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DEPARTMENT OF PARASITOLOGY

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The immune response of naïve mice infected with the neuropathogenic schistosome *Trichobilharzia regenti*

PH.D. THESIS SUMMARY

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ABSTRACT

Helminth neuroinfections represent a serious health issue, but the mechanisms of the host immune response often remain neglected despite the fact they might contribute to pathogenesis. This is partly due to the unavailability of clinical samples and the lack of suitable laboratory models. Herein, I focused on the characterization of several aspects of the immune response of mice infected with the neuropathogenic avian schistosome *Trichobilharzia regenti*.

After the percutaneous infection of mice (accidental hosts), most *T. regenti* schistosomula are entrapped and eliminated in the skin, but the parasite antigens initiating the protective immune reaction are not known. Our *in vitro* experiments revealed that *T. regenti* cathepsin B2, a cysteine peptidase used for the skin penetration, activates bone marrow-derived dendritic cells much stronger than the parasite homogenate, suggesting its role in initiating the mixed type1/2 host immune response. However, some schistosomula manage to escape from the skin and continue their migration to the spinal cord. Here they crawl preferentially within the white matter which we demonstrated by the robust 3D imaging techniques, ultramicroscopy and micro-CT. The invasion of the spinal cord is accompanied by striking hypertrophy of astrocytes and microglia. We showed that living schistosomula induce production of interleukin 6 in astrocyte cultures, but their homogenate or active isoforms of *T. regenti* cysteine peptidases trigger even stronger reaction, including the increased secretion of tumor necrosis factor α and nitric oxide by astrocytes and/or microglia. It seems that these glial cells actively participate in maintaining the neuroinflammation initiated by the infection. Finally, we examined the role of nitric oxide in the host immune response. Our data show that nitric oxide is produced early in the skin phase of the infection, but it does not directly kill the schistosomula. It rather continuously debilitates the parasite by disrupting its proteolytic machinery.

Taken together, the thesis markedly extends the knowledge of the host-parasite immune interactions between the neuropathogenic schistosomes and their accidental mammalian hosts. These novel data set a good starting point for further research on *T. regenti* neuropathogenicity and the impacts of helminth-caused neuroinflammation on the host. Such findings will be valuable not only in the field of parasitic neuroinfections but might also be appreciated in the research of (autoimmune) neurodegenerative diseases.

Key words: avian schistosomes, *Trichobilharzia regenti*, immune response, skin, spinal cord, astrocytes, microglia, nitric oxide, cysteine peptidases, 3D imaging.

1. INTRODUCTION

Parasitic helminths often invade the central nervous system (CNS) of mammals, including humans. Invasion of the CNS is either a natural part the helminth somatic migration or it represents an unwanted, ectopic localization (Finsterer and Auer 2013). The clinical manifestation of helminth neuroinfections ranges from mostly asymptomatic to very severe which leads to sensory or cognitive deficits and seizures or epilepsy (Carpio *et al.* 2016, Vezzani *et al.* 2016, Garcia *et al.* 2019).

Many factors, such as parasite burden, size, motility or localization within the CNS, influence the course and outcome of the neuroinfection. Moreover, the host immune response affects the helminth growth and survival but might also participate in pathogenesis (Adalid-Peralta *et al.* 2018). Thus, a better understanding of host-helminth immune interactions is essential to get a comprehensive insight into the biology of neuroinfections. It would allow us to develop better protective measures, treatment strategies and diagnostic tools. Additionally, the lessons learned from the neuroinfections could be potentially utilized in the study of immunopathological processes associated with (autoimmune) neurodegenerative diseases (Fan *et al.* 2015).

The theoretical part of the thesis briefly introduces the general concepts of the immune response in the CNS and addresses it specifically for selected helminth neuroinfections. Current knowledge about *Trichobilharzia regenti*, the neuropathogenic schistosome used in the practical part of the thesis, is then summarized. Original results of the practical part, dealing with the immune response of naïve mice infected with *T. regenti*, are presented within five articles published/accepted for publication in peer-reviewed journals.

2. AIMS

The thesis focused on the characterization of the host immune response of naïve mice infected with the neuropathogenic schistosome *T. regenti*. The specific aims were:

- Characterize the parasite-specific antibody response within four weeks after the infection and the effect of *T. regenti* antigens on bone marrow-derived dendritic cells.
- Examine the response of murine astrocytes and microglia exposed to *T. regenti* antigens with a special emphasis on the production of cytokines and NO.
- Elucidate the role of NO in the host immune response against *T. regenti*.
- Assess the suitability of fluorescence tracers and 3D-imaging techniques for studying *T. regenti* migration and the host immune response in the CNS of mice.

3. MATERIALS & METHODS

Materials & methods are described in detail in the respective publications. They include parasitological, immunological, biochemical, and imaging techniques. A brief overview is provided in publication abstract on pages 10–12.

4. RESULTS & DISCUSSION

The presented thesis aimed at the characterization of several aspects of the immune response of mice infected with the neuropathogenic schistosome *Trichobilharzia regenti*. The parasite serves as a suitable comparative model for human schistosomes and was established as a trigger of CD. It is also a promising tool for studying multicellular neuropathogens due to its strong inherent affinity towards the nervous tissue. However, the host-parasite immune interactions have insufficiently been explored, especially in naïve, previously uninfected mammals.

The penetration of the mouse skin by *T. regenti* cercariae triggers an influx of inflammatory cells that accumulate around the newly transformed schistosomula. This initial phase of the infection is well characterized histopathologically, especially in repeatedly infected mice suffering from CD, and resembles the reaction mounted against human schistosomes (Incani and McLaren 1984, Kouřilová *et al.* 2004a, Kouřilová *et al.* 2004b). Antigens present in parasite glycocalyx, ESP, and perhaps extracellular vesicles are expected to initiate the host immune response in the case of human schistosomes (Perona-Wright *et al.* 2006, Paveley *et al.* 2009, Kuipers *et al.* 2020), but no data have been available for bird species. Thus, we tested the effect of *T. regenti* antigens on murine bone marrow-derived dendritic cells (BMDCs) (Majer *et al.* 2020).

The cercarial homogenate, containing a mixture of surface and somatic antigens, only marginally affected maturation status and inflammation-promoting phenotype of BMDCs. The reason might be that dendritic cells do not normally encounter damaged schistosomula during the initial phase of the infection. They should rather recognize ESP released into the host tissues during the skin penetration as suggested but not tested by Kouřilová et al. (2004a). Accordingly, we demonstrated that the recombinant cysteine peptidase rTrCB2 activated mouse BMDCs and induced expression of *Ccl5*, *Cxcl10*, *Il12*, *Il33*, and *Il10* (Majer *et al.* 2020). CCL5 could promote the production of IL-6 which was present in the skin early after *T. regenti* infection (Fischer *et al.* 2001, Kouřilová *et al.* 2004a) and CXCL-10 might boost Th1 response (Bonecchi *et al.* 1998, Khan *et al.* 2000, Vasquez *et al.* 2008). The cytokine profile displayed by rTrCB2-stimulated BMDCs, including IL-10, IL-12, and IL-33, suggests the mixed type 1/2 response, which agrees with the already published data (Kouřilová *et al.* 2004a). The mixed response is also supported by the equal levels of parasite-specific IgG1 and IgG2 associated with Th2 and Th1, respectively (Finkelman *et al.* 1988, Majer *et al.* 2020).

The effects of rTrCB2 might be caused either by its direct immunogenicity or by its enzymatic activity which could be sensed by the protease-activated receptors (Bonnart *et al.* 2017, Cano *et al.* 2019). The latter explanation seems to be more probable as it accords with our report of the inability to stimulate immune cells by enzymatically inactive rTrCB1.6 (Dvořáková *et al.* 2020). Similarly, the proteolytic activity of *S. mansoni* cathepsin B1 is necessary for the induction of T cell response in mice (Soloviova *et al.* 2019). However, the hypothesis needs further experimental validation.

The strong skin inflammation is believed to impede *T. regenti* further migration. Indeed, 90% of schistosomula are estimated to be arrested and eliminated here in mammals (Kouřilová *et al.* 2004a). It seems to be a consequence of the host-parasite evolutionary incompatibility, specifically the inability of *T. regenti*, the avian schistosome, to evade the immune response of mammals. The latter is often seen in human schistosomes which are much better adapted to survive in mammalian hosts (Jenkins *et al.* 2005, Angeles *et al.* 2020). Nevertheless, the particular immune mechanisms responsible for the elimination of

the avian schistosomes in the mammalian skin have been unknown. Regarding both historical and recent reports of the harmful impact of NO on human schistosomes (James and Glaven 1989, Ahmed *et al.* 1997, Shen *et al.* 2017), we evaluated its effect on *T. regenti* infection in mice (Macháček *et al.* 2020).

We detected iNOS in the epidermal layer adjacent to the penetrating cercariae 8 hpi, but no signal was noticed later either in the epidermis or skin infiltrating leukocytes. We also did not observe any schistosomula damage after their *in vitro* treatment by NO-donors which suggested that NO did not cause acute cytotoxicity to *T. regenti* schistosomula. As the acute cytotoxicity is mostly related to the disruption of aerobic mitochondrial metabolism in human schistosomes (Ahmed *et al.* 1997), our data support the view that early *T. regenti* schistosomula rather rely on anaerobic/microaerobic energy metabolism (Leontovyč *et al.* 2016).

Unexpectedly, further experiments based on *in vivo* inhibition of NO formation suggested ambiguous actions of NO. It likely promoted the parasite growth in the early phase of the infection but prevented it later, which was accompanied by suspended schistosomula migration in the CNS. The latter effects might stem from continuous and chronic debilitation of the parasite, partly related to NO inactivation of its vital peptidases (Macháček *et al.* 2020). Specifically, we demonstrated that NO decreased activity of rTrCB1.1 and rTrCB2, the peptidases essential for parasite digestion and migration (Dvořák *et al.* 2005, Dolečková *et al.* 2009). Such NO-related disruption of the proteolytic machinery has already been described for parasitic protists (Colasanti *et al.* 2001, Bocedi *et al.* 2004), but to the best of author's knowledge, this is the first time the phenomenon is reported for parasitic helminths.

Activation of microglia and astrocyte hypertrophy are striking features of the mouse immune reaction in the spinal cord invaded by *T. regenti* schistosomula which were able to escape from the skin (Lichtenbergová *et al.* 2011). This is a common feature observed also in other helminth neuroinfections, but little is usually known about the function of these glial cells. Astrocytes are generally believed to participate in tissue repair (Sofroniew and Vinters 2010) while microglia are regarded as the major immune players participating in the parasite clearance (Rock *et al.* 2004). However, studies testing these assumptions are mostly lacking for helminth neuroinfections. To better understand the role of astrocytes and microglia in *T. regenti* infected mice, we examined their production of NO and cytokines after treatment by various *T. regenti* antigens (Macháček *et al.* 2016, Dvořáková *et al.* 2020).

We observed significant differences between the immunogenicity of living schistosomula (LS) and the soluble fraction of their homogenate (HSF). LS did not induce NO production in either astrocytes or microglia which corroborates with our *in vivo* data from the spinal cord, where iNOS was present no more than around 60% of schistosomula 3 dpi (Macháček *et al.* 2020). Considering the cytokines, LS induced production of IL-6 in astrocytes which might be associated both with initiating the CNS inflammation nut also nervous tissue repair (Swartz *et al.* 2001). On the contrary, HSF triggered the production of NO, IL-6, and TNF-α which might facilitate the inflammatory processes and nervous tissue damage occurring after the schistosomula death when intrinsic antigens are released (Chao *et al.* 1992, Lichtenbergová *et al.* 2011, di Penta *et al.* 2013).

The similar immunostimulatory properties towards astrocytes and microglia were recorded also for rTrCB1.1 and rTrCB2, but not the inactive isoform rTrCB1.6. It potentially makes the active cysteine peptidases strong PAMPs which trigger the host immune reaction both in the periphery (Majer *et al.* 2020) and in the CNS (Macháček *et al.* 2016, Dvořáková *et al.* 2020). This is generally in agreement with the view that the mammalian immune system can be very sensitive to helminth cysteine peptidases

as they are released into the tissues in large amounts contrary to the host own peptidases often tightly stored in intracellular compartments (Sokol *et al.* 2008, Soloviova *et al.* 2019). Of note, none of the tested antigens influenced the secretion of IL-10 or TGF-β which implies their limited capacity to trigger directly the immunoregulatory phenotype in astrocytes or microglia.

The host immune reaction against pathogens varies in different CNS compartments and tissues (Andersson *et al.* 1992, Stevenson *et al.* 1997, Schnell *et al.* 1999) so the accurate data on the parasite migration and distribution within the CNS is required for appropriate interpretation of the experimental outcomes. The conventional imaging methods, such as histology, usually provide a good resolution and overview of a small region of interest. However, they become laborious and time demanding if a comprehensive screening of bigger tissue samples or even entire organs is necessary. This is why various 3D imaging techniques (e.g. micro-computed tomography (μCT), ultramicroscopy, light sheet microscopy, etc.) have gained emerging popularity not only in parasitology (O'Sullivan *et al.* 2018). We applied some of these techniques to revise the previous histology-based data on *T. regenti* distribution within the vertebrate CNS tissues (Kolářová *et al.* 2001, Kouřilová *et al.* 2004b, Lichtenbergová *et al.* 2011). Additionally, we tested the suitability of fluorescence tracers for staining cercariae/schistosomula to enable better parasite tracking within the CNS.

Imaging of the murine spinal cord by ultramicroscopy and the duck spinal cord by μCT revealed that the schistosomula are predominantly localized in the white matter (Bulantová *et al.* 2016). The preference for this tissue, which tends to develop stronger inflammation than the gray matter (Andersson *et al.* 1992), is likely a consequence of the *T. regenti* adaptation to feeding on myelin that is the main constituent of the white matter (Leontovyč *et al.* 2019). If suitable contrasting agents were applied, there was no need to stain the parasite to enable its later identification in the samples imaged by μCT. On the contrary, pre-infection fluorescence staining was required to visualize the schistosomula by ultramicroscopy. For this purpose, we found suitable tracers that retain the fluoresce long enough to study *T. regenti* behavior in the spinal cord (Bulantová *et al.* 2016). The major advantage of the latter approach is that more tracers or fluorescently labeled antibodies can be used which would enable more focused spatial characterization of the host immune response (Ertürk *et al.* 2012).

5. CONCLUSIONS

The thesis presents the role of various *T. regenti* antigens in triggering the mouse immune response either in the periphery or within the CNS. Reciprocally, the effects of the anti-parasitic host immune effector molecule, NO, on *T. regenti* schistosomula, and the course of the infection were examined. Finally, the applicability of fluorescence tracers and 3D-imaging techniques for studying *T. regenti* migration in mice and the associated host immune response were tested. Taken together, the thesis markedly extends the knowledge of the host-parasite interactions between the neuropathogenic schistosomes and their accidental mammalian hosts. These novel data set a good starting point for further research on *T. regenti* neuropathogenicity and the impacts of helminth-caused neuroinflammation on the host. Such findings will be valuable not only in the field of parasitic neuroinfections but might also be appreciated in the research of (autoimmune) neurodegenerative diseases.

6. REFERENCES

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PUBLICATIONS

Publication #1

Bulantová J, Macháček T, Panská L, Krejčí F, Karch J, Jährling N, Saghafi S, Dodt H-U and Horák P (2016) Trichobilharzia regenti (Schistosomatidae): 3D imaging techniques in characterization of larval migration through the CNS of vertebrates. *Micron* **83**: 62–71.

ABSTRACT: Migration of parasitic worms through the host tissues, which may occasionally result in fatal damage to the internal organs, represents one of the major risks associated with helminthoses. In order to track the parasites, traditionally used 2D imaging techniques such as histology or squash preparation do not always provide sufficient data to describe worm location/behavior in the host. On the other hand, 3D imaging methods are widely used in cell biology, medical radiology, osteology or cancer research, but their use in parasitological research is currently occasional. Thus, we aimed at the evaluation of suitability of selected 3D methods to monitor migration of the neuropathogenic avian schistosome *Trichobilharzia regenti* in extracted spinal cord of experimental vertebrate hosts. All investigated methods, two of them based on tracking of fluorescently stained larvae with or without previous chemical clearing of tissue and one based on X-ray micro-CT, exhibit certain limits for *in vivo* observation. Nevertheless, our study shows that the tested methods as ultramicroscopy (used for the first time in parasitology) and micro-CT represent promising tool for precise analyzing of parasite larvae in the CNS. Synthesis of these 3D imaging techniques can provide more comprehensive look at the course of infection, host immune response and pathology caused by migrating parasites within entire tissue samples, which would not be possible with traditional approaches.

Publication #2

Macháček T, Panská L, Dvořáková H and Horák P (2016) Nitric oxide and cytokine production by glial cells exposed in vitro to neuropathogenic schistosome *Trichobilharzia regenti*. *Parasit Vectors* **9**: 579.

ABSTRACT: Background: Helminth neuroinfections represent a serious health problem, but host immune mechanisms in the nervous tissue often remain undiscovered. This study aims at *in vitro* characterization of the response of murine astrocytes and microglia exposed to *Trichobilharzia regenti* which is a neuropathogenic schistosome migrating through the central nervous system of vertebrate hosts. *Trichobilharzia regenti* infects birds and mammals in which it may cause severe neuromotor impairment. This study was focused on astrocytes and microglia as these are immunocompetent cells of the nervous tissue and their activation was recently observed in *T. regenti*-infected mice. Results: Primary astrocytes and microglia were exposed to several stimulants of *T. regenti* origin. Living schistosomulum-like stages caused increased secretion of IL-6 in astrocyte cultures, but no changes in nitric oxide (NO) production were noticed. Nevertheless, elevated parasite mortality was observed in these cultures. Soluble fraction of the homogenate from schistosomulum-like stages stimulated NO production by both astrocytes and microglia, and IL-6 and TNF-α secretion in astrocyte cultures. Similarly, recombinant cathepsins B1.1 and B2 triggered IL-6 and TNF-α release in astrocyte and microglia cultures, and NO production in astrocyte cultures. Stimulants had no effect on production of anti-inflammatory cytokines IL-10 or TGF-β1. Conclusions: Both astrocytes and microglia are capable of production of NO and proinflammatory cytokines IL-6 and TNF-α following *in vitro* exposure to various stimulants of *T. regenti* origin. Astrocytes might be involved in triggering the tissue inflammation in the early phase of

T. regenti infection and are proposed to participate in destruction of migrating schistosomula. However, NO is not the major factor responsible for parasite damage. Both astrocytes and microglia can be responsible for the nervous tissue pathology and maintaining the ongoing inflammation since they are a source of NO and proinflammatory cytokines which are released after exposure to parasite antigens.

Publication #3

Dvořáková H, Leontovyč R, Macháček T, O'Donoghue AJ, Šedo O, Zdráhal Z, Craik CS, Caffrey CR, Horák P and Mikeš L (2020) Isoforms of cathepsin B1 in neurotropic schistosomula of *Trichobilharzia regenti* differ in substrate preferences and a highly expressed catalytically inactive paralog binds cystatin. *Front Cell Infect Microbiol* **10**: 66.

ABSTRACT: Schistosomula (the post-infective stages) of the neurotropic schistosome *Trichobilharzia regenti* possess multiple isoforms of cathepsin B1 peptidase (TrCB1.1-TrCB1.6) with involvement in nutrient digestion. The comparison of substrate preferences of TrCB1.1 and TrCB1.4 showed that TrCB1.4 had a very narrow substrate specificity and after processing it was less effective toward protein substrates when compared to TrCB1.1. Self-processing of both isoforms could be facilitated by sulfated polysaccharides due to a specific binding motif in the pro-sequence. Trans-activation by heterologous enzymes was also successfully employed. Expression profiling revealed a high level of transcription of genes encoding the enzymatically inactive paralogs TrCB1.5 and TrCB1.6. The transcription level of TrCB1.6 was comparable with that of TrCB1.1 and TrCB1.2, the most abundant active isoforms. Recombinant TrCB1.6wt, a wild type paralog with a Cys^{29}-to-Gly substitution in the active site that renders the enzyme inactive, was processed by the active TrCB1 forms and by an asparaginyl endopeptidase. Although TrCB1.6wt lacked hydrolytic activity, endopeptidase, but not dipeptidase, activity could be restored by mutating Gly^{29} to Cys^{29}. The lack of exopeptidase activity may be due to other mutations, such as His^{110}-to-Asn in the occluding loop and Asp^{224}-to-Gly in the main body of the mature TrCB1.6, which do not occur in the active isoforms TrCB1.1 and TrCB1.4 with exopeptidase activity. The catalytically active enzymes and the inactive TrCB1.6 paralog formed complexes with chicken cystatin, thus supporting experimentally the hypothesis that inactive paralogs could potentially regulate the activity of the active forms or protect them from being inhibited by host inhibitors. The effect on cell viability and nitric oxide production by selected immune cells observed for TrCB1.1 was not confirmed for TrCB1.6. We show here that the active isoforms of TrCB1 have different affinities for peptide substrates thereby facilitating diversity in protein-derived nutrition for the parasite. The inactive paralogs are unexpectedly highly expressed and one of them retains the ability to bind cystatins, likely due to specific mutations in the occluding loop and the enzyme body. This suggests a role in sequestration of inhibitors and protection of active cysteine peptidases.

Publication #4

Majer M, Macháček T, Súkeníková L, Hrdý J and Horák P (2020) The peripheral immune response of mice infected with a neuropathogenic schistosome. *Parasite Immunol* **42**: e12710.

ABSTRACT: *Trichobilharzia regenti* (Schistosomatidae) percutaneously infects birds and mammals and invades their central nervous system (CNS). Here, we characterized the peripheral immune response of infected mice and showed how it was influenced by the parasite-induced inflammation in the skin and the CNS. As revealed by flow cytometry, T cells expanded in the spleen and the CNS-draining lymph nodes 7-14 days post-infection. Both T-bet+ and GATA-3+ T cells were markedly elevated suggesting a

mixed type 1/2 immune response. However, it dropped after 7 dpi most likely being unaffected by the neuroinflammation. Splenocytes from infected mice produced a high amount of IFN-γ and, to a lesser extent, IL-10, IL-4 and IL-17 after *in vitro* stimulation by cercarial homogenate. Nevertheless, it had only a limited capacity to alter the maturation status of bone marrow-derived dendritic cells (BMDCs), contrary to the recombinant *T. regenti* cathepsin B2, which also strongly augmented expression of *Ccl5*, *Cxcl10*, *Il12a*, *Il33* and *Il10* by BMDCs. Taken together, mice infected with *T. regenti* developed the mixed type 1/2 immune response, which was driven by the early skin inflammation rather than the late neuroinflammation. Parasite peptidases might play an active role in triggering the host immune response.

Publication #5

Macháček T, Šmídová B, Pankrác J, Bulantová J, Horák P (2020). Nitric oxide debilitates the neuropathogenic schistosome in mice, partially by inhibiting its vital peptidases. *Parasit Vectors* accepted.

ABSTRACT: Background**:** Avian schistosomes, causative agents of human cercarial dermatitis (or swimmer's itch) die in mammals, but the mechanisms responsible for parasite elimination are unknown. Here we examined the role of reactive nitrogen species, specifically nitric oxide (NO) and peroxynitrite, in the immune response of mice experimentally infected with *Trichobilharzia regenti*, a model species of avian schistosomes remarkable for its neuropathogenicity. Methods**:** Inducible NO synthase (iNOS) was localized by immunohistochemistry in the skin and the spinal cord infected by *T. regenti*. The impact of iNOS inhibition by aminoguanidine on parasite burden and growth was then evaluated *in vivo*. The vulnerability of *T. regenti* schistosomula to NO and peroxynitrite was assessed *in vitro* by viability assays and electron microscopy. Additionally, the effect of NO on the activity of *T. regenti* peptidases was tested using a fluorogenic substrate. Results**:** iNOS was detected around the parasites in the epidermis 8 hours post infection and also in the spinal cord 3 days post infection (dpi). Inhibition of iNOS resulted in slower parasite growth 3 dpi, but the opposite effect was observed 7 dpi. At the latter timepoint, moderately increased parasite burden was also noticed in the spinal cord. *In vitro*, NO did not impair the parasites, but it inhibited the activity of *T. regenti* cathepsins B1.1 and B2, the peptidases essential for parasite migration and digestion. Peroxynitrite severely damaged the surface tegument of the parasites and decreased their viability *in vitro*, but rather did not participate in parasite clearance *in vivo*. Conclusions**:** Reactive nitrogen species, specifically NO, do not directly kill *T. regenti* in mice. NO promotes the parasite growth soon after penetration (3 dpi) but prevents it later (7 dpi) when it also suspends the parasite migration in the CNS. NO-related disruption of the parasite proteolytic machinery is partly responsible for this effect.

CURRICULUM VITAE

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Education

2015–present **PhD in Parasitology** (Charles University, Prague, Czechia)
2012–2015 **MSc in Parasitology** (Charles University, Prague, Czechia)
2012–2015 **Special study to obtain teaching competence in biology** (lifelong learning profession-oriented programme; Charles University, Prague, Czechia)
2009–2012 **BSc (Hons.) in Biology** (Charles University, Prague, Czechia)

Employment

2017–present **Assistant lecturer** (Charles University, Prague, Czechia)
2015–2017 **Research assistant** (Charles University, Prague, Czechia)

Internships

2020 **Internship in Dr. Martina Sombetzki's Lab** (Division of Tropical Medicine and Infectious Diseases, University Medical Centre Rostock, Germany). Skin and liver immune response and pathology in *Trichobilharzia-Schistosoma* co-infected mice. Duration: 2 weeks.

2017 **Internship in Prof. Ingo Bechmann's Lab** (Institute of Anatomy, University of Leipzig, Germany). Isolation of immune cells from brain and spinal cord, multicolour flow cytometry – experimental design and data analysis. Duration: 2 months.

Courses

2019 BIOCEV Practical Flow Course (Charles University, Prague, Czechia)
2018 Detection of lymphocyte proliferation by flow cytometry (Exbio, Prague, Czechia)
2017 Introductory course on glial biology (European Meeting on Glial Cells, Edinburgh, UK)
2016 Multiplexing ELISPOT (Institute of Microbiology, Prague, Czechia)
2015 Super-resolution in confocal microscopy (Olympus, Prague, Czechia)
2014 Training course to obtain qualification and competence in the field of experimental animals (Charles University, Prague, Czechia)
2014 Summer school of primate parasitology (Veterinary and Pharmaceutical University Brno, Czechia)
2012 Motol cytometry course (Faculty Hospital Motol, Prague, Czechia)

Grants & awards

2016–2018 Principal investigator of the project "The role of nitric oxide in infections of vertebrate hosts by viscerotropic and neurotropic species of bird schistosomes" (Grant Agency of Charles University)
2018 Best oral presentation award, PhD category (Slovak & Czech Parasitological Days)
2017 Special award for the series of educational articles (Živa magazine)
2013 Prof. Heyrovský award for the best BSc graduates (rector of Charles University, Prague, Czechia)

Publications (full list)

Macháček T, Šmídová B, Pankrác J, Bulantová J, Horák P (2020). Nitric oxide debilitates the neuropathogenic schistosome in mice, partially by inhibiting its vital peptidases. *Parasit Vectors* accepted.

Dvořáková H, Leontovyč R, Macháček T, O'Donoghue AJ, Šedo O, Zdráhal Z, Craik CS, Caffrey CR, Horák P and Mikeš L (2020) Isoforms of cathepsin B1 in neurotropic schistosomula of *Trichobilharzia regenti* differ in substrate preferences and a highly expressed catalytically inactive paralog binds cystatin. *Front Cell Infect Microbiol* **10**: 66.

Majer M, Macháček T, Súkeníková L, Hrdý J and Horák P (2020) The peripheral immune response of mice infected with a neuropathogenic schistosome. *Parasite Immunol* **42**: e12710.

Macháček T, Turjanicová L, Bulantová J, Hrdý J, Horák P and Mikeš L (2018) Cercarial dermatitis: a systematic follow-up study of human cases with implications for diagnostics. *Parasitol Res* **117**: 3881–3895.

Novák J, Panská L, Macháček T, Kolářová L and Horák P (2017) Humoral response of mice infected with *Toxocara canis* following different infection schemes. *Acta Parasitol* **62**: 823–835.

Macháček T, Panská L, Dvořáková H and Horák P (2016) Nitric oxide and cytokine production by glial cells exposed in vitro to neuropathogenic schistosome *Trichobilharzia regenti*. *Parasit Vectors* **9**: 579.

Bulantová J, Macháček T, Panská L, Krejčí F, Karch J, Jährling N, Saghafi S, Dodt H-U and Horák P (2016) *Trichobilharzia regenti* (Schistosomatidae): 3D imaging techniques in characterization of larval migration through the CNS of vertebrates. *Micron* **83**: 62–71.

Conferences (selection)

Macháček T *et al*.: Transcriptomic profiling sheds new light on the invasion of the spinal cord by *Trichobilharzia regenti*. Helminthological Days 2019 (Czechia), oral presentation.

Macháček T *et al*.: Cercariae on the campus: a follow-up study of swimmer's itch acquired in a faculty pool. BSP Spring Meeting 2019 (UK), oral presentation.

Macháček T *et al*.: Dynamics of immune cells in the CNS of mice infected by *Trichobilharzia regenti* (Schistosomatidae): implications for parasite clearance. Molecular and Cellular Biology of Helminths 2018 (Greece), oral presentation.

Macháček T *et al*.: *Trichobilharzia regenti* (Schistosomatidae) in mice: Dynamics of immune cells in the CNS and implications for parasite clearance. Slovak and Czech Parasitological Days 2018 (Slovakia), oral presentation.

Macháček T *et al*.: Neuropathogenic bird schistosome *Trichobilharzia regenti* activates astrocytes and microglia of infected ducks and mice. European Meeting on Glial Cells in Health and Disease 2017 (UK), poster presentation.

Macháček T *et al*.: Astrocytes and microglia produce nitric oxide, IL-6 and TNF-α after exposure to stimuli from the neuropathogenic schistosome *Trichobilharzia regenti*. European Multicolloquium of Parasitology 2016 (Finland), oral presentation.

Science popularization (articles in Czech)

Macháček T and Parohová I (2020) Paraziti a roztroušená skleróza: spouštěči onemocnění, nebo nástroj terapie? *Živa* **68**: 129–32.

Bulantová J and Macháček T (2017) Dobrodružství mikroskopie – cesta ke třetí dimenzi. *Živa* **65**: 294–6.

Bulantová J and Macháček T (2017) Kapesní průvodce světem mikroskopů. *Živa* **65**: CLXII–V.

Macháček T and Bulantová J (2016) Na sever za parazity mořských ryb. *Přírodovědci.cz* **5**: 30–3.

Macháček T, Mikešová K, Turjanicová L and Hampl V (2016) Proměny vyšší systematiky eukaryot a její odraz ve středoškolské biologii. *Živa* **64**: 27–30.

Macháček T (2015) Hledání řádu v živém světě. *Přírodovědci.cz* **4**: 22–4.

Macháček T, Bulantová J, Jedličková L, Leontovyč R, Pankrác J, Skála V, Turjanicová L and Horák P (2015) Jekyll a Hyde: Máme se obávat parazitických helmintů člověka? *Živa* **63**: 215–9.

Professional affiliations

2018–present British Society for Parasitology
2013–present Czech Society for Parasitology

FUNDING

Charles University Grant Agency (Grant No. 729516): **The role of nitric oxide in infections of vertebrate hosts by viscerotropic and neurotropic species of bird schistosomes** (2016–2018)

Czech Science Foundation (Grant No. 18-11140S): **Mechanisms of host immunomodulation by *Trichobilharzia regenti*, an avian neuropathogenic schistosome** (2018–2020)

Czech Science Foundation (Grant No. 18-11140S): **The neurotropic schistosome *Trichobilharzia regenti* in vertebrates: immune response, pathology and diagnostic markers** (2013–2017)

European Regional Development Fund and Ministry of Education, Youth and Sports of the Czech Republic (CZ.02.1.01/0.0/0.0/16_019/0000759): **Centre for Research of Pathogenicity and Virulence of Parasites** (2018–2022)

Charles University **institutional grants** (PROGRES Q43 (2016-2020), UNCE/SCI/012 - 204072/2018 (2018-2023), SVV 260432/2018)

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