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Decreases in thymopoiesis of astronauts returning from space flight

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Following the advent of molecular assays that measure T cell receptor excision circles (TRECs) present in recent thymic emigrants, it has been conclusively shown that thymopoiesis persists in most adults, but that functional output decreases with age, influencing the maintenance of a diverse and functional T cell receptor (TCR) repertoire. Space flight has been shown to result in a variety of phenotypic and functional changes in human T cells and in the reactivation of latent viruses. While space flight has been shown to influence thymic architecture in rodents, thymopoiesis has not previously been assessed in astronauts. Here, we assessed thymopoiesis longitudinally over a 1-year period prior to and after long-term space flight (median duration, 184 days) in 16 astronauts. While preflight assessments of thymopoiesis remained quite stable in individual astronauts, we detected significant suppression of thymopoiesis in all subjects upon return from space flight. We also found significant increases in urine and plasma levels of endogenous glucocorticoids coincident with the suppression of thymopoiesis. The glucocorticoid induction and thymopoiesis suppression were transient, and they normalized shortly after return to Earth. This is the first report to our knowledge to prospectively demonstrate a significant change in thymopoiesis in healthy individuals in association with a defined physiologic emotional and physical stress event. These results suggest that suppression of thymopoiesis has the potential to influence the maintenance of the TCR repertoire during extended space travel. Further studies of thymopoiesis and endogenous glucocorticoids in other stress states, including illness, are warranted.

Introduction

The thymus is responsible for production of the diverse repertoire of naive T cells that are critical for effective adaptive immunity (1–4). Naive T cells are continuously produced by the thymus and exported to the periphery where they expand in response to homeostatic signals or antigen exposure. Maintenance of this naive T cell receptor (TCR) repertoire is essential for control of endogenous pathogens, including latent herpesviruses, and other opportunistic viral and fungal pathogens. The thymus is also critical to the neogenesis of regulatory T cells essential for maintenance of peripheral tolerance (5–7). The diversity of the naive T cell pool is diminished by a decrease in thymic activity and/or persistent egress of naive T cells into the peripheral memory pool following antigenic stimulation.

Thymic involution occurs after adolescence, and, until recently, the thymus was thought to be practically vestigial in adults. Following the advent of molecular assays that measure TCR excision circles (TRECs) present in recent thymic emigrants (RTE), it has been conclusively shown that the thymus remains active in most adults, although functional output decreases with age (8, 9). The molecular data demonstrate that persistence of thymopoiesis in healthy individuals is essential to maintenance of TCR repertoire diversity (1). Multiple clinical settings, including primary immunodeficiency states, HIV-1 infection (1, 10–12), and that after allogeneic hematopoietic stem cell transplantation (13–16), are characterized by deficiencies in human thymopoiesis and are associated with an increased risk of death due to infections normally controlled by a broad TCR repertoire. The recovery of thymopoiesis following chemotherapy and therapy of infection-related immunodeficiency is age dependent and governs diverse and functional TCR reconstitution (11, 13–15).

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Table 1. Subject characteristics

No. of subjects	16
Male	14
Female	2
Mean age in years (range)	48.6 (42–55)
Mean days in space (range)	182.6 (152–215)
Median days in space	184

Dexamethasone has been shown in model systems to diminish the numbers of thymus-derived naive T cells, which is followed by a rapid reseeding of the periphery after discontinuation of glucocorticoid therapy (22). In vitro, immature thymocytes have been shown to be susceptible to dexamethasone, resulting in apoptosis and a subsequent reduction in T cells (23).

Space travel exposes astronauts to factors including microgravity, solar and cosmic radiation, and other significant stressors. Immune system dysregulation has been documented in astronauts during and after space flight (24–28). Space flight has been shown to result in suppression of antigen-specific T cell function, altered T cell memory subset distribution, altered cytokine production profiles, decreased delayed-type hypersensitivity, and herpesvirus reactivation (29, 30). Physical and psychological stress (i.e., launch and landing stressors, microgravity, confinement, separation from family, and sleep deprivation) mediate these changes, presumably via activation of the hypothalamic-pituitary-adrenal axis. Accordingly, elevated levels of cortisol have been observed during and after spaceflight. Notably, glucocorticoids affect the immune system by altering leukocyte trafficking and migration as well as directly inhibiting cellular functions.

In prior studies of astronauts during short-term space shuttle flight, we found that increased endogenous glucocorticoid levels were associated with reactivation of CMV (29); similarly, we found that CMV reactivation occurring during glucocorticoid therapy after stem cell transplantation for graft-versus-host disease was associated with dysfunction, rather than lower numbers, of CMV-specific T cells (31). In addition to the documented suppression of thymopoiesis and functional pathogen-specific T cells, corticosteroid administration rapidly induces neutrophilia, with attendant relative, but not absolute, decreases in peripheral blood lymphocyte numbers (32).

We hypothesized that astronauts returning from long-term spaceflight aboard the International Space Station (ISS) would be exposed to emotional and physical stress, leading to increased production of endogenous glucocorticoids. We further hypothesized that stress-associated dysregulation of the neurologic-endocrine-immunologic axis would adversely affect human thymopoiesis. We tested these hypotheses in a prospective study wherein we measured RTE within peripheral blood mononuclear cells (PBMCs) of astronauts returning from flight aboard the ISS and performed parallel assessments of stress-associated glucocorticoids.

Results

Thymopoiesis, measured by assessment of TRECs, declines following return from space flight. Thymopoiesis was measured at 3 time points beginning 180 days before scheduled launch by analysis of TRECs known to be present in RTE (13). Baseline thymopoiesis varied significantly ($P < 0.001$) within the group, consistent with prior studies in healthy adults, but remained relatively stable within individuals prior to launch (Figure 1). Astronauts remained in space for variable periods, with a median duration of 184 days, and no sampling was possible during this interval. Upon return, samples were taken within 2 to 4 hours of landing and thymopoiesis was assessed within purified mononuclear cells, given the potential occurrence of stress-associated neutrophilia. In all 16 astronauts studied, thymopoiesis was lower at the time of return than during any measured interval prior to flight (graphical representation of original data in Figure 1 and Table 1 as Log_{10} -transformed data). Mean levels declined from a baseline of 3,155 to 1,734 TRECs/million PBMCs ($P < 0.001$), corresponding to a 45% decrease in the output of RTE from starting levels.

Following the consistent and significantly diminished thymopoiesis observed at landing, thymic output began a return to preflight levels within days to weeks of return to Earth and normalized to the preflight range, remaining there through the 180-day postflight observation period. Thymopoiesis rebounded from

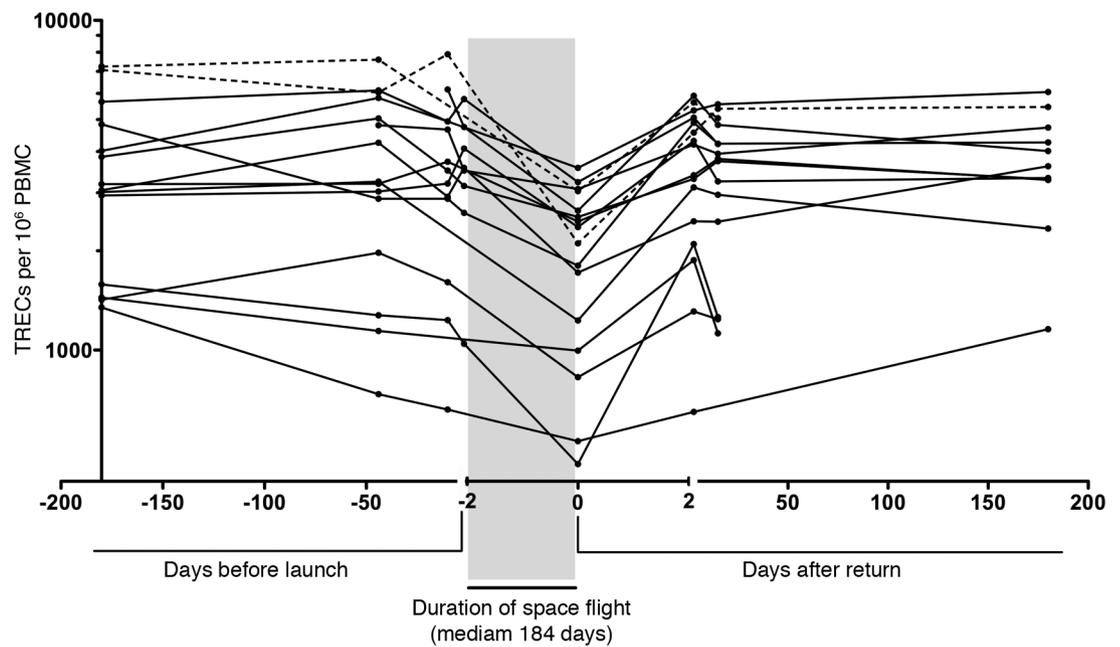


Figure 1. Thymopoiesis of 16 astronauts during a 1-year period, during which astronauts had an International Space Station (ISS) mission. The gray box indicates time astronauts spent in space (median of 184 days, with a range of 152–215 days). The dashed lines represent female astronauts. Discontinuous breaks in the x axis reflect change in scale for clarity.

nadir at the time of return to 2,825 TRECs/million PBMCs ($P < 0.001$) postflight, and the difference between the baseline and postflight TREC levels was not significantly different ($P = 0.12$) (Table 2). Results were consistent across the entire group of ISS astronauts, and the relative decline in thymopoiesis appeared to be independent of the baseline values. These results demonstrate that thymic function is altered in ISS astronauts and suggest that T cell homeostasis may be impaired in long-duration space flight.

Suppression of thymopoiesis is inversely correlated with a transient rise in cortisol levels. In chickens, in which thymocyte production may be measured directly using a specific surface marker, administration of endogenous glucocorticoids has a profound but reversible effect on thymopoiesis (22). We sought to determine whether alterations in endogenous glucocorticoids, known to be elevated in human stress, were similarly associated with changes in thymopoiesis. Because plasma levels of cortisol vary diurnally, we also assessed cortisol in a 24-hour urine collection for confirmation. We found that the transient decline in thymopoiesis was associated temporally with a transient rise in both plasma and urinary cortisol at return to Earth (Table 2 and graphical representation in Figure 2). In 15 of 16 astronauts, cortisol levels were strongly and inversely correlated to preflight to postflight changes in thymopoiesis (Table 2). In parallel to the significant declines in thymopoiesis seen at the time of return to Earth ($P < 0.001$), urine cortisol levels were significantly increased at return (36.2 to 73.5 $\mu\text{g}/\text{d}$; $P = 0.004$), with no differences between levels at baseline versus those after space flight ($P = 0.43$). Furthermore, stress responses in long-duration ISS astronauts were greater than those observed in shuttle astronauts spending relatively short amounts of time in space (28).

Discussion

This study is the first investigation to our knowledge of the effects of space travel on thymopoiesis in human astronauts. In 16 astronauts followed prospectively over a 1-year period, during which astronauts experienced space flight, we found consistent and significant decreases in thymopoiesis in all subjects upon return from space. Our results demonstrate that the stresses of prolonged space flight result in a significant ($P < 0.001$) decrease in T cell production, with a median decrease of 45% from baseline. To our knowledge, this is the first report to prospectively demonstrate a significant change in thymopoiesis in any group of healthy individuals in association with a defined and consistent event characterized by profound physiologic stress.

Prior avian studies demonstrated that administration of dexamethasone reversibly suppressed thymopoiesis and transiently diminished thymus-derived naive T cells with rapid T cell reseeding of the periphery

Table 2. Statistical summary of TREC and cortisol levels

	TREC		Urine cortisol		Plasma cortisol	
	Mean	SEM	Mean	SEM	Mean	SEM
Preflight	3.50	0.071	1.56	0.097	1.29	0.046
Return	3.24	0.068	1.87	0.12	1.42	0.073
Postflight	3.45	0.072	1.64	0.080	1.17	0.031
Overall mean difference <i>P</i> value	<0.0001		0.0049		0.0001	
Difference preflight vs. return	-0.26	0.032	0.31	0.078	0.14	0.072
Adjusted <i>P</i> value	<0.0001		0.0036		0.18	
Difference return vs. postflight	-0.21	0.030	0.23	0.077	0.25	0.066
Adjusted <i>P</i> value	<0.0001		0.027		0.0049	
Difference preflight vs. postflight	-0.023	0.023	0.077	0.061	-0.12	0.026
Adjusted <i>P</i> value	0.12		0.43		0.0013	

Log₁₀-transformed data with adjusted *P* value by Tukey's method under an ANOVA for single factor with repeated measures are shown. Cortisol and TREC data are displayed graphically in Figure 2, while untransformed TREC data displayed graphically in Figure 1.

following discontinuation of glucocorticoid therapy (22). In the present study, plasma and urinary cortisol were highly elevated in ISS crew members (50%–200% greater than Shuttle crew members) immediately after space flight. Our results demonstrate that stress-induced increases in endogenous corticosteroids are strongly associated with reversible suppression of thymopoiesis and confirm that the thymus exhibits plasticity of function in healthy individuals subjected to stress. Indeed, the kinetics of recovery were quite rapid and consistent with timing observed in the avian model, following cessation of supraphysiologic dosing of dexamethasone, a particularly potent glucocorticoid (22). The rapid return to baseline thymopoiesis raises the possibility that effects on thymic export, rather than suppression of production, cannot be ruled out as the cause of the decreases in peripheral RTE observed. While the rapid recovery of thymopoiesis observed was surprising, prior avian studies did reveal similar kinetics following the withdrawal of exogenously administered glucocorticoids. Future studies should examine effects of space travel on both thymic production and export, including the role of KLF2/S1P₁ in the emigration of thymocytes as it relates to the physiological stress of astronauts (33).

Studies have tried to explore the effect of microgravity on T cell development by utilizing microgravity organ culture systems (34). A block in T cell development was observed in mouse fetal thymus cultures subjected to vector-averaged gravity with a clinostat (35). This block occurs between the pre-T cell and double-positive T cell stage. Although in these systems there is an absence of stress steroids that are known to influence thymic function, as discussed above in the avian model. Additionally, mRNA changes, as detected by microarray, in thymus tissue taken from mice spending 13 days aboard a space shuttle demonstrate that there is alteration in gene expression occurring as a result of combination microgravity and increased stress. These alterations include genes that are involved in regulation of stress, glucocorticoid receptor metabolism, and T cell signaling activity (36). More recently, there is evidence that hypergravity has an effect on the TCRβ diversity, demonstrating an 85% difference in TCRβ repertoire in mice born under increased gravitation force (37).

Our prior studies (27, 29) demonstrated that elevated catecholamines at mission return reflected activation of hypothalamic-pituitary-adrenal and sympathetic-medullary-adrenal axes. While the mechanisms underlying changes of thymic output are incompletely understood, it is well known that developing thymocytes, and double-positive cells in particular, are sensitive to glucocorticoid activation of caspases, leading to apoptosis (38). These results demonstrate that stress-related changes in corticosteroids not only inhibit mature T cell function, but also are associated with suppression of thymopoiesis, which is essential for the maintenance of the naive TCR repertoire. Additionally, while the thymus is known to be sensitive to radiation exposure, the astronauts in this study remained in low Earth orbit and were not subjected to deep space radiation. Recently, NASA has implemented a radiation biodosimetry program. Early data suggests that ISS astronauts (similar space duration of 180 days) received doses of approximately 81 milligray equivalent, which is well below the established NASA limit of 250 milligray equivalent per 30 days (39). Future studies will include radiation dose received.

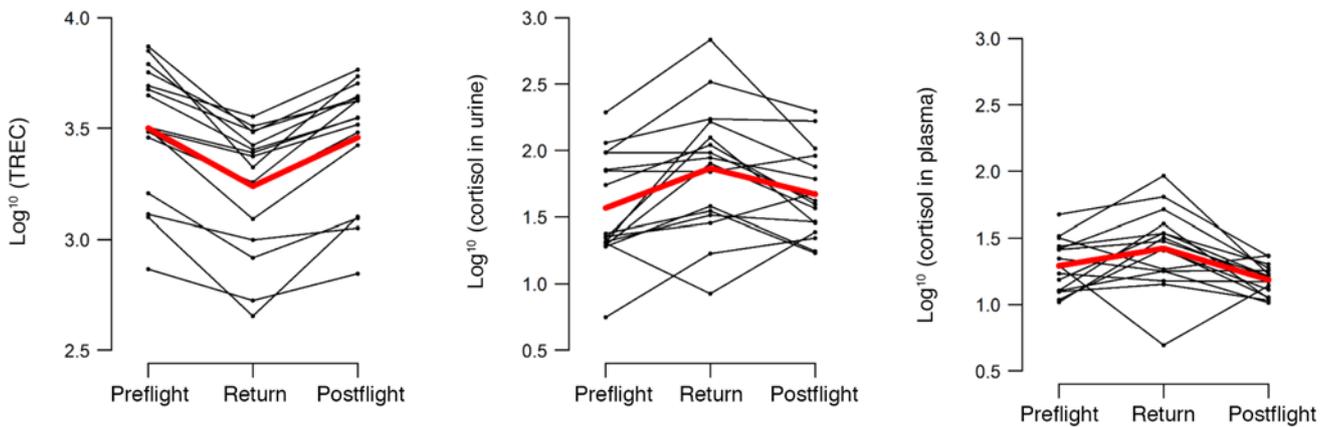


Figure 2. Thymopoiesis, as measured by TREC analysis, and cortisol in urine and plasma are summarized for the 3 intervals (preflight, return, and postflight). Mean preflight and mean postflight data are presented for each astronaut in black, and the mean across all 16 subjects is displayed in red. For each endpoint, an ANOVA for single factor with repeated measures using SAS mixed procedure, followed by pairwise means comparison with *P* value adjustment for multiple comparisons using Tukey's method was performed. A 2-sided *P* value less than 0.05 was considered significant.

These data add to a growing body of literature demonstrating immune system dysregulation in crew members and demonstrate that suppression of thymopoiesis consistently occurs following space flight. Future studies with greater numbers of subjects and analyses of samples obtained during space flight would enhance the breadth of understanding of mechanisms contributing to the observations presented here, including the influence of circadian effects, which are not clearly understood. The present data, while compelling, cannot definitively establish whether suppression of thymopoiesis occurs while in microgravity or due to physiologic stress associated with return to Earth. Our data raise concerns that peripheral effects and central effects leading to suppression of thymopoiesis may together significantly increase the risk of infection during long-term space flight, as would be necessary for interplanetary travel. Additionally, loss of thymic function may be associated with increased risk of autoimmunity due to increased homeostatic proliferation or loss of regulatory T cells following lymphocyte depletion (40, 41).

We acknowledge limitations in our study, which was constrained by the unique circumstances of space travel. Although we found consistent declines in thymopoiesis and glucocorticoid levels upon landing, we were unable to obtain samples during space flight to determine whether changes were associated with progressive time in space and/or caused by the stress and physiologic changes associated with reentry to the atmosphere. Additionally, the amount of blood we could sample was limited in this initial analysis, precluding a more detailed flow cytometric analysis of phenotypic markers of naive and memory T cells or putative surface markers of RTE. This also limited us from assessing whether increases in glucocorticoid levels induced dysfunction of antigen-specific T cells, as we previously demonstrated in CMV-specific T cells following the use of therapeutic glucocorticoids in stem cell transplantation (31). Despite the considerable logistic limitations, these findings suggest that expanded analyses of both in-flight and return samples should be conducted in future studies inspired by the present analysis.

Our results suggest that comprehensive health consequences for astronauts on exploration missions spending significant time in space deserve study, particularly as the control of endogenous pathogens, including the human herpesviruses, could be impaired due to the effects of space flight on the thymus. These alterations are particularly remarkable since astronauts are exceptionally healthy individuals due to the rigorous process associated with their selection for duty. It is notable that astronauts demonstrate significant decreases in thymic output and immune system dysregulation, despite anticipation and exceptional training for the physical and mental stressors they will face during space flight. Finally, the restoration of thymopoiesis is indicative of the plasticity of the central lymphoid compartment in individuals following suspension of the stress state. These data provide a foundation for future studies in normal thymic function in astronauts and in the setting of acute and chronic illness.

Methods

Subjects/cells. Peripheral blood samples were collected from 16 astronauts in the morning (7–8 am) starting at 180 days prior to launch (L–180) and continuing at defined intervals until 180 days after return (R+180) at Johnson Space Center, aside from those taken on R+0 and R+3, which were collected at Kennedy Space Center or Star City, Russia. The precise date of sampling varied on some crew members due to training schedules and other logistical considerations, and not every sample was collected from each subject. There was an average of 6.8 samples taken total per astronaut (range 5–8). However, a landing day sample was collected from all subjects (28). Sample size was limited by the number of astronauts traveling on ISS missions during this time period. PBMCs were isolated within 1 hour of collection by density-gradient centrifugation, frozen at less than -80°C , and held in liquid nitrogen until time of analysis. DNA was extracted from the PBMCs and resuspended in water at 35 μl per million PBMCs lysed.

Plasma were obtained after centrifugation and stored at -70°C until testing. Twenty-four-hour urine collections took place in parallel with every blood draw; aliquots of twenty-four-hour pools were stored at -20°C until analysis.

Measurement of stress hormones. The measurement of immunomodulatory hormones has been previously described (28). Urinary cortisol was measured by radioimmunoassay. Plasma cortisol was measured using commercially available kits (Cortisol ELISA kit (cat. # 11-CORHU-E01), Alpco Diagnostics). Samples were batch analyzed to minimize interassay variation.

Real-time PCR. Thymic function was assessed by amplification and quantification of the TCR δ -deletion TRECs using a Bio-Rad QFX96 Real-Time PCR machine. Briefly, DNA was amplified in 2X iQ Supermix (with additional MgCl_2 to a final concentration of 3.5 mM), and fluorescent probes were used to measure the reaction. Primers for the TREC sequence were 5'-CCCTTCAACCATGCTGACAC-3' (forward) and 5'-GGGTGCAGGTGCCTATGC-3' (reverse), which produced an 80-bp fragment that was detected with the probe 5'-FAM-TCTGGTTTTTGTAAAGGTGCCACTCCTG-BHQ-1-3'. TREC quantification was normalized for cell input by parallel quantification of the human B-globin gene. The primers for human B-globin were 5'-GAAGAGCCAAGGACAGGTACG-3' (forward) and 5'-CCTGGGAGTAGATTGGCCAA-3' (reverse), which produced an 85-bp fragment that was detected by the probe 5'-FAM-CTGTCATCACT-TAGACCTCACCTGTG-BHQ-1-3'. All standards were run in duplicate, and samples were run in triplicate.

Statistics. Statistical analyses were performed by the Biostatistics and Bioinformatics Core at Sylvester Cancer Center. Unequal spaced repeated-measures analysis was performed for TREC values. End-point analysis was performed on the TREC and cortisol levels in urine and plasma with an ANOVA for single factor with repeated measures using the SAS mixed procedure, followed by pairwise means comparison with *P* value adjustment for multiple comparisons using Tukey's method. Additionally, paired Student's *t* test was used to compare preflight versus postflight data. For all comparisons, 2-sided *P* values below 0.05 were considered significant. Analyses were conducted in SAS 9.3 and R version 15.0.

Study approval. The study protocol was approved in advance by the Committee for the Protection of Human Subjects at Johnson Space Center and by the Institutional Review Board of The University of Texas Medical Branch. Each subject provided written informed consent before participating.

Author contributions

CLB designed/performed experiments, analyzed/interpreted the data, and wrote the manuscript. RPS conceived/designed the experiment, collected samples, interpreted data, and reviewed/edited the manuscript. LSJ performed preliminary experiments and reviewed/edited the manuscript. CFS, SKM, BEC, and DLP collected/processed samples, performed cortisol experiments, and reviewed/edited the manuscript. KVK designed the experiment, interpreted the data, and reviewed/edited the manuscript.

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