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Leishmania tarentolae infecting dog macrophages exerts a protective effect against *Leishmania infantum* by modulating cytokines expression

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Background

Leishmaniases are neglected vector-borne tropical diseases distributed worldwide, mainly in East Africa, Southeast Asia, and Latin America, as well as in several Mediterranean countries. The genus *Leishmania* comprises more than 20 species which infect and replicate in dendritic cells and macrophages of vertebrate hosts. *Leishmania infantum* is the most widespread species of zoonotic concern with dogs as important reservoirs. Similar to *L. infantum*, the herpetophilic *Leishmania tarentolae* (subgenus *Sauroleishmania*) may persist not only in human and murine macrophages [2] but also in those of dogs [1]. The detection of this non-pathogenic species in sympatric areas where *L. infantum* is endemic raised questions regarding a protective effect exerted by *L. tarentolae* in asymptomatic dogs coinfected with *L. infantum* [3]. Thus, the study aimed to monitor the expression profile of pro- (IFN- γ; TNF-α; IL-12) and anti-inflammatory (IL-4; IL-6; IL-10) cytokines [4] in primary canine monocyte-derived macrophages infected by either *L. infantum*, or *L. tarentolae*, or both.

Materials and methods

Macrophage cells obtained from peripheral blood from a German shepherd dog negative for *Leishmania* spp were infected (parasite/macrophage ratio 10:1) by incubating *L. tarentolae* (field-isolated strain from *Tarentola mauritanica* RTAR/IT/21/RI-325, and laboratory strain RTAR/IT/81/ISS21-G.6c/LEM-124), and *L. infantum* (laboratory strain MHOM/TN/80/IPT1) promastigote cultures. Infected cells were monitored from 4 to 96 hours by bright-field microscopy to determine the percentage of infected cells and the number of amastigotes within 200 infected cells (a/infc). Each experiment was conducted in triplicate. The complementary DNA from scraped cells was analyzed by quantitative PCR (qPCR) to assess the gene expression of IL-6, IL-10, IL-4 and IFN- γ, TNF- α, IL-12 cytokines by the $2^{-\Delta\Delta Cq}$ value. Control genes *G3PDH* and *OAZ1* were used as housekeeping. Statistical analyses were performed by two-way ANOVA and Tukey's post hoc. Values were considered statistically significant when $p < 0.05$.

Results

Coinfection of RI-325 + IPT1 showed the highest percentual of infection (57.7%) in all time points followed by the LEM-124 + IPT1 (42.2%). IPT1 in single infection presented a higher medium value of a/infc (1.54) in comparison with single and coinfections. RI-325 (up to 6.681) and LEM-124 (up to 3.041) in single infection presented the lowest expressions of IL-4 in comparison with IPT1 (8.481 at 24 h), while coinfections showed a decrease of IL-4 until 96 h (0.003 for RI-325 + IPT1; 0.217 for LEM-124 + IPT1) (Figure 1A). All strains presented an overall reduction of IL-6 expression, but the expression was significantly lower in coinfections (0.172 for RI-325 + IPT1) than in single infections (e.g., 11.622 for IPT1) (Figure 1B). The highest gene expression value for IL-10 was noticed for all strains at 4 h in single and coinfection, followed by a constant decrease until 96 h (Figure 1C). The expression of IL-12 in coinfections was significantly higher (up to 6.705 for RI-325 + IPT1; up to 4.699 for LEM 124 + IPT1) than in single infections (e.g., 2.261 for IPT1) (Figure 1D). No expression was observed for pro-inflammatory cytokines IFN-γ and TNF-α.

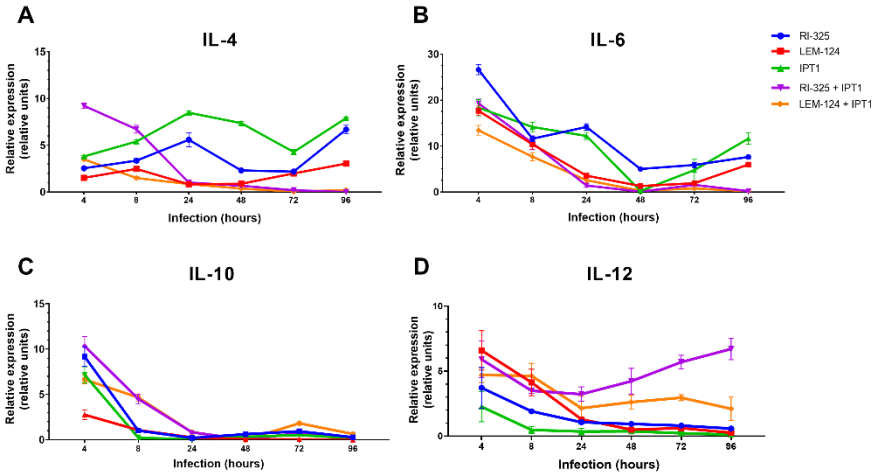

Figure 1: Relative mRNA gene expression (relative units) of anti-inflammatory cytokines IL-4 (A), IL-6 (B), and IL-10 (C), and pro-inflammatory cytokine IL-12 (D) from 4 to 96 h (standard deviation shown).

Conclusions

Data indicates that *L. tarentolae* may elicit the production of pro-inflammatory cytokine IL-12 in coinfection conditions that could ultimately exert a protective effect against *L. infantum*. The protective role of *L. tarentolae* is also highlighted by the reduced expression of anti-inflammatory cytokines (i.e., IL-4 and IL-6). The results may explain the asymptomatic status of dogs coinfected with *L. tarentolae* and *L. infantum* [3], as well as suggest a genetic exchange and hybridization events, opening new perspectives for vaccine development against canine leishmaniosis.

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Conflict(s) of interest: None declared.

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