Exploring Spaceflight-Associated Changes in Lung Macrophage Profiles

Takara O'Brien[1], Carol Mitchell[1], Eryn Riesberg[1], April E. Ronca[2,4] and Amber M. Paul[1,2,3]

[1] Embry Riddle Aeronautical University, Department of Human Factors and Behavioral Neurobiology, Daytona Beach FL, 32114
[2] NASA Ames Research Center, Space Biosciences Division, Moffett Field CA, 94035
[3] Blue Marble Institute of Science, Seattle WA, 98104
[4]Wake Forest Medical School, Dept of Obstetrics & Gynecology, Winston-Salem, NC 27101

ABSTRACT

Immune dysregulation is a recognized phenomenon during spaceflight, including impaired macrophage differentiation and function. Activated macrophages exist in polarized phenotypes, such as M1 macrophages, which produce primarily pro-inflammatory mediators and M2 macrophages that are involved in anti-inflammatory processes and tissue repair. Effective macrophage polarization processes are vital for generating appropriate immune responses and facilitating recovery on Earth and in spaceflight. To gain deeper insight into macrophage polarization processes in spaceflight, we analyzed open-sourced, GeneLab lung tissue transcriptional datasets (OSD-248) from mice previously flown on the Rodent Research (RR)-6 mission. Mice were euthanized on-board the ISS after a 60-day mission. Preliminary analysis revealed an overall decrease in both M1/M2 biosignatures in spaceflight compared to ground controls. Interestingly, select M2 biosignatures were significantly reduced compared to M1, suggesting deficits in tolerogenic/anti-inflammatory activity and a shift towards pro-inflammatory states. In a ground-based study simulating spaceflight conditions, male and female C57BL/6J mice were exposed to simulated galactic cosmic ray radiation combined with hindlimb unloading and social isolation. To assess M1/M2 predominance in the lung and to test the fidelity of a single cell isolation protocol, total macrophages were isolated from frozen-stored lung tissue two weeks post-irradiation exposure. Cells were positively selected for using the F4/80 biomarker, and lung resident and infiltrating macrophage subtypes (M1 and M2) were characterized by flow cytometry, including F4/80, CD170, Arginase-1 (M2), and iNOS (M1). Future studies using tissues from space-flown RR-20 mice will further validate the definition of M1/M2 macrophages in the lung. In summary, characterizing polarized macrophage populations within the lung microenvironment is crucial for advancing our understanding of immune responses in spaceflight, particularly for lunar missions, where astronaut pulmonary physiology will be challenged by unique lunar environmental soil.

INTRODUCTION

- Radiation exposure in the spaceflight environment can trigger immune-related responses, including oxidative stress and inflammation, which lead to immune suppression that is sexually dimorphic.
- (Alveolar macrophages) AM are lung tissue-resident cells that play a vital role in innate respiratory barrier immunity.
- AM functionality is compromised due to the heightened vulnerability of astronauts to infectious pathogens and the exacerbation of inflammatory responses from foreign molecules.
- M1 and M2 are polarized macrophage (AM and infiltrating) phenotypes, which can promote pro-inflammatory and tolerogenic/tissue repair processes, respectively.

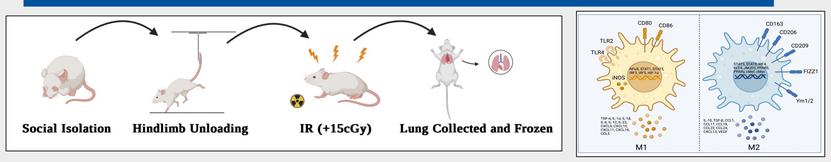
Hypothesis

Males and females will produce elevated inflammation through elevated iNOS promoting M1 vs M2 populations in the lung, which may be enhanced in males.

Objectives

1. Characterize lung tissue profiles through NASA's Open Science Repository: ground v. spaceflight
2. Develop an experimental protocol to isolate lung AM from ground and spaceflight studies, to prepare for upcoming Rodent Research (RR)-20 mission.

EXPERIMENTAL DESIGN



- Raw transcriptome differential gene expression (DEG) datasets from the RR-6 mission characterizing M1 and M2-related genes from ISS and ground controls (GeneLab OSD-248).
- All mouse lung tissues were shared from the parent HRP funded grant (PI: Dr. April Ronca) for these studies. Male and female mice (24-weeks old) were socially isolated for 7-days prior to experiment start date. Mice experienced simSpace via hindlimb unloading for 14 days, followed by 5-ion simplified galactic cosmic ray (GCRsim) radiation at 15 cGy, followed by euthanization and lung tissue collection/frozen on day 28.
- Frozen lung samples were mechanically digested and positively selected using the EasySep™ Positive Selection Kit and magnetic separation (F4/80).
- Macrophages subjected to fluorophore staining (M1 = proinflammatory (F4/80+, CD170^{high}, iNOS^{+}); M2 = anti-inflammatory (F4/80^{+}, CD170^{high}, Arginase-1^{+}) and acquired using a Sony SH800 flow cytometer with FlowJo post-processing analysis to identify population median fluorescence intensity (MFI) of M1 or M2 biomarkers per experimental cohort.

RESULTS

M1 Biomarkers	Log FC	Negative Log p value	M2 Biomarkers	Log FC	Negative Log p value
Tnf	-1.066	2.526	*Pparg*	-1.701	5.481
Hif1a	-0.322	1.397	*Cd163*	-1.735	4.781
Cxcl10	-0.819	0.986	*Arg1*	-1.70712	2.22184875
Il1b	0.543	0.902	*Ccl24*	-1.732	2.197
Cd86	-0.390	0.858	*Cxcl13*	-2.082	2.183
Cxcl9	-1.104	0.842	*Il10rb*	-0.14655	1.29319737
Il1a	-0.284	0.575	*Il10*	-1.304	1.236
Stat1	-0.168	0.573	*Mrc1/CD206*	-0.417	1.199
Nfkb1	0.123	0.461	*Cd209a*	-0.270	0.923
Tlr4	-0.225	0.451	*Retnla*	-0.433	0.725
Ccl5	0.234	0.395	*Cd68*	-0.308	0.58670024
Irf5	-0.184	0.297	*Ccl17*	0.444	0.577
Cxcl16	0.101	0.245	*Tgfb1*	0.260	0.378
Il6	-0.250	0.231	*Vegfa*	0.135	0.302
Il12b	-0.120	0.216	*Tlr8*	-0.1160802	0.226056327
Cd80	-0.129	0.168	*Chil3*	0.150	0.187
Il23a	-0.176	0.096	*Ccl1*	-0.526	0.139
Il12a	-0.078	0.095	*Stat3*	0.040	0.095
Irf3	0.042	0.092	*Stat6*	-0.039	0.070
Tlr2	0.023	0.078	*Ppard*	-0.045	0.048
Stat5b	-0.043	0.072	*Il10ra*	-0.03136	0.04261855
Nos2	-0.023	0.026	*Ccl22*	0.023	0.038
Cxcl11	0.020	0.007	*Klf4*	-0.008	0.015

Table 1. *Table indicate depiction of the differential expression of M1 and M2 genes in AMs under ISS conditions compared to ground controls.*

NASA GeneLab's Open Science Data (OSD)-248 RNA transcriptomic DEG profiling of female (32-week-old) *C57Bl/6NTac* mice lung tissue collected on board the ISS day 30 of mission duration were preliminarily characterized.

- Overall decreased M1 and M2 biosignatures
- M2 biomarkers were significantly reduced more so than M1 biomarkers
- Suggest predominance of M1 macrophages phenotypes in spaceflight.

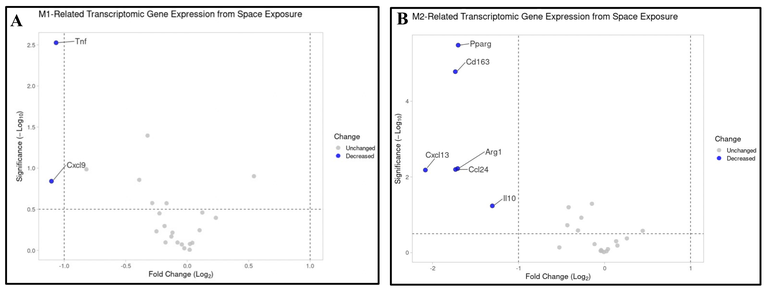


Figure 1. *Volcano plot displays differential gene expression profile of M1 under ISS conditions compared to ground control (A) and profile of M2 macrophages in response to ISS conditions relative to ground control (B). Plots demonstrates distribution of log2 fold change (x-axis) against -log10 of the adjusted p-value (y-axis). Genes displaying a fold change greater than -1 and a significance level of 0.5 are highlighted. Notably Tnf and Cxcl9, identified as significantly downregulated genes in M1 (A) and significant downregulation of crucial genes including Pparg, Cd163, Cxcl13 (B).*

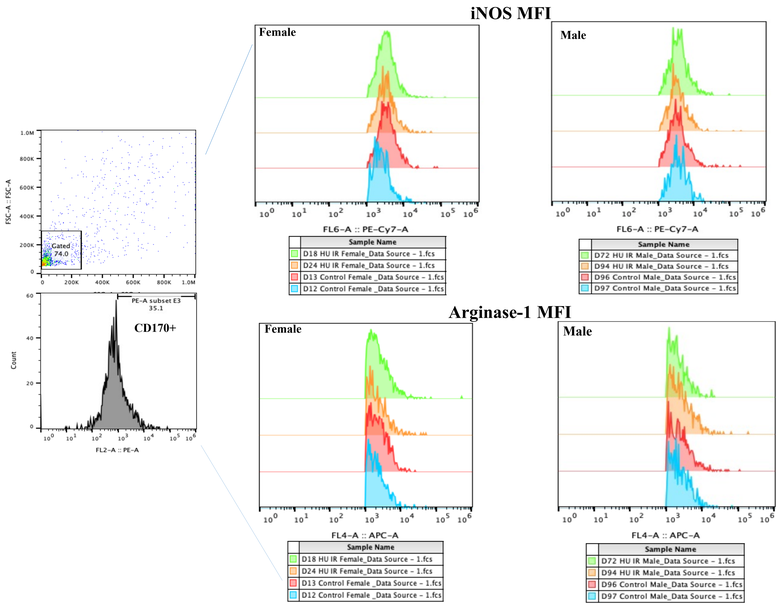


Figure 2. *Flow cytometric analysis gating scheme of M1 and M2 Median Fluorescence Intensity (MFI) of iNOS (M1) and Arginase-1 (M2), within CD170+ AM. All flow acquisitions were performed on a Sony SH800 instrument and FlowJo (v10).*

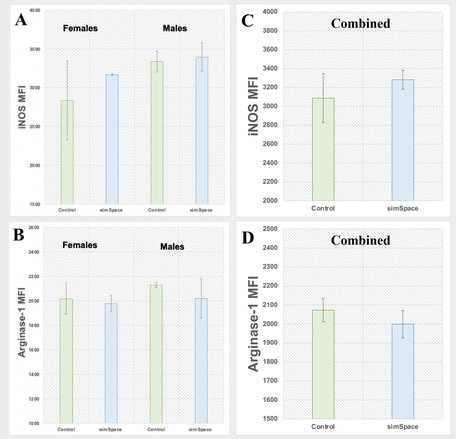


Figure 3. *Bar chart represents dimorphic male and female expression levels of iNOS (M1) and Arginase-1(M2) **(A and B)** and combined male and females are displayed **(C and D).** MFI results for M1 (iNOS) and M2 (Arginase-1) phenotypes within CD170^{+} AM.*

- Absence of statistically significant differences, due to low sample size (n=3M/3F per group) and variance.
- Observable decrease in the mean fluorescence intensity (MFI) of Arginase-1 was detected in the simSpace group when compared to the controls.
- Reduced trends in Arginase-1 MFI suggests potential predominance of M1 phenotypes within the simSpace environment, implying a shift towards a pro-inflammatory state within the lung in response to simSpace conditions.

CONCLUSION

- Analysis from the OSD-248 revealed downregulation in the expression of both Arginase-1 and iNOS between the control and simSpace conditions, suggesting impaired differentiation/polarization processes, with a predominance of M1 macrophages are noted.
- Ground simSpace studies displayed observable differences in M1 and M2 population shifting in simSpace, favoring M1 predominance.
- Observed dimorphism, with males displaying higher M1 mean fluorescence intensity (MFI) compared to females, suggests potential sex-specific responses post simSpace recovery.

LIMITATIONS OF STUDY

- Availability of a limited number of frozen lung samples constrained the depth of the analysis and restricted the ability to draw robust conclusions. Frozen cells have reduced cell viability, integrity, and staining variability potentially influencing the obtained results.
- Accessibility to fresh lung samples from spaceflown mice will be instrumental in advancing research in this area, allowing for the implementation of the newly developed protocol.

FUTURE STUDIES

- Future research aims to elucidate the precise molecular mechanisms underlying the observed phenotypic changes in lung macrophages in response to simulated microgravity.
- Ongoing investigation with space-flown RR-20 mice aims to further validate the definition of M1/M2 macrophages in the lung, emphasizing the role of characterizing polarized lung macrophage populations in comprehending immune responses during spaceflight and the challenges posed to astronaut pulmonary physiology by unique environmental conditions.

ACKNOWLEDGMENTS

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