**IV-Enrichment for Preferential Labeling of Perivascular Emigrating Thymocytes**

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### Abstract

T lymphocytes, a crucial component of the adaptive immune system, develop in the thymus and, once they have matured, emigrate to various locations in the periphery where they carry out their functions. It has been suggested that the T cells leaving the thymus are trafficked through the perivascular spaces (PVS) at the corticomedullary junction. Defining the contents of the PVS and therefore the types of cells that are emigrating from or recirculating to the thymus is the goal of this project. Specifically, we tested the validity of using IV-enrichment to selectively label the cellular contents of the PVS in the thymus. Our results show the IV-enrichment predominantly labels cells in the thymic PVS which are distinct from circulating cells in the blood. Using this technique in future experiments will contribute to the understanding of T cell development and thymic emigration patterns. A better understanding of T cell release from the thymus to the periphery will help to improve therapeutic approaches directed towards autoimmune diseases and cancer.

### Background

The thymus is a unique organ specialized to support the development of self-tolerant CD4 and CD8 T cells. Regulatory cells, intrathymic lymphocytes, and natural killer T cells, among others. Once mature, these T lymphocytes must leave the thymus and travel to various locations in the periphery to carry out their functions. It is thought that they emigrate from the thymus via the perivascular spaces (PVS) located near the corticomedullary junction. In order to know what types of cells are trafficked through the PVS, their stage of maturity, and whether they are entering or leaving the thymus, it is important to develop a technique to specifically identify the resident cells of this space. In a previous study conducted by Zadranah and Cyster, intravenous (IV) injection of fluorochrome-conjugated antibodies was used to selectively label CD4 single positive thymocytes in the PVS. The goal of this project was to confirm and optimize this technique using fluorochrome-conjugated antibodies against CD45, as lymphocyte marker, and amplify the cell population of interest by enrichment of the labeled cells. We characterized the labeled cells using various markers against antibodies to indicate cell maturity and cell type to better understand what types of cells occupy the unique environment of the PVS.

### Methods

The methods section would detail the procedures and techniques used in the study, including the IV-labeling and enrichment method. This would include information on how the samples were collected, treated, and analyzed.

![IV-labeling and enrichment method diagram](image)

- **Sacrifice mouse with isoflurane**
- **Collect peripheral blood...**
- **and thymus with or without perfusion**
- **Single-cell suspensions**
- **Stain (anti-PE) magnetic beads**
- **Sample to be enriched**
- **Anti-PE magnetic beads**
- **Flow cytometry analysis**

### Results

- **Does IV-enrichment label the contents of the PVS?**
  - A. IVx fraction from blood
  - IVx enriched sample (thymic)
  - IVx MACS enriched sample (PVS)

- **Further characterization of T cells in the PVS**
  - Fig. 5: Flow cytometric analysis of TCRβ+ cells from tissue samples from Rag2+/+ mice labeled with markers which indicate thymocyte maturity. Compared expression of CD45L, Qa2 and CD24 against expression of GFP. Red population represents cells in the blood, blue population represents cells in the thymic PVS (IV+) and orange represents cells in the thymic parenchyma (IV-).

- **Immunofluorescence microscopy**
  - Fig. 6: Immunofluorescence microscopy of thymic sections from a non-perfused NOD mouse. Sections were stained for the indicated antibody markers. B220 shows the difference between cortex and medulla. Cytokerin type IV stains the inner and outer basement membranes of the PVS. DAPI was used to stain nuclei. CD45.2-PE is the injected antibody that labels thymocytes of CD45.2 origin.

### Conclusions & Future goals

- IV-enrichment predominantly labels cells that are in the thymic PVS and are distinct from cells in the blood
- Using markers such as Rag2, Qa2, CD62L and CD24 we can determine the age of the thymocytes in the PVS and speculate on whether they are emigrating from or returning to the thymus from the periphery
- This methodology can be used in the future to study the cellular contents of the thymic PVS

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