Enhance the immunomodulatory capability of the bacterium *Asaia* with the expression of a *Wolbachia* protein: implication for the control of leishmaniases

DIPARTIMENTO DI BIOSCIENZE

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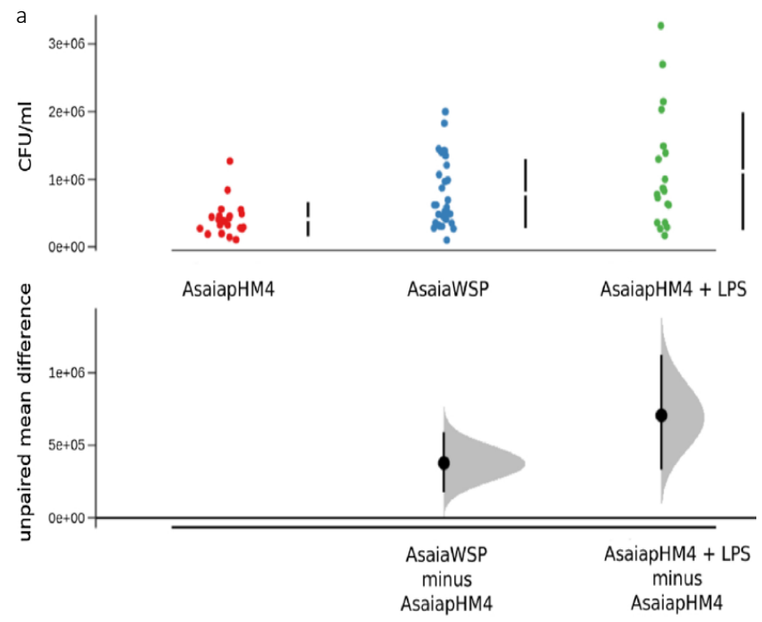
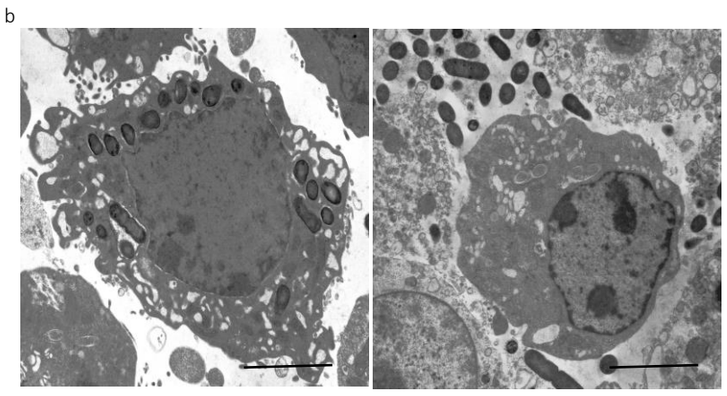
INTRODUCTION

The outcome of leishmaniasis is strongly impaired by the immune polarisation [1]. The identification of the factors involved in M1 polarization, which is protective for the resolution of the disease, is essential for the design of new strategies of intervention. Considering the role of the bacterium *Wolbachia* as a pro-M1 immunomodulator [2], we engineered the bacterium *Asaia* for the expression of a protein of *Wolbachia*, the *Wolbachia* surface protein (*Asaia*^{WSP}). The chimeric bacterium was tested for its capability to: i) stimulate a M1 macrophage polarisation; ii) to induce *Leishmania* killing.

M1 POLARIZATION: PHAGOCYTOSIS ACTIVITY



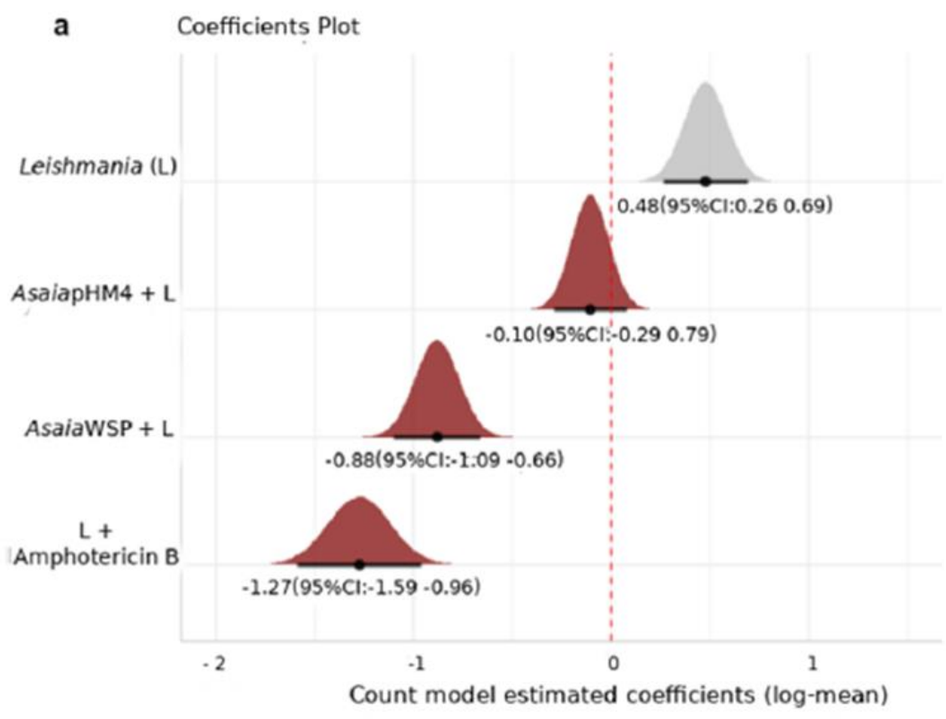
A higher phagocytosis activity is reported when macrophages are treated with *Asaia*^{WSP}. This result is also confirmed by TEM analysis: in macrophages exposed to *Asaia*^{WSP}, bacteria were mostly observed inside the cells and the presence of many vacuoles is suggestive of a strong digestive activity.

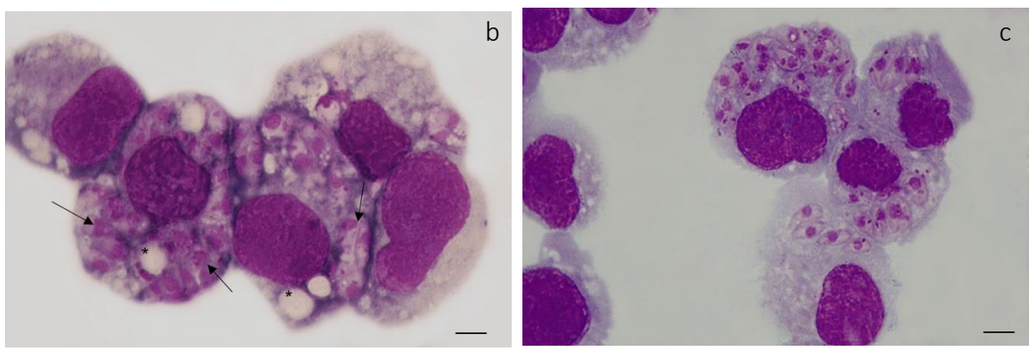


a) The numbers of bacteria internalized are shown as CFU/mL. Vertical error bars: mean and standard deviation. *Asaia*^{pHM4}: control bacterium (engineered with the empty plasmid). In the bottom part the unpaired mean differences between treatment groups are reported. b) TEM images of murine macrophages incubated with *Asaia*^{WSP} on the left and control bacterium (on the right). Bars: 5 µm.

LEISHMANICIDAL ACTIVITY

The pre-treatment of macrophages with the bacterium *Asaia*^{WSP} determines an anti-leishmanial effect, associated with a reduction of the number of live intracellular parasites after 48h of infection. In the Giemsa stain pictures macrophages infected with engineered bacterium present several vacuoles, and the amastigotes show morphological alteration.

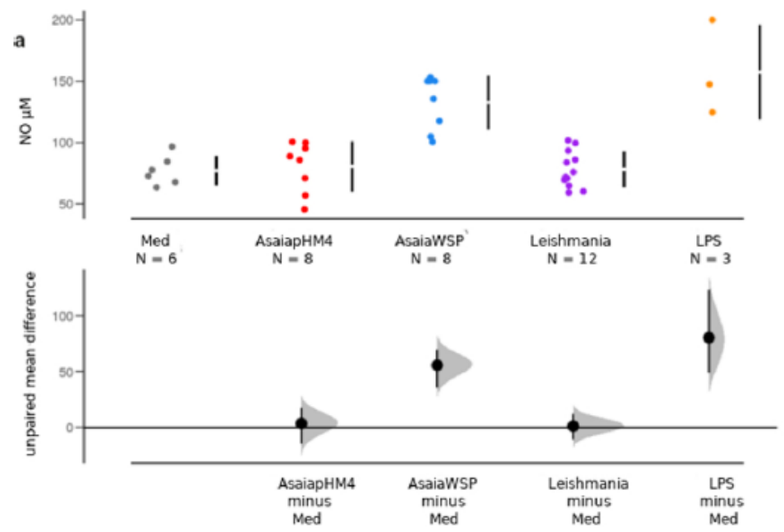




a) Horizontal bars indicate the 95% confidence interval; the exponential of the estimated coefficient in the control group only *Leishmania* (0.48) corresponds to the average number of amastigotes in this group. The other estimated coefficients (-0.10, -0.88, -1.27) correspond to the difference between the estimate of the reference group (L) and that of the treatment group. B-c) Giemsa staining of macrophages infected *Asaia*^{WSP} (b) and *Asaia*^{pHM4} (c). Bars: 5 µm

M1 POLARIZATION: M1 CYTOKINES, ROS AND NO RELEASE

The bacterium *Asaia*^{WSP} determines the expression of markers of classical macrophage activation, including M1 cytokines as TNF-α and IL12p40 and other factors like reactive oxygen species (ROS) and nitrites.



The estimation plot shows the observed data for nitrites (µM) production by macrophages incubated with the bacterium *Asaia*^{WSP} and controls. Vertical error bars: mean and standard deviation. In the bottom part, the unpaired mean differences between treatment groups compared to untreated macrophages (Med) are reported.

CONCLUSION

Our results confirmed the immunopolarising properties of the protein WSP and the capability of the bacterium *Asaia*^{WSP} to induce a M1 immune activation suitable to inhibit *Leishmania* vitality in *in vitro* assays.

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