REDUCED GRAVITY CONTRIBUTES TO NEUTROPHIL TO LYMPHOCYTE RATIO SHIFTING AND PROMOTION OF THE OXIDATIVE STRESS RESPONSE

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Introduction

Short-and long-term spaceflight missions can cause immune system dysfunction in astronauts. Studies indicate elevated white blood cells (WBC) and polymorphonuclear neutrophils (PMN) in astronaut blood, along with unchanged or reduced lymphocyte counts, and reduced T cell function, during spaceflight. A high PMN to lymphocyte ratio (NLR) can acts as a strong predictor of poor prognosis in cancer, and as a biomarker for subclinical inflammation in humans and chronic stress in mouse models, however, the NLR has not yet been identified as a predictor of astronaut health during spaceflight. For this, complete blood cell count data collected from astronauts and rodents that have flown for short- and long-term missions on board the International Space Station (ISS) was repurposed to determine the NLR and granulocyte to lymphocyte ratio (GLR) pre-, in-, and post-flight. Collectively, these results suggest a disrupted NLR/GLR profile in spaceflight, which may further disrupt immune homeostasis. Under the hypothesis that spaceflight induces a higher NLR due to aberrant oxidative stress response, this study aimed to; **1: determine the NLR and GLR of astronauts and rodents during short- and long-term missions, and 2: determine the contributing role of reactive oxygen species (ROS) in this immune profile shifting.** For this, the ground-based microgravity analog, hindlimb unloading (HU) mouse model and the high-aspect rotating wall vessel cell culture system (HARV-RWV) were utilized to determine the effects of microgravity on the generation of ROS and resulting effects on the maturation and function of PMNs in human blood samples.

Methods

- **Cell Culture:** Human whole blood was separated into cell types using ficoll- and histo- paque. Similarly, mouse blood was collected and separated. RBCs were lysed and WBCs were cultured in complete RPMI media.
- **Phagocytosis assay:** Isolated blood cells were cultured with pHrodo red/green bioparticles. Cells were fixed, washed and analyzed by flow cytometry.
- **Simulated microgravity:** 3D high-aspect rotating wall vessels (HARV-RWV, Synthecon) were used to simulate microgravity. Cells were suspended at 5 x 10^5 cells/ml in 10 ml and rotated at 20 RPM (µg) for 20 hr. Upright flasks were used as 1g controls. Following incubation cells were collected for appropriate assays.
- **Hindlimb unloaded mice:** Female, 4-month old mice were subjected to tail restrained hindlimb unloading for 30-days.
- **qPCR:** Total RNA was extracted from sorted granulocytes. qRT-PCR was performed using iQ SYBR green supermix. β-Actin, normalizing gene was used for calculating relative fold change (RFC).

Spaceflight

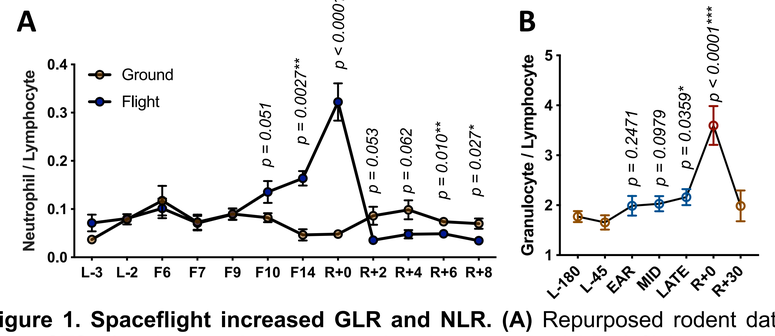

Figure 1. Spaceflight increased GLR and NLR. (A) Repurposed rodent data from STS-58 mission show progressive increase in NLR inflight, while a significantly increased NLR was displayed post-return. **(B)** Repurposed astronaut blood data shows progressive increase in GLR inflight and at post-return. Data indicates $p^*<0.05$

Simulated microgravity models

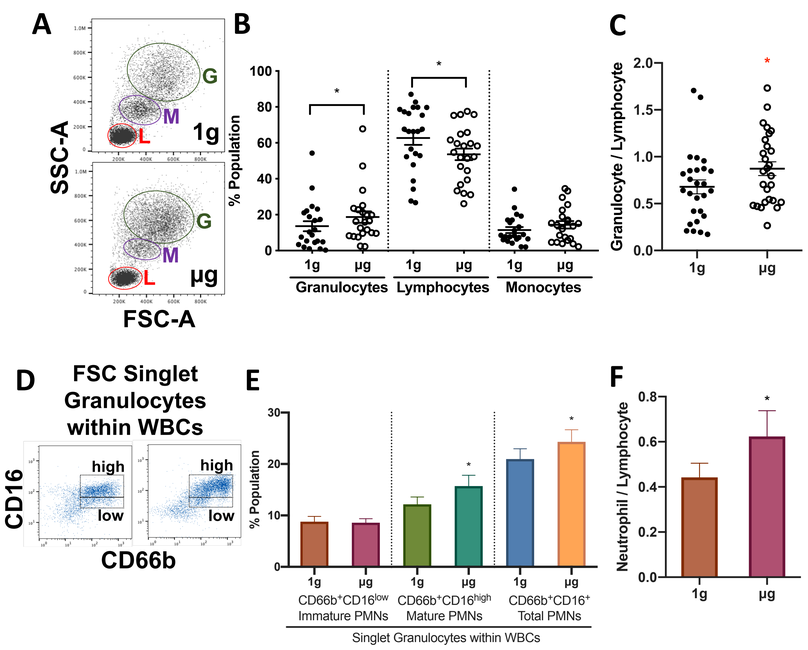

Figure 2. Simulated microgravity resulted in increased granulocyte and PMN maturation, myeloperoxidase expression, and increased NLR. (A) Flow scatter plot with different cell types marked, G, Granulocyte (green); M, Monocyte (purple); L, Lymphocytes (red) **(B)** Dot plot representing the % population of each cell type **(C)** Dot plot representing the granulocyte to lymphocyte ratio **(D)** Scatter plot marking immature ($CD66b^+CD16^{low}$) and mature ($CD66b^+CD16^{high}$) neutrophils **(E)** Graph depicting increased mature and total neutrophils in simulated microgravity **(F)** Graph shows increased total neutrophil to total lymphocyte ratio in simulated microgravity conditions. * indicates $p<0.05$

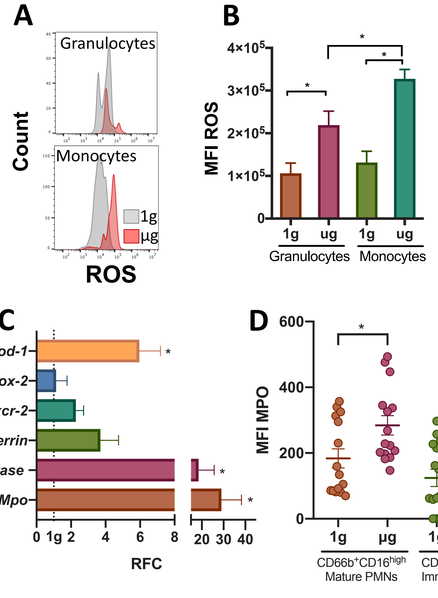

Figure 3. Simulated microgravity induced oxidative stress and Myeloperoxidase. (A) Histogram shows a shift in the intensity of CellROX suggesting increase in total ROS fluorescence in microgravity condition as compared to 1g controls **(B)** Quantification of mean fluorescence intensity of CellROX stain suggest increased ROS in granulocytes and monocyte populations **(C)** qPCR analysis of sorted granulocytes indicate increased neutrophil-specific and oxidative stress responsive genes represented as relative fold change (RFC). **(D)** Mean fluorescence intensity (MFI) of MPO in mature PMNs. * indicates $p<0.05$

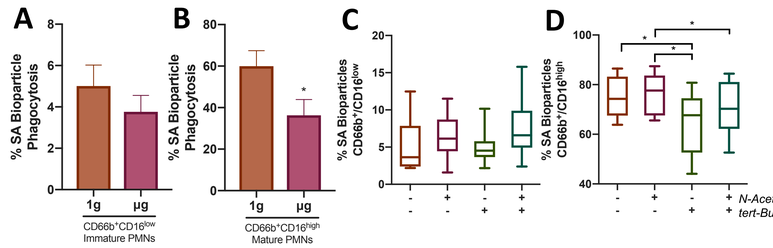

Figure 4. Simulated microgravity resulted in decreased phagocytosis. Phagocytic ability of cells under oxidative stress was determined using *S.aureus*-pHordo bioparticles in cells post simulated microgravity **(A and B)** and stimulated with *tert*-Butyl hydroperoxide (ROS inducer) and *N*-acetyl cysteine (antioxidant) **(C and D)**. We observe reduced phagocytic ability of mature PMNs under ROS inducer treatment. * indicates $p<0.05$.

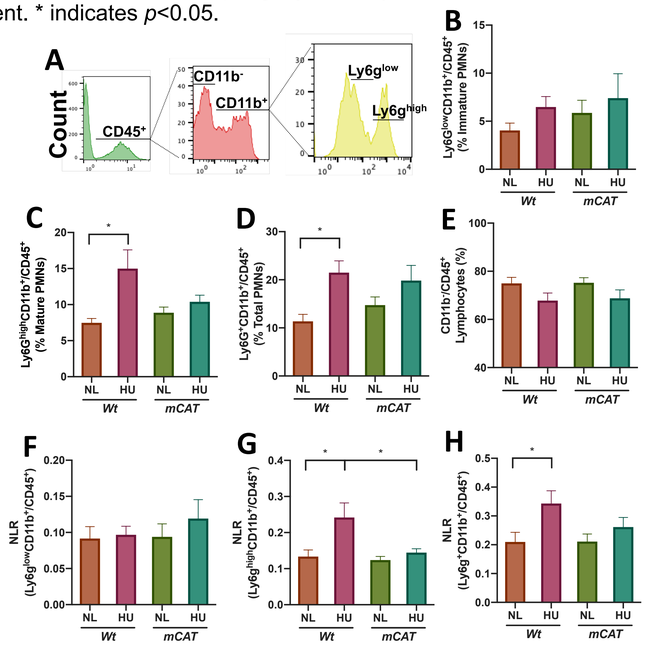

Figure 5. Hindlimb unloaded mice (30-days) displays increased NLR while human mitochondrial catalase (*mCAT*) mitigates this phenotype (A) Histograms display gating schemes flow cytometric plots. **(B)** Graphs display immature ($Ly6g^{low}CD11b^+/CD45^+$); **(C)** mature ($Ly6g^{high}CD11b^+/CD45^+$); **(D)** total ($Ly6g^+CD11b^+/CD45^+$) neutrophils; **(E)** lymphocytes ($CD11b^-/CD45^+$); **(F)** NLR with immature PMNs; **(G)** NLR with mature PMNs; and **(H)** NLR with total PMNs in mice blood.* indicates $p<0.05$

Conclusions

- Progressive increased in GLR (humans) and NLR (rodents) in spaceflight and at post-flight return.
- Increased GLR, NLR and ROS in human blood under simulated microgravity, resulting in dysfunctional phagocytosis and robust MPO expression.
- Increased NLR in hindlimb unloaded mice.

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