ABSTRACT

Phlebotomine sand flies (Diptera, Psychodidae) from genera *Phlebotomus* and *Lutzomyia* are proven vectors of *Leishmania* (Kinetoplastea, Trypanosomatida), causative agent of leishmaniases, tropical neglected diseases. To contribute on creating new control strategies we investigated the molecular aspects of interaction between the vector and pathogen on the immunity level.

Sand fly innate immunity is based on cellular and humoral events which work synergistically to secure effective protection against pathogens. Here we present our research on humoral aspects of sand fly immunity, specifically on main humoral pathways (Toll, Imd, and Jak-STAT), their genes and function under different conditions especially during *Leishmania* development in sand fly midgut.

We have described gene expression profiles of Toll and Imd – related genes in *Phlebotomus papatasi* larvae fed with different microbe loads and in adult females infected with *Leishmania major*. We have identified three antimicrobial peptides (AMPs) in *P. papatasi* and followed their expression profiles during parasite infection and described a gut-specific defensin upregulated by *Leishmania* infection. We have proven that the knockdown of *defensin* genes in *P. papatasi* supports *Leishmania* infection and negatively affects sand fly survival. Moreover, we identified a correlation between Imd pathway and expression of AMPs by silencing *relish* resulting in reduced expression of some AMPs. We discovered that *Leishmania* lipophosphoglycans or bacterial liposaccharides trigger the expression of AMPs, whereas *attacin* showed the earliest and most dramatic changes in both studied species, *P. papatasi* and *Lutzomyia longipalpis*. We have also investigated the role of Jak-STAT pathway during *Leishmania* infection in *L. longipalpis*. While in LL5 cell line, a co-culturing with *Leishmania infantum* led to overexpression of negative regulators of the pathway, the parasitic infection of adult females did not lead to significant change in Jak-STAT related genes. However, use of the gene silencing of STAT transcriptional factor in females reduced the gene expression of inducible oxide synthase and Dual oxidase leading to increased *Leishmania* growth.

Lastly, we attempted to establish a CRISPR-Cas9 gene editing protocol in our laboratory. With two different approaches using CRISPR plasmid constructs and direct sgRNA + Cas9 injection, we injected more than 14,000 sand fly's embryos. Unfortunately, we were not able to establish any edited sand fly line. However, we have made important steps on which future experiments can be built.