Decreased non-MHC-restricted (CD56⁺) killer cell cytotoxicity after spaceflight

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Mehta, Satish K., Indreshpal Kaur, Elizabeth A. Grimm, Christine Smid, Daniel L. Feeback, and Duane L. Pierson. Decreased non-MHC-restricted (CD56⁺) killer cell cytotoxicity after spaceflight. J Appl Physiol 91: 1814–1818, 2001.—Cytotoxic activity of non-major histocompatibility complex-restricted (CD56⁺) (NMHC) killer cells and cell surface marker expression of peripheral blood mononuclear cells were determined before and after spaceflight. Ten astronauts (9 men, 1 woman) from two space shuttle missions (9- and 10-day duration) participated in the study. Blood samples were collected 10 days before launch, within 3 h after landing, and 3 days after landing. All peripheral blood mononuclear cell preparations were cryopreserved and analyzed simultaneously in a 4-h cytotoxicity ⁵¹Cr release assay using K562 target cells. NMHC killer cell lytic activity was normalized per 1,000 CD56⁺ cells. When all 10 subjects were considered as one study group, NMHC killer cell numbers did not change significantly during the three sampling periods, but at landing lytic activity had decreased by ~40% (P < 0.05) from preflight values. Nine of ten astronauts had decreased lytic activity immediately after flight. NMHC killer cell cytotoxicity of only three astronauts returned toward preflight values by 3 days after landing. Consistent with decreased NMHC killer cell cytotoxicity, urinary cortisol significantly increased after landing compared with preflight levels. Plasma cortisol and ACTH levels at landing were not significantly different from preflight values. No correlation of changes in NMHC killer cell function or hormone levels with factors such as age, gender, mission, or spaceflight experience was found. After landing, expression of the major lymphocyte surface markers (CD3, CD4, CD8, CD14, CD16, CD56), as determined by flow cytometric analysis, did not show any consistent changes from measurements made before flight.

space immunology; microgravity; non-major histocompatibility complex-restricted killer cell

LIVING AND WORKING IN the relatively crowded, closed environment of a spacecraft using recycled air and water may increase the risk of contracting an infectious disease during spaceflight. As the typical duration of space missions increases from <20 days aboard the US space shuttle to ≥6 mo aboard the International Space Station, the potential risk to crew health is expected to increase.

Changes in human immune responses during short- and long-term spaceflights have been described (1, 4, 15, 18, 19, 21–23, 25, 27) and have been recently reviewed (24). Short-term spaceflight has been associated with reductions in absolute numbers of lymphocytes, eosinophils, and natural killer (NK) cells (1) and with elevations in white blood cell numbers, resulting primarily from increased numbers of neutrophils in peripheral blood (18, 23). In other studies, reduced lymphocyte mitogenic response (1, 23), diminished delayed-type hypersensitivity (23), changes in CD4⁺-to-CD8⁺ ratios, and reduced production of interleukin (IL)-2 and interferon-γ (24) have been observed. Russian investigators have reported significant decreases in NK cell cytotoxicity index after spaceflight of 8–366 days (4, 5, 15).

As National Aeronautics and Space Administration (NASA) begins long-term habitation aboard the International Space Station and perhaps the establishment of lunar outposts and eventually the human exploration of Mars, understanding the effects of spaceflight on the immune system grows in importance. NK cell function is an important element of the immune system, and monitoring NK cell cytotoxicity will be an essential part of assessing health effects of long-term space missions.

The objective of this study was to determine the effects of short-term space shuttle flights on non-major histocompatibility complex-restricted (CD56⁺) (NMHC) killer cell cytotoxicity.

MATERIALS AND METHODS

Subjects. A total of 10 crew members (9 men and 1 woman) from two space shuttle missions (9- and 10-day duration) participated in this study. Their ages ranged from 39 to 48 yr. All subjects signed informed consent forms, and the investi-
gation was approved by the Johnson Space Center Institutional Review Board.

Collection and cryopreservation of blood cells. Blood samples (10 ml) were collected from each crew member into EDTA-containing vacutainers at three time points: 10 days before launch (L−10), within 3 h after landing (R+0), and 3 days after landing (R+3). The blood was collected during the scheduled physical examination for crew medical assessment. Immediately after collection, the blood was diluted 1:1 with normal saline, underlain with 15 ml of Ficoll Hypaque (Pharmacia, Uppsala, Sweden), and centrifuged for 35 min at 400 g. The mononuclear layer [peripheral blood mononuclear cells (PBMCs)] was collected and washed by using Hanks’ balanced salt solution without Ca2+ or Mg2+ (BioWhittaker, Walkersville, MD). Cells were counted with a hemocytometer and resuspended in 1 ml of freezing medium consisting of 90% pooled human blood group AB serum (BioWhittaker) and 10% dimethyl sulfoxide (Sigma Chemical, St. Louis, MO). These cells were then cryopreserved (13) by placing them in a −70°C bath of 100% ethanol and then transferring their macromolecular liquid nitrogen, where they were stored until analysis for cytotoxicity.

51Cr release assay. Cryopreserved PBMCs were thawed. Their viability was determined by Trypan blue (0.2% Trypan blue, Sigma Chemical) exclusion. Viability of PBMCs was routinely >95%. Cytolytic activity of NMHC killer cells in the thawed PBMCs was measured by the standard assay of Lagoo-Deenadayalan et al. (6). Briefly, target K562 cells were labeled with Na251CrO4 (DuPont, Boston, MA), washed, and incubated for 4 h with effector cells (PBMCs) at effector-to-target ratios (E/T) of 100:1, 50:1, and 25:1. Each E/T demonstration was conducted in triplicate, and assay variation was ±5%. Percent cytotoxicity was calculated as 100 × ([counts/min released by targets with effectors − counts/min released by targets alone]/counts/min released by targets with 0.1 M HCl) − (counts/min released by targets alone)). Cytotoxicity values obtained from serial E/T were converted into lytic units (LU). One LU was defined as the number of effector cells required to achieve 20% specific lysis of 5 × 104 targets and is expressed per 107 effectors (PBMC). These LU were further normalized for 1,000 NMHC killer (CD56+ T-) cells. For these calculations, the percentage of NMHC killer cells in the PBMCs was calculated from the surface staining for CD56 described below.

A control group of 10 men and 1 woman (age matched) was included in the study. Three samples from each control subject were collected over a period of 3 wk to approximate the sample collection schedule of astronauts before and after spaceflight. After cryopreservation, PBMCs from control subjects showed viability levels (>95%) similar to those in astronauts.

Endocrinology. Plasma cortisol and ACTH were measured by radioimmunoassay (2, 16) from aliquots of blood samples collected over a period of 3 wk to approximate the sample collection schedule of astronauts before and after spaceflight. After cryopreservation, PBMCs from control subjects showed similar cortisol levels (95%) to those in astronauts.

Lymphocyte surface markers. PBMCs were resuspended at a concentration of 105 cells/100 μl of PBS containing 1% bovine serum albumin and 0.1% sodium azide. Aliquots were incubated for 30 min at 4°C with each of the following monoclonal antibodies: anti-CD3-FITC, anti-CD56-phycocerythrin (PE), anti-CD4-FITC, anti-CD8-PE, anti-CD14-FITC, and anti-CD16-PE (Becton Dickinson, San Jose, CA). Mouse IgG1-FITC and mouse IgG1-PE (Becton Dickinson, San Jose, CA) were used as isotype controls. After staining, the cells were washed with PBS, fixed in 0.5 ml of 1% paraformaldehyde, and analyzed on a FACScan (Becton Dickin-son) flow cytometer. Ten thousand events were collected for each sample, and the gates were set on the lymphocyte population based on forward and side scatter. The results are expressed as the percentage of gated cells positive for each surface marker.

Statistical analysis. NMHC killer cell cytotoxicity was calculated as LU per 1,000 NMHC killer cells. The data on NMHC killer cell cytotoxicity, cell numbers, and plasma and urine hormones from all 10 crew members, measured at the three time points [1 before flight (L−10) and 2 after landing (R+0 and R+3)], were tested for normality, and one-way repeated-measures ANOVA was performed. Multiple-comparison procedures (Tukey’s test) were used after initial ANOVA. When the test for normality failed, Friedman’s repeated-measures ANOVA on ranks was used. Differences were considered statistically significant if P ≤ 0.05.

RESULTS

Individual variation in the number of NMHC killer cells was observed among the astronauts at the different sampling points. However, after spaceflight, the mean number of NMHC killer cells of all 10 astronauts as a group was not significantly different from the preflight mean. The mean ± SE number of NMHC killer cells for L−10 was 204 ± 40 cells/mm3 of blood; for R+0, 237 ± 70 cells/mm3 of blood; and for R+3, 169 ± 32 cells/mm3 of blood. To ensure that any changes detected in NMHC killer cell cytotoxicity were not a function of absolute numbers of NMHC killer cells, data are expressed as LU per 1,000 NMHC killer cells. The cytotoxicity decreased (P < 0.05) at landing from preflight values by ~40% in the group of 10 astronauts (Fig. 1).

Individual NMHC killer cell cytotoxicity data are shown in Fig. 2. At landing, the NMHC killer cell cytotoxicity of 9 of the 10 crew members was reduced from preflight values. For seven of the nine crew members, these reductions in cytotoxicity ranged from 33% (crew member 10) to 95% (crew member 9). Crew member 3 had a slight increase (~5%) in his NMHC killer cell activity at landing, but a 60% reduction was evident by R+3. Three days after landing, the NMHC killer cell cytotoxicity of only three crew members increased toward their preflight values. The R+3 value of crew member 1 surpassed his preflight value. NMHC killer cell cytotoxicity of six crew members at R+3 was lower than their landing (R+0) values.

Flow cytometry was used to assess phenotypic changes in expression of CD3, CD4, CD8, CD14, CD16, and CD56 on PBMCs. Although individual variations in NMHC killer cell numbers existed, as measured by CD56+ cells (Table 1), the mean percentage of CD56+ cells did not change significantly after spaceflight. Their mean ± SE percentage at L−10 was 10.6 ± 2.6%; at R+0, 9.6 ± 1.5%; and at R+3, 10.4 ± 2.2%. Of the 10 crew members, 3 showed decreases in the percentage of CD16+ cells at landing compared with preflight values (Table 1). The percentage of CD14+ (LeuM3+ T-) cells (monocytes) of six crew members increased at landing from their preflight values and by R+3 returned to preflight values (data not shown). The total percentage...
of CD3+ T cells increased at landing in >50% of the crew members (data not shown).

Hormones measured in urine and plasma from these crew members before and after flight showed variable results. At landing, urinary cortisol levels from 24-h urine pools were significantly increased ($P < 0.001$) from preflight values. No significant differences were found between single preflight and single postflight plasma cortisol and ACTH levels (Fig. 1).

**DISCUSSION**

This is the first study to describe the effects of short-term spaceflight on the lytic activity of NMHC killer cell cytotoxicity, plasma and urine cortisol, and plasma ACTH from 10 crew members on 2 spaceflight missions. Values are means ± SE. Blood samples were collected 10 days before launch (L−10), on landing day (R+0), and 3 days after landing (R+3). Lytic units are normalized per 1,000 NMHC killer cells.
cells. An understanding of the human immune response during spaceflight has been hampered by severe constraints on access to critical blood specimens and lack of technology for in-flight analyses. Within these constraints, this study was undertaken to determine the effect of short-duration (9–10 days) missions aboard the space shuttle on NMHC killer cell cytotoxicity. We observed only small changes in the numbers of CD56+ (NMHC killer) cells, unlike previous reports (2, 10, 12). Most importantly, we found a decrease of ~40% in NMHC killer cell lytic activity in the group of 10 astronauts after spaceflight. Large variations in cytotoxicity were apparent in the astronauts during the three sampling periods, probably indicating the wide range of individual responses to the unique stresses associated with spaceflight (12). Rapid changes in the immune response, such as decreased cell-mediated immunity, have also been reported after short space shuttle flights (23).

Previous studies reported decreased NK cell cytotoxicity in cosmonauts after short-term (8–14 days) and long-term (30–366 days) spaceflights (4, 5, 10, 11, 15). Rykova et al. (17), using a murine NK cell target (YAC-1), reported decreases in rat NK cell cytotoxicity during a 14-day space mission. Direct comparison of data obtained from Russian cosmonauts and US astronauts was not practical because of substantial differences in methodology used. For example, in previous studies, NK cell cytotoxicity was defined as the ability of PBMCs to lyse K562 target cells. The phenotype of the study cells was not defined; therefore, the exact cell type studied is uncertain. The present study used 51Cr-labeled K562 target cells, whereas previous studies used 3H-labeled uridine K562 cells. Lesnyak et al. (10) identified substantial differences between results using the 3H-labeled uridine target cell assay used in previous studies and the 51Cr-labeled target cell assay employed in this study.

Decreased total NK cell cytotoxicity of some cosmonauts after long-term spaceflight returned to preflight values by 14 days after landing, whereas preflight cytotoxic activity of other cosmonauts did not return even after 2 mo (5). The present study also showed that the NMHC killer cell activity of some crew members returned to preflight levels much more slowly than that of others. Spaceflight constraints did not allow collection of blood samples beyond 3 days after flight, preventing us from determining the average time needed for return to preflight levels. We examined the age, gender (although only one woman participated in the study), flight experience (i.e., first-time or repeat flyers), mission factors, and mission role (e.g., pilot or scientist) of the crew members and found no correlation with individual NMHC killer cell function levels. Two crew members performed an extravehicular activity (space walk) with no observable effect on NMHC killer cell function.

It is probable that the reductions in NMHC killer cell cytotoxicity after both short- and long-term spaceflights are the result of stress. Many physical and psychosocial stresses are associated with spaceflight (12) and spaceflight analogs (13), and the effects of stress on immune function, including increased viral reactivation, are well recognized (20, 27). Spaceflight stress usually results in increased levels of stress hormones, such as cortisol, ACTH, growth hormone, and β-endorphin. Stein and Schluter (20) showed that urinary excretion of IL-6 and cortisol increased significantly on the first day of spaceflight (after launch) and on landing day. They did not observe additional changes before, during, or after flight. The present study showed no significant change in plasma cortisol or ACTH levels after the missions, but urinary cortisol was significantly increased at landing. This is consistent with our laboratory’s previous findings (14). Plasma cortisol, which has a short (~4-h) half-life, was collected only once before and once after spaceflight, giving a “snapshot” look at plasma levels, whereas each urinary cortisol value was obtained from a pool of urine collected over a 24-h period. The snapshot of plasma cortisol may have missed transient but significant changes that were detected in urine, which provided an integration of 24 h of collection. Previously, Leach et al. (9) reported significant reduction in plasma cortisol and elevation in urinary cortisol at landing. In another study, Leach (7) showed that plasma ACTH levels in a

Table 1. Number and percentage of immunophenotypic marker-positive cells from 10 space shuttle crew members

<table>
<thead>
<tr>
<th>Crew Member No.</th>
<th>CD56+ Cells (in the lymphocyte gate)</th>
<th>CD16+ Cells (in the lymphocyte gate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L - 10</td>
<td>R + 0</td>
</tr>
<tr>
<td>1</td>
<td>5.9</td>
<td>160</td>
</tr>
<tr>
<td>2</td>
<td>4.9</td>
<td>120</td>
</tr>
<tr>
<td>3</td>
<td>24.4</td>
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</tr>
<tr>
<td>10</td>
<td>1.3</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>L - 10</td>
<td>R + 0</td>
</tr>
<tr>
<td></td>
<td>14.0</td>
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</tr>
<tr>
<td></td>
<td>71.8</td>
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<td></td>
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<td></td>
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<td>52</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0</td>
</tr>
</tbody>
</table>

L - 10, 10 days before launch; R + 0, landing day; R + 3, 3 days after landing.
group of eight other space shuttle crew members were higher at landing than before flight; however, for crew members on the Spacelab Life Sciences missions (8), levels were increased on flight day 12 (of a 14-day flight) and not on landing day. Significantly raised levels of human growth hormone at landing have also been reported (8). Kay et al. (3) have shown that β-endorphins and several analogs (e.g., γ-endorphins) enhance human peripheral blood NK cell function. β-Endorphins have yet to be measured in astronauts.

The mechanisms responsible for the decrease in NMHC killer cell activity are not clear and may be the result of a combination of factors, including the inhibitory effect of glucocorticoids and the failure of NMHC killer cells to migrate from bone marrow to the periphery (26). The changes reported here in NMHC killer cell activity are consistent with stress-induced effects of spaceflight mediated through the hypothalamic-pituitary-adrenal axis (12). Future studies should investigate the effects of spaceflight on IL-2-activated NK (LAK) cells. These studies should include stress assessment and lead to the testing of stress countermeasures protocols.

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