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# Effects of Antimomium crudum 30cH and 200cH on the macrophage – *Leishmania* (L) *amazonensis* relation *in vitro*

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**Background:** Leishmaniasis is a chronic skin and systemic disease, whose treatment is related to important side effects and loss of life quality. In previous results [1], mice treated with *Antimonium crudum* (AC) 30cH presented reduction in local inflammatory process and modulation of B1 cell-phagocyte differentiation and migration, but also increase of free amastigotes number among the surrounding tissue. **Aims:** To know the mechanisms involved, a series of *in vitro* studies was done, using co-cultures of macrophages (RAW 264.7) and *Leishmania (L.) amazonensis* treated with AC 30cH and 200cH, in different times. **Methodology:** The morpho-functional features of macrophages (spreading, phagocytosis and oxidative activity), the number of free promastigotes in the supernatant and the cytokines measurement were evaluated. The spreading and phagocytosis assays were performed in quadruplicate, in three experiments, resulting in 12 datapoints for each dilution/time. Kruskal Wallis (for no parametric variables) and two-way ANOVA test (for parametric variables) were used to verify time to time differences and the interaction between treatment and time respectively, being $p \leq 0.05$ considered significant. Animals were not used in this study. **Results:** The treatment with AC 30cH and AC 200cH resulted in significant but transitory increase in spreading and phagocytosis activity after 2 to 24 hours of incubation, followed by increase in the number of free promastigotes in the supernatant (in AC 30cH treatment) and decrease in CD86 expression (in AC 200cH treatment). GMCSF, alpha-INF, IL1, IL6, IL10 and IL12 were reduced after 48 to 72 hours and CCL4 and RANTES were reduced after 120H, for both potencies. Two peaks of CCL2 were seen in *Leishmania sp* infected macrophages, at 24 and 120 hours, but only AC30cH inhibited them. A peak of VEGF was observed after 120 hours following the treatment with AC 200cH, together with the increase in the number of multinucleated cells. The morphology of macrophages at fluorescence microscopy after the staining with acridine orange in fresh unfixed cells showed severe reduction of acid vacuoles in AC 30CH treated cells at 2 hours of parasite-macrophage interaction, revealing possible macrophage anergy. No difference in peroxide / NO



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production and in apoptosis percentage of free promastigotes was seen among groups, in all evaluated times. **Conclusions:** Both potencies were able to decrease most of cytokines production, specially CCL2 in AC 30cH treated cells, which explains the inhibition of monocyte migration seen *in vivo [1]*. The late peak of VEGF observed in AC 200cH treated cells suggests a M1 - M2 polarization, whose biological meaning is still under discussion.

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**References**

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