde in 0.1 M PBS/0.1 M sucrose for 6 h and processed for standard paraffin embedding. Tissue sections (6 μm) were stained with haematoxylin and eosin (H&E) and checked for pathological changes under an optical microscope (Eclipse E800-Nikon, Japan).

2.5. Cytokines measurements

Blood was obtained from mice by ocular function and the pool of serum from each experimental group was assayed in triplicate. Popliteal and inguinal lymph nodes were excised from animals and single cell suspensions prepared in Dulbecco-modified minimum essential medium (Sigma Chemical Co., USA) containing 10% fetal bovine serum and antibiotics. Cells were plated in triplicate in 24-well culture plates at 4×10^6/ml and stimulated with 2.5 μg/ml Concanavalin A (Con A) (Sigma Aldrich, USA) for 48 h at 37 °C with 5% CO_2 in the atmosphere (Pinto et al., 2003). A mitogenic stimulus was used as *L. amazonensis* antigens induce T cell anergy in vitro (Pinheiro et al., 2004). The levels of interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α) and interleukin 10 (IL-10) were measured in two-fold dilutions of the supernatants and sera by ELISA, accord-

ing to the manufacturer instructions (R&D Systems, USA). The levels of cytokines were determined against standard curves using the respective recombinant murine cytokines (R&D Systems, USA).

2.6. Statistical analyses

Statistical significance between control and experimental groups were determined by the Student's *t*-test. Data are expressed as mean ± standard deviation of the mean (S.D.).

3. Results

Fig. 1 demonstrates that cutaneous lesions progressively increased in size in BALB/c infected with *L. amazonensis*. Mice exposed to HBO 1 h before *L. amazonensis* infection and daily for 20 days after infection showed delayed lesion development (Fig. 1A). By the end of HBO treatment none of HBO-exposed mice showed a visible lesion at the site of infection and footpad thickness was about four times smaller than that of the footpads of control animals (Fig. 1A, inset). The lesion size differences between HBO-exposed mice and HBO-unexposed mice were statistically significant for the first 70 days after infection (Fig. 1A). We compared the effect of the reference drug glucantime to that of HBO therapy. The results showed a significant decrease in the size of the lesion after glucantime treatment, although a complete reduction was not obtained, similar to the pattern observed in animals exposed to HBO (Fig. 1A). A combination therapy of HBO and glucantime revealed no additional effect on the course of the infection (data not shown).

To ensure that the reduction of lesion size observed in HBO-exposed mice was related to a reduction of parasite burden, amastigotes counts were performed in footpad lesions. As shown in Fig. 1B parasites were consistently less abundant in lesions of HBO-exposed mice than in lesions of HBO-unexposed mice at 3, 20 and 70 days after infection (3.8-, 4.6- and 13-fold lower than in untreated lesions, respectively). The parasite burden progressively increased with time in footpad lesions of HBO-exposed mice despite the fact that this increase was less pronounced than that observed in lesions of HBO-unexposed animals (Fig. 1B).

Significant differences in the tissue pathology between HBO-exposed mice and untreated mice were observed by the end of HBO therapy (20 days after infection) (Fig. 2). In the footpads of HBO-unexposed mice an infiltration consisting mainly of vacuolar macrophages within amastigotes were seen in the dermis. In con-

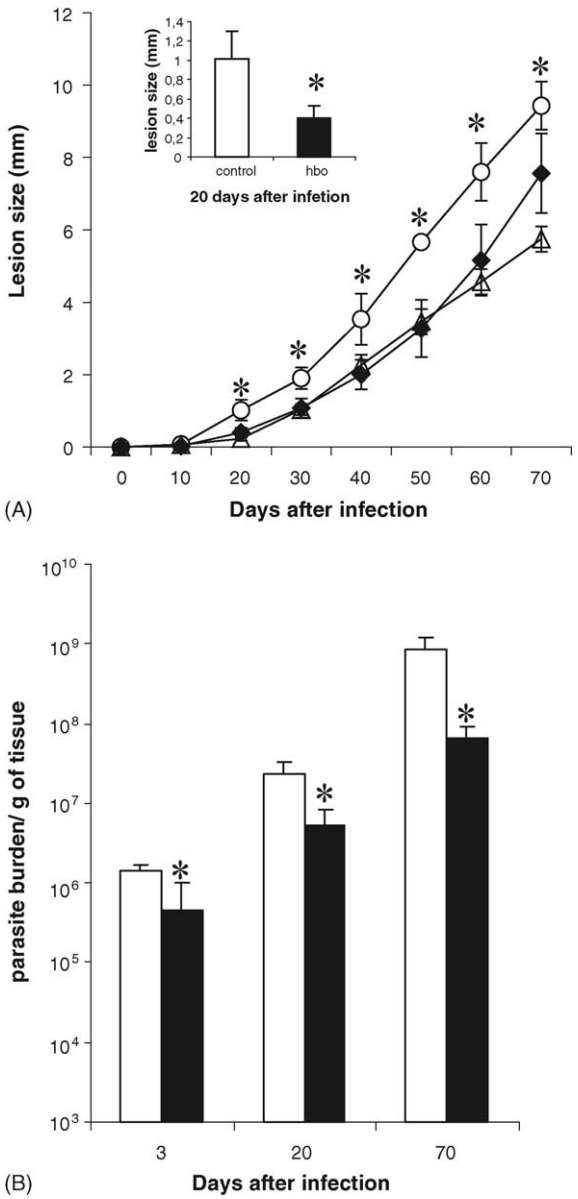

Fig. 1. Effect of HBO treatment on the course of *L. amazonensis* infection in BALB/c mice. Mice (10 per group) were left untreated (◯); treated with glucantime (100 mg/kg/day) (△); or exposed to HBO 1 h before and for 20 days after infection with 10^5 *L. amazonensis* amastigotes (♦). (A) Lesion size is expressed as the difference in size between the infected and contralateral, uninfected footpads. (B) Parasite burden in infected footpad from HBO-exposed (■) and HBO-unexposed (□) mice were determined on the days indicated after infection. The data shown represent the mean ± S.D. This experiment is representative of two independent repeats ($^*P < 0.01$ in comparison to HBO-unexposed mice).

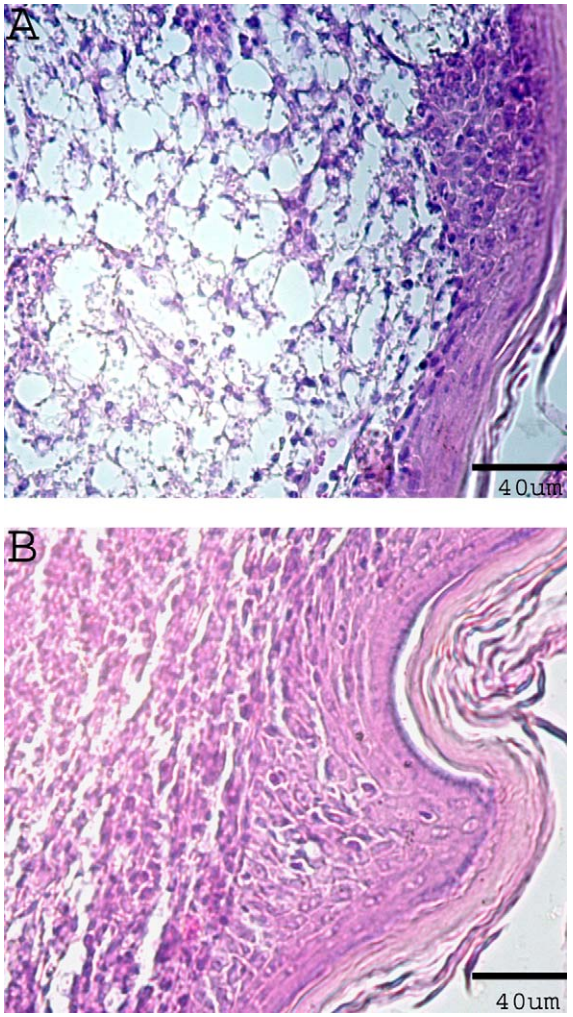

Fig. 2. Photomicrographs of footpad lesions from *L. amazonensis* infected BALB/c mice exposed to HBO and control mice. (A) Most of the cells seen in the lesion of a mouse unexposed to HBO are parasitized macrophages that show large parasitophorous vacuoles containing many amastigotes, after 20 days of infection. (B) A mixed inflammatory cell population infiltrating the lesion of a mouse exposed to HBO as in Fig. 1, after 20 days of infection.

trast, lesions of HBO-exposed mice showed a cellular population infiltration consisting of inflammatory cells and infected macrophages (Fig. 2). Later in the infection, untreated mice lesions were characterized by a massive number of heavily parasitized macrophages, whereas lesions of HBO-exposed mice had vacuolated macrophages containing parasites and few inflammatory cells (data not shown). A small increase in spleen, liver and lymph nodes was apparent in HBO-exposed mice, whereas considerable splenomegaly, hepatomegaly and popliteal/inguinal lymphadenopathy were observed in HBO non-exposed BALB/c mice during the course of infection (data not shown).

A Th1-type response has previously been associated with control of *L. amazonensis* infection (Beyrodt et al., 1997; Khaskhely et al., 2001, 2002). In this study we considered the production of IFN-γ and TNF-α as indicators of a Th1-type response and IL-10 production as an indicator of Th2-type response. The data shown in Fig. 3 indicate that the levels of IFN-γ produced by Con A-stimulated draining lymph nodes cells of HBO-exposed mice were significantly higher than the levels of this cytokine produced by cells from HBO-unexposed mice at 20 days after infection with *L. amazonensis*. The circulating IFN-γ levels were also higher in HBO-exposed mice (Fig. 3). The levels of TNF-α produced by draining lymph nodes cells from HBO-exposed animals indicated no significant differences compared with the level of cytokine produced by cells from HBO-unexposed mice (Fig. 3). However the serum levels of TNF-α of HBO-exposed mice were higher than those detected in the serum of HBO-unexposed mice (Fig. 3). Twenty days after infection, HBO-exposed mice showed a significantly lower IL-10 production in the lymph nodes cells compared with the levels of IL-10 produced by cells from HBO-unexposed animals (Fig. 3). Together these results indicate that the HBO therapy favored the development of Th1-type cytokine response during infection.

4. Discussion

HBO therapy is clinically applied in patients with soft tissue infections such as necrotizing fasciitis and gas gangrene, osteoradionecrosis and compromised skin grafts (Leach et al., 1998; Clark and Monn, 1999). Our previous studies demonstrated that HBO is toxic for promastigotes and amastigotes of *L. amazonensis* in in vitro experiments (Arrais-Silva et al., 2005a). On the basis of these facts the present study was designed to investigate whether HBO treatment would influence the development of clinical disease in *L. amazonensis* infection of an extremely susceptible mouse strain (Barral-Netto et al., 1987). We are not aware of any previous reports describing the effects of HBO therapy on leishmaniasis. We exposed BALB/c mice to HBO 1 h before infection and for the first 20 days after infection with *L. amazonensis* (1 h daily sessions). The results indicated that HBO treatment produces an effect similar to that of the classical antimonial drug, glucantime, in lesions of infected mice (Fig. 1A). While complete cure did not occur, all the parameters evaluated relevant to leishmaniasis changed significantly after HBO exposition, indicative of a reduced pathology. Mice exposed to

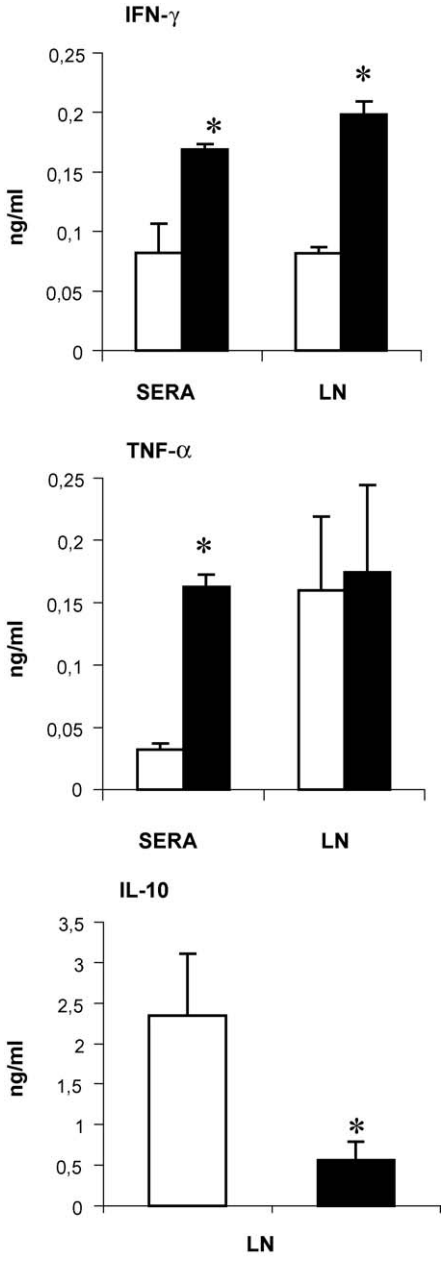

Fig. 3. Cytokine production in BALB/c mice infected with *L. amazonensis* and exposed to HBO. At 20 days after infection sera and/or supernatants from peripheral lymph nodes cells (LN) from mice unexposed to HBO (□) or exposed to HBO (■) as in Fig. 1 were assayed for INF-γ, TNF-α and IL-10 production by ELISA. The data shown represent the mean ± S.D. This experiment is representative of two independent repeats ($^{*}P < 0.01$ in comparison to HBO-unexposed mice).

HBO showed a significant delay in the development of cutaneous lesions compared with HBO-unexposed mice (Fig. 1A). In addition, HBO-exposed mice showed a significantly lower parasite burden in foot lesions compared that in unexposed HBO mice during the course of infection (Fig. 1B). A partial elimination of the parasites from the primary lesion induced by HBO treatment during the first 20 days of infection can explain the reduced parasite burden still observed 50 days after the last HBO exposure (Fig. 1B). Light microscopy of HBO-exposed mice lesions showed areas of mixed cell inflammatory reactions and fewer parasites compared with lesions of HBO-unexposed mice at 20 days of infection (Fig. 2). Administering only three HBO sessions (one prior and two after infection) had no effect upon infection (data not shown).

The protective mechanisms for *Leishmania* infection are considered to involve Th1-type response with the production of IFN-γ and TNF-α, potent inductors of the leishmanicidal macrophage function (Scott et al., 2004). The susceptibility to infection reflect the expansion of Th2 response with the production of IL-4 and IL-10, potent inhibitors of macrophage function (Scott et al., 2004). The observation that IL-10 is diminished, whereas IFN-γ and TNF-α are increased in the peripheral lymph nodes cells or serum of HBO-exposed mice (Fig. 3), is in accord with the inflammatory reactions seen in lesions (Fig. 2), and corresponds with previous reports of a Th1-type response associated with the control or delay of *L. amazonensis* infection in BALB/c mice pre-exposed to ultraviolet radiation (Khaskhely et al., 2001, 2002) or immunized with parasite antigens (Beyrodt et al., 1997; Campbell et al., 2003; Pinto et al., 2003).

A major question raised by these results is related to the mechanism by which HBO causes a reduction in cutaneous lesions. It is likely that the reduced parasite burden in the lesions of HBO-exposed mice (Fig. 1B) is the result of a direct effect of tissue high oxygen tension on parasites leading to their destruction (Arrais-Silva et al., 2005a), with a consequent shift in the Th cell response from a Th2- to a Th1-type response (Fig. 3). This notion is supported by studies showing that BALB/c mice given low doses of *L. major* are resistant to infection and establish a Th1 response. In contrast, high parasite doses promote susceptibility and a Th2 response in this mouse strain (Bretscher et al., 1992; Doherty and Coffman, 1996).

We cannot exclude the possibility that HBO has direct modulatory effects on the immune response of *L. amazonensis* infected mice. HBO and hyperoxia has been shown to alter various aspects of host defense (Smith and Mohideen, 1991; Brenner et al., 1999; Chen et al., 2003). However, given the variety of experimental models and HBO treatment protocols used to study the effects of HBO on the immune system, both immunosuppressive, as well as immunostimulatory effects, have been reported. For example, whether HBO exposure activates

or depresses proinflammatory cytokines is not yet clear. In a murine zymosan-induced shock model, circulating TNF-α levels were reduced in animals exposed to HBO compared with unexposed mice (Luongo et al., 1998). After exposing normal rats to HBO, Lahat and coworkers observed an increase in the spontaneous secretion of TNF-α by mononuclear cells from blood, spleen and lung (Lahat et al., 1995). Another study observed that macrophages isolated from normal mice exposed to HBO produce a decreased concentration of IL-1 and similar concentrations of IL-6 and phagocytic activity when compared to macrophages from unexposed mice (Inamoto et al., 1991). Mononuclear cells from humans exposed to HBO released less IFN-γ and similar quantities of IL-1 and TNF-α as compared with cells obtained before HBO treatment (Granowitz et al., 2002). Benson and coworkers examining the duration effects of HBO exposure demonstrated that suppression of proinflammatory cytokine synthesis by human macrophages was evident during the first 3 h of HBO exposure and after 12 h of HBO exposure, there was an augmentation of stimulus-induced proinflammatory cytokine production (Benson et al., 2003). The authors suggest that relatively short HBO treatments may be immunosuppressive while HBO prolonged exposure may induce proinflammatory cytokine synthesis (Benson et al., 2003). We propose that the HBO treatment used in our study (a total of 21 h of HBO exposure) induced IFN-γ and TNF-α production which activated macrophage leishmanicidal mechanisms in *L. amazonensis* infected mice.

In conclusion, the beneficial effect of HBO in murine leishmaniasis as attested by lesion size, parasite burden, histopathology and cytokine pattern, encourages further studies. Such studies should elucidate whether 100% oxygen at 1 ATA or 2.5 ATA at normoxia can exert an effect similar to the effect seen with HBO.

Acknowledgements

This work was supported by the Fundação de Amparo a Pesquisa do Estado de São Paulo, Conselho Nacional de Desenvolvimento Cientifico e Tecnológico e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

References

Arrais-Silva, W.W., Colhone, M.C., Ayres, D.C., Souto, P.C.S., Giorgio, S., 2005a. Effects of hyperbaric oxygen on *Leishmania amazonensis* promastigotes and amastigotes. Parasitol. Int. 54, 1–7.

Arrais-Silva, W.W., Paffaro Jr., V.A., Yamada, A.T., Giorgio, S., 2005b. Expression of hypoxia-inducible factor-1alpha in the cutaneous lesions of BALB/c mice infected with *Leishmania amazonensis*. Exp. Mol. Pathol. 78, 49–54.

Barbiéri, C.L., Giorgio, S., Merjan, A.J.C., Figueiredo, E.N., 1993. Glycosphingolipid antigens of *Leishmania* (*Leishmania*) *amazonensis* identified by use of a monoclonal antibody. Infect. Immun. 61, 2131–2137.

Barral-Netto, M., Cardoso, S.A., Barral, A., 1987. Different patterns of disease in two inbred mouse strains infected with a clone of *Leishmania mexicana amazonensis*. Acta Trop. 44, 5–11.

Benson, R.M., Minter, L.M., Osborne, B.A., Granowitz, E.V., 2003. Hyperbaric oxygen inhibits stimulus-induced proinflammatory cytokine synthesis by human blood-derived monocyte-macrophages. Clin. Exp. Immunol. 134, 57–62.

Berman, J., 1997. Human leishmaniasis: clinical, diagnostic, and chemotherapeutic developments in the last 10 years. Clin. Infect. Dis. 24, 684–703.

Berman, J.D., 2003. Current treatment approaches to leishmaniasis. Curr. Opin. Infect. Dis. 16, 397–401.

Beyrodt, C.G., Pinto, A.R., Freymuller, E., Barbieri, C.L., 1997. Characterization of an antigen from *Leishmania amazonensis* amastigotes able to elicit protective responses in a murine model. Infect. Immun. 65, 2052–2059.

Brenner, I., Shephard, R.J., Shek, P.N., 1999. Immune function in hyperbaric environments, diving, and decompression. Undersea Hyperb. Med. 26, 27–39.

Bretscher, P.A., Wei, G., Menon, J.N., Bielefeldt-Ohmann, H., 1992. Establishment of stable, cell-mediated immunity that makes "susceptible" mice resistant to *Leishmania major*. Science 257, 539–542.

Campbell, K., Diao, H., Ji, J., Soong, L., 2003. DNA immunization with the gene encoding P4 nuclease of *Leishmania amazonensis* protects mice against cutaneous leishmaniasis. Infect. Immun. 71, 6270–6278.

Chen, S.Y., Chen, Y.C., Wang, J.K., Hsu, H.P., Ho, P.S., Chen, Y.C., Sytwu, H.K., 2003. Early hyperbaric oxygen therapy attenuates disease severity in lupus-prone autoimmune (NZB × NZW) F1 mice. Clin. Immunol. 108, 103–110.

Clark, L., Monn, R.E., 1999. Hyperbaric oxygen in the treatment of life-threatening soft-tissue infections. Respir. Care Clin. N. Am. 5, 203–219.

Desjeux, P., 2004. Leishmaniasis: current situation and new perspectives. Comp. Immunol. Microbiol. Infect. Dis. 27, 305–318.

Doherty, T.M., Coffman, R.L., 1996. *Leishmania major*: effect of infectious dose on T cell subset development in BALB/c mice. Exp. Parasitol. 84, 124–135.

El-On, J., Sneier, R., Elias, E., 1992. *Leishmania major*: bacterial contamination of cutaneous lesions in experimental animals. Isr. J. Med. Sci. 28, 81–85.

Giorgio, S., Linares, E., Ischiropoulos, H., Von Zuben, F.J., Yamada, A., Augusto, O., 1998. In vitro formation of electron paramagnetic resonance-detectable nitric oxide and nitrotyrosine is not impaired during murine leishmaniasis. Infect. Immun. 66, 807–814.

Gonçalves, G.S., Fernandes, A.P., Souza, R.C., Cardoso, J.E., de Oliveira-Silva, F., Maciel, F.C., Rabello, A., Ferreira, L.A., 2005. Activity of a paromomycin hydrophilic formulation for topical treatment of infections by *Leishmania* (*Leishmania*) *amazonensis* and *Leishmania* (*Viannia*) *braziliensis*. Acta Trop. 93, 161–167.

Granowitz, E.V., Skulsky, E.J., Benson, R.M., Wright, J., Garb, J.L., Cohen, E.R., Smithline, E.C., Brown, R.B., 2002. Exposure to increased pressure or hyperbaric oxygen suppresses interferon-gamma secretion in whole blood cultures of healthy humans. Undersea Hyperb. Med. 29, 216–225.

Grimaldi Jr., G., Tesh, R.B., 1993. Leishmaniasis of the new world: current concepts and implications for future research. Clin. Microbiol. Rev. 6, 230–250.

Handman, E., 2001. Leishmaniasis: current status of vaccine development. Clin. Microbiol. Rev. 14, 229–243.

Herwaldt, B.L., 1999. Leishmaniasis. Lancet 354, 1191–1199.

Inamoto, Y., Okuno, F., Saito, K., Tanaka, Y., Watanabe, K., Morimoto, I., Yamashita, U., Eto, S., 1991. Effect of hyperbaric oxygenation on macrophage function in mice. Biochem. Biophys. Res. Commun. 179, 886–891.

Khaskhely, N.M., Maruno, M., Takamiyagi, A., Uezato, H., Kasem, K.M., Hosokawa, A., Kariya, K., Hashiguchi, Y., Landires, E.A., Nonaka, S., 2001. Pre-exposure with low-dose UVA suppresses lesion development and enhances Th1 response in BALB/c mice infected with *Leishmania* (*Leishmania*) *amazonensis*. J. Dermatol. Sci. 26, 217–232.

Khaskhely, N.M., Maruno, M., Uezato, H., Takamiyagi, A., Ramzi, S.T., Al-Kasem, K.M., Kariya, K., Toda, T., Hashiguchi, Y., Gomez Landires, E.A., Nonaka, S., 2002. Low-dose UVB contributes to host resistance against *Leishmania amazonensis* infection in mice through induction of gamma interferon and tumor necrosis factor alpha cytokines. Clin. Diagn. Lab. Immunol. 9, 677–686.

La Van, F.B., Hunt, T.K., 1990. Oxygen and wound healing. Clin. Plastic Surg. 17, 463–472.

Lahat, N., Bitterman, H., Yaniv, N., Kinarty, A., Bitterman, N., 1995. Exposure to hyperbaric oxygen induces tumor necrosis factor-alpha (TNF-α) secretion from rat macrophages. Clin. Exp. Immunol. 102, 655–659.

Leach, R.M., Rees, P.J., Wilmshurst, P., 1998. Hyperbaric oxygen therapy. Br. Med. J. 317, 1140–1143.

Luongo, C., Imperatore, F., Cuzzocrea, S., Filippelli, A., Scafuro, M.A., Mangoni, G., Portolano, F., Rossi, F., 1998. Effects of hyperbaric oxygen exposure on a zymosan-induced shock model. Crit. Care Med. 26, 1972–1976.

Macharia, J.C., Bourdichon, A.J., Gicheru, M.M., 2004. Efficacy of trypan: a diminazene based drug as antileishmanial agent. Acta Trop. 92, 267–272.

Park, M.K., Myers, R.A.M., Marzella, I., 1992. Oxygen tensions and infections: modulation of microbial growth, activity of antimicrobial agents and immunological responses. Clin. Infect. Dis. 14, 720–740.

Pinheiro, R.O., Pinto, E.F., Benedito, A.B., Lopes, U.G., Rossi-Bergmann, B., 2004. The T-cell anergy induced by *Leishmania amazonensis* antigens is related with apoptosis and defective antigen presentation. Anais Acad. Bras. Ciências 76, 519–527.

Pinto, E.F., de Mello Cortezia, M., Rossi-Bergmann, B., 2003. Interferon-gamma-inducing oral vaccination with *Leishmania amazonensis* antigens protects BALB/c and C57BL/6 mice against cutaneous leishmaniasis. Vaccine 21, 3534–3541.

Scott, P., Artis, D., Uzonna, J., Zaph, C., 2004. The development of effector and memory T cells in cutaneous leishmaniasis: the implications for vaccine development. Immunol. Rev. 201, 318–338.

Sitkovsky, M.V., Lukashev, D., Apasov, S., Kojima, H., Koshiba, M., Caldwell, C., Ohta, A., Thiel, M., 2004. Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors. Annu. Rev. Immunol. 22, 657–682.

Smith, R.M., Mohideen, P., 1991. One hour in 1 ATA oxygen enhances rat alveolar macrophage chemiluminescence and fungal cytotoxicity. Am. J. Physiol. 260, L457–L463.

Taha, S.M., Tabbara, K.S., 1997. Modulation of the immune response of Balb/c mice against *Leishmania major* by imuvert. Int. J. Immunopharmacol. 19, 443–449.

Tandara, A.A., Mustoe, T.A., 2004. Oxygen in wound healing-more than a nutrient. World J. Surg. 28, 294–300.