Scientific Progress Report Migrated - Scientific Progress Report Migrated

Award ID	**17PRE33410275**	Project Title	**Roles of cardiac and hepatic macrophage subsets in immune tolerance**
Grantee	**Soysa, Dilini Radika**	Institution	**University of Washington**
Award Start	**01/01/2017**	Award End	**06/30/2019**

***1. Please provide a concise summary of progress for each specific aim of your project during this reporting period. If this is your final report, include an overall summary of the project achievements. Note: The suggested length for the Progress Report is approximately 2 pages. You can copy and paste from another document or type responses in the boxes below.**

1. For each aim, describe or summarize the following:
a) Major completed or ongoing activities;
b) Significant results, including major findings, developments, or conclusions (both positive and negative);
c) Discussion of stated goals not met or problems you have encountered and how they were resolved.

Progress report

In this project, we aimed to understand the function of embryo-derived macrophages in heart and liver. Specifically, we proposed to test their response to acute inflammation and in tolerogenic settings.

In Aim1 we proposed to label the embryo-derived macrophages (emMQ) in liver and heart and analyze the immune functions through gene expression. Due to the lack of surface markers to identify ontogenetically distinct macrophage subsets in both organs we used an inducible fate-mapping model based on Cx3cr1CreER and RiboTag reporter to label each subset. Originally we proposed to do tamoxifen inductions in pregnant females during gestation to label the embryonic subset. Due to the complications at birth due to tamoxifen and not being able to produce sizable litters to do statistically comparable experiments we sought out an alternative strategy to label emMQs.

We have developed an alternative model, a neonatal induction strategy where 2- day old neonates are tamoxifen-induced to label emMQs that are long-lived into adulthood (Figure 1). Using this approach we now show that in the adult mouse, 45% cardiac MQs and 37% of hepatic MQs are derived during embryonic stages. We have used RiboTag approach in which mRNA is isolated in situ using polysome immunoprecipitation bypassing the cell isolation to retrieve the MQ specific mRNA. We used RNAseq to analyze the translatome in those embryo-derived cardiac and hepatic MQs (Figure 2). The embryonic MQ subsets in liver and heart have a tissue-specific identity at steady state. Pathway analysis indicated that emMQs in the heart are involved in extracellular matrix maintenance, and cardiac tissue development while emMQs of liver perform functions such as lipid metabolism, complement activation and phagocytosis (Figure 2). We further validated some of the key gene expressions using qRT-PCR.(Figure 3)

In Aim 2 we proposed to study the responses of each MQ subset in heart and liver during sterile inflammation. Specifically, we proposed to study the response of cardiac macrophages to Isoproterenol mediated stress and hepatic macrophages to carbon tetrachloride-mediated stress. In the process of designing the experiments, we learned that the best way to perform a comparative study of the two

subsets is to use a model where both organs are stressed using the same method. Thus we have switched to using a reversible systemic inflammation model using LPS to induce stress in mice. We administered LPS 2mg/Kg intra-peritonealy and at 4 hours and at 2 weeks harvested serum, heart and liver tissue for analysis. We intend to study the immune response to systemic inflammation and how this response resolves in embryo-derived macrophages. We show that at the gene expression level LPS stress elevated known cytokines Tnf, Il6 and Il18 validating the model. Furthermore, emMQs from heart and liver upregulated chemokines important for Monocyte recruitment (ccl2), lymphocyte recruitment (Ccl5) eosinophil recruitment (Ccl3) and showed elevation of macrophage activation markers (CD40) and MHC class I and II molecules. (Figure 4). To get a global view of the functional state we performed RNAseq on both heart and liver emMQs at 4 hrs and 2 weeks time points. (Figure 5). Volcanoplots clearly demonstrated that both embryo derived subsets equally responded to LPS-induced inflammation and they both resolved back to steady state by two weeks. These data indicate that these embryo-derived macrophages can be polarized to pro-inflammatory state when encountered with a pro-inflammatory signal.

***3. Indicate what percent of the project you estimate has been completed (e.g. 30%).**

75

***4. Are there any significant changes or delay in your training program and/or research plan?**

Yes

***5. Are there any changes in Sponsor (on fellowship or mentored awards)?**

No

***6. Are there any changes in Principal Investigator or collaborators?**

No

***7. Are there any changes in location or facilities?**

No

***13. No Changes.**

No

***14. Please describe any significant changes that have occurred, as noted above, or any anticipated changes that represent a significant deviation from the original plan. Briefly discuss the reason(s) for the changes and the implications.**

We have modified our initial research plan in the following ways.
1. Excluded the isoproterenol-induced cardiac inflammation model and carbon tetrachloride-induced inflammation models and replaced with a systemic LPS induced inflammation model to study the immune functions. This was done so that we can compare the macrophage subsets to the same stress and can drive conclusions that are unique to their origin- in our case the embryo origin.

2. We also adopted a method to label embryo-derived subset post birth instead of embryonic stage labeling due to the difficulty of having viable litters and enough mice for experiments. We have shown that the current model consistently labels long-lived embryo-derived macrophages in both the heart and the liver.

***15. Expenditures: (not applicable to fellowship awardees.) If you have a low rate of expenditures this fiscal period, please provide a brief explanation.**