Immune System Dysregulation, Viral Reactivation and Stress
During Short-Duration Space Flight

European Space Agency (ESA)
International Society for Gravitational Physiology (ISGP)
ISSBB Symposium
European Low Gravity Research Association (ELGRA)

‘Life in Space for Life on Earth’
13-18 June 2010
Trieste – Italy

Brian Crucian, Satish Mehta, Raymond Stowe, Peter Uchakin, Heather Quiriarte, Duane Pierson and Clarence Sams
**In-flight cell culture**
- Intracellular signaling, cytoskeleton rearrangement, microtubule organizing center orientation, generalized proliferative responses all altered during flight.

**Reactivation of latent herpesviruses**
- EBV, CMV, VZV reactivation during flight
- Infectious VZV particles secreted in saliva (Shuttle)

**Short duration**

**Post-flight observations**
- Altered circulating leukocyte distribution
- Altered cytokine production patterns (secreted, intracellular, Th1/Th2)
- Decreased NK cell function
- Decreased granulocyte function
- Decreased T cell function*
- Altered immunoglobulin levels
- Latent viral reactivation
- Altered virus-specific immunity
- Expression of EBV IE/late genes*
- Altered neuroendocrine responses

*Post-flight observations differ between long vs. short duration space flight.

**Long duration**

**Humoral immunity**
- Immunization with antigen generates normal antibody response during flight (MIR-18)

**Reduced cell mediated immunity**
- CMI Multitest, common recall antigens, long duration flight (long and short) (MIR missions)
Could spaceflight-associated immune system weakening preclude the expansion of human presence beyond Earth’s orbit?

Nathan Guiguiwine, Cécile Mail-Schroën, Matthieu Barone, Jean-Luc Barb, Erve Tichenon, Christine Lagrange-Francois, and Jean-Pol Frigui

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 constraints 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, Yuri Gagarin became the first human to leave the confines of Earth; since then, over 450 people have traveled into space, but so far, only 24 astronauts (those of the Apollo missions) have traveled beyond the first 500–800 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 belts, 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 on land on the moon. This summer, we celebrate 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]. The Barack H. Obama’s 2010 budget request, released on February 28, 2009, confirmed that NASA will stay on track to return the moon by 2020. A mission to Mars and back will take a minimum of 282 days, of which roughly 1 month will be spent on the martian surface, and the rest will be spent in transit. As in further, the crew will be some 500 million km away from home. Consequently, astronauts will have 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 expectation of performance, and risks of equipment failure or fault misjudgments.

The enormous distance and long travel times to Mars will also probably affect the astronauts psychologically. The crew will therefore endure increased stress levels, radiation, and, as neither the moon nor Mars has magnetic fields or dense atmospheres that could attenuate them, and microgravity-induced changes, such as alterations in body fluid distribution, which could influence their immune system. Gravity has shaped the architecture of all biological systems on our planet, it is reasonable to observe aberrations in normal functioning of life in weightlessness. A long-term spaceflight will also pose a multiplicity of health risks, not only those associated with spaceflight, such as bone demineralization, skeletal muscle atrophy, and immune system suppression. It also, but also common diseases that might cause specific problems under these circumstances. Another risk may be the development of pathogens in a closed environment, where air, food, and water are recycled. Concerns of the crew during flight can be reduced in the transfer of microorganisms among crew members [4, 5]. Finally, specific health risks might also be encountered on the lunar or martian surface, such as dust or chemicals dust that could irritate the respiratory tract, for example, or even new organisms. Indeed, 3 days on the moon during the Apollo mission in 1972 left astronauts Eugene Cernan weary and fitly with rock dust. A trip to Mars will certainly multiply the hazards of space travel.

Humans are ready so accept great risks so go where no one has gone before, but do we have sufficient and sound biological...
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

HRP-47069  13-1

**Objectives**

- Replace several recent immune studies with one comprehensive study that will include in-flight sampling.

- Address lack of in-flight data: determine the in-flight status of immunity, physiological stress, viral immunity/reactivation (short/long).

- 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.
Assays

JSC Immunology Laboratory
- Leukocyte subsets
- T cell function
- Intracellular/secreted cytokine profiles

Mercer University
- Plasma cytokine balance
- Leukocyte cytokine RNA

Microgen Laboratories
- Virus specific T cell number
- Virus specific T cell function
- Plasma stress hormones

JSC Microbiology Laboratory
- Latent herpesvirus reactivation (saliva/urine)
- Saliva/urine stress hormones
- Circadian rhythm analysis
### Samples - Timepoints

**Short Duration**

<table>
<thead>
<tr>
<th>BLOOD</th>
<th>SALIVA (liquid)</th>
<th>SALIVA (dry book)</th>
<th>URINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>L</td>
<td>D</td>
<td>U</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>L-180/A.M.E.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PRE-FLIGHT</th>
<th>IN-FLIGHT</th>
<th>POST-FLIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>L</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>U</td>
<td>U</td>
<td>U</td>
</tr>
</tbody>
</table>

**Long Duration**

<table>
<thead>
<tr>
<th>BLOOD</th>
<th>SALIVA (liquid)</th>
<th>SALIVA (dry book)</th>
<th>URINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>L</td>
<td>D</td>
<td>U</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>L-180/A.M.E.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PRE-FLIGHT</th>
<th>IN-FLIGHT</th>
<th>POST-FLIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>L</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>U</td>
<td>U</td>
<td>U</td>
</tr>
</tbody>
</table>

**LONG DURATION ISS MISSION**

<table>
<thead>
<tr>
<th>BLOOD</th>
<th>SALIVA (liquid)</th>
<th>SALIVA (dry book)</th>
<th>URINE</th>
<th>Health Survey</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>L</td>
<td>D</td>
<td>U</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>L</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-180/A.M.E.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PRE-FLIGHT</th>
<th>IN-FLIGHT</th>
<th>POST-FLIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>L</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>U</td>
<td>U</td>
<td>U</td>
</tr>
</tbody>
</table>

**Long Duration Timepoints**

<table>
<thead>
<tr>
<th>R-1</th>
<th>R+0</th>
<th>R+14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
DISTRIBUTION OF IMMUNE CELLS

- WHITE BLOOD CELLS
  - GRANULOCYTES
    - BASOPHILS
    - NEUTROPHILS
    - EOSINOPHILS
  - MONOCYTES
    - MACROPHAGE
  - LYMOPHOCYTES
    - B CELLS
    - T CELLS
    - NK CELLS
    - CD4+ ‘Helper’
    - CD8+ ‘Cytotoxic’
    - PLASMA CELLS
      - Memory
      - Naive
    - Th1
    - Th2
    - Th17
    - Treg
    - True Naive
    - Central Memory
    - Effector Memory
    - Terminal Diff.

- RED BLOOD CELLS

- MAST CELLS

- DENDRITIC CELL
Peripheral Leukocyte Distribution

Granulocytes 54.0%

Monoocytes 7.1%

Lymphocytes 38.1%

B1 35.0%
B2 2.3%
B3 3.3%
B4 59.4%

B1 8.9%
B2 4.1%

B3 15.2%
B4 71.8%

CD38 PE Log

MEMORY

NAIVE

CD45RA FITC Log
Peripheral Leukocyte Distribution

**Leukocyte Subsets**
- GRAN
- LYMPH
- WBC
- MONO

**Lymphocyte Subsets**
- T CELLS
- B CELLS
- NK CELLS

**T cell Subsets**
- CD4
- CD8

**Memory/Naive T cells**
- MEM CD4
- MEM CD8
**KINETICS OF T CELL ACTIVATION**

<table>
<thead>
<tr>
<th>Time</th>
<th>Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>0:00</td>
<td><strong>Ligand-receptor binding</strong></td>
</tr>
<tr>
<td>0-5 sec</td>
<td>Membranes increase permeability to ions  &lt;br&gt; Shifts in ions from one intracellular compartment to another  &lt;br&gt; Changes in membrane potential  &lt;br&gt; Changes in intracellular pH</td>
</tr>
<tr>
<td>0-5 min</td>
<td>Changes of state in membrane lipids and proteins  &lt;br&gt; Activation of adenylate cyclase, ATPase, and other membrane-associated enzymes  &lt;br&gt; Changes in cyclic nucleotide concentrations  &lt;br&gt; Changes in receptor distribution and motility occur  &lt;br&gt; Adhesion molecule conformational changes</td>
</tr>
<tr>
<td>T+30 min</td>
<td>Coalescence of patched receptors into cap at one pole of the cell (dependant on contraction of cytoskeletal microfilaments, ATP energy source)</td>
</tr>
<tr>
<td>T+6-12 hr</td>
<td>Expression of CD69 on T cell surface</td>
</tr>
<tr>
<td>T+24 hr</td>
<td>Secretion of IL-2, cell surface expression of IL-2 receptor (CD25)  &lt;br&gt; Upregulation of CD40L  &lt;br&gt; IL-2 binds to IL-2r (autocrine activation)  &lt;br&gt; CD40L binds to CD40 on APC, upregulating CD86/CD80  &lt;br&gt; APC CD86/80 binds to CD28 on T cell surface, results in additional cytokine expression, expression of BCL-x (anti-apoptosis, proliferation</td>
</tr>
<tr>
<td>36-72 hr</td>
<td>DNA synthetic activity  &lt;br&gt; Expression of HLA-DR</td>
</tr>
<tr>
<td>3-4 days</td>
<td>Blast transformation  &lt;br&gt; Differentiation into Th1/Th2/Th17 cell based on factors such as antigen dosage, local cytokine environment, other costimulatory molecules, APC involvement</td>
</tr>
</tbody>
</table>
Constitutively Activated T Cells

Kinetics of Expression of Activation Antigens on T Cells

Activated T Cells

- CD4/CD69
- CD8/CD69
- CD4/HLA-DR
- CD8/HLA-DR
T Cell Function: Intracellular Cytokine, Early Blastogenesis

Intracellular Cytokines

T Cell Function: A+B

CD4/IL-2
CD8/IFNγ
CD4/69+
CD8/69+
CD4/69/25+
CD8/69/25+
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

![Diagram of cytokine responses between Th1 and Th2 cells](image-url)

Th1 - Th2 balance
Secreted Cytokine Profiles (T cell stimulation)
Secreted Cytokine Profiles (PMA-I stimulation)
Secreted Cytokine Profiles (monocyte stimulation)
# In-flight Secreted Cytokine Summary (short-duration)

## T cells (CD3/CD28)

### Adaptive immunity:
- IFNg ↓
- IL-2 ↑
- IL-4 --
- IL-5 --
- IL-10 ↓
- IL-17 ↓

### Innate/Inflammatory:
- IL-6 nc
- TNFa ↑

## Monocytes (LPS)

### Innate/Inflammatory:
- IL-1b nc
- TNFa nc
- IL-6 nc
- IL-8 ↑
- IL-10 ↓

## All Cells (PMA+ion)

### Adaptive immunity:
- IFNg ↓
- IL-2 nc
- IL-4 ↓
- IL-5 ↓
- IL-10 ↓
- IL-17 var

### Innate/Inflammatory:
- IL-6 ↓
- TNFa ↓
Viral Antibody Titers

- **Anti-EBNA (Log2)**
  - Collection Time: L-180, L-10, 14d, R+0, R+14
  - EBNA

- **Anti-VCA (Log2)**
  - Collection Time: L-180, L-10, 14d, R+0, R+14
  - VCA
  - EA

- **Anti-CMV (Log2)**
  - Collection Time: L-180, L-10, 14d, R+0, R+14
  - CMV

- **VZV IgG**
  - Log
  - VZV
Routine detection of Epstein–Barr virus specific T-cells in the peripheral blood by flow cytometry

Brian E. Crucian*, Raymond P. Stowe†, Duane L. Fierston†, Clarence F. Samuel‡

*Hed Laboratories, Cell and Molecular Research Laboratories, Houston, TX, USA
†University of Texas Medical Branch, Department of Pathology, Galveston, TX, USA
‡NASA-Johnson Space Center, Life Sciences Research Laboratories, Matl Code: XXL, Houston, TX 77058, USA

Received 10 May 2000; revised in revised from: 1 November 2000; accepted 3 November 2000

Abstract

The ability to detect cytomegalovirus-specific T-cells (CD8+ T-cells) in the peripheral blood by flow cytometry has been recently described by Felciano et al. In this method, cells are incubated with viral antigens and responding (cytomegalovirus producing) T-cells are then identified by flow cytometry. To date, this technique has been relatively used to detect Epstein–Barr virus (EBV)-specific T-cells, primarily due to the expression ubiquitous properties of the virus which non-specifically activate T-cells. By modifying culture conditions under which the antigens are presented, we have overcome this limitation and demonstrated in assay to detect and quantify EBV-specific T-cells. The detection of cytokines producing T-cells by flow cytometry requires an extremely strong signal (such as culture in the presence of PMX and IL-2). Our data indicate that modified culture conditions (pre-removal of EBV antigen) non-specific activation of T-cells by EBV is reduced, but antigen presentation will continue unabated. Using this method, EBV-specific T-cells may be specifically detected using flow cytometry. No reduction in the number of EBV-specific T-cells was observed by the early removal of target antigen when using cytomegalovirus antigen as a T-cell activation property. In EBV-responsive individuals, the phenotype of the EBV-specific cytokines producing T-cells was evaluated using four-color flow cytometry and found to be CD4+ CD8+ CD45RA+ CD69-. This phenotype indicates the stimulation of circulating previously unactivated memory T-cells. No cytokine production was observed in CD4+ T-cells from EBV-non-responsive individuals, confirming the specificity of this assay. In addition, the use of four-color cytometry (CD4+ CD8+ IFN-γ IL-2) allows for the quantification of EBV-specific T-cells while excluding the interference of EBV non-specific cytokine activity. This method may have significant utility for monitoring the immune response to latent virus infection/infections.
Virus-specific T cell Number/Function

![Graph showing virus-specific T cell counts over time. The x-axis represents collection times (L-180, L-10, 14d, R+0, R+14), and the y-axis represents % EBV (BMLF) Tetramer Positive CD8+ T-cells and % IFN-producing EBV T-cells. Two lines are plotted: EBV Tetramer CD8+ T-cells (closed circles) and EBV Function (IFN) (open triangles).]
Virus-specific T cell Number/Function

Collection Time

- CMV Tetramer-positive T-cells
- CMV Function (IFN)
Virus Specific T Cells – Functional Percentage

- EBV T Cells
- CMV T Cells
EBV Viral Load per 1e6 PBMCs

Collection Time

EBV DNA Copies/mL

- L-180
- L-10
- 14d
- R+0
- R+14
EBV shed in 82% of crewmembers

Samples positive for virus - Pre: 16.2%, During: 23.4%, Post: 23.1%
VZV shed in 41% of crewmembers

Samples positive for virus - Pre: 0.0%, During: 16.0%, Post: 7.7%
Salivary VZV in Shingles patients & Astronauts

VZV copies/ml saliva

Shingles Patients
Days after treatment

0
7
14
Astronauts
CMV shed in 47% of crewmembers

Samples positive for virus - Pre: 17.7%, Post: 43.8%
Stress Hormone Levels

![Graph showing cortisol levels over time]

- **X-axis**: Collection Time
  - L-180
  - L-10
  - 14d
  - R+0
  - R+14

- **Y-axis**: Cortisol (ug/dL)
  - 0
  - 10
  - 20
  - 30
  - 40

- **Title**: Plasma Cortisol

The graph illustrates the cortisol levels over the specified collection times.
Conclusions

• Some measures of immune dysregulation are not merely related to landing stress/re-adaptation to gravity, but are present during flight.

• Bulk leukocyte subsets largely unaltered during flight. Some alteration within CD8+ T cell subsets occurs during short duration flight.

• Diminished T cell function, alterations in various cytokine production profiles (secreted, mRNA) occurs during short duration flight.

• CMV, EBV viral antibody titers trended to elevation during flight. EBV specific T cell number and function reduced during flight (correlated with EBNA), CMV specific T cells elevated, function unchanged.

• Reactivation of latent EBV (14/17), VZV (7/17) and CMV* (8/17) occurred during short duration flight.

• General plasma cortisol levels were elevated during flight. Circadian rhythm of cortisol was abnormal early in flight, tended to resolve later in flight.
ESA/NASA Immunology Flight Studies

Diagram showing innate immunity (rapid response) and adaptive immunity (slow response) with various immune cells and processes.
Questions?