Tissue Macrophages Relevant to Spaceflight

Fundamental Understanding Needed to Support the Artemis Program and Deep Space Exploration

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INTRODUCTION

Macrophages, the primary phagocytic cells in the body, play diverse roles in human physiology, ranging from host defense to tissue repair and remodeling, including regulation of inflammatory processes, participation in immune clearance and communication with cells of the immune system, etc. For many years, the accepted dogma was that macrophages are derived from circulating monocytes that originate in the bone marrow [1]. While many tissues are populated by monocyte-derived macrophages, at least in part (the gastrointestinal tract [2], the kidney [3] and the liver [4]), it is now understood that many macrophages are derived from embryonic yolk sac cells and that they become established in various tissues early on in the course of development [5,6]. An appreciation of tissue macrophage ontogeny provides an important foundation for viewing macrophages as cells with highly specialized functions that are determined by the localized tissue niches in which they reside. In thinking about spaceflight effects on humans, it is helpful to think about several examples of macrophages that play roles in key physiological processes that we know (or suspect) are relevant to the exposure of the human body to spaceflight conditions. While potentially all tissue macrophages are relevant to spaceflight, because of space limitations, this white paper focuses on examples that we believe have the strongest relevance to known spaceflight risks.

TISSUE MACROPHAGES RELEVANT TO SPACEFLIGHT—THREE EXAMPLES

1) Alveolar Macrophages. Alveolar macrophages are resident macrophages of the lungs and play an important role in the host response to invading pathogens as well as particulate matter that enters the lung [7]. Like other tissue-resident macrophages, alveolar macrophages become established in the lungs early in the course of development. Before birth, F4/80hiCD11blo primitive macrophages and Ly6ChiCD11bhi fetal monocytes sequentially colonize the developing lung around E12.5 and E16.5, respectively [8]. The establishment of alveolar macrophages in the lungs is dependent on the growth factor GM-CSF. In addition to pathogen clearance and response to particulate matter, alveolar macrophages play an important role in surfactant homeostasis [9], regulating surfactant protein and phospholipid clearance and catabolism. An important aspect of alveolar macrophage physiology relevant to exploration of the moon is the behavior of these cells in response to particulate matter, such as lunar dust. When particulate matter enters the lungs, alveolar macrophages respond with an *oxidative burst,* with increased oxygen consumption, production of superoxide anion O2•−, H2O2, and increased nitric oxide production [10,11]. These initial alveolar macrophage responses are the start of a cascade of events that determine how the lung responds to particulate materials. In the context of human exploration of the moon, where exposure to lunar dust can be expected, macrophages are expected to play a critical role in the response to lunar dust entry into the lungs [11–14].

Critical Question Relevant to Space Travel: ***Do spaceflight conditions, such as space radiation and other factors, alter the normal functioning of alveolar macrophages in a way that impairs their ability to respond appropriately to lunar dust exposure?***

2) Arterial, Cardiac and Venous Macrophages

Macrophages play critical roles in the cardiovascular system, in the arteries, veins, and heart. Macrophages are the most abundant immune cells in the arterial wall [15], and are involved in inflammatory disease, atherosclerosis and fibrosis. Macrophages can drive local inflammation and can also help to resolve inflammation. In the wall of the aorta and in the walls of other peripheral arteries, macrophages are derived, early in life, from erythroid-myeloid progenitor cells in the yolk sac and bone marrow-derived monocytes as well. Self-renewal accounts for the persistence of these cells into adulthood [15–17].

The epicardial coronary arteries, similar to other arteries in the body, possess an abundance of macrophages. Coronary artery macrophages play a critical role in the development of atherosclerotic coronary artery disease, including inflammation at sites of plaque, propagation of coronary plaques, and promotion of thrombus formation. In the context of coronary artery disease, classically-activated (M1) macrophages and alternatively activated (M2) macrophages are viewed as promoting and resolving inflammation, respectively, although this dichotomy may be an oversimplification [18]. Macrophages play a key role in repair of the heart after ischemic injury [19].

Macrophages are also an intrinsic part of the healthy functioning myocardium. Recent evidence points to a role of CCR2- macrophages in limiting infarct size in animal models of myocardial infarction [20]. Studies of cardiac macrophages implicate macrophages in normal and aberrant cardiac conduction, highlighting the importance of gap junction connections between macrophages and cardiomyocytes [21,22].

Human data from the radiation therapy literature demonstrates that radiation exposure can induce significant pathology in the heart [23] and in blood vessels [24]. Unavoidable irradiation of the heart occurs in radiation therapy for Hodgkin's Disease, lung cancer, and breast cancer. As a result, pericardial disease, restrictive cardiomyopathy, valvular abnormalities, premature coronary artery disease, cardiomyopathy, and arrhythmias can occur. Even low doses of radiation can cause problems [25]. *Can space radiation also affect the cardiovascular system?* Rodent studies are supportive of this notion [26]. ^{56}Fe ion-irradiation led to impaired cardiac function and more adverse remodeling in acute myocardial infarction-induced mice. Decreased angiogenesis and pro-survival factors in cardiac tissues were seen as well [27].

A recent study looked at an endogenous peptide, N-acetyl-Ser-Asp-Lys-Pro (Ac-SDKP), in a rat model of radiation cardiotoxicity. The peptide exerted a protective effect by inhibiting inflammation and fibrosis and reducing macrophage activation [23,28]. These findings raise the prospect that countermeasures to radiation cardiotoxicity, with the potential to benefit space travelers, may be soon become available.

Lastly, the function of macrophages in the veins deserves mention, in light of a recent episode of venous thrombosis in an internal jugular vein in a female astronaut aboard the ISS [29]. An internal jugular vein clot is highly concerning, because of the risk of pulmonary embolism, which can be fatal. In one recent review from a single institution, 10% of cases of internal jugular thrombosis resulted in pulmonary embolism, and 25% of those cases were fatal [30]. In

addition to their role in arteries, macrophages are important in veins. In a recent study of induced venous thrombosis in mice, macrophages with M2-like characteristics were found to be critical for thrombus resolution [31].

Critical Question Relevant to Space Travel: ***What macrophage functions in the heart and in blood vessels are altered by space radiation or other factors associated with spaceflight, and how can these functions be targeted for countermeasure development?***

3) Microglia

Microglia are macrophages present in the CNS, distinct from perivascular, meningeal, and choroid plexus macrophages in that they are the only true brain parenchymal immune cells. Microglia originate from embryonic yolk sac precursors, migrate to the brain at E9.5, and then differentiate into mature cells. Once in place, microglia maintain their population by self-renewal [32]. As mature cells, microglia are significant in both homeostasis and disease response and repair. Microglia are known to interact indirectly with other CNS cells and have been shown to physically interact with neurons as well [33]. Their cellular roles are defined by their abilities to function as antigen-presenting cells, capability of expression of toll-like receptors, and role in phagocytosis [34]. Microglia have been shown to be significant in synapse remodeling [35], synaptogenesis, neuronal survival, neuronal death, and in CNS infection [36].

Galactic cosmic radiation (GCR) will be a novel and significant risk in deep-space travel. Several studies have investigated HZE particles (GCR with high charge (Z) and high energy (E)) in mice. HZE exposure altered recognition, social memory, and spatial memory, in addition to increasing anxiety, in experimental animals [37]. Furthermore, simulated GCRs using helium ions were shown to affect attention in mice [38]. Thus, GCR has been demonstrated to broadly affect the murine CNS. HZE irradiation was also demonstrated to increase microglia activation for up to 12 months, perhaps by causing synapse loss [37]. Interestingly, when microglia were depleted one week after simulated helium cosmic radiation exposure, long-term memory issues were avoided [38]. These results suggest a complex role for microglia, the "macrophages of the CNS," in mediating the cerebral changes induced by spaceflight-specific radiation [35].

Critical Question Relevant to Space Travel: ***Given that 1) simulated GCR radiation negatively impacts CNS functions and 2) microglial depletion avoids long-term memory issues associated with simulated GCR radiation, can modifications of microglial populations serve as opportunities to combat GCR exposure?***

RECOMMENDED EXPERIMENTAL APPROACHES

We have highlighted three examples of macrophages that play critical roles in tissues (alveolar macrophages, cardiac and vascular macrophages, and microglia—CNS macrophages) that are relevant to spaceflight. These tissue-specific macrophage populations (and others, such as those in the liver and those in the kidney) warrant close examination under spaceflight conditions using rodents and other model systems (perhaps even non-human primate) in order to help anticipate spaceflight risk. As shown in **Fig. 1,** hindlimb unloading of rodents by tail suspension is a

simple ground-based experimental technique that can mimic many of the features of reduced gravity in rodents, and radiation exposure of rodents at the NASA Space Radiation Laboratory (at Brookhaven National Laboratory) can simulate galactic cosmic radiation (GCR), to enable macrophage studies in space-like environments. All of the (initial) experimental approaches suggested in this white paper would be carried out in rodents (mice or rats) with hindlimb unloading or radiation exposure as the primary independent variable.

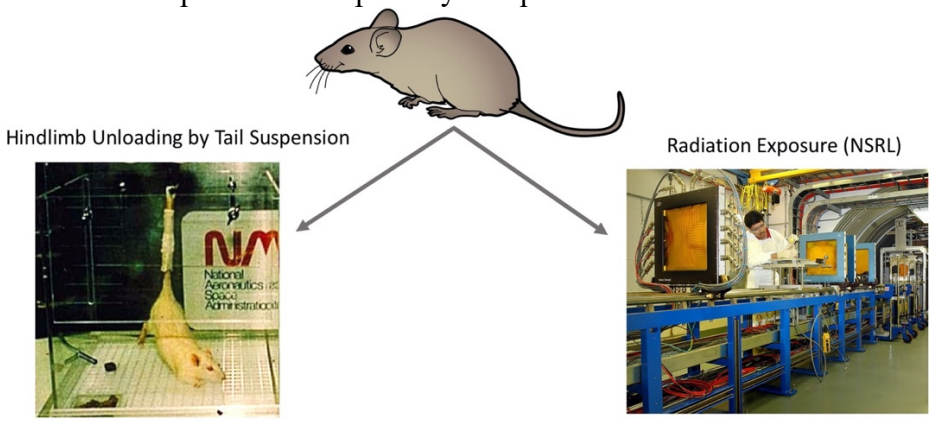

Figure 1. Ground-based techniques to mimic spaceflight risks. Hind-limb unloading of rodents by tail suspension is a simple technique that can mimic many of the features of reduced gravity in rodents. Radiation simulating galactic cosmic ray exposure of rodents can be accomplished at the NASA Space Radiation Laboratory (NSRL). Other radiation facilities, besides the NSRL, may be available for these experiments, too. (Images courtesy of NASA)

Recommended Approach to Alveolar Macrophage Studies

Rodent experiments involving exposure to simulated space radiation and/or hind-limb unloading would be appropriate for studies of alveolar macrophages from the lungs. Bronchioalveolar lavage is a technique that can be used to isolate alveolar macrophages (and other cells) from the alveoli. Valuable endpoints include enumeration of macrophages in bronchioalveolar lavage fluid, comparing experimental animals exposed to radiation (or hindlimb unloading) with control animals. Further insight would be provided by experiments that that involve exposure of rodents to lunar dust simulant, followed by bronchioalveolar lavage to assess macrophage (and other cell) counts, as well as the degree of activation of the macrophages by the Luminol assay [39,40]. Additional value would be provided by genomics studies of macrophages isolated by bronchioalveolar lavage, to better understand how macrophage gene expression may change as a function of radiation or modeled reduction in gravity of the experimental animals.

Recommended Approaches to Cardiac and Vascular Studies

We recommend that functional assays that demonstrate macrophage function in the vascular system be used in rodent studies that utilize space radiation and/or hindlimb unloading to mimic spaceflight stressors. One example is a mouse model that uses locally applied angiotensin II to mimic arterial inflammation [15]. Numbers of macrophages and degree of fibrosis would be

reasonable endpoints. For studies of venous thromboembolism, mouse models that induce thromboembolism could be used, with time-to-resolution of thrombus as a helpful endpoint [31]. Similar approaches could be used to probe cardiac macrophage function, looking at both epicardial coronary arteries, cardiac myocytes and the cardiac conduction system, all of which are dependent on macrophages. Cytokine signaling, an important downstream effect involving macrophages, should also be investigated. For all cardiovascular tissues, single-cell mRNA studies and epigenetic studies may be helpful as well, especially when functional tests are difficult to establish.

Recommended Approaches to Microglia Studies

Studies should be performed in rodents to determine if exposure of experimental animals to GCR (simulated) impacts microglia-mediated synaptic remodeling. Previously, interactions between microglia and synapses were investigated using stimulated emission depletion (STED) microscopy through fluorophore-conjugated cell types to visualize cellular localization. Synaptic engulfment by microglia was observed when conjugating against PSD95 and SNAP25 in synapses and microglia of the postnatal mouse hippocampus. Subsequent study of a KO-*Cx3crl* mouse observed the same colocalization but higher amounts of PSD95 puncta, suggesting a functional role for *Cx3crl* in microglial-induced synaptic pruning [41].

Single cell RNASeq (scRNASeq) is a recently developed genomics-based technology able to evaluate the expression of specific genes on an individual cell level. To study if increased synaptic pruning is responsible for GCR-caused effects on the CNS found to be ameliorated by microglial depletion, scRNASeq pre- and post-simulated GCR irradiation in mice can be investigated at various synaptic sites. *Cx3crl* and other putative microglial genes involved in synaptic remodeling should be investigated for upregulation post-irradiation. Furthermore, STED microscopy can be utilized to inspect if co-localization is altered by irradiation. If *Cx3crl* and other synaptic remodeling genes are upregulated after GCR exposure, then future work should study if KO-*Cx3crl* is independently able to protect against GCR-caused CNS complications. Alternatively, if upregulation is not observed or if synaptic engulfment is altered after GCR exposure, subsequent experiments should investigate an alternative role for microglia in GCR response. Follow-on studies may include spaceflight experiments to low Earth orbit, and, eventually, experiments beyond low Earth orbit.

SUMMARY

The authors of this white paper advocate a new Space Biology investment focused on the study of macrophages in specific tissues which are critical to the function of those tissues and are known or suspected to be affected by spaceflight conditions. Alveolar macrophages in the lungs (relevant to lunar dust exposure), cardiac and vascular macrophages, macrophages in the CNS (microglia), and liver, kidney and gut macrophages may all be relevant. Fundamental investigations in these arenas, including genomics-based studies and key functional assays, may lead to an improved understanding of spaceflight risk. These studies are expected to be highly relevant to NASA's Artemis Program, and will help to improve understanding of physiology relevant to Mars and other solar system destinations.

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