PLASMA CYTOKINE LEVELS DURING LONG-DURATION SPACEFLIGHT

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THE IMMUNE SYSTEM

- THYMUS GLAND
- LYMPH NODES
- LIVER
- SPLENE
- B CELLS & OTHER LYMPHOCYTES

Innate immunity (rapid response):
- Dendritic cell
- Mast cell
- Natural killer cell
- Macrophage
- Basophil
- Complement protein
- Eosinophils
- Neutrophils
- Gralocytes

Adaptive immunity (slow response):
- B cell
- T cell
- γδ T cell
- Natural killer T cell
- CD4+ T cell
- CD8+ T cell
- Antibodies
Could spaceflight-associated immune system weakening preclude the expansion of human presence beyond Earth’s orbit?

Nathan Gavish,1,8 Cécile Hain-Schlob,1 Matthias Baccar,1 Jean-Luc Barth,1 Erica Titcomb,1 Christine Legrand-Floster,1 and Jean-Pol Poppin2,3

1 Nancy University, Development and Immunomagnetic Team, Vandoeuvre-lès-Nancy, FR 54377, France; and 2 University of Luxembourg, Inst Research Unit, Luxembourg

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 an outpost there that will provide a technical basis for future manned missions to Mars. This paper summarizes major uncertainties associated with a trip to Mars, preventive 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. J. Leukoc. Biol. 86:1027-1038, 2009.

Introduction

In 1969, Neil Armstrong became the first human to leave the confines of Earth. Since then, over 450 people have ventured into space, but so far, only 24 astronauts (none of the Apollo missions) have traveled beyond the first 400-500 km of the low-Earth orbit, in which the magnetic field of the Earth deflects a significant fraction of 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 so become the starting point for manned missions to Mars [1]. President Barack Obama’s 2010 budget request, released on February 26, 2009, confirmed that NASA will no return to the moon by 2020. A mission to Mars and back will take a minimum of 360 days, of which roughly 1 month will spend on the martian surface, and the rest will be spent in space. As a preparation, the crew will experience a 900 million yen mission. Consequently, astronauts will have to undergo an unprecedented level of psychological and teamwork. During the mission, they will experience not only microgravity but also variable and stressful conditions, such as confinement, high exposure to radiation, and risks of equipment failure or fatal mishaps. The length of time and the long period in the Mars will also make the effect of psychological. The crew will therefore induce increased stress levels, radiation, as well as the moon and Mars have magnetic fields, or dense atmospheric that could accentuate them, and microgravity-induced changes, such as alterations in body fluid distribution, which could influence their immune system. As gravity has shifted the ambience of all biological systems on our planet, it is conceivable 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, nervous system and immune system suppression (Fig. 1), but also from common diseases that might cause specific problems under these circumstances. Another risk is the development of predispositions to a wide variety of disease, such as air, food, water, and air are recycled. Confined conditions of the crew during flight can and can result in the transfer of microorganisms among crew members [4, 5]. Specific health risks might also be encountered on the lunar or martian surface, such as dust or chemicals that could induce respiratory tract, for example, or even new organisms. Instead, 5 days on the moon during the final Apollo mission in 1972 let astronauts Eugene Cernan worry and adjust with rock dust. A trip to Mars will certainly multiply the hazards of space travel.

Human Research Program

Human Health Countermeasures Element

Evidence Book

Risk of Crew Adverse Health Event Due to Altered Immune Response

June 2009

National Aeronautics and Space Administration
Lyndon B. Johnson Space Center
Houston, Texas


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
<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>IFNγ, IL-2, TNFα, LT</td>
<td>Purpose: CMI, DTH, intracellular pathogens. Pro-inflammatory, cause organ-specific auto-immunity.</td>
</tr>
<tr>
<td>TGFβ+IL-6</td>
<td>Th17 (Neutrophil. Inf.)</td>
<td>IL-17a, IL-1f, 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>IL-21</td>
<td>Tfh</td>
<td>IL-6, IL-10, IL-21</td>
<td>Folicular 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>

Cytokines: Th1/Th2 (updated!)
**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 to 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

SMO-018

~2 weeks

FD15

FD30

FD60

Early

Mid

2-4 mos

FD120

FD180

Late

R-1-2 days

SMO-016

FD15

FD30

FD60
### Cytokine Categories

<table>
<thead>
<tr>
<th>INFLAMMATORY</th>
<th>ADAPTIVE/Th1</th>
</tr>
</thead>
<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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</tbody>
</table>

<table>
<thead>
<tr>
<th>anti-INFLAMMATORY</th>
<th>ADAPTIVE/Th17</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1ra/IL-1F3</td>
<td>IL-17</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>ADAPTIVE/Th2</th>
<th>CHEMOKINES</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>CXCL8/IL-8 (+/-)</td>
</tr>
<tr>
<td>IL-5</td>
<td>CCL2/MCP-1</td>
</tr>
<tr>
<td>IL-10</td>
<td>CCL3/MIP-1 alpha</td>
</tr>
</tbody>
</table>

|                                  | CCL4/MIP-1 beta (+)           |
|                                  | CCL5/RANTES (+++)             |
|                                  | CXCL5/ENA-78 (+++)            |

**GROWTH FACTORS**

<table>
<thead>
<tr>
<th>G-CSF</th>
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<tbody>
<tr>
<td>GM-CSF</td>
<td>(+/-)</td>
</tr>
<tr>
<td>FGF basic</td>
<td></td>
</tr>
<tr>
<td>Tpo</td>
<td>++</td>
</tr>
<tr>
<td>VEGF</td>
<td></td>
</tr>
</tbody>
</table>

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

IFNγ

IL-2

IL-17

IL-4

IL-5

IL-10
Plasma Cytokine Data – Growth Factors

G-CSF

GM-CSF

FGF basic

Tpo

VEGF

* * *
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.