Received Date : 10-Nov-2013

Revised Date : 17-Nov-2014

Accepted Date : 28-Nov-2014

Article type : Research Letter

Title: Th17 cytokines expression in leprosy skin lesions

Running head: Th17 cytokines in leprosy

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Funding sources: Conselho Nacional de Pesquisa – CNPq – Brazil.

Conflict of interest: None declared.

What's already known about this topic?

- Tissue immune response plays an important role in the clinical evolution of infectious dermatoses.
- The role of Th1 and Th2 cytokines in infectious skin diseases is well studied and described.
- Clinical forms of leprosy have a strong association with the host immune response.
- Few studies have shown the role of Th17 cytokines in leprosy.

What does this study add?

- New pattern of cytokines immune response have been described recently.
- Th17 cytokines have a key role in the immune response to infectious agents.
- The role of cytokines Th17 expressed in the lesion shows its relation to clinical form of leprosy.

Dear Editor, until recently, it was believed that Th1 and Th2 lymphocytes are the only effector cells of the immune response; however, another group of T cells producing cytokines that differ from those observed in the Th1-Th2 model has been identified recently. These cells comprise a third subtype of CD4+ effector T helper called type 17 T helper cells, or Th17[1]. Th17 lymphocytes produce IL-17, IL-17F, IL-6, IL-21, IL-22, and TNF-α, which play a role in both tissue inflammation and neutrophil activation[2]. IL-17 is a potent proinflammatory cytokine that exerts effects on a variety of cells[2]. In leprosy, the cytokine profile of the tuberculoid and lepromatous forms (stable forms) has been extensively

investigated and is characterized by a Th1 and Th2 response, respectively[3]. However, the dimorphic forms are highly unstable and complex and Th17 lymphocytes may play an important role in the immunopathology of these lesions.

The project was approved by the Ethics Committee Tropical Medicine Center-Federal University of Para-Brazil (Protocol No. 047/2008). A cross-sectional, analytical study was conducted at the Laboratory of Immunopathology of Tropical Medicine Center-Federal University of Para-Brazil. Fifty untreated patients from State of Para-Brazil with a confirmed diagnosis of leprosy were selected, including 10 cases of indeterminate leprosy, 16 of the TT (tuberculoid leprosy) form, 17 of the BB (borderline leprosy) form, and 7 of the LL (lepromatous leprosy) form. The diagnosis of leprosy was made based on WHO and Madrid classification. The skin biopsies were collected and embedded in paraffin and cut into 4-µm thick sections with a microtome.

Immunohistochemistry was performed using monoclonal antibodies against TGF-β (GENZYME/80-1835, Dilution 1:30), IL-6 (SANTA CRUZ/1265, Dilution 1:50) and IL-17 (ABCAM/79056, Dilution 1:30) [4]. Positive events were counted in five fields at high magnification (400X) and an area of .0625 mm^2. The hypotheses were evaluated using the Mann-Whitney and Kruskal-Wallis tests according to the values obtained (Fig. 01).

Most cases were classified as dimorphic (34%) according to the Madrid classification and 60% had the paucibacillary disease. Regarding TGF-β staining, according to the Madrid classification, the mean number (± SD) of positive events was 2.00 ± 2.21 in the group of indeterminate lesions (MHI), 5.53 ± 10.67 in the tuberculoid group (MHT) and 4.93 ± 6.30 in the dimorphic group (MHD), whereas a mean number of 9.00 ± 3.08 was observed in the group of lepromatous lesions (MHL). This difference was statistically significant (MHI p = 0.0285; MHD p=0.0453), i.e., TGF-β production was higher in patients with virchowian or lepromatous leprosy compared to indeterminate and dimorphic form.

With respect to IL-6 staining, according to the Madrid classification, the mean number (± SD) of positive events was 142.9 ± 79.48 in the MHI group, 439.9 ± 80.59 in the MHT group and 288.1 ± 154.2 in the MHD group, whereas a mean number of 139.6 ± 84.75 was observed in lesions of the MHL group, with a strongly significant difference (p = 0.0001), i.e., IL-6 production was higher in patients with tuberculoid leprosy compared to indeterminate form.

With respect to IL-17 lymphocytes, according to the Madrid classification, the mean number (± SD) of positive events was 132.4 ± 62.1 in the MHI group, 279.2 ± 109.6 in the MHT group and 255.9 ± 190.2 in the MHD group, whereas a mean number of 91.4 ± 18.85 was observed in lesions of the MHL group. This difference was statistically significant (p = 0.0066), with IL-17 production being higher in patients with tuberculoid leprosy compared to indeterminate form. This finding suggests an involvement of IL 17 in progression to tuberculoid form of the disease, but more research is needed to describe the role of IL17 in the immunopathogenesis of leprosy.

A significant difference in the concentration of IL-6 was observed between tuberculoid lesions and the indeterminate, dimorphic and lepromatous forms. IL-6 is involved in the modulation of inflammatory responses mediated by T cells and in the inhibition of Treg cell activation. An increase in the expression of IL-6 has also been reported by Scollard et al., who observed an immune response to the components of *M. leprae* both at the tuberculoid pole and in patients with the lepromatous form[5]. In addition, high levels of IL-6 in skin lesions of patients with type 1 leprosy reactions[5].

A higher concentration of TGF- β was observed in lepromatous lesions when compared to the tuberculoid, indeterminate and dimorphic forms. These results indicate an immunomodulatory activity of TGF-β in the immune responses to the agent, as supported by

the association of this cytokine with more severe and bacilliferous forms of the disease, suggesting a role as an inhibitor of macrophage activity[3,6].

IL-17 is the characteristic Th17 cytokine and is correlated with TGF-β and IL-6 since the latter are involved in the differentiation and maintenance of this profile[5,7,8]. The expression of IL-17 was higher in the tuberculoid and dimorphic forms, demonstrating its role in the inflammatory response and its importance for the unstable or dimorphic forms of the disease[9] (Fig. 02).

Comparative analysis of the histopathological findings and biological activity of Th17 cytokines seems to indicate their participation in the pathogenesis of leprosy reactions, since these cytokines are involved in neutrophil attraction, a characteristic histopathological finding in reactional leprosy lesions[10]. The Th17 profile is important for the progression of the tuberculoid forms since the concomitant presence of TGF-β and IL-6 activity induces Th17 development and the absence of the proinflammatory activity of IL-6 activates TGF-β to induce the differentiation of Treg lymphocytes which, in turn, negatively regulate immunological activity[10-12]. Few studies have demonstrated the involvement of IL-6 and IL-17 in the reactional states, but this role needs to be confirmed by further investigations[13]. The balance between proinflammatory and suppressive immunological response respectively will depend on the concomitant presence of TGF-β/IL-6 or only the TGF-β. Finally, in contrast to Treg cells, Th17 cells seem to play an important role in the development and maintenance of proinflammatory responses and, secondarily, exert a concatenate function with subsequent differentiation to proinflammatory responses mediated by cells (Th1) or antibodies (Th2)[7]. In conclusion, the balance of Th17/Treg seems to elicit more or less vigorous responses in *M. leprae* infections.

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FIGURE LEGENDS

Fig 01 – TGF-β, IL-17 and IL-6 immunohistochemistry pattern in skin lesions of leprosy. Dimorphic leprosy TGF-β (A), IL-17 (D), IL-6 (G). Tuberculoid leprosy TGF-β (B), IL-17 (E), IL-6 (H). Lepromatous leprosy TGF-β (C), IL-17 (F). Magnification 200x.

Fig 02 – Quantification of tissue expression of Th17 cytokines in accordance with the Madrid classification. II - Indeterminate form, TT - Tuberculoid form, DD- Dimorphic form, LL – Lepromatous form.