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Gene expression of IL-12 in the thymus of hamsters infected with *Leishmania infantum*

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Background

Visceral leishmaniasis is among the major human health problems in the Americas, East and North Africa and West and Southeast Asia. T cells, an important part of the adaptive immune system, are responsible for establishing Th1, Th2 and Th17 immune responses involved in resistance and susceptibility to leishmaniasis. Lymphocytes development and maturation occur in the thymus, through processes that depend on the action of cytokines. IL-12 acts on the apoptosis of immature thymocytes and, together with other cytokines, stimulates the proliferation of specific subsets of thymocytes. In the search for a better understanding of the mechanisms that lead to thymic alterations that may be correlated with interference in the maturation and differentiation of T lymphocytes during *L. infantum* infection, we evaluated the expression of interleukin 12 in the thymus of hamsters experimentally infected with *Leishmania infantum*.

Materials and methods

Fifteen male hamsters between six and eight weeks of age were infected by experimental intraperitoneal infection with 10^7 *L. infantum* promastigotes (MHOM/BR/1972/BH46). They were divided into three groups, each with five infected hamsters, and sacrificed 15, 60 and 120 days after infection. Control groups consisted of three groups of five healthy hamsters of the same age, sacrificed simultaneously with the infected ones. IL12 expression was evaluated in fragments of thymus by RTqPCR (Slope -2.64, Efficiency 1.39, R2 0.99), by the relative expression method using the geometric mean of the GAPDH genes (Slope -3.34, Efficiency 0.99, R2 0.99) and HPRT-1 (Slope -3.15, Efficiency 0.98, R2 0.98). Differences between infected groups and their corresponding controls, and between groups infected at different times were determined by Student's t-test or Man-Whitney test according to the parametric or non-parametric distribution of the data, respectively. Values of $p < 0.05$ were considered statistically significant. All statistical analyzes were performed using Prism software (v8.0.1, GraphPad, La Jolla, CA, USA).

Results

The relative gene expression of IL-12 showed a significant increase in Groups I-15 (p=0.0072) and I-60 (p=0.0079) compared to the paired control groups, C-15 and C-60 respectively (Figure 1A, B and C). This increase was not maintained throughout the infection, with a significant reduction in the relative expression of I-120 compared to I-15 (p=0.0081) (Figure 1D and 1E).

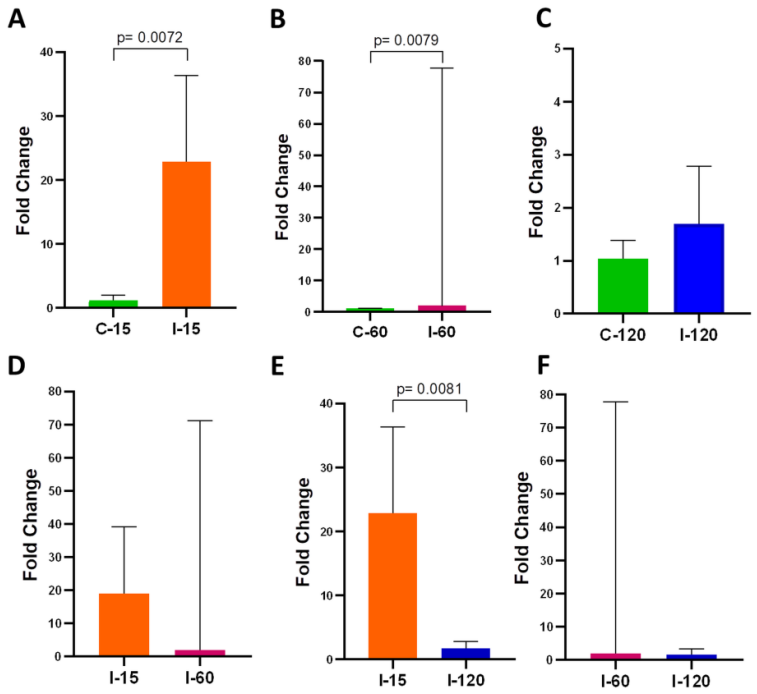

Figure 1- IL-12 gene expression. **(A)** Groups 15 DPIs mean ± standard deviation (p=0.0072); Student's t-test t. **(B)** Groups 60 DPIs median and range; Man-Whitney (p=0.0079). **(C)** Groups 120 DPIs mean ± standard deviation; T student (p>0.05). **(D)** Infected groups 15 and 60 DPIs median and range; Man-Whitney (p>0.05). **(E)** Infected groups 15 and 120 DPIs mean ± standard deviation; T student (p>0.0081). **(F)** Infected groups 60 and 120 DPIs median and range; Man-Whitney (p>0.05).

Conclusion

In this study, we confirmed that in a model of visceral leishmaniasis the expression of IL-12 in the thymus is altered. IL-12 has been implicated in the apoptosis of immature thymocytes and in stimulating the regulation of specific thymocyte subsets. Therefore, the changes observed in IL-12 gene expression suggest that thymocyte development and maturation may be affected during *L. infantum* infection which may interfere with the efficiency of the immune response of infected animals. These results highlight the importance of additional investigation of thymocyte subpopulations and cytokine expression during the infection.

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