Microbial Pathogenesis 78 (2015) 29–36

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Analysis of microvasculature phenotype and endothelial activation markers in skin lesions of lacaziosis (Lobomycosis)

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ARTICLE INFO

Article history:
Received 7 August 2014
Received in revised form
18 November 2014
Accepted 20 November 2014
Available online 20 November 2014

Keywords:
Jorge Lobo's disease
Microvasculature
ICAM-1
VCAM-1
E-selectin

ABSTRACT

Jorge Lobo's disease is a rare mycosis characterized by chronic inflammation, which causes skin lesions in the absence of visceral dissemination. The disease occurs mainly in hot and humid climates and most cases have been registered in the Brazilian Amazon region. This study investigated possible microvascular alterations in skin lesions caused by infection with *Lacazia loboi* which may interfere with the clinical progression of the disease. Immunohistochemistry was used to evaluate the density of blood and lymphatic vessels, as well as expression of the cell adhesion molecules ICAM-1, VCAM-1 and E-selectin. The results showed a reduced number of blood (62.66 ± 20.30 vessels/mm^2) and lymphatic vessels (3.55 ± 5.84 vessels/mm^2) in Jorge Lobo's disease when compared to control skin (169.66 ± 66.38 blood vessels/mm^2 and 8 ± 2.17 lymphatic vessels/mm^2). There were a larger number of vessels expressing ICAM-1 (27.58 ± 15.32 vessels/mm^2) and VCAM-1 (7.55 ± 6.2 vessels/mm^2). No difference was observed in the expression of E-selectin (4.66 ± 11 vessels/mm^2). Taken together, the results indicate changes in the local microvasculature which may interfere with the development of an efficient cell-mediated immune response and may explain restriction of the fungus to the site of injury.

1. Introduction

Lobomycosis is a chronic granulomatous infection caused uncultivable fungus by *Lacazia loboi*. The disease affects the skin and subcutaneous tissue of humans and members of the family Delphinidae (dolphins) through inoculation of the fungus, probably associated with trauma [1,2].

Lobomycosis was described for the first time in 1931 by the Brazilian dermatologist Jorge de Oliveira Lobo in a rubber tapper from the Amazon Valley [3]. The disease is therefore also known as Jorge Lobo's disease or Jorge Lobo's mycosis. Other synonyms include lacaziosis, keloidal blastomycosis, Jorge Lobo type blastomycosis, *miraip* or *piraip* ("that which burns" in Tupi language), Caiabi leprosy, false leprosy, blastomycoid granuloma, and Amazonia blastomycosis [2]. This mycosis is typical of the Neotropical region and most cases have been reported in the Brazilian Amazon region. Since notification is not mandatory, it is difficult to establish the exact number of cases of the disease; however, there is an estimated 490 cases in the world, 318 of them in Brazil, including 61 Indians of the Caiabi tribe [4,5].

Little is known about the physiopathological features of this disease, probably because it has so far been impossible to grow the etiological agent in artificial media [6,7]. The lesions in Jorge Lobo's disease generally exhibit marked fibrosis, absence of well-formed granulomas, and large numbers of the fungus. These features indicate a deficient cell-mediated immune response and a well-established humoral response, which seem to lead to clinical manifestation of the disease [5].

The maintenance of infection and establishment of a predominant cell-mediated response are influenced by the activation of vessels. Several inflammatory diseases are associated with a hyperactive or insufficient vasculature, including dengue, psoriasis, atopic dermatitis, and ultraviolet light-induced skin damage [8].

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http://dx.doi.org/10.1016/j.micpath.2014.11.013
0882-4010/

Endothelial and lymphatic vessels seem to play an important role in acute and chronic inflammatory diseases. In inflamed skin, vascular remodeling consists of an increase in permeability, expansion of the vessel network accompanied by increased blood flow, and inflammatory cell influx. During chronic inflammation, the activated endothelium expresses chemokines, cytokines and adhesion molecules that induce rolling stable adhesion and leukocyte migration to the skin [9].

The skin undergoes different histopathological changes during infection with *L. loboi* and establishment of the disease. In Jorge Lobo's disease, the activity of endothelial and lymphatic vessels during development of chronic inflammation in response to infection with the fungus has not been investigated. Therefore, the objective of the present study was to evaluate possible microvascular alterations associated with *L. loboi* infection in skin lesions of patients with a confirmed diagnosis of Jorge Lobo's disease.

2. Materials and methods

2.1. Patients

Paraffin-embedded skin lesion of the lower limbs biopsies from 24 patients with a diagnosis of Jorge Lobo's disease obtained before therapeutic intervention were selected from the archive of the Nucleus of Tropical Medicine, Federal University of Pará (Núcleo de Medicina Tropical, Universidade Federal do Pará). The diagnosis was based on the clinical presentation of the patients, direct mycological examination and histopathological analysis according to the report of each sample.

Excluded were samples of patients not diagnosed with Jorge Lobo's disease, patients co-infected with HIV, and patients receiving some type of treatment at the time of biopsy. 20 lower limbs skin biopsies, 10 of the young and 10 of the old subjects without histopathological alterations served as controls. These biopsies were selected from the archive of the Department of Pathology, University of São Paulo School of Medicine (Departamento de Patologia da Faculdade de Medicina da Universidade de São Paulo – FM-USP) and Tropical Medicine Center of Federal do Para University (Nucleo de Medicina Tropical da Universidade Federal do Para).

2.2. Sample processing

The paraffin blocks of cases of Jorge Lobo's disease and control cases were cut into 4-μm thick histological sections with a microtome and mounted on glass slides previously prepared with 3-amino-propyltriethoxy-silane adhesive (Sigma Chemical Co., St. Louis, MO, USA). Some slides were stained with hematoxylin-eosin for morphological analysis and the remaining slides were submitted to immunohistochemistry using specific antibodies against ICAM-1 (Novocastra/NCL-CD54/diluted at 1:50), VCAM-1 (RD Systems/BBA19/diluted at 1:1000), E-selectin (Novocastra/NCL-CD62E-382/diluted at 1:50), CD34 (Novocastra/NCL-END-CD34/diluted at 1:50), and podoplanin (D2-40) (Dakocytomation/M3619/diluted at 1:600).

2.3. Immunohistochemistry

Immunostaining for endothelial antigens and cell adhesion molecules was performed according to the method of Hsu et al. [10], partially modified and standardized by the laboratory of the Discipline of Pathology of Transmissible Diseases, Department of Pathology, FM-USP. For this purpose, the histological sections were deparaffinized by incubation in an oven at 56 °C for 24 h, immersed in xylene for 20 min at room temperature, and again immersed in xylene for 10 min at room temperature. Next, the sections were hydrated in a decreasing ethanol series (100%, 95%, 70%) for 5 min each and then washed under running water and in distilled water and phosphate-buffered saline (PBS), pH 7.4, for 5 min. Endogenous peroxidase was blocked by three incubations in 3% hydrogen peroxide for 10 min in a dark chamber. The slides were then washed as described above. Antigen retrieval, if necessary, was achieved by wet heat treatment for 20 min at 95 °C in Target Retrieval Solution (Dako), pH 9 or 6 according to antibody standardization. The sections were again washed as described above. Nonspecific proteins were blocked by incubating the sections in 10% skim milk for 30 min at 37 °C, followed by washing under running water, distilled water and PBS, pH 7.4, for an average of 5 min each. Next, the sections were incubated with the antigen-specific primary antibody diluted in 1% bovine serum albumin for 12 h at 4 °C.

After incubation with the primary antibodies, the sections were washed twice with PBS, pH 7.4, for 5 min each. The method of detection and amplification differed for each primary antibody. The LSAB method was used for slides labeled with the anti-VCAM-1 antibody. The sections were incubated with the biotinylated secondary antibody (Biotinylated link universal, Dako A/S, Denmark) for 30 min at 37 °C, followed by incubation with the streptavidin-biotin HRP complex (Dako A/S) for 30 min at 37 °C. For slides labeled with the anti-CD34, anti-ICAM-1 and anti-E-selectin antibodies, an indirect polymer method using the Novolink kit (Novocastra, USA) was applied. The sections were incubated with the secondary antibody for 30 min at 37 °C, followed by incubation with the tertiary antibody labeled with peroxidase-conjugated polymer at 37 °C. An indirect polymer method using the Envision kit (Dako) was used for slides labeled with the anti-D2-40 (podoplanin) antibody. The sections were incubated with the secondary antibody labeled with peroxidase-conjugated polymer for 30 min at 37 °C. After washing, the reaction was developed by incubating the slides with 0.03% 3.3′-diaminobenzidine as chromogen plus 1.2 ml 3% hydrogen peroxide. Color intensity was monitored under a light microscope using positive controls (amygdala) for each reaction. The slides were then washed under running water for 10 min and counterstained with Harris hematoxylin for 15 s, washed under running water, and dehydrated in ethanol. Finally, the slides were mounted in Permount resin (Fisher Scientific, Fair Lawn, NJ, USA).

2.4. Quantitative analysis of immunostaining

Positive staining for the antigens in all samples was defined as cells exhibiting brown staining in the cytoplasm. Immunoreactive vessels were quantified by a single observer. For this purpose, immunostained vessels were counted using a 1-cm^2 grid divided into 10 portions of 1 mm^2, which was adapted to the eyepiece of a Nikon Eclipse 200 light microscope. Vessels were counted at 400× magnification. The grid was positioned over the entire dermis and 12 fields were selected randomly per sample. The sum of the number of immunostained vessels was divided by the number of fields (12) and the result was divided by 0.0625 (area of the grid), providing the number of cells/mm^2.

2.5. Statistical analysis

Quantitative variables were compared between patients with Jorge Lobo's disease and controls using the nonparametric Mann–Whitney test. Differences between individual groups were considered to be significant when the probability of equality was less than 0.0001 ($p < 0.0001$) and when the probability of not having a relationship was less than 0.05. All statistical analyses

were performed with the GraphPad Prism 6.0 for Windows program (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Clinical and pathological characteristics of the sample studied

The mean age of the patients was 52 years (range: 22–89 years). There were 8.34% women and 91.66% men.

3.2. Anatomopathological analysis of cases of Jorge Lobo's disease

Large numbers of parasites with an individual double membrane were identified in all preparations. Individual chain-like forms were frequently observed. In addition, live and dead fungi were present in the lesions (Fig. 1). Histological analysis revealed cases exhibiting an atrophic epidermis, acanthosis/hyperkeratosis, or preserved epidermis (Fig. 2).

As shown in Table 1, an atrophic rectified epidermis was seen in 66.66% ($n = 16$) of cases which exhibited formation of the so-called grenz zone, a thin layer of subepidermal collagen. The opposite events, i.e., acanthosis and hyperkeratosis characterized by thickening of the epidermis due to a larger number of cells in the spinous layer (acanthosis) and corneal layer (hyperkeratosis), were observed at a lower frequency in 25% of cases ($n = 6$). Transepidermal elimination of the fungus was seen in some lesions. The epidermis was preserved in 8.33% ($n = 2$) of cases.

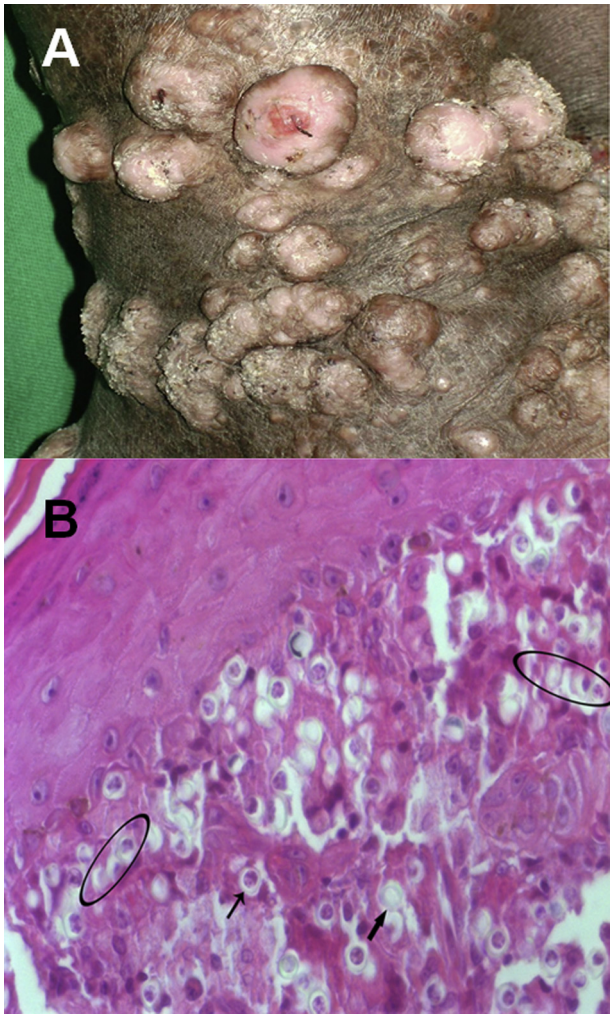

Fig. 1. Clinical aspects and histopathology with presence of the fungus in human *Lacazia loboi* injury. Intense presence of the fungus in the lesion in individual forms (arrows) and catenulada (ellipse). Note the presence of live yeast (thin arrow) and dead (large arrow). Histological sections stained with hematoxylin and eosin. Magnification: 400×.

Histological analysis of the dermis revealed intense and massive infiltration of macrophages in all cases. Other features included the presence of macrophages filled with finely granular material, the formation of giant cells in the absence of granuloma formation, and the sparse presence of lymphocytes. Asteroid bodies were observed in two cases. Plasma cells and eosinophils were present in some lesions (Fig. 3).

As can be seen in Table 2, macrophages filled with finely granular material were rare in 37.5% ($n = 9$) of cases, moderate macrophage infiltration was observed in 33.33% ($n = 8$), intense infiltration in 16.66% ($n = 4$), and macrophages were absent in 12.5% ($n = 3$). Giant cell formation was observed in all cases and was rare in 41.66% ($n = 10$), moderate in 29.16% ($n = 7$) and intense in 29.16% ($n = 7$). The formation of asteroid bodies was seen in two cases. Lymphocyte infiltration ranged from rare (41.66%, $n = 9$) to moderate (45.66%, $n = 11$) and was absent in 12.5% ($n = 3$) of cases. Eosinophils and plasma cells were identified at a lower frequency. Eosinophil infiltration was rare in 16.66% ($n = 4$) of cases, moderate in 4.16% ($n = 1$), and absent in 79.16% ($n = 19$). Plasma cells were mainly absent (83.33%, $n = 20$). A rare plasma cell infiltrate was observed in one case (4.16%), a moderate infiltrate in 2 (8.33%), and an intense infiltrate in one (4.16%). A moderate number of neutrophils were present in only one case (4.16%). Moderate fibrosis was observed in 37.5% ($n = 9$) of cases and collagen fibers were detected in 33.33% ($n = 8$).

3.3. Microvascular density

The density of blood and lymphatic vessels was determined by immunohistochemical staining of CD34 (pan-endothelial) and D2-40 (lymphatic endothelium) endothelial antigens (Fig. 4).

First, the total number of vessels stained was compared between lesions and normal skin. Vessel density was lower in lesions of patients with Jorge Lobo's disease (66.22 ± 20.65 vessels/mm^2) when compared to normal skin samples (177.66 ± 64.5 vessels/mm^2) (Fig. 5).

Next, the density of blood and lymphatic vessels was evaluated. Blood vessels were detected in all cases studied and were concentrated in the papillary layer of the dermis. Immunohistochemical quantification showed a mean blood vessel density of 62.66 ± 20.30 vessels/mm^2 in lesions of patients with Jorge Lobo's disease and of 169.66 ± 66.38 vessels/mm^2 in normal skin samples. Comparative analysis of blood vessel density between cases and controls showed a reduction in lesions caused by *L. loboi* infection (Fig. 6).

Lymphatic vessels were less frequent in patients with Jorge Lobo's disease. No lymphatic vessels were detected in 41.66% ($n = 10$) of cases. If present, lymphatic vessels were observed in the reticular layer of the dermis. Immunohistochemical quantification showed a mean density of 3.55 ± 5.84 lymphatic vessels/mm^2 in lesions of patients with Jorge Lobo's disease and of 8 ± 2.17 vessels/mm^2 in normal skin samples. A lymphatic vessel density of about 25 vessels/mm^2 was observed in only one case. Comparative analysis with the control group showed that infection with *L. loboi* reduced the density of lymphatic vessels in the lesions (Fig. 6).

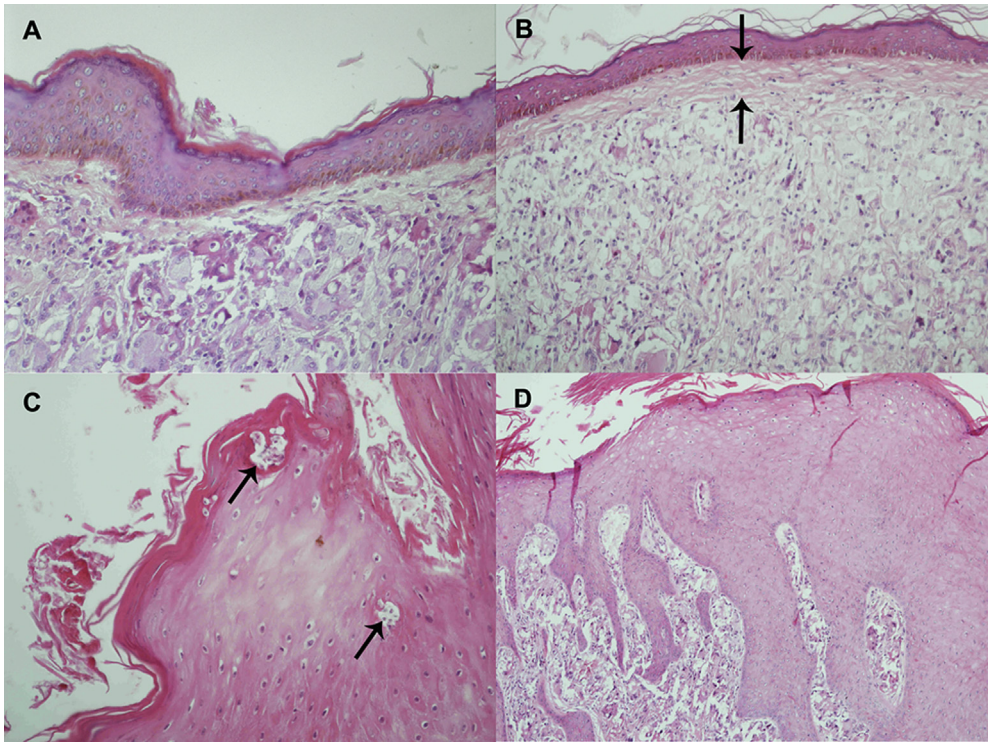

Fig. 2. Histological alterations found in the epidermis during Jorge Lobo's disease. (A) Epidermis conserved. (B) rectified atrophic epidermis with formation of subepidermal band Grenz (arrows). (C) Elimination epidermal transmigration of fungi. (D) acanthosis/hyperkeratosis. Histological sections stained with hematoxylin-eosin. Magnification 200× in (A), (B) and (D). 400× magnification in (C).

Table 1
Distribution of the histopathological aspects of the epidermis in the lesion of patients with Jorge Lobo's disease.

Histopathological aspects	Histopathological aspects of the epidermis		
	Atrophic epidermis rectified	Acanthosis/ hyperkeratosis	Normal epidermis
%	66.66	25	8.33
N	16	6	2

3.4. Evaluation of expression of cell adhesion molecules

Endothelial activation was evaluated by immunohistochemical staining for ICAM-1, VCAM-1 and E-selectin (Fig. 7). A significantly larger number of vessels with endothelial cells expressing ICAM-1 on their surface were observed in lesions of patients with Jorge Lobo's disease when compared to the control group ($p < 0.0001$). The mean number of vessels expressing ICAM-1 was 27.58 ± 15.32 vessels/mm^2 in lesions of patients, whereas no expression of this adhesion molecule was observed in normal skin (Fig. 8). In addition to endothelial cells, other cell types also exhibited ICAM-1 staining.

The mean number of vessels with cells expressing VCAM-1 was also significantly higher ($p = 0.0375$) in lesions of patients with Jorge Lobo's disease (7.55 ± 6.2 vessels/mm^2) compared to normal skin (1.6 ± 1.6 vessels/mm^2) (Fig. 8).

In contrast, the number of E-selectin-stained vessels did not differ significantly between control and infected skin. No E-selectin staining was observed in 46% ($n = 11$) of cases. The mean number of E-selectin-stained vessels was 4.66 ± 11 vessels/mm^2 in lesions of patients, whereas no stained vessels were detected in normal skin (Fig. 8).

For better evaluation of the level of expression of the cell adhesion molecules in vessels of patients with Jorge Lobo's disease, the percent expression of each molecule was determined in relation to total vessel density. The percent expression of ICAM-1 was significantly higher than the expression of VCAM-1 and E-selectin (Fig. 9).

4. Discussion

Jorge Lobo's disease is a rare mycosis of the skin caused by the fungus *L. loboi*. The dynamic interaction between the pathogenic agent and the host results in a chronic inflammatory disease whose physiopathological aspects are still not well understood. To our knowledge, no studies have investigated the cutaneous vascular system in Jorge Lobo's disease and some aspects of this system were therefore addressed in the present study.

The sample of Jorge Lobo's disease cases studied predominantly consisted of male patients with a mean age of 52 years. This finding agrees with a survey involving 125 cases, in which 92% ($n = 115$) of the patients were males and 8% ($n = 10$) were females [11]. With respect to age, according to Baruzzi et al. [12], a higher proportion of cases of Jorge Lobo's disease are found in the 21–40 year age group considering the onset of the disease. The mean age above 40 years observed in the present study might be attributed to the fact that it corresponded to the mean age of the patients at sample collection.

Histopathological analysis of the slides showed the presence of an atrophic rectified epidermis in most cases. The opposite event, i.e., acanthosis and hyperkeratosis, was observed in a smaller number of patients and some cases exhibited a preserved epidermis. Similarly, Opromolla et al. [13] found a higher frequency of atrophic rectified epidermis in 40 patients with Jorge Lobo's disease. Another event observed was transepidermal elimination of

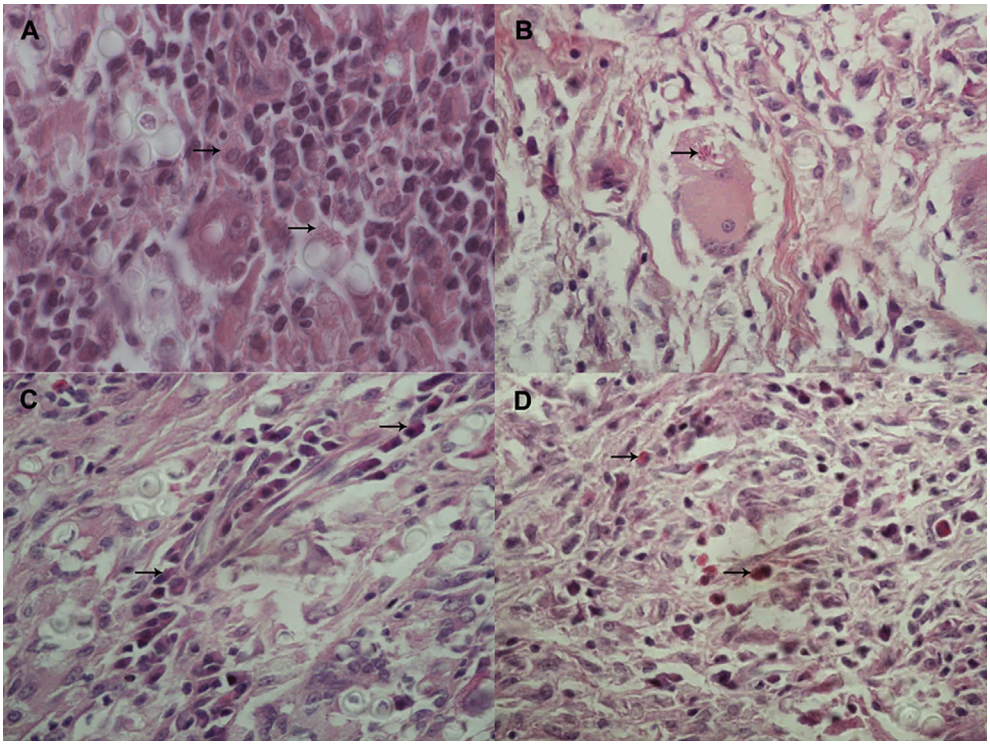

Fig. 3. Micrograph showing the cell infiltrate in the dermis during Jorge Lobo's disease. (A) rich in macrophages, the presence of finely granular macrophages (arrow), giant cells and lymphocytes infiltrate rare. (B) Giant cell containing asteroid bodies inside. (C) Presence of plasma cells. (D) Presence of eosinophils. Histological sections stained with hematoxylin-eosin. Magnification: (A) 630× and (B), (C) and (D) 400×.

the fungus, a process by which the skin sheds inflammatory cells, tissue components, foreign body material, and microorganisms [14]. This process has been reported in other studies on lacaziosis [13,15].

A large number of fungi and macrophages, some lymphocytes and other cell types, which were present in smaller numbers or absent, were seen in the dermis of patients with Jorge Lobo's disease. Similar histopathological features have been described in other studies [12,13].

Immunohistochemistry revealed microvascular alterations in lesions of Jorge Lobo's disease. The presence of a hyperactive or insufficient vasculature has been reported for different dermatological disease, including many inflammatory disorders such as psoriasis rosacea and contact dermatitis [16]. Microvascular alterations have also been described in other infectious diseases such as dengue, malaria, and hemorrhagic fever [17–19].

Quantitative analysis of immunostaining for D2-40 and CD34 showed fewer blood and lymphatic vessels in lesions of patients with Jorge Lobo's disease when compared to the control group. Among fungal diseases, the event of vessel regression or inhibition of angiogenesis has been observed in invasive pulmonary aspergillosis [20]. That study demonstrated that secondary metabolites of the fungus *Aspergillus fumigatus* inhibited the differentiation and migration of endothelial cells, as well as the *in vitro* formation of capillary tubes. In addition, gliotoxin was found to exert a specific role in the antiangiogenic activity of *A. fumigatus* [20].

With respect to lymphatic vessels, although inflammation can be characterized as a mechanism that promotes lymphangiogenesis, this phenomenon is not observed in certain situations [21]. In the present study, a marked reduction in the density of lymphatic vessels was observed in Jorge Lobo's disease lesions. This reduction is also seen in ultraviolet light-induced skin damage [22]. In contrast, in psoriasis, the number of lymphatic vessels is increased in the plaques [23]. This increase in lymphatic vessel density has also been described for arthritis and is intensified after standard therapy with infliximab [24].

Two hypotheses can be raised to explain the vascular regression in Jorge Lobo's disease: an immunological and a molecular hypothesis. Regarding the immunological hypothesis, immunohistochemical studies have shown intense staining for TGF-β in this disease [25,26]. At high concentrations, TGF-β can inhibit the proliferation and migration of endothelial cells, attenuating angiogenic stimuli and limiting vessel growth [27–29]. A probable molecular explanation for the regression of blood and lymphatic vessels in Jorge Lobo's disease is the loss of extracellular matrix and degradation of elastic fibers induced during the inflammatory process, leading to detachment of the endothelium [30,31].

Expanding the interpretation of the results obtained, the loss of blood vessels can cause tissue hypoxia that leads to fibrosis and, depending on its intensity, to necrosis, histopathological features characteristic of Jorge Lobo's disease [13]. Since *L. loboi* inhabits soil, the fungus is probably well adapted to low-oxygen environments

Table 2
Distribuição do infiltrado celular na lesão de pacientes com doença de Jorge Lobo. N = number of cases % = Percentage.

Cellular infiltrate	Absent		Rare		Moderate		Intense	
	N	%	N	%	N	%	N	%
Macrophages	–	–	–	–	–	–	24	100
Finely granular macrophages	3	12.5	9	37.5	8	33.33	4	16.66
Giant cells	–	–	10	41.66	7	29.16	7	29.16
Lymphocytes	3	12.5	10	41.66	11	45.66	–	–
Eosinophils	19	79.16	4	16.66	1	4.16	–	–
Plasma cells	20	83.33	1	4.16	2	8.33	1	4.16
Neutrophil	23	95.83	–	–	1	4.16	–	–

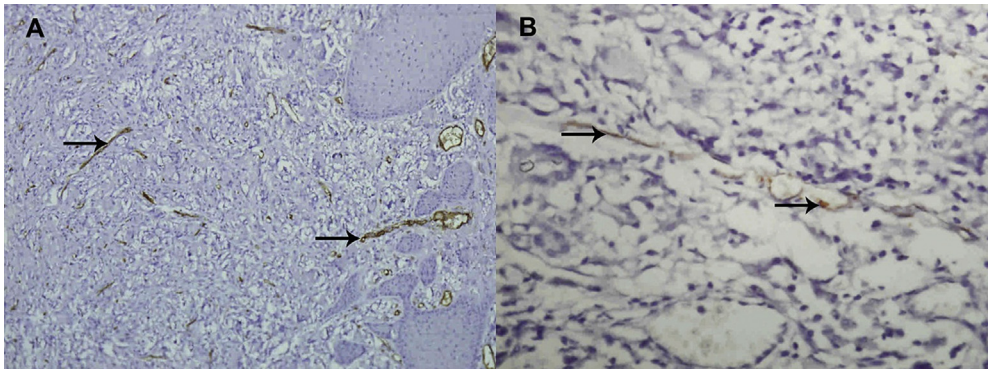

Fig. 4. Immunohistochemical staining of vessels in the microvasculature injury during Jorge Lobo's disease. (A) marking of CD34 (arrows), showing the vessel endothelium. (B) labeling the antigen podoplanin (D2-40) (arrows) of the lymphatic endothelial specific. Magnification: (A) 100× (B) 400×.

[2,32]. The loss of lymphatic vessels results in the accumulation of interstitial fluid which, in turn, interferes with the influx of dendritic cells, the establishment of an adequate adaptive immune response, and the maturation and migratory capacity of Langerhans cells, inhibiting these cells because its migrate to the site of infection via blood vessels [33].

The reduced vessel density in lesions of Jorge Lobo's disease may explain why dissemination to other organs is rare despite the large number of fungi in the lesion. The only case of dissemination was reported in Costa Rica in a man with lesions on the left leg for more than 47 years, which were associated with lymphangitis and a testicular tumor [34].

The present study also evaluated skin microvasculature function based on the detection of adhesion molecules on endothelial cells. It is known that microvascular endothelial cells at the site of inflammation are active components of the inflammatory process. These activated cells express adhesion molecules that play an important role in cell recruitment [35]. In this respect, expression of the adhesion molecules ICAM-1, VCAM-1 and E-selectin was investigated by immunohistochemistry and the density of vessels expressing these molecules was determined. The results showed a significantly larger number of vessels expressing ICAM-1 and VCAM-1, but not E-selectin, in lesions when compared to control, demonstrating the participation of these molecules in the recruitment of leukocytes to the site of infection in Jorge Lobo's disease. This expression pattern of adhesion molecules differs from that of infections caused by other fungi. In this respect, *Candida albicans* stimulates the expression of ICAM-1, VCAM-1 and E-selectin [36]. In infections caused by *A. fumigatus* hyphae, endothelial cells are stimulated to express E-selectin and VCAM-1, but not ICAM-1 [37]. These differences may reflect a fine regulation between these molecules and cytokines and chemokines produced during infection, interfering directly with cell recruitment and with the local immune response.

Evidence indicates that the cell-mediated immune response is compromised in Jorge Lobo's disease [38,39]. This fact might be explained by the low expression of E-selectin in the lesions. E-selectin is known to mediate the initial contact of Th1 and Th17, but not Th2 cells, with the activated endothelium and is expressed at high levels in the lumen of the plasma membrane of vascular endothelial cells at the site of inflammation [40–42]. Thus, if the cell-mediated response is compromised, the activity of E-selectin will be limited.

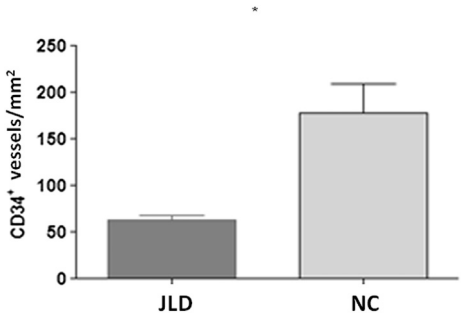

Fig. 5. Total vessel density in the lesion during Jorge Lobo's disease. $^{*}p < 0.05$ compared with the control. JLD: group of cases of Jorge Lobo's disease. NC: control group.

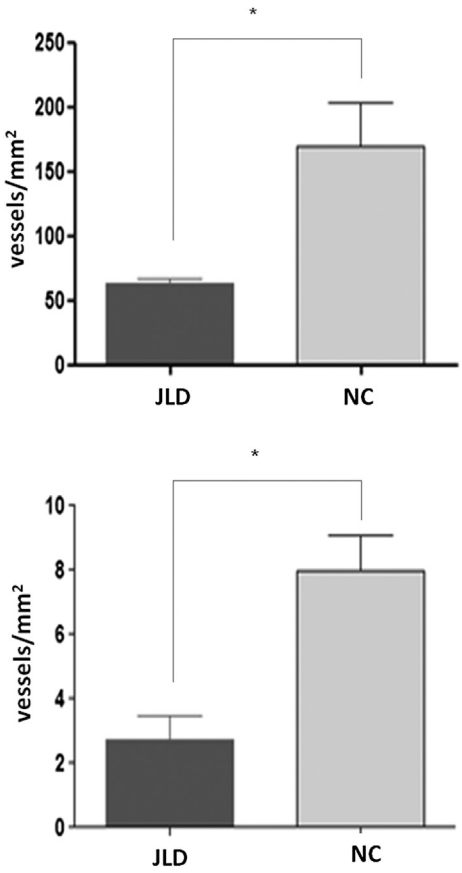

Fig. 6. The density of blood and lymphatic vessels of the lesion in Jorge Lobo's disease. $^{*}p < 0.05$ compared with the control. JLD: group of cases of Jorge Lobo's disease. NC: control group.

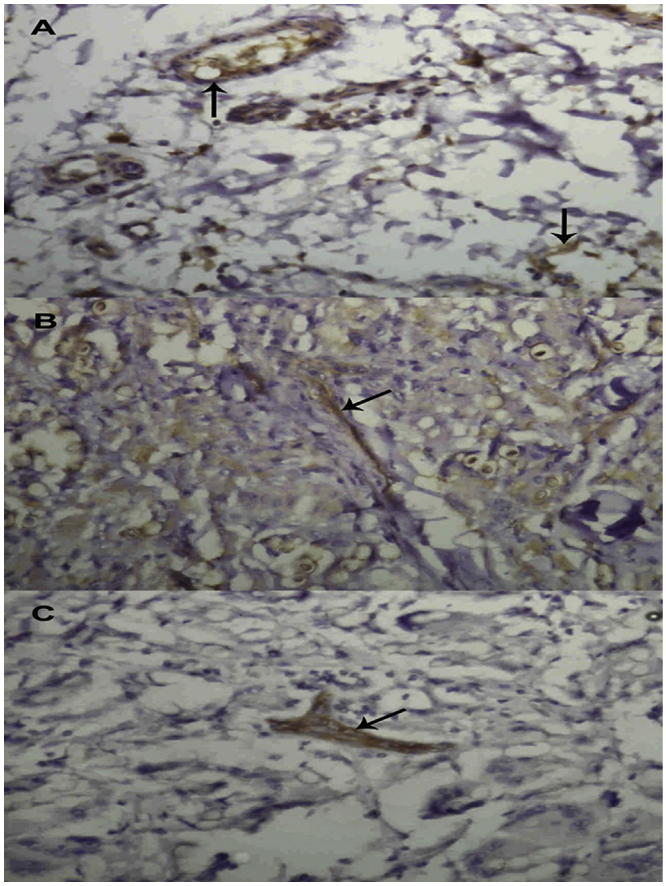

Fig. 7. Immunohistochemical staining of cell adhesion molecules in the lesion during Jorge Lobo's disease. (A) Endothelial cells expressing ICAM-1 (B) endothelial cells expressing VCAM-1. (C) endothelial cells expressing E-selectin. Magnification 400×.

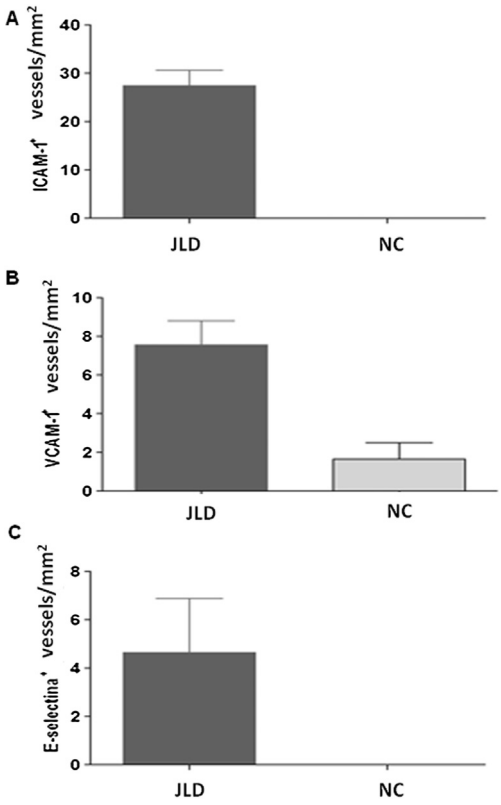

Fig. 8. Analysis of the expression of cell adhesion molecules in the lesion during Jorge Lobo's disease. (A) vessels expressing ICAM-1. (B) vessels expressing VCAM-1. (C) vessels expressing E-selectin. $^{*}p < 0.05$ compared with the control. JLD: group of cases of Jorge Lobo's disease. NC: control group.

ICAM-1 and VCAM-1 were significantly present in a larger number of vessels when compared to control, with the observation of a higher percent expression of ICAM-1. The binding of ICAM-1 and VCAM-1 to integrins present on the surface of leukocytes permits strong adhesion of these cells to the endothelial layer of vessels. These molecules are important modulatory components of cellular traffic to peripheral tissues and lymphoid organs, promoting an effector immune response [43].

The present results indicate ICAM-1 to be an important component for leukocyte recruitment in Jorge Lobo's disease. A study on experimental infection with *Paracoccidioides brasiliensis* demonstrated the participation of ICAM-1 in leukocyte recruitment, contributing to the initial inflammatory infiltrate which mainly consisted of neutrophils and macrophages [44]. Another study evaluating the recruitment of CD4+ and CD8+ T cells in the lungs of mice infected with *P. brasiliensis* showed that ICAM-1 is involved in the control of fungal replication [45].

As mentioned earlier, intense production of TGF-β occurs in lesions of Jorge Lobo's disease. In human endothelial cells, the elevated level of TGF-β has been shown to inhibit the expression of E-selectin, but does not interfere with the expression of ICAM-1 or VCAM-1 [45]. TGF-β seems to actively participate in the pathogenesis of Jorge Lobo's disease by interfering with microvascular density in the lesions and with the expression of E-selectin, thus highlighting the vast importance of TGF-β. Quaresma et al. [46] already called attention to the effect of TGF-β on fibrogenesis and local immunodeficiency.

Taken together, the results of the present study indicate the occurrence of microvascular dysfunction in lesions of Jorge Lobo's disease, which reflects on the clinical progression of the disease. The damaged skin contains a reduced density of vessels that seems to limit the fungus to the site of infection and interferes with adequate recirculation of cells and with the establishment of an adequate adaptive immune response. On the other hand, endothelial activation consisting of the expression of ICAM-1 and inhibition of E-selectin permits maintenance of the infectious process.

Therefore, immunohistochemical results of TGF-β in Jorge Lobo lesions already described in the literature [26,46] suggest a possible inhibition of the expression of E-selectin and the influx of immune cells at the site of infection, creating an immunosuppressive microenvironment characterized by a deficient cell-mediated immune response. In addition, endothelial activation, which is mainly demonstrated by the expression of ICAM-1 associated with the production of chemokines, is responsible for the marked presence of macrophages. Together with this process, TGF-β induces fibrosis that interferes with microvascular density, causing the destruction

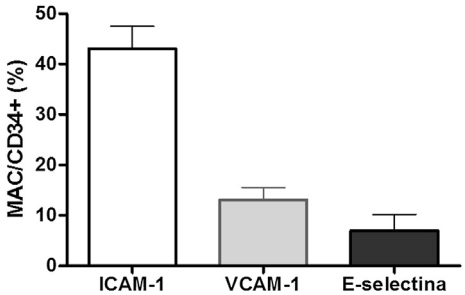

Fig. 9. Percentage of expression of cell adhesion molecules in the lesion during Jorge Lobo's disease. $^{*}p < 0.05$ compared with the control. %: Percentage.

of vessels, especially lymphatic vessels, edema formation, tissue hypoxia and cell retention, as well as maintaining the fungus restricted to the site of infection.

The present study expands the view on the immunopathogenic events involved in the chronicity of Jorge Lobo's disease and, at the same time, highlights the importance of more in-depth research on this topic. There is a need to better understand the process of vessel density reduction, to better characterize the production of cytokines and chemokines, and to identify possible positive and negative regulators of angiogenesis and lymphogenesis produced in the local microenvironment.

Funding source

This research was funded by Conselho Nacional de Pesquisa – CNPq-Brazil- grant 481020/2012.

Conflict of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Ethical aspects

The study was approved by the Ethics Committee for the Analysis of Research Projects (Comissão de Ética para Análise de Projetos de Pesquisa – CAPPesq) of the Clinical Board of the University Hospital, FM-USP (Protocol No. 191/12). The study was conducted in accordance with Resolutions 196/96 and 347/05 of the National Health Council regarding the guidelines and standards for research involving humans.

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