Presence of lactoferrin (LF) in lymphocutaneous sporotrichosis. Yeast-bound antimicrobial peptide

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Abstract

Sporotrichosis is a common subcutaneous mycosis in Latin America, produced by dimorphic fungi belong to Sporothrix schenckii sensu lato, a complex of cryptic species. Infection is acquired by traumatic inoculation with contaminated organic material. Host immune response includes polymorphonuclear neutrophils chemotaxis and release of granular components. Lactoferrin is a protein member of the transferrin family of iron-binding proteins, present inside polymorphonuclear granular structure, and has been reported to affect growth and development of infectious agents, including fungal organisms. Nevertheless, lactoferrin expression in sporotrichosis infections has not been reported yet. **Objective:** To determine the expression of lactoferrin using immunohistochemical staining in sporotrichosis human infection. **Material and methods:** The dermatology department’s files during a period of five years were reviewed; cases with a diagnosis of sporotrichosis were selected and lactoferrin immunostaining was performed when enough biological material was available. **Results:** Three cases with a diagnosis of sporotrichosis and adequate biological material on paraffin block were identified. In all cases, lactoferrin immunostaining was positive around yeast cell. (Gac Med Mex. 2016;152:743-6)

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Introduction

Sporotrichosis is a subcutaneous mycosis that is common in Mexico and highly prevalent in Latin America¹-⁴, which is acquired by traumatic inoculation with contaminated organic material⁵. Host immune response includes polymorphonuclear neutrophils (PMN) chemotaxis with subsequent release of their granular components⁶. Innate immune response is crucial in the control of the pathogen’s growth and subsequent activation of adaptive immunite⁷. In innate immunity the first defensive line is the natural barriers (skin and mucosae) that separate body from the environment; in these tissues are produced antimicrobial substances⁸. Once the fungus is introduced into the host, it is confronted with a series of defense mechanisms that include recognition by cell receptors, interaction with antimicrobial peptides (AMP) and phagocytosis, among others⁹. Currently, AMPs are thought to possess direct antimicrobial
properties; in addition, they activate and coordinate multiple innate and adaptive response components. The best characterized AMPs include β-defensins, cathelicidins, lactoferrin (LF), lysozyme, perforins, etc.\textsuperscript{10}.

LF is considered an important AMP that participates in the immune response against viruses, bacteria and fungi\textsuperscript{11-13}. It is an iron-transporting protein that lacks a hemo group, is a member of the transferrin family\textsuperscript{14} and it is characterized by its high affinity to iron, even in a highly acidic pH. Although the presence of LF has been identified in the immune response against \textit{Candida albicans}, it has not been reported in other fungi. The purpose of this work was to identify the LF expression in histological sections obtained from patients with sporotrichosis.

**Material and method**

The present work was approved by the ethics committees of the participating institutions and was carried out in compliance with stipulations of the Declaration of Helsinki. Dermatology Department medical files of a 5-year period (2003-2008) were reviewed. Cases with histological analysis and/or culture-confirmed sporotrichosis diagnosis were included (Fig. 1). In all cases, other skin infections were ruled out as the cause of the clinical lesions. Three cases with insufficient biological material available were excluded. The histological sections were independently reviewed by two observers. The technique to carry out the staining is next described:

- Hematoxylin-eosin staining. Harris hematoxylin was used for 1 min to stain the tissue; subsequently, it was washed with water. For differentiation, acid alcohol was used; the sample was washed and then ammonia water was applied for bluing; then, it was washed and restained with eosin for 30 s; subsequently, it was dehydrated and mounted.

- Technique for LF detection by immunochemistry. For the detection of LF, the Cell and tissue anti-goat staining kit HRP-AEC System was used (R&D Systems; cat. no. CTS009); the primary antibody was anti-human LF polyclonal immunoglobulin G produced in goat, at a concentration of 200 µg/ml, from Santa Cruz Biotechnology laboratories.

**Results**

The hematoxylin-eosin-stained histological sections confirmed previous reports found on medical files and showed images consistent with sporotrichosis, characterized by chronic granulomatous inflammatory infiltrate, comprised by epithelioid histiocytes, with or without the presence of multinucleated giant cells; clusters of neutrophils were observed at the central portion of the granuloma\textsuperscript{15,16}. Only in case number 3 asteroid bodies were observed. LF-staining in one case facilitated and
in the others improved yeast visualization (Fig. 2). Concomitantly, using immunofluorescence detection, the presence of CD4+ type lymphocytes was found in the inflammatory infiltrate to the epidermis, around dermal vascular structures and in subcutaneous cell tissue (Fig. 3).

**Discussion**

LF forms part of the innate immune response and constitutes a bridge between immune and adaptive response. The reported mechanisms of action against bacteria include membrane permeabilization, Fe$^{3+}$ ion sequestration, bacterial growth inhibition and expression of virulence factors$^{17,18}$. LF bactericidal function is attributed to its direct association on the bacterial surface. In Gram-negative bacteria, LF binding to lipopolysaccharide damages the external membrane$^{19}$; in other bacteria, it prevents adhesion to host cells. LF actions in fungi were reported with *Candida* spp., where two antifungal mechanisms were described: Fe$^{3+}$ ion sequestration and changes in the membrane permeability, similar to what occurs in bacteria$^{20,22}$. Actions on the cell membrane were observed with both bovine and human LF, as well as with peptides thereof derived. For example, permeabilization of fungal membrane was observed in *Candida* multiresistant strain, using a peptide constituted of the first eight residues bovine LF N-terminal extreme, which binding to lipid A presented in the fungal cytoplasmic membrane$^{23}$. Another reported effect was monocyte differentiation into macrophages, accompanied by an increase in pathogen recognition$^{24}$. On the other hand, under experimental conditions, LF action was associated with activation of a Th-1-type polarized immune response$^{25}$. LF is thought to favor dendritic cell maturation and T cell activation. LF oral administration increases interleukin 8, interleukin 10 and interferon γ production in intestinal epithelium and mesenteric lymph node lymphocytes; in addition, it increases the
number of CD4 and CD8 lymphocytes and natural killer cells. In the small bowel, it increases nucleotide-binding oligomerization domain-containing-2 (NOD-2), interferon β and interleukin 12p40 expression. From the clinical point of view, the reported benefits of LF oral administration include an improvement in intestinal microflora, an increase in serum ferritin and hematocrit, and a decrease in low respiratory tract disease occurrence. With regard to skin infections, LF administration has demonstrated beneficial effects on herpes virus and oral candidiasis in animal models, whereas in humans, a double-blind study demonstrated beneficial effects on tinea pedis.

In the sporotrichosis cases reported in the present work, LF was found to be present on the surface of S. schenckii yeasts, whereas CD4+ lymphocytes were presented on surrounding tissue as part of granulomatous reaction, just as reported for other microorganisms. This suggests that LF binds to the fungal wall. However, the wall composition in S. Schenckii is different to that of Candida spp., and, therefore, maybe in this case it probably acts as a chemotaxic marker and/or inducer of monocyte differentiation into macrophages rather than as direct fungicide.

Currently, there are different LF-derived AMPs under development with variable activity against C. albicans and in fungal membrane in vitro models. Some LF-derived peptides can alter the distribution of particles within the membrane, thus affecting its morphology and facilitating its destruction. Efficacy and toxicity analyses of LF and/or its peptide derivatives, on different administration modalities (cutaneous or systemic), appear promising for the generation of new antifungal drugs.

Conclusion

LF is present and adhered in S. schenckii yeasts, which suggests its fungistatic action and participation in the innate immune response in sporotrichosis.

References
