ABSTRACT

This Ph.D. thesis focuses on enzymes and other molecules that affect leishmania development in their vectors. It summarizes results of three published project and one publication in preparation.

It has been proposed that cysteine peptidase inhibitor (ICP) of *Leishmania mexicana* protects this protozoan parasite from insect proteolytic enzymes, and therefore promotes their survival in the sand fly vector. To test this hypothesis, *L. mexicana* mutants deficient in ICP were evaluated for their ability to develop in the sand fly *Lutzomyia longipalpis*. The experiments showed that ICP of *L. mexicana* has no major role in promoting leishmania survival in the vectorial part of its life cycle. In addition, recombinant *L. mexicana* ICP did not inhibit peptidase activity of *L. longipalpis* midgut extracts *in vitro*.

Another objective of this thesis was to study the attachment of leishmania to the midgut epithelium of their vectors. Laboratory studies examining the development of different *Leishmania* species in a range of phlebotomine species suggest that sand flies fall into two groups: specific and permissive vectors. In the specific vectors, successful parasite development is mediated by the parasite surface lipophosphoglycan (LPG), while this LPG is not required for the attachment in permissive vectors. Our experiments confirmed this hypothesis and showed that *L. major* LPG is not required for the parasite survival in permissive species *Phlebotomus perniciosus* and *P. argentipes*, but plays an important role in the specific vector *P. duboscqi.*

Using a novel method of competitive binding of fluorescently labeled leishmania promastigotes to the midguts *in vitro*, we studied stage- and species-specificity of the binding. Our results showed that leishmania binding is strictly stage-dependent; nectomonads and leptomonads bound, while procyclics and metacyclics did not. Furthermore, we showed, by comparing the binding of several leishmania species, that the natural parasite is not necessarily the species binding most efficiently *in vitro* to the midgut of its vector. We conclude that, although midgut binding seems to be necessary for the parasite establishment, in several parasite–vector combinations, the ability of the parasite to bind *in vitro* to sand fly gut is insufficient to explain the vectorial competence and parasite–vector specificity.

We also compared the results from experimental infections and *in vitro* binding assays using leishmania mutated in GPI-anchored surface molecules putatively involved in attachment to sand fly midgut: LPG and gp63. Taken together, our data suggest an important role of GPI-anchored proteins, including gp63, in *Leishmania* attachment to sand fly midgut. In addition, our data confirmed the presence of an LPG-independent parasite binding mechanism of *L. major* within the midgut of permissive vector *P. perniciosus* and provide a strong support for the crucial role of *L. major* LPG in specific vector *P. papatasi*. In contrast, the LPG role in permissive vector *P. perniciosus* was suprisingly disputable as this sand fly showed low susceptibility to *L. infantum lpg1-* mutants.