ZIBI Graduate School

Project description

Project Title: Comparative analysis of innate immune responses of intestinal organoids from different rodents infected with *Toxoplasma gondii*

Research Group: RKI Unit 16: Mycotic and Parasitic Agents and Mycobacteria
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Project Description:

***Toxoplasma gondii**'s* route of infection is via the oral/intestinal route but current knowledge how this proceeds in detail and which intestinal innate immune factors are determining the success of infection are only vaguely known. Almost all studies dealing with intestinal *T. gondii* infections were done in inbred C57/BL6 mice since they are highly susceptible to oral infection by cysts and which results in gut pathology. However, another inbred mouse strain, BALB/c, are resistant under the same conditions. This indicates large differences even in the same host species and requires careful consideration of rodent models for specific questions (1).
Nothing in this respect is known in wild house mice or **wild rodents** (voles and field mice, i.e. *Microtus* sp., *Myodes* sp., and *Apodemus* sp.). However, these rodents could be an important reservoir for so-called "virulent" *T. gondii* strains, as proposed for the house mouse, *Mus musculus*, since in Europe they are usually much more consumed as prey by cats (the definitive host for *T. gondii*) than house mice are. Consequently, their contribution to maintain these virulent strains in nature could be more important than the postulated role of house mice in this process (2).
Therefore, this project aims to study the infection with *T. gondii* (and later on with another intestinal parasite, *Giardia duodenalis*) of '**intestinal organoids' (IOs)** (i.e. intestinal small organ-like structures derived from intestinal stem cells) from named wild rodents in comparison to wild mice and lab mice with regard to innate immune responses. Using IOs over living animals for this purpose has several distinct experimental advantages, like microscopic imaging of infection processes or variation/manipulation of the intestinal microbiota (3).

Two major questions will be addressed:
(i) *What role do the IFNg-inducible immunity-related GTPases (IRGs) play during intestinal infection, and (ii) is the reported death of Paneth cells in T. gondii-infected C57/BL6 mice also seen in wild mice/rodents, and what is its mechanism?*

The approaches that will be taken are:
- generation of IOs from mentioned rodents using protocols and reagents well established in the lab
- analysis by microscopy (live cell imaging, IFA, EM) of the course of infection of rodent by *T. gondii* strains of different virulence for different parameters (e.g. which cell types are infected; replication in dependence on IFNg; induction of innate immune response genes like IRGs, etc.)
- generation and comparison of dual RNAseq transcriptomes of infected IOs; with initial analysis focused on IFNg-induced transcripts

References: (1) Ehret, T., Torelli, F., Klotz, C., Pedersen, A.B., and Seeber, F. (2017). Translational Rodent Models for Research on Parasitic Protozoa-A Review of Confounders and Possibilities. *Front Cell Infect Microbiol* 7**,** 238. (2) Lilue, J., Müller, U.B., Steinfeldt, T., and Howard, J.C. (2013). Reciprocal virulence and resistance polymorphism in the relationship between Toxoplasma gondii and the house mouse. *eLife 2, e01298*. (3) Klotz C, Aebischer T, Seeber F. (*2012)* Stem cell-derived cell cultures and organoids for protozoan parasite propagation and studying host-parasite interaction*. Int J Med Microbiol 302, 203-9.*