Plasmacytoid dendritic cells in cutaneous lesions of patients with chromoblastomycosis, lacaziosis, and paracoccidioidomycosis: a comparative analysis

Carla Pagliari1,2,3,*, Luciane Kanashiro-Galo1, Aline Alves de Lima Silva1,2, Tânia Cristina Barboza2,3, Paulo Ricardo Criado2, Maria Irma Seixas Duarte1, Arival Cardoso de Brito4, Marília Brasil Xavier4,5, Deborah Unger, Clivia Maria Moraes Oliveira4, Juarez Antonio Simões Quaresma4,5 and Mirian Nacagami Sotto2

1Universidade de São Paulo, Faculdade de Medicina, Departamento de Patologia, 2Universidade de São Paulo, Faculdade de Medicina, Departamento de Dermatologia, 3Instituto de Assistência Médica ao Servidor Público Estadual—Programa de pós-graduação em Ciências da Saúde, São Paulo, Brazil, 4Universidade Federal do Pará, Núcleo de Medicina Tropical and 5Universidade Estadual do Pará, Belém, Pará, Brazil

*To whom correspondence should be addressed. Carla Pagliari, Faculdade de Medicina da Universidade de São Paulo, Departamento de Patologia, Av Dr Arnaldo, 455, sala 1118, Cerqueira Cesar, São Paulo, Brasil, CEP 01246-903. Tel/Fax: (55 11) 3061-7238; E-mail: cpagliari@usp.br

Received 3 July 2013; Revised 9 October 2013; Accepted 29 November 2013

Abstract

Plasmacytoid dendritic cells (pDCs) are characterized by expression of CD123 and BDCA-2 (Blood Dendritic Cell Antigen 2) (CD303) molecules, which are important in innate and adaptive immunity. Chromoblastomycosis (CBM), lacaziosis or Jorge Lobo’s disease (JLD), and paracoccidioidomycosis (PCM), are noteworthy in Latin America due to the large number of reported cases. The severity of lesions is mainly determined by the host’s immune status and in situ responses. The dendritic cells studied in these fungal diseases are of myeloid origin, such as Langerhans cells and dermal dendrocytes; to our knowledge, there are no data for pDCs. Forty-three biopsies from patients with CBM, 42 from those with JLD and 46 diagnosed with PCM, were evaluated by immunohistochemistry. Plasmacytoid cells immunostained with anti-CD123 and anti-CD303 were detected in 16 cases of CBM; in those stained with anti-CD123, 24 specimens were obtained from PCM. We did not detect the presence of pDCs in any specimen using either antibody in JLD. We believe that, albeit a secondary immune response in PCM and CBM, pDCs could act as a secondary source of important cytokines. The BDCA-2 (CD303) is a c-type lectin receptor involved in cell adhesion, capture, and processing of antigens. Through the expression of the c-lectin...
receptor, there could be an interaction with fungi, similar to other receptors of this type, namely, CD207 in PCM and CD205 and CD209 in other fungal infections. In JLD, the absence of expression of CD123 and CD303 seems to indicate that pDCs are not involved in the immune response.

**Key words:** plasmacytoid dendritic cells, paracoccidioidomycosis, chromoblastomycosis, lacaziosis.

### Introduction

Some mycoses constitute a major public health problem in Latin America. Because diagnostic methods are not available in all medical centers, the lesions that result from such infections often become exacerbated and cause serious consequences in immunocompromised patients.

There are some mycoses that are noteworthy due to the relatively large number of reported cases and the severity of clinical manifestations. Here we describe the following three major mycoses because they share some epidemiological, clinical, and histological characteristics: chromoblastomycosis (CBM), lacaziosis, or Jorge Lobo’s disease (JLD), and paracoccidioidomycosis (PCM). The etiologic agents of these infections are *Fonsecaea pedrosoi* (the most common in Latin America), *Paracoccidioides brasiliensis*, and *Lacazia loboii*, respectively. There is no requirement to report the diseases caused by these agents; most primarily cause skin lesions, the severity of which is usually determined by the host’s immune status and the in situ response developed against the fungus. The main form of transmission is the traumatic implantation of fungal elements from contaminated soil or, in the case of PCM, the inhalation of propagules, which form a primary pulmonary focus and later spread to other organs including the skin [1–4].

CBM lesions are characterized by the presence of muriform cells. In warty lesions, a mixed inflammatory infiltrate composed of neutrophils, histiocytes, fewer lymphocytes, plasma cells, eosinophils, mast cells, and many Th2 cytokines is present. In addition, suppurative and poorly formed granulomas with extensive tracts of fibrosis, high parasitism, and fungi intact and dividing within microabscesses could be present. The plate-type atrophic lesions show inflammatory infiltrate in the upper dermis with well-organized granulomas, giant cells, and lymphocytes with Th1 cytokines [5–7].

JLD is a chronic disease that presents with keloid lesions. It is commonly found in tropical regions, with a large number of cases found in the Amazon region, especially among males. The dermal infiltrate is of the granulomatous type, with macrophages, giant Langhans-type, and foreign bodies with many fungal forms. Neutrophils and necrosis are rare; nerve structures are intact in the interstices of the tissue and the granuloma [8–11].

PCM cutaneous lesions also consist of granulomas, giant cells, and macrophages with fungal forms and lymphocyte infiltrate. The best tissue response is associated with Th1 cytokines, while the worst injuries are noted in the presence of Th2 cytokines [12–14].

Considering dendritic cells in these three mycoses in association with the skin, it has been verified in CBM that macrophages, in particular, and also Langerhans cells and factor XIIIa+ dermal dendrocytes function as antigen-presenting cells. Also, no numerical difference in the population of Langerhans cells has been noted between the forms of plate-type atrophic or verrucous lesions [15].

In JLD, it has been reported that Langerhans cells do not present trophy or numerical changes when compared with normal skin, which seems to represent some mechanism for escape and evasion of antigen presentation [16]. Factor XIIIa+ dermal dendrocytes are present in cutaneous lesions but seem to be related to a small number of yeast-like cells. The phagocytic or antigen presentation capacities seem to be altered in the presence of high fungal parasitism [C. Pagliari, personal communication]. In PCM, Langerhans cells demonstrate trophy and numerical reduction, whereas factor XIIIa+ dermal dendrocytes are activated to initiate phagocytic activity [17,18].

Plasmacytoid dendritic cells (pDCs) are characterized by expression of the CD123 molecule, which binds the receptor of interleukin (IL)-3α. This cell population is involved in the antiviral immune response by producing interferon-alpha (IFN-α) and is important in both innate and adaptive immunity. Another important marker that is typically expressed is CD303 (BDCA-2), a lectin type II [19].

Massone and colleagues studied such cells in leprosy, a granulomatous disease that shares some pathogenic characteristics with the mycoses we present here, such as granulomatous reaction, chronicity, and lymphohistiocytic infiltrate, among others [20]. This cell population was also described as participating in the immune response in American cutaneous leishmaniasis skin lesions [21].

To our knowledge, no data exist in the literature regarding pDCs in these three diseases. Thus, we explored this cell population and present our findings here.
Material and methods

Forty-three biopsies from CBM patients and 46 from PCM patients were retrieved from the files of the Departamento de Dermatologia, Faculdade de Medicina da Universidade de São Paulo (USP), Brazil. Forty-two biopsies from JLD patients came from the Núcleo de Medicina Tropical, Universidade Federal do Pará (UFPA), Belém, Brazil. The biopsy samples had been fixed in formalin and embedded in paraffin.

We observed the histopathological characteristics of lesions using hematoxylin-eosin staining. An immunohistochemical reaction based on polymers was performed. Briefly, following deparaffinization in xylene and hydration in ethanol, the antigen was recovered in a retrieval solution (pH 6.0); the process was conducted at 95°C. The primary monoclonal antibodies, that is, anti-CD123 (E-bioscience, 14–1239–82, CA, USA) and anti-CD303 (Dendritics, A546, Lyon, France), were applied and, following overnight incubation at 4°C, two polymer systems were used (Advanced Dako, Carpinteria, CA, USA and Novolink-Leica, Newcastle, UK), respectively. The chromogen used to develop the immunohistochemistry was 3,3-diaminobenzidine tetrahydroxychloride (Sigma, St. Louis, MO, USA) and the specimens were counterstained with hematoxylin.

The immunolabeled cells were quantified considering nine randomized high-power fields for each specimen using a ×10 ocular lens with a square grid and a ×40 objective. Results were expressed as number of cells per square millimeter, compared and statistically analyzed using Graph Pad Prism, version 6.0 for Windows, and the Mann-Whitney test was performed (P < 0.05).

The procedures used to obtain the biopsy materials were in accordance with the ethical standards of the responsible committees on human experimentation at Universidade de São Paulo, Faculdade de Medicina and Universidade Federal do Pará, Núcleo de Medicina Tropical.

Results

CBM lesions were clinically and histologically classified as verrucous and characterized by inflammatory infiltrates with neutrophils, macrophages, lymphocytes, plasma cells, and eosinophils. The granulomas were poorly organized, with extensive tracts of fibrosis and extensive parasitism (Fig. 1A). Plasmacytoid cells immunostained with anti-CD123 and anti-CD303 antibodies were observed in 16 cases (Fig. 2A and 2B).

The JLD lesions presented granulomatous inflammatory infiltrates with extensive parasitism, lymphocytes, and frequent asteroid corpuscles (Fig. 1B). We did not detect the presence of plasmacytoid cells in any specimen immunostained with either antibody.

Lesions of PCM were characterized by the presence of compact granulomas, loose granulomas, or both types of granulomas in the same lesion. There was infiltration of lymphocytes and macrophages (Fig. 1C). PDCs were visualized in 24 specimens with anti-CD123 (Fig. 2C), but cells expressing CD303 were rarely visualized.

The comparative analysis between groups using the nonparametric Mann-Whitney test demonstrated a similar number of pDCs when CBM and PCM groups were compared (P = 0.06). However, there was a statistically significant difference between CBM and PCM lesions when compared with those of JLD (P < 0.0001). Figure 3 presents the statistical analysis, showing the numerical similarity between CBM and PCM.

Discussion

The three fungal infections investigated here have a major impact on public health in Latin America; however, their immunopathogenic mechanisms have not been fully elucidated. These fungal infections are a frequent health problem in tropical and subtropical areas in the world and are difficult to control. The health programs do not require the notification of new or recurrent cases and so, they are considered neglected [1,22,23].

CBM progresses slowly and is usually limited to skin and subcutaneous regions. It develops with different clinical manifestations, such as nodular, plaque, and warty lesions that have the potential to develop into squamous cell carcinoma [24,25,26]. CBM is common among people who work in rural environments [23,25]. JLD also develops slowly, forming keloid lesions and is also common among those who work in rural environments. There is no effective treatment for JLD [20]. PCM is also common in rural areas and presents different clinical results of its infection.

Histologically, CBM lesions are characterized by chronic granulomatous inflammatory reaction and large numbers of neutrophils. The sclerotic bodies are seen in the cytoplasm of giant multinucleate Langhans cells or appear free in the tissue. In our evaluation, the lesions were of the verrucous type and characterized by the presence of eosinophils and plasma cells [27,28]. In the context of dendritic cells, in vitro studies demonstrate the inability of Langerhans cells to contain a primary infection [29], although by immunohistochemical method it was possible to demonstrate the interaction of this cell population with fungal forms [15].

The role of pDCs in host immune response against fungal infections has not been explored adequately. These cells were visualized in 37% of the CBM biopsies and about
Figure 1. Histological evaluation of lesions. Inflammatory infiltrate in chromoblastomycosis (A) with neutrophils, macrophages, lymphocytes, plasma cells, and eosinophils. Fungal forms with natural pigmentation (arrow) in the cytoplasm of giant cells. (B) Lesion from lacaziosis, presenting granulomatous infiltrate, lymphocytes, macrophages, and intense parasitism (arrow). (C) Lesion from paracoccidioidomycosis with granulomas and intense inflammatory infiltrate of lymphocytes and macrophages. Fungal forms in the cytoplasm of macrophages and giant cells (arrow). Staining with hematoxylin-eosin; ×200. This Figure is reproduced in color in the online version of Medical Mycology.

Figure 2. (A) Immunohistochemical detection of cells expressing CD123 in chromoblastomycosis distributed in the inflammatory infiltrate; (B) cells expressing CD303 in chromoblastomycosis; (C) cells expressing CD123 in paracoccidioidomycosis lesion in the inflammatory infiltrate near the dermal–epidermal junction. Immunohistochemistry (arrow), polymer system; ×200. This Figure is reproduced in color in the online version of Medical Mycology.

Figure 3. Quantitative analysis of cells expressing CD123 in chromoblastomycosis (CBM), paracoccidioidomycosis (PCM), and lacaziosis (JLD). The comparative analysis evidenced similar number of cells between CBM and PCM (P = 0.06) and a statistically significant difference between CBM and JLD or PCM and JLD (P < 0.0001). Mann-Whitney test was used. The values as mean ± standard deviation were CBM, 4.322 ± 11.17; PCM, 4.998 ± 7.94; and JLD, 0.00.

50% of PCM specimens. However, in JLD, there was no evidence of pDCs using both protocol to visualize reaction to CD123 or CD303 molecules. The populations of dendritic cells studied to date in these three fungal infections are classified as being of myeloid origin and, to our knowledge, there are no data in the literature about pDCs in these three diseases. pDCs function as antigen-presenting cells, expressing CD123 and BDCA-2 (CD303), among other markers. They are well studied in malignant melanoma, carcinoma of the head and neck, and ovarian cancer, in addition to inflammatory conditions such as systemic lupus erythematosus, psoriasis, and contact dermatitis [30–33]. Under these conditions, they are able to release cytokines and chemokines and activate lymphocytes [34]. Pathogen-mediated activation is seen in viral infections such as human immunodeficiency virus-1, herpes simplex, influenza virus, and others. There are also reports of plasmacytoid cells being activated by Plasmodium falciparum malaria and some bacteria [35,36]. As IFN-α/β producing-cells, pDCs would be able to participate in the modulation of immune response such as differentiation of myeloid dendritic cells, activation of Th1 cells, natural killer cell activation, and induction of antibody response [37,38]. The BDCA-2 (CD303) expressed by the pDCs is a c-type lectin receptor that is involved in cell adhesion, signaling and antigen capture and processing [39].
We believe that, albeit a secondary immune response to PCM and CBM, the pDCs, in response to lesions, could act as a secondary source of production of important cytokines, as previously described [5]. We suggest that, at least in CBM and considering expression of c-lectin receptor (CD303), pDCs could interact with fungal forms, similar to what has been described with other receptors of this type, namely, CD207 in PCM [18] and CD205 and CD209 in other fungal infections [40].

In JLD, as in leprosy, which shares some features of tissue injury, the absence of CD123 and CD303 expression seems to indicate that such cells are not involved in the immune response against *Lacazia lobo*. *Lacazia lobo* is not highly pathogenic, which relates to the high number of fungal forms, chronicity, number of the skin lesions, and absence of systemic compromise. The antifungal role of pDCs was previously demonstrated [41]. In JLD, the difficulty of eliminating the agent from skin lesions may be due to possible local immune-deficient mechanisms, which may include the absence of pDCs in the tissue response. Added to the immune issues discussed above, pDCs displayed in CBM and PCM could also be important to the inhibition of fungal growth in skin.

Although we made numerous efforts to detect CD303 in lesions of PCM, the results were negative. This may be due to the length of time the specimens were conserved. However, after performing the same protocol with a newer group of PCM lesions, the results were also negative. We intend to study this molecule in other specimens from PCM patients, such as lungs and lymph nodes.

**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper. The findings and conclusions of this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

**References**

10. Salgado CG. Fungal x host interactions in chromoblastomycosis. What we have learned from animal models and what is yet to be solved. *Virulence* 2010; 1: 3–5.


