Training Report

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This PhD project is based on a partnership and a collaboration agreement between the University of Surrey, Guilford, UK and the University of Balamand, koura, Lebanon. I work under the supervision of Dr. Kamalan Jeevaratnam (k.jeevaratnam@surrey.ac.uk), Head of Department of Veterinary Pre-Clinical Sciences & Programme Director BVMSci, as main supervisor and Dr Kikki Bodman-Smith (K.Bodman-Smith@surrey.ac.uk) , School of Biosciences and Medicine Director of Learning and Teaching, Senior Teaching Fellow in Immunology, as co-supervisor. Since I am an overseas student, most of the experiments will be performed at the University of Balamand (the host institution), Faculty of Arts and Sciences, under the supervision of Dr. Marc Karam(marckaram1@gmail.com), an Immunologist and Associate professor, as local supervisor from the University of Balamand and Dr. Mirna Chahine (mirnachahine01@gmail.com), a Professor of Cardiovascular Physiology and Director of Basic Sciences Research & Coordinator at F-MRI, as local co-supervisor from the Lebanese University, Faculty of Medical Sciences, Al Hadath.

Project Overview:

- **Title:** The effect of *Leishmania major* infection on atherogenesis and cytokine responses in resistant and susceptible mice

- **Abstract:** Atherosclerosis, the major underlying pathology responsible for cardiovascular diseases, was traditionally considered as a lipid disorder in which the vascular wall becomes filled up with lipids. Currently, it is widely accepted that an immune response, mediated mainly by pro-inflammatory cytokines, plays an important role in atherogenesis. Therefore, any condition (such as infection) that involves such cytokines might induce or exacerbate atherosclerotic plaque formation. In this project, we will use the parasitic protozoa *Leishmania major* as the infectious model because many hosts, such as humans and C57BL/6 mice are resistant to such infection, so, the production of pro-inflammatory cytokines during infection, may promote atherogenesis. In the current study, C57BL/6 mice fed with high fat diet will be infected with *L. major* in the presence or the absence of TH1 blocker which suppresses the production of Th1 cytokines (proinflammatory cytokines). The plasma, aorta, spleen, heart and paw skin will be collected from these mice for analysis. The levels of IFN-γ, TNF-α, IL-4, IL-10, IL-13 and IL-17 will be determined using ELISA along with the measurement of the aortic atherosclerotic lesions. We expect to prove an up-regulation of some pro-inflammatory cytokines such as TNF-α, IFN-γ and IL-17 and a down-regulation of some anti-inflammatory cytokines such as IL-4, IL-10 and IL-13 in aortas, paws skin, and spleen of the mice. This cytokine interplay would favor atherogenesis which will be clear when assessing the atherosclerotic lesion size in the aorta of the different groups.

- **Research plan:**

Phase 1:

1. Induction of atherosclerosis for 15 weeks in 4 groups of C57BL/6 mice:
   NF →7 males and 7 females fed on normal/low fat diet
   NFL → 7 males and 7 females fed on low fat diet + infected with *Leishmania major* parasites
   HF → 14 mice (7 males and 7 females) fed on high fat diet (TD90221, Teklad)
   HFL → 14 mice fed on high fat diet+ infected with *Leshmania major* parasites
2. Sacrifice at week0: 14 mice= 7 males and 7 females as controls for all the groups
   Week5: 14 mice from each group =>14*4= 56 mice
   Week10: 14*4= 56 mice
   Week15: 14*4= 56 mice
3. Removal of blood, hearts, spleens, aortas, and paw skin
4. Isolation and storage of plasma for ELISA
5. Performance of ELISA for the abdominal part of the aorta, paws skin, hearts and spleens in order to measure the level of both Th1 and Th2 cytokines
6. Performance of Immunohistochemistry on aortas and hearts
7. Haematoxylin/ Eosin staining to assess degree of necrotic cardiac lesions on hearts samples embedded in O. C. T medium, frozen at -20°C, sectioned transversally using cryostat into consecutive 5µm thick sections and thaw-mounted onto glass slides.
8. Assessment of atherosclerosis

In the **phase 2** of the project we will follow the same procedures with the same number of mice **but** with the addition of Th1 blocker in order to suppress the production of Th1 proinflammatory cytokines and study its effect on atherogenesis.

Project timeline:

1. September 2019 to March 2020- ordering Kits+ Diets+ Blockers+ Antibodies + C57 mice
2. April to May 2020- Inducing atherosclerosis (high fat diet) and cutaneous Leishmaniasis in 8 groups of C57BL/6 mice with/ without administration of Th1 cells blocker
3. End of May 2020: Sacrificing of all 8 groups and storage of plasma and tissues in -80^{0}C.
4. June 2020- March 2021- Proceeding with all the required tests (Immunohistochemistry, staining, western blot, ELISA …)
5. April 2021 to August 2021: Writing of PhD thesis and 2 papers

The outcomes:

According to the scheduled timetable for the project and the objectives that were previously set, we were supposed to order the diets and mice between September 2019 and March 2020 and start the first phase of the project in January 2020. Unfortunately, due to the difficulties and the latest events we have faced in Lebanon, I did not have access to the laboratory during October and November 2019. In addition, because of the economic crisis and the inability to transfer money abroad, the order of the diets was delayed until the end of January 2020.
In addition, the institution that provides us with mice experienced logistical complications in the animal house, which has affected the normal breeding of mice and, as a result, it has delayed the set-up of our experiments. To address this issue and to save time, we built a partnership with the American University of Beirut to supply us with the required quantity of

mice and facilities. But, according to their policies it is forbidden to take female C57BL/6 mice outside their animal facility,so we were provided with only males. Finally, the COVID-19 pandemic caused the suspension of this collaboration until December 2020. During this period, to reduce the spread of this pandemic we underwent a total lockdown (since March 2020) and, as a result I suspended my registration at the University of Surrey a few times, but have participated in a few training sessions including: how to handle animals, blood and tissue extraction from mice and *Leishmania major* parasite culture. In addition, I have attended some online workshops such as: "Data Management plans", "Intellectual Property Master class: infection and immunity", "Research Funding: the basics", "Introduction to SPSS", and "Introduction to R studio". Furthermore, during this period I have taken an online course from Harvard University: "Principles, Statistical and Computational Tools for Reproducible Data Science".

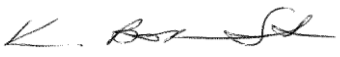
Finally, between January and February this year, we procured the required number of mice (84 males) for the first phase of the project; I have divided them into 4 groups:

1. NF= 21 mice, control, fed normal/ low fat diet, untreated
2. NFL= 21 mice, fed on normal/low fat diet but injected with *L. major* parasites
3. HF= 21 mice fed on high fat diet only
4. HFL= 21 mice fed on high fat diet+ injected with *L. major* parasites

At three different time points, 5 weeks, 10 weeks and 15 weeks, 7 mice from each group will be sacrificed; their blood and tissues will be removed for further analyses.

At time t0 of the experiment, I have sacrificed 7 mice that are considered as control for this phase and I have collected their blood, hearts, spleens, aortas and paw skin and stored them for further usage.

At time t= 5 weeks (2 April 2021), I have sacrificed 28 mice (7mice from each group) and I have repeated the same procedure.

Supervisor at the time of the award
Dr Kikki Bodman-Smith signature: 
Student's signature: *Amany Chahine*
Date: 11th May 2021