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

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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?

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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 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. J. Leukoc. Biol. 86:1027-1038; 2009.

Introduction
In 1969, Yuri Gagarin became the first human to leave the con-

tours of Earth. Since then, over 650 people have traveled into

space, but so far, only 21 astronauts (those of the Apollo mis-
sions) have traveled beyond the far 500-800 km of the low

Earth orbit, in which the magnetic field of Earth deflects a

significant fraction of radiation. Beyond the Van Allen radiation

belt, where charged particles are trapped in the magnetic field

of 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 cel-

ebrated 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 to become the staging post for manned

missions to Mars [1]. President Barack Obama’s 2010 budg-

et request, released on February 26, 2009, confirmed that

NASA will stay on track to return to the moon by 2020. A mis-

sion 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 it moves, the crew

will be some 300 million km away from home. Consequently,

astronauts will have to exercise an unprecedented level of

anxiety and teamwork [2]. During the mission, they will expe-

rience not only microgravity but also various forms of stress,

such as confinement, high expectations of performance, and

risks of equipment failure or fatal mishaps. The enormous dis-

tance and long travel time to Mars will also probably affect

the astronauts psychologically. The crew will therefore endure

increased stress levels, radiation, as neither the moon nor Mars

has magnetic fields or dense atmospheres that could asemine

them, and microgravity-induced changes, such as alterations

in body fluid distribution, which could influence their immune

system. As 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 multitude of health risks, not only

those associated with spaceflight, such as bone demineraliza-

tion, skeletal muscle atrophy, and immune system suppression

(Fig. 1), but also from common diseases that might cause spe-

cific problems under these circumstances. Another risk may

be the development of pathogen in a closed environment,

where air, food waste, and water are recycled. Components of

the crew during flight can and has resulted in the transfer of

microorganisms among crew members [4, 5]. Finally, specific

health risks might also be encountered on the lunar or mar-

tian surface, such as dust or chemicals dust could irritate the

respiratory tract, for example, or even new organisms. Indeed,

3 days on the moon during the Final Apollo mission in 1972

left astronauts Eugene Cernan weary and ill with rock dust.

A trip to Mars will certainly multiply the hazards of space

travel.

Humans are ready to accept great risks so go where no one

has gone before, but do we have sufficient and sound biologi-

References:
[1] Correspondence: Development and Immunomigration Team, JR 3357, 9

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Risk of Crew Adverse Event Due to Altered Immune Response

Human Research Program
Human Health Countermeasures Element

Evidence Book
Risk of Crew Adverse Health Event Due to Altered Immune Response

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Lyndon B. Johnson Space Center
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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

![Diagram](chart.png)

- **PHYSIOLOGICAL STRESS**
- **Immune System Changes (Status and Function)**
- **Adverse clinical outcomes (Latent Viral Reactivation)**
Samples - Timepoints

Short Duration

Long Duration
Peripheral Leukocyte Distribution
Peripheral Leukocyte Distribution

Leukocyte Subsets

Lymphocyte Subsets

T cell Subsets

Memory/Naive T cells
T Cell Activation

KINETICS OF T CELL ACTIVATION

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

Activation of naive T4-lymphocyte

Kinetics of Expression of Activation Antigens on T Cells

Activated T Cells

Expression on Activated T Cells

CD69
CD25
CD71
HLA-DR

Time (Hours)
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
Secreted Cytokine Profiles (T cell stimulation)

- **IFNγ**
- **IL-17a**
- **IL-4, 5, 10**
- **IL-2**
- **TNFα**
- **IL-6**
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 $\downarrow$
  - IL-2 $\uparrow$
  - IL-4 --
  - IL-5 --
  - IL-10 $\downarrow$
  - IL-17 $\downarrow$
- **Innate/Inflamatory:**
  - IL-6 nc
  - TNFa $\uparrow$

#### Monocytes (LPS)
- **Innate/Inflamatory:**
  - IL-1b nc
  - TNFa nc
  - IL-6 nc
  - IL-8 $\uparrow$
  - IL-10 $\downarrow$

#### All Cells (PMA+ion)
- **Adaptive immunity:**
  - IFNg $\downarrow$
  - IL-2 nc
  - IL-4 $\downarrow$
  - IL-5 $\downarrow$
  - IL-10 $\downarrow$
  - IL-17 var
- **Innate/Inflamatory:**
  - IL-6 $\downarrow$
  - TNFa $\downarrow$
Viral Antibody Titers

- Anti-EBNA (Log2)
- Anti-VCA (Log2)
- Anti-CMV (Log2)
- VZV IgG
Virus-specific T cell Number/Function

Tetramer Assay

Peptide Stimulation Assay

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

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Abstract

The ability to detect cryptogammavirus-specific T-cells (CD4+) in the peripheral blood by flow cytometry has been recently described by Feigen et al. In this method, cells are incubated with viral antigen and responding (cytotoxic production) T-cells are then identified by flow cytometry. To date, this technique has not been reliably used to detect Epstein-Barr virus (EBV)-specific T-cells. T-cells are primarily due to the up-regulation of an immune system properties of the system which non-specifically activate T-cells. By modifying culture conditions under which the antigens are presented, we have overcome this limitation and developed a method to detect and quantify EBV-specific T-cells. The detection of cytotoxic T-cells by flow cytometry requires an extremely strong signal (such as culture in the presence of PMA and ionomycin). Our data indicate that in modified culture conditions (early removal of viral antigen) the non-specific activation of T-cells by EBV is reduced, but antigen presentation will continue unaffected. Using this method, EBV-specific T-cells may be specifically detected using flow cytometry. No reduction in the numbers of antigen-specific T-cells was observed by the early removal of target antigen when using cryopreserved antigen (a system with non-specific T-cell activation properties). In EBV-responsive individuals, the phenotype of the EBV-specific cytotoxic T-cells was evaluated using four-color flow cytometry and found to be CD8+CD4+CD45RA−CD28+CD25+. This phenotype indicates the activation of circulating pre-existing autoreactive T-cells. No cytotoxic production was observed in CD4+ T-cells from EBV-seronegative individuals, confirming the specificity of this assay. In addition, the use of four color cytometry (CD4+CD8+CD25+IFN-α) allows the total quantification of EBV-specific T-cells while monitoring the interference of EBV non-specific autoreactive activity. This method may have significant utility for the monitoring of the immune response to lower virus infection.
Virus-specific T cell Number/Function

Graph showing the percentage of CMV (pp65) CD8+ T-cells and CMV Function (IFN) over collection time. The x-axis represents collection time (L-180, L-10, 14d, R+0, R+14), while the y-axis shows the percentage of CMV T-cells.
Virus Specific T Cells – Functional Percentage

- EBV T Cells
- CMV T Cells
EBV Viral Load

![Graph showing EBV Viral Load per 1e6 PBMCs over different collection times: L-180, L-10, 14d, R+0, R+14. The graph indicates a peak at R+14 with a trend of increasing EBV DNA copies/mL.]
EBV shed in 82% of crewmembers

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

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
Viral Reactivation: Urine CMV DNA

CMV shed in 47% of crewmembers

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

![Graph showing plasma cortisol levels over time.](image)
Stress Hormone Levels – Circadian Rhythms

Collection Time

CORT (nmol/L) +/- 1 SEM

- 180
- 10
Early
Late
Early
Late
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.
Questions?