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Deciphering the role of ZBTB7B in the transcriptional regulation of macrophage inflammatory responses

Research problem and hypothesis:

Macrophages play key roles in both innate and adaptive immunity and are a part of the first line of defense against infectious diseases. Understanding macrophage regulation and the mechanisms of their immune responses is key to understanding the pathogenesis of, and developing treatment for, infectious diseases. Zinc Finger And BTB Domain Containing 7B (**ZBTB7B, also known as ThPOK**), is a transcription factor well known to be essential to lineage determination in T helper (Th) lymphocytes[1]. Previous work by the lab has shown that ZBTB7B is also involved in inflammation and **macrophage-mediated disease processes**. ZBTB7B is required for disease pathogenesis in a mouse model of experimental cerebral malaria caused by excessive neuroinflammation[2], while mice carrying a loss of function *Zbtb7b* mutation are more susceptible to infection by *Mycobacterium bovis* (BCG)[2]. Moreover, preliminary ChIP-seq studies by Dr. Langlais have identified 4240 binding sites specific to mouse bone marrow-derived macrophages (BMDMs) that are not found in Th lymphocytes. Thus, **we hypothesize that ZBTB7B is a key transcriptional regulator of proinflammatory responses in macrophages.**

Research objective:

The objective of the proposed project is to decipher the role of ZBTB7B in regulating proinflammatory responses in macrophages. This will be done through two specific aims:

1. Evaluate the *in vitro* functional capacity of BMDMs from *Zbtb7b*$^{-/-}$ mice
2. Profile the transcriptomic alterations in BMDMs from *Zbtb7b*$^{-/-}$ mice

Preliminary Results:

Supported by the altona Canada research award, I have conducted the proposed research project. Below is a summary of the experiments conducted and results obtained toward my aims:

1. **To evaluate the functional capacity of *Zbtb7b*$^{-/-}$ BMDMs *in vitro*** as compared to wild type (WT) BMDMs, I challenged these primary cells with acute activators, lipopolysaccharide (LPS) and BCG bacteria, assessing the pro-inflammatory and activation profiles of the cells using flow cytometry. To this end, I have learned to culture BCG in liquid and solid media, as well as to differentiate primary macrophages from mouse bone marrow. Initial results showed no significant difference in activation and cytokine markers (MHCII, CD86, iNOS) between *Zbtb7b*$^{-/-}$ BMDMs and WT BMDMs in response to LPS stimulation. However, I found that *Zbtb7b*$^{-/-}$ BMDMs produced significantly less NO than did WT BMDMs did in response to BCG infection. Repeat experiments will be done to confirm this result in BCG infections and to quantify bactericidal activity.

2. **To profile the transcriptomic alterations in *Zbtb7b*$^{-/-}$ BMDMs** as compared to WT BMDMs, we performed bulk RNA sequencing (RNAseq) to identify the transcriptional changes after 24hours infection with BCG at two doses. Starting from the raw sequencing results, I have performed the quality control analyses, alignment of the sequences to the mouse genome, quantification of gene expression and started the comparative analysis between the

experimental groups. A principal component analysis showed that while treatment effects in a dose dependent manner are the greatest contributor to variance (gene expression changes) in the dataset, genotype effects do contribute as well. Initial differential expression analysis, between WT and *Zbtb7b*$^{-/-}$ BMDMs, showed dysregulation in acute immune response genes and lipid metabolism pathways. Further analysis will be done to examine this effect and probe differences that are enhanced by increased dose of BCG infection.

Contribution to the advancement of knowledge:

This study will contribute to our understanding of a new player in macrophage biology, ZBTB7B. As a largely preliminary project, the data generated would provide a context in which to investigate the role that ZBTB7B plays in macrophage immune responses *in vivo*, with the goal of ultimately furthering our understanding of infectious disease processes. Should ZBTB7B be shown to be required for macrophage-mediated immunity, it would also be interesting to begin to probe whether certain as yet idiopathic clinically inflammatory diseases are mediated by ZBTB7B dysfunction.

References:

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2. Kennedy JM, Georges A, Bassenden AV, et al. ZBTB7B (ThPOK) Is Required for Pathogenesis of Cerebral Malaria and Protection against Pulmonary Tuberculosis. *Infect Immun*. Jan 22 2020;88(2)doi:10.1128/IAI.00845-19
3. Muroi S, Naoe Y, Miyamoto C, et al. Cascading suppression of transcriptional silencers by ThPOK seals helper T cell fate. *Nat Immunol*. Oct 2008;9(10):1113-21. doi:10.1038/ni.1650