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JORGE LOBO'S DISEASE IMMUNOPATHOLOGY: CELLULAR COMPOSITION OF THE INFLAMMATORY INFILTRATE AND CYTOKINE QUANTIFICATION IN THE SUPERNATANT OF MONONUCLEATED CELL CULTURES AND IN BLOOD SERUM

THESIS. F. R. Vilani-Moreno submitted this thesis for her Doctorate in Tropical Diseases at Botucatu School of Medicine, São Paulo State University, UNESP, Botucatu, São Paulo, Brazil, 2002.

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ABSTRACT: Jorge Lobo's disease is a cutaneous or subcutaneous mycosis of chronic evolution, caused by the fungus *Lacazia loboi*. This mycosis occurs predominantly in the Amazon region and affects mainly rural workers who live in straight contact with the soil and plants, such as the rubber plantation workers. There are scarce studies about the immunopathological aspects of this disease. Until now, the cellular components of the granuloma induced by the fungus *L. loboi* are not known, neither the role of the immune response in the genesis and development of this granuloma. The present study, therefore, aimed to identify the mononuclear cell population in cutaneous lesions and to quantify macrophagic and lymphocytic cytokines in the cell culture supernatants and in the serum.
The study participants were 15 Jorge Lobo's disease patients from Acre State, and 15 healthy adults as the control group. Blood samples were collected for serum and isolation of mononuclear cells. The monocytes were cultured for 24 hours in the presence or absence of LPS (10 μg/ml) and *L. loboi* (5 cells:1 fungus). Lymphocytes were cultured for 48 hours in the presence or absence of PHA (8 μg/ml) and *L. loboi* (5 cells:1 fungus). The supernatants were collected after predetermined time periods and then stored at -70°C until use. The cytokines IL-1β, TNF- α, and IL-6 were quantified by ELISA in the monocyte cultures supernatant and in the sera. The cytokines IL-2 and INF-γ (Th1 profile), and IL-4 and IL-10 (Th2 profile) were quantified by ELISA in the lymphocyte cultures supernatant and in the sera.
Histology sections obtained from the patients cutaneous lesions were stained with hematoxylin-eosin and methenamine silver. The following mononuclear cells were identified by immunohistochemical methods: lymphocytes T ($CD3^+$), lymphocytes T helper ($CD4^+$), lymphocytes T citotoxic ($CD8^+$), lymphocytes B ($CD20^+$), plasma cells ($CD79^+$), NK cells ($CD57^+$), histiocytes ($CD68^+$), and Langerhans and interdigitating reticular cells ($S100^+$).
The results showed that the inflammatory infiltrate was composed predominantly by histiocytes and multinucleated giant cells (MGC), besides a large number of fungi, many of them presenting morphological features of nonviable cells. The number of

lymphocytes was discreet to moderate, and neutrophils were rarely found. The histopathological picture of this mycosis is characteristic of foreign body granulomas. The frequency of cells in the inflammatory infiltrate was: histiocytes $CD68^+$ > lymphocytes T $CD3^+$ (lymphocytes T $CD4^+$ > lymphocytes T $CD8^+$) > NK cells $CD57^+$ > plasma cells $CD79^+$ > lymphocytes B $CD20^+$ = Langerhans and interdigitating reticular cells $S100^+$. The lymphocytes were present close to histiocytes and MGC, forming small foci, or grouped around vessels; most of them were from the helper sub-population ($CD4^+$), being the ratio $CD4^+$:$CD8^+$ of approximately 3:2. The NK cells were frequently found in the lesion, being the third cell type identified; they were seen near histiocytes. The number of plasma cells was superior to that of lymphocytes B, and could be found near lymphocytes T and around vessels. The histopathology of patients with the localized (09 patients) and non-localized form of the disease (06 patients) demonstrated similar features in respect to cell type and distribution.
The quantification of cytokines from the cultures supernatant showed higher production of IL-4 and IL-6, and lower levels of IL-2 in patients when compared to the control group. The production of IL-1β, TNF-α, IL-10, and INF-γ was similar for patients and controls. There was no significant difference in the quantification of serum cytokines.
The mononuclear cells of patients with the non-localized form of the disease produced higher levels of INF-γ when compared to the patients with the localized form. Considering the results obtained we may suggest that Jorge Lobo's disease patients show cytokine profile changes, represented by the predominance of Th2 profile. Moreover, further studies are needed to evaluate the *in situ* role of cytokines in the cell-fungus interaction and the possible mechanisms involved in the lysis of *L. loboi*, so that a better understanding of the Jorge Lobo's disease pathogenesis may be achieved.

KEY WORDS: Jorge Lobo's disease, *Lacazia loboi*, cytokines, peripheral blood mononucleated cells, immunopathology, immunohistochemistry

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