Effects of spaceflight on innate immune function and antioxidant gene expression

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1Department of Radiation Medicine Molecular Radiation Biology Laboratories and 2Department of Basic Sciences, Division of Microbiology and Biochemistry, Loma Linda University, Loma Linda, California; 3BioServe Space Technologies, University of Colorado at Boulder, Boulder, Colorado; and 4Kansas State University, Manhattan, Kansas

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Baqai FP, Gridley DS, Slater JM, Luo-Owen X, Stodieck LS, Ferguson V, Chapes SK, Pecaut MJ. Effects of spaceflight on innate immune function and antioxidant gene expression. J Appl Physiol 106: 1935–1942, 2009. First published April 2, 2009; doi:10.1152/japplphysiol.91361.2008.—Spaceflight conditions have a significant impact on a number of physiological functions due to psychological stress, radiation, and reduced gravity. To explore the effect of the flight environment on immunity, C57BL/6NTac mice were flown on a 13-day space shuttle mission (STS-118). In response to flight, animals had a reduction in liver, spleen, and thymus masses compared with ground (GRD) controls (P < 0.005). Splenic lymphocyte, monocyte/macrophage, and granulocyte counts were significantly reduced in the flight (FLT) mice (P < 0.05). Although spontaneous blastogenesis of splenocytes in FLT mice was increased, response to lipopolysaccharide (LPS), a B-cell mitogen derived from Escherichia coli, was decreased compared with GRD mice (P < 0.05). Secretion of IL-6 and IL-10, but not TNF-α, by LPS-stimulated splenocytes was increased in FLT mice (P < 0.05). Finally, many of the genes responsible for scavenging reactive oxygen species were upregulated after flight. These data indicate that exposure to the spaceflight environment can increase anti-inflammatory mechanisms and change the ex vivo response to LPS, a bacterial product associated with septic shock and a prominent Th1 response.

immunity; cytokines; stress; microgravity

SINCE THE FIRST HUMAN FLIGHTS to space, there has been a focus on the health effects of spaceflight-related environmental stressors such as isolation, nutrition, microgravity, radiation, and changes in the circadian rhythm (30, 63). Studies have shown that the spaceflight environment can impact several physiological systems, potentially resulting in serious consequences for immunity. As more astronauts travel to and occupy the International Space Station, and with future interplanetary exploration and space tourism on the rise, the physiological effects of spaceflight environment will necessarily receive greater attention.

In vivo flight studies performed in rat models have shown changes in mass (5, 22, 40) as well as hypoplasia (11) in both the spleen and thymus. Rats flown on both the Cosmos and space shuttle platforms have shown decreases in peripheral leukocyte and lymphocyte counts (1). Functionally, exposure to the spaceflight environment has resulted in decreases in the proliferative responses of lymphocytes isolated from lymph nodes (44) and spleen (23). Other studies have shown changes in cell-mediated immunity (69), cytokine production (8, 18, 35, 62), signal transduction (6), and natural killer cell activity (29). Furthermore, these findings are not limited to animal models. Blood collected from astronauts selected from 11 of the first 12 U.S. space shuttle missions indicated that the spaceflight environment leads to decrements in both the numbers of circulating leukocytes and the proliferative response to mitogenic challenge with phytohemagglutinin (PHA) (70).

Although these studies have been critical in understanding the effects of spaceflight, many of the results have been inconsistent due to differences in genetic backgrounds, mission duration, degrees of environmental stress, housing conditions, and ambient CO₂ levels (19). The results presented in this study are from the second of two space shuttle experiments using identical animal models and housing conditions with similar flight durations. Data from our previous space shuttle experiment (STS-108) showed decreases in both body mass and spleen mass of flight animals. Although there were no changes in white blood cell (WBC) counts, decreases in monocyte and granulocyte percentages in flight mice were observed. The spleen of flight mice had an increase in B cells and a decrease in T cells, which secreted lower levels of IFN-γ, IL-1, and IL-4 when stimulated with PHA (19, 48). Results from this study 1) verify the reproducibility of spaceflight results using similar models and analyses, 2) establish a baseline allowing future investigators to take advantage of transgenic mouse models, and 3) expand on our previous findings by exploring gene expression patterns related to oxidative stress and reactive oxygen metabolism.

C57BL/6 mice were flown on the space shuttle Endeavor (STS-118) in August 2007 on a 13-day mission. The primary aim of the study was to examine the effectiveness of an experimental therapeutic agent by the biotechnology company Amgen (Thousand Oaks, CA), as a countermeasure to the muscle atrophy known to be associated with spaceflight. However, as part of a tissue-sharing program, organized through BioServe Space Technologies at the University of Colorado at Boulder, we were able to obtain samples for investigating changes in immune parameters.

To date, there have only been two shuttle flights that have characterized immune parameters in a mouse model. A common criticism of spaceflight research is the lack of opportunity to replicate experiments. However, through the generosity of Amgen and BioServe, we were able not only to repeat the experiment but also to expand on the previous findings. Results from our bone marrow and T cell analyses have been published previously (21, 46). This article focuses on changes in splenic leukocyte population distributions, expression of liver genes responsible for maintaining redox balance, and ex vivo mito-
gen-induced proliferation and cytokine secretion. These were important experiments, because previous studies reported that microgravity episodes and space shuttle flight can affect oxidative bursts and protein kinase C activation (14, 74), and we have seen changes in spleen cell subpopulations in both rats and mice in response to space flight (20, 21, 47, 49). These current experiments explored some of the underlying causes for these changes. The gene expression and LPS-induced cytokine expression data presented in this study are novel observations that establish a new data set on transcriptional activation in response to spaceflight.

**MATERIALS AND METHODS**

**Animals and housing.** Female C57BL/6 mice were ordered from Taconic Laboratories (Hudson, NY), delivered to the National Aeronautics and Space Administration (NASA) Space Life Sciences Laboratory (SLSL) at Kennedy Space Center at 7 wk of age, and adapted to the facility and experiment cage conditions. Female mice were used because they are less aggressive and have been used in previous spaceflight studies (48). However, it is important to note that the estrous cycle was not characterized in this study. At 9 wk old, mice were assigned to experimental groups by matching total body mass and lean body mass. Twenty-four flight (FLT) and 24 ground (GRD) control mice were housed in animal enclosure module (AEM) hardware equipped with solid food bars and a water dispenser. An additional 12 control mice were housed in standard vivarium (VIV) conditions. By using telemetry from the shuttle, the AEM-housed GRD controls were exposed to environmental conditions comparable to flight animals (i.e., temperature, humidity, CO₂) on a 48-h delay. The mice were treated once with placebo or therapeutic agent ~24 h before launch (n = 12 mice/group). Only placebo-treated mice were used for immunological assessments. The FLT groups were flown in space shuttle Endeavor (STS-118) for 13 days. Within 3–6 h after landing of the space shuttle, FLT and GRD mice were evaluated for muscle strength and scanned with nuclear magnetic resonance imaging to assess lean and fat mass composition. Mice were then euthanized with 100% CO₂ (51). This animal study was approved by Loma Linda University, NASA Life Sciences, University of Colorado, and Amgen Institution Animal Care and Use Committees.

**Specimen collection.** At euthanasia, spleen, liver, and thymus were harvested and weighed. A quarter of the spleen was used to obtain leukocyte numbers at the SLSL. The rest of the spleen was shipped on wet ice via overnight courier to Loma Linda University in screw-cap tubes containing RPMI 1640 (Cellgro; Mediatech, Herndon, VA) supplemented with 20% fetal bovine serum (FBS; ATCC, Manassas, VA). The other organs were placed in sterile cryovials, snap frozen in liquid nitrogen, placed on dry ice, and shipped to Loma Linda University.

**Leukocyte numbers in spleen.** At the SLSL, a single-cell suspension of splenocytes was obtained by grinding one-quarter of the spleen in liquid nitrogen, placed on dry ice, and shipped to Loma Linda University.

**Spontaneous and mitogen-induced blastogenesis.** Upon arrival at Loma Linda University, single-cell suspensions of splenocytes were obtained following the method described above. After the first wash, erythrocytes were lysed in 2 ml of lysing buffer containing 5 mM potassium bicarbonate, 77 mM ammonium chloride, and 49 mM EDTA at 4°C for 10 min. The lysing reaction was terminated by adding 2 ml of RPMI 1640. Cells were then centrifuged, washed twice, and resuspended in 1 ml of RPMI 1640.

For spontaneous blastogenesis, 50 µl of cells were dispensed into 96-well, flat-bottom microculture plates in triplicate. One hundred fifty microliters of RPMI 1640 (150 µl) supplemented with 10% FBS and 50 µl of tritiated thymidine ([³H]Tdr; specific activity = 50 Ci/mmol; ICN Radiochemicals, Irvine, CA) were added to the cells, and the plates were incubated for 4 h at 37°C. Readings were later normalized to 2 × 10⁶ cells/ml based on the original cell counts.

For mitogen-induced blastogenesis in the spleen, cells were adjusted to 2 × 10⁶ cells/ml, and 100-µl aliquots were plated in triplicate into wells of 96-well, flat-bottom microculture plates. One hundred microliters of 3.3 × 10⁻³ mg LPS (Sigma Chemical, St. Louis, MO) were added in triplicate; control wells with no mitogens were included. Fifty microliters of [³H]Tdr (specific activity = 50 Ci/mmol) were added to each well during the last 4 h of a 48-h incubation at 37°C.

For both assays, cells were collected with a multiple sample harvester (Harvard 96 Mack III-m, Tomtec, Hamden CT), and the incorporated radioactivity was quantified on a 1450 Microbeta Trilux liquid scintillation and luminescence counter (EG&G-Wallac, Turku, Finland). Stimulation index (SI) for the mitogen was calculated as follows: SI = [cpm without mitogen] – [cpm with mitogen]/[cpm without mitogen].

**LPS-induced cytokine secretion.** Spleen leukocytes were diluted with RPMI 1640 supplemented with 10% FBS and adjusted to 2 × 10⁶ cells/ml. One hundred-microliter aliquots were plated into 96-well, flat-bottom microculture plates, and 100 µl of 3.3 × 10⁻³ mg LPS was immediately added to each well. Cells were incubated at 37°C for 48 h. Supernatants were aspirated and centrifuged to remove debris. Levels of interleukin (IL)-1β, IL-6, IL-10, IL-12, and tumor necrosis factor (TNF)-α were quantified using enzyme-linked immunosorbent assays (ELISA) according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN) using the appropriate dilutions.

**RNA purification.** Frozen liver tissue was pulverized into a powder in a mortar and pestle submerged in liquid nitrogen. RNA was extracted using Trizol (Invitrogen, Carlsbad, CA). After incubation of homogenized samples at room temperature for 5 min, 0.2 ml of chloroform was added and samples were incubated for 3 min. This mixture was then centrifuged at 12,000 g for 15 min at 4°C. Isopropyl alcohol (0.5 ml) was added to the aqueous phase in a sterile RNase-free tube, and samples were incubated at room temperature for 10 min before centrifugation at 12,000 g for 15 min at 4°C. The pellet was washed with 1 ml of 70% ethanol and recentrifuged at 7,500 g for 5 min at 4°C. Ethanol was removed with a pipette, and the pellet was allowed to air dry for 5 min. Pellet was dissolved in 50 µl of nuclease-free water. The RNA was further purified using an RNAeasy purification kit (Qiagen) following the manufacturer’s instructions.

**RNA microarrays.** RNA quality was assessed using the Agilent 2100 Bioanalyzer (Santa Clara, CA). The integrity of the RNA was determined by looking at 18S and 28S RNA peaks and by RNA integrity number. RNA concentrations were measured using the nano drop, and all samples had 260/280 ratios above 2.0 and 260/230 ratios above 1.7. An equal amount of RNA (1 mg) was taken for all samples, and reverse transcription was done using the RT² First Strand kit (SuperArray Biosciences, Frederick, MD). Polymerase chain reactions (PCR) were performed to evaluate expression of 84 genes using RT² profiler PCR array PAMM-065 (Mouse Oxidative Stress and Antioxidant Defense SuperArray) on the ABI Fast 7900 with RT² Real-Time SYBR green PCR master mix PA-0101. Thermocycler parameters were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Relative changes in gene expression were calculated using the comparative threshold cycle (DDCt) method. This method first subtracts the Ct (threshold cycle number) of the gene
average Ct of the three housekeeping genes on the array (HPRT1, GAPDH, and ACTB) to normalize to the RNA amount. Finally, the DDCt was calculated: normalized average Ct of the FLT group — normalized average Ct of the GRD group. This DDCt value was raised to the power of 2 to calculate the degree of change. The genes of interest included those involved in oxidative stress. Six mice per group were used for this analysis.

Statistical analysis. The data were evaluated using Student’s t-test (Systat; Systat Software, Richmond, CA). Organ masses were normalized to both body mass and brain mass. Studies have shown that spaceflight has significant effects on body weight (19), reflecting changes in muscle weight and body water content. However, the brain is considered to be the most stable organ and has been used to normalize organ masses (5). Since the FLT and GRD mice were evaluated on separate days, the VIV control mice were used to normalize the data, thus minimizing any day-to-day variability. One-half of the VIV mice were euthanized on each day of assessment. For example, the FLT data were normalized using the following equation: 

\[
\text{Normalized organ mass} = \frac{\text{individual data point from FLT day}}{\text{average of all VIV controls on both FLT and GRD days}} \times \text{average of all VIV controls on both FLT and GRD days}
\]

A similar, corresponding equation was used to normalize the data from the GRD controls. P values <0.05 and <0.1 were selected to indicate significance and trend, respectively.

RESULTS

Organ masses. There was no significant change in the brain masses between the FLT and GRD groups. As indicated in Table 1, there were significant decreases in masses of spleen (P < 0.005), liver (P < 0.001), and thymus (P < 0.001). When normalized to body mass, there was a significant decrease in only the thymus (P < 0.001) in FLT group compared with GRD group. However, significant decreases were observed for liver (P < 0.001) and thymus (P < 0.001) when normalized to brain mass, with a similar trend observed for spleen (P = 0.065).

Leukocyte subpopulations in spleen. As shown in Fig. 1, there was a significant effect of spaceflight conditions on the WBC counts (P < 0.05). In addition, there were significant space-induced decreases in all leukocyte subpopulations: lymphocytes (P < 0.001), monocyte/macrophages (P < 0.005), and granulocytes (P < 0.05). There were no significant changes in lymphocyte, monocyte/macrophage, and granulocyte percentages (Table 2); however, a trend (P = 0.065) was observed with monocyte/macrophages of FLT animals.

Table 1. Organ masses normalized to body mass and brain mass

<table>
<thead>
<tr>
<th></th>
<th>GRD</th>
<th>FLT</th>
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<tbody>
<tr>
<td>Organ mass, mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>431±4.0</td>
<td>435±3.8</td>
</tr>
<tr>
<td>Spleen</td>
<td>55±1.2</td>
<td>47±1.9*</td>
</tr>
<tr>
<td>Liver</td>
<td>736±22.2</td>
<td>634±13.9†</td>
</tr>
<tr>
<td>Thymus</td>
<td>108±2.0</td>
<td>65±1.7†</td>
</tr>
<tr>
<td>Normalized to body mass, mg/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>3.0±0.2</td>
<td>3.0±0.5</td>
</tr>
<tr>
<td>Liver</td>
<td>39.4±3.4</td>
<td>37.4±3.2</td>
</tr>
<tr>
<td>Thymus</td>
<td>5.8±0.4</td>
<td>4.3±1.1†</td>
</tr>
<tr>
<td>Normalized to brain mass, mg/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.1±0.0</td>
<td>0.1±0.0</td>
</tr>
<tr>
<td>Liver</td>
<td>1.7±1.4</td>
<td>1.5±0.1†</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.3±0.0</td>
<td>0.2±0.0†</td>
</tr>
</tbody>
</table>

Values are means ± SE for 12 mice/group of flight (FLT) or ground control (GRD) mice. *P < 0.05; †P < 0.001, GRD vs. FLT.

Spontaneous and mitogen-induced blastogenesis. Splenic spontaneous blastogenesis was elevated in the FLT mice (P < 0.001). There was a decrease in cpm of LPS-induced blastogenesis (P < 0.001) (Fig. 2). The stimulation index of LPS-induced blastogenesis was also significantly reduced (P < 0.005; data not shown).

Cytokine secretion by activated splenocytes. Flight conditions had a significant effect on the levels of LPS-induced TNF-α, IL-6, and IL-10 levels (Fig. 3) but not on IL-1β and IL-12 levels (data not shown). TNF-α in FLT mice was...
significantly lower than in GRD mice \((P < 0.001)\). On the other hand, IL-6 and IL-10 levels were significantly higher in FLT mice compared with the GRD mice \((P < 0.05\) and \(P < 0.005\), respectively).

**Gene expression.** The genes that were statistically significant \((P < 0.05)\) and varied between FLT and GRD groups by \(>1.5\)-fold are presented in Table 3. Of the 84 evaluated genes, 8 were upregulated and 4 were downregulated. The greatest enhancement occurred in \(Mb\) (almost 143-fold), whereas the greatest suppression was noted in \(Serpinb1b\) \((-3.6\)-fold).

**DISCUSSION**

The spaceflight environment can have a dramatic impact on a variety of immune parameters \((1, 8, 29, 43)\). However, differences in flight platforms, mission durations, animal models, and housing conditions have sometimes made interpretation of the results difficult. The data reported in this study are the result of a rare opportunity in which we were able to repeat, and expand on, a previous flight experiment using a mouse model \((19, 48)\).

The organs of interest in these studies have been the spleen, thymus, and liver. The spleen is the largest secondary lymphoid organ, and the decrease in mass reported presently is similar to that of previous spaceflight experiments using both rat and mouse models \((11, 19, 44)\). In contrast, the thymus, site of T cell maturation, has proven to be more variable, decreasing \((11, 27)\), increasing \((50)\), or remaining constant \((5, 7, 18)\) depending on the flight. Interestingly, there was a similar trend in thymus mass after our previous flight, although the differences did not reach significance \((19)\). Finally, the liver is responsible for numerous biological activities that include production of complement components and acute stress proteins. We found a significant decrease in liver mass. Although, to our knowledge, liver mass has not been reported after other spaceflight studies, a decrease in Kupffer cells and hepatic cytochrome \(P-450\) cells in rats has been reported \((57, 58)\). Similar results were found when we normalized organ masses to brain mass. In contrast, when normalized to body mass, there were no significant differences in spleen and liver. We believe that normalizing to brain mass more accurately reflects changes due to animal growth, since the brain is less susceptible to transient effects (e.g., dehydration) compared with total body mass.

**Table 2. Percentage of leukocytes in spleen**

<table>
<thead>
<tr>
<th>Subpopulation</th>
<th>GRD</th>
<th>FLT</th>
</tr>
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<tbody>
<tr>
<td>%Lymphocyte</td>
<td>64.80 ± 0.4</td>
<td>64.05 ± 0.5</td>
</tr>
<tr>
<td>%Monocyte</td>
<td>10.93 ± 0.2</td>
<td>10.32 ± 0.2</td>
</tr>
<tr>
<td>%Granulocyte</td>
<td>24.51 ± 0.2</td>
<td>25.31 ± 0.5</td>
</tr>
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</table>

Values are means ± SE for 12 mice/group.

**Fig. 2. Effects of spaceflight on spontaneous and mitogen-induced blastogenesis in spleen.** Data were obtained by quantifying the amount of tritiated thymidine \(([^3]H)TdT\) incorporated (cpm, counts per minute). Each bar represents mean ± SE \((n = 12 \text{ mice/group})\). \(* P < 0.001, \text{GRD vs. FLT}\).

**Fig. 3. Effects of spaceflight on cytokine secretion by activated splenocytes.** Supernatants from splenocytes were obtained 48 h after incubation with LPS. Cytokine concentrations were evaluated using ELISAs. Each bar represents mean ± SE \((n = 12 \text{ mice/group})\). \(* P < 0.05; ** P < 0.001; *** P < 0.005, \text{GRD vs. FLT}\).

**Table 3. Oxidative stress gene expression in liver**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upregulated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqr</td>
<td>Aquarius</td>
<td>1.71</td>
</tr>
<tr>
<td>Cyyb</td>
<td>Cytoglobin</td>
<td>1.85</td>
</tr>
<tr>
<td>Fmo2</td>
<td>Flavin-containing monooxygenase 2</td>
<td>1.72</td>
</tr>
<tr>
<td>Gpx6</td>
<td>Gultathione peroxidase 6</td>
<td>1.96</td>
</tr>
<tr>
<td>Mb</td>
<td>Myoglobin</td>
<td>142.98</td>
</tr>
<tr>
<td>Nox1</td>
<td>NADPH oxidase 1</td>
<td>2.58</td>
</tr>
<tr>
<td>Tmod1</td>
<td>Tropomodulin 1</td>
<td>2.94</td>
</tr>
<tr>
<td>Ucp3</td>
<td>Uncoupling protein 3</td>
<td>13.62</td>
</tr>
<tr>
<td>Downregulated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idh1</td>
<td>Isocitrate dehydrogenase 1 (NADP(^+)), soluble</td>
<td>1.69</td>
</tr>
<tr>
<td>Nox4</td>
<td>NADPH oxidase 4</td>
<td>2.09</td>
</tr>
<tr>
<td>Scd1</td>
<td>Stearyl-coenzyme A desaturase 1</td>
<td>3.00</td>
</tr>
<tr>
<td>Serpinb1b</td>
<td>Serine peptidase inhibitor, clade B, member 1b</td>
<td>-3.64</td>
</tr>
</tbody>
</table>

Values represent fold change between GRD and FLT \((P < 0.05)\).
The changes in organ masses suggest that the mice have been subject to psychological and/or physiological stress in flight or during landing. Unfortunately, since we were unable to measure catecholamine or glucocorticoid levels, we cannot conclude whether this was an acute or chronic response. However, exposure to the spaceflight environment has already been shown to induce the release of hormones and neurotransmitters such as catecholamines and glucocorticoids (41, 68, 71). Ground-based studies have shown that chronic elevation of these factors is damaging, often leading to the atrophy of lymphoid tissues (32, 61, 72) and decreases in organ mass (10, 60). Spaceflight studies on astronauts also have shown a link between stress in stress hormones and EBstein-Barr reaction (54).

Splenic WBC and leukocyte subpopulation counts also were significantly reduced after flight. A decrease in granulocyte count is consistent with our previous study (48), and similar results were found after STS-57 using a rat model (23). However, there were no significant differences in splenic leukocytes after a shorter, 9-day flight (1), suggesting that flight duration may be a critical factor. These losses likely reflect changes in leukocyte trafficking patterns due to flight-induced muscle atrophy (50), changes in fluid volume and distribution (43), and/or stress. Ground-based studies already have shown that stress can result in splenocyte apoptosis (72).

To determine the recovery and proliferative capacity of lymphocytes, we characterized spontaneous blastogenesis. The observed increase in [3H]TdR incorporation by splenic lymphocytes demonstrates that ex vivo DNA synthesis was increased after flight and suggests that the cells were capable of shifting to a proliferative (or recovery) state once removed from any stress-induced inhibition in vivo. In contrast, LPS-induced proliferation was decreased in the FLT mice, indicating that the ability to respond to a potent B cell mitogen may be compromised. There has been at least one study where investigators reported increases in the LPS response (43), suggesting that this response may be dependent on the animal model. Interestingly, decreases in the response to T cell mitogens (PHA and concanavalin A) have consistently been reported (40, 43, 64). This, combined with our results, would suggest that although there is an increase overall proliferative capacity, there is a downregulation of adaptive immune mechanisms. However, further studies are required to confirm this downregulation.

Ex vivo cytokine production has been shown to alter during and after flight in cells obtained from mice, rats, nonhuman primates, astronauts, and cosmonauts (4, 18, 19, 65). Cytokine concentrations in the extracellular milieu dictate proliferation and activity of immunocytes, as well as numerous other cell types. In the present study, LPS was used for activation of splenocytes that consisted of a mixture of leukocytes. Although LPS is often referred to as a B cell mitogen, interactions among B cells, T cells, and macrophages are inevitable in a cellular mixture.

IL-6 is a cytokine with both pro- and anti-inflammatory effects that is typically secreted by T cells and macrophages. Its ability to induce TNF-α and IL-1β antagonists indicates that it is a major factor needed for resolution of inflammation and the switch from innate to adaptive immunity (28). Recent reports indicate that IL-6 is also released by contracting muscles (13, 53), suggesting that the secretion of this cytokine may be influenced by the postflight muscle tests. Another, albeit unlikely, possibility is that the smooth muscle of blood vessels within the spleen may be secreting IL-6. However, IL-6 levels in this study were quantified ex vivo, and any cytokines present in the spleen and/or circulation were likely washed away in the splenocyte processing procedure before stimulation. Because IL-6 is also associated with increases in IL-10 and other anti-inflammatory cytokines in the presence of stress and an immune challenge (9, 26, 52, 75), we believe our changes in secretion are due to persistently activated anti-inflammatory mechanisms induced by spaceflight-related stress.

IL-10, produced by macrophages and, to a lesser extent, Th2 cells, inhibits adaptive immunity by downregulating Th1 cytokine expression, major histocompatibility complex class II molecules, and costimulatory proteins on antigen-presenting cells (42). However, the ultimate effect of both IL-6 and IL-10 is to limit inflammation (26). In contrast, TNF-α is a potent proinflammatory cytokine secreted primarily by macrophages (37). The increased capacity to produce IL-6 and IL-10 seen in the present study, coupled with the low level of TNF-α and the absence of any change in IL-1β, suggests an overall decrease in inflammatory mechanisms. In our previous study on STS-108, we found an increase in PHA-induced proinflammatory cytokines TNF-α and IL-2 (19). Because PHA stimulates T cells, this apparent discrepancy is likely due to differences in the target populations of the mitogen. Further research is required to determine whether this mitogen-dependent difference in responses is due to a spaceflight-induced shift away from B cell to T cell mechanisms.

Perhaps not surprisingly, there are ground-based studies suggesting that these differences in cytokine production may be attributed to a stress response. For instance, adrenocorticotropin hormone and catecholamines both have been shown to influence cytokine production in the presence of LPS (31). Glucocorticoids and catecholamines regulate Th2 cytokine production via stimulation of the glucocorticoid and β2-adrenergic receptors. Through the stimulation of β2 receptors, catecholamines upregulate production of anti-inflammatory cytokines IL-6 and IL-10 (12, 25, 39), leading to a decrease in TNF-α levels (2).

An important aspect of spaceflight is the production of reactive oxygen species (ROS), since they play vital roles in cell signaling, mitogen response, cell differentiation, and apoptosis. Furthermore, sustained exposure to high concentrations of ROS can lead to tumorigenesis, diabetes, and sepsis (17, 55). Increases in lipid peroxidation of human erythrocytes and reductions in circulating antioxidant levels after long-duration spaceflights have been reported (66). Using the hindlimb suspension model, Guillet et al. (24) found that HS may alter ROS metabolism, and Fleming et al. (15) reported an increase in superoxide responses by murine polymorphonuclear neutrophilic leukocytes when exposed to short periods of microgravity. Exposure to chronic stress has also been reported to chronically increase ROS production and oxidative stress (67).

These previously published findings are consistent with our liver gene expression data. We found that many of the genes responsible for scavenging ROS were upregulated. These genes included Fmo2, involved in ROS metabolism, and peroxidases such as glutathione peroxidase 6 and cytoglobin. Cytoglobin is also responsible for intracellular oxygen storage and transfer and sensing of O2. By far, we saw the greatest
increase in myoglobin transcript, an important ROS transporter. The upregulation of these genes in the FLT mice can be attributed to cells attempting to scavenge excess ROS. These changes also may be a result of readaptation to nominal G rather than an actual spaceflight effect. Although the majority of the genes responsible for ROS scavenging were upregulated, several genes involved in ROS production were also upregulated. For instance, we found an increase in a gene in the NADPH oxidase complex Nox1. However, this gene has been shown to be involved in normal cell metabolism, including cell proliferation (33). The elevation of Nox1 in the liver of FLT mice may be due to an increase in proliferation consistent with our splenic blastogenesis results. This, in turn, could be due to a stress-induced decrease in liver mass, in combination with an increased demand for hepatocytes involved with clearing dead/dying cell debris from the blood (16). Nox4 expression, on the other hand, decreased. This homolog of Nox1 has been shown to be elevated in response to hypoxia (36), and studies have suggested that microgravity and spaceflight lead to hypoxia (38, 56, 59).

The data confirm that immune parameters are influenced by the spaceflight environment. The results suggest that the stressors inherent to spaceflight missions lead to a shift toward anti-inflammatory responses shortly after landing. The upregulation in ROS scavenging genes, suppression of LPS-induced blastogenesis, and changes in the cytokine expression profile all indicate an overall activation of anti-inflammatory signaling. This shift may very well be necessary to regain homeostasis upon return to Earth. Furthermore, these data also suggest that the response to an infection with gram-negative bacteria may be compromised during the time needed to regain homeostasis. However, further research is necessary to verify this possibility (e.g., characterize changes in Toll-like receptor 4 expression) (34, 45).

It remains to be determined whether homeostasis can be efficiently achieved after chronic stress exposure experienced during extended journeys in space. Although causality has not yet been established, at least three recently published reviews suggest a possible link among muscle atrophy, oxidative stress, and inflammatory responses in disease, disuse, and exercise models (3, 52, 73). We believe that several aspects of the spaceflight environment (e.g., microgravity, low-dose/low-dose-rate radiation, chronic psychological and physiological stress) lead to chronic, systemic increases in ROS. If kept unchecked, this would lead to chronic inflammatory disease. As a consequence, all mechanisms involving increasing ROS as part of normal cellular function, such as that which occurs during an infection, have been downregulated. This would explain the decreases in oxidative stress related genes and increases in anti-inflammatory cytokine expression. However, this idea is speculative and, to the best of our knowledge, has not yet been considered in the context of spaceflight. Because long duration missions are being planned, it is important to better understand the mechanisms responsible for the observed changes in the immune parameters to reduce health risks to astronauts.

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REFERENCES

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