Stress Induced Immune Dysregulation: A Continuum spanning Antarctica Winterover, Spaceflight, and Terrestrial Patients

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INTRODUCTION

- Spaceflight is a unique environment characterized by stress, microgravity, isolation, circadian misalignment, and radiation exposure and impacts immune health.
- Planned long duration missions to Mars are a top priority for NASA and mitigating the negative health consequences of spaceflight is particularly important. Terrestrial analogs are a vital aspect of spaceflight research since data from astronauts is limited and it is costly to receive samples from ISS. The most relevant ground analog would include station lifestyle, stress, disrupted circadian rhythms and isolation.
- This analysis compares various aspects of immune dysregulation in astronauts during long-duration orbital spaceflight to ground-analogs. Astronaut data were also compared to a clinical immunodeficiency population, shingles patients, to help interpret clinical risks during deep space missions.
- A comprehensive evaluation was performed across hypoxic interior Antarctica, normoxic coastal Antarctica, HERA, and astronauts which included plasma and mitogen stimulated cytokine profiles, T-cell function, and peripheral leukocyte distribution. A cross platform analysis was then performed to define in-flight immune alterations, determine analog appropriateness, and interpret clinical risk.

A Continuum of Immune Dysregulation

METHODS

Blood samples were collected before, during and after mission ISS, Antarctica winterover, and HERA crewmembers. Whole blood was stained with monoclonal antibodies (CD45, CD3, CD4, CD8, CD14, CD19, CD56, CD69, etc.), lysed, and washed for phenotype analysis by flow cytometry. Whole blood was centrifuged and plasma removed, aliquoted, and frozen for later cytokine analysis. Peripheral blood mononuclear cells (PBMCs) were isolated using standard density gradient centrifugation techniques. Whole blood (100 uL) was used for cultures of HERA samples. Cultures were set up as described in **Table 1**.

Table 1: Cultures Set-up

Mitogen	Mitogen Concentration	Number of PBMCs	Incubation Time
SEA+SEB	10 µg SEA 10 µg SEB	1×10^6	24 hours
Anti-CD3/CD28	0.125 µg CD3 0.25 µg CD28	1×10^6	24 hours
LPS	10 µg	1×10^6	48 hours
Anti-CD3/CD28	0.125 µg CD3 0.25 µg CD28	1×10^6	48 hours
PMA+I	10 ng PMA 2 µg Ionomycin	1×10^6	48 hours

Supernatant was removed following a 24-hour incubation and cells were stained with monoclonal antibodies, CD3, CD4, CD8, CD25, and CD69. Samples from Concordia were then frozen with 10% DMSO and shipped back to NASA Johnson Space Center for analysis upon conclusion of the mission, ISS and HERA samples were analyzed immediately. The proportions of CD4+ and CD8+ T-cells expressing CD25 and CD69 were assessed using a Beckman Coulter Gallios flow cytometer.

Following the 48-hour incubations, supernatant was removed and frozen for subsequent batch analysis. The concentrations of 13 cytokines were measured in the supernatant using a commercially available magnetic multiplex assay (EMD Millipore) and a Luminex® MAGPIX® instrument.

Blood samples were collected from Shingles patients upon presentation, centrifuged, and plasma removed and frozen for subsequent batch analysis of cytokines.

Paired Student's *t* tests were performed comparing each mission day to the baseline sample, to determine if the in mission and post mission samples were significantly different ($p < 0.05$) from the baseline sample.

RESULTS

Peripheral Leukocyte Distribution

Figure 1

Figure 1. Whole blood was stained with monoclonal antibodies and analyzed by flow cytometry to determine leukocyte distribution. Concordia blood samples were frozen with 10% DMSO and shipped to NASA Johnson Space Center upon mission completion for processing and analysis.

Mitogen-Stimulated Cytokine Production

Figure 2

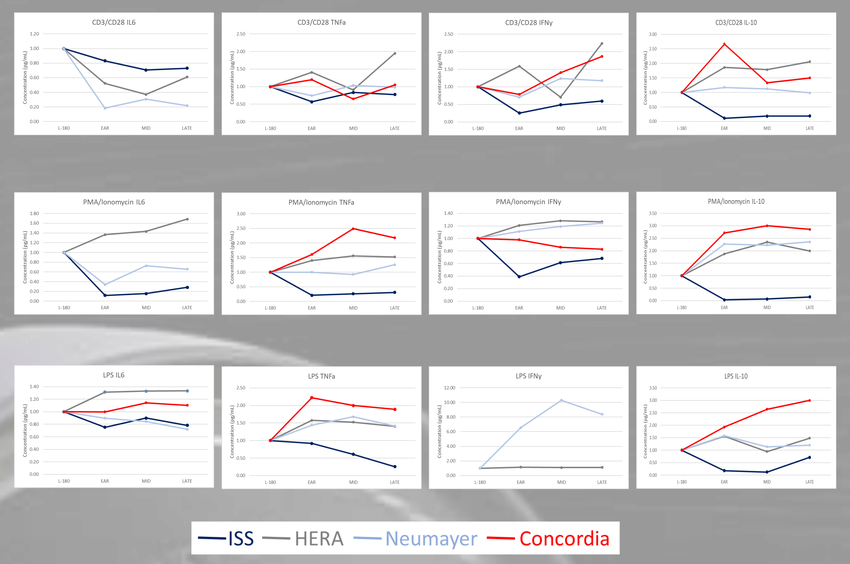

Figure 2. Secreted cytokine concentrations measured in culture supernatant following a 48-hour culture of PBMC or whole blood with the indicated mitogen. Samples were collected and processed before, during and after mission. Line graphs are presented as mean concentration (pg/mL) normalized to baseline

Plasma Cytokine Concentration

Figure 3

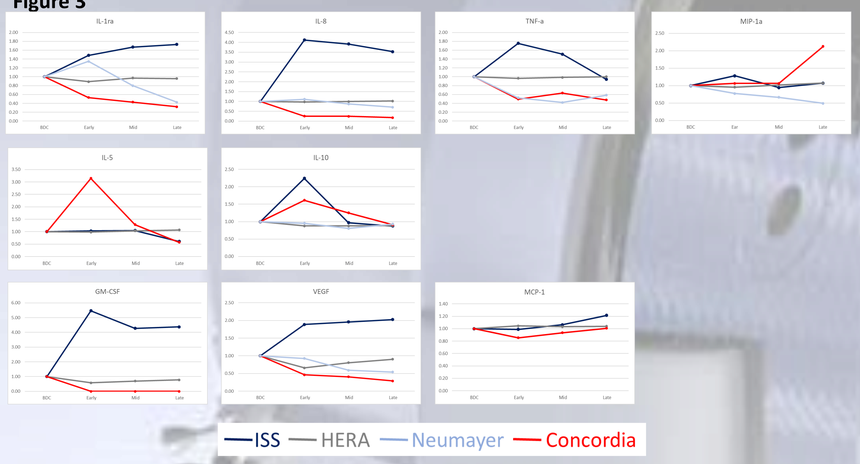

Figure 3. Plasma cytokine concentrations were measured before, during and after mission. Data in line graphs are presented as mean plasma concentration (pg/mL) normalized to baseline.

Terrestrial Patients

Figure 4

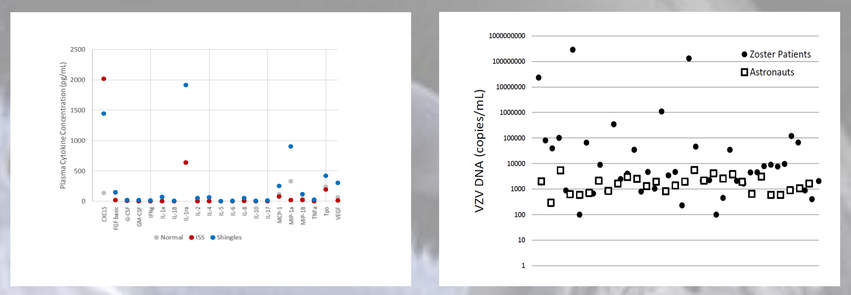

Figure 4. Plasma was collected from Shingles patients at presentation and cytokine analysis was performed. This data was then compared to plasma cytokine data from normal, healthy adults and ISS crewmembers at flight day 30.

T-Cell Activation

Figure 5

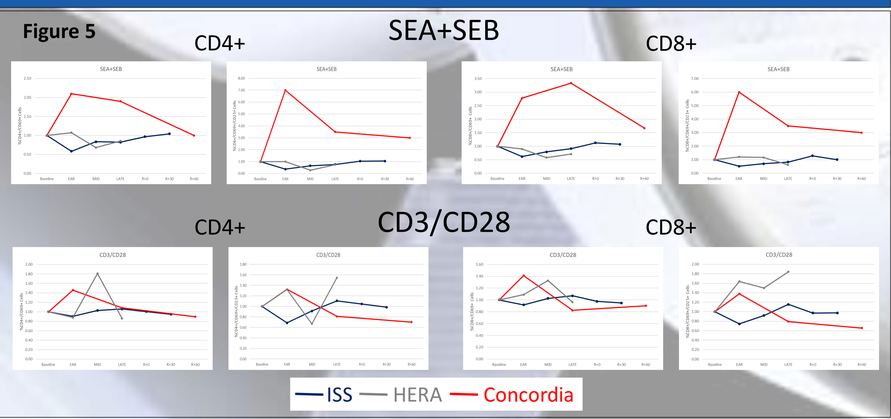

Figure 5. CD4+ and CD8+ T-cell early (CD69+) and late (CD69+/CD25+) activation following a 24-hour culture of PBMCs or whole blood with the indicated mitogen. Samples were processed before, during and after mission. Data are presented as mean.

DISCUSSION

- Astronauts manifest a distinct pattern of immune alterations, including relatively unaltered leukocyte distribution, reduced T/NK cell function, and increases in plasma cytokine concentrations leading to the reactivation of latent herpesviruses.
- Immune alterations during interior Antarctic winterover at Concordia station were dissimilar from spaceflight, likely due to hypobaric hypoxia. Normoxic winterover at Neumayer station, to date only cytokine data exist, appears more homologous to spaceflight.
- The Human Exploration Research Analog (HERA) at NASA Johnson Space Center shows the least similarities to spaceflight.
- The pattern is similar to that observed in shingles patients, but reduced in magnitude.
- Stress induced reductions in immunity can lead to clinical disease. This phenomenon may represent a continuum, where alterations in astronauts may represent more subtle variations which precede the development of disease. Antarctica data, at a magnitude between flight and disease, suggest that stress and circadian issues may be a primary contributor.