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Short communication

# Transforming growth factor β and apoptosis in leprosy skin lesions: possible relationship with the control of the tissue immune response in the *Mycobacterium leprae* infection

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**Abstract**

The course of leprosy depends of the host immune response which ranges from the lepromatous pole (LL) to the tuberculoid pole (TT). A comparative study was conducted in 60 patients with the LL and TT. The results showed a mean expression of TGF-β of 339 ± 99.4 cells/field for TT and of 519.2 ± 68.2 cells/field for LL. Frequency of apoptosis was 6.3 ± 1.8 in TT and 14.0 ± 6.1 in LL. A correlation ($p = 0.0251$) between TGF-β and caspase-3 in the LL was found. This finding indicates a role of TGF-β and apoptosis in the immune response in leprosy.


*Keywords:* Leprosy; TGF-β; Apoptosis; Cytokines; Immunopathology; *Mycobacterium leprae*

## 1. Introduction

Leprosy is a chronic infectious disease that has plagued mankind for many centuries and continues to be a public health problem in some countries like Angola, Brazil, Central African Republic, Democratic Republic of Congo, India, Madagascar, Mozambique, Nepal, and the United Republic of Tanzania. According with World Health Organization (OMS), official reports received during 2010 from 141 territories, the global registered prevalence of leprosy at the beginning of 2010 stood at 211,903 cases.

The clinical presentation of the infection depends on the immunological susceptibility of the host and includes the TT, borderline and LL. Leprosy is caused by the alcohol-acid resistant bacillus (AARB), *Mycobacterium leprae*. After the initial infection, the bacilli are phagocytosed by antigen-presenting cells that secrete interleukin-1 (IL-1) and activate T lymphocytes [1]. In parallel, antigens present in the type II major histocompatibility complex (MHC) are recognized by T lymphocytes that undergo clonal proliferation, producing effector and memory cells. The LL is characterized by selective depression of Th1 CD4+ T lymphocytes that produce IFN-γ and tumor necrosis factor-α (TNF-α), important cytokines for the activation of the cellular immune response against intracellular pathogens. On the other hand, in these

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patients exacerbation is observed in the response of Th2-like CD4+ T cells producing IL-4 and IL-5, cytokines that activate the production of antibodies and also exert inhibitory effects on the Th1 response [2].

During the development of the immune response immediately after mycobacterial entry into macrophages, *M. leprae* itself induces the production of TNF-α and transforming growth factor-β (TGF-β) by the infected macrophages. On the one hand, TNF-α activates macrophages for the intracellular destruction of the infectious agent and potentiates the Th1 effect and, on the other hand, TGF-β deactivates macrophages, increases bacillary proliferation and counteracts the effects of TNF-α with the predominance of a Th2 response. In the humoral response, elimination of the bacillus is inefficient, as demonstrated by the detection of antibodies (anti-PGL-1) specific for a cell wall phenolic glycolipid of *M. leprae*. High antibody concentrations in peripheral blood are associated with the marked bacillary load observed in the borderline-lepromatous and LL, in contrast to patients with the TT whose titers are similar to those of healthy controls [3,4].

The destruction or multiplication of the bacillus inside macrophages is defined by immune mechanisms that involve MHC and HLA histocompatibility-antigen presentation which are both genetically determined. In the TT, the HLA-DR2 and HLA-DR4 phenotype predominates. In LL and borderline-lepromatous leprosy there is a predominance of the HLA-DQ1 phenotype which is related to susceptibility [5].

The role of apoptosis in leprosy is still poorly understood. The first study on apoptosis in leprosy was published by Cree et al. [6]. The authors observed apoptotic bodies scattered in lepromatous lesions and concentrated at the borders of tuberculoid lesions in areas predominantly containing epithelioid cells, a finding that might be related to the resolution of tuberculoid lesions. Expanding their studies, Cree et al. [7] suggested that apoptosis might be involved in cell renewal in leprosy granulomas which are characterized by high cell turnover. In 1999, Niang et al. [8] reported an increase in the rate of spontaneous apoptosis of peripheral blood mononuclear cells in patients with paucibacillary and multibacillary leprosy, especially CD8+ T and B lymphocytes, and suggested that this might be a mechanism for the elimination of infected cells. This increase in the spontaneous apoptosis rate was confirmed by Hernandez et al. [9].

The authors reported that the efficiency of *M. leprae* in inducing apoptosis was lower than that observed for *Mycobacterium tuberculosis*. The findings reported so far in the literature are still insufficient to effectively characterize the role of apoptosis in the immunopathogenesis of infection with *M. leprae*, or its correlation with the tissue expression of TGF-β, an immunosuppressive cytokine and inducer of apoptosis, in the clinical forms of leprosy. Thus, the main objective of the present study was to investigate the role of apoptosis and TGF-β in the immunopathogenesis of leprosy by immunohistochemistry technique in tissue from lesions of the polar forms of the leprosy.

## 2. Materials and methods

### 2.1. Patients and tissue samples

A study was conducted in the Nucleus of Tropical Medicine, Federal do Para University. Sixty untreated patients with leprosy, hiv negative, 30 with the LL and 30 with the TT of the disease, were selected. All patients were from the Para State, Brazil. Patient age ranged from 25 to 57 years. Twenty patients were females and 40 were males. The diagnosis of leprosy was made according to WHO criteria and the collection of biopsy samples for histopathological analysis. Patients were assigned to one of the poles of the disease based on clinical data, bacilloscopy and characteristic histopathological findings of the lesion.

### 2.2. Histological and immunohistochemistry processing

Histological sections (5 μm thick) were cut with a microtome, mounted on silanated slides and submitted to hematoxylin-eosin staining and histochemistry for Ziehl–Nielsen technique. After staining, the sections were dehydrated in a solution containing increasing alcohol concentrations and xylene and mounted in Entellan (Merck). The sections were then analyzed under a light microscope and classified according to the histopathological aspect of the lesions. The streptavidin-biotin peroxidase (SABC) method employing monoclonal antibodies against caspase-3 and TGF-β using protocol described Hsu et al. [10]. The quantification of positive events was made to count for positive events in 05 high magnification fields (400×) using graduated graticule with 10 × 10 subdivisions and 0.0625 $mm^2$, using optical microscope Nikon Eclipse E200. Getting the result of the number of events per unit area.

### 2.3. Statistical analysis

Statistical analysis was performed using the BioEstat 3.0 program described by Libonati et al. [11]. The results of the diagnostic methods were analyzed descriptively. Pearson's linear correlation test was used to evaluate the correlation between variables.

### 2.4. Ethical aspects

The study was approved by the Ethics Committee of the Nucleus of Tropical Medicine, UFPA.

## 3. Results

Histopathological analysis revealed the characteristic findings which agreed with the clinical aspects of the lesion. The LL was characterized by the presence of macrophages intensely parasitized by bacilli demonstrated by staining for Ziehl–Nielsen technique. These bacilli were generally scattered in the cytoplasm but frequently clustered forming globoid bodies, and macrophages with a foamy cytoplasm,