Sample SWEP Proposal

Assessing cardiac macrophage phenotypes in a monocyte deficient mouse model of myocardial infarction

Project Overview

In the heart specialized innate immune cells, known as macrophages, play important roles in mediating the inflammatory and anti-inflammatory response to injury, such as a heart attack. A subset of tissue resident macrophages are important for maintaining proper function of the heart and participate in the healing response by limiting adverse remodelling. These resident macrophages were originally thought to come from, and be continuously replaced by, circulating monocytes originating from the bone marrow. Thanks to recent advancements in our ability to label and track these cells, we and others have shown that a majority of macrophages come from embryonic sources such as the yolk sac and fetal liver and are maintained independent of monocytes over an entire lifespan via local self-renewal. Self-renewal capacity is most often associated with stem cell populations that replace fully differentiated cells for homeostatic maintenance and repair. However, we have now redefined this paradigm by identifying a fully differentiated cell type that is capable of life-long self-renewal capacity.

Recently, we have identified colony stimulating factor 1 (CSF1) as the major growth factor used by tissue resident macrophages for their self-renewal and expansion in the setting of injury. Blocking this signalling axis via a small molecule inhibitor of the CSF1 receptor (CSF1R) demonstrated a loss of resident macrophage numbers and cardiac function in a mouse model of a heart attack, as known as myocardial infarction (MI). We hypothesized exogenous CSF1 administration would enhance resident macrophage numbers and cardiac repair, however recruited, inflammatory monocytes and monocyte-derived macrophages also depend on CSF1 signalling for their survival and differentiation. Thus, there is competition for resources in the inflamed myocardium between reparative resident macrophages and inflammatory recruited macrophages. In support of this, when we limited monocyte numbers in a genetic mouse model of monocyte depletion (CCR2KO), we observed an increase in resident macrophage proliferation and expansion following MI, suggesting monocytes are competing for the same growth factor to elicit very different functions. We predict this competition for resources is a major contributing factor by which monocyte recruitment limits myocardial repair following injury. To experimentally address this, we developed a monocyte specific CSF1R KO mouse [$CCR2^{CreER/+}$:$CSF1R^{flx/flx}$] which we will use to limit monocytes ability to bind this growth factor locally in the heart. By using the newly developed tamoxifen inducible CCR2-CreER line we can specifically address the requirement of CSF1R signalling during emergency hematopoiesis (induced by tissue injury) without the developmental defects seen with global CSF1R KO.

This project will involve *in vivo* experiments to assess the requirement of CSF1R signalling for monocyte survival, differentiation, and proliferation during a state of emergency hematopoiesis. The student will be responsible

for the initial characterization of the CCR2$^{CreER/+}$:CSF1R$^{flx/flx}$ mouse model with the ultimate goal of understanding the different ways resident and recruited macrophage respond to the same growth factor in the setting of injury, and to introduce novel tools to the field to limit monocyte differentiation while promoting resident macrophage expansion. This is important, as a major limitation in CSF1-based therapies is that they promote both the pro-reparative functions of resident macrophages while also promoting the differentiation of inflammatory recruited macrophages, leading to confounding results. Overall, this research will address the gap in our knowledge of the specific function of monocyte CSF1R signalling in emergency hematopoiesis in a cell-specific inducible manner.

Relevance to Science, Queen's and the community:

Heart failure is a chronic debilitating condition affecting at least 1 million Canadians, with almost 50% mortality in those affected within 5 years of diagnosis. One major cause of heart failure is myocardial ischemia brought on by narrowing of the coronary arteries or atherosclerotic plaque rupture and complete occlusion of the arteries that carry blood to the heart, also known as a heart attack. It is critical to understand the role of the immune system in cardiac healing following a heart attack, as chronic inflammation can worsen heart failure outcomes. However, generalized anti-inflammatory drugs often fail to have a positive effect. We identified a novel subset of these cells that are critical for limiting the adverse remodeling of the heart, such as fibrosis and hypertrophy, that is important to limit the progression to heart failure. This project will advance our understanding of these novel cell types and how they are maintained in the heart, using sophisticated genetic mouse models, which is a prerequisite for our ability to enhance the expansion of these pro-reparative cells in the setting of disease. The long-term goal of this project will be to accelerate our knowledge of tissue immunology to develop the next generation of immunotherapies to treat heart disease.

This project will benefit from close involvement with the Queen's Cardiopulmonary Unit (Q-CPU), a $10 million CFI-funded centre for translational research in cardiovascular disease, providing students access to cutting-edge infrastructure, including state-of-the-art facilities for microscopy. The lab also has access to several core facilities and equipment withing the Department of Biomedical and Molecular Science including flow cytometry, Imaging, and single cell RNA sequencing. These cores provide additional expert training and support to trainees in these techniques.

Role:

The student will perform experiments on mouse tissues to determine the relative number, phenotype, and proliferation rate of macrophage subsets in the heart. This will be accomplished using intercellular BrdU staining by flow cytometry during a state of emergency hematopoiesis stimulated by a surgical model of myocardial infarction.

Specifically, the student will be required to (1) inject mice with Tamoxifen to induce CSF1R deletion in CCR2+ monocytes; (2) collect blood and isolate RNA for qPCR analysis to confirm gene knockdown; (3) Coordinate with animal technician to perform MI surgeries and participate in post-op care of the animals; (4) on day of tissue isolation, inject mice with BrdU to label actively dividing DNA and isolate heart tissue from mice and either fix for histology or digest to get a single cell suspension for flow cytometry; (5) Flow: stain the single cell suspension with antibodies to label the BrdU and macrophage cell surface markers and perform flow cytometry; (6) Histology: embed and section the fixed tissue and label with H&E or Sirius Red/Fast Green stain for measure hypertrophy and fibrosis, respectively; (7) compile all data and perform statistical analysis.

Responsibilities:

The student will be responsible for managing and executing their project in a professional and timely manner. This includes planning of experiments, learning required techniques, performing the experiments, and presenting their data at lab meetings.

Through these activities the student will gain valuable first-hand experience in mouse handling; preparing reagents; tissue dissection and dissociation; flow cytometry; microscopy; and data and statistical analysis. They will benefit from 1-on-1 training in the lab to ensure their success in all aspects of their project and mentored on experimental design and critical thinking.

Required Qualifications:

At the time of holding the award, the student must have completed the first two years of a program in Life Sciences, Biochemistry, or a related discipline.

- Have strong attention to detail and be able to integrate new knowledge into daily activities.
- Self-motivated with a high degree of independence.
- Ability to manage time effectively and meet deadlines
- Some undergraduate coursework in Biology and Life Sciences, including basic understanding of cell biology and immunology is required
- Must be willing to handle mice and perform tissue dissection

Learning Plan

The student will be able to develop hands-on practical lab skills as well as knowledge in immunology and cardiac biology which will form a foundation for understanding the role for immunotherapies for treating cardiovascular disease. This will provide them with the skills and training required for a career in biomedical research, including very specialized experience in tissue immunology. They will be a part of a diverse team in the Dick lab, which is a collaborative and inclusive environment conducive to learning and research. In the lab each member will be treated and treat others with respect. This will ensure the lab remains a place where everyone feels welcome and supported.

During their 16 weeks in the lab, the student will acquire the following **technical** skills:

1. Animal handling and injection
2. Tissue dissection and processing
3. Analytical flow cytometry
4. qPCR
5. Histology

They will also acquire the following **professional** skills through their participation in weekly lab meetings and journal clubs where the candidate will be expected to summarize the results of their research and discuss recent publications in the field:

1. Data analysis and interpretation
2. Critical thinking
3. Oral presentation
4. Time management
5. Research method development

Project Aims

The student will begin to understand the importance of CSF1R signalling in recruited monocytes during periods of emergency hematopoiesis such as during a myocardial infarction. This will be done by **first** confirming the knockdown of the CSF1R in monocytes using qPCR. **Second**, we will use flow cytometry to look at monocyte and macrophages in the blood and heart tissue to determine affect of knockdown on numbers, polarization markers, and proliferation. And **third**, we will isolate some tissue for histology to look at how CSF1R depletion within the recruited subsets affects myocyte hypertrophy and interstitial fibrosis.

We hypothesize that monocyte depletion of CSF1R will limit their differentiation into inflammatory macrophage subsets and increase the available growth factor for resident subsets resulting in their enhanced proliferation, as we have previously shown in the CCR2KO mouse model. This has important implications, as we continue to elucidate the *in vivo* functions of CSF1 signalling in emergency hematopoiesis in a cell type-dependent manner. Future work will involve the development of monocyte-specific delivery of drugs to inhibit this pathway for treatment in individuals suffering from a heart attack.

Detailed learning plan divided into 5 discrete segments:

- Segment 1 (2 weeks): Complete all training. Become familiar with mouse lines, tissue dissection, dissociation, and flow cytometry.
- Segment 2 (2 weeks): Perform tamoxifen injection to adult mice and isolate blood to confirm knockdown of CSF1R by flow cytometry and qPCR.
- Segment 3 (6 weeks): Once knockdown is confirmed, induce a myocardial infarction (surgery to be perform by animal technician) and isolate hearts from mice at early (D3), mid (D7), and late (D14) time points post-injury and perform intercellular BrdU staining on single cell suspensions and analyze on flow cytometer to determine cell numbers and proliferation rate.
- Segment 4 (4 weeks): Use fixed tissue from previous isolations to perform histology using H&E and Sirius Red/Fast Green staining. This includes tissue embedding, sectioning, staining, and imaging.
- Segment 5 (2 weeks): Compile results and perform statistical analysis.

The candidate will be provided with substantial support to ensure the successful completion of this project. Being a recently established lab, they will have the added benefit of learning directly from the supervisor and get to experience the excitement of starting a new academic lab. Furthermore, integration with the QCPU and core facilities at DBMS will give the students access to the best training on state-of-the-art equipment. They will also have the opportunity to participate in group meetings to discuss their project and get feedback from a broad range of faculty at Queen's University, further enhancing their presentation and critical thinking skills.