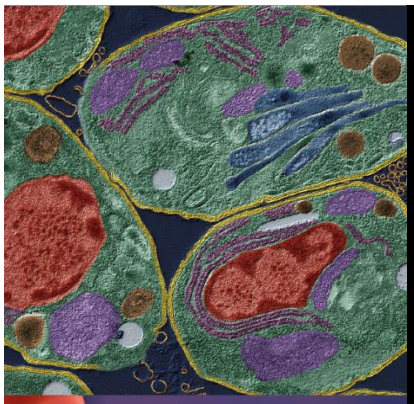
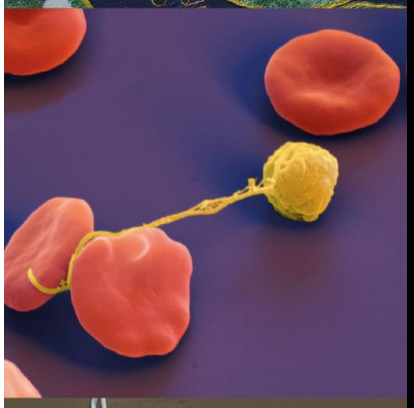




GRK 2046 "Parasite Infections: From Experimental Models to Natural Systems"

Annual Retreat 2019

Veterinarium Progressum

VetMed faculty, Freie Universität Berlin

17th September 2019

Notes:

Location:

Veterinarium Progressum, VetMed faculty, FU Berlin, campus Düppel
Oertzenweg 19B, 14163 Berlin

Organization:

Alexander Gerhard: alexander.gerhard@fu-berlin.de

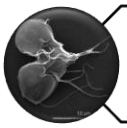
Benedikt Fabian: tilbenbru@gmail.com

Miguel Veiga: veiga@izw-berlin.de

Coordination:

Marko Janke: Marko.Janke@fu-berlin.de

Retreat program

	**8.45 am**	Welcome Address
	**9 - 10.30 am**	**Session 1: Protozoan parasites**
	**10.30 - 11 am**	Coffee break
	**11 am - 12.15 pm**	**Session 2: Vectors & Vector-borne diseases**
	**12.15 - 1.30 pm**	Lunch
	**1.30 - 2.30 pm**	**Session 3: Wildlife parasitology**
	**2.30 - 2.45 pm**	Short break
	**2.45 - 3.45 pm**	**Session 4: Nematode infections**
	**4 - 5 pm**	Principal Investigators meeting
	**5 - 6 pm**	Doctoral Researchers meeting
	**6.30 pm - open end**	Social evening & BBQ

SCHEDULE OVERVIEW

Welcome Address
Speaker GRK 2046: Susanne Hartmann
8.45 – 9.00 am

Session 1: Protozoan parasites

9.00 – 10.30 am

Moderators: Ivet Yordanova and Benjamin Hamid

Coffee Break (retreat picture)
10.30 – 11.00 am

Session 2: Vectors and vector-borne diseases

11 am – 12.15 am

Moderators: Bhavya Kapse and Irina Diekmann

Lunch Break (during which there will be a Steering Committee Meeting)
12.15 – 1:30 pm

Session 3: Wildlife parasitology

1.30 – 2.30 pm

Moderators: Alex Katelas and David Holthaus

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Alexander Gerhard & Ankur Midha	Serengeti Field Training Course	13
Miguel Veiga	Using non-invasive measures of intestinal immune response to better understand parasite heterogeneity and its impact on Darwinian fitness	14
Vivian Schüller	*Nosema ceranae*, a deadly emerging parasite of bees	15

Session 4: Nematode infections

2.45 – 3.45 pm

Moderators: Estefanía Delgado Betancourt and Nina Militzer

Name	Title	Page
Hongwei Zhang	High susceptibility to intestinal nematode infections is associated with delayed gut homing of Th2 cells and poor parasite-specific adaptive immune responses	16
Benjamin Hamid	Response of porcine dendritic cell subsets to *Toxoplasma gondii* and *Ascaris suum*	17
Irina Diekmann	Cyathostomin population diversity in treated and untreated equine hosts with different geographical background	18
Bhavya Kapse	Th2/1 hybrid cells seem to limit the control of gastrointestinal nematodes via IFN-γ production and the regulation of innate immune cells	19

Principal Investigator Meeting (1st floor Veterinarium Progressum)
4 – 5 pm

Doctoral Researcher Meeting (1st floor Veterinarium Progressum)
5 – 6 pm

Closing session
6 – 6.30 pm

Social evening & BBQ (all PIs and PhD student are welcome!)
6.30 pm – open end

Apical-out polarized enteroids to study *Toxoplasma gondii* intestinal infection in wild rodents and laboratory mice

Estefania Delgado Betancourt[1], Francesca Torelli[1], Marion Joncic[2], Michael Beekes[2], Christian Klotz[1], Frank Seeber[1]
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Natural reservoirs for *Toxoplasma gondii*, such as the bank vole *Myodes glareolus*, are important for the transmission to cats. This rodent has been shown, among other wild rodent species, to be a more relevant prey to cats in Europe than *M. musculus* and also potentially be more resistant to infection with T. gondii. Oral infection with tissue cysts or oocysts is the natural route of infection and the initial encounter of the parasite with its host is at the intestinal epithelium. The mechanism by which the parasites invade intestinal epithelial cells and the cellular responses to invasion have only been studied in laboratory inbred mice to a minor extent, but nothing is known in this regard in other hosts of *T. gondii.*

To study the early events of intestinal *T. gondii* infection, I have established protocols for the generation, cultivation and differentiation of small intestinal 3D-organoids from *M. glareolus*. Moreover, organoids presenting an inverted polarity (‘apical-out’ phenotype) have also been generated, providing facile experimental access of the parasite to the apical side of the intestinal epithelial cells and thus mimicking in vivo cell invasion. Wild rodent organoids were successfully infected with different *T. gondii* strains and will be compared to infected organoids from laboratory mice, to determine possible differences in infection success between hosts.

Delgado Betancourt, E.; Hamid, B.; Fabian, B. T.; Klotz, C.; Hartmann, S.; Seeber, F. (2019). From Entry to Early Dissemination-Toxoplasma gondii's Initial Encounter With Its Host. *Front Cell Infect Microbiol.*; 9:46. doi: 10.3389/fcimb.2019.00046

Oocyst-molecules of *Toxoplasma gondii:* their role for survival in the environment and their diagnostic potential

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The environmental stage of *T. gondii,* the oocyst, plays a key role in the spreading of the parasite. To improve detection of oocysts, I aim to investigate new agents for binding to the oocyst surface and to develop new methods to efficiently enrich oocysts from environmental samples for subsequent analysis by PCR. One approach to achieve this is the development of camelid-derived nanobodies. In addition, I investigate other possible molecules with regards to their oocyst binding capacities such as C-type lectin binding receptors. Since oocysts are only shed by felids, access to them is limited. Thus, to analyse the different interaction partners, I developed an immunofluorescence assay that allows investigating small numbers of valuable oocysts without losing significant amounts of them during the assay. Using this assay I could demonstrate the successful immunisation of an alpaca to generate nanobodies.

In a second project, I aim to characterize oocyst-specific proteins to determine their role in the survival of oocysts in the environment. Oocysts are known to remain viable in the environment for up to 24 months. Several genes in the *T. gondii* genome are annotated as so called **L**ate **E**mbryogenesis **A**bundant proteins (LEA). LEA proteins are known to confer resistance to stressors such as desiccation and low temperatures in plants, nematodes and some microorganisms. LEA proteins seem to be intrinsically disordered, employing a defined secondary structure only upon induction (e.g. through removal of water), thus protecting cellular components from damage. To assess the protecting potential of the TgLEAs, growth assays in *Escherichia coli* under different stressing conditions have been conducted and analysed. In addition, I performed biochemical characterization of these proteins to gain insight into their mode of action and their function on a molecular level. Using techniques such as thermal shift analysis and lactate dehydrogenase aggregation assays, I gained further proof that TgLEAs possess characteristics of intrinsically disordered proteins, thereby strengthening our assumption that they might protect oocysts from environmental stress.

Delgado Betancourt, E.**;** Hamid, B.; Fabian, B. T.; Klotz, C.; Hartmann, S.; Seeber, F. (2019). From Entry to Early Dissemination-Toxoplasma gondii's Initial Encounter With Its Host. *Front Cell Infect Microbiol.*; 9:46. doi: 10.3389/fcimb.2019.00046

Effects of sphingolipids derivatives on *Leishmania* spp. viability and virulence

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INTRODUCTION: Leishmaniasis is a neglected tropical disease that is caused by the protozoan parasite *Leishmania* a unicellular parasitic genus of kinetoplastids. Around 12 million people are affected by this disease worldwide (Vieneetha and Shailza, 2016). The parasite is transmitted by the bite of infected female phlebotomine sandflies that feed for blood. *Leishmania's* life cycle alternates between flagellated promastigotes living in the midgut of sandflies and non-flagellated amastigotes residing in mammalian phagocytes (Bates et al., 2004). Current disease treatment relies on substances characterized by significant toxicity, limited efficacy, and high cost (Zhang et al., 2012). Understanding parasite biology and host interaction in greater detail is expected to result in the discovery of new drug candidates. Sphingolipids (SL) are essential components of eukaryotic cell membranes with key roles in cell processes like signal transduction, intracellular membrane trafficking and the regulation of cell growth and survival (Denny et al., 2010). *Leishmania* species synthesize inositol phosphoryl ceramide (IPC) which is derived from ceramide as their primary phosphosphingolipids (PSL) which is distinct to the host cell SL (Denny et al., 2010). IPC metabolizing parasite enzymes such as Inositol phosphosphingolipid phospholipase C-like (ISCL) proteins are homologous to human neutral SMase and have a role in hydrolysis of both IPC and (host) SM (Zhang et al., 2012). Reverse genetic studies with parasites deficient in ISCL synthesis revealed a loss of pathogenic capacity (Zhang et al., 2010). Preliminary study of ceramide-based synthesized compounds against *Leishmania* showed that the drugs inhibit parasite's proliferation in vitro.

PURPOSE: Therefore, this study evaluates whether synthetic sphingolipid-derivatives can be used to affect *Leishmania* viability and pathogenicity and, if true, will aim at elucidating the molecular basis of this effect.

METHODS: Four candidate compounds (Ala-007, Ala-Diazirine, ES048, and C11AG) were synthesized and tested against both, promastigotes (extracellular form) and amastigotes (intracellular form) in the model of in vitro infected bone-marrow derived macrophages of C57BL/C mice. Furthermore, inhibitors cytotoxic effects evaluated at the concentration higher than IC50 values.

RESULTS: All four compounds inhibit leishmanial proliferation with IC50 between 0.8 and 1.9 µM. The results demonstrated that ceramide analogues did not show significant toxicity on host at the concentration above IC50.

CONCLUSION and OUTLOOK: Since candidate compounds targeting the SL metabolism show in vitro reactivity against *Leishmania* spp., the hypothesis that the ISCL enzyme may be the target is and will be tested and the mechanism of action further elucidated. In addition, parasite cell death will be studied at the ultrastructural level.

A base-exchange-type Phosphatidylserine synthase is essential for the lytic cycle of *Toxoplasma gondii*

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Phosphatidylserine (PtdSer) is a universal lipid, which is involved in a variety of functions such as, apoptosis, membrane potential, protein sorting and secretion in mammalian cells. Among these multiple functions, some depend on the acidic nature and negative charge of PtdSer, which allows it to interact with calcium and cationic domains of several proteins. In addition to these characteristics, its biophysical features help maintain the membrane potential across organelle membranes. Herein, we have identified the parasite enzyme underlying PtdSer synthesis in tachyzoites of *Toxoplasma gondii*, phosphatidylserine synthase (PSS). The protein localizes in the membranes of the endoplasmic reticulum. Our futile efforts to delete the PSS gene suggest its essential nature during the lytic cycle in accordance with its phenotypic score (-4.8) in ToxoDB. Conditional regulation of PSS by tetracycline or Shield1 significantly reduced the synthesis and amount of PtdSer in tachyzoites, corroborating its role in PtdSer biogenesis. Surprisingly, however, both mutants survived almost complete depletion of PSS concurrent with a reduction in PtdSer synthesis, likely due to compensation by other lipids including phosphatidylthreonine and posttranslational control of PSS activity. In future work, the PSS gene will be tagged with an auxin-inducible degradation (AID) domain to get a strain with a severe phenotype that will be useful for further characterization. Additionally, we are implementing a Cre-LoxP-based gene excision method to demonstrate the essentiality of the PSS gene. Our work also expands towards making a double mutant of PSS and PtdThr synthase to resolve the functional relationship between the two related lipids. Finally, importance of both phospholipids in regulation of calcium will be tested through a series of lipid/protein assays.

Giardia duodenalis induces barrier breakdown in human small intestinal organoids

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Giardia duodenalis represents a species complex of protozoan parasites causing acute and chronic intestinal disease worldwide. It is assumed that alterations of the intestinal barrier determine disease pattern and severity but current models to study this in vitro, such as CACO-2 cells, are limited and often show no or little direct impairment of barrier function. An improved in vitro model system for *G. duodenalis* infection is therefore highly desirable. For this purpose, we developed a two-dimensional, compartmentalized infection model based on human intestinal organoids. Here, we show that our organoid-derived monolayers are polarized, differentiated and form a physiological barrier between both compartments by expression of tight junctions. Infections of monolayers with *G. duodenalis* lead to barrier breakdown by alteration of tight junction complex. Transcriptome data using RNASeq has been generated and is currently analyzed to identify potential targets responsible for the parasite mediated effect on barrier function.

The role of bacterial communities in structuring mosquito populations

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Malaria is one of the most threatening infectious disease, causing about half a million deaths per year. This disease is caused by the unicellular eukaryotic parasite *Plasmodium* transmitted by *Anopheles* mosquitoes. The environment is a key factor influencing malaria transmission. By having an impact on mosquito`s life history traits, it influences vector competence, growth and development. One important environmental factor are the microbes which are mainly acquired from the environment. The microbial composition is among others made up of bacteria. Bacteria are necessary for the aquatic stages of mosquito development, and mosquito development is arrested in sterile or germ-free conditions [Coon KL et al., 2014]. Moreover, the bacterial community not only exerts a strong effect on the mosquito, but also on the parasite itself consequently affecting the transmission of the parasite.

Until now the composition and seasonal dynamics of bacterial communities of mosquitoes have not been investigated. To explore how bacterial communities and other factors drive mosquito populations, we performed time-series collections of aquatic stages of *Anopheles* mosquitoes during two consecutive rainy seasons in Mali. The bacteria composition, species, sex and *TEP1* will be determined. The results will be finally used in a mathematical modelling to determine relationships between mosquitoes and several ecological and genetical factors.

Ultimately, a better understanding of all aspects of vector ecology will inevitably lead to numerous new insights in the structuring of mosquito populations and such knowledge will also improve our understanding of malaria transmission.

Contribution of a CD8+ T cell epitope on pre-erythrocytic stage protection in a malaria mouse model

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Malaria is a vector-borne disease caused by the intracellular *Plasmodium* parasites, and is responsible for about half a million of deaths worldwide annually. This pathogen is transmitted among mammalian hosts *via* infected female *Anopheles* mosquitos, which are highly prevalent within the tropical and sub-tropical regions. Due to unsuccessful vaccine approaches, limitations in vector control, and the development of anti-malarial drug resistances over the years, malaria remains as one of the major threats to public health.

An important aspect of identifying vaccine candidate is to validate specific antigens as targets of acquired immunity. The expression profiles, roles and locations of the proteins that harbour antigens, which induce a $CD8^+$ T cell response, are very diverse. Previous studies have identified and described several H2-K^b-restricted $CD8^+$ T cell epitopes (including the pre-erythrocytic stage antigen epitope *P. berghei* sporozoite-specific gene 20; $S20_{318\text{-}326}$), but their contribution to protection has not yet been demonstrated. In this study, we developed a novel approach by immunizing a H2-K^b-restricted rodent model with irradiated *s20*(-) *Plasmodium berghei* sporozoites followed by a challenge with wild-type sporozoites in order to evaluate the significance of the epitope in protection against malarial infection.

Functional genome analysis in *Ixodes ricinus* ticks and tick cell lines

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Ixodes ricinus is arguably the most important tick species in Europe, with medical as well as veterinary relevance. It acts as a vector for a wide range of pathogens, including the causal agents of Lyme borreliosis and tick-borne encephalitis. Since the incidence of tick-borne diseases is predicted to rise further in the near future according to the European Center for Disease Prevention and Control, strategies to control tick-borne diseases are imperative. The elucidation of biological mechanisms associated with pathogen acquisition, maintenance and transmission by the tick, as well as tick biology in general, could facilitate the development of novel control strategies.

Currently, gene function analysis in ticks is mainly performed using gene silencing by RNA interference (RNAi). Although well established and successful, RNAi does have certain disadvantages as the effect is transient, difficult to establish in juvenile life stages and complete gene silencing is rarely achieved. The aim of this project is to improve methods of dsRNA delivery in tick larvae and nymphs for RNAi and to develop a CRISPR/Cas9-based gene editing method in tick cell lines and ticks as a novel tool for functional genome analysis. The CRISPR system has several advantages over RNAi and various ways to establish CRISPR in tick cell lines and ticks will be evaluated. Since the micro-injection of tick embryos is complicated due to the presence of a hard chorion and a unique wax layer, alternative delivery methods are looked at. This includes the microinjection of adult engorged females with CRISPR components followed by electroporation, as well as the direct electroporation of tick embryos. If successful, relevant tick genes will be evaluated in more detail to further study their function and role in host-pathogen interactions.

Keywords: Ixodes ricinus, CRISPR/Cas9, ticks, tick cell lines

Artificial feeding and infection of the hard tick *Ixodes ricinus*

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Ixodes ricinus is the most common hard tick species in Europe and a primary vector for zoonotic tick-borne diseases such as Lyme borreliosis and tick-borne encephalitis. A promising option for further investigations into the vector biology is the establishment of a robust artificial tick feeding system (ATFS). Although these are well established for soft ticks, which feed for a relatively short period, ATFS for hard ticks such as *I. ricinus* need to be refined further before they can form an appropriate alternative for the feeding of ticks on experimental animals. Furthermore, ATFS are a promising option for infection and transmission studies.

Laboratory-reared larvae and nymphs were fed by ATFS on sterile bovine blood. Briefly, the modified ATFS consists of a tick feeding unit, which measures 20 x 2.5 x 40mm and can be placed in a 12-well plate. The membrane on which ticks feed is based on a matrix of goldbeater's skin coated with a silicone mixture. The initial aim of this study is to close the life cycle of *I. ricinus in vitro* and compare the ATFS to the conventional *in vivo* feeding on experimental animals to evaluate the suitability of the ATFS for *I. ricinus* tick colony maintenance. During this study, critical points of the ATFS became apparent and will be analyzed to further optimize the system. Further studies are conducted to examine changes in the tick microbiome of ticks fed *in vivo* and *in vitro*. Studies to establish an *in vitro* (co-)infection model of *I. ricinus* larvae and nymphs with *Borrelia afzelii* and *Anaplasma phagocytophilum* will also be performed. Applications such as these form promising alternatives to *in vivo* models and may help to reduce the use of experimental animals in the future.

Serengeti Field Training Course

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On 23rd February a group of eight GRK Ph.D. students and four PIs set off on a journey on which they would explore a range of parasites in wild animals of the Serengeti National Park, Tanzania for 10 days within a framework of a field training course.

We present a brief overview of the individual projects – the Zebra nemabiome project (Irina Diekmann, Alexander Gerhard, Lubomir Bednar, Prof. Georg von Samson-Himmelstjerna, Prof. Emanuel Heitlinger), the tse-tse/trypanosomes project (Benjamin Hamid, Ankur Midha), the ELISA project (Ivet Yordanova, Benedikt Fabian, Prof. Susanne Hartmann) and the hyena project (Miguel Veiga), all benefitting from the experience of Richard Lucius. Technological highlights were the nanopore MinION sequencer and the DIY mobile app-based fluorescence ELISA system used for screening for *Toxoplasma* and *Giardia*.

We conclude that this course was a great success, most importantly due to our hosts Prof. Heribert Hofer and Dr. Marion East, but also as a result of the creative approaches arising from the initiative to apply cutting-edge technology to push the boundaries of conventional field research. Besides the training in field work the course was also a valuable cultural and ecological experience sharpening our perspective on the human-wildlife interface and the complexity and diversity of ecology and parasitology of wild animals.

Using non-invasive measures of intestinal immune response to better understand parasite heterogeneity and its impact on Darwinian fitness

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Studies on wild mammals often report heterogeneity across individuals in both parasite infection load and richness. Factors such as social status, lactation and age contribute to this heterogeneity in our study population of free-ranging spotted hyenas in the Serengeti National Park, Tanzania. Higher infection loads in low-ranking individuals during energetically costly life history stages such as lactation suggest that resource allocation trade-offs may influence an individual's ability to produce immune effectors. Other factors such as age, sex and allostatic load may also affect immunocompetence. To describe the immune phenotype of an individual across its life-span we need to determine how factors such as social status, age and life-history stage alter immune function and also how immune function alters in relation to parasite load and richness. To assess immune function non-invasively, we have developed and validated several faecal immune assays that measure different aspects of the mammalian intestinal immune system. These include measures of faecal secretory antibodies (sIgA and sIgG), *O*-linked oligosaccharides (mucin) and lysozyme concentration. We have also developed a faecal assay validated for the spotted hyena to measure glucocorticoid metabolites (fGCM). For each individual hyena in our study population, we have data on factors such as age, sex, social status and life-history data plus measures of gastrointestinal parasite load and richness. We will use this extensive data set to statistically investigate which factors shape immune function across the life-span of an individual and how immune function affects different components of Darwinian fitness (e.g. survival to adulthood, reproductive success and longevity).

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Gisder S, Schüler V, Horchler LL, Groth D, Genersch E (2017). Long-term temporal trends of *Nosema spp*. infection prevalence in Northeast Germany: Continuous spread of *Nosema ceranae*, an emerging pathogen of honey bees (*Apis mellifera*), but no general replacement of *Nosema apis*. *Front Cell Infect Microbiol.*; 7:301. doi: 10.3389/fcimb.2017.00301

High susceptibility to intestinal nematode infections is associated with delayed gut homing of Th2 cells and poor parasite-specific adaptive immune responses

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Gastrointestinal nematode infections are highly prevalent in nature, lead to considerable morbidity in infected humans and cause considerable economical loss in animal husbandry. While it is clear that type 2 responses orchestrated by $CD4^{+}GATA\text{-}3^{+}$ Th2 cells are pivotal for the control of GI nematodes, the basis for differential susceptibility of distinct host genetic backgrounds is less well understood. We hence investigated Th2 responses and innate type 2 associated effector mechanisms in inbred mouse lines displaying slow, fast or rapid expulsion of adult worms in infections with the small intestinal nematode *H. polygyrus*. Surveying the phenotype of CD4+ T cells in lymphatic organs, we unexpectedly detected similar or even higher magnitudes of Th2 responses in infected slow responder C57BL/6 mice compared to fast and rapid responder mice (BALB/c, SJL). However, infected BL/6 mice displayed a delay in the migration of Th2 cells to the small intestine, possibly related to the poor expression of the a4b7 integrin by Th2 cells in this mouse line. Slower mucosal homing of Th2 cells coincided with the poor expression of the M2 markers arginase-1/RELM-a and a delayed upregulation of RELM-b in C57BL/6 mice early during infection. The accumulation of small intestinal Th2 cells reached similar levels in BL/6 mice as in the fast and rapid responder lines as the infection proceeded, but BL/6 mice maintained high worm burdens. We hence assessed adaptive immune responses for functional differences and found that the Th2 cell population generated by BALB/c and SJL mice comprised significantly higher proportions of parasite-specific cells allowing for the accelerated generation of parasite-specific antibody responses. In conclusion, susceptibility versus resistance to primary worm infections appears to be less dependent on the overall magnitude of Th2 responses, but rather result from differential kinetics of Th2 cell gut homing and the quality of the parasite-specific adaptive responses.

Affinass, N.; Zhang, H.; Löhning, M.; Hartmann, S.; Rausch, S. (2018). Manipulation of the balance between Th2 and Th2/1 hybrid cells affects parasite nematode fitness in mice. Eur J Immunol 48:1958-64. doi:10.1002/eji.201847639

Response of porcine dendritic cell subsets to *Toxoplasma gondii* and *Ascaris suum*

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B04 Gen 1: Norus Ahmed

Toxoplasma gondii tachyzoites can invade any nucleated cell, however their interaction with dendritic cells (DCs) is particularly important. On one hand, the parasite is able to modulate DCs to induce an ameboid-like hypermigratory phenotype, which they utilize to disseminate throughout the host. On the other, DCs are among the first immune cells to encounter the parasite in the intestinal lamina propria and are essential for initiation of the host immune response. Thus, understanding the interaction between *T. gondii* and host DCs is vital for understanding the early stages of Toxoplasmosis.

To date the majority of research into *T. gondii* infection has been conducted in mice. However, the interaction between the parasite and host DCs differs considerably between mice and humans. In mice, the cDC2 and pDC subsets produce IL-12 and TNF-a following parasite recognition, whereas in humans only the cDC1 subset responds. Furthermore, murine DCs are able to recognize the soluble *T. gondii* antigen profilin extracellularly via TLRs 11 and 12. In contrast these TLRs are absent in humans, with parasite recognition requiring phagocytosis of a live tachyzoite. These differences necessitate the development of a more human-like animal model, for which the pig is an ideal candidate.

Here I aim to characterize the responsiveness of porcine peripheral blood DC and monocyte subsets to *T. gondii* infection, as well as identify the likely recognition mechanism. Porcine PBMCs were MACS-depleted for lymphocyte lineage marker expressing cells, and cDC1, cDC2 and pDC subsets isolated by FACS. MoDCs were also generated. Each cell subset will be cultured and treated *in vitro* with live and heat-killed tachyzoites, soluble *T. gondii* antigen and recombinant profilin. This will determine whether the response of porcine DCs resembles that of humans, therefore representing a more relevant model than the mouse. I am also working to characterise the phenotype of each porcine DC subset following exposure to the helminth *Ascaris suum.* Ultimately, any immunomodulatory effect of *T. gondii* or *A. suum* pre-exposure on the subsequent response to the other parasite will be evaluated.

Delgado Betancourt, E.; Hamid, B.; Fabian, B. T.; Klotz, C.; Hartmann, S.; Seeber, F. (2019). From Entry to Early Dissemination-Toxoplasma gondii's Initial Encounter With Its Host. *Front Cell Infect Microbiol.*; 9:46. doi: 10.3389/fcimb.2019.00046

Cyathostomin population diversity in treated and untreated equine hosts with different geographical background

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Cyathostomins belong to the nematoda and represent an exceptional multi-species complex. Based on morphological classification 14 genera exist with in total 50 species. Mixed infections are the rule with up to 29 species described for a single horse. To date, little is known about the species-specific pathogenicity or the interdependencies between these species concerning prevalence or pathogenicity. The morphological determination of cyathostomins is limited to the identification of adult worms and can reliably performed only by few experts worldwide. One part of the project will be the continuation of the previous project, which aimed at the single species identification with more universally applicable methods to extend the currently available molecular reference database. To identify the population composition of the different species, 169 cyathostomin larval cultures were prepared from different equines originating from different countries (Germany, Brazil, Kentucky, Scotland, France and Ukraine) with different treatment history. The DNA of the larval pool sample of each equine was amplified with a metabarcode primer. During larval culturing potential biases can occur due to species-specific differences in larval development. To examine such potential biases, samples from 10 horses coming from ten different farms in Germany were collected. After larval culture the DNA of the larval pool sample but also of the eggs was extracted and also amplified with a metabarcode primer. All samples will be then sequenced using Illumina MiSeq 2x300 bp sequencing, value package 20- 25 Mio read pairs. The processing of the MiSeq system with 13x13 different index primers will make it possible to identify to which animal each individual sequence belongs. All haplotypes in the entire data set will be identified, the number of different haplotypes per animal will be determined and haplotypes associated with different parasite species will be identified. Next generation sequencing enables an analysis of population dynamics and diversity in cyathostomin populations with different geographical and treatment background.

Th2/1 hybrid cells seem to limit the control of gastrointestinal nematodes via IFN-γ production and the regulation of innate immune cells

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Infections by gastrointestinal nematodes elicit type 2 immune response characterized by the differentiation of GATA-3+ Th2 cells producing IL-4, IL-5 and IL-13. Our group has previously shown that next to classical Th2 cells, *H. polygyrus* infections also leads to the differentiation of Th2/1 hybrid cells characterized by the co-production of IFN-γ with type 2 cytokines. Th2/1 cells can be expanded via IFN-γ supplementation during the priming phase of anti-parasite T cell responses, which leads to impaired control of the infection in C57BL/6 mice. Hence, we addressed the following questions: i) Are Th2/1 cells restricting the control of *H. polygyrus* infection via IFN-γ production? ii) Can we convert relatively resistant BALB/c mice to a more susceptible phenotype by expanding their Th2/1 responses? iii) Which type 2 immune parameters are affected by differential Th2/1 responses?

Comparing C57BL/6 wild type (WT) and IFN-γ $^{-/-}$ mice we found that the application of rIFN-γ early during infection drastically increased Th2/1 cell differentiation in both mouse lines. However, stronger Th2/1 hybrid responses only impaired control of the parasites in WT mice. By contrast, IFN-γ $^{-/-}$ Th2/1 cells although expanded failed to affect the control of infection. We next applied rIFN-γ to BALB/c mice, which normally display relatively poor Th2/1 responses and control the infection more efficiently compared to C57BL/6 mice. Again, Th2/1 responses were sharply increased in response to treatment and coincided with higher parasite egg production. Hence, Th2/1 cells appear to restrict the control of the parasite, at least in part, by the production of IFN-γ and seem to convert BALB/c mice to a more susceptible phenotype. Furthermore, our preliminary data on type 2 effector mechanisms suggest that strong Th2/1 hybrid responses are associated with reduced PD-L1 expression by macrophages and poor mast cell degranulation, possibly creating an environment permissive for increased parasite fitness and prolonged worm infection.

Acknowledgements

CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN

ROBERT KOCH INSTITUT

Freie Universität Berlin

HUMBOLDT-UNIVERSITÄT ZU BERLIN

MAX-PLANCK-GESELLSCHAFT

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DFG Deutsche Forschungsgemeinschaft

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