Testing IV-Enrichment as a Technique to Define Contents of Thymic Perivascular Space (PVS)

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Intravenous injection of Labeled Antibody

Thymus

- Front view
- Structure
  - Thymic corpuscle
  - Interlobular septum
  - Cortex
  - Medulla

Perivascular Space

- CD45+

CD45+ cells include:
- T lymphocytes
- B lymphocytes
- Neutrophils
- Monocytes/macrophages
- Dendritic cells
**Method**

IV+ fraction from blood

IV(-) non-enriched sample $\rightarrow$ intrathymic cell population

IV+ MACS enriched sample $\rightarrow$ PVS cell population

αCD45.2-PE

3 min

Sacrifice mouse with isofluorane

Collect Cardiac blood...

...and thymus with or without perfusion

Single-cell suspensions

Non-enriched control

Sample to be enriched

Stain (+) fraction from column, blood, and non-enriched control and analyze with flow cytometry

IV+ TCRβ+

Total IV+ 99.1

Total IV++ 99.9

Total IV++ 1.13

TCRβ

CD45.2-PE
Does IV-enrichment label the contents of the PVS?

**Blood (IV+)**
- CD8:CD4 = 0.66

**Intrathymic (IV-)**
- CD8:CD4 = 0.23

**PVS (IV+)**
- CD8:CD4 = 0.22

**CD8:CD4 Ratio**
- Non-perfused
- perfused (IV+)
- PVS (IV+)

**% neutrophils**
- Non-perfused
- perfused (IV+)
- PVS (IV+)

**% B Cells**
- Non-perfused
- perfused (IV+)
- PVS (IV+)
Perfusion does not change IV-labeled cell population

Blood (IV+)

CD4

CD8

GR1

CD11b

MHCII

CD19

Intrathymic (IV-)

PVS(IV+)

CD8:CD4 Ratio

% Neutrophils

% B Cells
Does the population of IV+ T cells in the thymus represent emigrating thymocytes?

Blood

Thymic parenchyma

Non-perfused PVS

Perfused PVS

Rag2

Qa2

Rag2\text{GFP}^+$

8:4=0.26$

Rag2\text{GFP}^*$

8:4=0.25$

Rag2\text{GFP}^+$

8:4=0.23$

Rag2\text{GFP}^*$

8:4=0.18$

CD4

CD8
Conclusions

• In the thymus, the majority of IV-labeled cells represent cells in the PVS, but there is likely some blood contamination
  – Future work: Quantify amount of blood contamination

• This methodology can be used in the future to study the cellular contents of the thymic PVS
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Effect of Perfusion

IV-labeled cells in non-perfused vs. perfused thymi

![Bar chart showing IV-labeled cells in different conditions.](Image)
Additional Rag2 vs Qa2 Data

Non-perfused Thymus

Perfused Thymus