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IL-37 and leprosy: A novel cytokine involved in the host response to *Mycobacterium leprae* infection

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ABSTRACT

Leprosy is a chronic infectious granulomatous disease caused by *Mycobacterium leprae*, in which the clinical outcome depends on the pattern of the host immune response. Because it is a spectral disease, leprosy is a good model for studying the immunology of the pathogen–host relationship. Although previous studies have characterized the participation of cytokine profiles such as Th1, Th2, Th7, Treg, Th9, and Th22 responses in leprosy, the role of new cytokines such as IL-37 have not yet been described for the spectral model of the disease. Here, we used an immunohistochemical technique to evaluate IL-37 expression in the skin of patients with leprosy. The expression of this cytokine was observed in the keratinocytes, endothelial cells, macrophages, and lymphocytes. Moreover, the IL-37 expression level was increased in patients with the tuberculoid (TT) form when compared to those with the lepromatous leprosy (LL) form in keratinocytes, endotheliocytes, and lymphocytes. However, in the macrophages, the cytokine expression was more intense in the LL form of the disease. These results point to the effective participation of IL-37 in the immunopathogenesis of leprosy, which is expressed in both the epidermal cells and the dermis.

1. Introduction

Leprosy is a chronic disease of infectious origin caused by *Mycobacterium leprae*, an intracellular bacillus that causes granulomatous and demyelinating lesions in the peripheral nerves [1,2]. Leprosy still constitutes an important public health problem in some regions of the world, mainly because of its high severity and disabling potential [3–5].

As leprosy is a spectral disease, according to the Ridley and Jopling classification, the disease presents in five clinical forms: two stable forms that constitute the poles of the disease, tuberculoid leprosy (TT) and lepromatous leprosy (LL), and three forms that are considered as intermediary, including borderline tuberculoid (BT), borderline borderline (BB), and borderline lepromatous (BL) [6]. This classification is based on clinical, histopathological, and immunological criteria, and thus leprosy is considered to represent an immunocomplex disease whose evolution depends on the host immune response. Considering these characteristics, leprosy has been considered a useful model for the study of pathogen–host relationships in bacterial diseases, and to examine the extent to which the immune response influences the evolution of these diseases [6–12].

Increasing evidence has helped to broaden the immunological discussions surrounding the response of subpopulations of T lymphocytes (Th1, Th2, Th9, Th17, Th17, Th22, and Treg) as well as macrophages (M1 and M2) in the pathogenesis of the disease [7–12]. In this context, the study of new cellular subtypes has contributed to the investigation of new markers belonging to the interleukin (IL) families of IL-1, IL-10, and IL-17 [13–15].

IL-37 is a member of the IL-1 family belonging to the IL-18 subfamily that has several isoforms (IL-37a, IL-37b, IL-37d, IL-37e) and is produced by macrophages, natural killer cells, neutrophils, dendritic cells, and helper T (Th) cells. In the development of mechanisms that induce the production of IL-37, the formation of the immunological cascade has been shown to be directly related to the transforming growth factor-beta (TGF-β) response as well as to activation of SMAD3 [16–18].

Previous studies investigating the role of IL-37 have described the cytokine as a potent immunosuppressive factor of the innate and

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Table 1
Quantitative analysis of IL-37 expression in keratinocytes, endothelial cells, macrophages, and lymphocytes in the polar forms of leprosy.

IL-37								
Group	Keratinocyte	*p*	Endothelial Cells	*p*	Macrophage	*p*	Lymphocyte	*p*
Tuberculoid	16.38 ± 3.94	.0414*	14.23 ± 1.92	.0104*	19.92 ± 4.19	.0001***	32.31 ± 3.44	.0001***
Lepromatous	13.38 ± 3.09		11.46 ± 3.55		48.31 ± 6.42		19.00 ± 2.16	

* $p < 0.05$.
*** $p < 0.0001$.

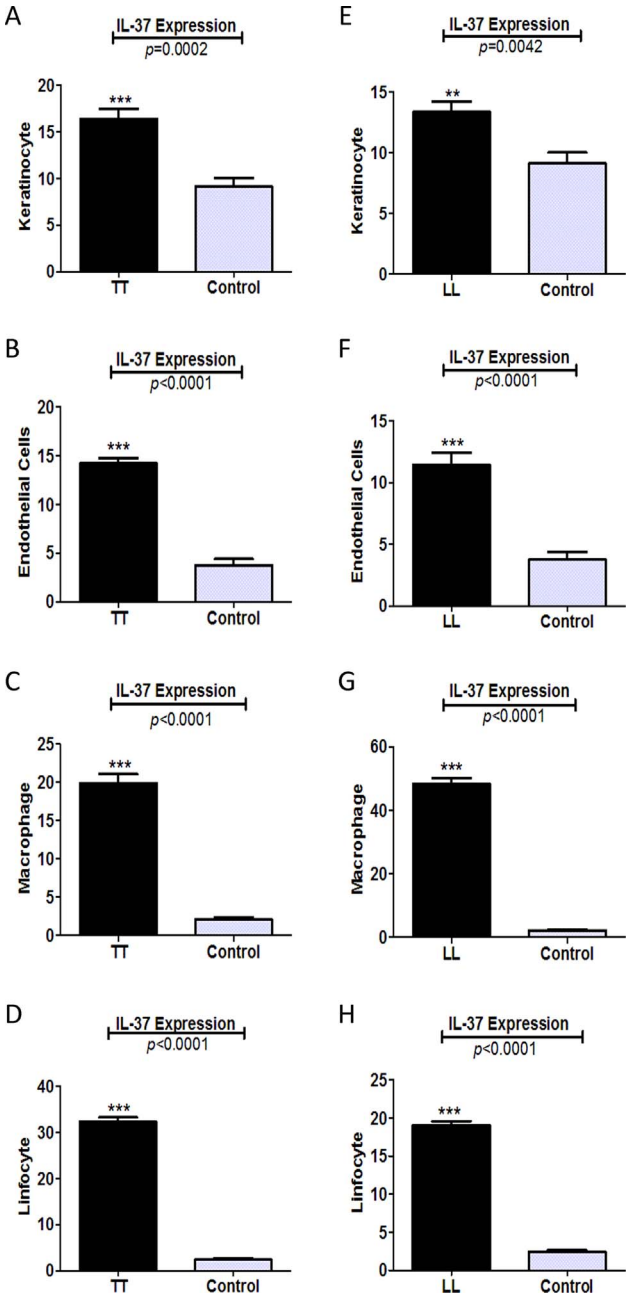

Fig. 1. Quantitative analysis of IL-37 expression in the keratinocytes, endothelial cells, macrophages, and lymphocytes in patients with the TT and LL forms of leprosy compared to those of the control group.

adaptive responses [17,18]. In the innate immune response, IL-37 can reduce the activity of IL-18, interferon-gamma, tumor necrosis factor-alpha, IL-1β, IL-6, as well as neutrophils and macrophages [19,20]. In adaptive immunity, in addition to inhibiting the production of proinflammatory cytokines, IL-37 also contributes to the production of anti-inflammatory cytokines such as IL-10, IL-13, TGF-β [21,22].

Moreover, IL-37 expression has been shown to be extremely high in certain autoimmune diseases, including lupus and rheumatoid arthritis [23]. In the development of neoplastic processes, IL-37 is able to inhibit cell growth and proliferation, and increase apoptotic activity by inhibiting the production of Bcl2, cyclin D1, and HIF-1α [24]. In fungal infections such as aspergillosis, IL-37 expression in the lung inhibits inflammasome activation and recruitment of neutrophils to the infectious site [25]. In the lung lesions caused by *Mycobacterium tuberculosis*, IL-37 expression in epithelioid cells has been shown to be increased via recognition of lipoarabinomannan by Toll-like receptor 2 and positive regulation of the p38 MAPK and ERK1/2 pathways [26].

However, no study has addressed the possible role of IL-37 in the immunopathogenesis of leprosy to date. Therefore, the aim of the present study was to investigate the response of IL-37 in leprosy lesions. These results could provide new insights into the mechanisms that guide the immune response associated with leprosy *in situ*.

2. Materials and methods

2.1. Ethics

Ethical approval was obtained from the Tropical Medicine Center Ethics Committee and patients provided written informed consent.

2.2. Characterization of the samples

Thirty-four tissue samples were evaluated in this study, including those obtained from control subjects (normal skin, n = 8), patients with TT (n = 13), and patients with LL (n = 13) from the archives of the Nucleus of Tropical Medicine of the Federal University of Pará. For histopathological analysis, 5-μm-thick histological sections of tissue biopsies were stained by hematoxylin-eosin, and tissue immunostaining was conducted with the anti-IL-37 antibody by an immunohistochemistry technique as described below.

2.3. Immunohistochemistry

Immunostaining of the tissue with the IL-37 antibody (Abcam, ab116282) was based on the method involving the formation of the biotin-streptavidin peroxidase complex. Initially, the tissue samples were dewaxed in xylol and hydrated in ethyl alcohol at a lower concentration. The endogenous peroxidase was then blocked with 3% H_2O_2 for 45 min, and antigenic recovery was performed with citrate buffer (pH 6.0) for 20 min at 90 °C. Subsequently, non-specific proteins were blocked with skim milk concentrated at 10% for 30 min. The histological sections were incubated with diluted primary antibodies and 1% bovine serum albumin for 14 h. The slides were immersed in a solution containing 1× phosphate-buffered saline (PBS) and then incubated with the biotinylated secondary antibody labeled streptavidin biotin (LSAB; DakoCytomation) in an oven for 30 min at 37 °C. The slides were then immersed in 1× PBS and incubated with streptavidin peroxidase (LSAB; DakoCytomation) for 30 min at 37 °C. The cuts were revealed