T-Cell Shenanigans: The Impact of MHC Pathway & Lipid Metabolism Genes on T-Cell Differentiation in the Thymus

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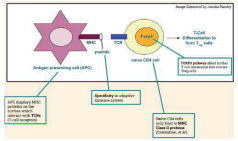
Abstract

Astronaut health and proper immune function are key to the success of sustainable long-term missions in space. While previous studies have observed adaptive immune dysfunction such as diminished white blood cell (WBC) counts during spaceflight, the underlying mechanisms behind loss of immune function are poorly understood. Using transcriptomic data analyzed from mouse thymus tissues we used a standardized RNA-Seq pipeline to identify four largely dysregulated genes (p<0.05): lipid metabolic genes *Hpgd* and *Pgr* and cell cycle regulator genes *Cenpe* and *Kif11*. We analyzed how these genes relate to the MHC-TCR interaction, and propose a novel mechanism for spaceflight-induced alterations to T-cell differentiation. We hope this methodology will improve our knowledge of MHC-TCR interaction, contribute to a better understanding of the mechanisms of autoimmune diseases, and improve the efficacy of newer cancer treatments such as CAR-T cell therapy.

Background

Scientific Background

- The thymus is a small endocrine organ that serves as the site for the maturation and development of T cells
  - developing T-cells known as thymocytes differentiate into mature T-cells of various types utilizing the MHC-TCR interaction (Costantino et al., 2012)



- T-cell differentiation is a **biologically unfavored** and **complex** process
  - **Strong Recognition =** APOPTOSIS
  - **Weak Recognition =** APOPTOSIS

Is it possible that spaceflight causes dysregulation of this interaction between TCRs and MHC proteins in differentiation of Treg cells?

- While the effects of space flight on the thymus have been well documented, the mechanisms behind the T-cell differentiation have yet to be identified.

Dataset Background

- Our data was obtained from the GLDS-421 experiment, Rodent Research-9 mission
- The mice were launched aboard the SpaceX-12 rocket to the International Space Station before being returned to Earth for analysis 33 days later
- The mouse thymus cells were then sampled and sequenced at GeneLab Sample Processing Lab

Preliminary RNA-Seq Analysis

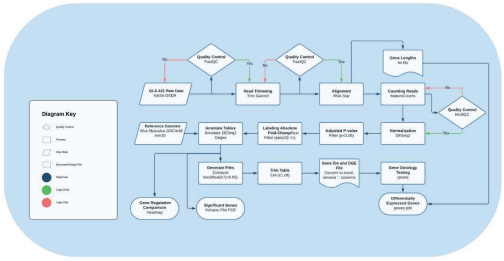


Figure 1. RNA-Seq Pipeline (UseGalaxy.org)
- RNA sequencing was performed at GeneLab Sample Processing Lab on Illumina NovaSeq6000

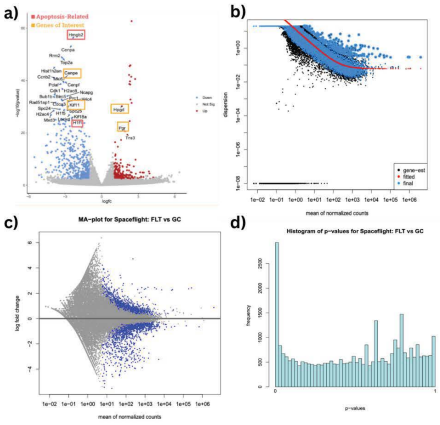


Figure 2. Analysis of Genes of Interest
- Lipid metabolic genes (Hpgd & Pgr) are upregulated
- Cell cycle genes (Cenpe and Kifll) are downregulated

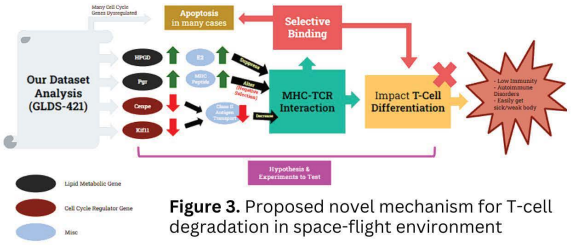


Figure 3. Proposed novel mechanism for T-cell degradation in space-flight environment

Hypothesis

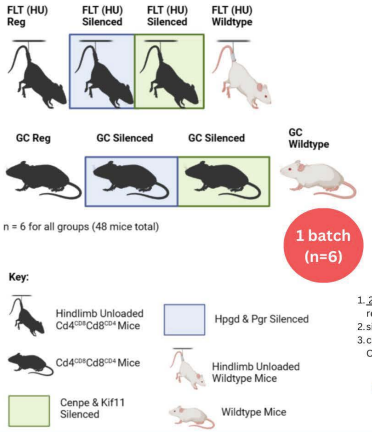
IF there is dysregulation of key genes in the lipid metabolism and MHC pathway during spa and MHC pathway during spaceflightceflight **THEN** it will disrupt the MHC binding interaction with naive TCRs and impair Treg cell differentiation in the thymus.

Genes of Interest

- MHC Pathway Genes
  - Generate pro-inflammatory cytokines and can induce thymocyte apoptosis
- Lipid Metabolism Genes
  - Affects the ability of TCRs to bind to the MHC Class II molecules

Research Design and Aims

Overall Experimental Design:



Experimental Design Overview:

- First 14 Days:
  - 4 FLT groups vs 4 GC groups (n=6)
    - Hindlimb unloading will be used to simulate microgravity (14 day duration)
    - 2 subgroups in each - containing either silenced genes or wildtype
      - Gene silencing via the usage of custom small interfering RNA primers
  - Total of 36 CD4CD8 knock-in/knock-out transgenic mice & 12 wild-type mice
- Thymic tissue from the mice will be dissected and snap frozen for analysis

Experimental Aims/Procedure

Aim 1
effect of **MHC pathway disruption** in microgravity on T-cell differentiation

1. 2 batches of transgenic mice (6 silenced and 6 regular) and 1 batch of wildtype mice in FLT & GC
2. silence Cenpe & Kif11 genes
3. conduct **Flow Cytometry** and compare ratio of naive CD4 cells to differentiated T-reg cells

Expected Results: *Highest CD4+ to Treg Ratio in FLT Silenced Group (LEAST differentiation)*

Aim 2
effect of **lipid metabolic gene disruption** in microgravity on T-cell differentiation

1. 2 batches of transgenic mice (6 silenced and 6 regular) and 1 batch of wildtype mice in FLT & GC
2. silence Hpgd & Pgr genes
3. conduct **Flow Cytometry** and compare ratio of naive CD4 cells to differentiated T-reg cells
4. perform **Alamar Blue Assay** to compare metabolism

Expected Results: *Highest CD4+ to Treg Ratio (LEAST differentiation) & LOWEST Metabolic Activity in FLT Silenced Group*

Aim 3
effect of **dysregulated T-cell differentiation** on immunity

1. 1 batch of transgenic mice and 1 batch of wildtype mice in FLT & GC
2. conduct **Flow Cytometry** and compare ratio of naive CD4 cells to differentiated T-reg cells
3. conduct **ELISA** to measure the general immune response (using chemokine and cytokine levels)

Expected Results: *Highest CD4+ to Treg Ratio (LEAST differentiation) & Abnormal IL levels in FLT Silenced Group*

Conclusion

We proposed a novel mechanism of immune system dysregulation in spaceflight. The proposed experiment and field of study has essential long-term scientific benefits such as:

- Contributing to a more comprehensive of understanding immune system (especially in the context of long-term manned missions where there is limited access to healthcare and smaller crews)
- Significant implications for managing autoimmune disorders as we continue space exploration and prioritize astronaut health
- Knowledge about the MHC-TCR interaction can contribute to current mechanisms on Earth such as cancer CAR-T therapy and HLA-related organ transplant rejections

References

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