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**Astroimmunology: Uncovering Neutrophil Biology and Function in Spaceflight**

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*“Your immune system is a window to your physiology” – Dr. Elizabeth Blackburn NASA Ames Seminar, October 20, 2021*

**The problem**

With the advent of exploration missions to the Moon and Mars, a critical need for a better understanding of the spaceflight factors that impact immunity are required. While many groups have provided overviews of immune dysregulations that are caused from spaceflight there is still a lack of mechanistic underpinnings for immune disparities. Although T cells have been the focus of research for the past two decades, with excellent evaluations on adaptive function, concentration on other immune cells in particular innate immune cells, such as granulocytes have been limited. This may be due to a limited number of neutrophil biology-specific immunologists to study this specialized field, or because of the difficulty to study these cells in spaceflight, due to their short-life span and sensitivity to freeze/thaw processes during storage. Yet, their importance to directing and contributing to immunity are ever apparent and require more attention particularly in the fields of space biology and space biosciences.

**Background**

Neutrophils are part of the innate immune system, constitute up to 60% of human leukocytes in circulation, and are one of the ‘first responders’ to sites of infection or injury. Neutrophils enter these sites following chemokine recruitment via neutrophil extravasation from circulation, and ultimately from the bone marrow, where they mature. Systemic inflammation causes emergency granulopoiesis, or massive influx of neutrophils from the bone marrow into circulation, which is caused by a number of different challenges, including those found during spaceflight exposure. Neutrophils participate in phagocytosis and degradation of pathogens, while killing themselves in the process. They secrete reactive species and are typically short-lived, however in pathophysiological conditions, their persistence can cause bystander tissue damage, enhancing acute inflammation and contributing to chronic inflammation. Therefore, clearance of these neutrophils is important to maintain immune homeostasis.

Spaceflight-induced immune dysfunction includes, increased rash formation and allergic like responses. Reactivation of herpesviruses, has been consistently observed during spaceflight concomitant with decreased cellular immunity. Bacterial species are more virulent inflight and with combined immune dysfunction this may leave crew susceptible to commensal-reverted microbial species, in addition to threating Earth-bound microorganisms that may have breached shuttle access. Mild, non-resolving inflammation and inflammaging are described, which is similar to elder immunity, resulting in immune senescence, bystander tissue damage and mitochondrial dysfunction. T cell function and proliferation are impacted, thereby development of effector/memory functions are altered, along with impaired production of cytokines, including IL-2. An elevated neutrophil-to-lymphocyte ratio (NLR), a biomarker for subclinical inflammation, is also elevated in-flight resulting in redox imbalance. Thus, immune dysfunction in space is a combination of leukocyte disparity, non-resolving inflammation, and functional impairments.

Seminal studies, on neutrophil biology in spaceflight have identified population shifting occurs at landing. These results may be in response to the highly stressful impacts of hypergravity experienced during landing. Neutrophils (granulocytes) were increased 1.5-fold to pre-flight counts ([DOI: 10.1002/jlb.65.2.179](https://jlb.onlinelibrary.wiley.com/doi/10.1002/jlb.65.2.179)), while another group identified neutrophils were 85% elevated compared to preflight levels following short duration missions ([DOI: 10.1016/j.bbi.2003.10.005](https://www.sciencedirect.com/science/article/abs/pii/S0889159103002010?via%3Dihub)). Determining altered neutrophil distributions occurred in-flight were not identified until 16 years later, when in-fight blood collections on the International Space Station (ISS) and rapid return to Earth for analyses (within 72 hours) post-collection were performed. These studies determined that in-flight granulocytes populations were elevated, which persisted up to 6 months ([DOI: 10.1038/npjmgrav.2015.13](https://www.nature.com/articles/npjmgrav201513)). Functional studies including neutrophil phagocytic impairment and dysfunctional redox balance have been reported post-flight and in ground models of spaceflight ([DOI: 10.1016/j.bbi.2003.10.005](https://www.sciencedirect.com/science/article/abs/pii/S0889159103002010?via%3Dihub), [DOI: 10.3389/fimmu.2020.564950](https://www.frontiersin.org/articles/10.3389/fimmu.2020.564950/full), [DOI: 10.1016/j.isci.2020.101747](https://www.cell.com/iscience/fulltext/S2589-0042(20)30944-5?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS2589004220309445%3Fshowall%3Dtrue)), however there is a current need to understand the effects of neutrophil functions and roles in-flight.

**Recommendations**

**Research-driven scientific recommendations:** *Due to paucities in understanding neutrophil function in spaceflight the following research recommendations that require support through the next decade are necessary to elucidate immune kinetics important for future exploration-crew and civilian missions.*

1. Flow cytometry or hematology analyzer technologies must be onboard spaceships or the ISS for in-flight immune differential and complete blood count (CBC) monitoring. Integration of this requirement for overall health assessment on all missions (long- and short-term missions and exploration-class missions).
2. Aside from differential profiling, the impacts of in-flight stressors on neutrophil function is limited. While this requires crew time and encounters fluidics-issues we propose funding then be directed towards tackling these important assays. With the advent of 3D tissue and chip-based techniques that can be performed in-flight, with limited crew intervention, the implementation of these technologies would limit the caveats associated with astronaut sampling. Therefore, collectively more funding should be dedicated to in-flight immune research to assess functional studies.
3. Sex- and age-specific research: with the upcoming Artemis missions, there is a critical need to assess immune differentials and functions between males and females, and other metadata such as age-specific effects. This is critical to differentiate as the immune system is functionally unique to sex- and age-dependency. Therefore, studies directed to understanding the diversity of the immune system is crucial for personalized medicine.
4. Due to their contribution to inflammation and elevated numbers in-flight, assessment of dampening inflammation with countermeasures that target these cells may be necessary to shift immunity during spaceflight. Therefore, countermeasure research should be highlighted in the next 10 years of astroimmunology results, with a focus on neutrophil targeting.
5. Immune functions during circadian cycles is another avenue for interest. Since neutrophil numbers fluctuate throughout the day suggesting functional consequences, studies that assess these outcomes in-flight are required to survey immune status. Furthermore, the immune and neuroendocrine systems highly influence each other. Therefore, considerations of neutrophil counts and functions during these diurnal timepoints, along with neuroendocrine peptide/hormone concentration assessments, should be noted in future research. A preference for funding should be provided to PI’s that consider the convolution of these physiological systems and their impacts on immunity.
6. While there are multiple reports that characterize the immune profile, the effects of these profiles in response to challenges are lacking. For example, herpesvirus reactivation in terrestrial settings is dependent on CD8 T cell cytotoxicity, however although viral reactivation is experienced in-flight, there is no causal link directed to CD8 T cell function. Therefore, addressing these types of functional assays are absolutely critical to better equip crew with proper countermeasures.
7. Mechanisms of leukocyte/neutrophil migration are limited as well. Therefore, assessment of migratory behaviors of neutrophils in-flight are important along with sampling timepoints, as microgravity can impact lymphatics and circulatory system vasculature.

**Logistical recommendations:**

1. Onboard flow cytometry or hematology analyzer technologies should be readily available for use by multiple PIs conducting research on these crafts, and consent and open-sourced to a repository such as Life Sciences Data Archive (LSDA) for data sharing.
2. Request for information from astronauts from repositories, such as LSDA, should be more streamline. There is currently a paucity in data availability for astronauts that requires a more accessible system. For example, requested retrospective data from LSDA may take up to a year to acquire, which severely impacts research queries and proposals that have deadlines for submission.
3. There are currently only, a few PIs that can acquire astronaut data. This should be much more explicit and open for multiple PI involvement. This open accessibility would be more beneficial for science and interpretation.
4. On board mouse/rodent habitats would provide a viable research tool to assess in-flight immune differentials, migration assays, as well as perform pathogen challenge experiments.
5. Data science integration with NASA GeneLab or other bioinformatic mechanisms would be beneficial to assess systems biology queries and physiological contributions to immunity. Therefore, continuity of programs that support open data science would be necessary for the next 10 years of astroimmunology research.
6. More civil servants, scientists, contractors, postdoctoral fellows, and undergraduates in astroimmunology to join NASA centers are crucial to study this research area. The immune system is a complicated system that requires basic immune knowledge to assess functional underpinnings, therefore trained scientists/students in immunology are important to enhance NASA’s workforce in astroimmunology research.

**Additional analog, ground-based recommendations:**

1. In addition, enhanced support to research that assesses neutrophil function via ground-based models including high-aspect rotating wall cell culture vessels (HARV-RWV) and/or 3D clinostats in combination with ionizing radiation doses and dosing schemes (LEO and BLEO). For example, enhanced support for GCR simulation modeling at Brookhaven National Laboratories (or other research facilities that address LEO/BLEO irradiation types) should be supported in the next decade.
2. In addition, ground-based analog supported research (i.e. Antarctica, NEEMO, HERA) is relevant for studying immune system differentials and function, especially where longitudinal stressor effects can be assessed. For example, although lacking microgravity effects, the impact of social isolation and hypercapnia can be assessed which may serve as considerations for future in-flight directed studies.
3. Along these lines, standardization of ground studies must also be achieved, as combined stressors of spaceflight produce very distinct immune profiles and function, making this biological system difficult to study. Therefore, standard models (that mimic the spaceflight environment, in particular BLEO) are required.

In brief, with advancements in human exploration and civilian missions, a critical need to assess immunity is required. As arguably the most sensitive system of physiological defense, it is absolutely necessary to determine neutrophil kinetics and functions in response to spaceflight stressors, which can be diagnostically evaluated in-flight following the aforementioned recommendations. We stress the importance of understanding immune function is vital to developing effective countermeasure that will be required for long duration deep space missions, and an invaluable area of research in the next decade.