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

Ph.D. Thesis

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The present thesis consists of a literature overview and results presented in the form of original papers published in peer-reviewed journals.

The research work was performed at the Department of Parasitology, Faculty of Science, Charles University in Prague, Czech Republic; at the Division of Cellular Allergology, Research Center Borstel, Germany; at the Department of Neuroimmunology, Center for Brain Research, Medical University of Vienna, Austria.

I declare, that the present thesis summarizes the results of experimental work done by my own or in collaboration with co-authors of the presented original papers. All the other data cited from literature are referred to in list of references.

I declare, that handed in printed version of the thesis is identical to the version electronically loaded in Student Information System (SIS 3. LF UK).

Prague, 2011 Lucie Lichtenbergová

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1. INTRODUCTION

Unlike human schistosomes (genus *Schistosoma*) that occur in tropical and subtropical areas, 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 and Mehl 2010). It was generally accepted that human infections caused by bird schistosomes do not result in severe health disorders and, therefore, they attracted less attention comparing with infections by *Schistosoma* spp. In the past, human infections by avian schistosomes were mostly associated with skin symptoms, however, recent results suggest that also other health disorders might occur (Horák and Kolářová 2011).

Although, bird schistosomes can mature only in the specific bird host, their infective larval stage (cercaria) posses an ability to invade skin of mammals, including humans. In humans, repeated exposures to cercariae lead to development of skin inflammatory reaction called cercarial dermatitis or "swimmer's itch" which is a disease considered to emerge all over the world (Horák and Kolářová 2001; Horák and Kolářová 2005; Horák and Kolářová 2011).

The most intensively studied genus *Trichobilharzia* includes parasites, adults of which inhabit either visceral or nasal areas of their bird hosts (Blair and Ottesen 1979). The present thesis focuses on species *Trichobilharzia regenti*. In comparison with other species of the genus, *T. regenti* exhibits an unusual mode of behavior within the definitive host (Horák *et al*. 1999). After penetration into the skin, schistosomula are able to invade peripheral nerves and continue *via* the central nervous system (CNS) to the nasal cavity of the birds. Study on experimentally infected mice revealed that schistosomula are able to migrate also into the CNS of non-specific mammalian host. During CNS involvement, the infections of both specific and non-specific hosts can result in leg paralysis, balance and orientation disorders and even death (Horák *et al*. 1999; Kolářová *et al*. 2001).

Objective of the introduction is to summarize knowledge about immuno-pathogenic effect of bird schistosome *Trichobilharzia regenti*. The first two parts deal with the life cycle of bird schistosomes and the process of skin penetration of the definitive host by schistosome larvae (cercariae). Further, interactions of mammalian immune system and bird schistosomes in comparison with human schistosomes of the genus *Schistosoma* are discussed. Pathogenic effect of helmith neuroinfections with emphasis on *T. regenti* schistosomula is characterized in section 1.6. The last part shortly outlines the potential of schistosomes in modulation of immune response to prevent autoimmune diseases development.

1.1. Life cycle

In the life cycle of bird schistosomes of the genus *Trichobilharzia*, water snails and waterfowl are employed as intermediate and definitive hosts, respectively. The development in snails ends by production of free swimming cercariae; in definitive hosts the parasites mature and lay eggs. The eggs of visceral *Trichobilharzia* species are deposited in capillaries of the target organs, then pass through tissue to lumen of the intestine and, subsequently, leave the host with feces; when they come into water, miracidia hatch from the eggs and actively search for intermediate hosts (snails) (Horák *et al*. 2002). In case of the nasal species *T. regenti*, the eggs are deposited in the nasal mucosa, where they mature and miracidia hatch directly within the host tissue (i.e. without water-contact stimulus) (Horák *et al*. 1998).

Miracidia of the genus *Trichobilharzia* usually have a narrow specificity towards the intermediate hosts in which miracidium penetrates and multiplies *via* two generations of sporocysts to thousands fork-tailed infective larvae (cercariae). Leaving the snail, cercariae start to search for the skin of vertebrate hosts; they swim with positive phototactic and geonegative orientation, and react sensitively to physical and chemical stimuli, e.g. shadow, water turbulence, warmth and duck-foot skin lipids - cholesterol and ceramides (Feiler and Haas 1988a; Feiler and Haas 1988b). After initial contact with the hosts, the penetration of *T. ocellata* cercariae into the host skin is mainly stimulated by fatty acids (Haas and van de Roemer 1998).

In the skin of definitive host (birds), schistosomula move through the skin layers towards their definitive location and, therefore, require again an information for their orientation. The studies on visceral schistosomes showed that schistosomula use negative photo-orientation to move away from the light source towards darker locations (Grabe and Haas 2004a). The other stimulus involved in navigation of visceral schistosomula is represented by concentration gradient of chemicals, such as D-glucose and L-arginine (Grabe and Haas 2004b). Unfortunately, data about the orientation of nasal species *T. regenti* are not complete and published at present.

After escaping the skin, further development in a host body depends on the biology of particular schistosome species. Parasites find specific routes for migration through the host body in order to reach the site of their final localization. Schistosomula of visceral *Trichobilharzia* species enter the blood vessels and continue their migration into the lungs of avian definitive host (Bourns *et al*. 1973) as well as experimental mammalian hosts (Olivier

1953; Haas and Pietsch 1991). The worms persist several days in the host lungs, and then they migrate *via* blood vessels to the place of final location in the intestinal-hepatic area. Frequently in the veins and tissue of the gut, parasites develop to adult worms and lay eggs (Bourns *et al.* 1973). The eggs are released from the host intestine with feces (for a review see Horák *et al*. 2002). The passage of schistosomula through the host body may cause tissue injury and trigger inflammatory reaction in different tissues, and the eggs represent pathogenic agent causing enteritis of the definitive hosts (van Bolhuis *et al*. 2004).

The small group of nasal schistosomes is represented by species, adult worms of which occur in the nasal tissue of their definitive host. Location in the nasal area has been documented for nine species till present; one mammalian species represented by *Schistosoma nasale* and eight species of the genus *Trichobilharzia* – *T. nasicola*, *T. rodhaini*, *T. spinulata*, *T. aureliani*, *T. duboisi*, *T. australis*, *T. arcuata*, *T. regenti* (Horák *et al*. 1998). The life cycle of nasal *Trichobilharzia* species has not been completely described yet, except for *T. regenti*. Transformed schistosomula of *T. regenti* seek after the peripheral nerves, and migrate through/along them into the spinal cord. Then the parasites continue migration through the brain to the nasal area of the definitive host, where they mature and lay eggs (Horák *et al*. 1999; Hrádková and Horák 2002). Penetrating cercariae and migrating schistosomula represent important pathogenic agents causing skin inflammatory reaction, and injury and immune reactions in the host CNS, respectively (Kolářová *et al*. 2001; Hrádková and Horák, 2002; Kouřilová *et al*. 2004a; Kouřilová *et al*. 2004b). Similarly to other schistosomes, the eggs of *T. regenti* trigger inflammatory reaction in adjacent tissue in the nasal area (Chanová and Horák 2007).

Except for compatible hosts (waterfowl), bird schistosomes are able to infect non-compatible mammalian hosts. However, the parasites never mature and complete their development in mammals and they die at some point after the infection.

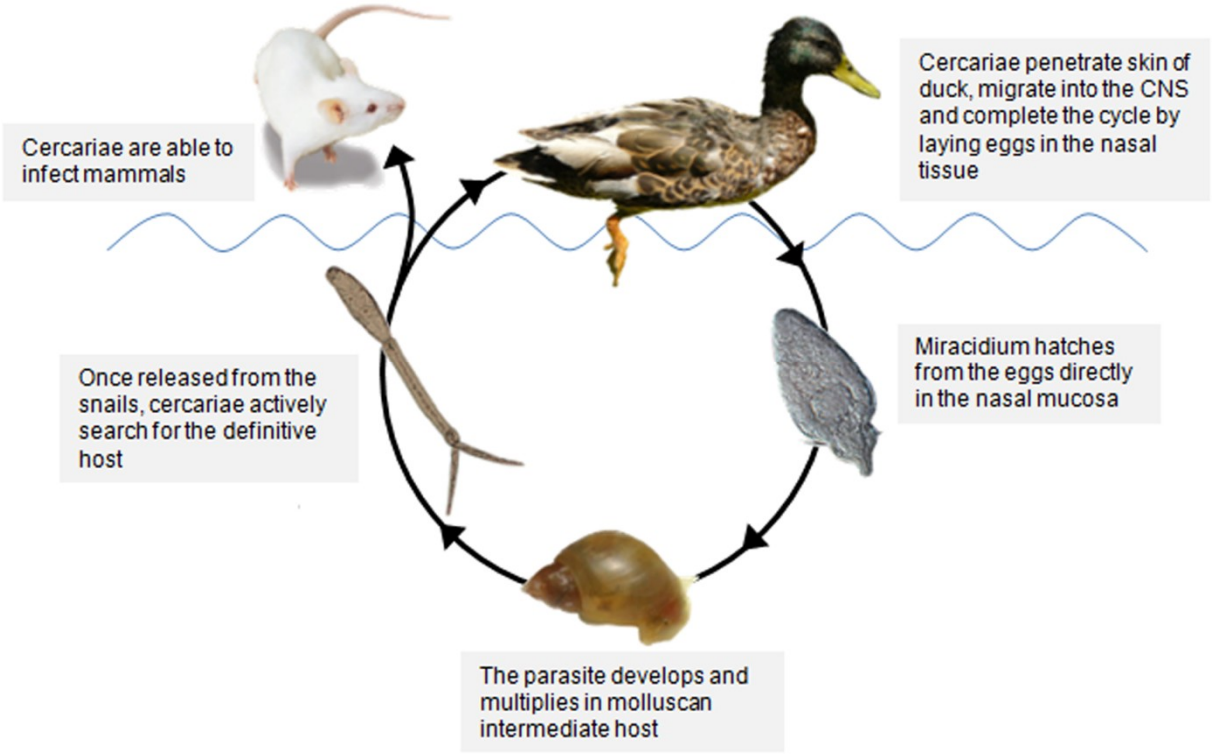

Fig.1. Life cycle of *T. regenti*

1.2. Invasion of the host skin

Cercariae of bird schistosomes actively find and penetrate skin of their definitive hosts. However, they are also able to invade skin of mammals, including human skin. After being released from the snail intermediate hosts, bird schistosome cercariae tend to cling to the water surface in a resting posture. They react sensitively to minor shadow stimuli and start to swim with negative phototactic orientation from the water surface toward the definitive host (Feiler and Haas 1988a).

After attachment to duck or human skin, cercariae perform leech-like creeping behavior in order to find out a suitable penetration site (Haas and van de Roemer 1998). In contrast to human schistosomes of *S. mansoni* cercariae of which penetrate into smooth skin areas, bird schistosome cercariae prefer wrinkles and hair follicles as suitable entry sites (Haas and Haeberlein 2009). Study on *S. mansoni* and *S. japonicum* revealed differences in speed of their migration through the mammalian skin (Wang *et al*. 2005). Migration from the epidermis into the dermis was significantly quicker in *S. japonicum* than *S. mansoni*, and *S. japonicum* did not stay in the skin of experimental mice longer than a day (He *et al*. 2002;

Wang *et al*. 2005). Similarly, *T. szidati* cercariae invade the human skin with a higher efficiency than *S. mansoni*. They are able to find the entry sites much faster than cercariae of *S. mansoni* and also their penetration through the skin is more rapid (Haas and Haeberlein 2009).

Cercarial penetration of the skin is stimulated by fatty acids (Haas and van de Roemer 1998). According to study of Haas and Haeberlein (2009), *T. szidati* cercariae respond to linolenic acid more sensitively if compared with *S. mansoni*. This feature seems to represent an adaptation to invade the duck skin with a lower content of free fatty acids than human skin (Haas and Haeberlein 2009). Therefore, human skin rich in surface lipids is likely more attractive for bird schistosome cercariae than duck-foot skin (Haas and van de Roemer 1998).

Penetration through the skin is supported and facilitated by a number of proteolytic enzymes released from cercarial circum- and post-acetabular glands immediately after attaching the host skin. In case of bird schistosomes, secretion of the glands is stimulated mainly by fatty acids and also by ceramides and cholesterol (Haas and Haeberlein 2009). These secretions account for about one-third of the cercarial body mass (Harrop and Wilson 1993) and contain many of potentially antigenic proteins. The most important penetration enzyme of *S. mansoni* is probably serine protease, elastase (Salter *et al*. 2000). Nevertheless, similarly as for *S. japonicum* Mikeš *et al*. (2005), Kašný *et al*. (2007) and Dvořák *et al*. (2008) did not find any elastase activity in the secretions of *T. szidati* and *T. regenti* cercariae. However, cathepsin B-like activity was detected in the three species mentioned above. This enzyme from cercarial penetration glands is considered to be the main component in penetration process (Mikeš *et al*. 2005; Kašný *et al*. 2007; Dvořák *et al*. 2008). The same type of enzymes could be the reason for similar speed of *S. japonicum* and *Trichobilharzia* cercariae (Haas and Haeberlein 2009). Cathepsin B identified in extracts of *T. regenti* schistosomula is believed to facilitate migration of the parasite through the nervous tissue due to its ability to degrade myelin basic protein (Dvořák *et al*. 2005).

Fatty acids seem to stimulate not only penetration into the host skin, but also transformation of the tegument as a part of parasite immune evasion (Haas and van de Roemer 1998). Penetration of larvae into the host skin is accompanied by cercaria/schistosomulum transformation with reconstruction of the tegumental surface. Transformation starts with the loss of tail, a process supported by a sphincter muscle in the cercarial hindbody (Haas and van de Roemer 1998), then cercariae shed glycocalyx and start to form a surface double

membrane. Creation of a new surface is accompanied by disappearance of lectin and antibody targets on the surface of schistosomula (Horák *et al*. 1998).

1.3. Cercarial dermatitis

In humans, the skin infection leads to development of inflammatory reaction known as swimmer's itch or cercarial dermatitis. Cercarial dermatitis can occur in any location all over the world where people come in contact with water bodies containing snails infected by bird schistosomes (Brant and Loker 2009). During the past several years the infections with bird schistosomes have been documented in new regions, e.g. in the southwest of the United States (Brant and Loker 2009), central Chile (Valdovinos and Balboa 2008) and the United Kingdom (Fraser *et al*. 2009). Cercarial dermatitis is regarded to be a re-emerging infection (Horák a Kolářová 2011), nevertheless, the incidence of cercarial dermatitis is still unknown. In Iceland, cercarial dermatitis has been reported several times since the first confirmation in 1997 (Skírnisson *et al*., 2009). Schets *et al*. (2008) reported about cases of presumptive cercarial dermatitis in freshwater lakes in the Netherlands that occurred each summer from 2000 to 2008 (Schets and de Roda Husman 2005, Schets *et al*. 2008). Also in France (Vuong *et al*. 2002) and Switzerland (Chamon *et al*. 1998) cercarial dermatitis seems to occur more frequently during the last years. Caumes *et al*. (2003) reported about many cases of cercarial dermatitis developed after swimming in Lake Annecy in France and Fraser *et al*. (2009) brought short report about cercarial dermatitis which manifested after bathing in a freshwater loch in eastern Scotland. The northernmost cases of cercarial dermatitis in Europe originate from Norway, where an increasing number of cases has been recorded since the first report in 1980 (Soleng and Mehl 2010).

Risk of the onset of cercarial dermatitis increases with the number of water exposures. Higher incidence of the infection is connected with bathing in shallow water which is a typical habitat for water snails and, therefore, a place where cercariae tend to accumulate (Verbrugge *et al*. 2004). Penetration of cercariae into the skin may lead to immediate prickling sensation that lasts for about 1 hour (Chamot *et al*. 1998). Severity and intensity of cercarial dermatitis depends on various factors including the number and duration of exposures to the cercariae, and host immune status, i.e., history of cercarial dermatitis, and individual susceptibility to the infection by the cercariae (Chamot *et al*. 1998). After primary infection, the skin reaction is inapparent or mild with small and transient macules or

maculopapules which develop after 5-14 days (Chamot *et al*. 1998). The main phase of the disease occurs after repeated infections and results in a strong inflammatory reaction against the parasites (Augustine and Weller 1949) when the skin disease manifests by maculo-papulo-vesicular eruptions accompanied by intensive itching and, occasionally, by erythema, fever, local lymph node swelling, oedema; massive infections may also cause nausea and diarrhea (for a review see Horák *et al*. 2002). Skin lesions always develop only on parts of the body that were in contact with cercariae, including the parts under swimming suits (Kolářová *et al*. 1989).

Diagnosis of cercarial dermatitis is based on anamnestic data and clinical findings (Meinking *et al*. 2003). Some work has been done using serological tests for confirmation of the diagnosis (Bechtold *et al*. 1997). Nevertheless, the immunological tests are not routinely available and laboratory confirmation of causative agents of the dermatitis remains difficult.

1.4. Host immune response

Clinical pattern of cercarial dermatitis is linked with histopathological reactions to the infection. Ramaswamy *et al*. (1997) observed that inflammation is one of the earliest signs of the host response against human schistosomes in the skin of immunized animals. Histopathological study showed a low number of infiltrating neutrophils and mononuclear cells in the skin of naive mice infected with *S. mansoni*. In contrast, more severe cellular reaction was observed in the mouse skin after repeated infections with *S. mansoni* (Ramaswamy *et al*. 1997). Similarly as for human schistosomes, infections of mice with the bird schistosomes *T. szidati* (*T. ocellata*) and *T. regenti* initiate development of skin immune reaction (Bahgat *et al*. 2001; Kouřilová *et al*. 2004a). Moreover, repeated infections with bird schistosomes have less harmful impact on hosts, whereas pathogenic effect of repeated infection with human schistosomes often leads to dead of experimental animals. Therefore, bird schistosomes represent useful model which enable study of the skin immune response against repeated infections (Mountford and Trottein 2004).

Primary mouse infections with *T. regenti* lead to an acute inflammatory reaction with oedema, vasodilatation and tissue infiltration by neutrophils, macrophages, mast cells, MHC II antigen presenting cells (APCs) and a weak infiltration by $CD4^+$ lymphocytes; repeated infections cause substantially elevated infiltration of all cells mentioned above (Kouřilová *et al*. 2004a; Kouřilová *et al*. 2004b). Immunohistochemical staining showed that, within the first 48 h p.i.,

proliferation of CD4+ lymphocytes and macrophages was restricted only on the site of penetration of *T. regenti* cercariae into the mouse skin; 8 d.p.i. it was possible to detect increased tissue infiltration by CD4+ lymphocytes and macrophages around the penetration site (L. Lichtenbergová, unpublished).

Repeated infections of mice led to massive infiltration of the skin by CD4+ lymphocytes and macrophages. 6 h post the last infection, an increased proliferation of both types of the cells was detected mainly around the penetration site of *T. regenti* cercariae, whereas within the next days the entire tissue area was infiltrated by a high number of these cells (L. Lichtenbergová, unpublished) (Fig.2, 3). $CD4^+$ cells, number of which significantly increases in the skin after challenge infections, are potential source of IL-4 associated with Th2 response (Kouřilová *et al*. 2004a). Skin immune response to the infection lead to capture and elimination of the majority of larvae in the skin. Decline in the number of parasites able to escape from the skin and migrate further to the CNS is much faster in repeatedly infected animals (Kouřilová *et al*., 2004a). Massive influx of the cells to the area around parasites potentially inhibits their movement through the skin, and thereby delays their migration (Kumar and Ramaswamy, 1999).

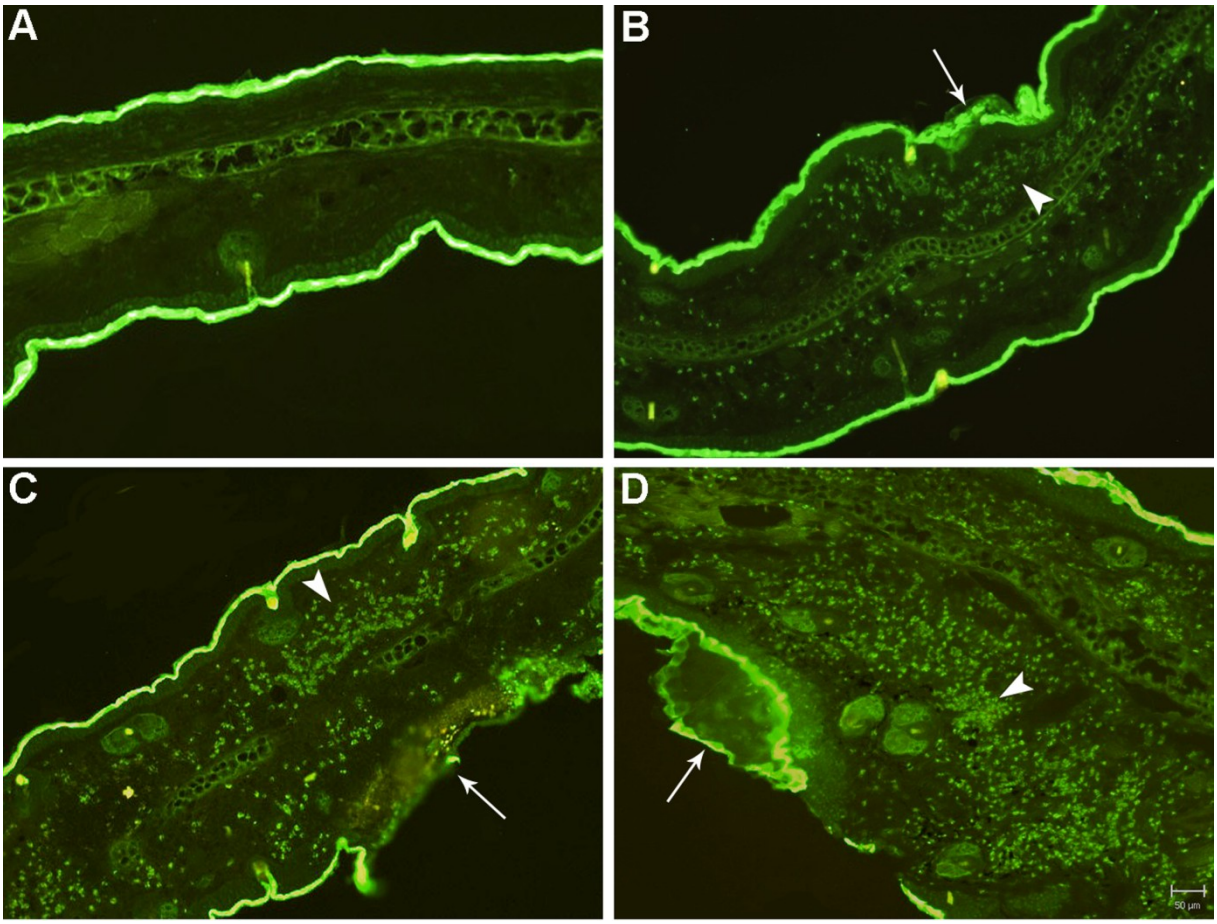

Fig. 2. CD4+ lymphocyte infiltrates in the skin of mice experimentally infected with *T. regenti*. Cercariae invaded the skin; they initiated influx of CD4+ lymphocytes (arrowhead) and development of lesion in the site of penetration. Mouse skin: **A** – skin tissue of non-infected mouse; skin tissue of re-infected mouse: **B** – 6h after the 4thinfection; **C** –24h after the 4th infection; **D** – 4 days after the 4th infection. Arrows indicate the site of penetration of cercariae. In all cases, unspecific reaction with the skin surface was detected. Rat anti-mouse CD4+, purified antibody (AbD Serotec), Anti-rat IgG, FITC conjugated antibody (Abcam). Scale bar = 50 μm.

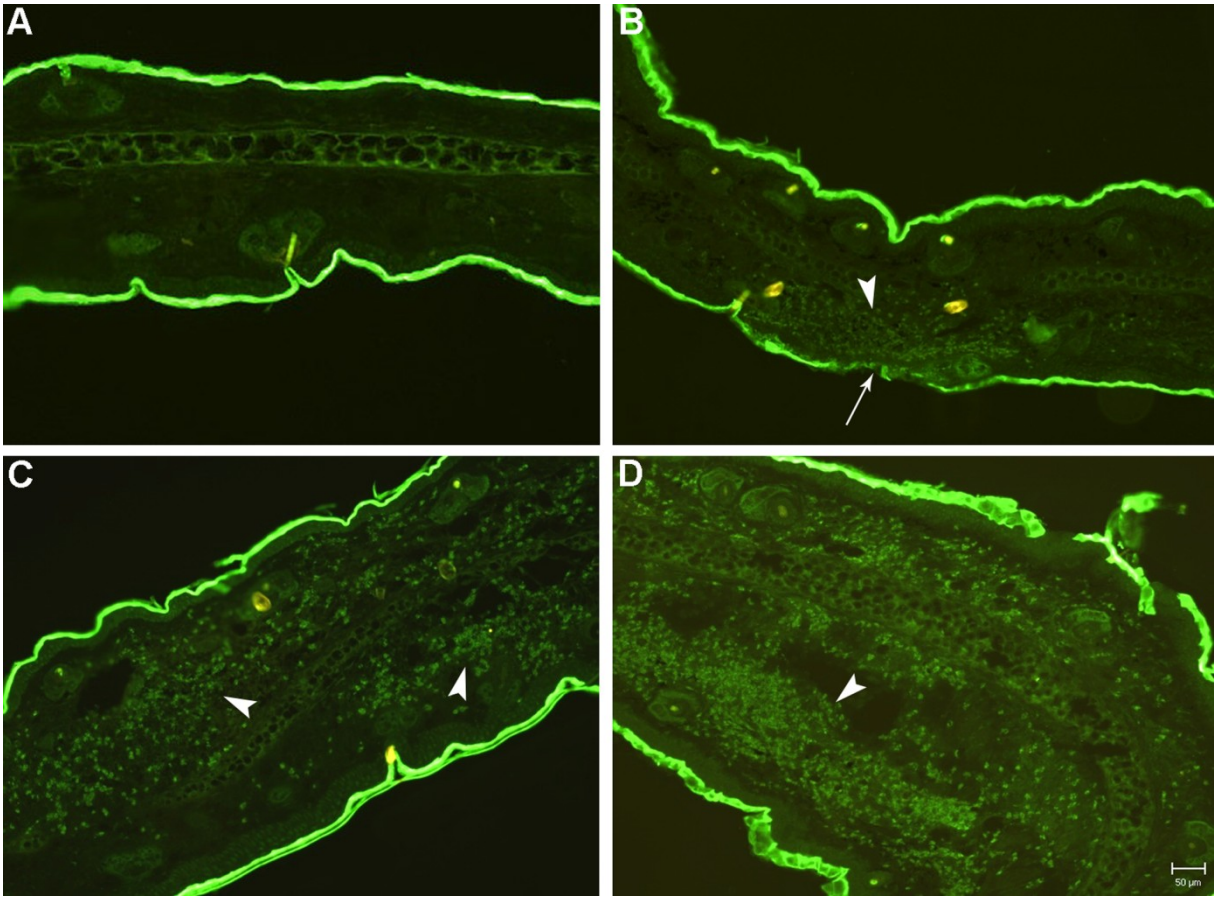

Fig. 3. Accumulation of macrophages in the skin of *T. regenti* re-infected mice. Penetration of cercariae led to macrophage infiltration (arrowhead) of the mouse skin: **A** – skin tissue of non-infected mouse; skin tissue of re-infected mouse: **B** – 6h after the 4th infection; **C** – 24h after the 4th infection; **D** – 4 days after the 4th infection. Arrows indicate the site of penetration of cercariae. In all cases, unspecific reaction with the skin surface was detected. Rat anti-mouse F4/80, purified antibody (Biolegend), Anti-rat IgG, FITC conjugated antibody (Abcam). Scale bar = 50 µm.

Entry of inflammatory cells (lymphocytes and neutrophils) from systemic circulation into the skin is facilitated by cytokine-induced expression of adhesion molecules, especially intercellular adhesion molecule-1 (ICAM-1), on the surface of endothelial cells (Steeber and Tedder 2000). Binding and subsequent migration through the endothelium is mediated predominantly by the LFA-1/ICAM-1 interactions (Steeber and Tedder 2000). Ramaswamy *et al*. (1997) detected an increased expression of ICAM-1 in the dermis and hypodermis of immune mice (repeatedly infected or vaccinated by *S. mansoni*). The highest expression of ICAM-1 was observed on cutaneous cells around *S. mansoni* schistosomula, where extensive cellular reaction occurred. Expression of ICAM-1 was also detected on epidermal cells around the migratory trails of schistosomula, and endothelial cells lining the capillaries in the dermis and hypodermis of immune mice (Ramaswamy *et al*. 1997). In contrast, ICAM-1 expression

was low or absent on cutaneous cells adjacent to *S. mansoni* schistosomula in the skin of naive mice (Ramaswamy *et al*. 1997). It is possible that the low or none ICAM-1 expression in the skin of naive mice may contribute to the diminished cutaneous inflammatory reaction against the parasite (Ramaswamy *et al*. 1997).

Trichobilharzia regenti primo-infection leads to the development of inflammatory reaction in the murine skin within 1-6 h after exposure (Kouřilová *et al*. 2004a). The inflammation is accompanied by a transient release of acute phase cytokines (IL-1β and IL-6) and steadily increasing amounts of IL-12 which correlates with higher Th1-associated IFN-γ production by cells from skin-draining lymph nodes (Kouřilová *et al*. 2004a). INF-γ and IL-12 promote Th1 cell differentiation, whereas IL-6 is cytokine associated with Th2 polarization (Brunet *et al*. 1998) and it is detected as one of the first cytokines after *T. regenti* infection (Kouřilová *et al*. 2004a). Similarly, the study of Hogg *et al*. (2003) on *S. mansoni* illustrates rapid host immune response to parasite penetration with production of acute–phase cytokines, such as IL-1β and IL-6. In the early phase of re-infection with *T. regenti* cercariae, infiltration of mouse skin with inflammatory cells was also accompanied by oedema caused by local vascular permeability that was initiated by histamine produced by activated mast cells and basophils (Kouřilová *et al*. 2004a). Degranulation of mast cells and basophils with release of histamine and IL-4 is realized after the binding of IgE-antigen complex *via* high affinity receptors FcεRI on the cell surface (Falcone *et al*. 2000; Kawakami and Galli 2002). Histamine has been previously described as a potent effector of both Th1 and Th2 responses as well as immunoglobulin synthesis (Banu and Watanabe 1999; Jutel *et al*. 2001). Repeated infections evoke dominant production of Th2-type cytokines, and the first and most abundant cytokine detected after repeated infections in the skin is IL-6, which can initiate Th2-type polarization *via* induction of IL-4 (Rincón *et al*. 1997). Within 1 hour after the penetration of *T. regenti* cercariae a massive upregulation of IL-4 and IL-10 can be observed, and the level of these cytokines declines after 48 h. This upregulation during *T. regenti* infection is accompanied by release of histamine and proliferation of mast cells (Kouřilová *et al*. 2004a). IL-4 plays also a crucial role in the development of Th2-type immune responses to *S. mansoni* antigens and regulation of immunoglobulin isotype switching to IgE (Falcone *et al*. 1996).

Helminths are considered to be the most effective and reliable inducers of IgE and Th2 responses, although this ability can be restricted to a specific developmental stage of the parasitic worms (Bell 1996, Pritchard *et al*. 1997). For example, implantation of different developmental stages of the nematode *Brugia malayi* into mice showed that the presence of

adult worms induced IL-4 production, whereas microfilariae promoted production of IFN-γ (Lawrence *et al*. 1994). Similarly, infections of mice with *S. mansoni* lead to a development of Th2 response elicited by the eggs, whereas schistosomulum stage induces a Th1-like response (Falcone *et al*. 1996). It is of interest that cercariae of the bird schistosome *T. regenti* induce a mixed Th1/Th2 response in mice after primary infection, but after repeated infections cercarial penetration into the skin leads to the development of Th2 polarized immune response which is a probable cause of parasite elimination (Kouřilová *et al*. 2004a). A possible explanation can be found in the ability of E/S products of both human and bird schistosome cercariae to induce mast cell and basophil degranulation, and production of IL-4 (Machado *et al*. 1996; Kouřilová *et al*. 2004a; Lichtenbergová *et al*. 2008).

Domination of Th-2 polarization of the immune response after repeated *T. regenti* infections was confirmed by detection of antigen-specific antibody levels. Increase of Th2-associated antigen-specific IgG1 and IgE antibodies was demonstrated in sera of mice repeatedly infected with *T. regenti* (Lichtenbergová *et al*. 2008). Similarly as in the study of Kouřilová *et al*. (2004a), the elevated levels of antigen-specific IgG1 and IgG2b antibodies after the *T. regenti* primary infection indicated development of a mixed Th1/Th2 antibody response, whereas repeated infections induced increase of IgG1 antibody level, and the level of Th1-associated IgG2b antibody declined (Lichtenbergová *et al*. 2008).

During *T. regenti* primary infection, IgM response against glycan structures of cercariae and their E/S products was observed. This indicates that the early antibody response is directed against the components of highly antigenic cercarial glycocalyx as well as against glycoproteins contained in excretory/secretory (E/S) products of the circum- and post-acetabular glands of cercariae (Lichtenbergová *et al*. 2008). It seems that helminth glycans generally drive anti-inflammatory responses and promote Th2-type responses (Thomas and Harn 2003).

Examination of the mouse skin biopsies showed an elevated histamine production detected within 1 h after *T. regenti* re-infection which correlated with an increase in number of degranulating mast cells and elevated levels of serum IgE in re-infected mice (Kouřilová *et al*. 2004a). Mast cells together with basophils are the major source of granule-stored histamine (Jutel *et al*. 2002) and are considered to be important effector cells in Th2-associated allergic reactions (Kawakami and Galli 2002) as well as in immune responses associated with Th2 cells and IgE production during helminth infections (Bell 1996). Both cells can be rapidly recruited to the sites of infection and draining lymph nodes where they produce IL-4 and/or

IL-13 (Cadman and Lawrence 2010). Re-infection with *T. regenti* was also accompanied by increase in IL-4 released from mast cells in the mouse skin. It is suggested that the production of histamine and IL-4 immediately after the last infection of re-infected mice was realized *via* IgE-dependent mast cell degranulation (Kouřilová *et al*. 2004a).

Like mast cells, basophils possess high-affinity IgE surface receptors FcεRI that, after antigen-specific cross-link, induce production and release of mediators such as histamine and IL-4 (Turner and Kinet 1999). In contrast to mast cells and eosinophils, basophils are not found in healthy tissues, but their level in the tissue rapidly increases during inflammatory reaction (Falcone *et al*. 2001). Basophil migration into the sites of inflammation is facilitated by several resident cells which release factors initiating adhesion to the microvascular endothelium, transendothelial migration and finally locomotion through the tissues (Gibbs 2005). Basophils may play a crucial role in modulation of the immune response to helminth infections associated with elevated levels of IgE due to their ability to rapidly produce and release cytokines (IL-4 and IL-13) involved in Th2 responses (Falcone *et al*. 2000; Gibbs *et al*. 2000). In mice infected by *Nippostrongylus brasiliensis* or human filarial infections basophils have been identified as the main source of IL-4 production (Min *et al*. 2004; Mitre *et al*. 2004). Stimulation of basophils from uninfected (parasite naive) individuals with *S. mansoni* egg antigens (Falcone *et al*. 1996; Haisch *et al*. 2001) and extracts from metacestodes of *Echinococcus multilocularis* (Aumüller *et al*. 2004) led to basophil degranulation and secretion of histamine, IL-4 and IL-13. Similarly, stimulation of basophils from healthy (non-sensitized) humans by homogenate of cercariae and excretory/secretory (E/S) products of *T. regenti* cercariae revealed that these antigens induce basophil degranulation and release of IL-4 (Lichtenbergová *et al*. 2008). The antigens stimulated basophil release of IL-4 in a dose-dependent manner, and antigens from E/S products were more potent inducers of IL-4 release than cercarial homogenate (Lichtenbergová *et al*. 2008). Elevated levels of skin mast cells (Kouřilová *et al*. 2004a) and high titres of IgE in *T. regenti* repeatedly infected mice (Lichtenbergová *et al*. 2008) showed that the cells of mast cell/basophil lineage play an important role in the development of Th2 responses during *Trichobilharzia* infections.

1.5. Mechanisms of schistosome immune response evasion

1.5.1. Schistosomulum transformation

After penetration into the host skin, cercariae transform to schistosomula by getting rid of tails, releasing E/S products and rebuilding their surface (Samuelson and Caulfield 1985). E/S products of human as well as bird schistosome cercariae, mainly the products released by the transforming larvae, are rich in components of the glycocalyx and secretions of the circum- and post-acetabular glands (Samuelson and Caulfield 1982; Mikeš *et al*. 2005). Although substantial part of the glycocalyx is removed by cercariae during their penetration (Samuelson and Caulfield 1982), some of the glycocalyx components remain on the surface of schistosomula for some time after the transformation (Samuelson and Caulfield 1982; Wang *et al*. 2005). Loss of residual glycocalyx is accompanied by decrease in antibody binding and complement fixation (Samuelson *et al*. 1980). Glycocalyx represents the major moiety on cercarial surface, and is likely the major component responsible for complement activation (Samuelson and Caulfield 1986). Proteases secreted by transforming schistosomula of *S. mansoni* play a role in shedding of the glycocalyx, and also are able to cleave C3b component of the human complement system. Cleavage of C3b molecules bound on schistosomulum surface prevents activation of the complement system and opsonization of the parasite (Marikovsky *et al*. 1988). Therefore, shedding of the glycocalyx might represent an important evasion strategy (Abath and Werhauser 1996).

Tegument of schistosomes is a dynamic host-interactive layer involved in nutrition, immune evasion and modulation, excretion, osmoregulation, sensory reception and signal transduction (Jones *et al*. 2004). Tegument is represented by a syncytium of fused cells surrounding the entire worm (van Hellemond *et al*. 2006). Electron micrograph analysis of the tegument transformation revealed large quantities of vacuoles which rapidly appear in the tegument during the first hours of cercarial transformation (Hockley and McLaren 1973). These vacuoles had a double bilayer membrane and contained additional membraneous content. Many of the vacuoles fused with the outer membrane of the tegument and their membraneous content became spread out over the surface of the worm. Therefore, it seems that these membraneous vacuoles are involved in formation of the double bilayer of the outer tegumental membrane (Hockley and McLaren 1973). Second type of membraneous bodies, elongate or discoid bodies, was observed in large numbers in the tegument, and these discoid bodies appeared 3 h following schistosome transformation when the double-bilayer

membrane has been formed (Hockley and McLaren 1973). Localization of glucose transport protein SGTP4 in tegumental multilamellar bodies and discoid bodies as well as the surface lipid bilayers suggest that these bodies are involved in the biogenesis of the tegumental surface (Jiang *et al*. 1996). Hockley (1973) observed elongated bodies and membraneous vesicles also in the tegument of the adult male of *S. mansoni*. Electron microscopy examination of the adult tegument showed presence of elongated bodies (100 to 150 nm in length, 20 to 30 nm in width) in the area close to the tegumental outer membrane (Gobert *et al*. 2003). Within the tegumental matrix, large spherical bodies (150-200 nm in diameter) were observed. Further, small spherical vesicles (75 to 150 nm in diameter) closely associated with the base of infolding of the surface membrane were detected (Gobert *et al*. 2003). Both bodies are probably synthesized in Golgi region of the subtegumental cells and continuously transported to the tegument by cytoplasmic channels (bridges) (Wilson and Barnes 1974).

Two lipid bilayers form the surface membrane with many pits and invaginations that enlarge the surface of schistosomes (Gobert *et al*. 2003) and may facilitate nutritional uptake or prevent host immune response by internalizing antibodies (Skelly and Wilson 2006). Dynamic changes of the tegument may help the parasite to avoid immune-mediated damage by reducing antigenicity of the worm outer surface (Skelly and Wilson 2006). Continual shedding of the surface, particularly of the damaged areas, could lead to removal of immune complexes formed on the external membrane of schistosomes (Abath and Werhauser 1996). This syncytial tegument is a successful adaptation to parasitism that enables parasite survival in the hostile internal environment of their hosts (Mulvenna *et al*. 2010).

1.5.2. Other evasion mechanisms

Parasites of the family Schistosomatidae possess several successful adaptations for survival in the hostile internal environment of their hosts. Generally, parasites use mainly these mechanisms of immune evasion: 1) modification of the host immune response by parasite molecules or by changes of host effector cell regulation, 2) antigenic variation - fast changes of antigen expression in order to avoid any efficient host immune response, or antigen mimicry - expression of surface epitopes identical or similar to host molecules, 3) acquisition of host products onto the tegument to mask its foreign origin (Salzet *et al*. 2000; Loukas et al 2001). Characterization and description of the escape mechanisms is not the aim of the thesis, but these mechanisms are closely related to the development of the host immune response and, therefore, some of these evasion strategies are described below.

During the penetration into the host, cercariae release (except for penetration enzymes) many molecules serving for different purposes; one of these molecules is a phospholipid identified as lysophosphatidylserine (Kusel *et al*. 2007) which stimulates innate immune responses in peripheral blood mononuclear cells of naive hosts, and leads to maturation of dendritic cells by interaction with Toll-like receptor 2 (Van der Kleij *et al*. 2002). Fully matured dendritic cells are capable to induce Th2 response and production of IL-10 (Van der Kleij *et al*. 2002). In addition, lysophosphatidylserine can also act as a detergent and thus, it may probably react with membranes of the host effector cells and stimulate their lysis; and thus it may play a role in immune evasion (Kusel *et al*. 2007).

Other molecules employed in stimulation of IL-10 production are eicosanoids. Eicosanoids are synthesized from arachidonic acid that may be derived from lipids of the host skin (Fusco *et al*. 1986). Eicosanoids are not only produced by cercariae; prostaglandin E_2 (PGE_2) can be synthesized from free fatty acids by skin schistosomula of *S.mansoni* (Salafsky and Fusco 1987). Moreover, secretory products of *S. mansoni* schistosomula contain a factor that can potentially induce PGE_2 production in human keratinocytes (Ramaswamy *et al*. 2000). Schistosomes may use eicosanoids as immunomodulators of the host immune response (Salafsky and Fusco 1987). Cercariae of *T. ocellata* and *S. mansoni* secrete similar types and amounts of eicosanoids produced *via* arachidonic acid, and the eicosanoids from both parasites have similar inhibitory effect on superoxide production by human neutrophils (Nevhutalu *et al*. 1993). PGE_2 is known as a potent stimulator of IL-10 from keratinocytes (Enk and Katz 1992), macrophages, dendritic cells or even B1 lymphocytes (Jenkins *et al*. 2005). Also CD4+ cells being present in a high number after multiple exposures to *T. regenti* (Kouřilová *et al*. 2004a) or *S. mansoni* (Jenkins *et al*. 2005) represent likely source of IL-10 in the skin (Jenkins *et al.* 2005). IL-10 has wide-ranging regulatory effects upon antigen presentation, co-stimulation and the development of acquired T-cell responses (Moor *et al*. 2001), and, therefore, plays an important role in regulation of schistosome-induced dermal inflammation (Hogg *et al*. 2003).

During cutaneous infections, skin-penetrating pathogens (in)directly activate Langerhans cells to migrate to the skin-draining lymph nodes (Arnoldi and Moll 1998; Wu *et al*. 2000). Nevertheless, study on mouse models showed that schistosomula of *S. mansoni* activate Langerhans cells, but inhibit their migration from the epidermis to the skin lymph nodes (Angeli *et al*. 2001). This inhibition is directly mediated by excreted/secreted lipophilic factors, particularly by prostaglandin D_2 (PGD_2), produced by cercariae (Angeli *et al*. 2001).

PGD_2 has multiple effects on the immune system; it enhances release of mediators by eosinophils and mast cells, reduces production of superoxide in neutrophils, and suppresses T cell mitogenesis (Raible *et al*. 1992; Kanamori *et al*. 1997). During inflammatory reaction, PGD_2 may control migration of antigen presenting cells (APCs) from the site of antigen capture to the lymph nodes. In the skin, particularly in the epidermis, PGD_2 belongs to the major arachidonic acid metabolites produced. Study on *S. mansoni* revealed enzyme responsible for PGD_2 synthesis which was excreted by cercariae during penetration through host skin (Angeli *et al*. 2001). PGD_2 synthase was later identified as 28kDa glutathione-S-transferase (termed Sm28GST) (Hervé *et al*. 2003). Sm28GST and its product PGD_2 play a crucial role in Langerhans cell homeostasis and regulation of the immune response during the early phases of schistosome infection (Mountford and Trottein 2004). Sm28GST is also important for parasite survival, because it actively participates in detoxification of parasite- and host-derived products; Sm28GST may neutralize immune attack by inhibiting lipid peroxidation and removing hydroxyalkenals produced (Taylor 1988).

One of the immune evasion strategies described in human schistosomes is the binding of host proteins onto the schistosomulum surface tegument in order to mask it against the host immune response. Studies on human schistosomes showed that these molecules include immunoglobulins (Loukas *et al*. 2001), β2-microglobulin (Loukas *et al*. 2001), blood group antigens (Goldring *et al*. 1976), MHC products (Sher *et al*. 1978), and a number of complement components (Horta *et al*. 1991; Inal and Schifferli 2002; Deng *et al*. 2003). Binding of antibodies is mostly realized *via* their Fc domain that makes them unavailable for subsequent immune interaction, e.g., with complement components or immune effector cells (Loukas *et al*. 2001).

Paramyosin was identified as a schistosome Fc-receptor (Loukas *et al*. 2001). McIntosh *et al*. (2006) confirmed that paramyosin of *S. mansoni* is an Fc-receptor for IgG, but exhibits a low affinity. Non-filamentous membrane-bound form of paramyosin was detected in the tegumental outer layer of *S. mansoni* (Matsumoto *et al*. 1988; Loukas *et al*. 2001; Deng *et al*. 2003). In *S. japonicum*, paramyosin was immunolocalized using electron microscopy on the surface of lung schistosomula and adult worms (Gobert *et al*. 1997; Gobert 1998). Due to the ability of paramyosin to modulate host immune response by inhibition of C1, paramyosin is a promising vaccine candidate against schistosomiasis (Laclette *et al*. 1992). Fc-binding is not exclusively realized by paramyosin, but other parasite proteins probably play a role in the binding of IgG (McIntosh *et al*. 2006). Glycoprotein secreted from *S. mansoni* eggs termed

IL-4-inducing principle of *S. mansoni* eggs (IPSE) binds human IgE in a non-specific manner, involving both Fc and Fab regions (Schramm *et al*. 2003). Metabolically active schistosomes secrete proteases which may also cleave antibodies that bind to the worm surface. The cleaved Fc fragments may remain associated with the membrane, they get into the body of parasite, or due to the low affinity binding, they are released to the host circulation (McIntosh *et al*. 2006). Extracts of *S. mansoni* schistosomula possess ability to cleave Fc-region of host IgE, and thus prevent its interaction with FcεRII present on a number of host inflammatory cells including monocytes, eosinophils, platelets and B cells (Pleass *et al*. 2000). Similarly, recombinant cathepsin D aspartic protease of *S. japonicum* can cleave human IgG (Verity *et al*. 2001). 28kDa serine protease, detected in a soluble form in circum- and post-acetabular glands of *S. mansoni* cercariae, can inhibit complement attack by cleavage of C3, C3b and C9 complement molecules (Marikovsky *et al*. 1990). The same protease is expressed on the surface membrane of schistosomulum; therefore, this enzyme is likely involved in transformation processes and parasite protection against host immune response (Marikovsky *et al*. 1990). It seems that binding and cleavage of host effector molecules is important for schistosomes as immune evasion mechanism as well as nutrient supply and tegumental repair mechanism (McIntosh *et al*. 2006).

Another successful strategy of schistosome immune evasion is the formation of serpine-enzyme complexes. Serpins are serine protease inhibitors produced by mammalian hosts which are able to inactivate proteolytic activity from endogenous as well as exogenous sources, e.g. of parasites (Travis and Salvesen 1983). Interestingly, parasite proteases in a serpin-enzyme complex become non-immunogenic. Schistosomes thus may exploit formation of these complexes to render their proteases invisible to the host immune system (Modha *et al*. 1996).

In case of avian schistosomes, the immune evasion mechanisms have not been studied in details. A precise study of surface antigens and other molecules involved in masking processes could bring new data clarifying failure of bird schistosomes in mammalian host.

1.6. CNS infections

Neuroinfections caused by parasitic worms are known from all parts of the world. Most of the neurotropic helminths appear in the central nervous system (CNS), where they usually occur in the subarachnoid space. The pathways from the site of worm entry (gastrointestinal tract or

skin) to the host the CNS differ from species to species (for a review see Katchanov and Nawa 2010). The CNS involvement occurs either accidentally during atypical migration of parasites or during infections by helminths with innate neurotropic behavior. For example, *Angiostrongylus cantonensis* is considered to be a neurotropic helminth with obligatory intracerebral migration which requires a passage through the subarachnoidal space for completion of its life cycle in the definitive host - rat (Mackerras and Sandars 1954; Jindrák 1968; Prociv *et al*. 2000). Another known parasitic helminth with obligatory migration through the host CNS is the bird schistosome *Trichobilharzia regenti* (Horák *et al*. 1999).

Parasitic worms can invade the CNS *via* the systemic circulation, *via* the Batson's paravertebral venous plexus or they penetrate the blood brain barrier (BBB) in parenchymal microvessels or blood-cerebral spinal barrier in choroid plexus. Another entry site is represented by connective tissues of the skull and invertebral foramina (Katchanov and Nawa 2010). Haematogenous spread to the CNS can be used by e.g. *Taenia*, *Echinococcus*, *Strongyloides*, *Angiostrongylus* and *Toxocara*. After ingestion of the eggs or larvae, the parasites penetrate the intestinal wall, reach the portal vein and then they enter in the systemic circulation. In case of *Strongyloides stercoralis*, the infective filariform larvae penetrate the skin and directly invade into the subcutaneous venous system (Katchanov and Nawa 2010).

Human schistosomes may reach the CNS at any time from the moment when egg laying starts (Pittella 1997). Two ways have been postulated for the CNS invasion by schistosome eggs (Nascimento-Carvalho and Moreno-Carvalho 2005). During the first type of entry route into the CNS, eggs passage is realized through the valveless vertebral venous plexus of Batson (Batson 1940; Pittella and Lana-Peixoto 1981; Scrimgeour and Gajdusek 1985). The eggs deposited in the inferior mesenteric veins are carried by blood flow through rectal portocaval anastomoses into deep iliacal veins. These veins communicate with the Batson's plexus through the lumbar and lateral sacral veins (van der Kuip *et al*. 1999). The pressure around zero in the Batson's plexus facilitates a retrograde transport to the spinal venous system and thereby making the CNS invasion by schistosome eggs possible. Furthermore, the internal part of Batson's plexus, the epidural vertebral venous plexus, communicates with the occipital and basilar venous sinuses and, therefore, represents a venous route for schistosome eggs to enter the brain (Katchanov and Nawa 2010). This route is probably utilised by *Schistosoma japonicum* and *S. haematobium*, but not by S. *mansoni*. This may be explained by the size of *S. japonicum* eggs which are smaller (70-100 μm long by 55-64 μm wide) in comparison with *S. mansoni* (114-180 μm long by 45-70 μm wide) eggs and shape of *S. haematobium* which

bear a terminal spine, whereas, *S. mansoni* eggs have a prominent lateral spine. Therefore, combination of these features leads to trapping of *S. mansoni* eggs in the spinal venous plexus (Bruijning 1964). The second way how human schistosomes reach CNS could be represented by the *in situ* deposition of eggs after anomalous migration of adult worms (Pittella 1997; Artal *et al*. 2006).

Direct penetration into the CNS is common for *Spirometra* spp. larvae (*sparganum*), adults of the lung parasite *Paragonimus* spp. and larvae of *Gnathostoma* spp. These parasites reach the organ through connective tissue of the neural foramina of the skull base and intervertebral foramina of the spine along the cranial and spinal nerves, and vessels (Katchanov and Nawa 2010). Peripheral nerves as an alternative route to the CNS were observed in experimental rat angiostrongylosis (Jindrák 1968) as well as in human angiostrongylosis (Clouston *et al*. 1990); it is suggested that the human infection can be associated with radiculomyelopathy (Wood *et al*. 2001). In case of *T. regenti* infection, schistosomula migrate mostly through or along peripheral nerves, further reach the spinal cord by the spinal roots and continue to the brain (Hrádková and Horák, 2002; Lichtenbergová *et al*. 2011); similar type of migration was also reported for *Gnathostoma* (Herman and Chiodini 2009).

This direct invasion is realized mechanically by active movements of the parasites and it is also facilitated by parasite products which are released into the surrounding environment. It has been observed, that some helminth larvae release proteases that degrade extracellular matrix and macromolecules and, presumably, participate on histolysis (McKerrow 1988; Hotez *et al*. 1990). Cysteine proteases detected in secretions of *Paragonimus westermani* (Na *et al*. 2006) and *Gnathostoma binucleatum* (Caballero-García *et al*. 2005) were found to play a key role in the invasion and migration through the host tissue. Similarly, in case of *T. regenti*, predominant cysteine protease identified in migratory schistosomula degrades myelin basic protein, and it is believed to be an adaptation of the parasites for migration through the CNS (Dvořák *et al*. 2005).

1.6.1. Pathogenesis and disease manifestation

Migration of relatively large worms and release of their E/S products usually evoke directly and/or indirectly pathological changes in the host CNS. Severity of the nervous tissue damage and subsequent clinical syndromes differs from species to species. One of the most health threatening helminthic neuroinfection is caused by *Baylisascaris procyonis*, the common roundworm of raccoons (Sorvillo *et al*. 2002). In humans, the larvae tend to invade the CNS

and eyes, and the infection has been associated with human fatal eosinophilic meningoencephalitis (reviewed in Gavin *et al*. 2005). Severe clinical manifestation of *B. procyonis* infection in humans and other paratenic hosts results from mechanical damage of the nervous tissue by migratory larvae which continue to grow and release a large amount of antigenic components (Kazacos 2001). In addition, the CNS damage can be also caused by severe host inflammatory reaction against *B*. *procyonis*, accompanied by release of toxic proteins by eosinophils (Kazacos 2001; Moertel 2001).

Radicular pain and paresthesias of the trunk and extremities and, less frequently, paresis or paralysis belong to the observed clinical symptomes in another helminthic infection - gnathostomosis (Rusnak and Lucey 1993; Re and Gluckman 2003). The untreated CNS infection with *Gnathostoma* spp. may be fatal (Herman and Chiodini 2009). Clinical symptoms are related to the mechanical damage of nervous tissue associated with larval migration within cranial or peripheral nerves to the spinal cord (Rusnak and Lucey 1993; Re and Gluckman 2003). Direct invasion into the nervous tissue can result in radiculitis, radiculomyelitis, meningeal inflammation or encephalitis, and damage of cerebral vasculature can cause subarachnoid haemorrhages (Schmutzhard 1988; Rusnak and Lucey 1993; Re and Gluckman 2003). The severe damage of the CNS is due to direct mechanical and toxin-mediated destruction of the nervous tissue and its vascular structures, as well as by local host inflammatory response to the parasites and their products (Rusnak and Lucey 1993; Herman and Chiodini 2009). In comparison with *Angiostrongylus*, *Gnathostoma* larvae are more invasive and cause more frequent focal neurological pathologies. Although *Angiostrongylus* is a neurotropic parasite, the infections are rarely fatal (Herman and Chiodini 2009). Clinical spectrum in angiostrongylosis can range from mild disease to meningitis or, less frequently, encephalitis (Slom *et al*. 2002). Natural course of the disease often results in spontaneous resolution of symptoms after a few weeks, although the symptoms like headache and paresthesias can persist for weeks or months (Slom *et al*. 2002).

Clinical presentation of neurocysticercosis is variable and depends on the number, size, and location of tapeworm cysts within the CNS, and on the host immune response (Hawk *et al*. 2005). The most common clinical symptom of neurocysticercosis is the late-onset of epilepsy (Burneo *et al*. 2009); seizures occur in over of 70% of patients with neurocysticercosis (Shandera and Kass 2006). Patients may also suffer from headache associated with intracranial hypertension which is most commonly caused by hydrocephalus related to granular ependymitis, arachnoiditis or ventricular cysts (Burneo *et al*. 2009). When

symptomatic, neuroschistosomiasis is one of the most severe presentations of schistosomal infections (Ferrari 2004). Pathogenesis depends basically on the presence of schistosome eggs in the nervous tissue and on the host immune response (Ferrari 2004). When the eggs are deposited in the nervous tissue, similarly as in other organs and tissues, the miracidium secretes antigenic molecules that leave the egg through the pores of the shell, and thus triggers cell-mediated granulomatous reaction around the eggs (Ferrari *et al*. 2008).

1.6.2. Immune response in CNS

The CNS response to inflammatory insults differs from that of other organs. The organ is equipped by cellular barriers that maintain the CNS homeostasis for proper electrical activity and communication of neurons and hamper infiltration of immune cells; therefore, CNS was considered to be immunologically privileged organ. However, recent results revealed that there is an efficient immuno-surveillance and the immune cells are able to overcome the BBB under certain circumstances (Allan and Rothwell 2003; Engelhardt 2008). Besides immune cells also resident nervous cells participate in the immune processes in the nervous tissue. Neurons, astrocytes and microglia are able to produce a great repertoire of immune and inflammatory molecules, including cytokines, chemokines and their receptors, complement molecules and their inhibitors, coagulating factors, proteases and protease inhibitors (McGeer and McGeer 2001).

Microglial cells are resident phagocytic immune cells in CNS, which are activated in response to infection, inflammation and injury. Together with macrophages, microglia form the first line of defense (Kreutzberg 1996; Streit 2002; Chavarria and Alcocer-Varela 2004). Based on histological and immunophenotypic characteristics microglia can be subdivided into two major classes: a stable pool of cells residing in parenchyma, and cell population termed pericytes or perivascular macrophages located within BBB (Hickey and Kimura 1988; Ford *et al*. 1995). Microglia are sensitive to changes in the CNS microenvironment and rapidly become activated by systemic inflammation (Kreutzberg 1996). The process of microglia activation involves both morphological changes with hypertrophy and proliferation, and functional differentiation with increased expression of MHC molecules. In the CNS of healthy humans, expression of MHC class II molecules is restricted to some microglia. Upregulation of MHC class II and adhesion molecules, such as leukocyte function-associated molecule 1 (LFA-1; CD11a) and intercellular adhesion molecule 1 (ICAM-1; CD54), occurs early after microglia activation in response to the most CNS injuries (Kreutzberg 1996). Activated

microglia are capable to secret a wide range of different immune regulating peptides such as cytokines and chemokines. But they also produce non-specific inflammatory mediators, e.g., reactive oxygen species and nitric oxide. In the final phase of activation microglia become phagocytic and, therefore, the also serve as latent scavenger cells of the CNS (reviewed in Aloisi *et al*. 2000). During *Toxoplasma gondii* infection of mice, microglia are major producers of IFN-γ within the CNS and trigger an activation of glial cells to control the parasite in the early stage of infection (Suzuki *et al*. 2005). Therefore, microglia are the most important cells in prevention against *T. gondii* tachyzoite proliferation in the brain (Rock *et al*. 2004). Similarly, microglia and macrophages are responsible for schistosomula destruction and elimination in the CNS during *T. regenti* experimental infections of mice (Lichtenbergová *et al*. 2011).

Under normal conditions, astrocytes are more important than microglia in the processes providing normal functions of nerve cell. Astrocytes play a role in neurotransmitter regulation, electrical transmission, ion homeostasis, BBB maintenance, and production of extracellular matrix molecules destined for the basal lamina and perineuronal net (Chen and Swanson 2003; reviewed in Fitch and Silver 2008). By their foot processes, astrocytes protect CNS vascular endothelial cells and thus support integrity of BBB (Allt and Lawrenson 1997). Astrocytes are also activated by inflammation, although with some delay compared with microglia response. After the CNS injury, astrocytes become hypertrophied and ramified, they increase production of glial fibrillary acidic protein (GFAP), proliferate and migrate to the site of injury (Norenberg 1994). They play the main role in separation of the damaged areas from the healthy tissue by formation of glial scars, and thus protect the fragile nervous tissue from further destruction (Fitch *et al*. 1999; Myer *et al*. 2006). Formation of glial scar by astrocytic processes that surrounded adjacent inflammatory infiltrate was observed in the brain of human patients infected by *Taenia solium* metacestodes (Alvarez *et al*. 2002). Presence of the parasites was accompanied by astrocytic activation and increased expression of GFAP (Alvarez *et al*. 2002). Similar activation of astrocytes was detected in the brain of *Toxocara*-infected mice (Othman *et al*. 2010) as well as in the spinal cord and brain of the mice infected by *T. regenti* (Lichtenbergová *et al*. 2011).

The CNS injury initiates a series of cellular and molecular events. Following the damage, the endothelial cells express selective adhesion molecules that attract circulating cells participating further in the inflammatory response (Nordal and Wong 2004). Studies on animal models revealed that activated T cells are delivered to the CNS intravenously, and in

contrast to resting T cells, they are able to cross an intact BBB and play a role in immune surveillance (Perry *et al*. 1997). ICAM-1 is an important molecule participating in immune-mediated cell-cell adhesive interactions (Springer 1994); it is predominantly involved in lymphocyte adhesion and migration through the CNS endothelium (Male *et al*. 1994; Greenwood *et al*. 1995). ICAM-1 increases expression on the luminal surface of endothelial cells is associated with release of proinflammatory mediators, such as IL-1β, TNF-α, TGF-β and IFN-γ (Jiang *et al*. 1997). Some of the bacterial or parasitic CNS infections causing meningitis or encephalomeningitis are frequently associated with the occurrence of BBB disruption (Drevets and Leenen 2000; Lee *et al*. 2006; Nikolskaia *et al*. 2006). Higher permeability of BBB accompanied by leukocyte migration from the peripheral blood was observed during the CNS infections of laboratory mice infected by *Mesocestoides corti* (Alvarez and Teale 2006), *Angiostrongylus cantonensis* (Lee *et al*. 2006) and *Toxocara canis* (Liao *et al*. 2008).

Macrophages and microglial cells play the main role in destruction of schistosomula in the nervous tissue of mice experimentally infected by *T. regenti*; host defense against the parasite is also supported by CD 3+ lymphocytes (Lichtenbergová *et al*. 2011). Lymphocyte infiltration in the CNS has been also demonstrated for many disorders including neurocysticercosis (Cardona and Teale, 2002), cerebral malaria (Hansen *et al*. 2007) and toxoplasmic encephalitis (Wilson *et al*. 2005). It has been observed that T cells potentiated immune defense against *Mesocestoides corti* infection of mice by indirect activation of other immune cells (i.e. macrophages) and the resident CNS cells (i.e. microglia, astrocytes) (Cardona and Teale, 2002).

1.7. Immunomodulatory potential of schistosomes

Nowadays, developed countries are being confronted with an increase in the incidence of most immune disorders, including autoimmune and allergic diseases. Epidemiological evidence indicates that this increase is linked to improvement of high socio-economic standards (Wills-Karp *et al*. 2001; Reddy 2010). In contrast, several autoimmune disorders have reduced incidence and severity in geographical regions with higher occurrence of parasite infections (Weinstock and Elliott 2009). According to the Hygiene Hypothesis it is believed that parasites and microbes have been important for shaping and tuning the evolution of human immune system (Dunne and Cooke 2005).

The studies on human schistosomes revealed that infections by these parasites results in Th2 polarized response (Pearce and McDonald 2002). It is believed that persons infected with helminths tend to develop a diminished Th1 response when challenged with other antigens. Sewell *et al*. (2003) developed a model to study the effect of Th2 preconditioning on the course of experimental autoimmune encephalomyelitis (EAE) after immunization with *S. mansoni* eggs. EAE is mouse model for human multiple sclerosis (MS), an inflammatory disease of the CNS that causes demyelination and axonal degeneration leading to neurological function impairment (Noseworthy *et al*. 2000). In a simplified way, EAE as well as MS is associated with infiltration of the CNS by Th1 cells producing proinflammatory cytokines (Merrill 1992). Sewell *et al*. (2003) demonstrated significant protection against EAE in *S. mansoni* eggs preimmunized mice, and similar amelioration of EAE was observed after experimental infection of rats by *Trichinella spiralis* (Gruden-Movsesijan *et al*. 2008). It seems, therefore, that infection by parasitic helminths can positively influence the CNS autoimmune reactions (Sewell *et al*. 2003; Gruden-Movsesijan *et al*. 2008). Identification and characterization of mechanisms by which schistosomes modulate immune response and prevent autoimmune disease might, therefore, bring a novel approach to discover effective drugs for the treatment of allergies and chronic inflammatory diseases like MS. Also, further study of immunomodulatory molecules of bird schistosomes could contribute to this research effort.

2. AIMS OF THE THESIS

Neuroinfection represents the most severe complication of helminthic infections which may lead to development of various neurological symptoms. Parasitic helminths can occur in host nervous tissue in different stages of their life cycle, and only some of them require an obligatory passage through the host nervous system in order to complete their life cycle.

Bird schistosome, *Trichobilharzia regenti*, has been recognized as an interesting model for study of such behaviour pattern in central nervous system (CNS). Experiments on mice revealed that immature flukes are able to migrate to the host CNS where they can survive for weeks (as described in Introduction). Surprisingly, detailed information about pathogenic impact of *T. regenti* schistosomula on the host nervous tissue and immune response against the parasite in the CNS are still missing. The infection by *T. regenti* arises in water bodies after penetration of human skin by infective larvae (cercariae). Repeated infections lead to development of skin inflammatory reaction termed cercarial dermatitis which manifests as a maculopapular skin eruptions accompanied by intensive itching. Although cercarial dermatitis is annoying skin disease, in fact it does not represent a serious health problem for humans. This cutaneous immune reaction attracts an attention because it may lead to capturing of cercariae and thus prevent the further migration of the parasites. The complete understanding of this process is believed to provide new targets for a protective vaccine against human schistosomes. Mammals experimentally infected with bird schistosomes may serve as a suitable model for studies on skin immune response in comparison with human schistosomes which are highly pathogenic for mice and cause severe organ disorders.

The present thesis deals with mammalian infection by bird schistosomes of *T.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 is also focused on description of 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 identified.

Particular aims of the thesis:

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

3. 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., 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.

- **<u>Lichtenbergová L.</u>, 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.** (2011). Immunolocalization of antigenic structures of intravertebrate stages of neuropathogenic schistosome *Trichobilharzia regenti*. *Parasitology Research* (submitted manuscript)

4. SUMMARY OF THE RESULTS

Experiments presented in the Thesis characterized the immuno-pathological effect of *Trichobilharzia regenti* on mammalian host and contributed to knowledge of host immune response to the infection. **The main results of the experimental work are as follows.**

Mammalian humoral immune response

- Analysis of sera from mice multiply infected with *T. regenti* revealed development of antigen-specific IgM antibody directed mainly against glycoproteins of the cercarial glycocalyx as well as glycoproteins contained in the cercarial excretory/secretory (E/S) products.

- Elevated levels of antigen-specific IgG1 and total IgE serum antibodies indicated domination of Th2 polarized immune response after repeated infections.

- Reaction of sera from re-infected mice with corpuscular antigens of *T. regenti* cercariae showed that both IgG and IgE antibodies recognized with high sensitivity and specificity the antigen of 34 kDa; other antigens (14.7, 17, 28 and 50 kDa) were recognized with lower sensitivity. Two of these antigens (34 kDa and 50 kDa) were also identified in cercarial E/S products.

- Analysis of sera from patients with a history of cercarial dermatitis showed elevated levels of anti-cercarial IgG and most of the serum samples specifically recognized a protein of 34 kDa.

- 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 Th2 response.

Mouse CNS infection

- Neuroinfections of immunocompetent (BALB/c) and immunodeficient (SCID) mice led to development of cellular immune response against migrating schistosomula in the nervous tissue which resulted in formation of inflammatory lesions.

- 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, 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 host cellular response.

- The schistosomula destruction in the nervous tissue was mainly dependent on activation of macrophages and microglial cells. Elimination of the worms by these cells was more efficient with contribution of CD3+ lymphocytes.

- Immunohistochemical staining revealed axonal damage around the schistosomula and in places of their previous migration; the damage was likely caused mechanically by migrating parasites.

- Use of specific antibody against components of the mouse nervous tissue revealed presence of immunoreactive material in the lumen of *T. regenti* gut and, therefore, it suggests that the parasites ingest host nervous tissue during their migration.

Immunolocalization of antigenic structures

The studies were performed on *T. regenti* cercariae, schistosomula developed under different conditions (in duck and mouse, and in culture) and adult worms.

- Antibodies from sera of mice repeatedly infected with *T. regenti* specifically bound to cercarial surface and subtegumental structures of all investigated schistosomula.

- TEM observation of immunolabeled sections of different life stages 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 the 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 antibodies.

- Comparison of 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 both specific and non-specific hosts.

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