Effects of Mission Duration on Neuroimmune Responses in Astronauts

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Background: Spaceflight poses a unique stress to humans that can impair cellular immune responses and reactivate latent herpes viruses. Notably, prior studies have suggested that mission length may be an important factor in the variability of immune alterations observed after spaceflight. In this study, adrenocortical responses and circulating leukocytes were compared between astronauts who participated in either 9- or 16-d missions. ***Hypothesis:*** Mission duration will differentially affect neuroimmune responses after spaceflight. ***Methods:*** Blood and urine samples, collected from 28 crewmembers who flew on 5 Space Shuttle missions, were analyzed for levels of plasma and urinary cortisol, urinary catecholamines, leukocyte and lymphocyte subsets, and total IgE. ***Results:*** After spaceflight, plasma cortisol was significantly decreased after the 9-d missions but increased after the 16-d missions. In contrast, urinary epinephrine and norepinephrine levels were greater after the 9-d missions than the 16-d missions. Total IgE was significantly increased after the 16-d missions and correlated with urinary cortisol. The number of white blood cells, polymorphonuclear leukocytes, and $CD4^+$ T cells were significantly increased postflight. After the 9-d missions, monocytes were increased while natural killer cells were decreased. However, monocytes were decreased after the 16-d missions; no change occurred in natural killer cells. ***Conclusions:*** These results suggest that sympathetic nervous system responses predominate after shorter spaceflights, while longer flights are characterized by glucocorticoid-mediated changes at landing that may result from the accumulative effects of microgravity (i.e., physiological deconditioning) over time.

Keywords: spaceflight, microgravity, neuroimmunity, catecholamines, adrenocortical response, glucocorticoids, leucocytes, lymphocytes.

AN UNDERSTANDING OF the mechanisms underlying immune alterations in astronauts is vital to the development of effective countermeasures for long-duration spaceflight. A body of literature now exists which shows that cellular immune responses are reduced during and after spaceflight in which multiple stressors (e.g., launch and landing stresses, microgravity, confinement, separation from family, sleep deprivation, etc.) are thought to be involved (12,18,20,23). As previously pointed out (18,24), this may result in opportunistic infections developing within a crewmember, transfer of potential pathogens between crewmembers, and/or increased risk of infection on return to Earth.

Alterations in neuroendocrine hormones such as cortisol and catecholamines have been implicated as causal factors in immune changes in astronauts (8,13,22,25). Glucocorticoids affect the immune system by altering leukocyte trafficking and migration to areas of inflammation as well as directly inhibiting individual cellular functions. Corticosteroid administration rapidly induces neutrophilia as a result of demargination and early release from the bone marrow (6,7). Lymphocyte number in peripheral blood decreases owing to retention of recirculating lymphocytes within the bone marrow, spleen, and lymph nodes; a transient monocytopenia with kinetics similar to those of lymphocytes have been reported after corticosteroid administration (6,7). Catecholamines, in contrast, induce a short-lived lymphocytosis [predominantly natural killer (NK) cells] followed by mobilization of granulocytes from the marginated pool; T and B cells do not change appreciably after epinephrine administration (2).

While it is generally agreed that spaceflight results in increased white blood cell counts and elevated neutrophils (10,13,22,25), there are conflicting data regarding other circulating leukocytes subsets. For instance, a decrease in monocytes was found postflight in one study (25), whereas another study reported an increase after spaceflight (13). Subsequently, this discrepancy was attributed to varying mission lengths, which may have differentially affected levels of neuroendocrine hormones (12,23). Unfortunately, many parameters including mission duration cannot be controlled for in spaceflight experiments. Published studies often group results from multiple missions that vary in duration, which may mask significant findings.

In light of data from studies comprised of different mission lengths, we analyzed plasma and urinary cortisol and urinary catecholamines in samples from 28 astronauts who flew on either three 9-d missions or two 16-d missions in order to better understand the effects of mission duration on neuroendocrine responses. In addition, we measured leukocyte subsets and IgE levels to correlate with the changes in neuroendocrine hormones.

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METHODS

Subjects: The Johnson Space Center Institutional Review Board approved this investigation, and informed consent was obtained from all participants. There were 3 separate samples of peripheral blood collected from each of 28 astronauts who flew aboard 5 U.S. space shuttle flights. The mission lengths were 9 d (n = 16) and 16 d (n = 12). Peripheral blood was collected 10 d before launch (L-10), within 4 h after return (R + 0), and 3 d after return (R + 3); these collections coincided with the Medical Operations physicals that occurred before and after spaceflight.

Preflight blood draws were typically performed early in the morning (around 7:30 a.m.) and processed at the Johnson Space Center, Houston, TX. Blood was collected within 2–4 h of landing (R + 0) at the Kennedy Space Center, Cape Canaveral, FL. Plasma was obtained after centrifuging and was stored at −70°C until testing. Urine voids were collected from crewmembers before (L-10) and after (R + 0) spaceflight; aliquots of 24 h pools were stored at −20°C until analysis.

Neuroendocrine hormones and total IgE: The measurement of hormones has been previously described in detail (22). Plasma adrenocorticotropic hormone (ACTH) and plasma and urinary cortisol were measured by radioimmunoassay. Urinary catecholamines (epinephrine, norepinephrine) were measured by high-performance liquid chromatography. IgE was measured by microparticle enzyme immunoassay (22). Samples were batch analyzed to minimize interassay variation.

Leukocyte subset analysis: Absolute numbers of leukocytes per milliliter of blood were determined by a Coulter blood cell analyzer (Coulter Electronics, Hialeah, FL). Relative numbers of lymphocytes, monocytes, and granulocytes were determined in Wright-stained blood smears by standard techniques. These data were used to calculate the absolute numbers and percent of circulating leukocytes.

Immunofluorescent staining: Phenotypic analysis of whole blood was performed by flow cytometry as previously described (20). Fluorescent antibodies conjugated to various fluorochromes (obtained from Becton-Dickinson, Mountain View, CA) were added to whole blood (100 μl) and incubated at room temperature for 30 min. Red blood cells were lysed using FACSLyse (Becton-Dickinson). The cells were then washed once, resuspended in paraformaldhyde, and analyzed on a FACSCalibular flow cytometer (20). A minimum of 20,000 events was collected for each sample.

Statistical analysis: Normality was assessed using the Kolmogorov-Smirnov normality test. Data not normally distributed were subjected to natural log transformations to normalize the distributions prior to analyses. Statistical analysis was performed using the Student's *t*-test for paired (intragroup) data. Data from multiple timepoints were analyzed by ANOVA followed by multiple-comparison procedures (Tukey's test). Correlation between variables was described by Pearson's product moment test. The percent change in each hormone for each subject was calculated as follows: (R + 0 level − L-10 level)/L-10 level × 100. Results are expressed as mean values ± SE, and $p < 0.05$ was considered significant.

RESULTS

In order to investigate the effects of mission duration on adrenocortical responses, data were stratified into 9-d (n = 16) and 16-d (n = 12) spaceflights. **Fig. 1** shows the percent changes in neuroendocrine hormone levels at R + 0 as compared with L-10 values for both the 9-d and 16-d missions. A significant decrease was observed in plasma cortisol after the 9-d missions (18.4 ± 2 $\mu g \cdot dl^{-1}$ preflight vs. 11.8 ± 2 $\mu g \cdot dl^{-1}$ postflight). There were no significant changes in plasma ACTH or urinary cortisol after spaceflight. In contrast to the 9-d missions, there was a statistically significant increase in plasma cortisol after the 16-d missions (16.4 ± 1 $\mu g \cdot dl^{-1}$ preflight vs. 21.3 ± 3 $\mu g \cdot dl^{-1}$ postflight); plasma cortisol then returned to preflight levels at R + 3 (data not shown). A significant increase in urinary cortisol was also observed after the 16-d missions (80.3 ± 12 $\mu g \cdot 24\ h^{-1}$ preflight vs. 121.9 ± 17 $\mu g \cdot 24\ h^{-1}$ postflight). No significant change was observed in plasma ACTH, although two crewmembers on the 16-d mission had large increases (128% and 345%) (Fig. 1).

When combining astronauts in both groups, urinary epinephrine and norepinephrine were significantly elevated at R + 0 (data not shown). However, since a prior study showed that mission duration affected catecholamine levels (15), we grouped the data according to mission length. Urinary norepinephrine levels were significantly elevated on the 9-d missions (82.2 ± 7 $\mu g \cdot 24\ h^{-1}$ preflight vs. 110.2 ± 11 $\mu g \cdot 24\ h^{-1}$ postflight); urinary epinephrine was increased but not significantly (14.0 ± 2 $\mu g \cdot 24\ h^{-1}$ preflight vs. 18.6 ± 3 $\mu g \cdot 24\ h^{-1}$ postflight). For the 16-d missions, marginal increases were found for urinary epinephrine and norepinephrine (21% and 26%, respectively).

The percent change in total IgE for the 9- and 16-d missions is shown in **Fig. 2**. IgE was not significantly increased in the 9-d group, but IgE was significantly increased after the 16-d missions ($p < 0.01$). Notably, one astronaut had over a 100% increase in total IgE. However, excluding this individual did not change the significance of the analysis ($p = 0.014$). Overall, a significant correlation was found between urinary cortisol and total IgE in the 16-d flights ($r = 0.82$, $p < 0.01$).

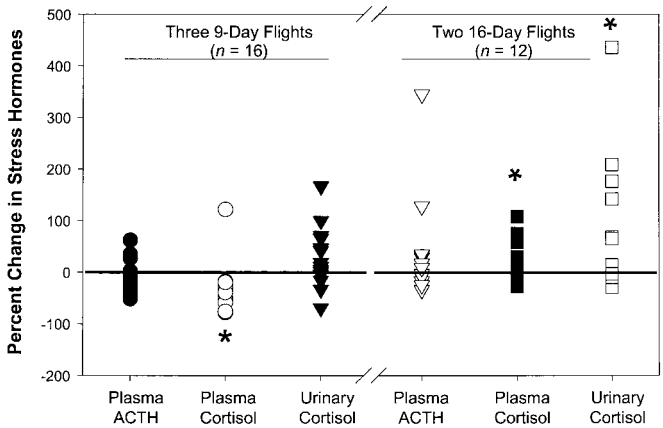

Fig. 1. Changes in plasma ACTH and plasma and urinary cortisol after 9- and 16-d spaceflights. Percent change in stress hormones at landing (R + 0) is expressed as the difference compared with individual preflight (L-10) values. * indicates $p < 0.05$.

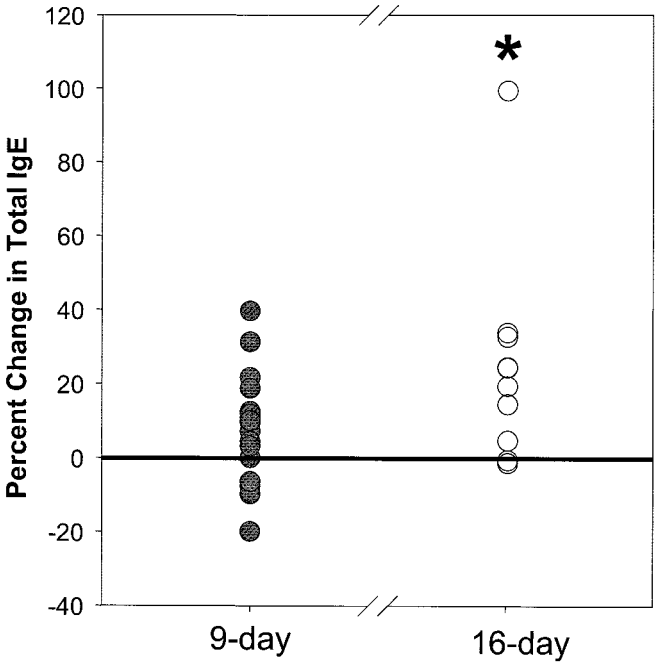

Fig. 2. Percent change in total IgE at landing according to mission duration. Data from 28 astronauts were grouped into 9-d (n = 16) and 16-d (n = 12) flights. * $p < 0.05$.

As shown in **Table I**, significant changes in specific leukocyte and lymphocyte subsets were observed after flight in the 9- and 16-d missions alike. In both groups, significant increases were observed for white blood cells (WBCs) and neutrophils. The increase in neutrophils for the 9-d missions was significantly greater than the increase observed in the 16-d missions ($p < 0.05$). A nonsignificant increase was found in circulating lymphocytes in the 16-d missions, whereas a significant postflight decrease ($p < 0.05$) occurred in the monocyte number. Phenotypic analysis of lymphocytes was performed on six crewmembers from a 16-d mission and five crewmembers from a 9-d mission. When all 11 astronauts were included in the analysis, a significant increase was found in the $CD4^+$ T cell population (data not shown). Further analysis revealed that significant increases in $CD4^+$ T cells only occurred in the 9-d mission ($p < 0.05$). A significant decrease was observed in NK cells after the 9-d mission ($p < 0.05$), whereas no change occurred in the 16-d mission. No significant change was found in any of the other lymphocyte subsets.

DISCUSSION

In our previous studies of spaceflight (grouped missions of 14 d or less), plasma cortisol was decreased after spaceflight but 24-h urinary cortisol was significantly increased (14,22). These seemingly contradictory findings can be explained by the fact that cortisol has a short half-life (approximately 30 min) in the circulation (9), and most landing day blood samples were taken 2–4 h after landing. Thus, our prior findings underscore the importance of measuring 24-h urinary cortisol levels, especially in the context of interpreting postflight immunological data. Notably, complete measurements of neuroendocrine hormones (i.e., both plasma and urinary values) have not been included in most immunological studies to date.

In the present study, we were fortunate enough to have multiple missions of either 9- or 16-d flights that allowed a better examination of the effects of flight duration on adrenocortical responses. In agreement with prior studies (10,14,22), plasma cortisol was decreased after the 9-d missions. Urinary cortisol was not significantly elevated in this group, although we found increased catecholamines at landing. These results closely mirror those from the 9-d Spacelab Life Science-1 mission (11) and suggest that sympathetic nervous system responses predominate after shorter spaceflights.

Contrary to the results from the 9-d missions, plasma cortisol was significantly increased after the 16-d flights. Urinary cortisol was also significantly increased, whereas urinary catecholamines were not significantly increased. The biological relevance of increased glucocorticoids was underscored by the changes in circulating monocytes and total IgE. Monocytes are particularly sensitive to the effects of glucocorticoids, and a marked monocytopenia occurs after glucocorticoid administration (4). Likewise, we also found a decrease in circulating monocytes after the 16-d flights. Importantly, glucocorticoids inhibit IL-12 production in monocytes but increase production of IL-10 (3,5). Notably, IL-10 levels were elevated in the first few days of the SLS-1 and SLS-2 missions (19). Furthermore, glucocorticoids enhance IL-4 and IL-10 synthesis in T cells resulting in increased circulating IgE levels in vivo (1,17,26). Consistent with these studies, total IgE was sig-

TABLE 1. LEUKOCYTE[a] AND LYMPHOCYTE[b] SUBSETS AFTER 9- AND 16-D SPACEFLIGHTS.

	L−10		R+0		R+3	
	9d	16d	9d	16d	9d	16d
WBCs	5335 ± 347	5185 ± 284	8300 ± 491*	7242 ± 565*	4900 ± 262	5392 ± 348
Neutrophils	3145 ± 348	2858 ± 210	6186 ± 405*	4917 ± 523*	2585 ± 242	3878 ± 268
Lymphocytes	1533 ± 101	1648 ± 141	1536 ± 159	2123 ± 343	1625 ± 77	1597 ± 124
Monocytes	450 ± 42	429 ± 32	498 ± 59	382 ± 64*	459 ± 23	488 ± 37
$CD3^+CD4^+$	31 ± 6	46 ± 4	42 ± 6*	49 ± 4	29 ± 4	40 ± 5
$CD3^+CD8^+$	13 ± 2	25 ± 3	14 ± 2	23 ± 4	14 ± 2	23 ± 4
$CD19^+$	8 ± 1	12 ± 2	9 ± 1	13 ± 2	9 ± 1	13 ± 2
$CD3^-$ $CD16^+56^+$	10 ± 2	10 ± 2	5 ± 1*	10 ± 3	7 ± 1	10 ± 2

[a]White blood cell counts and differentials were performed on 28 crewmembers and grouped according to missions length ($n = 16$ for 9-d missions, $n = 12$ for 16-d missions). Values are mean ± SE (cells/mm^3). [b]Phenotypic analysis of lymphocyte subsets was performed on one 9-d flight ($n = 5$ crewmembers) and one 16-d flight ($n = 6$ crewmembers). Values are percentage ± SE of total cells analyzed. * indicates $p < 0.05$.

nificantly increased after the 16-d flights. Overall, these results indicate that glucocorticoid-mediated changes predominate in longer missions, and these changes may modulate cytokine production in favor of Th2-type cytokines.

Interestingly, a recent study (15) reported an association between mission length, catecholamine levels, and leukocyte subsets. The authors found greater increases in the number of WBCs and $CD4^+$ T cells after missions of 4–7 d duration than in missions of 11–16 d duration; these increases were associated with higher catecholamine levels. In our study, which included a larger subject number, we also found larger increases in catecholamines, WBCs, neutrophils, and $CD4^+$ T cells in the shorter 9-d missions. Notably, the percentage of NK cells were decreased after the 9-d flights but showed no change after the 16-d missions. Although epinephrine administration results in an increase in NK cells, we speculate that the decreased NK cells after the 9-d mission may have reflected a "rebound" effect since the blood was drawn 2–4 h after the stressor (i.e., reentry and landing). This interpretation is supported by lack of change in both urinary catecholamine levels and NK cells after the 16-d flights. Overall, these findings support those of Mills and coworkers (15) and suggests that in shorter missions (i.e., less than 11 d), activation of the sympathetic nervous system and subsequent elevations of catecholamines may be primary mediators in the postflight changes in leukocytes.

In summary, these results support the role of both catecholamines and glucocorticoids in mediating immune changes in astronauts. We propose that the accumulative effects of microgravity (i.e., muscle loss and generalized physiological deconditioning) associated with longer duration missions may have resulted in diminished sympathetic nervous system activation and increased hypothalamic pituitary adrenal axis activation at landing. These results may have important health implications for crewmembers on exploration-class missions, in which the landing site (i.e., lunar or Mars surface) is characterized by lack of accessibility to specialized medical facilities in case of injury or illness. This data also further supports our hypothesis that a Th1-to-Th2 shift in cytokine production may be partially responsible for many of the immune alterations in astronauts (20). To determine the clinical relevance of these findings, we are investigating latent herpes virus reactivation as an in vivo marker of decreased immunity in astronauts (14,16,20,21). Our current study, which includes both Shuttle and International Space Station crewmembers, will allow us to further assess the effects of mission duration on neuroendocrine responses, immune alterations, and latent herpes virus reactivation.

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REFERENCES

1. Akdis CA, Blesken T, Akdis M, et al. Glucocorticoids inhibit human antigen-specific and enhance total IgE and IgG4 production due to differential effects on T and B cells in vitro. Eur J Immunol 1997; 27:2351–7.
2. Benschop RJ, Rodriguez-Feuerhahn M, Schedlowski M. Catecholamine-induced leukocytosis: early observations, current research, and future directions. Brain Behav Immun 1996; 10:77–91.
3. Blotta MH, DeKruyff RH, Umetsu DT. Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ lymphocytes. J Immunol 1997; 158:5589–95.
4. Cupps TR, Fauci AS. Corticosteroid-mediated immunoregulation in man. Immunol Rev 1982; 65:133–55.
5. Elenkov IJ, Papanicolaou DA, Wilder RL, Chrousos GP. Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: clinical implications. Proc Assoc Am Physicians 1996; 108:374–81.
6. Fauci AS, Dale DC. Alternate-day prednisone therapy and human lymphocyte subpopulations. J Clin Invest 1975; 55:22–32.
7. Fauci AS, Dale DC, Balow JE. Glucocorticosteroid therapy: mechanisms of action and clinical considerations. Ann Intern Med 1976; 84:304–15.
8. Gmunder FK, Konstantinova I, Cogoli A, et al. Cellular immunity in cosmonauts during long duration spaceflight on board the orbital MIR station. Aviat Space Environ Med 1994; 65:419–23.
9. Kehrl JH, Fauci AS. The clinical use of glucocorticoids. Ann Allergy 1983; 50:2–8.
10. Leach CS. Biochemical and hematologic changes after short-term space flight. Microgravity Q 1992; 2:69–75.
11. Leach CS, Alfrey CP, Suki WN, et al. Regulation of body fluid compartments during short-term spaceflight. J Appl Physiol 1996; 81:105–16.
12. Meehan R, Whitson P, Sams C. The role of psychoneuroendocrine factors on spaceflight-induced immunological alterations. J Leukoc Biol 1993; 54:236–44.
13. Meehan RT, Neale LS, Kraus ET, et al. Alteration in human mononuclear leucocytes following space flight. Immunology 1992; 76:491–7.
14. Mehta SK, Stowe RP, Feiveson AH, et al. Reactivation and shedding of cytomegalovirus in astronauts during spaceflight. J Infect Dis 2000; 182:1761–4.
15. Mills PJ, Meck JV, Waters WW, et al. Peripheral leukocyte subpopulations and catecholamine levels in astronauts as a function of mission duration. Psychosom Med 2001; 63:886–90.
16. Payne DA, Mehta SK, Tyring SK, et al. Incidence of Epstein-Barr virus in astronaut saliva during spaceflight. Aviat Space Environ Med 1999; 70:1211–3.
17. Ramierz F, Fowell DJ, Puklavec M, et al. Glucocorticoids promote a TH2 cytokine response by CD4+ T cells in vitro. J Immunol 1996; 156:2406–12.
18. Sonnenfeld G. Immune responses in space flight. Int J Sports Med 1998; 19(Suppl 3):S195–202; discussion S-4.
19. Stein TP. Nutrition and muscle loss in humans during spaceflight. Adv Space Biol Med 1999; 7:49–97.
20. Stowe RP, Mehta SK, Ferrando AA, et al. Immune responses and latent herpesvirus reactivation in spaceflight. Aviat Space Environ Med 2001; 72:884–91.
21. Stowe RP, Pierson DL, Barrett AD. Elevated stress hormone levels relate to Epstein-Barr virus reactivation in astronauts. Psychosom Med 2001; 63:891–5.
22. Stowe RP, Sams CF, Mehta SK, et al. Leukocyte subsets and neutrophil function after short-term spaceflight. J Leukoc Biol 1999; 65:179–86.
23. Taylor GR. Immune changes during short-duration missions. J Leukoc Biol 1993; 54:202–8.
24. Taylor GR, Konstantinova I, Sonnenfeld G, Jennings R. Changes in the immune system during and after spaceflight. Adv Space Biol Med 1997; 6:1–32.
25. Taylor GR, Neale LS, Dardano JR. Immunological analyses of U.S. Space Shuttle crewmembers. Aviat Space Environ Med 1986; 57:213–7.
26. Zieg G, Lack G, Harbeck RJ, et al. In vivo effects of glucocorticoids on IgE production. J Allergy Clin Immunol 1994; 94:222–30.