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

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• 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
- LIVER
- LYMPH NODES
- BREAST LYMPHOCYTES
- ANTI BODIES
- ANTIBIOTICS
- LYMPH NODES
- B CELLS & OTHER LYMPHOCYTES

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

Adaptive immunity (slow response):
- B cell
- T cell
- γδ T cell
- B cell
- T cell
- Natural killer T cell
- Antibodies

IL-12, STAT4, T-bet
IL-4, STAT6, GATA3

Th0

Th1
- IFN-γ
- TNF-β
- IL-2

Th2
- IL-10
- IL-4, IL-5
- IL-13

Th1

Th2
Integrated Immune mid-study long duration data (n=10)

Immune dysregulation during long-duration spaceflight (SMO-015 mid-study data; n=10)

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

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>
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<tbody>
<tr>
<td>Normal iron diet</td>
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<td>High iron diet</td>
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- **Starts**

- **Ends**
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**: Controls show a noticeable increase in FE++/IRR compared to other groups.
- **IL-10**: Significant differences are observed between Control and FE++/IRR.
- **IL-4**: FE++/IRR group shows a marked increase compared to other groups.
- **IL-1b**: FE++/IRR group shows a notable increase.
- **TNFa**: FE++/IRR group shows a significant increase.
- **IL-6**: FE++/IRR group shows a marked increase compared to other groups.
Cytokine Production Profiles (anti LPS, 48hr)
Cytokine Production Profiles (anti PMA-I, 48hr)

Graphs showing cytokine production profiles:
- **IFNg**
- **IL-10**
- **IL-2**
- **IL-4**
- **IL-1b**
- **TNFa**
- **IL-6**

Each graph compares cytokine production across different conditions: Control, FE++, IRR, and FE++/IRR.
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.

Lutetia (Rosetta)  
Gaspar (Galileo)  
Ida (Galileo)
ALTERED INNATE AND LYMPHOCYTIC IMMUNITY IN MURINE SPLENOCYTES FOLLOWING SHORT-DURATION SPACEFLIGHT

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STS-135 Study Design

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

↓

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

↓

**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

Intense of marker expression

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

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

Differences in MHC II observed.
Post-flight splenocytes demonstrate increased CD25^+ in CD4+/CD8+CD28^+ cells when stimulation bypasses the 2° signal.

*** = p<0.001

* = p<0.05
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
- TNFα
- IL-17a

![Graphs showing cytokine production profiles](image-url)
Data – Cytokine Production Profiles (anti PMA-I, 48hr)
Data – Cytokine Production Profiles (anti LPS, 48hr)
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.