CHARLES UNIVERSITY IN PRAGUE

Third Faculty of Medicine

Department of Medical Microbiology

Study on neuropathophysiological changes in mammalian host caused by bird schistosome infection.

Ph.D. Thesis Summary

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CURRICULUM VITAE

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EDUCATION

1998-2003: M.S. study - Department of Zoology, Faculty of Science, Masaryk University in Brno; Theme of master thesis: Diversity of perch (*Perca fluviatilis*) parasites in condition of fragmented habitats.

2003- present: PhD. Study - Department of Microbiology, 3rd Faculty of Medicine, Charles University in Prague; Theme of PhD. Thesis: Study of neuropathophysiological changes of mammalian host caused by bird schistosome infection.

FOREIGN STAYS

2005 and *2006*: Division of Cellular Allergology, Research Center Borstel, Germany, laboratory of Prof. Helmut Haas (4 and 3 weeks).

2010: Department of Neuroimmunology, Center for Brain Research, Medical University of Vienna, Austria, laboratory of Prof. Hans Lassmann (5 weeks).

COOPERATION ON GRANT PROJECTS (research team member)

- *IGA MZ CR* (*Grant n*. NJ/7545-3): Bird schistosomes in nature ponds as risk factor of development of neurophysiological disorders (2003-2005).

- *The Wellcome Trust – Collaborative Research Initiative Grant (Grant n. 072255/Z/03/Z)*: The biochemical and immunological properties of *Trichobilharzia* proteases (2003-2007).

- *Czech Ministry of Education (Grant n. MSM LC06009)*: Centrum of molecular ecology of vectors and pathogens (2006-2010).

- *Czech Science Foundation (Grant No. P502/11/1621)*: Neuropathology of *Trichobilharzia regenti* infections in birds and mammals (2011-2012).

INTRODUCTION

Human schistosomiasis is a parasitic diseases caused by blood-dwelling flukes of the genus *Schistosoma*. More than 207 million people are currently infected worldwide and approximately 700 million people may be at risk of infection in 74 endemic countries (WHO, 2010). Schistosomiasis occurs in tropical and subtropical areas, especially in poor rural communities with bad hygienic conditions.

Contrary to human schistosomes, bird schistosomes are cosmopolitan and can be found even in cold areas of northern Europe (Thors and Linder 2001; Larsen *et al*. 2004; Aldhoun *et al*. 2009; Soleng *et al*., 2010). Despite their worldwide occurrence, avian schistosomes were neglected by parasitologists due to commonly accepted opinion that they have no or minor pathogenic impact for birds as well as mammals, including humans. Nowadays, many studies started to focus on these parasites, because it has been recognized that they are severe pathogens of birds. Moreover, their larval stages (cercariae) frequently infect humans causing cercarial dermatitis, a disease which is considered to emerge all over the world. The most reported agents of swimmer's itch are cercariae of the genus *Trichobilharzia* (Horák and Kolářová 2011).

Human infections by bird schistosomes are mostly associated with development of cercarial dermatitis (swimmer's itch), an allergic skin response which develops after repeated contacts with the cercariae penetrating into the skin. For a long time, it was assumed that the reaction is able to eliminate the majority of parasites which penetrated into the skin. However, the studies on mice experimentally infected with bird schistosomes showed that soon after the penetration, cercariae transform to schistosomula and this stage is able to resist the host immune response. Under certain circumstances, schistosomula are able to escape from the skin and migrate further to target organs (Kouřilová *et al*. 2004a). In mammals, bird schistosomes can survive for several days or weeks but they never mature. Despite of limited life-span in mammals, bird schistosomes can cause various organ disorders.

The studies on bird schistosomes revealed a new species with unusual behavior in compatible as well non-compatible hosts - *Trichobilharzia regenti* (Horák *et al*. 1999). In comparison to majority of bird schistosome species living in the blood system of visceral organs, mature *T. regenti* flukes occur in the definitive host nasal area where they lay eggs. Before worms reach the nasal area, they migrate from the skin through the spinal cord and brain (Horák et al. 1999). Studies on mice models showed that *T. regenti* schistosomula can also evade capture

by immune cells in the skin of mammalian host and they migrate further to the central nervous system (CNS) where they are able to survive for several days (Hrádková and Horák 2002; Kouřilová *et al*. 2004b). Migration of the parasites through the CNS causes severe tissue injuries (Kouřilová *et al*. 2004b). During the organ involvement, the infections can lead to leg paralysis, balance and orientation disorders and even the host death (Horák *et al*. 1999; Kolářová *et al*. 2001). Kouřilová *et al*. (2004b) described histopathological picture of the injuries CNS developing during *T. regenti* infection, however, detailed information on pathogenic impact of the schistosomula on the nervous tissue and the immune response against the migratory schistosomula in mammals (including man) are still missing. Since humans are frequently exposed to these parasites, therefore, intensive studies in immunopathological aspects of the infections caused by the parasites are required.

AIMS OF THE THESIS

The present thesis deals with mammalian infections by bird schistosomes of *Trichobilharzia regenti*. On a mouse model, development of the host humoral and cellular responses against the cercariae and schistosomula in the skin and CNS, respectively, are characterized. The study presents pathological changes in the nervous tissue caused by the migrating schistosomula. Last but not least, antigenic structures from different developmental stadia of the flukes are described.

Particular aims of the thesis:

1) Description of pathologic effect of the schistosomula on the nervous tissue and characterization of the host immune cell involvement in destruction of the parasites in the CNS of experimentally infected non-specific host.
2) Characterization of antibody response against *T. regenti* during primo- and re-infections.
3) Detection of the main parasite antigens recognized by host antibody response.
4) The study on the role of cercarial antigens in stimulation of human basophils degranulation.
5) Localization and description of antigenic substances in *T. regenti* cercariae, schistosomula and adult worms.

RESULTS AND CONCLUSIONS

Experiments presented in the Thesis characterize the immuno-pathophysiological effect of *Trichobilharzia regenti* on mammalian host and contributed to the recent knowledge of non-specific host immune against the parasites. **The main results of the experimental work are as follows.**

Mammalian humoral immune response

In sera of mice (C57BL/6) primarily infected or re-infected (4x) by *T. regenti*, a time course of antibody response was described. Using ELISA, a development of antigen-specific IgM antibody directed mainly against glycoproteins of the cercarial glycocalyx as well as glycoproteins contained in the cercarial E/S products was noted. The detected elevated levels of antigen-specific IgG1 and total IgE serum antibodies in the re-infected mice indicated domination of Th-2 polarized immune response. Preliminary study on analysis of sera from patients with a history of cercarial dermatitis showed elevated levels of anti-cercarial IgG.

Western blot analysis revealed that IgG and IgE antibodies in the sera of both mice and humans strongly and specifically recognized the antigen of 34 kDa. The 34 kDa protein was present in cercarial homogenate as well as cercarial excretory/secretory (E/S) products, and it seems that it may represent a major immunogen responsible for development of Th2-immune response.

Stimulation of purified human basophils with cercarial corpuscular antigens and cercarial E/S products induced dose-dependent basophil degranulation and IL-4 release. Cercarial E/S products were more potent inducers of the IL-4 release than antigens of cercarial homogenate. It seems that *Trichobilharzia* releasing E/S products can induce activation of host basophils, and thus initiate the development of Th-2 response.

The mouse CNS

Investigation on the CNS of immunocompetent (BALB/c) and immunodeficient (SCID) mice invaded by the *T. regenti* showed that the schistosomula migrating through nervous tissue initiated an influx of immune cells, activation of astrocytes and microglial cells and development of inflammatory lesions. Challenge infections induced strong inflammatory

reaction around schistosomula, which resulted in destruction of the parasites. The main role in destruction of schistosomula belonged to macrophages and microglial cells. However, comparison of infections of immunocompetent and immunodeficient mice revealed that elimination of the worms by these cells was more efficient with the contribution of CD3+ lymphocytes.

Immunohistochemistry showed axonal damage around schistosomula and in places of their previous migration; the injuries were likely caused mechanically by the migrating parasites. Presence of schistosomula in the *epineurium* of peripheral nerves as well as in subarachnoid space of the spinal cord and brain led to mild inflammation and, moreover, it did not cause pathological changes in the surrounding nervous tissue. It implied that schistosomula occurring in cavities outside of solid tissue were less susceptible to destruction by the host cellular response.

Using of specific antibody against components of the mouse nervous tissue revealed presence of immunoreactive material in the lumen of the intestine of immature flukes, indicating that the parasites may use the host nervous tissue for nutrition during their migration. Nevertheless, ingestion of the tissue by schistosomula had only a minor pathologic effect on the host CNS.

Immunolocalization of antigenic structures

The studies were performed on *T. regenti* cercariae, schistosomula developed under different conditions (in duck and mouse, and *in vitro*) and adult worms. Immunohistochemical staining showed that antibodies in sera of mice repeatedly infected with *T. regenti* bound to cercarial surface and subtegumental structures of all investigated schistosomula.

Transmission electron microscopy (TEM) observation of immunolabeled sections of cercariae showed a strong antibody reaction with cercarial glycocalyx and less intensive reaction with penetration glands of cercariae. After the cercaria/schistosomulum transformation, surface recognition by mouse antibody decreased and in the adult worms antibody reaction with tegumental surface was weak. In case of all types of schistosomula, positive reaction was detected within spherical bodies originated from the subtegumental cells. These bodies were transported *via* cytoplasmic bridges to the tegumental syncytium, where they probably

released the immunoreactive content. Therefore, the antigenic molecules can be recognized by mouse immune system.

A comparison of various immunolabeled sections of schistosomula produced under different conditions showed a similar pattern of antibody binding. The results seem to indicate, therefore, that schistosomula are able to form tegument of similar composition in the environment of both specific and non-specific hosts.

LIST OF PUBLICATIONS

Original papers

- **LICHTENBERGOVÁ L.**, KOLBEKOVÁ P., KOUŘILOVÁ P., KAŠNÝ M., MIKEŠ L., HAAS H., SCHRAMM G., HORÁK P., KOLÁŘOVÁ L., MOUNTFORD A.P. (2008). Antibody responses induced by *Trichobilharzia regenti* antigens in murine and human hosts exhibiting cercarial dermatitis. *Parasite Immunology* 30, 585-595.
- **LICHTENBERGOVÁ L.**, LASSMANN H., MALCOLM K.J., KOLÁŘOVÁ L., HORÁK P. (2011). *Trichobilharzia regenti*: Host immune response in the pathogenesis of neuroinfection in mice. *Experimental Parasitology*, doi:10.1016/j.exppara. 2011.04.006.
- CHANOVÁ M., **LICHTENBERGOVÁ L.**, BULANTOVÁ J., MIKEŠ L., HORÁK P. (2001). Immunolocalization of antigenic structures of intravertebrate stages of neuropathogenic schistosome *Trichobilharzia regenti*. *Parasitology Research* (submitted manuscript)

- **LICHTENBERGOVÁ L.**, KOLÁŘOVÁ L. (2006). Immune reaction of mammalian host to infection with bird schistosome genus, *Trichobilharzia regenti*. 8-12 May, 14th Helminthological Days, Ředkovec at Světlá nad Sázavou, Czech Republic. *Book of Abstract*: 36-38.

Conferential abstract

- **LICHTENBERGOVÁ L.**, KOLÁŘOVÁ L., KOUŘILOVÁ P. (2004). *Trichobilharzia regenti*– causative agent of histopathological changes of nervous tissue of bird and mammalian hosts. 7-12 May, VI Czech and Slovak Parasitological Days, Ostravice, Czech Republic, *Book of Abstracts*: 46.
- **LICHTENBERGOVÁ L.**, KOLÁŘOVÁ L. (2005). *Trichobilharzia regenti* - a causative agent of histopathologic changes of mammalian nervous tissue. 7-9 April, Leiden International Medical Student Congress, Leiden, The Netherlands. *Book of Abstract*: 153.
- **LICHTENBERGOVÁ L.**, KOLÁŘOVÁ L. (2005). *Trichobilharzia regenti*, a causative agent of histopathologic changes of non-specific host nervous tissue. 9-13 May, 13th Helminthological Days, Ředkovec at Světlá nad Sázavou, Czech Republic. *Helminthologia*, 42, 3: 171–186.
- **LICHTENBERGOVÁ L.**, KOLÁŘOVÁ L., HAAS H., MOUNTFORD A.P. (2006). *Trichobilharzia regenti* cercariae stimulate human basophils to degranulation and IL-4 release. 6-11 August, ICOPA XI, Glasgow, Scotland, UK. *Book of Abstract*
- **LICHTENBERGOVÁ L**. KOLÁŘOVÁ L., MOUNTFORD A.P**.** (2007). Immune response induced by *Trichobilharzia regenti* antigens. 14-18 May, 15th Helminthological Days, Ředkovec at Světlá nad Sázavou, Czech Republic. *Book of Abstract*: 56.
- **LICHTENBERGOVÁ L.**, KAŠNÝ M. AND KOLÁŘOVÁ L. (2007). Cellular and antibody response to *Trichobilharzia regenti* larvae. 9-14 September, 10th International Helminthological Symposium, Stará Lesná, Slovak Republic, *Book of Abstracts*: 47.
- **LICHTENBERGOVÁ L.**, CHANOVÁ M., KOLÁŘOVÁ L., HORÁK P. (2008). Mouse antibody response against *Trichobilharzia regenti* cercariae and schistosomula. 12-16 May, 16th Helminthological Days, Suchá Rudná, Czech Republic. *Book of Abstract*: 80.
- **LICHTENBERGOVÁ L.**, KOUŘILOVÁ P., KAŠNÝ M., MOUNTFORD A.P., KOLÁŘOVÁ L. (2008). Antibody response of mammalian host initiated by *Trichobilharzia regenti* antigens. 19-23 May, VIII Czech and Slovak Parasitological days, Sezimovo Ústí, Czech Republic. *Book of Abstract*: 56.

- **LICHTENBERGOVÁ L.** (2009). Histopathology of CNS caused by *Trichobilharzia regenti* migration in mouse. 11-15 May, 17th Helminthological Days, Vranov nad Dyjí, Czech Republic. *Book of Abstract*: 26.
- **LICHTENBERGOVÁ L.**, HORÁK P., KOLÁŘOVÁ L. (2009). The pathogenic effect of *Trichobilharzia regenti* migration on nervous tissue. 6-10 July, 3rd Workshop on Bird Schistosomes and Cercarial Dermatitis, Rejčkov, Ledeč nad Sázavou, Czech Republic. *Book of Abstract*: 20.
- **LICHTENBERGOVÁ L.**, HORÁK P., KOLÁŘOVÁ L. (2010). Pathogenesis of neuroinfection caused by migration of *Trichobilharzia regenti* schistosomula. 24-28 May, IX Slovak and Czech Parasitological Days, Liptovský Ján, Slovak Republic. *Book of Abstract*
- **LICHTENBERGOVÁ L.**, LASSMANN H., KOLÁŘOVÁ L., HORÁK P. (2010). Pathogenesis of *Trichobilharzia regenti* neuroinfection in mammalian host. 16-20 August, ICOPA XII, Melbourne, Australia. *Book of Abstract*
- **LICHTENBERGOVÁ L.**, CHANOVÁ M., BULANTOVÁ J., HORÁK P. (2011). Immunoreactivity of neuropathogenic schistosome *Trichobilharzia regenti*. 12-14 April, BSP Annual Spring Meeting, Nottingham, United Kingdom. *Book of Abstract*

Invited presentation

- **LICHTENBERGOVÁ L.**, KOLÁŘOVÁ L. (2006). Immune reaction of mammalian host to infection with bird schistosome genus, *Trichobilharzia regenti*. 8-12 May, 14th Helminthological Days, Ředkovec at Světlá nad Sázavou, Czech Republic. *Book of Abstract*: 36-38.
- **LICHTENBERGOVÁ L.**, HORÁK P. (2011). Pathogenicity of bird schistosomes. 9-13 May, 19th Helminthological Days, Kunžak, Czech Republic, *Book of Abstract*: 21.

REFERENCES

Horák P, Dvořák J, Kolářová L, Trefil L. (1999). *Trichobilharzia regenti*, a pathogen of the avian and mammalian central nervous system. *Parasitology* 119:577-581.

Hrádková K, Horák P (2002). Neurotropic behaviour of *Trichobilharzia regenti* in ducks and mice. *Journal of Helminthology* 76:137-141.

Aldhoun JA, Faltýnková A, Karvonen A, Horák P. (2009) Schistosomes in the North: A unique finding from a prosobranch snail using molecular tools. *Parasitology International* 58: 314-317.

Larsen AH, Bresciani J, Buchmann K (2004). Increasing frequency of cercarial dermatitis at higher latitudes. *Acta Parasitologica* 49:217-221.

Horák P, Kolářová L (2011). Snails, waterfowl and cercarial dermatitis. *Freshwater Biology* 56:779-790.

Kouřilová P, Hogg KG, Kolářová L, Mountford AP (2004a). Cercarial dermatitis caused by bird schistosomes comprises both immediate and late phase cutaneous hypersensitivity reactions. *Journal of Immunology* 172: 3766-3774.

Kouřilová P, Syrůček M, Kolářová L (2004b). The severity of mouse pathologies caused by the bird schistosome *Trichobilharzia regenti* in relation to host immune status. *Parasitology Research*. 93:8-16.

Soleng A, Mehl R (2010). Geographical distribution of cercarial dermatitis in Norway. *Journal of Helminthology* 12:1-8

Thors C, Linder E (2001). Swimmer's itch in Sweden. *Helminthologia* 38:244.