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The Regulation of Interleukin-10 by CD4+ T-Cells in a Murine Model
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Goals and Objectives

This project is a cellular immunology study of a conditional genetic knockout mouse model with modified immune cells lacking a key receptor, Toll-Like Receptor-2 (TLR2), on the CD4+ T-cells. The goal of this project is to demonstrate the effects of this conditional knockout on production of cytokines, and explain in part the underlying immunological basis of this mouse model's increased susceptibility to tuberculosis infection. Conducting this project will give the student exposure to and familiarity with the handling of animal models, in particular the murine (mouse) model which is widely used for genetic, immunological, and disease research. It will also provide exposure to and familiarity with molecular, cellular, and immunological research techniques including immune cell sorting, purification via flow cytometry, harvesting animal tissues, tissue culture with *in vitro* stimulation, enzyme-linked immunosorbent assays (ELISA), multiplex cytokine analysis, and intracellular cytokine staining (ICS). This project is a continuation of a collaboration from this past summer extending throughout the academic year between the student and the Boom lab, and will build off the substantial familiarity and training already present.

The objective is to carry out kinetic and functional experiments to evaluate the production of a particular cytokine, IL-10, within the conditional knockout in comparison to wild-type mice and a TLR2 flox control in order to ascertain the regulation of IL-10 by TLR2 stimulation. The wild-type mouse is a general control as it has functional TLR2 as normal, and the flox control is used to rule out that the mutated flox gene in the conditional knockout model is operating aberrantly and impacting MTB control instead of just the lack of functional TLR2. Through conducting this project, the role of TLR2 in cytokine regulation will be further elucidated. These efforts will contribute to a larger-scale project assessing the impacts of this TLR2 conditional knockout on multiple cytokines and the interplay between these cytokines and regulation of tuberculosis infection, as IL-10 has been demonstrated as a regulator in control of MTB infection. After conducting the experiments, the student is expected to analyze and interpret the data and results and present findings in a paper and presentation to an assembly of several lab groups within the School of Medicine's Department of Infectious Diseases.

Project Description

A flox/cre mouse model with a conditional knockout of the Toll-Like Receptor-2 (TLR2) on CD4+ helper T-cells and CD8+ cytotoxic T-cells has previously been developed within the Boom lab. The TLR gene within these mice is excised only in said immune cells, and the rest of the mouse TLR2 functions normally. Previous work has demonstrated that this conditional knockout murine model is compromised in its control of infection with *Mycobacterium tuberculosis* (MTB) at late timepoints. This mouse model thus provide a unique chance to study cytokine regulation by TLR2 and immune response to MTB infection.

Previous studies within the Boom lab have demonstrated that Interleukin-9 (IL-9) and Interferon-Gamma (IFN- γ) play important roles in protection against and control of MTB infection (Karim *et al.,* 2017). Recently, literature has suggested that Interleukin-10 (IL-10) is promoted by TLR2 stimulation and plays a role in control of MTB infection (Jun *et al.,* 2017). IL-10 is an inhibitory cytokine that can shut down overactive T-cells in order to prevent dangerously high levels of inflammation. It's possible that a lack of IL-10 within these TLR2 conditional knockout mice is in part responsible for the weakened response to MTB infection.

The goal of this experiment is to evaluate the production of IL-10 within the TLR2 conditional knockout mouse model and compare it to IL-10 expression within wild-type and flox control mice. Our hypothesis is that TLR2 co-stimulation regulates IL-10, so there will be less IL-10 within the conditional knockout mice. If this hypothesis is proven incorrect, this may suggest IL-10 is independent of TLR2 and it is rather a lack of IFN- γ and IL-2 increasing the conditional knockout model's susceptibility to MTB infection (Reba *et al.,* 2014)

Methodology

To test this hypothesis, CD4+ T-cells will be isolated from the spleens of flox/cre TLR2 conditional knockout mice as well as wild-type and flox control mice. These resting spleen CD4+ T-cells will be isolated then stimulated by platebound CD3 +/- TLR2 ligands. The effector and resting T-cells will then be purified via flow cytometry. After isolating, stimulating with TLR2 ligands Pam3Cys-KKKK, sorting, and plating the cells on CD3 coated plates, they will be stained for purity. At 48 and 72 hours, the cells will be measured for IL-10 and IFN- γ. The time point 24 hours will not be measured as previous studies have demonstrated that IL-10 is not present at 24 hours in the conditional knockout or wild-type mice, as IL-10 accumulates later on during infection order to reduce T-cell activity and inflammation. Thus, at 48 and 72 hours, supernatants will be collected to later use in enzyme-linked immunosorbent assays (ELISA) to quantify the presence of these cytokines.

Time Commitment

First, a week will be required for safety training for animal handling. Once the experiments begin, the amount of weeks required for the experiments depends on the number of experiments. Once a rhythm gets established, a week can be broken down as follows:

Day 1 - Coat plate anti-CD3; run ELISA from previous week if applicable
Day 2 - Cell harvest, plate cells, add antigen.
Day 3 - 24 hours (null day); run ELISA from previous week if applicable
Day 4 - 48 hours (take supernatants for ELISA)
Day 5 - 72 hours (take supernatants for ELISA)

After the first week, Day 1 and Day 3 will be used to do the ELISAs prepared during the previous week. Taking the supernatants for ELISA's doesn't take very long itself, but an ELISA usually takes up the majority of a day. Thus, each experiment takes two weeks to finish but overlaps with the next week, making the total amount of weeks n+1 if the number of experiments is n. To do a statistical amount of experiments, at least three will be needed. For the project to have a total of three to four experiments would require four to five weeks for CD3 ELISA experiments alone, assuming that all goes smoothly and no trials of experiments need to be redone.

Once this portion of the project is done, we will move on from the CD3 ELISA-based experiments to intracellular cytokine staining (ICS) experiments. The ICS experiments would have the same timeline outlined above, but the cells will be harvested and stained with no supernatants taken and larger bulk cultures used. To do three experiments would require three weeks. More time, perhaps about a week, may also need to be allotted afterwards for data interpretations and calculations. The final week will be spent preparing a write-up and the presentation. So, assuming no issues or complications arise, the project timelines at around nine to ten weeks.

Connection with Educational and Career Goals

The student's long-term educational goals are to obtain a PhD in a subdiscipline of biology, likely microbiology, immunology, molecular cell biology, or genetics. The techniques and principles utilized throughout this project will directly relate to and inform this career path. First, experience working with animal models, in particular the mouse model, will be invaluable as models such as this are utilized extensively throughout immunological, genetic, and disease research. In addition, the multiple techniques employed throughout this project such as ELISAs, flow cytometry, and cell isolating and purification will be directly applicable to nearly all the subfields of biological research in which the student's interests lay. Completion of this project will allow for immense growth and development as a researcher by equipping the student with the skills, knowledge, and confidence to conduct a complete research study from the generation of a hypothesis, conduction of trials and experiments, analysis and interpretation of data, and communication of findings. Research is in and of itself a skill, and like any skill, it takes time, dedication, and patience to develop. To be able to devote an entire summer to the completion of this singular project will allow not just familiarity with these advanced molecular and cellular techniques and animal models, but also the further development of the rudimentary lab skills and capabilities that will serve as the foundations for any successful long-term career in research.

Budget Summary

All materials, mice, and supplies will be provided and do not need to be funded by the SOURCE office. The only funds requested would be the stipend.

Bibliography

Jun, J. C., Jones, M. B., Oswald, D. M., Sim, E. S., Jonnalagadda, A. R., Kreisman, L. S., & Cobb, B. A. (2017). T cell-intrinsic TLR2 stimulation promotes IL-10 expression and suppressive activity by CD45Rb(Hi) T cells. *PLOS One.* doi:10.1371/journal.pone.0180688

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