Immune System Dysregulation and Herpesvirus Reactivation Persist during Long-Duration Spaceflight
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**BACKGROUND**

Immune system dysregulation occurs during spaceflight. It is currently unknown if this phenomenon persists during long duration flight. This may represent a clinical risk to crewmembers for exploration-class missions.

The current evidence base regarding spaceflight and immunity may be found in the NASA Human Research Program Evidence Book: http://humanresearch.nasa.gov/elements/immunoEvidenceBook.pdf. This phenomenon was also recently reviewed by Gueguenou et al.: *Lab Med* 2009 Nov;40(1):1027-38

This study, Integrated Immune (SMO-015), will address the following objectives:

- Determine the status of adaptive immunity, physiological stress, viral immunity, latent herpesvirus reactivation, in Astronauts during 6-month ISS missions
- Determine the clinical risk related to immune dysregulation for exploration class spaceflight.
- Determine an appropriate monitoring strategy for spaceflight-associated immune dysfunction, that could be used for the evaluation of countermeasures
- The anticipated n' for this study will be 17 subjects. For this presentation, mid-point study data are presented (n = 10).

**METHODS**

Blood and saliva samples were collected early, mid and late in-flight and returned for immediate analysis according to the following schedule. Functional assays were performed on ACD anticoagulated blood, which maintained viability for 48-72 hours until analysis. Specific mission dates could vary somewhat, as samples were required to be collected near a vehicle undocking for immediate sample return.

Specific assays were as follows:
- Leukocyte subsets
- T cell function
- Intracellular secreted cytokine profiles
- Plasma cytokine balance
- Leukocyte cytokine RNA
- Virus specific T cell number
- Virus specific T cell function
- Plasma stress hormones
- Latent herpesvirus reactivation (saliva/urine
- Saliva/urine stress hormones
- Circadian rhythm analysis

All methods used for this study were performed as previously described: *Aviation and Space Environmental Medicine, 2009 May; 80(5 Suppl): A17-44.

**RESULTS**

- Some shifts in leukocyte distribution occurred during flight (table 1): percentages of 8 cells, bulk memory T cells, and active cytotoxic CD8+ T cells were elevated; monocyte percentages were decreased. No increase in constitutively activated peripheral T cells was observed during spaceflight.
- General T cell function, defined as early MIP-1g expression in response to stimulation, was consistently reduced early in-flight following SEA-SEB stimulation (table 3, figure 2). Function following CD3/CD28 stimulation was generally unaltered.
- The percentage of IL-10 producing CD4+ T-cells were depressed early in-flight, and immediately upon landing (table 1). There was no alteration in the percentage of CD4+ T cells capable of secreting IFN-g.
- Persistent nitrogen-dependent reductions were observed in T cell bulk secretion of IFN-γ, IL-17a, IL-5, IL-10, TNFα and IL-6 (table 1). Monocyte production of IL-10 was reduced, whereas IL-8 levels were increased.
- Selected immune function parameters showed remarkably consistent deficiencies among most all subjects during flight (figure 1). Generally this dysregulation persisted for the entire 6-month mission.
- Levels of mRNA for the TNFα, IL-6 and IFN-γ were transiently elevated early in-flight, and the dynamics of TNFα and IL-6 gene expression were somewhat antagonistic to their corresponding receptors during flight (data not shown).
- 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 T-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 variable (figure 2).
- Viral (EBV) load in blood was elevated post-flight (data not shown).
- Anti-EBV VCA antibodies were significantly elevated by IgG; anti-EBV antibodies were not significantly elevated at landing; and anti-CMV antibodies were somewhat elevated during flight (figure 1).
- Higher levels of salivary EBV DNA were found during flight. EBV DNA reactivation occurred in ~37% of astronauts during flight, continuing for up to 30 days post-flight. CMV was shed in 33% of the in-flight and 30% of post-flight salivary samples of the crewmembers (figure 3).
- There was generally a higher level of cortisol as measured in urine and saliva in the astronauts during flight, but plasma cortisol was relatively unchanged during flight. Circadian rhythm of salivary cortisol was altered during flight (figure 4).

**CONCLUSIONS**

- Some alterations in adaptive immunity (leukocyte distribution, T cell function, cytokine production profiles) do not resolve during six month spaceflight.
- Spaceflight induces a broad functional deficiency, not restricted to expansion or contraction of specific cytokine-producing subsets. Spaceflight immunosuppression spams TH1, TH2 and TH17 profiles.
- Herpesvirus reactivation was generally found to persist during six month ISS flight in most crewmembers.
- Increased percentage of cytotoxic CD8+ T-cells may be associated with (attempted) control of virus reactivation. However, tetramer-specific T cell levels also decreased, and this discordant finding must be further investigated. The observed reduction in CD8+ T cell function is potentially associated with (in a cause-effect relationship), latent herpesvirus reactivation.
- Confirmation of these findings will require the full sample size to be completed. Upon study completion, specific adaptive immune system decrements will have been identified that are associated with spaceflight, and a monitoring strategy developed that may be used to validate potential countermeasures.
- Concurrent ground-analogue studies are underway, to identify an appropriate ground-analogue for spaceflight immune dysregulation (see SPIE/NSAC, NIMOD/underscope posters). A validated ground analogue would be an appropriate location to field-test immune countermeasures. A successful countermeasures development would mitigate this clinical risk for exploration class space missions.