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.jsc.nasa.gov/elements/smo/hrp_evidence_book.asp*. This phenomenon was also recently reviewed by Gueguinou et al: *J Leukoc Biol. 2009 Nov;86(5):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

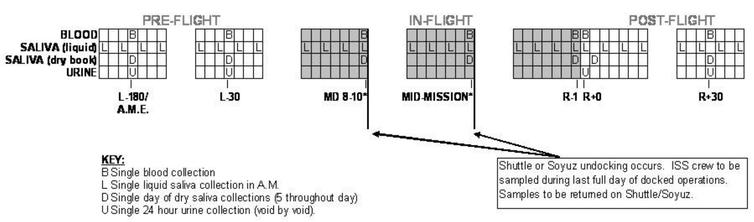
-Determine the clinical risk related to immune dysregulation for exploration class spaceflight.

-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.

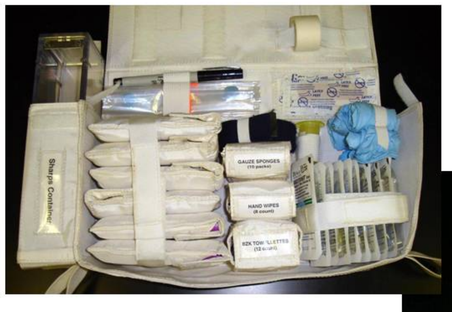


Specific assays were as follows:

-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): A37-44*.

Integrated Immune blood collection onboard the International Space Station



Integrated Immune on-orbit blood collection kit (top) and liquid/dry saliva collection kit (right)

Table 1: Mean general immune data, ISS, n=10 (red = consistent mission trend; * = statistically significant; p<0.05)

PERIPHERAL LEUKOCYTE SUBSETS

	L-180	L-45	Early	Mid	Late	R+0	R+30
WBC	4.6	4.9	5.4	4.9	4.9	7.6	4.7
Granulocytes	59	56	61	62	62	74*	59
Lymphocytes	33	36	32	32	30	19*	33
Monocytes	5.9	7.0	4.5	4.5*	3.9*	4.9*	7.1
T Cells	72	72	72	73	70	68	71
B Cells	8	9	14*	10	12*	14*	9
NK Cells	8	6	6	9	10	9	9
CD4+ T Cells	62	63	63	62	64	61	63
CD8+ T Cells	33	32	30	32	31	32	32
Bulk Memory CD4+	60	61	62	64	64	66*	60
Bulk Memory CD8+	43	43	46*	47	46	46	44
CD8+ Non-differentiated	47	46	43	46	44	42	42
CD8+ Active Cytotoxic	25	26	32	40*	38	40*	32
CD8+ Senescent	24	23	19	10*	15	15	20
CD8+ True Naïve	26	33	26	26	28	29	30
CD8+ Central memory	20	19	24	17	21	19	20
CD8+ Effector Memory	35	32	39	39	39	34	34
CD8+ Term Differentiated	17	16	13	19	17	19	20
CD4/CD69	1	1	6	3	2	2	3
CD8/CD69	2	2	6	4	5	4	3
CD4/HLA-DR	3	2	2	2	1	2	2
CD8/HLA-DR	7	4	4	3	3	3	4

INTRACELLULAR CYTOKINE PROFILES

	L-180	L-45	Early	Mid	Late	R+0	R+30
CD4/IL-2	48	47	39	48	49	35*	49
CD8/IFNg	26	34	27	32	29	33	29

T CELL FUNCTION

SEA+SEB 24hr

	L-180	L-45	Early	Mid	Late	R+0	R+30
CD4/69+	44	45	26*	36	34	44	45
CD8/69+	42	43	29*	32	35	49	43
CD4/69/25+	27	28	10*	17*	17*	29	29
CD8/69/25+	19	21	10*	13*	15	26	18

3H 24hr

	L-180	L-45	Early	Mid	Late	R+0	R+30
CD4/69+	54	49	50	52	49	53	44
CD8/69+	60	57	59	60	57	56	53
CD4/69/25+	39	37	27	32	30	36	32
CD8/69/25+	34	35	25	27	29	29	33

SECRETED CYTOKINE PROFILES

T cells: CD3+CD28--48hr

	L-180	L-45	Early	Mid	Late	R+0	R+30
IFNg	85	91	18*	34*	35	47*	89
TNFa	30	35	33	41	37	26	61
IL-10	27	32	4*	5*	6*	20	25
IL-4	1	1	0	0	0	1	1
IL-5	3	4	1*	1*	2	2	4
IL-2	7	20	23	14	13	5	21
IL-17a	2	2	0	0	0	1	2
IL-6	396	436	389	298	267	364	413

All leukocytes: PMA+Ionomycin--48hr

	L-180	L-45	Early	Mid	Late	R+0	R+30
IFNg	199	190	95*	118	153	162	202
TNFa	189	127	50*	40*	64*	97*	155
IL-10	17	22	1*	2*	4*	12	19
IL-4	8	7	1	2	3	4	9
IL-5	16	14	2*	4*	7	9	16
IL-2	701	607	459*	413	588	550	596
IL-17a	34	23	1*	1*	4*	3*	68
IL-6	246	221	71*	40*	30*	130*	232

Monocytes: LPS--48hr

	L-180	L-45	Early	Mid	Late	R+0	R+30
IL-12	0	0	0	0	0	0	0
TNFa	7	5	15	15	8	10	12
IL-10	19	25	7*	8*	11*	36	36
IL-6	256	257	268	233	279	297	305
IL-1b	99	97	41	104	28*	126	67
IL-8	315	301	389*	387*	432	345	349

Figure 1: General immunity, selected individual crewmember data

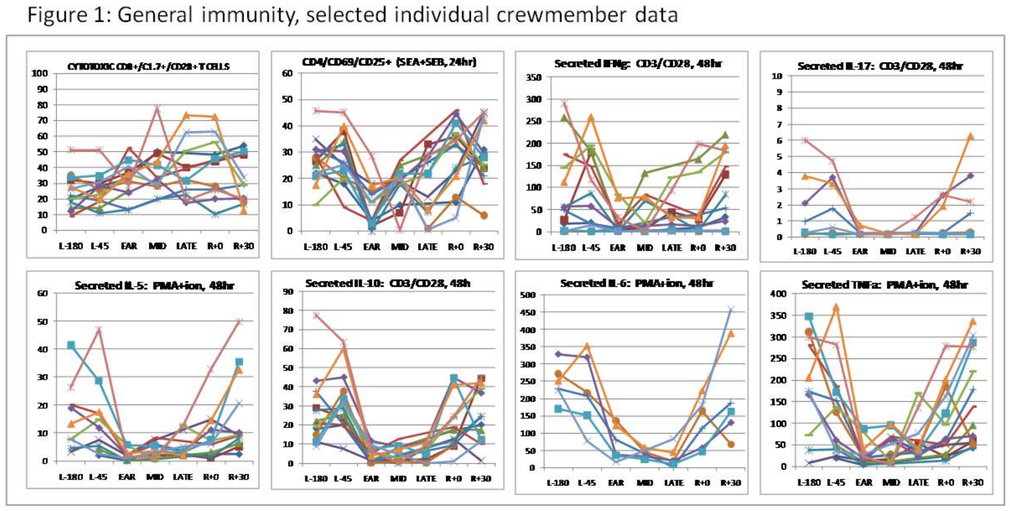


Figure 2: Mean virus-specific T cell number/function; mean viral antibody titers

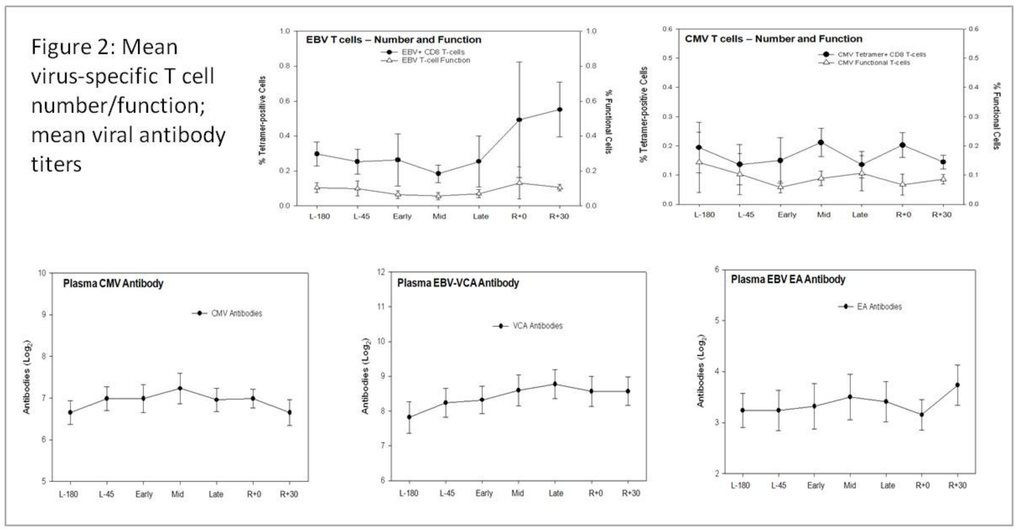


Figure 3: Latent herpesvirus reactivation; ISS crewmembers, n-10

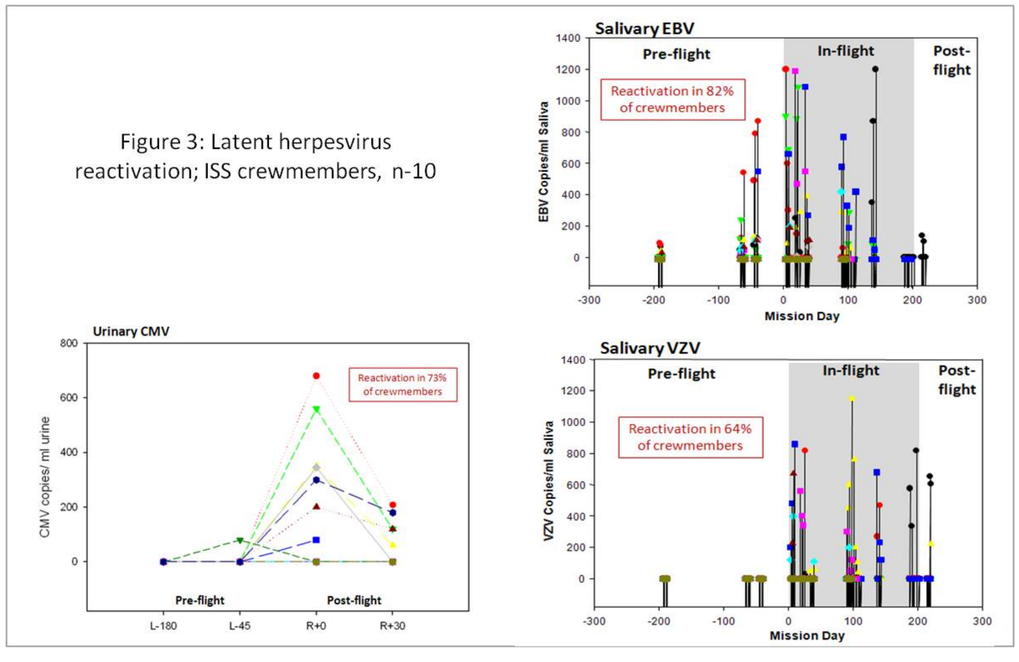
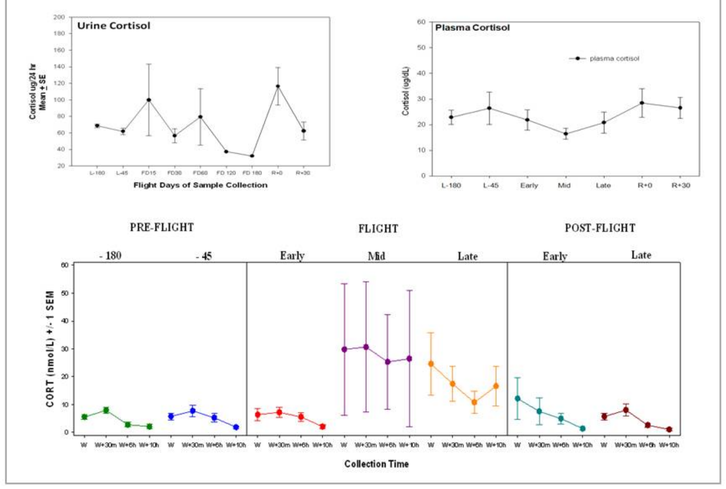


Figure 4: Mean plasma, urinary cortisol levels, mean salivary circadian rhythm of cortisol (4x daily saliva samples)



RESULTS

•Some shifts in leukocyte distribution occurred during flight (table 1): percentages of B 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 blastogenesis (CD69/25 expression in response to stimulation), was consistently reduced early in-flight following SEA+SEB stimulation (table 1, figure 1). Function following CD3/CD28 stimulation was generally unaltered.

•The percentage of IL-2+ producing CD4+ T cells were depressed early in-flight, and immediately upon landing (table 1). There was no alteration in the percentage of CD8+ T cells capable of secreting IFNg.

•Persistent mitogen-dependant reductions were observed in T cell bulk secretion of IFNg, IL-17a, IL-5, IL-10, TNFa 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 TNFa, IL-6 and IFNg were transiently elevated early in-flight, and the dynamics of TNF 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 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 postflight (data not shown).

•Anti-EBV VCA antibodies were significantly elevated by R+0; anti-EA antibodies were not significantly elevated at landing; and anti-CMV antibodies were somewhat elevated during flight (figure 2).

•Higher levels of salivary EBV DNA were found during flight. VZV DNA reactivation occurred in ~37% of astronauts during flight, continuing for up to 30 days post-flight. CMV was shed in 35% the in-flight and 30% of postflight urine 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 spans 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-analog investigations are underway, to identify an appropriate ground-analog for spaceflight immune dysregulation (see CHOICE/Antarctica, NEEMO/undersea posters). A validated ground analog would be an appropriate location to field-test immune countermeasures. A successful countermeasures development would mitigate this clinical risk for exploration class space missions.