Shifts in bone marrow cell phenotypes caused by spaceflight

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Ortega MT, Pecaut MJ, Gridley DS, Stodieck LS, Ferguson V, Chapes SK. Shifts in bone marrow cell phenotypes caused by spaceflight. J Appl Physiol 106: 548–555, 2009. First published December 4, 2008; doi:10.1152/japplphysiol.91138.2008.—Bone marrow cells were isolated from the humeri of C57BL/6 mice after a 13-day flight on the space shuttle Space Transportation System (STS)-118 to determine how spaceflight affects differentiation of cells in the granulocytic lineage. We used flow cytometry to assess the expression of molecules that define the maturation/activation state of cells in the granulocytic lineage on three bone marrow cell subpopulations. These molecules included Ly6C, CD11b, CD31 (platelet endothelial cell adhesion molecule-1), Ly6G (Gr-1), F4/80, CD44, and c-Fos. The three subpopulations were small agranular cells [region (R1)], larger granular cells (R2), which were mostly neutrophils, and very large, very granular cells (R3), which had properties of macrophages. Although there were no composite phenotypic differences between total bone marrow cells isolated from spaceflight and ground-control mice, there were subpopulation differences in Ly6C (R1 and R3), CD11b (R2), CD31 (R1, R2, and R3), Ly6G (R3), F4/80 (R3), CD44high (R3), and c-Fos (R1, R2, and R3). In particular, the elevation of CD11b in the R2 subpopulation suggests neutrophil activation in response to landing. In addition, decreases in Ly6C, c-Fos, CD44high, and Ly6G and an increase in F4/80 suggest that the cells in the bone marrow subpopulation of spaceflight mice were more differentiated compared with ground-control mice. The presence of more differentiated cells may not pose an immediate risk to immune resistance. However, the reduction in less differentiated cells may forebode future consequences for macrophage production and host defense. This is of particular importance to considerations of future long-term spaceflights.

BONE MARROW IS A COMPLEX MICROENVIRONMENT that is necessary for the generation of red and white blood cells (hematopoiesis) in adult animals (1, 58, 61). This organ can be disrupted by numerous factors including stress, bone changes, alterations in circadian rhythm, and irradiation (4, 35, 44, 45), all of which occur during spaceflight. Therefore, it is not surprising that spaceflight decreased the number of bone marrow-derived colony-forming units [CFU-monoocyte (M) or CFU-granulocyte/monocyte (GM)] during some spaceflights (26, 49, 50, 57). Spaceflight also has an impact on monocytes, cells that have recently emerged from the bone marrow. The number of monocytes in circulation declined (53), and some lacked the expression of insulin-like growth factor (IGF) receptors (34). A mouse model used to simulate some of the physiological changes associated with spaceflight, antithostatic suspension (56), also diminished the number of macrophage progenitor cells (CFU-M) in the bone marrow (6–8, 19, 20, 49). This strengthens the hypothesis that physiological changes that occur in response to spaceflight affect bone marrow cells.

Although many spaceflights have been done with rats, only Space Transportation System (STS)-108 addressed the impact of spaceflight on immune parameters in adult mice. Indeed, there was a decrease in the number and percentage of blast cells and an increase in the number of CD34+ cells in the bone marrow in flight animals assessed after the landing of STS-108 (41). However, there were no differences in the numbers of mature granulocytes or monocytes in the bone marrow of those same animals (41).

Several countries are preparing for space travel in the future (29, 40). Given the importance of bone marrow to the maintenance of host red and white blood cell populations, additional information is needed on the changes that occur in bone marrow cells in response to spaceflight. The invasive nature of the collection of human bone marrow specimens precludes extensive study of human bone marrow. Therefore, rodent models are essential to revealing clues about how spaceflight impacts this critical system. To this end, we had access to normal mouse bone marrow that was part of the Commercial Biomedical Test Module-2 (CBTM-2) payload experiment. Past spaceflight rodent studies indicated that there are effects on early blast cells (CFU-GM) in bone marrow (49, 50). Therefore, we assessed bone marrow cells for the expression of differentiation and activation molecules to determine whether spaceflight affects specific subpopulations of cells in the granulocytic lineage, e.g., macrophages and neutrophils.

MATERIALS AND METHODS

Antibodies used for phenotyping. Fluorescein isothiocyanate (FITC)-conjugated anti-Ly6C (Clone AL-21), FITC-anti-IgM (Clone RA-2A), phycoerythrin (PE)-conjugated anti-CD31 (Clone MEC13.3), PE-anti-IgG2a (Clone R35-95), PE-anti-CD44 (Clone IM7), and PE-anti-IgG2b (Clone A95–1) were purchased from BD Pharmingen. PE-anti-CD11b (Clone M1-70), PE-anti-IgG2b (Clone eB149/0H5), PE-anti-Ly6G (Clone RB6–8C5), PE-anti-IgG2b (Clone eB149/0H5), allophycocyanin (APC)-conjugated anti-F4/80 (Clone BM8), and APC-anti-IgG2a (Clone eBR2a) were purchased from eBioscience. PE-anti-c-Fos (Clone 4) and PE-anti-IgG2b (clone not categorized) were purchased from Santa Cruz Biotechnology.

Mice. Female C57BL/6 mice (n = 28) were 9 wk old at the beginning of the experiment. The mice were obtained from Charles River Laboratories (Wilmington, MA) and were housed at the National Aeronautics and Space Administration (NASA) Space Life...
All mice were acclimated to the food bar diet (39, 63) before their selection for the Flight or Ground-control group (16, 48). Mice were housed in animal enclosure module (AEM) housing for Ground-control (n = 12 mice) and Flight (n = 12 mice) groups and were given the same food bar diet. The Flight group was flown on the Space Shuttle Endeavour (STS-118), which flew for 13 days. Ground-control mice were exposed to environmental settings (temperature, relative humidity, and atmospheric CO2 levels) similar to the space-control mice. Mice were exposed to the same food bar diet. The Flight group was flown on the Space Shuttle Endeavour (STS-118), which flew for 13 days. Ground-control mice were exposed to environmental settings (temperature, relative humidity, and atmospheric CO2 levels) similar to the space-flight animals with telemetry from the shuttle on a 48-h time delay. Additional mice were housed in standard vivarium conditions (control; n = 4 mice). Mice had access to food and water ad libitum. Lighting was on a 12:12-h light-dark cycle.

Sample collection. Muscle strength testing and nuclear magnetic resonance imaging were performed before euthanasia with 100% CO2. Mice were euthanized within 3–4 h of landing. The humeri were recovered and cleaned of all nonosseous tissue for the control, Flight, or Ground-control mice in three consecutive sampling days. Because of the experimental design of the primary experiments of the CBTM-2 payload, only humeri were available for this secondary experiment. This was an acceptable alternative to obtaining bone marrow from hindlimb bones because in response to skeletal unloading the CFU-M colony formation was similar between bone marrow isolated from humeri and bone marrow isolated from hindlimbs (femora and tibiae) (6, 7). The marrow cavity was flushed with a sterile PBS solution. Red blood cells were lysed with ACK lysing buffer reagent (0.15 M NH4Cl, 10 mM KHCO3, and 0.1 mM Na2EDTA). Cells were centrifuged (300 g, 5 min) and washed twice with PBS to remove serum components. Cells were counted on a hemacytometer and suspended in 5% formalin in PBS at a concentration of 4 × 106 cells/ml. These cells were shipped at 4°C overnight to Kansas State University for further analyses.

Bone marrow-derived cell labeling and fluorescence-activated cell sorting analysis. After arrival at Kansas State University, bone marrow cells were recounted and cell concentrations were adjusted to 1 × 107 cells/ml. Phenotypic analysis of bone marrow-derived cells was performed by fluorescence-activated cell sorting (FACS) on two successive days. Bone marrow cells were blocked with PBS-goat serum (50:50; 50 μl) at 4°C for 0.5 h. FITC-anti-Ly6C or anti-IgM (0.5 μg), PE-anti-CD11b or anti-IgG2b (0.1 μg), APC-anti-CD31 or anti-IgG2a (0.5 μg), PE-anti-Ly6G or anti-IgG2b (0.1 μg), APC-anti-F4/80 or anti-IgG2a (1.4 μg), PE-anti-CD44 or anti-IgG2b (1 μg), and PE-anti-c-Fos or anti-IgG2b (3.8 μg) were added to the cell suspensions and incubated at 4°C for 1 h. In some instances, multiplexing of antibodies with compatible fluorochromes was done (e.g., c-Fos and CD31 or Ly6C and CD64). The cells were then washed twice in Hanks’ balanced salt solution and resuspended in 1% formalin. A FACSCalibur flow cytometer (Becton Dickinson, Rockville, MD) was used for the analyses. A minimum of 20,000 events were collected for each sample. Sample gating was done so that ≥95% of the isotype control was in the lower left quadrant (e.g., Figs. 3–5) or was in the left gate on histograms (e.g., Fig. 2). Since the Flight and Ground-control bone marrow cell surface antigens were analyzed on different days (the day after shipping), the day-to-day differences in cell staining were normalized by using control cells that were prepared at the SLSL at Cape Canaveral and were stained at the same time as Flight or Ground samples (2 mice per labeling day, Controllight and Controlground). Ground-control data were normalized to Flight data with the calculation normalized Ground data = (Controllight/Controlground) × Ground-control sample value.

Statistical analysis. Data were evaluated by factorial ANOVA with Tukey pairwise multiple comparison test and χ2-test (Statmost, Detaxiom Software, Los Angeles, CA). P values of <0.05 were selected to indicate significance. Data are presented as means ± SE for 12 mice per treatment group.

RESULTS

Bone marrow cell numbers and subpopulations. Flight and Ground-control mice had body masses of 18.1 ± 0.2 g before flight. At landing, all mice appeared well groomed, with a normal coat appearance, but they appeared disoriented. Video documentation obtained in space on flight days 5 and 6 indicated that the mice adapted well to the space environment. They exhibited normal eating, grooming behavior, and active movement throughout the AEM, including running and “flying.” Veterinary examination at landing found the mice to be in good health despite weight loss. Ground-control mice weighed 18.7 ± 0.2 g (+3.3%) and Flight mice weighed 16.5 ± 0.3 g (−8.8%). We isolated an average of 6.9 ± 0.5 × 106 bone marrow cells per mouse from 12 Flight mice and similar numbers of bone marrow cells from 12 AEM-housed Ground-control mice (6.5 ± 0.3 × 106). There were no significant differences in gross bone morphology between treatment groups or changes in the bone marrow cell numbers between Flight and Ground-control mice.

Bone marrow cells were identified by size (forward scatter) and granularity (side scatter) (Fig. 1). Region (R1) identified small, agranular cells. R2 identified cells that were larger and more granular. As shown by the expression of Ly6G (Gr-1) and CD11b, this region contains many granulocytes (neutrophils) (Table 1). The largest, most granular cells were identified in R3. These cells had the highest level of staining for macrophages based on the macrophage phenotypic expression of F4/80 and CD11b, which are expressed by monocytes and macrophages in various tissues throughout the mouse (23) (Table 1).

We compared AEM-housed Ground-control mice and the mice that were flown in space. R1 contained 38 ± 1% of the bone marrow cells from Flight or Ground-control mice. R2 contained 38 ± 1% and 36 ± 1% of the bone marrow cells from Flight and Ground-control mice, respectively. R3 contained 11 ± 0% of the total bone marrow cell population for both treatment groups. Therefore, spaceflight did not radically disrupt the distribution of bone marrow cell subpopulations.
Marrow cells were 3% of high numbers of cells in the granulocytic lineage in bone marrow, respectively. The high levels of Ly6C are consistent with the presence of high numbers of cells from Flight animals compared with Ground-control animals (7 ± 1% vs. 3 ± 0%, *P < 0.01). CD44 is another cell surface protein involved in cell-cell interactions, migration, cell adhesion, and lymphocyte activation (28). Some isoforms bind hyaluronic acid (32), which may be important in organizing and regulating the function of bone marrow cells (36, 46). Most bone marrow cells, especially granulocytes, express CD44 (55). Therefore, CD44 was also examined. In the total bone marrow, there were two subpopulations of CD44+ cells (Fig. 2). Therefore, for our analyses, we assessed the “CD44high” and “CD44low” populations separately. There were no differences in treatment groups in the percentage of cells that were CD44low. For CD44high cells, Flight and Ground-control bone marrow cells were 62 ± 2% and 69 ± 2% (*P < 0.05), respectively.

**R1 subpopulation phenotypic assessment.** The assessment of Ly6C and CD31 in the R1 population showed that overall there was a significant increase in expression of Ly6C from 9 ± 1% in Ground-control samples to 14 ± 1% (*P < 0.01) for Flight samples. The same trend was seen with Ly6C+CD31− cells (Fig. 3) from Ground-control and Flight samples (3% and 5%, respectively, *P < 0.01). However, there were no differences in Ly6C+CD31+ or Ly6C−CD31- cell percentages in the R1 subpopulation. Therefore, since Ly6C may discern differentiation (18), or may be important in the trafficking of CD8+ T lymphocytes (24), spaceflight may have an effect on the small agranular cells in the R1 subpopulation.

There was also a decrease in CD31 from 14 ± 2% on Ground-control mouse bone marrow cells to 3 ± 0% on Flight mouse bone marrow cells (*P < 0.01) in the R1 subpopulation. Because CD31 is involved in the transmigration of cells into tissue (10), these data also support the hypothesis that R1 cell trafficking may have been affected by spaceflight.

There was ≤2% expression of F4/80 and Ly6G in the R1 population. For c-Fos, bone marrow cells from Flight mice had slightly lower expression (2 ± 0% vs. 5 ± 1%; *P < 0.01) than bone marrow cells from Ground-control mice. CD44low expression was 59 ± 1% and 57 ± 1% for Ground-control and Flight mouse bone marrow, respectively. CD44high expression (28 ± 1% and 29 ± 2% for Ground-control and Flight bone marrow, respectively) was significantly lower than CD44low groups (Table 1). Overall, expression of F4/80 (a macrophage-specific marker; Refs. 11, 25) was higher on bone marrow cells from Flight animals compared with Ground-control animals (7 ± 1% vs. 3 ± 0%, *P < 0.01).

### Table 1. Effect of spaceflight on bone marrow-derived cell phenotypic markers

<table>
<thead>
<tr>
<th>Cell Marker</th>
<th>Subpopulation</th>
<th>Spaceflight</th>
<th>Ground Control</th>
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<tr>
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<td>TBM</td>
<td>35 ± 2%</td>
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<td>R1</td>
<td>14 ± 1%</td>
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<td>R3</td>
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<td>72 ± 3%</td>
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<tr>
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<td>14 ± 1%</td>
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<td>Ly6G</td>
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<tr>
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<td>R3</td>
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<tr>
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<tr>
<td></td>
<td>R3</td>
<td>64 ± 4%</td>
<td>83 ± 2%</td>
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</tbody>
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Values are % means ± SE for 12 mice/group. TBM, total bone marrow; R1, region 1; R2, region 2; R3, region 3. t-Test: *P < 0.05, †P < 0.01.

**Total bone marrow phenotypic assessment.** We analyzed the level of Ly6C expression on the total bone marrow population. Ly6C expression is related to differentiation and identifies cells at an intermediate stage within the myeloid lineage, but it is absent from cells of the erythroid lineage (30). Its expression is completely lost by cells on final maturation into mature macrophages (17). Overall, 35 ± 2% of bone marrow cells from Flight animals were positive compared with 21 ± 3% (*P < 0.01) positive for bone marrow cells from Ground-control mice. The high levels of Ly6C are consistent with the presence of high numbers of cells in the granulocytic lineage in bone marrow developing for host innate immune responses. Bone marrow cells were 3 ± 2% and 5 ± 1% (*P < 0.05) positive for CD31 (macrophages early in differentiation; Refs. 17, 18, 62) for Flight and Ground-control mice, respectively.

When we examined bone marrow cell expression of CD11b, a β-integrin that participates in strong adhesion and trafficking (27), we saw that the overall expression was low in both mouse treatment groups compared with the expression in the R2 or R3 subpopulations. Bone marrow cells were 11 ± 1% and 14 ± 1% (*P < 0.05) positive from Flight animals and Ground-control animals, respectively. These data are consistent with the low-level expression of CD11b in the whole bone marrow (33).

Ly6G is expressed predominantly on neutrophils. However, it can also be expressed on differentiating monocytes as well as other cells (3, 21, 31). There were no significant differences in Ly6G expression between the two treatment groups (Table 1). Overall, expression of F4/80 (a macrophage-specific marker; Refs. 11, 25) was higher on bone marrow cells from Flight animals compared with Ground-control animals (7 ± 1% vs. 3 ± 0%, *P < 0.01).
expression in the R1 subpopulation. These data are consistent with observations that CD44 expression is higher on granulocytic cells (55). However, there was no statistically significant spaceflight effect on the expression of CD44 in the R1 subpopulation.

**R2 subpopulation phenotypic assessment.** There were no significant differences in the expression of Ly6C, Ly6G, or F4/80 on bone marrow cells in the R2 subpopulation between Flight and Ground-control animals. There was a significant increase in the expression of CD11b on bone marrow cells from Flight mice (50 ± 3%) compared with Ground-control mice (4 ± 0%; P < 0.01). This reversedly correlated with the expression of CD31 and c-Fos protein on bone marrow cells. Flight mice had 2 ± 1% and 4 ± 1% CD31- and c-fos-expressing cells compared with 10 ± 2% (P < 0.01) and 13 ± 2% (P < 0.01) expression by bone marrow from Ground-control mice. Interestingly, there was expression of CD44\textsuperscript{high} by the R2 bone marrow cell subpopulation but no CD44\textsuperscript{low} expression. Flight mice had 92 ± 1% CD44\textsuperscript{high}-expressing cells compared with 95 ± 1% (P < 0.05) expression by bone marrow from Ground-control mice.

**R3 subpopulation phenotypic assessment.** There was little difference in the R3 bone marrow cell expression of CD11b between Flight and Ground-control mice. Flight mice had 40 ± 3% CD11b-expressing cells compared with 43 ± 3% expression by bone marrow cells from Ground-control mice. There was a small but significant difference in the cell expression of Ly6C and CD31 between Flight and Ground-control mice. Flight mice had 63 ± 3% Ly6C-expressing cells compared with 72 ± 3% expression by bone marrow from Ground-control mice (P < 0.01). Flight mouse bone marrow was 1 ± 2% positive for CD31, whereas bone marrow from Ground-control mice was 10 ± 1% positive (P < 0.05). These data were consistent when these two phenotypes were looked at in combination. There was a decrease in the Ly6C-CD31\textsuperscript{−} subpopulation (Fig. 4). There also was a decrease in the Ly6C-CD31\textsuperscript{+} subpopulation in cells from Flight mice compared with cells from Ground-control mice (Fig. 4). The evaluation of c-Fos in the R3 subpopulation showed that there was a significant decrease in its expression (15 ± 3%) in Flight mice compared with Ground-control mice (45 ± 3%, P < 0.01). The same trend was seen with c-Fos\textsuperscript{−}CD31\textsuperscript{+} cells from Flight and Ground-control mice (9 ± 4% and 29 ± 4%, respectively, P < 0.01). Bone marrow cells from flight that had the c-Fos\textsuperscript{−}CD31\textsuperscript{+} phenotype were 48 ± 4% positive. However, c-Fos\textsuperscript{−}CD31\textsuperscript{−} cells were 0 ± 6% (P < 0.01) for Ground-control mice (Fig. 5). No differences in c-Fos\textsuperscript{−}CD31\textsuperscript{−} cell percentages were seen in the R3 subpopulation (Fig. 5). Ground-control mouse bone marrow cells were 8 ± 1% positive for F4/80 compared with 24 ± 3% positive cells in the Flight samples (P < 0.05). This inversely correlated with the expression of Ly6G and CD44\textsuperscript{high}, which were 67 ± 4% and 83 ± 2% positive, respectively, in Ground-control mouse bone marrow cells compared with 26 ± 1% and 64 ± 4% positive cells in Flight samples (P < 0.05).

**DISCUSSION**

Bone marrow cells from the CBTM-2 payload were analyzed in this study as a complete bone marrow population and as cell subpopulations distinguished by size and granularity. Ly6C was the only surface molecule significantly elevated on whole bone marrow cells from Flight mice compared with Ground-control mice. This contrasts with the observations of Pecaut et al. (41), who did not see changes in Ly6A/E in their analysis of mouse marrow after a 12-day flight on STS-108.
This difference was probably because Ly6A/E is expressed on different cell populations than Ly6C, which was targeted in this study because of its higher specificity for cells in the granulocytic lineage than the other Ly6 family members (9, 42). This hypothesis is supported by the observations from STS-108 (41) and STS-118 (this report) in which there were no differences in total numbers of bone marrow cells isolated from Flight and Ground-control mice yet there were differences between bone marrow subpopulations (this report). It is possible that we might have observed a different outcome had we

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**Fig. 4.** Ly6C and CD31 surface expression by bone marrow-derived cells in the R3 subpopulation. 

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**Fig. 5.** c-Fos and CD31 surface expression by bone marrow-derived cells in the R3 subpopulation. 
examined bone marrow from the femur instead of the humeri. However, experience with skeletally unloaded mice and antiorthostatic suspension suggests that the changes in bone (47) and bone marrow (6, 7) are systemic in response to unloading. Therefore, we do not think this is a serious concern.

Although Ly6C expression was elevated on whole bone marrow isolated from Flight mice compared with Ground-control mice, whole bone marrow composite phenotype expression demonstrated no significant difference between Flight and Ground-control mouse bone marrow (P > 0.2 by χ²-test). This is not surprising because bone marrow contains a heterogeneous population of cells that share phenotypes. Therefore, we analyzed subpopulations of cells that were distinguished on the basis of their size and granularity. Three subpopulations of cells were identified and assessed for the expression of Ly6C, CD11b, CD31, Ly6G, F4/80, CD44, and c-Fos.

Cell surface antigen expression differences were present within spaceflight subpopulations. For example, Ly6C expression was elevated by spaceflight in the small agranular R1 subpopulation but was lower in the R3 subpopulation. The R1 population contained the fewest numbers of macrophages and neutrophils based on the expression of F4/80, CD11b, and Ly6G. Given that Ly6C is expressed on cells at less mature stages within the myeloid lineage (30), these data suggest that the impact of spaceflight was on less mature cells compared with more mature cells in the granulocytic lineage. This hypothesis is supported by the expression of CD31 as well. CD31 expression distinguishes macrophages early in differentiation (17, 18, 62). c-Fos is also expressed during early myeloid cell development (38, 60). Therefore, c-Fos expression is normally expected concurrently with CD31 expression at initial macrophage differentiation stages under normal conditions. As differentiation proceeds, c-Fos expression ceases as CD31 levels begin to decline. The depression in the c-Fos CD31 population and the increase in the c-Fos CD31 population in R3 bone marrow cells from Flight mice suggest that the STS-118 spaceflight increased macrophage development, resulting in the presence of more differentiated cells than in the Ground-control mice.

CD11b was significantly elevated on bone marrow cells in the R2 population after spaceflight. CD11b is involved in trafficking and strong adhesion of macrophages and granulocytes in trafficking. Therefore, the elevation of this marker on the R2 subpopulation but not the R1 and R3 subpopulations may reflect the activation of the enriched neutrophil population in the R2 group in response to landing. Unfortunately, the experimental design of this study precluded the collection of blood for differential analysis. Therefore, we cannot make a direct comparison between what was occurring in the bone marrow and the peripheral blood cell populations. However, neutrophilia has been seen as a consequence of landing stress in past rodent flights (2, 13, 14, 26). Consequently, the specific impact on the R2 subpopulation would be consistent with those observations.

The greater decrease in expression of CD44<sup>high</sup> and Ly6G in the R3 bone marrow subpopulation from Flight mice but not the R1 and R2 subpopulations was also notable. In fact, there was a reverse correlation between the expression level of CD44<sup>high</sup> and Ly6G, which are strongly expressed on polymorphonuclear neutrophils (PMNs), and the increased expression of F4/80 in R3. Because F4/80 identifies mature macrophages, these data suggest that spaceflight caused increases in the percentage of mature macrophages in the bone marrow or decreased the percentage of PMNs. The stress of landing could induce the mobilization of bone marrow PMNs into circulation and cause such an impact (2, 13, 14, 26). Although this is an attractive hypothesis that would be consistent with data from other flights, there were no differences in bone marrow cell numbers between Flight mice and Ground-control mice to account for margination. Therefore, other factors may also be affecting the distribution of macrophages in the R3 subpopulation. The loss of cell numbers and a decrease in the percentage of monocytes/macrophages in the spleen of these flight mice (Baquai FP, Gridley DS, Slater JM, Luo-Owen X, Stodiek LS, Ferguson V, Chapes SK, Pecaut MJ, unpublished observations) would be consistent with increased trafficking of those cells to the bone marrow. Alternatively, the decrease in Ly6C, c-Fos, and/or Ly6G and increase in F4/80 might suggest that the cells in the R3 subpopulation were more differentiated in the Flight mice compared with the Ground-control mice. This hypothesis is consistent with the diminished expression of IGF-I receptor (IGF-1R) on monocytes during spaceflight (34), since the expression of IGF-1R is reduced as macrophages differentiate (5).

The shift in cellular phenotypes is a complex process. For this reason, we compared the composite phenotypes of each of the three subpopulations of Flight bone marrow cells and Ground-control bone marrow cells with both parametric (factorial ANOVA) and nonparametric (χ²-test) statistics. R1, R2, and R3 comparisons between Flight and Ground-control AEM-housed mice suggest that there are significant differences (P < 0.01) between cells in the two treatment groups for all three subpopulations with either type of analysis. The mice were analyzed after landing. Therefore, it is not possible to separate the landing effects from the flight effects. As shown by behavioral observations made at days 5–6 during flight (normal grooming, eating, drinking, and movement) and the veterinary examination after flight, the animals were healthy. The weight loss most likely occurred in the first 3 days of habitation to the change in gravity during the spaceflight (37, 51, 59). Nevertheless, the loss in body weight and the significant thymic atrophy (Baqui et al., submitted for publication) that were seen in these mice suggest that the mice were subjected to chronic stress beyond what was endured during landing. Therefore, it is not unreasonable to suggest that there are significant changes in bone marrow phenotype in response to the stress of the spaceflight experience. Bone marrow cells are plastic in their response to host factors (52). Furthermore, not only are changes in bone marrow cell distribution seen in rats and mice in response to spaceflight (41, 50), bone marrow cell distribution is responsive to other stressors such as reactive oxygen species (43), ionizing radiation (15), ovariectomy (22), and glucocorticoid administration (54). It is also not clear whether these differences translate into changes in immune competence (12), but the data are consistent with observations that the number of CFU-M and CFU-GM are decreased during spaceflight (26, 49, 50). The shift toward more differentiated cells in the R2 and R3 subpopulations would reduce the number of early CFU-GM available to respond to colony-stimulating factor to differentiate into the appropriate cell lineage. If humans aspire to interplanetary space travel, appropriate concern and planning...
are needed to manage the possible consequences of a long-term spaceflight on hematopoiesis.

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