Fiscal Year:	FY 2018	**Task Last Updated:**	FY 11/20/2017
PI Name:	Porada, Christopher Ph.D.		
Project Title:	Effects of Microgravity on the Risks of Space Radiation-induced Leukemogenesis		
Division Name:	Space Biology		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Cell & Molecular Biology (2) Animal Biology: Vertebrate		
Space Biology Cross-Element Discipline:	(1) Immunology		
Space Biology Special Category:	(1) Translational (Countermeasure) Potential		
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Comments:			
Project Type:	Ground,NASA GeneLab	**Solicitation / Funding Source:**	2016 Space Biology (ROSBio) NNH16ZTT001N-GeneLab. Appendix A: Translational Systems Biology and Informatics Research Using the GeneLab Data System
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COI Name (Institution):	Almeida-Porada, Maria Graca M.D., Ph.D. (Wake Forest University) Walker, Steve Ph.D. (Wake Forest University) Wilson, Paul Ph.D. (University of California, Davis)		
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Task Description:	We will specifically be making use of data generated as part of GeneLab experiment sets GLDS-53, GLDS-55, and GLDS-25 as the basis for the novel hypothesis to be tested in the current proposal: microgravity (µG) acts in concert with solar particle event (SPE) and galactic cosmic ray (GCR) radiation to produce deleterious effects on the human hematopoietic system, which may lead to an enhanced risk of leukemogenesis, as a result of both increased genomic damage to cells of the hematopoietic system, and a reduced ability of the immune system to recognize and clear hematopoietic cells that have undergone malignant transformation as a result of exposure to SPE/GCR radiation and conditions of microgravity. Data generated from the aforementioned GeneLab studies support this hypothesis, as these data have shown that µG: 1) induces higher levels of spontaneous DNA damage in human hematopoietic cells; 2) markedly alters the ability of mature human immune cells to respond appropriately to stimuli; 3) diminishes the ability of human lymphocytes to efficiently repair DNA damage in response to ionizing radiation; and 4) leads to alterations in the levels of multiple miRNAs that have been implicated in a variety of human hematopoietic malignancies. We have also generated a wealth of data to support the hypothesis that µG and space radiation likely act synergistically to increase astronaut risk of leukemogenesis during a prolonged mission beyond LEO (low Earth orbit). In the present proposal, we will build upon these data by performing studies to directly test the ability of µG to increase the risk of leukemic transformation in human hematopoietic stem/progenitor cells (HSC) , while simultaneously reducing the ability of generated immune cells from recognizing and removing any malignant clones that arise.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Our research has thus far revealed that conditions of microgravity leads to marked alterations in the ability of human hematopoietic stem/progenitor cells (HSC) to repair DNA double strand breaks (DSBs) that are characteristic of the damage that occurs following exposure to ionizing radiation. Moreover, microgravity also appears to impair the ability of human HSC to generate functional dendritic cells, which act as critical sentinels within the immune system, detecting infectious invaders and cells that have undergone malignant transformation, and alerting/priming immune effectors to eliminate these threats. Further adding to these deleterious effects, we have also found that microgravity negatively effects the ability of human natural killer (NK) cells to recognize and lyse human leukemic cells. Taken together, our results to date have shown that conditions of microgravity present during spaceflight could add to the risks of leukemogenesis as a result of exposure to space radiation, both by impairing the ability of human HSC to repair the induced damage and by crippling the generation and function of the immune cells needed to recognize and eliminate cells damaged by the radiation. An understanding of the mechanism(s) by which microgravity effects and impairs these different processes could lead to the development of novel methods to target and augment these pathways, and thereby enhance the processes of DNA repair and anti-tumor immunity. Such developments could have a profound impact on the treatment of cancer and on the lives of patients suffering from this disease.
Task Progress:	One of the major factors limiting manned spaceflight beyond low-Earth orbit is the poorly defined health risks as a result of exposure to space radiation in the form of solar energetic particles (SEP) and galactic cosmic rays (GCR) that could potentially increase cancer morbidity/mortality in astronauts. We have recently demonstrated seriousness of this risk by showing that exposing human hematopoietic stem cells (HSC) to simulated GCR (at high dose-rate) results in the generation of leukemia when these cells repopulate the hematopoietic system of mice (Leukemia, 31(6), 1398-1407, 2017). In addition to being exposed to high energy radiation, astronauts are subjected to conditions of microgravity (µG) during spaceflight, which also exerts a wide range of untoward effects on the body, including altering immunity and the response to radiation. We therefore hypothesized that conditions of microgravity µG present during spaceflight may act in concert with SEP and GCR radiation to produce deleterious effects on the human hematopoietic system, leading to an enhanced risk of leukemogenesis, as a result of both increased genomic damage to cells of the hematopoietic system, and a reduced ability of the generated immune system to recognize and clear hematopoietic cells that have undergone malignant transformation. In the 1st set of studies to test this hypothesis, we treated the human HSC-like KG1a cell line with an acute dose (10 µg/ml) of the radiomimetic drug bleomycin to mimic the damaging effects of SEP/GCR radiation. We then took the treated cells, in addition to an identical batch of untreated cells, and split them in half. Half of the cells from each aliquot were cultured under conditions of normal gravity (1G) in a 370C incubator, and the other half was cultured in the Synthecon Rotary Cell Culture System (RCCS) to create a state of continual freefall, and thereby mimic µG. At 1 hour and 4 hours, half of the cells were harvested from the 1G and µG cultures, and we quantitated the extent of double-strand breaks (DSBs) and the kinetics of repair, using flow cytometry and confocal imaging to monitor the formation and disappearance of gamma-H2AX foci. Interestingly, both the bleomycin-treated and untreated cells experienced a moderate increase in their median fluorescence intensity (MFI) when cultured in a µG environment, indicating that just the presence of conditions of simulated µG may lead to an increase in DNA damage. To determine the impact conditions of µG exerted on the ability of KG1a cells to repair DSBs induced by the radiomimetic bleomycin, we calculated the ratio of bleomycin-treated cells' MFI to untreated cells' MFI under conditions of 1G vs. those in µG between the 1 hr and 4 hr time points. We reasoned that, if DNA repair proceeds normally, this ratio should decrease from the 1 hr to 4 hr time point, since gamma-H2AX foci disappear as DNA is repaired. When we first examined the cells cultured in 1G, this ratio did indeed decrease, by 26%, from the 1 hr time point to the 4 hr time point for cells, demonstrating successful repair of the bleomycin-induced DNA damage during this time. In marked contrast, cells maintained in µG experienced a 20% increase over this same period, suggesting they were unable to repair the bleomycin-induced DNA damage, and this damage accumulated during the 4 hr incubation. Since the "classical" method for assessing DSBs induced by bleomycin or ionizing radiation is to perform immunofluorescence microscopy on cells following staining with gamma-H2AX, we used this traditional method to confirm that fluorescence events that we were detecting on the flow cytometer were indeed due to the presence of gamma-H2AX foci within the nuclei of the human HSC-like KG1a cells maintained in conditions of simulated µG during the period of DNA repair following bleomycin exposure. From the studies we have performed to-date, we conclude that HSC DNA damage repair is compromised in conditions of µG, leading to an accumulation of DSBs that cannot be resolved over time. These findings thus support our hypothesis that conditions of µG may enhance the genotoxic effects of space radiation, and increase the risk of leukemogenesis. These findings were recently reported at annual meeting of the North Carolina Tissue Engineering and Regenerative Medicine Society. These studies were performed by Egil Brudvik, an undergraduate student who worked on this project as part of WFIRM's annual Summer Scholars Program. Egil has submitted an abstract detailing our findings for consideration for presentation at the Council on Undergraduate Research's 22nd annual undergraduate poster session to

	be held in the Spring of 2018 on Capitol Hill. In our 2nd set of experiments, we tested the hypothesis that μG alters the development and functionality of critical regulators of the immune system, which could further enhance cancer risk during spaceflight. The generation of an effective immune response requires that antigens be processed and presented to T cells by antigen presenting cells (APC), the most potent of which are dendritic cells (DC), which stimulate naïve T cells and initiate primary immune responses. DC also function as effector cells in innate immunity. Because DC have influence over both the innate and acquired arms of immunity, a defect in their production and/or function would be expected to result in broad impairment of immunity. We therefore tested whether DC could be generated from human HSC in μG, again using the RCCS. CD34+ cells were cultured in 1G or μG using cytokine-supplemented serum-free media, and were analyzed for the presence of plasmacytoid (CD123+) and myeloid (CD11c+) DC by flow cytometry. While culture in both 1G and μG produced higher numbers of myeloid (CD11c+) vs. plasmacytoid (CD123+) DC, the HSC cultured in 1G differentiated into DC in significantly higher numbers than those cultured in μG. Moreover, culture in μG significantly delayed the generation of DC; while DC were generated within 7 days in 1G, they only appeared after 14 days in μG. Our results thus suggest that μG delays the production of DC and reduces the number of these important APC that are generated. A manuscript detailing these findings is currently being written for submission to Dr. Elizabeth Blaber's Special Issue of Stem Cells and Development that is focused on Microgravity. In our 3rd set of experiments, we have begun exploring the effects that conditions of μG exert on the generation and functionality of NK cells, focusing on their ability to recognize and lyse leukemic cells. To begin these studies and optimize the methods/assays to be used, we began with the human IL-2-independent NK cell line NK-92MI, testing the effects that a brief (48 h) exposure to conditions of μG had on the ability of these cells to subsequently recognize and lyse the human erythroleukemic cell line K562. Even this relatively brief exposure to conditions of μG dramatically reduced the ability of this human NK cell line to recognize and lyse these human leukemic targets, irrespective of the effector:target (E:T) ratio used. Studies are currently ongoing with primary human peripheral blood NK cells, and samples are being prepared for transmission electron microscopy (TEM) to define the subcellular alterations that occur in the NK cells exposed to μG that so dramatically effects their ability to recognize and/or lyse leukemic cells. Studies are focused on the alterations μG induces in the actin cytoskeleton of the NK cells, as the actin filaments are known to play a critical role in both the formation of the immune synapse and the movement and ultimate release of the cytolytic granules from the NK cells. Combining the data we have generated thus far on the immune-altering effects of μG with those of the preceding experiments on DSB repair, we feel that our studies to-date support our overall hypothesis that conditions of μG present during spaceflight may enhance the risk of SEP/GCR-induced leukemogenesis, highlighting the importance of developing means of simulating gravity during spaceflight to reduce astronaut risk.
Bibliography Type:	Description: (Last Updated: 01/30/2023)
Abstracts for Journals and Proceedings	Brudvik E, George SK, Almeida-Porada G, Porada CD. "Simulated Microgravity Impairs DNA Damage Repair in a Primitive Human Leukemic Cell Line." Presented at the 19th Annual Meeting of the North Carolina Tissue Engineering and Regenerative Medicine Society, Winston-Salem, NC, November 10, 2017. 19th Annual Meeting of the North Carolina Tissue Engineering and Regenerative Medicine Society, Winston-Salem, NC, November 10, 2017. , Nov-2017