PLASMA CYTOKINE LEVELS DURING LONG-DURATION SPACEFLIGHT

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

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ABSTRACT
This year, we celebrate the 40th birthday of the first landing of humans on the moon. By 2020, astronauts should return to the lunar surface and establish a outpost there that will provide a technical basis for future manned missions to Mars. This paper summarizes major conclusions associated with a trip to Mars, presents immunological hazards associated with this type of mission, and shows that our current understanding of the immunosuppressive effects of spaceflight is limited. Weakening of the immune system associated with spaceflight is therefore an area that should be considered more thoroughly before we undertake prolonged space voyages.

Introduction
In 1969, Neil Armstrong became the first human to leave the craters of the moon. Since then, over 460 people have walked into space, but so far, only 12 astronauts (five of the Apollo mission) have explored beyond the first 40,000 km of the low-Earth orbit, in which the magnetic field of the Earth deflects a significant portion of the radiation. Beyond the Van Allen radiation belt, where charged particles are trapped in the magnetic field of the Earth, astronauts are exposed to solar and cosmic radiation.

On July 20, 1969, Neil Armstrong and Edwin Aldrin became the first humans to land on the moon. This summer, we celebrated the 40th birthday of this historic event. A few years ago, President George W. Bush proposed a manned return to the moon, with the moon as the staging post for manned missions to Mars [1]. President Barack H. Obama’s 2014 budget requests, released on February 26, 2013, confirmed that NASA will move on to return to the moon by 2020. A mission to Mars and back will take a minimum of 550 days, of which roughly 1 month will be spent on the surface, and the remainder will be spent in transit. At 120 km/s, the crew will be some 3.5 million km from home. Consequently, astronauts will need to exercise an unprecedented level of autonomy and teamwork [2]. During the mission, they will experience not only microgravity but also various forms of stress, such as confinement, high exposure to performance, and risks of equipment failure or failure of existing systems. The enormous distance and long travel time to Mars will also affect the psychological. The crew will therefore endure increased stress levels, radiation, as well as the moon and Mars has magnetic fields that deter astronauts due to the increased stress levels, radiation, and microgravity-induced changes, such as alterations in body fluid distribution, which could influence their immune system. As gravity has helped to enhance the robustness of all biological systems on our planet, it is reasonable to observe alterations in normal functioning of life in weightlessness. A long-term spaceflight will also pose a multitude of health risks, not only those associated with spaceflight, such as bone demineralization, renal muscle atrophy, and immune system suppression (Fig. 1), but also from common diseases that might cause specific problems under these circumstances. Another risk may be the development of cancers to a doses enlargement, where air, food, waste, and water are recycled.

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

**Immune Parameters**

- Cytotoxic CD8+
- CD4+ T cell function
- CD8+ T cell function
- IL-10 (CD3/CD28)
- IL-6 (PMA-I)
- TNFa (PMA-I)

**VZV Reactivation**

- Reactivation in 64% of crewmembers

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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.
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

**Cytokines: Th1/Th2**

![Diagram showing cytokines and their interactions between Th1 and Th2 cells.](image)
<table>
<thead>
<tr>
<th>Environment</th>
<th>Response type</th>
<th>Products</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12+IL-18</td>
<td>Th1 (Monocytic Inf.)</td>
<td>IFN-γ, IL-2, TNF-α, LT</td>
<td>Purpose: CMI, DTH, intracellular pathogens. Pro-inflammatory, causing organ-specific autoimmunity.</td>
</tr>
<tr>
<td>TGFβ+IL-6</td>
<td>Th17 (Neutrophil. Inf.)</td>
<td>IL-17a, f, 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>IL-4+IL-2</td>
<td>Th2 (Baso/Eo/Mast inf.)</td>
<td>IL-4, IL-5 (IL-10, 9, 12)</td>
<td>Purpose: humoral immunity, extracellular organisms. Disease: allergy, atopy.</td>
</tr>
<tr>
<td>TGFβ + IL-4</td>
<td>Th9</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>TGFβ</td>
<td>Treg</td>
<td>TGFβ</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>TGFβ+IL-27</td>
<td>Tr1</td>
<td>IL-10, TGFβ (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>IL-21</td>
<td>Th3</td>
<td>TGFβ, 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>Tfh</td>
<td>IL-6, IL-10, IL-21</td>
<td>Folicular helper T cells. Regulate step-wise development of ag-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

Late
R-1-2 days
FD180

SMO-018

SMO-016
## Cytokine Categories

<table>
<thead>
<tr>
<th>INFLAMMATORY</th>
<th>ADAPTIVE/Th1</th>
<th>ADAPTIVE/Th17</th>
<th>ADAPTIVE/Th2</th>
<th>CHEMOKINES</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1 alpha</td>
<td>IFN-gamma</td>
<td>IL-17</td>
<td>IL-4</td>
<td>CXCL8/IL-8</td>
</tr>
<tr>
<td>IL-1 beta</td>
<td>IL-2</td>
<td></td>
<td>IL-5</td>
<td>CCL2/MCP-1</td>
</tr>
<tr>
<td>TNF-alpha</td>
<td>+/-</td>
<td></td>
<td>IL-10</td>
<td>CCL3/MIP-1 alpha</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
<td>CCL4/MIP-1 beta</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CCL5/RANTES +++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CXCL5/ENA-78 +++</td>
</tr>
</tbody>
</table>

### anti-INFLAMMATORY
- IL-1ra/IL-1F3 ++

### GROWTH FACTORS
- G-CSF
- GM-CSF +/-
- FGF basic
- Tpo ++
- VEGF

### CHEMOKINES
- CXCL8/IL-8 +/-
- CCL2/MCP-1
- CCL3/MIP-1 alpha
- CCL4/MIP-1 beta +
- CCL5/RANTES +++
- CXCL5/ENA-78 +++

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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**

**TNFa**

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

IFNg

IL-2

IL-17

IL-4

IL-5

IL-10
Plasma Cytokine Data – Chemokines

- **CCL2**
- **CCL3**
- **CCL4**
- **CXCL5/ENA-78**
- **RANTES**
- **IL-8**
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.