

Annual Progress Report 2009

- Respectfully submitted by Dr. Laura Sly in compliance with CCFC Grant Guidelines and Conditions (May, 2009 version)

Macrophage Phenotype in Inflammatory Bowel Disease

i. Technical update

I began my position as an Assistant Professor at the University of British Columbia and a Principal Investigator at the Child & Family Research Institute in January, 2008. My laboratory space became available on June 30, 2008, immediately before initiation of availability of funding for this project from the CCFC. During the past year, I have established my research program including the hiring and training of two lab personnel for this project funded by the CCFC. In addition, significant progress has been made on this project re-enforcing the original hypotheses and generating exciting new data and hypotheses. Specifically, my laboratory has established mouse colonies at my new institute, developed and set up biological assays for the evaluation of macrophage phenotype and function *in vitro* and *in vivo*, and established mouse models of acute intestinal inflammation, as outlined in my original grant proposal. There are three main accomplishments and observations (a-c) that I would like to highlight.

a. SHIP deficient mice on a BALB/c background (Objective 1A). SHIP deficient mice (SHIP^{-/-}) are profoundly M2 skewed but are on a mixed background (F2 generation of 129Sv x C57BL/6 mice). Mixed backgrounds can be difficult in animal models because of variability between mice. BALB/c mouse macrophages are alternatively activated relative to C57BL/6 mouse macrophages. SHIP^{-/-} mice on a homogeneous BALB/c background were predicted to be an excellent whole mouse model of profoundly alternatively activated macrophages. SHIP heterozygote male mice were mated with female BALB/c mice for four generations and then the heterozygote male and female progeny were mated to generate F4 SHIP^{+/+} and SHIP^{-/-} littermates.

In vivo differentiated macrophages harvested by peritoneal and alveolar lavage from these mice were not alternatively activated compared with their wild type counterparts. This is in marked contrast to the results seen with SHIP^{-/-} genotype on a mixed or a C57BL/6 background. Alternative activation was assessed by looking for markers including Western Blot analyses of arginase, Ym1 and FIZZ1 protein levels. With regard to function, SHIP^{-/-} BALB/c macrophages were not alternatively activated in that they had similar levels of arginase activity constitutively expressed as well as similar levels of nitric oxide, and anti-inflammatory IL-10 produced in response to lipopolysaccharide (LPS) stimulation. Pro-inflammatory cytokine production in response to LPS, including IL-12p40 and TNF α , was elevated in the SHIP^{-/-} BALB/c mice compared to their wild type counterparts, opposite to anticipated results.

Upon further examination of these mice, unlike the SHIP^{-/-} on a mixed or C57BL/6 background, SHIP^{-/-} BALB/c mice had a 100% survival rate at 14 weeks of age (compared to 75% and 40% for the mixed or C57BL/6 background mice). There was no infiltration of myeloid cells into lungs, no asthmatic-like lungs and there was no increase of granulocyte/macrophage progenitors from bone marrow in colony forming unit assays. In summary, many of the features of the SHIP^{-/-} mice myeloid compartment that have been

described, are only apparent on the C57BL/6 or mixed background mice. Proposed experiments with the SHIP^{-/-} mice and macrophages will be continued with a line of SHIP mice on a mixed background (Objectives 1B and 2B). The *in vivo* alternatively activated macrophage phenotype has been confirmed in the mice that we received from Dr. Gerry Krystal and breeding will continue from one line to minimize variability in animal experiments.

b. SHIP and the phosphatidylinositol 3-kinase (PI3K) pathway in alternative activation of macrophages (Objective 2A – extension). One of the objectives of the proposed research was to examine which type of macrophage might also act as myeloid suppressor cells. Based on the unexpected results described in **a.**, we wanted to ask whether SHIP played a role in alternative activation of macrophages derived in different ways. Comparing C57BL/6 mouse and BALB/c mouse macrophages, SHIP levels and activation were similar but there was no difference between these mouse strains for the expression of arginase activity. In other forms of alternatively activated macrophages, M-CSF- or IL-3-derived, IL-4 treated, or isolated from tumours, SHIP levels were always inversely proportional to arginase protein levels and activity. Arginase expression in response to IL-4 was inhibited by the pan PI3K inhibitor, LY294002, thus likely dependent on the enzymatic activity of SHIP. To understand this better, I established a collaboration with Dr. Kevan Shokat (University of California San Francisco). Dr. Shokat's laboratory is a biochemistry laboratory that designs, produces and assays isoform-specific inhibitors of PI3K. PI3K isoform inhibitors (10 in total covering inhibition of catalytic subunits p110 α , β , γ , and δ) have been screened and titrated to determine their specificity. PI3Kp110 δ is critical for alternative activation of macrophages. Interestingly, macrophage skewing to an alternatively activated phenotype was not just inhibited, but blocked, by PI3Kp110 δ inhibition suggesting that this, like the well-known role of STAT6, is absolutely required for alternative activation of macrophages. This provides a novel target and pathway for intervention in affecting macrophage phenotype *in vitro* and *in vivo*. To that end, I have just established a collaboration with Dr. Bart Vanhaesebroek (University of London), to obtain and study the PI3Kp100 δ deficient mice in murine models of intestinal inflammation. I have just received the completed material transfer agreement and mice will be imported as soon as available (MTA from the Ludwig Institute For Cancer Research). These mice will provide a new model of profoundly classically activated macrophages and are predicted to have directly opposing phenotype to the SHIP^{-/-} mixed background mice.

c. Titration of acute dextran sodium sulfate- (DSS-) induced colitis in Child & Family Research Institute (CFRI) animal facility (Objectives 1, 2B and 3). C57BL/6, BALB/c and SHIPF2 (129Sv x C57BL/6 mixed background mice) were subjected to DSS-induced colitis. Each mouse background listed (n=4) were subjected to 3 concentration of DSS in their drinking water for 5 days and euthanized 3 days later. Weight loss and rectal bleeding were monitored. Colon sections were harvested for H&E staining to score tissue damage, and immune cell infiltration. C57BL/6 mice and (129Sv x C57BL/6) will be treated with 2.5% DSS for 5 days. This led to an average weight loss of 14%, rectal bleeding, extensive tissue damage and immune cell infiltration (average score 7/8) and no deaths or required euthanasia during the experiment. BALB/c mice will be treated with 5% DSS for 5 days. This led to an average weight loss of 11%, barely detectable rectal bleeding, tissue damage and immune cell infiltration (average score 5/8). These parameters will be used for future DSS-*induce* colitis experiments including

macrophage depletion experiments and macrophage swapping experiments described in objectives 1-3 in the project proposal.

ii. Lay translation

My research laboratory opened on June 30, 2008, with a focus on macrophages and their role in inflammatory bowel disease (IBD). Macrophages are white blood cells that are critical in our ability to fight off invading pathogens. After their role in defense, they are equally important in the healing process cleaning up damaged cells and promoting wound healing. These are called “healer” macrophages. During inflammatory bowel disease, the macrophages are geared up and remain in their fighting mode. My lab aims to better understand the role of these fighters in IBD and to determine whether we can turn them into healers to stop inflammation and lessen disease.

We have a genetic model of healer macrophages that we have proposed to use to investigate the role of healers in IBD. This healer model is due to targeted deletion of a gene called SHIP. Macrophages without SHIP have a strong healer phenotype. It is also known that different genetic backgrounds have enhanced healing properties so we began by putting the SHIP deficient macrophages on a healer background. To our surprise, the macrophages that were created were not healers at all. This led us to ask questions about why SHIP was able to create healers only in specific situations.

Traditionally, healer macrophages are made by treatment with an agent called interleukin-4 (IL-4) so we asked how macrophages that are missing SHIP respond to IL-4. We found that macrophages missing SHIP are much more prone to become healers when treated with IL-4. SHIP normally blocks the activity of a family of enzymes called PI3 kinases. At the same time that my lab was doing this work, we met Dr. Kevan Shokat, who has developed very specific inhibitors to members of this family. We screened these inhibitors and identified one form of PI3 kinase that was required for macrophages to become healers. Boosting this enzyme activity has the potential to push macrophages to a protective healing phenotype in IBD and a loss of this activity may be something that contributes to inflammation in IBD. This work has identified a new potential target that may be useful in the treatment of IBD.

In my laboratory, we have spent much of the past year establishing protocols to make healer macrophages, to characterize the properties important in each of the different types and to test them for their anti-inflammatory properties. In so doing, we have validated one target for intervention and identified a new target for intervention. Our next step is to see if we can manipulate macrophages in models of inflammation to dampen down inflammation and to lessen disease.

iii. Abstracts and papers presented, published and/or submitted for publication

none to report

iv. Supplementary funding

In the Fall of 2008, I applied for, and have subsequently received, the CIHR/CAG/CCFC New Investigator Salary Award. The award provides \$75,000 per year for 4 years towards the investigators salary and \$40,000 per year for 4 years towards lab expenses. The application called for a new project initiative

but it was built on observations made in the SHIP-/- mouse during chronic inflammation caused by *Helicobacter hepaticus*. While this project has no overlap with the current CCFC project, they complement one another well. My successfully competing in a salary award competition was certainly enhanced because of the investment that the CCFC had already made in my laboratory in the form of this operating grant.

Data that we have collected on the role of PI3Kp110 isoforms in macrophage skewing, phenotype and function will be used as preliminary data in a proposal that I am preparing for CIHR for submission in the Sept 2009 competition. Hence unexpected and preliminary data arising from this project will be the jumping off point for further funding supporting basic and applied research in IBD.

v. Research assistants and other staff

1 technologist: Ms. Nicole Voglmaier. Ms. Nicole Voglmaier is a Level III Research Technologist in my laboratory. She has an Associate Degree in Science from the University College of the Fraser Valley and 10 years of work experience. Nicole has spent the past year establishing protocols in the laboratory including mouse maintenance, genotyping and DSS-induced colitis. She is responsible for the TGIF deficient mouse breeding colony and will perform deletion and replacement experiments with those mice.

1 graduate student: Ms. Shelley Miller is just finishing up the first year of her PhD studies in my laboratory. She has Bachelor of Science and Master of Science Degrees from the University of British Columbia. Shelley has spent her first year examining the role of SHIP in macrophage phenotype including setting up bioassays and using them to evaluate macrophage phenotype and function in macrophages derived in a wide range of conditions. She is responsible for the now established SHIP deficient mouse breeding colony and will perform deletion and replacement experiments with those mice.