Contents available at **PubMed**
www.anmm.org.mx

PERMANYER

Gac Med Mex. 2016;152:743-6

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

*Alejandro Palma-Ramos[1], Laura E. Castrillón-Rivera[1], María Elisa Vega-Mémije[2], Roberto Arenas-Guzmán[3] and Lucía Rangel-Gamboa[4]**

[1]Laboratory of Immunopotentiators, Department of Biological Systems, Universidad Autónoma Metropolitana, Unidad Xochimilco; [2]Subdirección de Investigación, Hospital General Dr. Manuel Gea González; [3]Department of Mycology, Hospital General Dr. Manuel Gea González; [4]Department of Pathogenic Agents Ecology, Hospital General Dr. Manuel Gea González. Mexico City, Mexico

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)

Corresponding author: *Lucía Rangel-Gamboa, draluciarangel@yahoo.com.mx*

KEY WORDS: *Innate immunity. Lactoferrin. Sporothrix schenckii.*

Introduction

Sporotrichosis is a subcutaneous mycosis that is common in Mexico and highly prevalent in Latin America[1-4], which is acquired by traumatic inoculation with contaminated organic material[5]. Host immune response includes polymorphonuclear neutrophils (PMN) chemotaxis with subsequent release of their granular components[6]. Innate immune response is crucial in the control of the pathogen's growth and subsequent activation of adaptive immunite[7]. In innate immunity the first defensive line is the natural barries (skin and mucosae) that separate body from the environment; in these tissues are produced antimicrobial substances[8]. 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 others[9]. Currently, AMPs are thought to possess direct antimicrobial

Correspondence:
*Lucía Rangel-Gamboa
Departamento de Ecología de Agentes Patógenos
Hospital General Dr. Manuel Gea González
Av. Calzada de Tlalpan, 4800
Col. Sección XVI, Del. Tlalpan
C.P. 14080, Ciudad de México, México
E-mail: draluciarangel@yahoo.com.mx

Date of reception: 15-07-2015
Date of acceptance: 02-08-2015

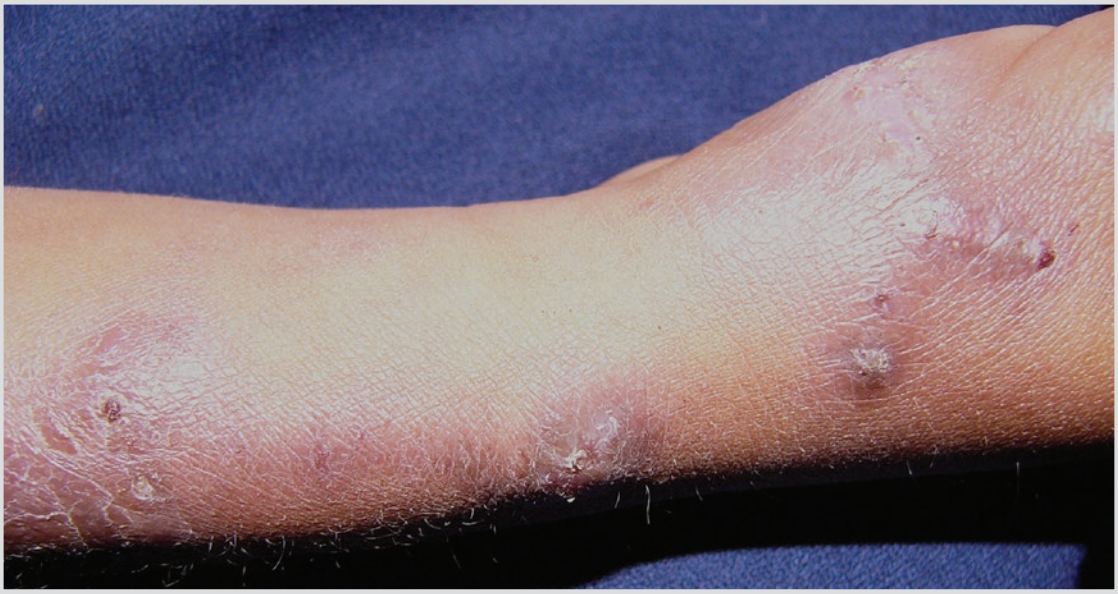

Figure 1. *Clinical image: lymphocutaneous sporotrichosis.*

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.[10].

LF is considered an important AMP that participates in the immune response against viruses, bacteria and fungi[11-13]. It is an iron-transporting protein that lacks a hemo group, is a member of the transferrin family[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 *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 blueing; 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[15,16]. Only in case number 3 asteroid bodies were observed. LF-staining in one case facilitated and

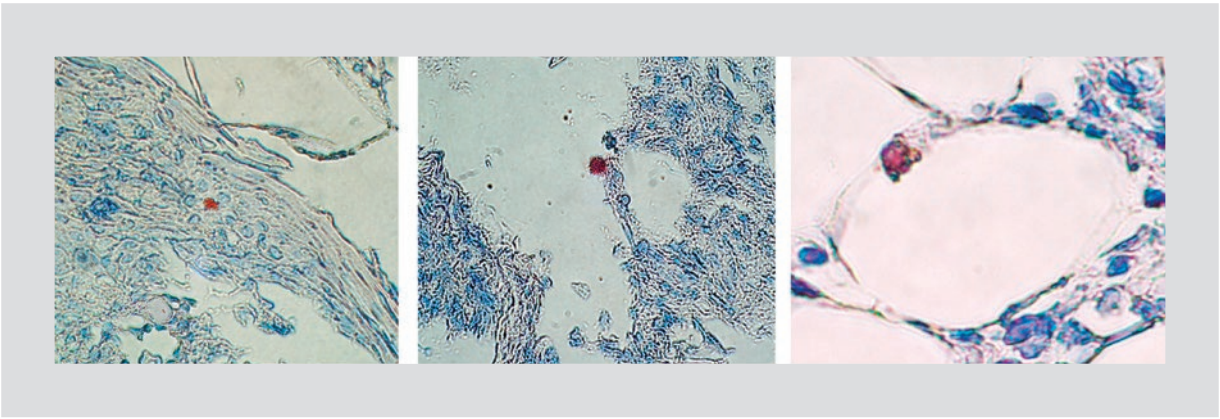

Figure 2. *Immunohistochemical staining for LF on epidermis, dermis and subcutaneous cell tissue (each picture corresponds to a different patient).*

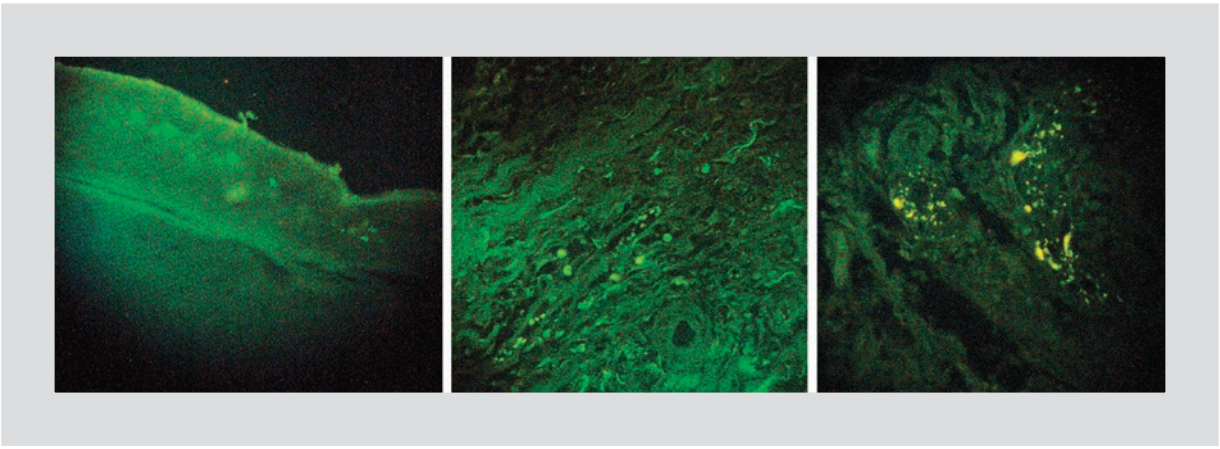

Figure 3. *CD4 lymphocytes present in epidermis, dermis and perivascularly.*

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 antifugal 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 firts 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[26]. 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[27], 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. schenkii* yeasts, whereas CD4+ lymphocytes were presented on surrounding tissue as part of granulomatous reaction, just as reported for other microorganisms[28]. This suggests that LF binds to the fungal wall. However, the wall composition in *S. Schenkii* is different to that of *Candida* spp.,[29] and, therefore, maybe in this case it probably acts as a chemotaxis marker and/or inductor 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[30-32]. 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. schenkii* yeasts, which suggests its fungistatic action and participation in the innate immune response in sporotrichosis.

References

1. Campos P, Arenas R, Coronado H. Epidemic cutaneous sporotrichosis. Int J Dermatol. 1994;33(1):38-41.
2. Coti IA. Epidemiology of sporotrichosis in Latin America. Mycopathologia. 1989;108(2):113-6.
3. Bada-del-Moral M, Arenas R, Ruiz J. Esporotricosis en Veracruz. Estudio de cinco casos. Dermatol Rev Mex. 2007;51:9-13.
4. Oliveira M, Almeida R, Muniz M, Gutierrez M, Zancope R. Phenotypic and Molecular Identification of Sporothrix isolates from an epidemic area of sporotrichosis in Brazil. Mycopathologia. 2011;172(4):257-67.
5. Nicot J, Mariat F. [Morphological characteristics and systematic position of Sporothrix schenkii, the causative agent of human sporotrichosis]. Mycopathol Mycol Appl. 1973;49(1):53-65.
6. Traynor TR, Huffnagle GB. Role of chemokines in fungal infections. Med Mycol. 2001;39(1):41-50.
7. Loures FV, Pina A, Felonato M, Araújo EF, Leite KR, Calich VL. Toll-like receptor 4 signaling leads to severe fungal infection associated with enhanced proinflammatory immunity and ampaired expansion of regulatory T cells. Infect Immun. 2010;78(3):1078-88.
8. Traynor TR, Huffnagle GB. Role of chemokines in fungal infections. Med Mycol. 2001;39(1):41-50.
9. Blanco JL, Garcia ME. Immune response to fungal infections. Vet Immunol Immunopathol. 2008;125(1-2):47-70.
10. Schauber J, Gallo RL. Antimicrobial peptides and the skin immune defense system. J Allergy ClinImmunol. 2008;122(2):261-6.
11. Schaible UE, Collins HL, Priem F, Kaufmann SH. Correction of iron overload defect in β-2microglobulin knockout mice by lactoferrina abolishes their susceptibility to tuberculosis. J Exp Med. 2002;196(11):1507-13.
12. Rodriguez-Franco DA, Vazquez-Moreno L, Ramos-Clamont G. [Antimicrobial mechanisms and potential clinical application of lactoferrin]. Rev Latinoam Microbiol. 2005;47(3-4):102-11.
13. Drago-Serrano M. Actividades antibacterianas de la lactoferrina. Enf Inf Microbiol. 2006;26:58-63.
14. Gonzalez-Chávez SA, Arévalo-Gallegos S, Rascón-Cruz Q. Lactoferrin: structure, function and applications. Int J Antimicrob Agents. 2009;33(4):301.e1-8.
15. Barhill R, Crowson N. Dermatopathology. 2.ª ed. McGraw-Hill; 2004. p. 496.
16. Lurie HI. Histopathology of sporotrichosis. Notes on the nature of the asteroid body. Arch Pathol. 1963;75:421-37.
17. Parrow NL, Fleming RE, Minnick MF. Sequestration and Scavenging of Iron in Infection. Infect Immun. 2013;81(10):3503-14.
18. Skaar EP. The battle for iron between bacterial pathogens and their vertebrate hosts. PLoS Pathogens. 2010;6(8):e1000949.
19. Drago-Serrano ME, Garza-Amaya M, Serrano-Luna JS, Campos-Rodriguez R. Lactoferrin-lipopolysaccharide (LPS) binding as key to antibacterial and antiendotoxic effects. IntImmunopharmacol. 2012;12(1):1-9.
20. Kirkpatrick CH, Green I, Rich RR, Schade AI. Inhibition of growth of Candida albicans by iron-unsaturated lactoferrina: relation to host-defense mechanisms in chronic mucocutaneous candidiasis. J Infect Dis. 1971;124(6):539-44.
21. Bellamy W, Wakabayashi H, Takase M, Kawase K, Shimamura S, Tomita M. Killing of Candida albicans by lactoferricin B, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrina. Med MicrobiolImmunol. 1993;182(2):97-105.
22. Samaranayake YH, Samaranayake LP, Wu PC, So M. The antifungal effect of lactoferrina and lysozyme on Candida krusei and Candida albicans APMIS. 1997;105(11):875-83.
23. Mishra B, Leishangthem GD, Gill K, et al. A novel antimicrobial peptide derived from modified N-terminal domain of bovine lactoferrina: Design, synthesis, activity against multidrug-resistant bacteria and Candida. Biochim Biophys Acta. 2013;1828(2):677-86.
24. Van der Does AM, Bogaards SJ, Ravensbergen B, Beekhuizen H, van Dissel JT, Nibbering PH. Antimicrobial peptide hLF1-11 directs granulocyte-macrophage colony-stimulating factor- driven monocyte differentiation toward macrophages with enhanced recognition and clearance of pathogens. Antimicrob Agents Chemother. 2010;54(2):811-6.
25. Spadaro M, Caorsi C, Ceruti P, et al. Lactoferrin, a major defense protein of innate immunity, is a novel maturation factor for human dendritic cells. FASEB J. 2008;22(8):2747-57.
26. Tomita M, Wakabayashi H, Shin K, Yamauchi K, Yaeshima T, Iwatsuki K. Twenty-five years of research on bovine lactoferrin applications. Biochimie. 2009;91(1):52-7.
27. Takakura N, Wakabayashi H, Ishibashi H, et al. Effect of orally administered bovine lactoferrina on the immune response in the oral candidiasis murine model. J Med Microbiol. 2004;53(Pt 6):495-500.
28. Palma A, Castrillon L, Espinosa V, Becerril D, Padilla M, Arenas-Guzman R. Existencia de lactoferrina en granos de micetoma: estudio de ocho actinomicetomas humanos. Dermatol Rev Mex. 2014;58:142-9.
29. Martinez-Álvarez JA, Pérez-García LA, Flores-Carreón A, Mora-Montes HM. The immune response against Candida spp. and Sporothrix-schenckii. Rev Iberoam Micol. 2014;31(1):62-6.
30. Bolscher J, Nazmi K, van Marle J, van't Hof W, Veerman E. Chimerization of lactoferricin and lactoferrampin peptides strongly potentiates the killing activity against Candida albicans. Biochem Cell Biol. 2012;90(3):378-88.
31. Silva T, Adão R, Nazmi K, et al. Structural diversity and mode of action on lipid membranes of three lactoferrincandidacidal peptides. Biochim Biophys Acta. 2013;1828(11):1329-39.
32. Viejo-Diaz M, Andrés MT, Fierro JF. Different anti-Candida activities of two human Lactoferrin-derived peptides, Lfpep and kaliocin-1. Antimicrob Agents Chemother. 2005;49(7):2583-8.