

## SUMMARY OF RESULTS

The thesis sums up the results of three projects I was involved in during my PhD study. Specifically, I addressed the putative effect of sand fly salivary hyaluronidase on transmission and establishment of *Leishmania* infection. The second project was dealing with the kinetics of anti-saliva antibody response in dogs exposed to *Lutzomyia longipalpis* sand flies and with the characterization of salivary antigens recognized by these dogs. Finally, I constructed and annotated a cDNA library from *Phlebotomus arabicus* and characterized *P. arabicus* salivary antigens reacting with antibodies of mice exposed to this sand fly species. The results of the projects are briefly outlined here.

We detected hyaluronidase activity in saliva of various bloodsucking Diptera, including sand flies. In coinoculation experiments with BALB/c mice we proved a positive correlation between the size of the cutaneous lesion caused by *Leishmania major* and the presence of hyaluronidase in the infective inoculum. In hyaluronidase-coinoculated mice, the lesions were significantly larger from week 3 post infection (infection dose  $10^5$  parasites) or week 4 (infection dose  $10^4$ ). On the other hand, hyaluronidase did not affect early visceralization of *L. major* at 24 hrs post infection. Thus, we demonstrated that hyaluronidase promotes *Leishmania* establishment in murine skin and we hypothesize that immunomodulatory effects of hyaluronan fragments generated at infection site are responsible for the effect. We suggest that hyaluronidase is one of the factors responsible for infection-enhancing ability of saliva in New World and Old World sand flies alike.

We studied the antibody response in dogs experimentally exposed to *Lutzomyia longipalpis* females to find out whether the level of specific anti-saliva antibodies reflects the intensity of exposure. Dogs experimentally exposed to feeding of *L. longipalpis* sand flies developed specific anti-saliva IgG and IgE antibodies and their sera recognized up to six salivary protein bands in *L. longipalpis* salivary gland lysate. The levels of anti-saliva IgG, IgG1 and IgG2 were related to numbers of fed *L. longipalpis* females and elevated antibody levels in bitten animals were found throughout the study. Differences in the strength of antibody response between high-exposed and low-exposed dogs were detected as late as 29 weeks after the last exposure. In contrast, specific IgE response developed in some dogs only and no correlation was observed between its level and the intensity of exposure. Therefore, anti-saliva IgG was found as a useful marker of exposure of dogs to sand flies. Moreover, anti-saliva IgG persists long enough to allow monitoring of canine exposure to sand flies even in regions which show marked fluctuations in numbers of sand flies throughout the year.

A cDNA library was constructed from salivary glands of *Phlebotomus arabicus* females. From this cDNA library, we sequenced 985 randomly selected clones from which 395 clusters of related sequences were obtained. The most abundant transcripts were those coding for putative secretory proteins; 74 clusters were generated from these sequences, with an average number of 7,65 sequences per cluster. Members of 21 different families were found among putative secretory proteins; most of these proteins have known homologs in other sand fly species. The most abundantly represented families were SP15-like proteins, 27 kDa-like proteins, D7-related proteins, yellow-related proteins, PpSP32-like proteins, antigen 5-related proteins, 34 kDa-like proteins, and the apyrases. Sequences coding for putative secreted enzymes were also found in the cDNA library, including hyaluronidase, endonuclease, pyrophosphatase, amylase and trehalase. Eight to ten antigens reacting with sera of mice exposed to *P. arabicus* feeding were detected using different serum samples. In addition, we confirmed our previous findings that the antibody response to sand fly salivary antigens is species-specific. Sera from mice bitten by *P. arabicus* specifically recognized antigens of *P. arabicus* and not those of *P. papatasi*.