Charles University in Prague
Faculty of Science
Department of Parasitology

Antigenic and enzymatic properties of sand fly saliva

Jitka Hostomská

Summary of PhD Thesis

Supervisor: Prof. RNDr. Petr Volf, CSc.

Prague, 2008

CURRICULUM VITAE

Mgr. Jitka Hostomská

Address:

Department of Parasitology
Faculty of Science
Charles University in Prague
Viničná 7, 128 44 Prague 2
Czech Republic

tel.: +420 221951814
email: jitka.hostomska@centrum.cz

Personal data:

Date of Birth: June 6, 1980
Place of Birth: Prague, Czech Republic
Nationality: Czech
Marital Status: single

Education:

2004 – present:
Postgraduate study in Parasitology, Charles University in Prague
PhD thesis: „Antigenic and enzymatic properties of sand fly saliva"
supervisor: Prof. RNDr. Petr Volf, CSc.

1999-2004:
Master of Science in Immunology, Charles University in Prague
M.Sc. thesis: „Mechanisms of immune response to xenografts"
supervisor: Doc. RNDr. Vladimír Holáň, DrSc.

Interships:

2007:
Unidade de Leishmanioses, Instituto de Higiene e Medicina Tropical, Lisboa, Portugal
Dr. Lenea Campino; 3 weeks

2006:
Laboratory of Malaria and Vector Research, NIAID / NIH, Rockville, MD, USA
Dr. Jesus G. Valenzuela; 1 month

Erasmus-Socrates fellowship
Vector and Parasite Physiology Unit, Department of Zoology, University of Aberdeen, UK
Dr. Peter F. Billingsley; 3 months

Courses:

2008:
Integrated Consortium on Ticks and Tick-borne Diseases 3
Bioinformatics Workshop
June 8-15, České Budějovice, Czech Republic

2005:
Summer School on Molecular Vector Biology
June 20-25, České Budějovice, Czech Republic

Employment:

2005-present:
research worker, Department of Parasitology, Faculty of Science, Charles University in Prague

2003-2004:
laboratory technician, Department of Physiology and Developmental Biology, Faculty of Science, Charles University in Prague

Membership in scientific societies:

2003-present:
Czech Society for Immunology

INTRODUCTION

Sand flies (Diptera: Phlebotominae) are tiny insects that can be found in tropical and subtropical regions. Female sand flies require bloodfeeding for the production of eggs. Two sand fly genera, *Lutzomyia* and *Phlebotomus*, transmit protozoan parasites of the genus *Leishmania* and are therefore of considerable importance for public health. Apart from transmitting *Leishmania* upon feeding, an infected sand fly female delivers sand fly saliva and parasite-derived promastigote secretory gel into host dermis. Both compounds were shown to promote *Leishmania* infectivity. Once in the vertebrate host, *Leishmania* parasites are taken up by phagocytic cells. Macrophages are the target cells for intracellular replication; eventually, parasite multiplication in host tissue together with immunopathological processes causes leishmaniasis. The clinical presentation varies from mild cutaneous affection to visceral disease leading to death if untreated. Currently, leishmaniases are endemic in 88 countries, with estimated 12 million people infected and 2 million new cases considered to occur annually. In my thesis, I focused on the *Leishmania* transmission-enhancing and antigenic properties of sand fly saliva.

Sand fly saliva was reported to have multiple enzymatic properties. In our laboratory, hyaluronidase activity was detected in salivary glands of several sand fly species. Hyaluronan (HA) is a glycosaminoglycan abundant in connective tissues. Under inflammatory conditions low molecular weight HA fragments are generated locally which may affect gene expression and motility of immune cells. Therefore, we were keen to investigate whether sand fly salivary hyaluronidase activity is capable of such modulation of local immune milieu at the inoculation site that would result in enhanced *Leishmania* infection establishment. A model of intradermal coinjection of *L. major* and hyaluronidase was used to evaluate the effect of the enzyme presence on the infection.

The capacity of salivary components of bloodfeeding insects to elicit hypersensitivity reactions in susceptible individuals is notorious. However, for the purposes of clinical and veterinary medicine the antigenic properties of insect saliva are beneficial in a sense. First, it was shown that animals previously exposed to insect bites or individual salivary components are partially protected against subsequent disease transmission. Leishmaniasis is by far the most intensively studied vector-borne disease in this respect and sand fly salivary proteins may hold promise as future vaccine components. Second, anti-saliva antibodies were suggested as markers of host exposure to vector insect species. Research on this topic is easier to translate into practice than current vaccine studies: such markers would be readily useful for evaluating the effectiveness of anti-vector campaigns. However, two points to be addressed are the identity and cross-reactivity of salivary antigens eliciting the antibody response, and the time course of antibody production. Having such information at hand would help greatly in development anti-saliva antibody detection kits for the field.

It was shown in previous work of our laboratory that immune response to sand fly salivary antigens is sand fly species-specific. Although much effort was put into analyzing salivary repertoire of *Lutzomyia longipalpis*, the widespread New World vector of visceral leishmaniasis, and *Phlebotomus papatasi*, one of the most important vectors of Old World cutaneous leishmaniasis, it is clear that salivary composition and properties of other vectors of leishmaniases have to be determined as well. *Leishmania tropica* is the causative agent of Old World cutaneous leishmaniases, although viscerotropic cases have also been described. As results from our laboratory have elucidated the life cycle of *L. tropica* in Israeli foci of cutaneous leishmaniases and identified both *P. sergenti* and *P. arabicus* as local vectors of the disease, we sought to characterize salivary composition of *Phlebotomus arabicus* to complement our previous work on this vector.

OBJECTIVES

My study was aimed at enzymatic and antigenic properties of sand fly saliva. I was interested in the role of salivary hyaluronidase in *Leishmania* transmission. In certain aspects, I focused specifically on sand fly species *Lutzomyia longipalpis* and *Phlebotomus (Adlerius) arabicus*, vectors of visceral and cutaneous leishmaniases, respectively. I studied the salivary antigens of these species and the immune response in animals exposed to sand fly bites. So far, nothing is known about the composition of saliva in any *Adlerius* species and our colony of *P. arabicus* is at present the only laboratory colony of subgenus *Adlerius* in the world. Therefore I sought to characterize salivary components of this species.

The main objectives of this thesis were as follows:

- to assess the effect of hyaluronidase coinoculation on the size of lesions in BALB/c mice infected with *Leishmania major*
- to determine whether hyaluronidase coinoculation affects the numbers of parasites in lymph nodes draining the site of infection with *L. major*
- to describe the long-term kinetics of anti-saliva antibody response in dogs experimentally exposed to *Lutzomyia longipalpis* females
- to detect and characterize salivary antigens of *L. longipalpis* reacting with antibodies of dogs repeatedly bitten by this sand fly
- to prepare and annotate a cDNA library from *P. arabicus* salivary glands
- to characterize the number and approximate molecular weight of *P. arabicus* salivary antigens reacting with antibodies of mice exposed to *P. arabicus*.

SUMMARY OF RESULTS

The thesis sums up the results of three projects I was involved in during my PhD study. Specifically, I adressed 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*.

PUBLICATIONS

Two original articles and one review in peer-reviewed scientific journals, and 10 conference abstracts.

Scientific papers:

Hostomská J., Rohoušová I., Volfová V., Stanneck D., Mencke N., Volf P., 2008. Kinetics of canine antibody response to saliva of the sand fly *Lutzomyia longipalpis*. Vector-Borne and Zoonotic Diseases 8 (4)
doi:10.1089/vbz.2007.0214
accepted for publication 30th November, 2007

Volf P., **Hostomská J.**, Rohoušová I., 2008. Molecular crosstalks in *Leishmania*-sandfly-host relationships. Parasite 15 (3)
accepted for publication 11th April, 2008

Volfová V., **Hostomská J.**, Černý M., Votýpka J., Volf P. Hyaluronidase of bloodsucking insects and its enhancing effect on *Leishmania* infection in mice. PLoS Neglected Tropical Diseases, in press
accepted for publication 25th July, 2008

Presentations at conferences:

Vlková M., **Hostomská J.**, Rohoušová I., Volfová V., Volf P.: Sand fly saliva, host immune system and *Leishmania*. 8th Czech and Slovak Parasitological Days, 19th-23th May 2008, Sezimovo Ústí, Czech Republic

Vlková M., **Hostomská J.**, Rohoušová I., Volfová V., Volf P.: Sand fly saliva, host immune system and *Leishmania*. Annual meeting of Czech Society for Parasitology, 5th-9th May 2008, Jáchymov, Czech Republic

Hostomská J., Volfová V., Černý M., Votýpka J. and Volf P.: Hyaluronidase in bloodsucking insect saliva: effect on pathogen transmission? Annual meeting of Czech Society for Parasitology, 5th-9th May 2008, Jáchymov, Czech Republic

Hostomská J., Volfová V., Votýpka J. and Volf P.: Salivary hyaluronidase of bloodsucking insects and its effect on pathogen transmission. British Society for Parasitology - Trypanosomiasis and Leishmaniasis Seminar, 30th March-2nd April 2008, Newcastle, UK

Rohoušová I., **Hostomská J.**, Vlková M., Volf P.: Antibodies against sand fly saliva: risk marker of *Leishmania* transmission. British Society for Parasitology, Trypanosomiasis and Leishmaniasis Seminar, 30th March-2nd April 2008, Newcastle, UK

Rohoušová I., **Hostomská J.**, Volf P.: Anti-sand fly saliva antibodies: marker of exposure and transmission risk of leishmaniasis. 56th Annual Meeting of American Society of Tropical Medicine and hygiene, 4th-8th November 2007, Philadelphia, PA, USA

Hostomská J., Rohoušová I., Volf P.: Yellow protein from sand fly saliva – a role in leishmaniasis transmission? 4th Meeting of Biomedical Doctoral Schools, Louis Pasteur University, Strasbourg & Charles University in Prague, 10th-11th May 2007, Prague

Hostomská J., Volfová V., Votýpka J. and Volf P.: Hyaluronidase activity in saliva of bloodsucking insects and its role in pathogen transmission. 11th International Congress of Parasitology, 6th-11th August 2006, Glasgow, Scotland

Rohoušová I., **Hostomská J.**, Vlková M., Volfová V., Volf P.: Host immune response to sand fly saliva and its influence on *Leishmania* infection development. 7th Czech and Slovak Parasitological Days, 23th-27th May 2008, Modra, Slovakia

Rohoušová I., **Hostomská J.**, Volfová V., Volf P.: Time course of the canine antibody response against *Lutzomyia longipalpis* saliva. Annual meeting of Czech Society for Parasitology, 24th-28th April 2006, Sedlice, Czech Republic

FINANCIAL SUPPORT

Grant Agency of the Charles University

GAUK 139/2006/B-BIO/PrF (2006-2008), Immunomodulatory and immunogenic properties of salivary Yellow protein from *Phlebotomus papatasi* (Diptera: Psychodidae); principle investigator J. Hostomská

GAUK 191/2006/B-BIO/PrF (2006-2008), Saliva of sand flies (Diptera: Phlebotominae) and host antibody response; principle investigator I. Rohoušová

Ministry of Education, Youth and Sports

MSM0021620828 (2005-2011), Ecological processes in the evolution of model organisms; coordinator P. Volf

LC06009 (2006-2010), Centre of molecular ecology of vectors and pathogens; coordinator J. Kopecký

Mobility Fund of Charles University in Prague

partial funding of the internship in the NIAID/NIH, USA

Socrates/Erasmus Programme

partial funding of the internship at the University of Aberdeen, UK

THESIS REVIEWERS

RNDr. Martin Bilej, DrSc.
Division of Immunology and Gnotobiology
Institute of Microbiology
Academy of Sciences of the Czech Republic

RNDr. Jiří Salát, Ph.D.
Department of Parasite Immunology
Institute of Parasitology
Academy of Sciences of the Czech Republic

COMMITEE MEMBERS

Prof. RNDr. Jan Tachezy, Ph.D. (head)
Department of Parasitology
Faculty of Science, Charles University in Prague

Prof. RNDr. Petr Horák, Ph.D.
Department of Parasitology
Faculty of Science, Charles University in Prague

Prof. RNDr. Jaroslav Kulda, CSc.
Department of Parasitology
Faculty of Science, Charles University in Prague

RNDr. Martin Bilej, DrSc.
Division of Immunology and Gnotobiology
Institute of Microbiology
Academy of Sciences of the Czech Republic

MUDr. RNDr. František Stejskal, Ph.D.
3rd Department of Infectious and Tropical Diseases
1st Faculty of Medicine, Charles University in Prague

RNDr. Vladimír Hampl, Ph.D.
Department of Parasitology
Faculty of Science, Charles University in Prague

The thesis will be defended on 16th September, 2008, at the Department of Parasitology, Faculty of Science, Charles University in Prague