Combined effects of Gamma Radiation and High Dietary Iron on Peripheral Leukocyte Distribution and Function

Brian E. Crucian,
Jennifer L.L. Morgan
Heather A. Quiriarte
Clarence F. Sams
Scott M. Smith
Sara R. Zwart
• Both radiation and increased iron stores can independently increase oxidative damage, resulting in protein, lipid and DNA oxidation.

• Oxidative stress increases the risk of many health problems including cancer, cataracts, and heart disease.

• This study, a subset of a larger interdisciplinary investigation of the combined effect of iron overload on sensitivity to radiation injury, monitored immune parameters in the peripheral blood of rats subjected to gamma radiation, high dietary iron or both.

• Specific immune measures consisted of:
  - peripheral leukocyte distribution
  - plasma cytokine levels
  - cytokine production profiles following whole blood mitogenic stimulation
THE IMMUNE SYSTEM

- Thymus Gland
- Lymph Nodes
- Liver
- Spleen
- Breast lymphocytes and antibodies
- Antibodies against viruses
- B cells and other lymphocytes

Innate immunity (rapid response):
- Dendritic cell
- Mast cell
- Macrophage
- Natural killer cell
- Complement protein
- Neutrophil
- Basophil
- Eosinophil
- Granulocytes

Adaptive immunity (slow response):
- B cell
- T cell
- Antibodies
- CD4+ T cell
- CD8+ T cell

Cytokines and transcription factors:
- Th0
- Th1
- Th2
- IL-12, STAT4, T-bet
- IL-4, STAT6, GATA3
- IL-10
- IL-4, IL-5, IL-13
- IFN-γ
- TNF-β
- IL-2
Integrated Immune mid-study long duration data (n=10)

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.
Current Study Design

Irradiation - Cs-137 source, .375 Gy

Sacrifice
Starting after week 2, irradiation every other day (8 doses); 3 Gy total

<table>
<thead>
<tr>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal iron diet</td>
<td>Normal iron diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High iron diet</td>
<td>High iron diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal iron diet</td>
<td></td>
<td>Irradiation</td>
<td></td>
</tr>
<tr>
<td>High iron diet</td>
<td></td>
<td>Irradiation</td>
<td></td>
</tr>
</tbody>
</table>

Irradiation - Cs-137 source, .375 Gy
Starting after week 2, irradiation every other day (8 doses); 3 Gy total
Specific Immunology Assays

• WBC

• Neutrophil, Lymphocyte, Monocyte

• T cell subsets: CD4+/CD8+ (Flow cytometry)

• Cytokine Profiles (cytometrix bead array)
  Adaptive immunity: IFNg, IL-10, IL-4, IL-2
  Innate/inflammatory: TNFa, IL-1b, IL-6)
Leukocyte Distribution

- **WBC**
  - Control
  - FE++
  - IRR
  - FE++/IRR

- **Grans**
  - Control
  - FE++
  - IRR
  - FE++/IRR

- **Lymph**
  - Control
  - FE++
  - IRR
  - FE++/IRR

- **Mono**
  - Control
  - FE++
  - IRR
  - FE++/IRR

- **CD4+**
  - Control
  - FE++
  - IRR
  - FE++/IRR

- **CD8+**
  - Control
  - FE++
  - IRR
  - FE++/IRR
Constitutive Plasma Cytokine Levels
Cytokine Production Profiles (*anti-CD3/28, 48hr*)

- IFNg
- IL-10
- IL-4
- IL-1b
- TNFa
- IL-6
Cytokine Production Profiles (anti LPS, 48hr)
Cytokine Production Profiles (anti PMA-I, 48hr)
Conclusions
(and places we can go...)

• **Gamma-radiation treatment:** Demonstrable alterations in peripheral leukocyte distribution and leukocyte cytokine production following mitogenic stimulation.

• **High iron diet:** Resulted in an elevated WBC but with a normal subset distribution; minimal direct immune effects; abrogated some of the radiation-induced functional alterations but not the phenotypic alterations.

• **Summary:** Radiation induced demonstrable changes in peripheral immunity. Generally, the high iron diet did not, yet did abrogate many of the radiation effects.
ALTERED INNATE AND LYMPHOCYTIC IMMUNITY IN MURINE SPLENOCYTES FOLLOWING SHORT-DURATION SPACEFLIGHT

Brian E. Crucian
Shen-An Hwang
Jeffrey K. Actor
Heather Quiriarte
Clarence F. Sams

UTHealth
The University of Texas
Health Science Center at Houston

NASA
**STS-135 Study Design**

**STS-135** (13 day mission)

Ground controls

½ spleen; n=6
Splenocytes harvested 3-4 hours post-landing

24 or 48hr cell culture

**Phenotype**

T cells: CD4:CD8
Dendritic cells: CD11c, CD86, MHC-I

**Activation marker** expression and cytokine profiles

**DC Function:**
Zymosan (TLR-2)
LPS (TLR-4)
Flagellin (TLR-5)
Expression of MHC-I, CD86

**Cytokine Profiles**
CD3/CD28
PMA/I
LPS
(7 cytokine array)

**T-cell Function:**
Anti-CD3/28
SEA/SEB
PMA/Ionomycin
CD69 and/or CD25
Altered expression of lymphocytic markers post-flight

% positive cells

Intensity of marker expression

*** = p<0.001
Post-flight splenocytes have decreased expression of antigen presentation and co-stimulatory molecules

% positive cells

Intensity of marker expression

** = p<0.01
*** = p<0.001
Post-flight DCs show decreased expression of presentation and co-stimulatory molecules after TLR stimulation.

** = p<0.01
*** = p<0.001

No differences in MHC II observed.
Post-flight splenocytes demonstrate increased CD25 in CD4+/CD8+CD28+ cells when stimulation bypasses the 2° signal.
Data – T Cell Function, 24h culture, CD69/25 dual positive

SEA+SEB

αCD3+CD28
Data – Cytokine Production Profiles (anti CD3/28, 48hr)

**IFNg**

**IL-10**

**IL-4**

**IL-6**

**TNFa**

**IL-17a**

**IL-2**
Data – Cytokine Production Profiles (anti PMA-I, 48hr)

- IFNγ
- IL-10
- IL-4
- IL-6
- TNFα
- IL-17a
Data – Cytokine Production Profiles (anti LPS, 48hr)

- **TNFα**
  - Ground: Lower values
  - Flight: Slightly higher values

- **IL-6**
  - Ground: Average values
  - Flight: Higher values with an asterisk

- **IFNγ**
  - Ground: Lower values
  - Flight: Higher values with a marked increase at 234
Conclusions

• These data indicate that alterations in splenocytes phenotype, function and cytokine production patterns are evident following spaceflight.

• The pattern suggests that some innate immune functions are possibly enhanced, whereas some adaptive immune parameters may be inhibited.

• Follow up human and in-flight studies will determine if a clinical risk related to immune dysregulation exists for astronauts.

Acknowledgement: