

Genes encoding cytokines implicated in the modulation of American cutaneous leishmaniasis. Study in skin lesion biopsies embedded in paraffin

Daise Damaris Carnietto De Hippólito¹; Cristina Silva Meira-Strejevitch¹; Marta Marques Maia¹; Cristina Kanamura²; Aparecida Helena De Souza Gomes³; José Angelo Laulleta Lindoso^{4,5} Vera Lucia Pereira-Chiocola¹

¹Laboratório de Biologia Molecular de Parasitas e Fungos, Centro de Parasitologia e Micologia; e ²Centro de Patologia, Instituto Adolfo Lutz, São Paulo. 01246-000 São Paulo, SP, Brasil. ³Laboratório Regional de Sorocaba; Instituto Adolfo Lutz, Sorocaba, SP, Brasil. ⁴Instituto de Infectologia Emilio Ribas, SP, ⁵Instituto de Medicina Tropical SP, Brasil

Email: daise.hippolito@usp.br

The immune T cell response is almost predominant in all clinical forms of American cutaneous leishmaniasis (ACL). Thus, the knowledge of the immunological mechanisms responsible for formation, progression, and cure of this infection is important. This study investigated whether patients with ACL were able to express genes encoding cytokines such as IFN- γ , IL-10, TNF- α , IL-27, TGF- β and IL-6. A total of 50 skin biopsy samples (formalin fixed and paraffin embedded) from patients with ACL (with positive immunohistochemistry and PCR) was studied. RNA molecules were extracted using RNeasy FFPE isolation kit (Qiagen), according to the manufacturer's instructions. Next, RNA samples were treated with DNase, quantified by fluorimetry (Quantus). After cDNA synthesis, relative expression of each gene expressing cytokine was determined (in duplicate) by real time PCR. The expression of each target gene and the endogenous gene (GAPDH) was determined by "C_T comparative" ($2^{-\Delta\Delta C_T}$) using a normal skin samples of a group of 5 individuals without ACL. The results were expressed as median. The statistical analysis was performed using ANOVA test (GraphPad Prism). According results, three Group of patients were formed. Group I, 35 patients with ACL, which relative expression of IFN- γ was below 100. Group II, 5 patients with ACL which relative expression of IFN- γ was over 100. Group III, 10 patients with mucosal leishmaniasis. Compared to normal individuals, the expression levels for IFN- γ were 33.34, 214.1 and 134.7-fold higher for Groups I, II and III, respectively. For TNF- α were 7.82, 12.59 and 4.98-fold higher than normal in Groups I, II and III respectively. For IL-10 were 5.67, 21.54 and 2.44-fold higher than normal in Groups I, II and III, respectively. For IL-27 were 18.64, 85.29 and 15.71-fold higher than normal in Groups I, II and III, respectively. For TGF- β were 3.67, 4.32 and 4.40-fold higher than normal in Groups I, II and III, respectively. For IL-6 were 6.49, 5.69 and 1.90-fold higher than normal in Groups I, II and III, respectively. These data are in concordance with others already published in literature that showed that the cutaneous form produce moderate production of IFN- γ and TNF- α in a typical Th1 response. However, the uncontrolled increase in IFN- γ plus the presence of TNF- α as showed in Groups II and III could led to an exacerbated response and consequently tissue destruction. IL-10, related to suppression of effector T cell response as well as, inhibition of macrophage function and IL-27, with anti-inflammatory activity causing a local and systemic effect on parasite growth were over expressed in Group II patients. In conclusion, these results can suggest that patients from Group II can develop more severe form of ACL when compared from those of Group I.

Keywords: ACL, gene expression, cytokines

Support: FAPESP (2015/04803-6)