SHORT REPORT
PERSISTENCE OF TUMOR NECROSIS FACTOR-\(\alpha\) IN SITU AFTER LESION HEALING IN MUCOSAL LEISHMANIASIS

VALDIR S. AMATO, HEITOR F. ANDRADE JR., VICENTE AMATO NETO, AND MARIA IRMA S. DUARTE
Department of Infectious and Parasitic Diseases, and Laboratory of the Discipline of Pathology of Transmissible Disease, School of Medicine, University of Sao Paulo, Sao Paulo, Brazil; Institute of Tropical Medicine of Sao Paulo, Sao Paulo, Brazil

Abstract. Mucosal leishmaniasis (ML) is a disease characterized by intense activation of inflammatory cells and extensive tissue destruction. Among the cytokines involved in the immune response to ML, tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) has attracted strong interest because of its roles in the modulation of the immune response. We studied 20 patients with ML who provided biopsy specimens before treatment and after lesion healing obtained by specific therapy. The biopsy specimens were subjected to immunohistochemical analysis for in situ quantification of cellular and extracellular TNF-\(\alpha\). The amount of TNF-\(\alpha\) was significantly lower in the healed lesions compared with pretreatment biopsy specimens, although TNF-\(\alpha\) persisted at the tissue level even after lesion healing. This relevant finding demonstrates for the first time an in situ tissue reduction of TNF-\(\alpha\) after treatment and shows persistence of TNF-\(\alpha\) in healed lesions may be related to the maintenance of an immunopathologic background for relapses observed in ML.

Mucosal leishmaniasis (ML), caused by Leishmania (Vian- nia) braziliensis, is characterized by chronic involvement of nasal septa, but may also affect the oropharynx, larynx and trachea, causing various complications that may lead to severe destruction of upper airway tissues. This disease is considered to be an immunopathologic hyperimmune reaction to parasites and their antigens, but the mechanism of tissue destruction remains unknown. Tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), a cytokine involved in the development of lesions in several infections, has also been implicated in ML because of high serum levels of TNF-\(\alpha\) in ML patients during active disease and greater production in vitro by stimulated blood mononuclear cells. The production of TNF-\(\alpha\) in ML patients could be related to regulatory genetic polymorphisms. The local production of this cytokine has been demonstrated in active ML lesions, but no data concerning post-therapy was evaluated. Here, we report the in situ identification of TNF-\(\alpha\) and its production by cells in a sequential biopsy study before and after therapy with lesion cicatrization.

We studied 20 patients (16 men) 19–78 years old (mean age = 54.4 years). Fourteen of them had restricted septal lesions and six had restricted palate involvement. The diagnosis was confirmed by epidemiology, positive reactions on Montenegro skin tests, and characteristic histologic findings in biopsy specimens with the presence of Leishmania amastigotes or antigens detected by immunohistochemical analysis using a 1:1,000 dilution of cross-reactive mouse anti-Leishmania (L.) amazonensis polyclonal serum detected with an appropriate conjugate. After diagnosis, specific treatment included antimony salts or, alternatively, pentamidine or amphotericin B when antimony was contraindicated by electrocardiographic abnormalities. Lesions were considered healed when complete mucosal re-epithelialization was observed by careful endoscopic evaluation. A post-therapy biopsy was performed at least six months after complete healing. The study was reviewed and approved by the Ethical Board of the School of Medicine of the University of Sao Paulo (Protocol no. 742/98), and informed consent was obtained from all participants.

Tumor necrosis-\(\alpha\) was detected by immunohistochemical analysis in lamina propria cells and extracellular spaces in 5-\(\mu\)m biopsy sections using a specific anti-human TNF-\(\alpha\) rabbit polyclonal antibody (IP300; Genzyme Diagnostics, Cambridge, MA) (Figure 1). The presence of cells expressing TNF-\(\alpha\) was semiquantified in the lamina propria by scoring as

![Figure 1](image-url) Immunohistochemical detection of tumor necrosis factor-\(\alpha\) in biopsy specimens from patients with mucosal leishmaniasis. A, Typical cell showing staining of the cytoplasm. B, Staining in extracellular spaces without definition of the stained cells. (Magnification \(\times\) 400.)
0 (absence of stained cells), 1 (less than 4 stained cells in 10 fields, 400 x), 2 (less than 9 stained cells), and 3 (at least 10 stained cells). Extracellular TNF-α was quantified by morphometric analysis using a 1-cm² (100 points) square grid adapted to a 10 x eyepiece and observed in a lamina propria area with a 40 x planachromatic objective. Points over immunohistochemically stained areas and their percentage in relation to the total number of points were determined. At least five lamina propria fields and 500 points were scored in blind preparations of each biopsy specimen by two independent observers. These data are listed in Table 1. Despite clear reduction after therapy and cicatrization, staining of TNF-α persisted in cells and in extracellular spaces, probably due to sustained immunopathologic reactions, except in one patient, who showed no extracellular staining for TNF-α in the post-therapy biopsy specimen.

Previous reports have shown that serum TNF-α levels decreased after antimonial therapy in patients with cutaneous leishmaniasis and ML.7,11 Additionally, the use of pentoxifylline, a TNF-α inhibitor, has yielded promising results in the treatment of ML,12 indicating a specific role of this cytokine in the development of ML lesions. Our data clearly show that expression of TNF-α and its release into extracellular spaces were maintained in the lamina propria of ML patients despite cicatrization. The presence of extracellular TNF-α may represent the persistence of a local immune reaction that could result in disease reactivation, despite a complete absence of intact amastigote forms in post-therapy biopsy specimens.

Leishmania antigen was detected by immunohistochemical analysis in 8 of 20 biopsy specimens obtained after treatment in this study, but no differences were observed in the quantitative mean levels of extracellular TNF-α and antigen. This antigen may represent quiescent parasitic infections, as previously suggested,13 or residual antigen in resting macrophages, and extracellular TNF-α could be the result of both effective immunity and disease activity. Extracellular detection of TNF-α in post-therapy healed lesions could be the result of the maintenance of an immunopathologic background that may be implicated in the relapses frequently observed after treatment of ML, as previously described.14,15

Table 1

<table>
<thead>
<tr>
<th>Event</th>
<th>Before treatment median (range)</th>
<th>After treatment median (range)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular TNF-α immunohistochemically stained lamina propria, %</td>
<td>14.17 (0.4–74.4)</td>
<td>5.02 (0–14.4)</td>
<td>0.007</td>
</tr>
<tr>
<td>Semiquantitative score of cellular TNF-α</td>
<td>3 (1–3)</td>
<td>2 (0–2)</td>
<td>0.05</td>
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</tbody>
</table>

* By Wilcoxon test.

REFERENCES