Discovery Day Research Symposium

Investigating the Relationship between Neuroendocrine and Immune Function following Simulated Ionizing Radiation

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Introduction

The neuroendocrine stress response can regulate immune function, which may impact immune dysfunction in deep space ionizing radiation environments. DNA damage from radiation induces apoptosis, can impact immune cell numbers and function. Therefore, identifying radiation dose depth damage within adrenal tissues can assess the types and amount of hormone biosynthesis gene expression impacts that regulates immunity.

Approach

- 24-week-old, male and female *C57Bl/6J* mice adrenals and blood samples will be assessed at 2-weeks post-exposure to simulated galactic cosmic ray (GCR) radiation at 5, 15, and 50 cGy, compared to sham controls.
- Whole transcriptome shotgun RNA sequencing of adrenals will be correlated to whole blood leukocyte differential analyses.
- Comparative immunohistochemistry of adrenals and brain tissues to identify protein phenotypes post-irradiation will be assessed, along with ELISA measurements of hormones types/concentrations in plasma.

Hypothesis

- Dose depth damage is predicated to reach the medulla of the adrenals.
- Male and female radiation dose differences in hormone biosynthesis.
- Deficits in hormone feedback mechanisms that will impact immune differentials and functions.
- CNS impacts - based off immune and endocrine deficits.

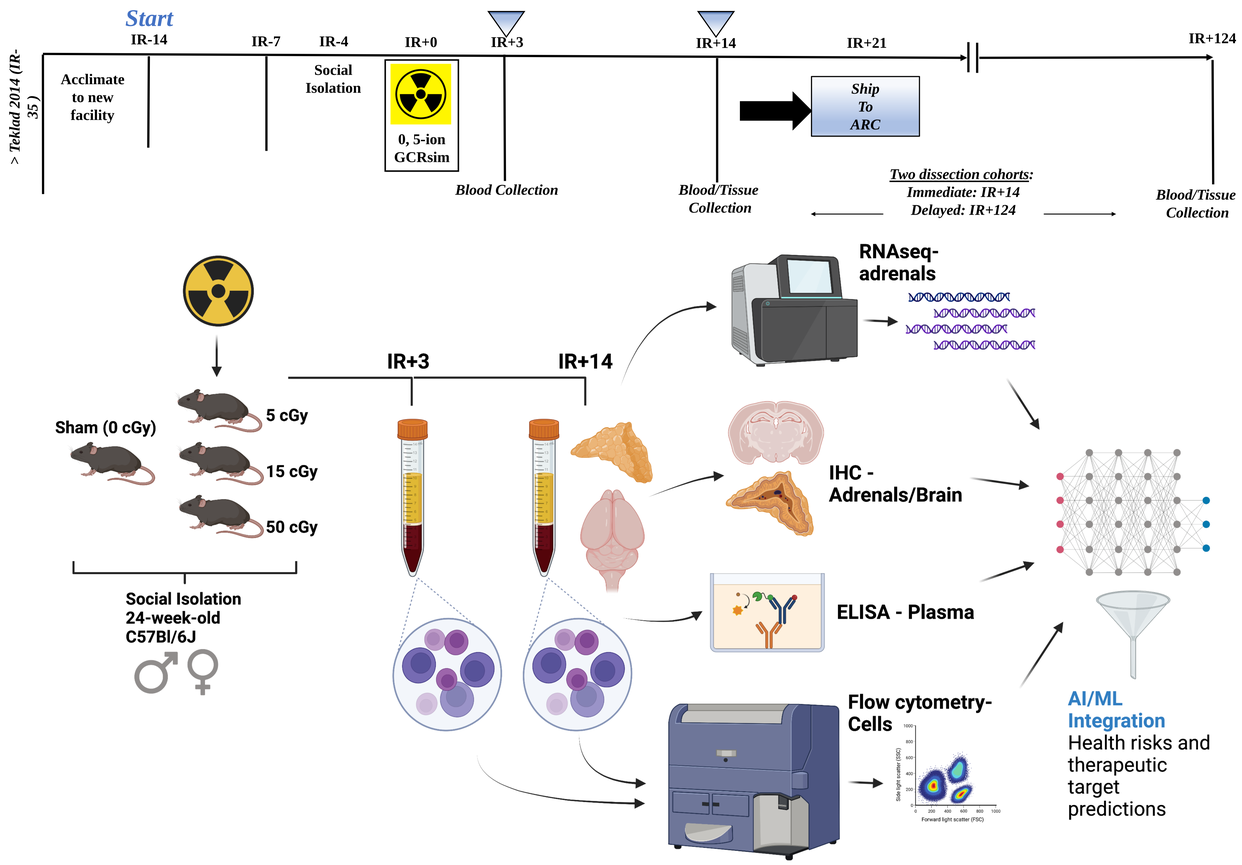

Figure 1. Utilizing 5-ion GCRsim at NASA Space Radiation Laboratory to assess the dose-dependent impacts of 5, 15, and 50 cGy of GCRsim on socially-isolated female and male mice. Collection of blood and tissues occur at 3- and 14-days post-irradiation RNAseq, immunohistochemistry (IHC) of tissues, ELISA of plasma hormone concentrations, and flow cytometry of leukocytes. Image was created BioRender

Results and Discussion

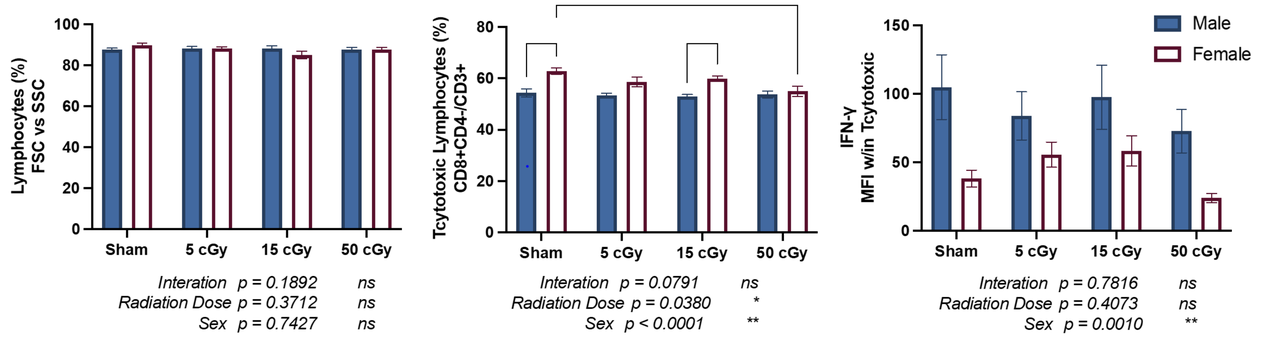

Figure 2. $T_{cytotoxic}$ responses following whole body GCRsim in mice. White blood cells were isolated from whole blood collections of male and female C57Bl/6J mice at 2-weeks post-whole-body exposure to simplified galactic cosmic ray (GCRsim) at 5 cGy, 15 cGy, and 50 cGy, compared to sham controls (w/out GCRsim). Cells were stimulated with ionomycin and PMA for 16hr to determine cytotoxic T cell responses as measured by the $T_{cytotoxic}$ differential and IFN-γ production. A two-way ANOVA was performed comparing the means across all data sets. Data represent ±SEM ($p < 0.05$*).

No significant difference in overall lymphocyte population.

Female mice exposed to 50 cGy have lower $T_{cytotoxic}$ cell counts compared to control, and elevated counts compared to males at 0 and 15cGy.

Lower $T_{cytotoxic}$ T cell counts did not impact functional IFN-γ production post-irradiation, however sex-specific effects were observed.

Future Directions

- **Hormone types & concentrations** in plasma will be determined by ELISA, confirming if these hormones correspond to alterations in adrenal tissues.
- Cell reduction at 50cGy in females can be analyzed through H&E, revealing overall cell density to determine homogenous dose depth, and offering clues to **regions affected in hormone biosynthesis**.
- Left and Right adrenals (n=10/group and sex) will be combined, total RNA will be isolated, and cDNA will be generated. Libraries will be prepared, sequencing will be performed, and raw data will be processed and quantified to **determine differentially expressed genes.**
- Immunohistochemistry of brain tissues biomarkers to **confirm HPA axis involvement** →CRH, CRHR1/2, and GnRH.
- Bioinformatic analysis to integrate data and **correlation with human genomes**.

Impact and Conclusions

Pharmacology

- *Spaceflight:* to manipulate the HPA axis hormones to minimize persistent inflammation and sharpen cytotoxic immunity.
- *Terrestrial:* guidelines and immune kinetics information for cancer patients undergoing proton therapy.

Personalized Medicine

- Unique hormonal regulations are important to consider for future therapeutics.
- Sex-specific immune responses are regulated by hormone production, therefore personalizing medications to each sex may be important considerations.

Systems Biology

- Understanding the contribution of other hormone-immune systems, i.e. aldosterone-immune axis, that impacts physiological systems, such as bone.