



EFFECTS OF SPACEFLIGHT STRESS ON ANTI-INFLAMMATORY RESPONSE

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ABSTRACT

Spaceflight conditions have a significant impact on a number of physiological functions. Preliminary studies indicate that many of the changes noted in immune parameters are due to spaceflight-induced oxidative stress. We hypothesize that exposure to the spaceflight environment causes an overall increase in ROS (reactive oxygen species) that, in turn, down-regulates inflammatory mechanisms. In August of 2007, female C57BL/6 mice were flown aboard the Space Shuttle *Endeavour* for 13 days using NASA's animal enclosure modules (AEMs). Ground controls were maintained under conditions similar to those experienced by flight mice on a 48 hour delay using telemetry from the shuttle. Within 3 hours of landing, the mice were euthanized and organs were harvested. After an initial assessment at Kennedy Space Center (KSC), tissues were sent to Loma Linda University via overnight courier for further processing. Splenocytes were counted via flow cytometry and automated hematology analyzer, plated at 1e6 cells/mL, and incubated with 0.017mg/mL lipopolysaccharide (LPS, 0111:B5) for 48 hours. We found spaceflight-induced decreases in spleen, liver, and thymus mass (p<0.005). There were also significant reductions in all major leukocyte populations (p<0.05). Similarly, overall T and B cell counts decreased (p<0.01). Although spontaneous blastogenesis increased, there were significant decreases in mitogen-induced blastogenesis (p<0.001). Finally, there were decreases in tumor necrosis factor- α (TNF- α , p<0.001) with corresponding increases in interleukins- 6 and 10 (p<0.05). These results suggest that exposure to the spaceflight environment leads to an anti-inflammatory response. These changes may cause a disturbance in the homeostasis maintained by the CNS and immune system.

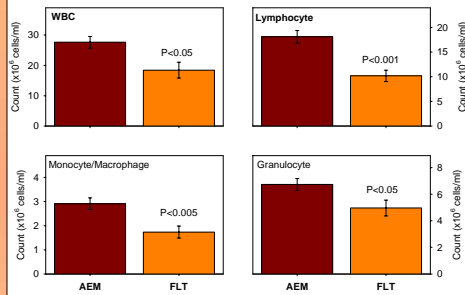


Figure 1. Leukocyte counts in spleen. Values represent Means \pm SEM.

MATERIALS AND METHODS

Mice and treatment: 24 nine week old female C57BL/6 mice flew aboard Endeavour in August 2007 for 13 days. 24 hrs prior to launch, mice were treated with placebo or the therapeutic agent. Mice were housed in three animal enclosure modules (AEMs), each containing 8 mice. 3 additional AEMs were kept on ground with control mice kept under same conditions as flight mice.

Tissue handling: Animals were euthanized and brain, lung, spleen, liver, adrenals and lungs were harvested, weighed, and processed at the Kennedy Space Center (KSC) and shipped to LLU via overnight courier.

Cell stimulation: Splenocytes were counted via flow cytometry and automated hematology analyzer. Cells were plated at 1e6 cells/mL and incubated with 0.017mg/mL of lipopolysaccharide (LPS, 0111:B5) for 48 hours.

Cytokine analysis: Supernatants from LPS stimulated cells were removed for cytokine analysis of IL-1b, IL-6, IL-10, IL-12 and TNF- α with ELISA kits (R&D).

Blastogenesis: Spontaneous blast was quantified with and without mitogens (phytohemagglutinin (PHA) 0.0063mg/mL and lipopolysaccharide (LPS) 0.017mg/mL) using thymidine.

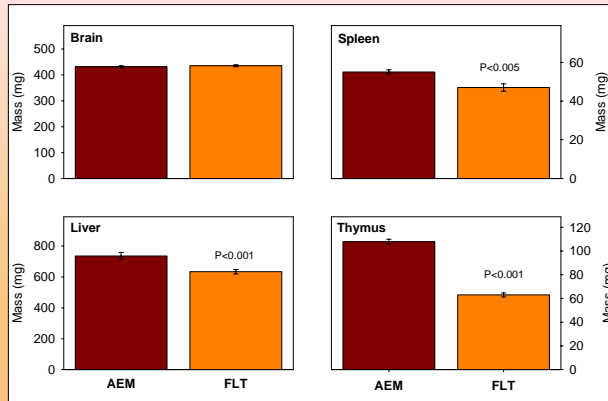


Figure 2. Organ masses. Values represent Means \pm SEM.

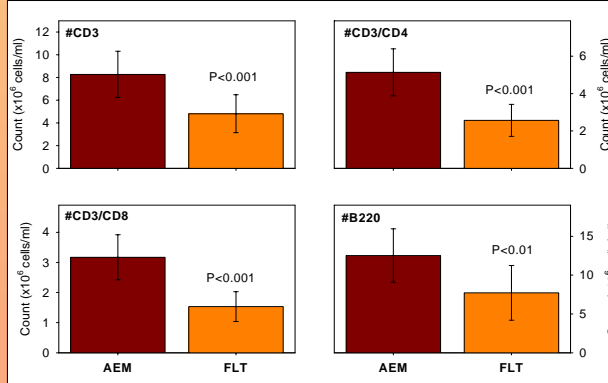


Figure 3. T cell, helper T cell, cytotoxic T cell and B cell populations. Values represent means \pm SEM.

Table 1. Leukocyte percentages. Values represent means \pm SEM.

	AEM	Flight	P value
CD3	38.9 \pm 2.3	36.6 \pm 3.1	0.050
CD3/CD4	24.5 \pm 1.7	19.9 \pm 5.1	0.001
CD3/CD8	15.0 \pm 1.0	12.1 \pm 0.7	0.001
B220	58.5 \pm 2.2	57.4 \pm 3.0	

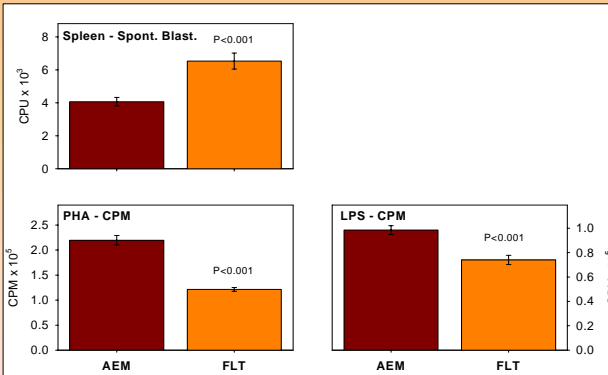


Figure 4. Spontaneous blastogenesis and mitogen induced blastogenesis. Values represent means \pm SEM.

BACKGROUND

Astronauts are exposed to a very stressful environment as a result of isolation, radiation, physical exertion (during extravehicular activities), anxiety and microgravity. Spaceflight leads to adverse pathological changes in many systems. Some of these changes include cardiovascular deconditioning, muscle atrophy, decreased plasma volume and altered hormonal and electrolyte levels (Sonnenfeld et al, 1998, Zayzafoon et al 2005). In addition, spaceflight conditions cause disturbances in the immune system such as changes in lymphocyte proliferation, cytokine production, natural killer cell cytotoxicity, cell-mediated immunity and signal transduction (Kaur et al 2004). These changes in the immune system may predispose astronauts to infectious illness as they are exposed to ecologically closed, crowded conditions. The microgravity allows infectious agents to remain suspended, facilitating the transfer of infectious agents. As astronauts are now continuously occupying ISS and several nations are planning lunar and interplanetary missions, it is important to understand the effects of spaceflight on the immune system. This study focuses on the changes of the immune system of female mice flown on the Space Shuttle *Endeavour* in August of 2007. Our data suggest that exposure to spaceflight environment causes a down-regulation of the inflammatory mechanisms, favoring an anti-inflammatory response.

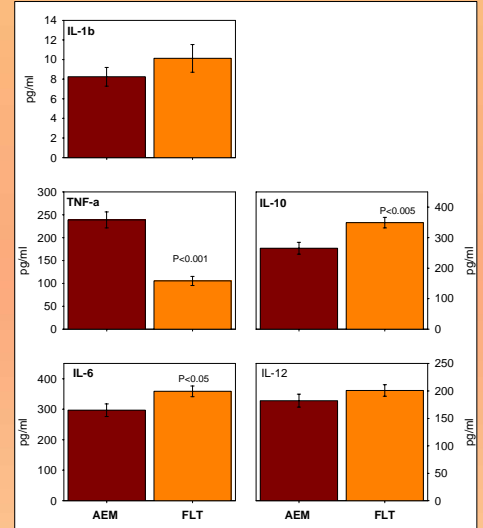


Figure 5. IL-1b, TNF- α , IL-6, IL-10 and IL-12 in spleen supernatant. Values represent means \pm SEM.

SUMMARY/ CONCLUSIONS

There were significant decreases in spleen white blood cells, lymphocyte, monocyte/macrophage and granulocyte counts (ps<0.05) of flight mice (Fig.1). Flight mice also had significant decreases in spleen, liver and thymus masses (ps<0.005) (Fig.2). There were reductions in overall B and T cells, as well as in the helper T and cytotoxic T cell subpopulations (ps<0.001) (Fig.3 and Table.1). Despite an overall increase in spontaneous blastogenesis (p<0.001), there were significant decreases in PHA and LPS induced blastogenesis (ps<0.001) (Fig.4). Flight animals had significant increases in IL-6 and IL-10 (ps<0.05) and there was a significant decrease in TNF- α (p<0.001). These results leads us to conclude that stress caused by space flight has an effect on the immune system and elicits an anti-inflammatory response.

