

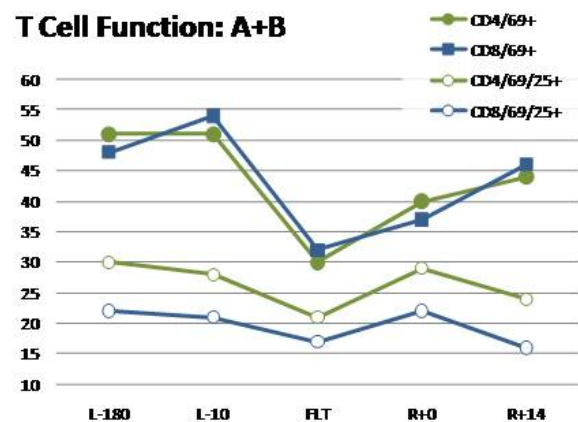
## Immune System Dysregulation, Viral Reactivation and Stress *During* Short-Duration Spaceflight

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**Background:** The objective of this NASA Short-Duration Bioastronautics Investigation (SDBI) was to assess spaceflight-associated immune dysregulation. Many previous studies have investigated this phenomenon post-flight, and found altered distribution and function of the peripheral leukocyte populations. Alterations in cytokine production profiles have also been reported. Unfortunately, post-flight data may be altered by the stress associated with high-G re-entry and readaptation to unit gravity following deconditioning. Therefore, the current study collected blood and saliva samples from crewmembers immediately before landing, and returned those samples to Earth for terrestrial analysis. Assays include peripheral comprehensive immunophenotype, T cell function, cytokine profiles, viral-specific immunity, latent viral reactivation (EBV, CMV, VZV), and stress hormone measurements. A total of 18 short duration crewmembers completed the study and the final data will be presented.

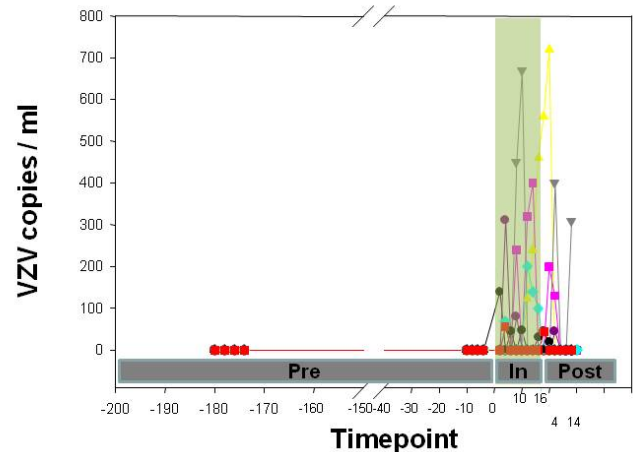
**General Immune Status:** The constitutive distribution of most peripheral leukocyte subset populations is largely unaltered during flight. Exceptions include a mild increase in levels of memory CD4+ T cells, and a decrease in naïve CD8+ T cells accompanied by a corresponding increase in central/effector memory CD8+ T cells. No increase in constitutively activated T cells was observed during flight. Various functional measurements were employed. Cytokine producing T cells (intracellular measurement; both CD4+/IL-2+ and CD8+/IFN $\gamma$ +) were mildly reduced during flight and further reduced upon landing. General T cell function (early blastogenesis response to mitogenic stimulation), yielded varying results. T cell stimulation with Staphylococcal enterotoxins was dramatically reduced in-flight, whereas T cell stimulation with anti-TCR antibodies was unchanged in-flight (and elevated post-flight). The post-flight elevation is in concurrence with previously published findings for that mitogen. Bulk secreted Th1/Th2 cytokines were measured following T cell activation, and mitogen-dependant in-flight reductions were observed in IFN $\gamma$ , IL-10, TNF $\alpha$  and IL-6 production. Secreted inflammatory cytokines levels were also measured following monocyte stimulation (LPS), however a flight-associated decrease was only observed for IL-10, whereas IL-8 levels were increased during flight.

**T Cell Function: A+B**



**Viral Specific Immunity:** The number of virus-specific CD8+ T-cells was measured using MHC tetramers, while their function was measured using intracellular cytokine analysis following peptide stimulation. Both the number and function of EBV-specific cells decreased during flight as compared to preflight levels. The number of CMV-specific T-cells generally increased as the mission progressed while their function was generally unaltered. Viral (EBV) load in blood was elevated postflight. Elevated anti-EBV VCA antibodies were evident in ~40% of the astronauts; anti-CMV antibodies generally increased during and after flight.

**Latent viral reactivation:** Samples collected from crewmembers before, during and after space flight were analyzed by the real time polymerase chain reaction (PCR) for the presence of virus. Epstein-Barr virus (EBV) and Varicella zoster virus (VZV) reactivation was measured in saliva, and Cytomegalovirus (CMV) was measured in urine. Higher levels of salivary EBV were found in during the flight-phase than before and after the flight. VZV was detected in about 50 % of the astronauts during and up to 5 days after space flight. No VZV was found in any preflight or control samples. There was also no CMV detected in any of the urine samples collected pre-flight from astronauts, however CMV was shed in 35 % the inflight samples and 30% of postflight urine samples of the crewmembers. The in-flight viral reactivation data may be summarized as follows: EBV (14/17 crewmembers), VZV (7/17 crewmembers) and CMV (8/17 crewmembers) occurred during flight.



**Physiological Stress:** Stress hormones were measured in plasma, urine and saliva before, during and after the spaceflight. There was generally a higher level of cortisol as measured in blood, urine and saliva in the astronauts during flight. Circadian rhythm of salivary cortisol was normal before and after flight in most of the astronauts, however, changes were observed during the flight phase.

**Conclusion:** Immune dysregulation, consisting of altered leukocyte distribution, diminished T cell function, and altered cytokine production profiles occurs during short duration spaceflight. This is accompanied by increases in the reactivation of latent herpesviruses. For some individual crewmembers, correlation may be observed between redistribution of CD8+ T cells, T cell function and latent virus reactivation. This is now defined as a legitimate in-flight condition, distinct from the stress of landing and readaptation. If this phenomenon were found to persist for the duration of exploration-class missions, it may represent specific clinical risks to crewmembers. These risks could include persistent infections due to reduced T cell function and altered bacterial virulence, hypersensitivities or autoimmunity due to persistent Th2 cytokine shifts, the consequences of persistent VZV reactivation (potentially shingles), and malignancies due to reduced NK cell function or persistent EBV reactivation. In such case, immune countermeasures development would be necessary to mitigate this risk.