Evaluation of the phagocytic activity of peripheral blood monocytes of patients with Jorge Lobo's disease

Avaliação da atividade fagocítica dos monócitos do sangue periférico de pacientes com doença de Jorge Lobo

Fátima Regina Vilani-Moreno[1], Luciana Moreira Silva[1] and Diltor Vladimir Araújo Opromolla[2]

ABSTRACT

Studies on host-parasite interaction in Jorge Lobo's disease are scarce, with no report in the literature on the phagocytosis of Lacazia loboi *by phagocytic mononuclear cells. Thus, the objective of the present study was to assess the phagocytic activity of blood monocytes in the presence of* L. loboi *in patients with the disease and in healthy subjects (controls) over 3 and 24 hours of incubation. Statistical analyses of the results showed no significant difference in percent phagocytosis of the fungus between patient and control monocytes. With respect to incubation time, however, there was a significant difference, in that percent phagocytosis was higher at 3 hours than at 24 hours (p <0.01). These results suggest that monocytes from patients with the mycosis are able to phagocyte the fungus, as also observed in control individuals.*

Key-words: *Jorge Lobo's disease. Phagocytosis. Monocyte.* Lacazia loboi.

RESUMO

Os estudos envolvendo a interação hospedeiro-parasita na doença de Jorge Lobo são escassos; até o momento, não existe nenhum trabalho abordando a fagocitose do fungo Lacazia loboi pelas células mononucleares fagocitárias. Assim, o presente estudo teve como objetivo avaliar a atividade fagocítica dos monócitos sangüíneos frente ao L. loboi *tanto em pacientes portadores da doença como em indivíduos sadios (grupo controle), utilizando 3 e 24 horas de incubação. A análise estatística dos resultados revelou que não houve diferença significante entre o percentual de fagocitose do fungo pelos monócitos de pacientes e do grupo controle, porém em relação aos tempos de incubação, houve diferença significante, isto é, às 3 horas o percentual de fagocitose foi maior que o obtido às 24 horas (p<0,01). Estes resultados sugerem que monócitos de pacientes portadores da micose são hábeis em fagocitar o fungo, à semelhança do que foi visto em indivíduos do grupo controle.*

Palavras-chaves: *Doença de Jorge Lobo. Fagocitose. Monócito.* Lacazia loboi.

Jorge Lobo's disease is a cutaneous-subcutaneous mycosis with a chronic evolution caused by the fungus *Lacazia loboi* (*L. loboi*)[8,18]. The mycosis predominantly occurs in Brazil but cases have been reported, in decreasing order, in Colombia, Surinam, French Guyana, Venezuela, Panama, Costa Rica, Peru, Ecuador, Bolivia, Mexico, and Europe[12]. According to Opromolla *et al*[11], the estimated number of cases of the disease is 458, 295 and those that occurred in Brazil (295 cases) were predominantly in the Amazon region.

Jorge Lobo's mycosis mainly attacks people from forest areas close to rivers and flood land with a warm and humid climate, as is the case for workers tapping rubber trees in the Amazon region, and is markedly more frequent among males[6]. The disease has been detected in white, black and Indigenous

1. Equipe Técnica de Imunologia do Instituto Lauro de Souza Lima, Bauru, SP, Brasil. 2. Divisão de Pesquisa e Ensino do Instituto Lauro de Souza Lima, Bauru, SP, Brasil.

This research has been approved by the Ethical Committee of the Instituto Lauro de Souza Lima, process 21/00.

Address to: Drª Fátima Regina Vilani Moreno. Equipe Técnica de Imunologia do Instituto Lauro de Souza Lima. Rod. Comte. João Ribeiro de Barros, km 225/226, 17034-971 Bauru, SP, Brasil.
Fax: 14 3103-5914, Tel: 14 3103-5872
e-mail: imunologia@ilsl.br

Recebido para publicação em 3/4/2003
Aceito em 16/2/2004

individuals, with no evidence of greater susceptibility to infection in a given ethnic group[7].

Clinically, the mycosis is characterized by cutaneous lesions of with a cheloid-like, wart-like infiltrative, ulcerous or gummy aspect, with the possible occurrence of more than one type of lesion in the same patient[16]. Ulcerated lesions seem to be a result of traumatic injuries more than to primary spontaneous ulceration and therefore are clinically secondary[11].

Histopathologically, Jorge Lobo's disease is characterized by an intense diffuse histiocytic reaction containing large numbers of foreign body giant cells and Langhans' cells, and numerous intra or extracellular parasites. Small numbers of lymphocytes, plasmocytes and neutrophils are also present[7 10].

Ultrastructural studies of the cutaneous lesions have revealed that, after the fungus is endocytosed by the phagocytic cell and suffers the action of lysosomal enzymes, remnants of the cell wall of *L. loboi* can be detected in the cytoplasm. These cell wall remnants progressively accumulate inside the macrophages, conferring a foamy aspect, as observed by light microscopy[14 15].

Macrophages are cells commonly associated with the defense mechanisms of the host against invading microorganisms. They belong to the mononuclear phagocytic system and, in addition to defending the host through phagocytosis and cell digestion, they also interact with and stimulate lymphocytes to participate in the defense mechanisms, thus playing a fundamental role both in nonspecific mechanisms of defense and in the specific immune response[1].

Few studies on the host-parasite interaction are available for Jorge Lobo's disease and no study exists about the phagocytosis of *L. loboi* by mononuclear phagocytic cells. However, this topic has been studied in other mycoses such as paracoccidioidomycosis[3 4] and candidiasis[13], demonstrating the important role of macrophages in host defense. Thus, the aim of the present study was to assess the phagocytosis of the fungus *L. loboi* by peripheral blood monocyte of patients with Jorge Lobo's disease.

PATIENTS AND METHODS

Patients. The study was conducted on 10 patients with Jorge Lobo's disease from the State of Acre and examined by the clinical staff of the Lauro de Souza Lima Institute, in Bauru-SP, with a diagnosis of the mycosis confirmed by anatomicopathological examination. Fifteen healthy adults, employees of the Lauro de Souza Lima Institute, with similar age to that of the patients, were used as control group.

Blood collection. Twenty ml of venous blood was collected from each patient and control subject with 2 Vacutainer (BD, Minas Gerais, Brazil) tubes containing heparin as an anticoagulant.

Mononuclear cell preparation. Mononuclear cells were separated on a Ficoll-Hypaque density gradient[2] (Sigma, Missouri, USA). The cells were resuspended in 1ml RPMI medium containing L-glutamine and 15mM HEPES buffer (Gibco BRL, São Paulo, Brazil) supplemented with penicillin (100UI/ml)-streptomycin (100μg/ml) (Gibco BRL). Monocytes were counted in a Neubauer chamber by 1:8 dilution of the cell suspension with 0.02% neutral red dye in saline solution. The preparation was left to stand at 37ºC for 20 min and the final concentration of the suspension was adjusted to 5×10^5 monocytes/ml.

Monocyte culture. One ml of the monocyte suspension was distributed in duplicate among the wells of flat-bottomed 24-well tissue culture plates (Corning, New York, USA) containing a previously sterilized coverslip measuring 13mm in diameter (Glasstécnica, São Paulo, Brazil). After 2 hours, the supernatants were removed and each well was washed with RPMI medium to remove non-adherent cells. Next, 1ml of RPMI medium supplemented with 15% fresh AB^+ pooled serum (obtained by combining sera from 5 healthy donors under conditions preserving complement activity) and with antibiotics was added. A 100ml aliquot of the *L. loboi* suspension containing 5×10^5 fungi at the proportion of 1 cell for 1 fungus was added to each well.

The *Lacazia loboi* suspension was obtained from skin lesions previously collected by surgical removal and stored frozen. The biopsies were processed in 0.85% saline solution and the fungal suspension obtained was filtered through gauze for the removal of tissue remnants and then autoclaved. The fungi present in the suspension were counted in a Neubauer chamber.

The plate was incubated at 37ºC in a 5% CO_2 atmosphere for 3 and 24 hours. After these incubation periods, the coverslips were removed, washed with 0.85% saline and stained with Giemsa.

Evaluation of phagocytic activity. Two hundred monocytes were examined in coverslips at 1000x magnification for enumeration of the cells containing fungus or not.

Statistical analyses. Two-way analyses of variance was used to determine the significance of the difference in percent fungus phagocytosis, comparing patient and control monocytes[19]. The same analyses was also used to determine the effect of time on the percentage of phagocytosis regardless of the group studied. In all circumstances, the level of significance was set at $p < 0.01$.

RESULTS

The percentages of peripheral blood mononuclear cells phagocyting *L. loboi* from patients and controls are presented in Table 1. Statistical analyses of the results showed no significant difference in percent phagocytosis by the monocytes of patients and controls. However, there was a significant difference between the time points evaluated, with a higher percentage of phagocytosis at 3 hours than at 24 hours ($p < 0.01$). Figure 1 illustrates *L. loboi* phagocytosis by peripheral blood monocytes from a patient with Jorge Lobo's mycosis.

We had also the opportunity to assess the phagocytic index of two patients with Jorge Lobo's disease by adding fresh and inactivated serum of the patient himself to the cultures. We observed a higher percentage of phagocytosis with not inactivated serum (33%) than with inactivated serum (24%).

Table 1 - Percent phagocytosis of L. loboi by blood monocytes from patients with Jorge Lobo's disease and controls.

	Percent phagocytosis (mean ± SD)	
	3 hours	24 hours
Patients	39.3 ±6.5	34.0 ±7.0
Control	37.5 ±4.9	29.3 ±8.1

Statistical analyses: patient = control (F = 1.83667; p = 0.189741)

3 hours>24 hours (F = 20.11371; p = 0.000204)*

* statistically significant difference (p <0.01)

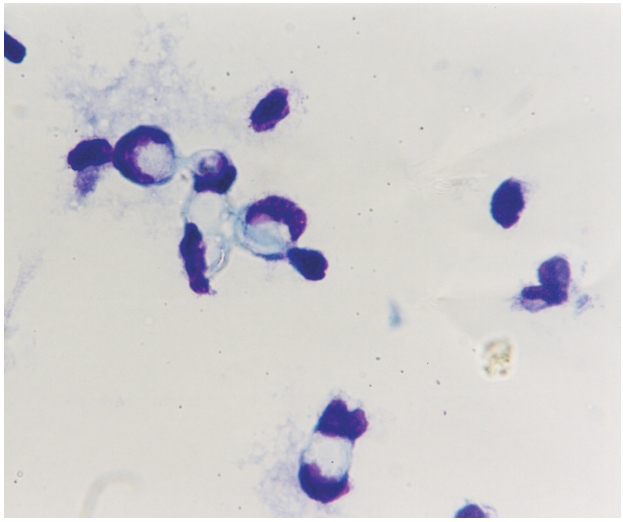

Figure 1 - Phagocytosis of L. loboi by peripheral blood monocytes. Culture time: 3 hours (Giemsa, 1000x).

DISCUSSION

The phagocytosis of microorganisms represents one of the nonspecific defense mechanisms of vital importance for the host. The monocyte/macrophage cell lines, commonly referred to as professional phagocytes, can neutralize, engulf and destroy particles, including infectious agents, thus presenting a high phagocytic potential[1]. In this respect, these cells have been frequently evaluated for phagocytic and lytic capacity against pathogenic microorganisms.

In the present study, the evaluation of the phagocytic capacity of blood monocytes from patients with Jorge Lobo's disease revealed that the monocytes of both patients and healthy individuals (controls) presented a similar percentage of phagocytosis, with no statistically significant difference between them.

A survey of the literature did not locate any report concerning the phagocytosis of *L. loboi* by monocytes of patients with the mycosis, although this topic has been investigated for other mycoses. In paracoccidioidomycosis, a study on the phagocytosis of *P. brasiliensis* by mouse alveolar macrophages revealed that 64.2% of the macrophages phagocytized the fungus. When the cultures were treated with interferon gamma (INF-γ) there was no significant difference in percent phagocytosis over a period of 4 hours, but the percentage increased significantly after 24 hours. Similarly, the fungicidal capacity of activated macrophages was also higher after 24 hours[3].

In a later study, Brummer *et al*[4] evaluated the phagocytic and fungicidal potential of murine peritoneal macrophages activated or not with INF-γ and observed that after 4 hours 92% of the fungi had been phagocytized by macrophages regardless of whether or not they had been activated. However, only activated macrophages were able to fully eliminate *P. brasiliensis* within a period of 48 hours. According to the cited investigators, activated macrophages play a decisive role in the resistance to *P. brasiliensis*. More recently, Moscardi-Bacchi *et al*[9] evaluated the phagocytosis of blood monocytes from patients with paracoccidioidomycosis and detected a phagocytic rate of 80%. They also observed that monocytes activated with INF-γ markedly inhibited the multiplication of *P. brasiliensis*.

In the present study, the phagocytosis of *L. loboi* was assessed at 3 and 24 hours and statistical analyses of the results showed that, regardless of the group studied, phagocytosis of the fungus was higher at 3 hours than at 24 hours. These results are similar to those obtained by Brummer *et al*[4] who detected 92.7% phagocytosis of *P. brasiliensis* by mouse peritoneal macrophages at 4 hours, 61% at 24 hours, and 10.2% at 48 hours.

In the literature, most studies on fungus phagocytosis used periods of time ranging from half an hour to 4 hours[3 5 13 17]. Thus, in the present study we opted for the evaluation of phagocytosis after 3 hours and then at 24 hours.

The design of the present study took into consideration what the ideal proportion of phagocytic cell:fungi would be for the cultures. Literature data have revealed wide variations[5 13 17] and therefore we opted for the use of 1 cell:1 fungus. In this respect, Sawyer[13] assessed the phagocytic activity of mouse alveolar macrophages against *C. albicans* using 1:1 and 5:1 phagocytic cell:fungus proportions and their results showed that the rates of phagocytosis were higher when the 1 cell:1 fungus proportion was used.

In another study, Gaziri *et al*[5] used the proportions of 1:3, 1:6 and 1:9 cell:fungus for the evaluation of *C. albicans* phagocytosis by mouse peritoneal macrophages and obtained better results when the 1:9 proportion was used. These investigators demonstrated the important role of the complement system in the opsonization of *C. albicans* and later phagocytosis by macrophages. They showed that phagocytic activity was much higher when fresh serum was added to the cultures as a source of complement. When fresh serum was added, phagocytosis was 50.6% and when heat-inactivated serum was used, phagocytosis was 5.4% using the proportion of 1 cell:3 fungi.

In the present study, monocyte cultures contained fresh AB^+ serum as a source of complement. As also done by Gaziri *et al*[5], we had the opportunity to assess the phagocytic index of two patients with Jorge Lobo's disease by adding fresh and inactivated serum of the patient himself to the cultures. The results obtained suggest that the antibody and complement favor the opsonization and phagocytosis of the fungus. However, a larger number of patients is necessary for a better study of the participation of these opsonins in *L. loboi* phagocytosis.

Whatever, the results reported here represent a first assay for the evaluation of one of the nonspecific defense mechanisms of the host and reveal that the monocytes of patients with Jorge Lobo's disease are able to phagocyte *L. loboi*, there was no

significant difference between patients and controls. They also show that the rate of fungal phagocytosis was higher during the period of 3 hours compared to 24 hours, suggesting that during the later period the phagocytes may be involved in the process of lysis of the phagocytized fungus.

ACKNOWLEDGMENTS

We wish to thank Prof. José Roberto Pereira Lauris, Dental School, University of São Paulo, Bauru, for statistical analyses of the data.

REFERENCES

1. Aderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. Annual Review of Immunology 17: 593-623, 1999.
2. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Scandinavian Journal of Clinical and Laboratory Investigation 21: 77-89, 1968.
3. Brummer E, Hanson LH, Restrepo A, Steven SA. *In vivo* and *in vitro* activation of pulmonary macrophages by IFN-gamma for enhanced killing of *Paracoccidioides brasiliensis* or *Blastomyces dermatitidis*. The Journal of Immunology 140: 2786-2789, 1988.
4. Brummer E, Hanson LH, Restrepo A, Stevens DA. Intracellular multiplication of Paracoccidioides brasiliensis in macrophages: killing and restriction of multiplication by activated macrophages. Infection and Immunity 57: 2289-2294, 1989.
5. Gaziri G, Gaziri LCJ, Kikuchi R, Scanavacca J, Felipe I. Phagocytosis of *Candida albicans* by concanavalin-A activated peritoneal macrophages. Medical Mycology 37: 195-200, 1999.
6. Guimarães FN, Macedo DG. Contribuição ao estudo das blastomicoses na Amazônia (blastomicose sul-americana). Hospital 38: 223-253, 1950.
7. Lacaz CS, Baruzzi RG, Rosa MCB. Doença de Jorge Lobo. Universidade de São Paulo-IPSIS, São Paulo, 1986.
8. Lobo J. Um caso de blastomicose, produzido por uma espécie nova, encontrada em Recife. Revista Médica de Pernambuco 1: 763-775, 1931.
9. Moscardi-Bacchi M, Brummer E, Stevens DA. Support of *Paracoccidioides brasiliensis* multiplication by human monocytes or macrophages: inhibition by activated phagocytes. The Journal of Medicine Microbiology 40: 159-164, 1994.
10. Opromolla DVA, Belone AFF, Taborda PRO, Taborda VBA. Correlação clínicopatológica em 40 casos novos de lobomicose. Anais Brasileiros de Dermatologia 75: 425-434, 2000.
11. Opromolla DVA, Taborda PR, Taborda VBA, Viana S, Furtado JF. Lobomicose: relato de 40 casos novos. Anais Brasileiros de Dermatologia 74: 135-141, 1999.
12. Rodriguez-Toro G. Lobomycosis. International Journal of Dermatology 32: 324-332, 1993.
13. Sawyer RT. Experimental pulmonary candidiasis. Mycopathologia 109: 99-109, 1990.
14. Sesso A, Azevedo RA, Baruzzi RG. Lanthanum nitrate labeling of the outer cell wall surface of phagocytized *Paracoccidioides loboi* in human lobomycosis. Journal of Submicroscopic Cytology and Pathology 20: 769-772, 1988.
15. Sesso A, Baruzzi RG. Interaction between macrophage and parasite cells in lobomycosis. The thickened cell wall of *Paracoccidioides loboi* exhibits apertures to the extracellular milieu. Journal of Submicroscopic Cytology and Pathology 20: 537-548, 1988.
16. Silva D. Micose de Lobo. Revista da Sociedade Brasileira de Medicina Tropical 6: 85-98, 1972.
17. Sugar AM, Picard M. Macrophage and oxidant mediated inhibition of the ability of live *Blastomyces dermatitidis* conidia to transform to the pathogenic yeast phase: implications for pathogenesis of dimorphic fungal infections. Journal of Infectious Diseases 163: 371-375, 1991.
18. Taborda PR, Taborda VA, Mcginnis MR. *Lacazia loboi* gen. nov., comb. nov., the etiologic agent of lobomycosis. Journal of Clinical Microbiology 37: 2031-2033, 1999.
19. Zar JH. Biostatistical analysis. Prentice-Hall, New Jersey, 1996.