

Controlled T-Cell Development with Specialized Stromal Cells and Receptors

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Abstract

The goal of these experiments is to find differences in how stem cells develop and what makes them commit to a T cell. We extracted mouse fetal liver and created an artificial environment to act as the thymus to test differences in signaling with or without T cell receptors. Our results through flow cytometry verified the creation of specified T cells.

Introduction

Stem cells have the potential to become any type of cell in the body (ex. blood, skin, heart, etc.). Hematopoietic Stem Cells (HSCs) are specialized stem cells that can develop into any type of immune cell, such as B cells. B cells originate and mature in the bone marrow (BM) or the fetal liver (FL) in mammals. T cells (white blood cells) originate in the BM but mature in the thymus (located on top of the heart). The thymus provides a unique environment for T cells to grow, predominantly through Delta-Notch signaling. Studying T cells is important because of their ability to protect the body from pathogens such as killing cells infected with a virus.

Background

OP9 stromal cells are from the BM in mice and they can mimic the BM environment for cells to grow *in vitro*. Thymic stromal cells support T cell development because they express high levels of Delta-Like-1 (DLL1) ligand, which signal to Notch receptors on developing T cells (Figure 1). Stromal cells from the thymus are hard to culture so we use BM OP9 stromal cells that are modified to express DLL1. The signals made in T cells by Delta-Notch signaling are vital to the development, commitment, and survival of T cells. The thymus normally secretes three cytokines (proteins) that are important to T cell development. To mimic this *in vitro*, three cytokines (IL-7, SCF, and FLT3L) are added to culture.

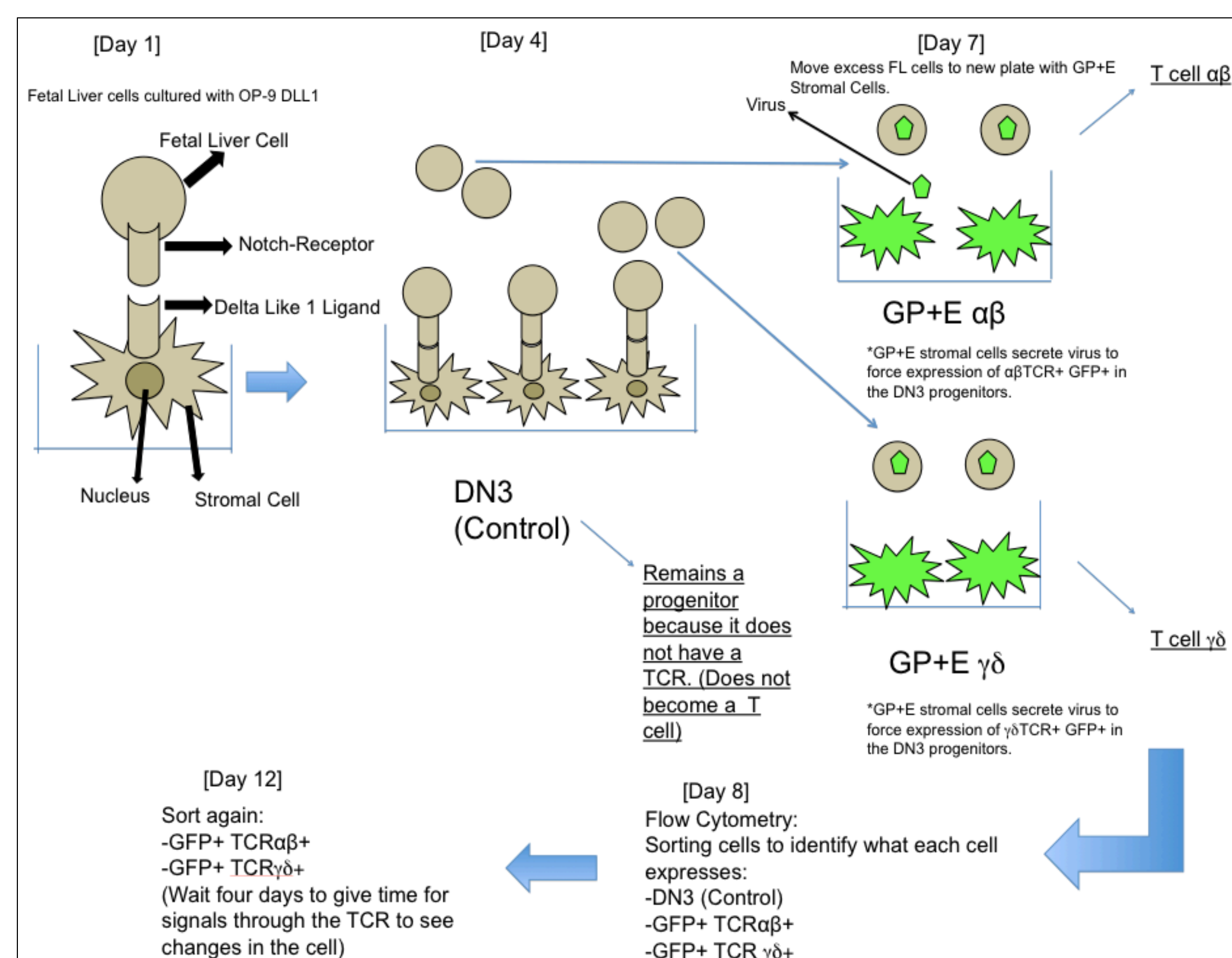


Figure 1: HSCs are cultured into three different types of cells followed by flow cytometry to verify the T cell state.

Methods

Projects and experiments:

- Plating and tissue culture
- Extracting fetal liver cells
- Purifying HSC's from FL cells
- Flow cytometry
- Virus-mediated gene overexpression

Process for extracting fetal liver cells:

Take FL (red tissue) from embryonic mice time point 14.5.

Fetal liver consists of:

- *Red Blood Cells (Ter119+)
- *B cells (CD19+)
- *NK cells (NK1.1+)
- *Myeloid cells (F4180+)
- *Granulocytes (Gr1+)
- HSCs [Ter119-, CD19-, NK1.1-, F4180-, Gr1-]
- *Mature, fully developed, and committed cells

To purify HSCs that can develop into T cells:

Dissect FL and pipette with media to transform tissue into single cells. Add primary antibody (anti-Ter119-Bio, anti-NK1.1-Bio, anti-CD19-Bio, anti-F4/80-Bio, anti-Gr1-Bio). The antibodies will attach to the corresponding receptors (only found on mature cells). Add anti-biotin beads. The beads will attach to biotin on each antibody. Pass cells through a magnetic column. The beads will stick to the side of the column through magnetic force and only the HSCs will pass through. Because the mature cells include RBCs, the pellet will be red. The HSCs will appear white or beige in color because they are white blood cells. Once separated, discard all mature cells and keep HSCs.

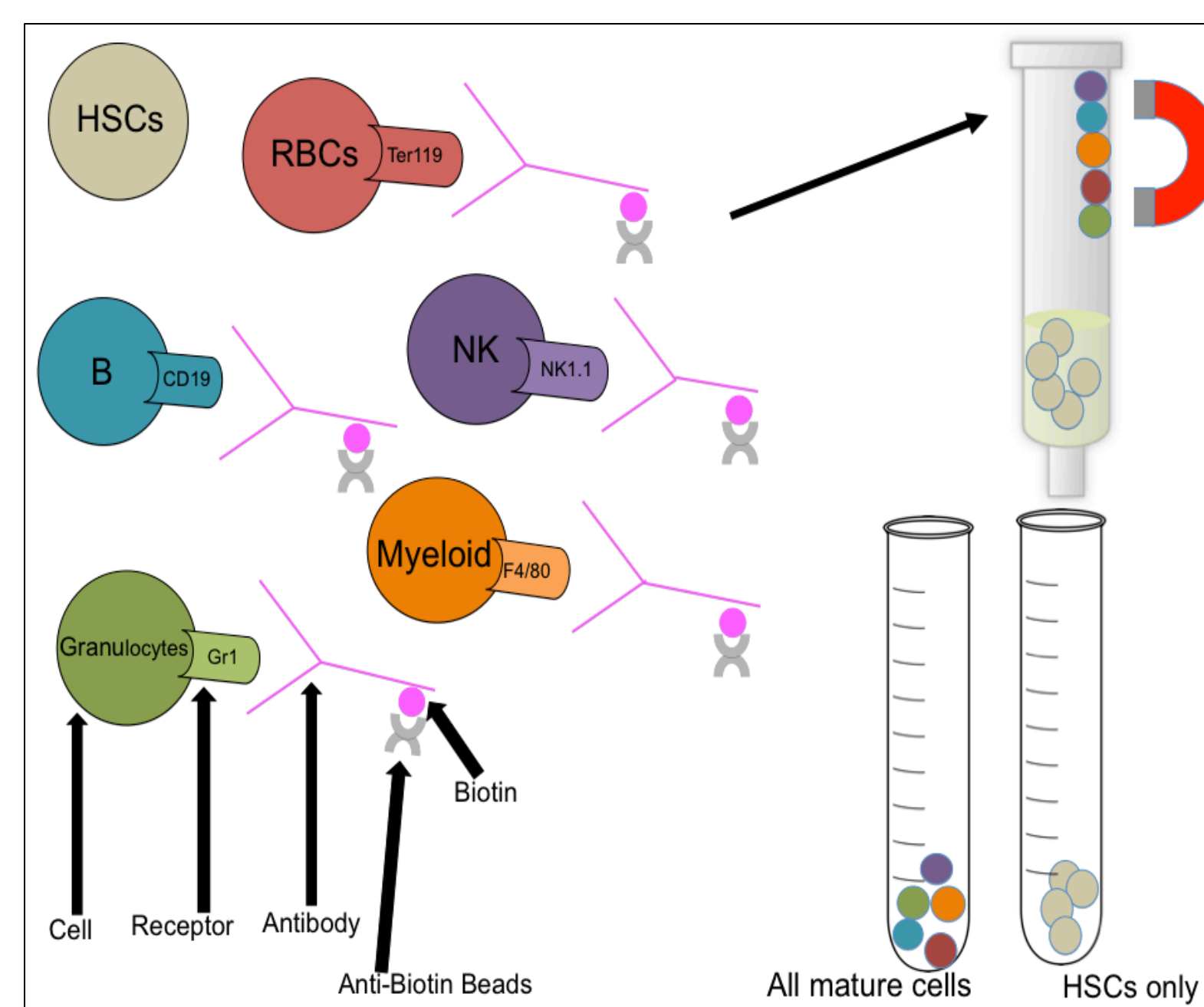
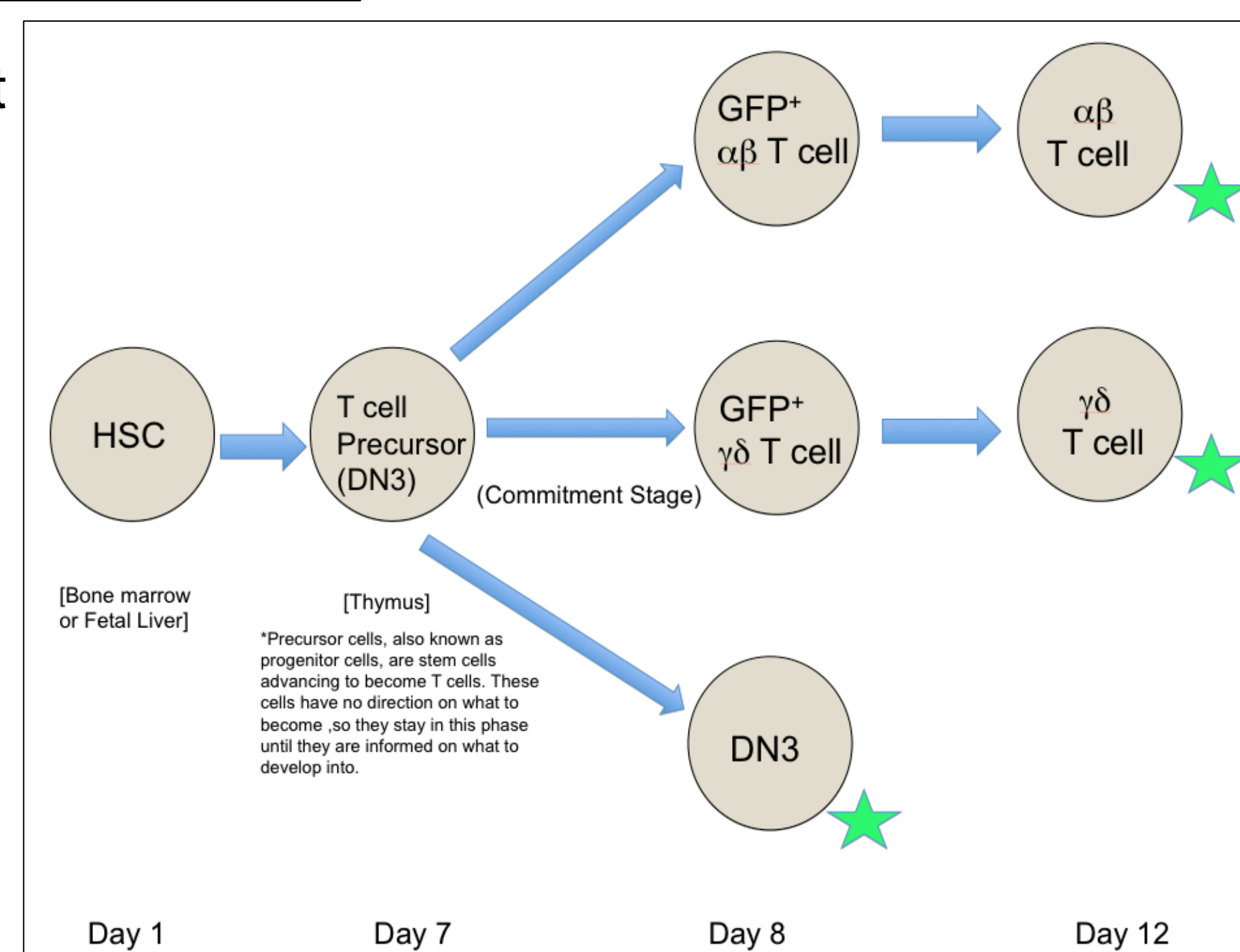


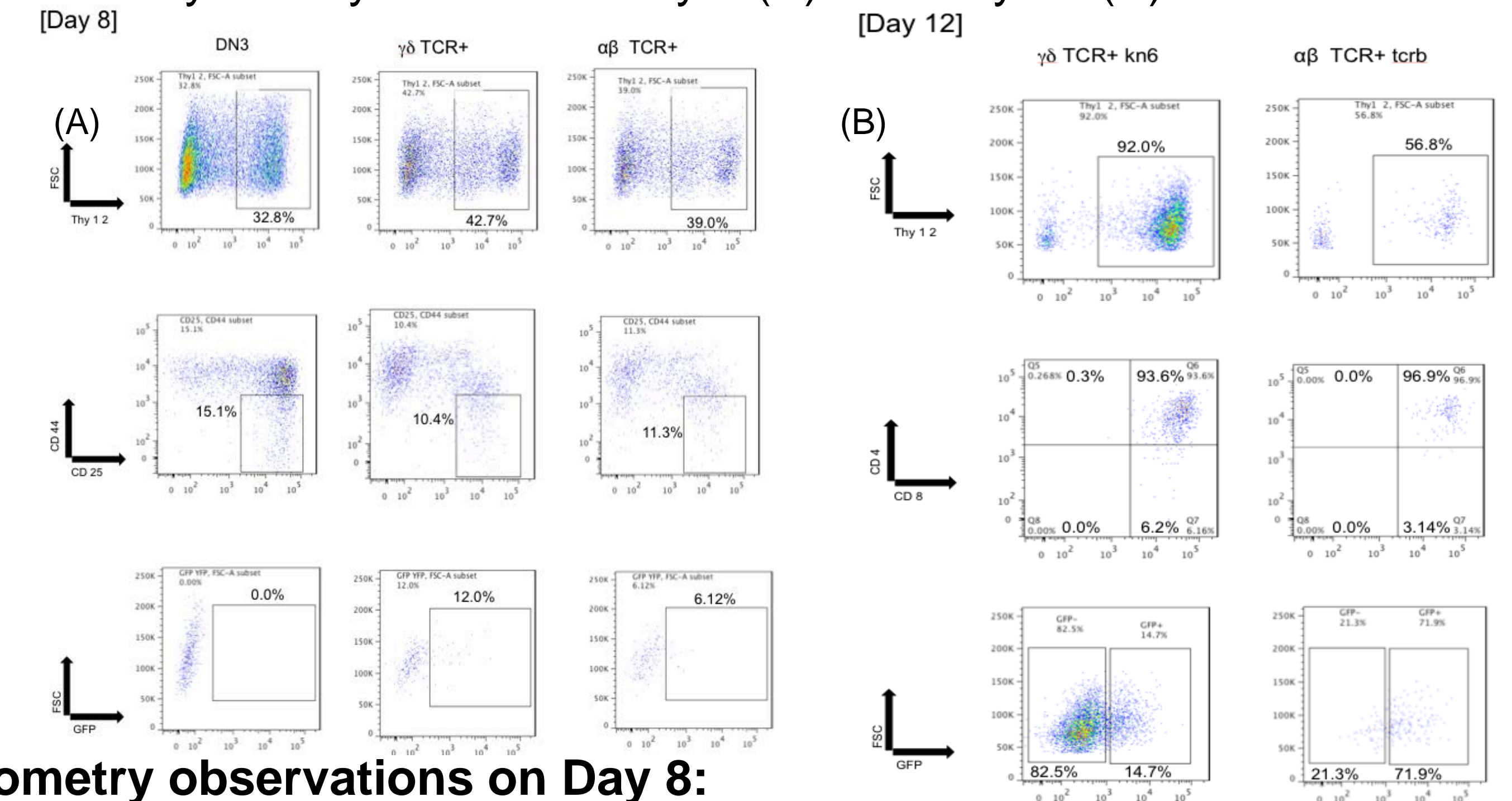
Figure 2: Separation of HSCs from mature FL cells. All mature cells are not needed because they cannot develop into anything else. Only HSCs are capable of further development.

Figure 3: The development of HSCs into committed T cells. DN3 cells are generated after 7 days of culturing HSCs with OP9-DLL1. $\alpha\beta$ and $\delta\delta$ are types of T cells with their own unique function. Once precursors commit to $\alpha\beta$ or $\delta\delta$ lineage they cannot differentiate into another specialized T cell.



Data/Results

Figure 4: Flow cytometry results on Day 8 (A) and Day 12 (B) of T cell culture



Flow cytometry observations on Day 8:

- Thy1.2+ cells were first gated. Only T cells express Thy1.2 receptor.
- All cells on Day 8 were CD4- CD8- (Double Negative (DN)).
- DN3 cells (CD44- CD25+) were gated next.
- After, we looked to see if GFP was expressed. No GFP+ cells (0%) were present for DN3 controls because DN3 cells were not exposed to the GP+E-producing virus.

Flow cytometry observations on Day 12:

- Mature T cells express CD4 and CD8. The figure shows $\delta\delta$ TCR+ and $\alpha\beta$ TCR+ cells develop into mature CD4+ CD8+ cells. Both receptors present indicates the cells have matured.
- DN3 cells will die without receptors by day 12 without instructions.

Conclusions

T cells are very important to the immune system because of their ability to effectively kill pathogens. T cells have many functions that are useful for modern medicine. For example, T cell count is used to monitor the health of patients with HIV/AIDS. A possible application of this project in T cell activation is this method of creating cells *in vitro* and adding specific T cell receptors to target pathogens (HIV and cancer). Instead of using mice HSCs, human HSCs would be experimented on. This project is related to a study that won a Nobel Prize in Physiology or Medicine in 2012 that showed how mature cells can be reprogrammed to become stem cells again. Strengthening our knowledge in how a T cell develops and what they can target can help support T cell therapy for cancers to lead to remission and programming of T cells.

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