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Gene expression profile of cytokines and chemokines in skin lesions from Brazilian Indians with localized cutaneous leishmaniasis

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ABSTRACT

Cutaneous leishmaniasis (CL) is a chronic inflammatory disease caused by dermotropic *Leishmania* species belonging to the *Viannia* subgenera, with *Leishmania* (*V.*) *braziliensis* considered the main agent in Brazil. After infection, a local inflammatory process is initiated, inducing the expression of several cytokine/chemokine genes. We evaluated the immunity to CL of patients living in the indigenous community Xakriabá, Minas Gerais state, Brazil, by performing detailed analyses of the mRNA expression of different cytokines and chemokines in CL lesions, considering the time evolution (recent or late). We also studied the profile of the inflammatory infiltrate by histopathological analysis. The histopathological features of recent CL lesions showed an intense inflammatory reaction, characterized by the presence of both mononuclear and polymorphonuclear cells, whereas late CL lesions exhibited a predominance of mononuclear leukocytes. The gene expression of cytokines/chemokines in skin biopsies from the CL group showed higher transcript levels of modulatory (IL10 and TGFB1), anti-inflammatory (IL4), and pro-inflammatory (TNF, IFNG, IL12B, CCL2, CCL3, CCL5, CXCL10) biomarkers in recent lesions than in late lesions. Our findings suggest that differential gene expression of cytokines and chemokines found in skin lesions from CL patients is associated with time evolution of lesions.

1. Introduction

Leishmaniasis is considered an emerging and re-emerging disease. Its incidence has increased in the last few decades (Reithinger et al., 2007; Goto and Lindoso, 2010), mainly due to human migration, deforestation, urbanization, and adaptation of the *Leishmania* parasite to additional vectors and mammalian hosts (Ferro et al., 2011). Leishmaniasis has a global estimated prevalence of 12 million cases, with approximately 2 million new cases each year (1.5 million cases of tegumentary leishmaniasis and 500,000 of visceral leishmaniasis). The disease occurs in 88 countries throughout Europe, Africa, Asia, and the Americas, and 350 million people are at risk of contracting leishmaniasis (Murray et al., 2005; Ameen, 2010; WHO, 2013).

The disease is caused by different species of protozoa of the genus *Leishmania*, which are transmitted by phlebotomine sand flies (Goto and Lindoso, 2010). The spectrum of clinical manifestations is large: in the skin, these range from localized cutaneous and mucocutaneous leishmaniasis to diffuse cutaneous leishmaniasis (Teixeira et al., 2006). *Leishmania Viannia braziliensis* is the primary cause of tegumentary leishmaniasis in Brazil. Cutaneous leishmaniasis (CL), the most common form of tegumentary leishmaniasis, is

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characterized by the appearance of a single or a few ulcerated skin lesions (Bittencourt and Barral, 1991; Giudice et al., 2012).

The occurrence of CL in Brazilian Indian tribes has been little explored. The first reports were described in the 1950s and 1960s in the Mato Grosso state (Forattini and Dos Santos, 1956; De Carneri et al., 1963). Pena and Heller (2008) conducted a study on the indigenous Xakriabá tribe (Minas Gerais, Brazil), demonstrating that the Indians' precarious sanitary and housing conditions contribute to the spread of the disease. To aggravate the situation, autochthonous cases of CL have been recorded since 2001 (MS, 2007a). Quaresma et al. (2011) reported a prevalence of 8.6% cases of CL in the Xakriabá tribe.

As part of their cycle life, *Leishmania* are injected into the vertebrate host as a promastigote, which is phagocytosed by different phagocytic cells in the host. Within cells of the mononuclear phagocyte system, promastigotes differentiate into amastigotes and then proliferate, establishing the infection (Goto and Lindoso, 2010). After infection, a local inflammatory process is initiated (Müller et al., 2001). According to Ritter et al. (1996), a major characteristic of *Leishmania*-infected skin lesions is the massive infiltration of macrophages with multiple functions. They serve as host cells for the intracellular replication of parasites, as modulators of the specific immune activity by presenting parasite antigen to T cells, and as the ultimate mediators of the host response.

In addition, several reports have shown that antimicrobial effectors are activated in macrophages and, as a consequence, resistance or susceptibility of the host to *Leishmania* infections correlates with distinct patterns of cytokine production in the infected skin (Pirmez et al., 1993; Cáceres-Dittmar et al., 1993; Melby et al., 1994). In fact, infection with *Leishmania* induces the expression of several cytokine/chemokine genes (Melby et al., 1994 Racoosin and Beverley, 1997; Ritter and Körner, 2002; Antoniazi et al., 2004). This could be beneficial to the parasite through recruitment of host cells that it can infect and survive within while it proliferates (van Zandbergen et al., 2004). For example, *L. major* has been shown to actively modify the chemokine profile at the infection site and thus recruit cells that will favor the development of persistent infection (Katzman and Fowell, 2008). Additionally, the virulence of some species of *Leishmania* is partly due to their ability to repress the induction of pro-inflammatory cytokines and chemokine genes, making their entry less detectable to the host (Matte and Olivier, 2002; Ji et al., 2003).

Many aspects of the immune response to *Leishmania* have been studied, particularly cytokine and chemokine interactions with cells in different cellular compartments. It is well established in mouse models that resistance to CL is associated with the development of T-helper type 1 (Th1) response, characterized by the production of interleukin (IL)-12 and gamma interferon (IFN-γ), whereas genetic strains that mount the Th2 response with IL4 and IL10 cytokine production are susceptible (Awasthi et al., 2004).

However, the clear Th1/Th2 dichotomy observed in murine CL has not been demonstrated in humans, because the host immune response and disease outcome during leishmaniasis are far more complex than in mice (Castellano et al., 2009). It is clear, however, in both human and experimental models of the disease that cytokines and chemokines play a critical role in shaping the nature of the host immune response to *Leishmania* infection (Teixeira et al., 2006; Cummings et al., 2010).

In the present study, we evaluated the localized immune response in CL patients living in the indigenous community of Xakriabá, Minas Gerais state, Brazil. We performed detailed analyses of the mRNA expression of different cytokines and chemokines in CL lesions, considering the time evolution, the number, and the type of lesions. We also studied the profile of the inflammatory infiltrate by histopathological analysis. The characterization of the localized immune response in the inflammatory infiltrate of CL lesions provided us with a better understanding of the mechanisms involved in the establishment and maintenance of this disease. Also, this study provided a rare opportunity to study immunological aspects related to CL in an indigenous population.

2. Population, materials, and methods

2.1. Study area

The Xakriabá Indigenous Reserve is located in São João das Missões municipality (14°53′01″S, 44°05′26″W) in northern Minas Gerais State, Brazil. The reserve covers 78% of the entire municipality and has a population of 7813 inhabitants (MS, 2012). Between 2001 and 2008, 224 cases of CL were recorded in Xakriabá Territory (Quaresma et al., 2011). Since 2008, a large study has been conducted in this reserve, addressing measures to prevent and control the transmission of CL. All actions have been conducted by the Family Health Program, currently adopted by the Brazilian Unified Health System, which is also located in Xakriabá Territory.

2.2. Data of the studied population

Eighteen indigenous patients with a diagnosis of CL were included in this 4-year study (2009–2012). The selection criteria for inclusion was patients with skin lesions suggestive of CL who had presented a positive Montenegro skin test (Biomanguinhos/FIOCRUZ); presence of *Leishmania* parasites by Giemsa-stained imprints of biopsy fragments; and/or parasite isolation in the culture medium (tubes containing blood agar-enriched LIT medium maintained at 25 ± 1 °C) and detection of *Leishmania* DNA by PCR. The identification of *Leishmania* species was performed through both biochemical and molecular techniques, according to protocols described previously (Cupolillo et al., 2004; Garcia et al., 2004). The exclusion criteria were patients with alcohol addiction; the presence of clinically manifested systemic disease; HIV infection or other immunosuppressive disease; use of immunosuppressive agents; pregnancy; patients with severe anemia; and patients with previous specific leishmaniasis treatment. All patients received specific treatment for CL at the Brazilian Family Health Program (meglumine antimoniate, Glucantime, 20 mg kg/day for 20 days), as recommended by the Brazilian Ministry of Health guidelines (MS, 2007b). Skin biopsies were taken before treatment and snapped frozen in liquid nitrogen.

Table 1 shows demographical and clinical data of the studied population. The patients were categorized into two groups according to the time of lesion appearance, which ranged from 1 to 49 months: (1) recent, those in whom lesions appeared within the previous 3 months, and (2) late, those in whom lesions appeared more than 3 months previously (Jara et al., 2013). This categorization was based on a heat map analysis that showed a strong association between time evolution of lesion and gene expression profile of cytokines and chemokines. This finding suggests that 3 months would be an appropriate time to classify recent and late CL lesions. Furthermore, the number (one or more) and type of lesion (typical or atypical) were evaluated following the criteria described previously (MS, 2006, 2007b; Guimarães et al., 2009).

Normal skin tissues were also collected from five indigenous individuals (Table 1), who constituted a control group, and preserved in RNA*later* (Life Technologies, Carlsbad, CA, USA). These subjects underwent surgery at a hospital in São João das Missões, which supplies health services to the Xakriabá Reserve.

2.3. Ethical issues

The study was conducted in agreement with the Helsinki Declaration and Resolution no. 446/2012 of the National Health Council