TITLE: TLR2 IS IMPORTANT FOR THE INDUCTION OF IDO IN LEPROSY

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ABSTRACT

The enzyme Indoleamine 2,3 dioxygenase (IDO) is involved in the first stage of tryptophan catabolism and may have a dubious effect and has been described in microbicidal or tolerogenic environments. Recent data from our group have shown that IDO is differentially regulated in the different clinical forms of leprosy, being able to mediate the tolerogenic mechanisms observed in the multibacillary form. In contrast, IDO contributes for the microbicidal activity observed in the cells from paucibacillary and reactional patients. The present study aims to investigate the mechanisms associated with IDO expression and activity in human dendritic cells differentiated from monocytes (mDCs) after stimulation with irradiated *Mycobacterium leprae* and its fractions: MLMA (membrane fraction) and MLSA (soluble fraction). Monocytes were obtained from *buffy coats* by positive selection. To differentiate the mDCs, monocytes were stimulated with GM-CSF (50 ng/mL) and IL-4 (10 ng/mL) for 6 days at 37^0C/5% CO_2. The phenotype of these cells and the expression of IDO were evaluated by flow cytometry. IDO activity was assessed by HPLC and cytokine production in the supernatants was determined by ELISA. *M. leprae* and its fractions induced the expression and activity of IDO in human mDCs. Among the stimuli studied, MLMA induced the production of proinflammatory cytokines, TNF and IL-6, in mDCs, whereas MLSA induced an increase in IL-10. As the 19-kD and 33-kD lipoproteins of *M. leprae* activate monocytes and DCs through TLR2, we investigated if TLR2 activation was necessary to IDO induction in mDCs. We observed that in the cultures treated with a neutralizing anti-TLR2 antibody, there was a decrease in IDO activity and expression induced by *M. leprae* and MLMA, showing the involvement of TLR pathway in the induction of IDO. We used a MyD88 inhibitor and observed that it led to a decrease in IDO activity in mDCs cultures. Our data demonstrated that co-culture of mDCs with autologous lymphocytes induced an increase in Treg cell frequency in MLSA-stimulated cultures, showing that the *M. leprae* constituents themselves may play opposite roles that may possibly be related to the the dubious effect of IDO in the different clinical forms of disease. Our data show that *M. leprae* and its fractions are able to differentially modulate the activity and functionality of IDO in mDCs by a pathway that involves TLR2, suggesting that this enzyme may play an important role in leprosy immunopathogenesis.

Key-words: Indodelamine 2,3 dioxygenase, dendritic cell and leprosy.

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