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Training on *in vitro* culture of immortalised human skeletal muscle cells and infection with *Toxoplasma gondii*, Robert Koch-Institut, Berlin, Germany, 13th – 24th May 2024

Attending the 17th International Congress on Toxoplasmosis, Berlin, Germany, 26th – 29th May 2024

I was fortunate to receive a grant from the Royal Society of Biology, which enabled me to travel to Berlin and pursue two invaluable opportunities. This included a laboratory visit with the Blume research group based at the Robert Koch-Institut, wherein I learnt key skills supporting the advancement of my PhD project. It simultaneously facilitated my attendance of the 17th International Congress on Toxoplasmosis, the leading conference in the field that my PhD falls within.

I am a first year PhD student at the University of Edinburgh and my project focuses on host-pathogen interactions during the chronic stage of *Toxoplasma gondii* infection. Approximately a third of the global population is chronically infected with the parasite *Toxoplasma gondii*, and there are currently no vaccines for use in humans or approved drugs against the chronic stage. Improving our understanding of this stage is vital, as re-emergence of the parasite in immunocompromised people can be fatal. Within my project, I aim to identify *Toxoplasma* proteins that are essential for chronic infection, as this will inform future efforts to control the chronic stage of infection. I aim to achieve this using a CRISPR screen of parasite mutants in conjunction with an improved *in vitro* model for chronic *Toxoplasma* infection which uses immortalised human skeletal muscle cells (KD3 cells).

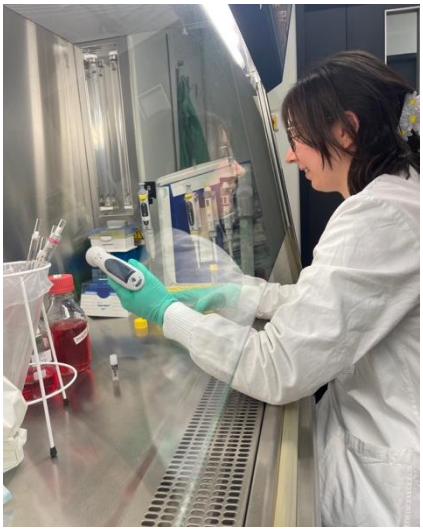
This method was developed by the Blume research group and has allowed them to study chronic *Toxoplasma* infection for a longer period (3+ months) compared to the standard *in vitro* method, which utilises alkaline pH to promote parasite conversion and thus limits growth to 1-3 weeks. However, this cell culture is complicated to maintain and differentiate; therefore, having the opportunity to train directly from the laboratory which developed a successful protocol was an invaluable opportunity for my PhD project.

During my two weeks training with the Blume research group, I learnt how to perform:

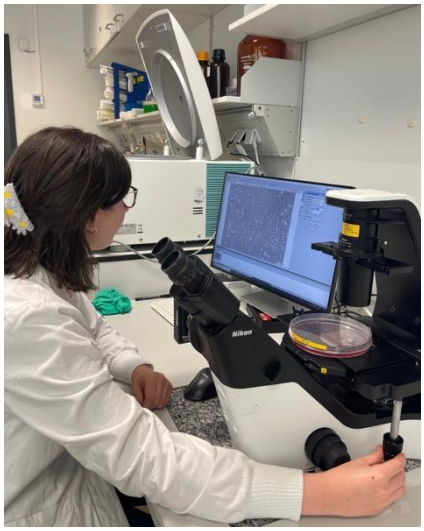
- Thawing, continuous passage and freezing of KD3 myoblasts (immature muscle cells)
- Induction of differentiation to KD3 myotubes (mature muscle cells)
- Pepsin digestion of 3-month-old *Toxoplasma* cysts within KD3 myotubes and recovery of viable parasites

This has enabled me to feel confident in performing these techniques and allow me to successfully implement them in my laboratory in Edinburgh. More specifically, this training will be particularly valuable for the CRISPR/Cas9-based genetic screen I will perform on *Toxoplasma*-infected skeletal muscle cells, which is central to my PhD project.

Outside the Robert Koch-Institut, Berlin



Culturing the KD3 myoblasts within the lab



Imaging of KD3 myotubes under the microscope within the lab

While in the lab, I was also able to look at the KD3 cells under the microscope daily and get a good understanding of what the myoblasts should look like, as they need to be kept at a low coverage to maintain their ability to rapidly divide and eventually successfully differentiate to myotubes. I was also able to follow the process of myotube differentiation and observed the morphological changes that take place during this process, enabling me to determine when they successfully achieve a mature state ready for infection with *Toxoplasma* when I implement this system in Edinburgh.

During my time spent with members of the Blume research group, I had the opportunity to deeply discuss the process of culturing this specialised cell system and learn the intricacies of working with this system, which are not necessarily clear in the published literature. This allowed me to understand what difficulties they faced whilst setting up this culture system and how they overcame them, which was incredibly beneficial as it will cut down on the optimisation and troubleshooting time required to set up the system in my laboratory in Edinburgh.

Since returning to Edinburgh, I plan to create a standard protocol and system for working with this cell line, for both myself and other lab members interested in working with it in the future, create stocks of the KD3 cells, generously provided by the Blume group, and then use this model for my CRIPSR screen which I am eager to commence. Additionally, through my time spent with their group, I have developed strong connections for future collaborations and an open communication link for any further questions I may have whilst initiating the system within my research base in Edinburgh. Furthermore, my time within the Robert Koch-Institut has provided me with experience working within a foreign laboratory, wherein I was able to observe similarities and differences between both lab and community cultures, expanded my horizons on academia globally, and developed my professional skillset.

In addition to this training, I had the opportunity to attend the 17th International Congress on Toxoplasmosis which also took place in Berlin. This is the leading conference in the field, which occurs biannually, and has grown from just 20 people when it originated to now hosting over 230 people in attendance. It was a privilege to hear presentations on ongoing and unpublished research, which covered a range of topics including epidemiology, parasite-host interactions, immunology, drug development and stage conversion of *Toxoplasma*. Due to the extensive research presented, I was

exposed to areas which I have limited experience in, including the state of Toxoplasmosis in South America and how different clonal strains which are distributed differentially across the globe impact symptoms of infection, which I found fascinating. I also listened to several presentations which discussed research involving CRISPR screens and found this valuable to inform my own research project.

Multiple poster sessions over the course of the conference enabled me to network with a range of other early career researchers based in laboratories across the world. This facilitated the exchange of knowledge and ideas regarding different aspects of *Toxoplasma* research, beyond what I have had the opportunity thus far to consider. It was furthermore uplifting and encouraging, despite my initial nerves, to have conversations about my PhD project with leading members of the community who conveyed great enthusiasm about my work, ideas, and future.

Overall, I had an incredible experience in my first international academic trip and have gained much from both the training at the Robert Koch-Institut and the Toxoplasmosis conference. I am now looking forward to implementing everything I have learnt from this opportunity afforded to me through funding from the Royal Society of Biology to my PhD research project.