PLASMA CYTOKINE LEVELS DURING LONG-DURATION SPACEFLIGHT

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Could spaceflight-associated immune system weakening preclude the expansion of human presence beyond Earth’s orbit?

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Evidence Book

Risk of Crew Adverse Health Event Due to Altered Immune Response

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Houston, Texas

*Integrated Immune* mid-study long duration data (n=10)

Immune dysregulation during long-duration spaceflight (SMO-015 mid-study data; n=10)

Survival of Human Naïve T Cells: L-180 L-45 Early Mid Late R+0 R+30

Cytotoxic CD8+ T cell function CD4+ T cell function CD8+

IL-10 (CD3/CD28) IL-6 (PMA-I) TNFα (PMA-I)

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Immune dysregulation during deep space missions has the capacity to synergize with other variables such as oxidative damage or radiation exposure. This would further enhance clinical risk to crewmembers.
**Cytokines: Th1/Th2**

**Th1** - Immunity to intracellular pathogens, viruses

*Normal Function*
- Cell Mediated ‘Inflammatory’ Response
- Fight intracellular pathogens (viruses)
- Control DTH response to skin viral/bacterial antigens
- Fight tumor formation
- Phagocyte dependent inflammation

*Disease correlations:*
- Rheumatoid arthritis
- Organ specific immune disorders
- Chohn’s disease
- Sarcoidosis
- Acute allograft rejection
- Unexplained recurrent abortions
- Multiple sclerosis

**Th2** - Antibody response to extracellular pathogens, parasites

*Normal Function*
- Humoral (Antibody) Responses
- ‘Anti-Inflammatory Response

*Disease correlations:*
- Rapid progression of HIV to AIDS
- Chronic graft vs. host disease
- Systemic autoimmune diseases
- Atopic asthma
- Scleroderma
- Serum lupus erythematosus
- Chronic allergies/sensitization
- Atopic dermatitis
<table>
<thead>
<tr>
<th>Environment</th>
<th>Response type</th>
<th>Products</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-12+IL-18</strong> (Monocytic Inf.)</td>
<td><strong>Th1</strong></td>
<td>IFNγ, IL-2, TNFα, LT</td>
<td>Purpose: CMI, DTH, intracellular pathogens. Pro-inflammatory, cause organ-specific auto-immunity.</td>
</tr>
<tr>
<td><strong>TGFB+IL-6</strong> (Neutrophil. Inf.)</td>
<td><strong>Th17</strong></td>
<td>IL-17α, IL-21, IL-22</td>
<td>Purpose: clear gut bacteria, other pathogens not handled by Th1/2 (citrobacter, k. pneumoniae, candida). Disease: arthritis, MS, psoriasis, EAE.</td>
</tr>
<tr>
<td><strong>IL-4+IL-2</strong> (Baso/Eo/Mast Inf.)</td>
<td><strong>Th2</strong></td>
<td>IL-4, IL-5 (IL-10, 9, 12)</td>
<td>Purpose: humoral immunity, extracellular organisms. Disease: allergy, atopy.</td>
</tr>
<tr>
<td><strong>TGFB + IL-4</strong></td>
<td><strong>Th9</strong></td>
<td>IL-9, IL-10</td>
<td>IL-9 stimulates proliferation, prevents apoptosis. Effector subset (not regulatory subset). Subset of Th2? Plastic, can switch to Th1 or Th17.</td>
</tr>
<tr>
<td><strong>TGFB</strong></td>
<td><strong>Treg</strong></td>
<td>TGFB</td>
<td>Natural Tregs control inflammation, secrete anti-inflammatory cytokines. Reduced Treg function associated with many autoimmune disorders. Express CD25, CD152, icFoxp3.</td>
</tr>
<tr>
<td><strong>TGFB+IL-27</strong></td>
<td><strong>Tr1</strong></td>
<td>IL-10, TGFB (IL-21 autoc)</td>
<td>Regulatory type 1 cell: potent immunosuppressive properties, do not express Foxp3. Main tolerance, control autoimmunity, prevent graft rejection, GVH disease.</td>
</tr>
<tr>
<td><strong>IL-21</strong></td>
<td><strong>Th3</strong></td>
<td>TGFB, IL-10</td>
<td>Th3 cells are involved in mucosal immunity. Mediate non-inflammatory environment. Promote switch to IgA (non-inflammatory, does not activate c', not involved with phagocytosis). Responsible for ‘oral tolerance’?</td>
</tr>
<tr>
<td></td>
<td><strong>Tfh</strong></td>
<td>IL-6, IL-10, IL-21</td>
<td>Follicular helper T cells. Regulate step-wise development of antigen-specific B cells in vivo. CXCR5⁺ Deployed to B cell zones of lymphoid tissues.</td>
</tr>
</tbody>
</table>
RADIATION
Immune cells generally susceptible to radiation damage. Peripheral T and B cells via apoptosis induction; and via lethal damage to marrow stem cells

BONE
Within the bone marrow cavity, cytokines produced by immune cells also have important effects on regulating bone homeostasis. RANKL, M-CSF, TNF, ILs, and IFNs, affect the differentiation and activity of osteoclasts and bone resorption. During chronic inflammation, the balance of bone modeling and remodeling can be greatly affected.

NEUROLOGY
A reciprocal flow of information and functional connection exists between the nervous and immune systems. Communication occurs via soluble mediators and cell-cell contacts.

MICROBIOLOGY
Host-pathogen interactions determine susceptibility to disease. Microbial virulence in conjunction with immune status determines the magnitude and outcome of infection

NUTRITION
Proper nutrition is a requirement for a normal immune response. Deficiencies in any of several dietary requirements have been linked to diminished immune function and/or clinical illness.

EXERCISE
Research is uncovering a link between moderate, regular exercise and a strong immune system. However, there is also evidence that too much intense exercise can reduce immunity and may even make you sick
Specific Study Objectives

• Determine the in-flight status of immunity, physiological stress, viral immunity/reactivation.

• Specific measurements include leukocyte distribution, T cell function, cytokine production profiles (mRNA, intracellular, secreted, plasma), virus-specific T cell number/function, latent herpesvirus reactivation, stress hormone levels.

• Determine the clinical risk related to immune dysregulation for exploration class spaceflight, as well as an appropriate monitoring strategy for spaceflight-associated immune dysfunction, that could be used for the evaluation of countermeasures.

Determine the nutritional status of astronauts before, during, and after spaceflight ensure adequate intake of energy, protein, and vitamins during missions.

The Clinical Nutritional Status Assessment measures dietary intake, body composition, protein, bone, iron, mineral, vitamin, and antioxidant status (60 total analytes). Currently, it is a medical requirement for U.S. crewmembers on-board the ISS.

The results of data analysis are used both to understand the connections between nutrition and human health during space flight, and to develop effective dietary strategies to reduce adverse health impacts (including bone loss, loss of important vitamins and minerals, and increased genetic damage from radiation).
SAMPLING SCHEDULE

Early
~2 weeks

FD15

FD30

FD60

Mid
2-4 mos

FD120

SMO-016

FD180

Late
R-1-2 days

SMO-018

FD150
Cytokine Categories

<table>
<thead>
<tr>
<th>INFLAMMATORY</th>
<th>ADAPTIVE/Th1</th>
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<tbody>
<tr>
<td>IL-1 alpha</td>
<td>IFN-gamma</td>
</tr>
<tr>
<td>IL-1 beta</td>
<td>IL-2</td>
</tr>
<tr>
<td>TNF-alpha</td>
<td>+/-</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
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<table>
<thead>
<tr>
<th>anti-INFLAMMATORY</th>
<th>ADAPTIVE/Th17</th>
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<tbody>
<tr>
<td>IL-1ra/IL-1F3</td>
<td>IL-17</td>
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</table>

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<tr>
<th>GROWTH FACTORS</th>
<th>CHEMOKINES</th>
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<tbody>
<tr>
<td>G-CSF</td>
<td>CXCL8/IL-8</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>CCL2/MCP-1</td>
</tr>
<tr>
<td>FGF basic</td>
<td>CCL3/MIP-1 alpha</td>
</tr>
<tr>
<td>Tpo</td>
<td>CCL4/MIP-1 beta</td>
</tr>
<tr>
<td>VEGF</td>
<td>CCL5/RANTES</td>
</tr>
</tbody>
</table>

For a 22-cytokine array, assuming a qualitative data, there are **4,194,304** possible outcomes.
Plasma Cytokine Data – Inflammatory Cytokines

**IL-1a**

**IL-1b**

**IL-6**

**TNFα**

**IL-1ra**
Plasma Cytokine Data – Adaptive Immunity

IFNg

IL-2

IL-17

IL-4

IL-5

IL-10
Plasma Cytokine Data – Growth Factors

- G-CSF
- GM-CSF
- FGF basic
- Tpo
- VEGF

* denotes significant differences.
Conclusions

• In general, levels of inflammatory and adaptive immunity cytokines are not elevated during long-duration spaceflight.

• Reduced T cell, granulocyte, NK and monocyte function have all been reported following both long and short duration spaceflight, however no systemic inflammatory or adaptive immune activation evident during spaceflight.

• Increases in growth factors and chemokines may indicate other types of adaptation occurring during spaceflight, such as attempts to overcome diminished immunocyte function.

• Are there localized inflammatory processes that result in a downstream peripheral manifestation (IL-1ra, CXCL5, IL-8)?

• There appear to be varied individual crew responses, and specific relationships between cytokines and markers of iron status and muscle turnover that warrant further evaluation.