The Effects of Long-Duration Spaceflight on Salivary and Blood Markers of Innate Immunity

Science Symposium Presentation
PI: Richard J Simpson, PhD 05/30/2013

MTL#725 - Salivary Markers

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# Spaceflight and the Immune System

- Immune system dysregulation has been documented during and after spaceflight, but it is not known if these changes increase infection susceptibility or pose a significant health risk to crewmembers.

- Infectious episodes reported among crewmembers aboard the Mir station (i.e. conjunctivitis, acute respiratory and dental infections).

- Many microorganisms have been identified in space vehicles including *Escherichia coli*, *Salmonella enterica*, *Bacillus subtilis*, *Staphylococci*, *Micrococci*, and *Coryneform*.

- Crewmembers have been found to shed live Varicella Zoster Virus (VZV) and other latent viruses both in-flight and on return to Earth.

- Impaired immunity coupled with increased pathogen exposure could trigger a number of adverse events that could compromise crew safety and mission objectives.
The Effects of Spaceflight on Immunity

- Difficult to control for the many confounding factors known to impact the immune system
- Pre and post-flight changes perhaps due to the stressors associated with landing and re-adaptation to gravity
- Most in-flight immune data are limited to short-duration spaceflight missions
- Small subject numbers used for in-flight research, logistical difficulties associated with the transportation of specialized equipment to space and viable sample return
- Studies investigating the impact of spaceflight on the innate arm of the immune system are scarce

Important to determine if long-duration spaceflight impairs immunity before planetary exploration class missions can be considered
Virus Load Increases while Immune Cell Function Decreases during Spaceflight

CMV (cytomegalovirus) Reactivation after spaceflight

Few In-Flight data exist on immune function, particularly in relation to:

1. long duration spaceflight (i.e. ISS mission)
2. Innate host immune defense
POTENTIAL CONSEQUENCES OF PROLONGED IMMUNE DYSREGULATION DURING EXPLORATION-CLASS SPACEFLIGHT

• Increased incidence of infection
• Allergy/hypersensitivity reactions
• Consequences of viral reactivation
• Impaired tumor surveillance, increased incidence of malignancies
• Impaired wound healing

Any adverse clinical event related to immune dysfunction would likely have an immediate mission impact.
Recent Spaceflight Immune Studies

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Pre-Flight</th>
<th>In-Flight</th>
<th>Post-Flight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Russian Med-Ops/</td>
<td>L-60</td>
<td>L-10</td>
<td>R+0</td>
</tr>
<tr>
<td>CYTOKINE DSO-501 (ISS)</td>
<td></td>
<td></td>
<td>R+7</td>
</tr>
<tr>
<td>Latent Virus/</td>
<td>L-180</td>
<td>L-10</td>
<td>saliva</td>
</tr>
<tr>
<td>DSO-493 (SHUTTLE)</td>
<td></td>
<td>saliva</td>
<td>saliva</td>
</tr>
<tr>
<td>Immune Function/</td>
<td>L-10</td>
<td></td>
<td>R+0</td>
</tr>
<tr>
<td>DSO-498 (SHUTTLE)</td>
<td></td>
<td></td>
<td>R+7</td>
</tr>
<tr>
<td>Japan Immunology/</td>
<td>L-60</td>
<td>L-30</td>
<td>R+0</td>
</tr>
<tr>
<td>DSO-206 (SHUTTLE)</td>
<td></td>
<td></td>
<td>R+3 R+7 R+30 R+90</td>
</tr>
<tr>
<td>Epstein Barr/</td>
<td>L-120 L-65</td>
<td>L-10 L-3/2</td>
<td>R+0</td>
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<tr>
<td>DSO-500 (SHUTTLE)</td>
<td></td>
<td></td>
<td>R+3 R+7</td>
</tr>
<tr>
<td>Epstein Barr/</td>
<td>L-60 L-10</td>
<td>L-3/2</td>
<td>R+0</td>
</tr>
<tr>
<td>AME/ L-180 (ISS)</td>
<td></td>
<td></td>
<td>R+3 R+7</td>
</tr>
<tr>
<td>Immuno-ESA/</td>
<td>L-30</td>
<td>L+90 to L+120</td>
<td>R+1 R+7 R+28</td>
</tr>
<tr>
<td>(ISS/SOYUZ)</td>
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<tr>
<td>Integrated Immune/</td>
<td>AME**/ L-10**</td>
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<td>R-1 R+0** R+14</td>
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<tr>
<td>SDBI-1900 (SHUTTLE)</td>
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<tr>
<td>Integrated Immune/</td>
<td>AME**/ L-30**</td>
<td>MD 8-10</td>
<td>R-1 R+0** R+30**</td>
</tr>
<tr>
<td>SMO-015 (ISS)</td>
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</table>

Very few studies have obtained samples In-Flight. Integrated Immune was the first study to comprehensively examine immune system changes In-Flight, although this study focused mostly on adaptive immunity.
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**Salivary Markers Specific Aims:**

- **Project Goal:** This project will address the PRD Risk title: Risk of Adverse Health Event Due to Altered Immune Response and the IRP Gap – M1: Does spaceflight alter immune function?

- **Specific Aim#1:** Longitudinally examine the impact of long-term spaceflight (up to 6 months) on salivary and cellular markers of innate immune function and latent viral reactivation

- **Specific Aim#2:** Examine the relationship between changes in salivary and cellular markers of innate immune function and changes in other stressors associated with the spaceflight environment (i.e. circadian desynchronization, sleep loss/disruption, mood state disturbances, stress, infection incidence).
Objectives of Salivary Markers:

Address current lack of data regarding immune status *during* flight, particularly in relation to innate immunity

Determine the in-flight status of antimicrobial immunity, latent viral reactivation, cellular innate immune function and physiological stress

Delineate the long-term effects of spaceflight from the effects of acute stress on immune function

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

Determine the appropriate monitoring strategy for spaceflight-associated immune dysfunction, that could be used for the evaluation of countermeasures
Salivary Markers Specific Assays

Salivary Antimicrobial Proteins/peptides

<table>
<thead>
<tr>
<th>Salivary Peptide/Protein</th>
<th>Origin</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-defensins HNP 1-4</td>
<td>Neutrophils, gingival sulcus, salivary duct cells</td>
<td>Antibacterial, antifungal, antiviral</td>
</tr>
<tr>
<td>β-defensins (HBD-1-3)</td>
<td>Epithelia, salivary ducts</td>
<td>Antibacterial, antifungal, antiviral</td>
</tr>
<tr>
<td>cathelicidin (LL-37)</td>
<td>Neutrophils, gingival sulcus, salivary glands and ducts</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>Histatins</td>
<td>Salivary ducts/glands</td>
<td>Antifungal</td>
</tr>
<tr>
<td>lysozyme</td>
<td>Neutrophils, salivary ducts</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>lactoperoxidase</td>
<td>Salivary glands</td>
<td>Antibacterial</td>
</tr>
<tr>
<td>lactoferrin</td>
<td>Neutrophils, salivary glands</td>
<td>Antibacterial, antifungal, antiviral</td>
</tr>
<tr>
<td>Immunoglobulin A</td>
<td>Salivary glands</td>
<td>Immune cell activation</td>
</tr>
</tbody>
</table>

Latent Viral Reactivation:
- EBV (saliva)
- VZV (saliva)
- CMV (urine)

Antibacterial capacity of saliva:
- E. coli inhibition assay

Detailed leukocyte phenotype assays:
- T-cells
- NK-cells
- Monocytes
- B-cells
- Dendritic cells

Flow Cytometry

Cellular Functional assays:
- NK-cell cytotoxicity
- Neutrophil degranulation
- TLR ligand stimulations
- Mitogenic T-cell stimulations
Salivary Antimicrobial proteins

- Evolutionary conserved component of the innate immune system
- Subdivided into subgroups based on their amino acid composition and structure
- Generally between 12 and 80 amino acids
- Have been demonstrated to kill a broad range of pathogens, including Gram Negative and Gram positive bacteria, enveloped viruses, fungi and malignant cells.
- Concentration and secretion of specific SAPs can change in the presence of infection and may be predictive of immunocompromise
- e.g. low concentrations of lysozyme and lactoferrin in saliva have been linked with oral thrush (candidiasis), periodontitis (gingivitis) and rhinovirus
Latent Viral Reactivation

- There are 8 known herpesviruses that infect humans and establish lifelong latency
- Latent viral reactivation has consistently been observed among crewmembers before, during and after space flight
- Latent viral reactivation coupled with immunosuppression could be clinically relevant in crewmembers

<table>
<thead>
<tr>
<th>EBV copies/ml saliva</th>
<th>200-140</th>
<th>139-60</th>
<th>59-1</th>
<th>2-4</th>
<th>5-7</th>
<th>8-14</th>
<th>1-30</th>
<th>31-45</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV frequency: 16%</td>
<td></td>
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<tr>
<td>EBV copies: 417 ± 31/ml</td>
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<td>EBV frequency: 29%</td>
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<tr>
<td>EBV copies: 40 ± 2/ml</td>
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<tr>
<td>EBV frequency: 16%</td>
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<td>EBV copies: 44 ± 5/ml</td>
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</table>

- CMV: Mostly Asymptomatic
- VZV: Shingles
- EBV: Infectious Mononucleosis
- HSV: Cold Sores
Experiment Design Overview

**KEY:**
- B: Single blood collection (29ml) collected
- S: Saliva sampling (3x oral swab)
- U: Single 24h urine collection.
- P: Health assessment

* On FD10, only 19ml of blood will be collected and frozen
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Saliva Sampling

![Saliva Sampling Image]

21 saliva samples collected over a 7-day period (3/day, all in the morning)

Samples collected at:
- **Pre-flight:** L-180, L-60
- **In-flight:** early (FD-10), mid (FD-90), and late (R-1)
- **Post-flight:** R+0, R+18, R+33, R+63

Inert polymer cylindrical swab (10 mm x 30 mm). Soft and comfortable (doesn’t taste too bad either)

Placed under the tongue for a defined period of time (usually 2-minutes). Saliva is passively absorbed in the swab

One of the three daily saliva samples is a timed collection. This is so we can determine salivary flow rate
Blood Sampling

9 intravenous blood samples collected over the entire duration of the study

Samples collected at:
**Pre-flight**: L-180, L-60
**In-flight**: early (FD-10), mid (FD-90), and late (R-1)
**Post-flight**: R+0, R+18, R+33, R+63

Three blood tubes (total of 29ml) will be collected from a single needle stick at each of the 9 sampling schedules with the exception of FD when only 19ml of blood will be collected and frozen.
## Urine Sampling

9x 24h urine samples collected over the entire duration of the study

Samples collected at:
- **Pre-flight**: L-180, L-60
- **In-flight**: early (FD-10), mid (FD-90), and late (R-1)
- **Post-flight**: R+0, R+18, R+33, R+63

Scheduled in conjunction with the blood draws
**Health Assessment**

“pen and paper” test designed to monitor adverse medical events, impairments in wound healing, sleep quality and mood state

9 assessments will be made over the entire duration of the study:
- **Pre-flight**: L-180, L-60
- **In-flight**: early (FD-10), mid (FD-90), and late (R-1)
- **Post-flight**: R+0, R+18, R+33, R+63

Each assessment should take approximately 10-minutes to complete. Scheduled at the end of the 7-day saliva sampling period

Of particular concern are conditions related to immunology, such as allergies, rashes, hypersensitivities, infections and wound healing. Recording such events will allow us to correlate them to any observed changes in biological immune markers
Questions?

Laboratory Peer Pressure