Adaptive co-evolution in visceral leishmaniasis: the role of host MIF cytokine and parasite mimicry

Wong S.[1], Ahmad R.[1], Hendrickx S.[1], Baeza Garcia A.[1], Caljon G.[1]
[1]Laboratory of Microbiology, Parasitology and Hygiene, University of Antwerp, Antwerpen (Wilrijk), Belgium, sin-ting.wong@uantwerpen.be, rokaya.ahmad@uantwerpen.be, sarah.hendrickx@uantwerpen.be, alvaro.baezagarcia@uantwerpen.be, guy.caljon@uantwerpen.be.

INTRODUCTION: Visceral leishmaniasis (VL) is transmitted by sand flies and varies from an asymptomatic to a life-threatening condition with limited treatment options. The *Leishmania*-host interaction is shaped by an evolutionary arms race involving inflammatory host responses and parasite immunomodulatory molecules. The macrophage migration inhibitory factor (MIF) is a key immune regulator produced by both the mammalian host (hMIF) and the parasite (pMIF). Upon infection, hMIF induces an anti-parasitic, proinflammatory state, whereas in cutaneous leishmaniasis, *L. major mif* (*Lm*MIF) supports parasite survival and persistence in macrophages. Although *Lm*MIF has been extensively studied, research regarding the role of hMIF and its parasite orthologs in VL is lacking. To address this knowledge gap, a search for orthologs in *L. infantum* identified two genes, *Linf*259 (LINF_330025900) and *Linf*260 (LINF_330026000). Overexpression and deletion of these genes were used to elucidate their individual roles.
METHODOLOGY: Single overexpressors (OE) were generated through gene cloning and introduction into the chromosomal *ssu* locus, whereas single and double knockout (KO) lines were obtained through CRISPR-Cas9 gene editing. All transgenic lines were characterized *in vitro* by assessing (i) their growth and metacyclogenesis rates, and (ii) their ability to infect, differentiate into amastigotes and multiply in primary peritoneal macrophages.
RESULTS: No distinct differences could be observed in promastigote proliferation, metacyclogenesis, and parasite survival in culture. Deletion of *Linf*MIF caused profound suppression of macrophage infection and *in situ* proliferation.