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Sand fly saliva & host immune response

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INTRODUCTION

Sand flies (*Diptera: Phlebotominae*) are hematophagous arthropods transmitting the important vector-borne disease - leishmaniasis. Within the vertebrate host, *Leishmania* reside in phagocytic cells and induce a spectrum of diseases ranging from a single self-healing cutaneous lesion to lethal visceral forms. Leishmaniases currently affect about 12 million people, with 1.5 – 2 million new cases estimated to occur annually; more than 350 million people in tropical and subtropical regions are at risk.

During the blood feeding, sand fly females inject *Leishmania* parasites into the host skin together with saliva. In naive hosts, presence of sand fly saliva enhances the initial phase of *Leishmania* infection. The pharmacological activities increase the chance of successful transmission since *Leishmania* co-injected with saliva become more virulent.

Sand fly saliva modulates the host immune response at different levels including both the innate and acquired immune response. It affects complement activation, T cell proliferation, haematopoiesis, and various functions of antigen presenting cells such as cytokines secretion, NO synthesis, and the expression of co-stimulatory molecules. Saliva of *Lutzomyia* and *Phlebotomus* species has some common as well as specific effects on host immune response. The principal immunomodulator known in *L. longipalpis* saliva is the vasodilatator maxadilan. The immunomodulatory molecule(s) in *Phlebotomus* saliva are less well defined.

In pre-exposed hosts, sand fly saliva stimulates an immune response, which may block this enhancing effect. Such data indicates the potential usage of sand fly saliva in a transmission blocking vaccine. However, an explanation of this anti-*Leishmania* effect is not straightforward. There are two hypotheses, not mutually exclusive, to explain this protective effect. First, a cellular immune response (mainly DTH) to salivary proteins may create an inhospitable site for pathogen establishment at the site of bite. Second, an antibody response to sand fly saliva may neutralize its enhancing effect on pathogen establishment. It is possible that both the enhancing and protective effects of sand fly saliva can be achieved by more than one mechanism and are influenced by *Leishmania* species, dose, and culture conditions, the genetic background as well as by sand fly species.

A charcterization of the immune response to sand fly saliva is useful in epidemiological studies. It also help us to better understand the interplay between vectors, parasites, and hosts. Last, but not least, these studies are important in considering immunoprophylaxis strategy.

AIMS OF THE THESIS

Saliva of sand fly vectors plays an important role in transmission of *Leishmania* parasites by modulating host immune response. It has been shown previously, that the protein composition and the immunomodulatory factors in sand fly saliva vary among species. Therefore, the species-specificity of the interactions between the sand fly saliva and the host immune response were in the focus of this study. There are 3 main aims of the thesis:

1) Characterization of salivary antigens and antibody response of bitten hosts

In nature, the history of exposure and frequency of sand fly bites are probably important for the outcome of infection. We used sera from animals experimentally bitten by sand flies (mice, hamsters, and rabbits) to characterize antigens in sand fly saliva by dot blot and western blot techniques. Using different sand fly species we determined the species-specificity of salivary antigens among three *Phlebotomus* species: *P. halepensis*, *P. papatasi*, and *P. perniciosus*.

The second part of the work was focused on human antibody response. Sera were obtained from inhabitants living in the endemic area for *Leishmania tropica* in SanliUrfa, Turkey. Using ELISA and westen blot methods, we tested whether human sera specifically react with two local sand fly species, *P. papatasi* and *P. sergenti*, and whether they recognise the same salivary antigens, as do the sera of experimental mice. In addition, we compared the antibody response against *P. papatasi* and *P. sergenti* in patients with active *Leishmania* lesions and healthy individuals living in the same houses.

2) Evaluation of the specificity of the pre-immunization effect

We studied the protective effect of sand fly saliva on *Leishmania* infection development. We tested the hypothesis whether the protection given by preexposure to sand fly saliva is species-specific (similarly to the antibody response) and whether the feeding of different sand fly species results in cross-protection against *Leishmania* transmission. To test this, saliva-naive or saliva-immunized mice were co-inoculated in the ear dermis with *Leishmania* plus sand fly saliva. Subsequently, parasite load was determined and cells recovered from ear tissues were characterized by flow cytometry.

3) Description of the effects of saliva on lymphocyte functions

We studied proliferation and cytokine production (IFN-γ, IL-2, and IL-4) of spleen cells from saliva-naive mouse by lymphocyte proliferation assay and ELISA, respectively. We compared these effects among three sand fly species: *L. longipalpis* and *P. papatasi*, two the most studied species, and another important vector *P. sergenti*, whose saliva has not been tested for immunomodulatory effects yet.

SUMMARY OF THE RESULTS

- Saliva inoculated by sand fly females stimulated production of high levels of anti-saliva antibodies. Using sera of experimentally bitten animals (rabbits, hamsters, and mice), we showed that salivary antigens are species-specific. Week cross-reactivity was detected only between some *Phlebotomus* species (Volf & Rohoušová, 2001, Rohoušová et al., 2005).

- Human antibody response against saliva of various sand fly species is specific as well. Sera from individuals living in the endemic area of *Le. tropica* in Sanliurfa (Turkey) showed high IgG levels against saliva of *P. sergenti* and *P. papatasi*, two the most abundant sand fly species in this area, but did not react with saliva of the New World sand fly, *L. longipalpis*. Patients with active *Le. tropica* lesions possessed significantly higher IgG levels against the saliva of *P. sergenti* (the vector) than the healthy individuals from the same place. The levels of IgG against the non-vectorial species of *Le. tropica* (*P. papatasi*) were equal in both groups. Major protein bands in *P. papatasi* and *P. sergenti* saliva reacted with both mouse and human sera; some differences were found only in the intensity of reactions (Rohoušová et al., 2005).

- Mice immunized with saliva of *L. longipalpis* developed partial protective immunity against the challenge with *Le. amazonensis* co-inoculated with *L. longipalpis* saliva. There is a correlation of this immunity with a lower number of mononuclear and polymorphonuclear phagocytes at the site of parasite inoculation. Pre-immunization with *P. papatasi* or *P. sergenti* lysates were ineffective against the co-inoculation of the parasite with *L. longipalpis* saliva (Thiakaki et al., 2005).

- Spleen cells from BALB/c mice were incubated with SGL of *P. papatasi, P. sergenti* or *L. longipalpis*. Both spontaneous and concanavalin A-stimulated lymphocyte proliferation were significantly suppressed with SGLs of all three sand fly species and all SGL doses tested (1, 1/4, and 1/16 of salivary gland). In parallel experiments, we analysed the effect of sand fly saliva on IFN-γ, IL-2, and IL-4 production. In mitogen-stimulated cells SGLs markedly inhibited IFN-γ production in all intervals tested and to a lesser degree impaired production of other two cytokines as well. Saliva of all sand fly species modulated cell proliferation and the cytokine production in a similar way (Rohoušová et al., *in press*).

Our results suggest that the antibody response to sand fly saliva could be used for monitoring the exposure to sand flies and might be used as a marker of risk for *Leishmania* transmission in endemic areas. The specificity of vector salivary antigens should be bear in mind when designing the vector-based transmission blocking vaccine against leishmaniasis.

At present, my research on sand fly saliva continues by studies aimed at the individual variability of salivary components, the dynamic of antibody response to sand fly saliva, and the specificity of cell-mediated immunity against sand fly saliva.

PUBLICATIONS

Four original publications in international scientific journals and 15 abstracts in journals or proceedings from conferences.

List of scientific papers

Rohoušová I., Volf P., and Lipoldová M.: Modulation of murine cellular immune response and cytokine production by salivary gland lysate of three sand fly species. *Parasite Immunology – in press*.

Rohoušová I., Ozensoy S., Ozbel Y., and Volf P., 2005: Detection of species-specific antibody response of humans and mice bitten by sand flies. *Parasitology* **130**: 443-449.

Thiakaki M., **Rohoušová I.**, Volfova V., Volf P., Chang K.P., and Soteriadou K., 2005: Sand fly species-specificity of saliva-mediated protective immunity in Leishmania amazonensis-BALB/c mouse model. *Microbes and Infection* **7**: 760-766.

Volf P. and **Rohoušová I.**, 2001: Species-specific antigens in salivary glands of phlebotomine sandflies. *Parasitology* **122**: 37-41.

List of abstracts in journals or proceedings from conferences

Rohoušová I., Volfová V., and Volf P. (2005) Antigenic variability in sand fly saliva. Fifth International Symposium on Phlebotomine Sandflies ISOPS V, April 17-21, 2005, Gammarth-Tunis, Tunisia. Abstract OP-48 in *Archives de l'Institut Pasteur de Tunis* 82(1): 62.

Rohoušová I., Thiakaki M., Volfová V., Chang K.P., Soteriadou K., and Volf P. (2005) Evaluation of immune response to sand fly bites as a risk factor for leishmania transmission. Third World Congress on Leishmaniosis Worldleish3, April 10-15, 2005, Palermo-Terrasini, Sicily, Italy. Abstract book p. 199.

Rohoušová I., Lipoldová M., and Volf P. (2005) Modulation of murine cellular immune responses and cytokine production by sand fly saliva. Czech Section of Protozoology Meeting, April 26-30, 2004, Josefův Důl, Czech Republic. Abstract 123A in *Journal of Eukaryotic Microbiology* 52(2): 37S.

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Rohoušová I., Lipoldová M., and Volf P. (2004) Vliv slin flebotomů (Diptera: Phlebotominae) na imunitní systém hostitele. 21. pracovní imunologická konference (PIK) České imunologické společnosti, October 22-24, 2004, Rožnov pod Radhoštěm, Czech Republic. Abstract book p. 59-63.

Rohoušová I., Thiakaki M., Soteriadou K., and Volf P. (2004) The protection against leishmania transmission given by pre-imunization to sand fly saliva is species-specific. British Society for Parasitology, Trypanosomiasis and Leishmaniasis Seminar, August 27-30, 2004, České Budějovice, Czech Republic. Abstract 97 in abstract book.

Rohoušová I., Lipoldová M., and Volf P. (2004) Sliny flebotomů a jejich vliv na imunitní systém hostitele. České a Slovenské parazitologické dny, May 18-21, 2004, Ostravice, Czech Republic. Abstract book p. 66.

Rohoušová I. and Volf P. (2004) Specificity of sand fly salivary antigens and host antibody response. Czech Section of Protozoology Meeting, May 12-16, 2003, Seč, Czech Republic. Abstrakt 72 in *Journal of Eukaryotic Microbiology* 51(2): 19A.

Rohoušová I. and Volf P. (2003) Antibody response of hosts bitten by sand flies. 15th European Immunology Congress (EFIS 2003), June 8-12, 2003, Rhodes Island, Greece. Abstract W21.22 in *Immunology Letters* 87: 185.

Rohoušová I., Černá P., Mikeš L., and Volf P. (2003) Sand fly salivary proteins and antibody response of bitten hosts. Czech Section of Protozoology Meeting, April 8-12, 2002, Kunín, Czech Republic. Abstrakt 69 in *Journal of Eukaryotic Microbiology* 50(2): 20A.

Rohoušová I., Černá P., Mikeš L., Ozensoy S., Ozbel Y., and Volf P. (2002) Immunogens and enzymes in sand fly saliva and antibody response of bitten hosts. International Symposium on Phlebotomine Sandflies ISOPS IV, August 3-7, 2002, Salvador – Bahia, Brazil. Abstract P-71 in *Entomologia y Vectores* 9(1): 174.

Volf P., **Rohoušová I.**, Mikeš L., and Kalvachová P. (2001) Characterization of antigens and hyaluronidase activities in sand fly saliva. WORLDleish 2, May 20-24, 2001, Crete, Greece. Abstract 200 in Abstracts book p. 52.

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Volf P. and **Rohoušová I**. (2000) Sandfly salivary antigens and antibody response in bitten animals. 13th European SOVE Meeting of Society for Vector Ecology, September 24-29, 2000, Belek, Antalya, Turkey. *Proceedings of the 13th European SOVE Meeting*, pp. 17-20.

Volf P., **Rohoušová I.**, and Lipoldová M. (1999) Sandfly salivary proteins and their effect on host immunity. International Symposium on Phlebotominae Sandflies ISOPS III, August 23-27, 1999, Montpellier, France. Abstract W18.

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