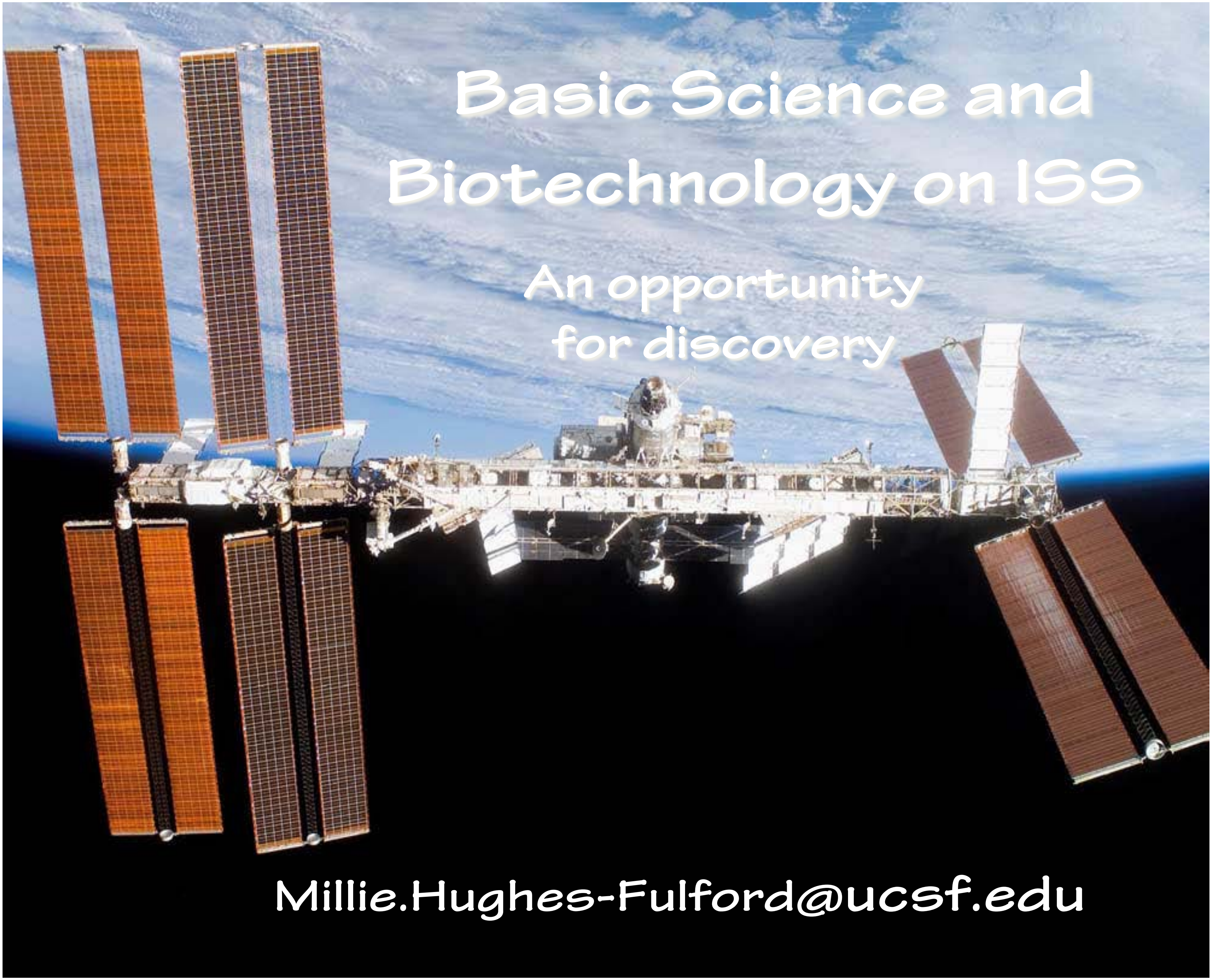
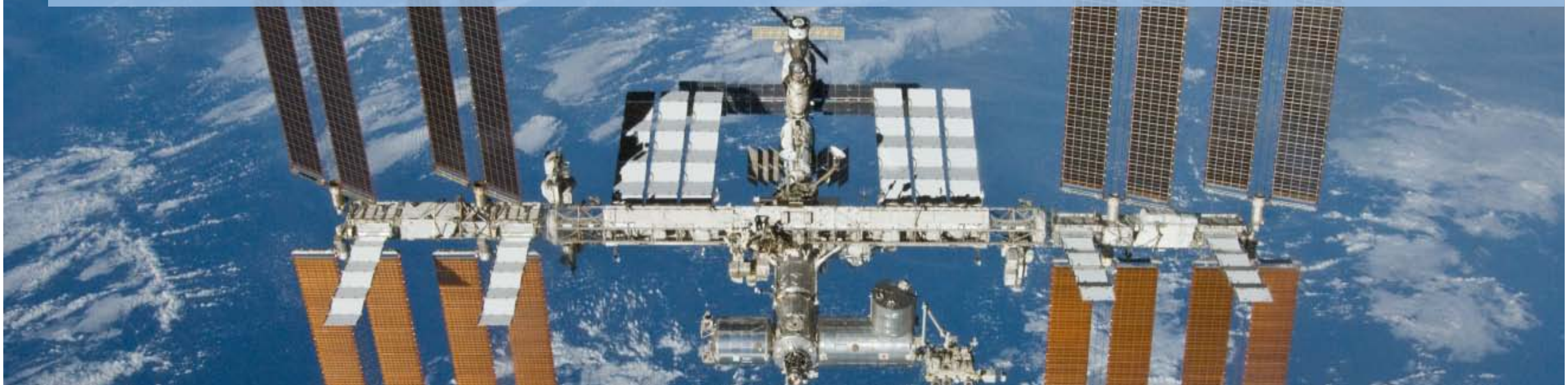

Basic Science and
Biotechnology on ISS
An opportunity
for discovery
Millie.Hughes-Fulford@ucsf.edu

Rationale for studying biological process in the unique environment offer by ISS a other space platforms

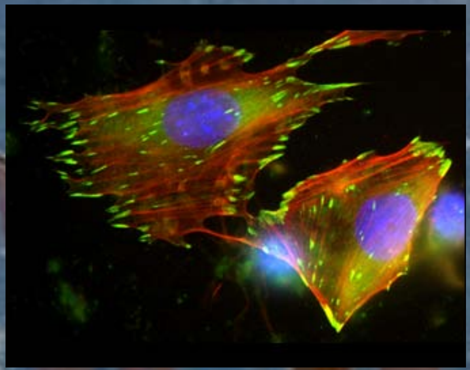


Basic and Applied Research

- What role does gravity play in biological processes at the molecular, cellular , organ and whole organism level?
- Manipulation of gravity level as a tool to study biological processes
- Transfer of knowledge to study of terrestrial problems and applications

Support long duration space exploration and application to health issues

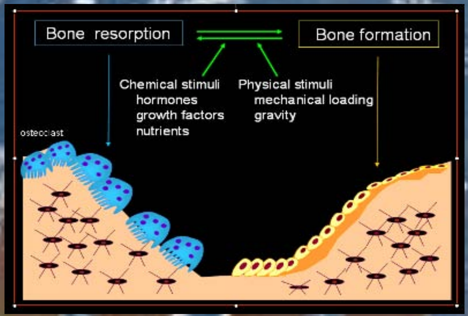
- Understand the mechanism by which spaceflight affects processes relevant to human physiology including bone loss, Immune system, cardiovascular dysfunction
- Support development of countermeasures
- Biological effects of cosmic radiation & detailed radiation dosimetry
- Application of acquired knowledge to terrestrial health issues associated with an ageing population including osteoporosis, immune dysfunction, orthostatic intolerence, and cancer



Cell, Molecular and Developmental Biology

Role of gravity in essential cell functions, including cytoskeleton, intracellular signaling and gene expression and identify any critical periods / process in development

Understanding cellular mechanisms of health issues associated with spaceflight including immune function, bone loss, cardiovascular function

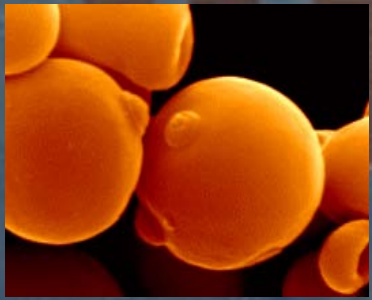


Plant Biology

Elucidate mechanisms of gravitropism and study weak phototropisms through g-dose experiments

Develop and test methodologies for spacecraft biological life support systems

Biological Science Research Fields studied aboard ISS

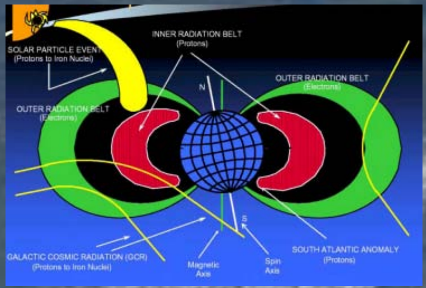


Microbiology

Role of gravity and radiation environment in bacterial and fungal growth & differentiation

Characterization of microbial environment of ISS and health risks to crew

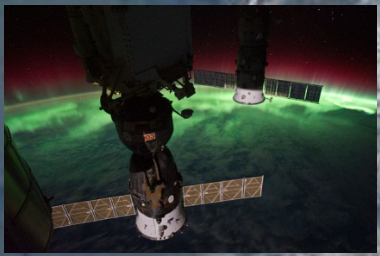
Test elements of biological life support systems in actual spaceflight environment



Radiation Biology & Dosimetry

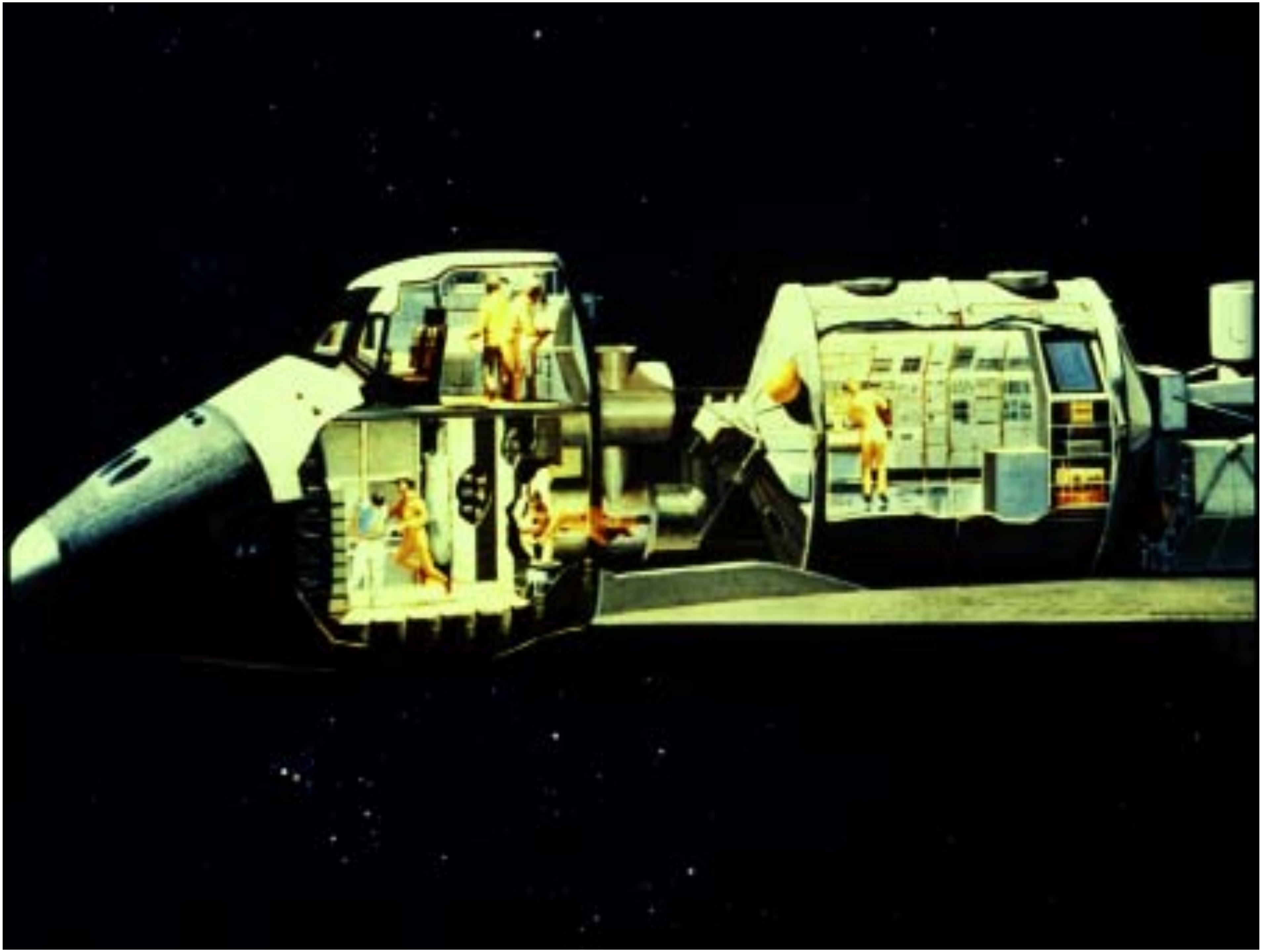
Understand the biological effects of high energy cosmic ray particles

Detailed characterization of the ISS internal radiation environment, which support development of appropriate shielding techniques



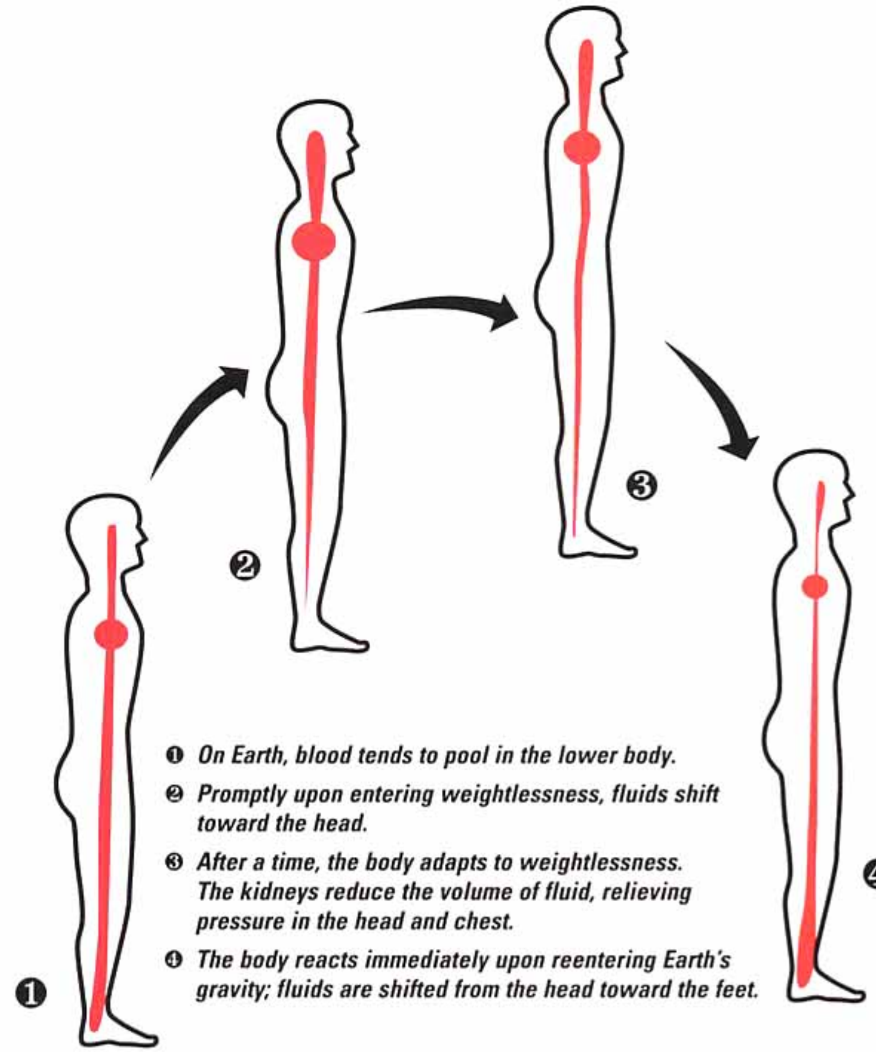
STS 40 Crew


O'CONNOR JERNIGAN GUTIERREZ
STS
40
SEDDON GAFFNEY HUGHES-FULFORD BAGIAN

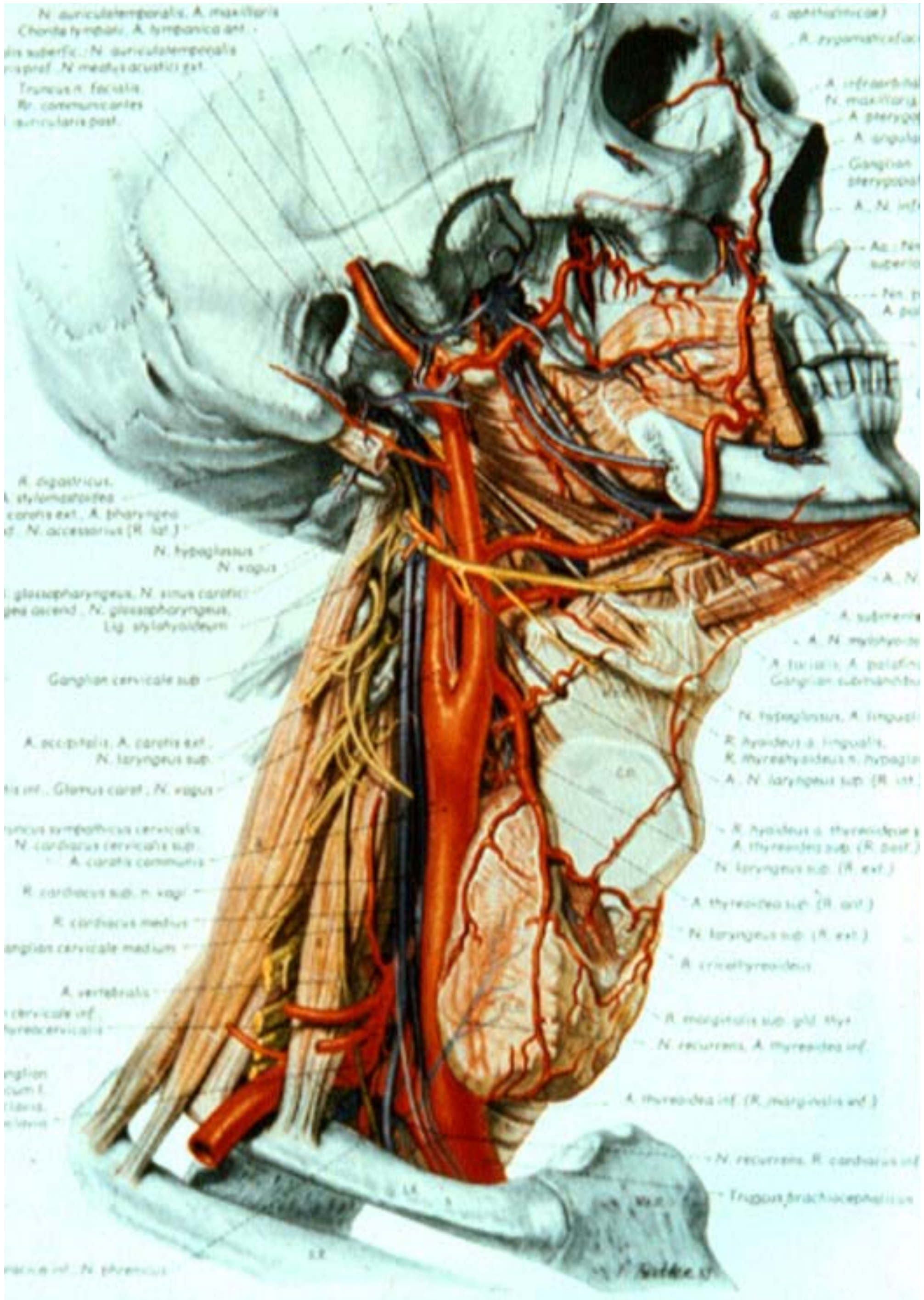


Physiological Changes That Occur in Astronauts in Spaceflight

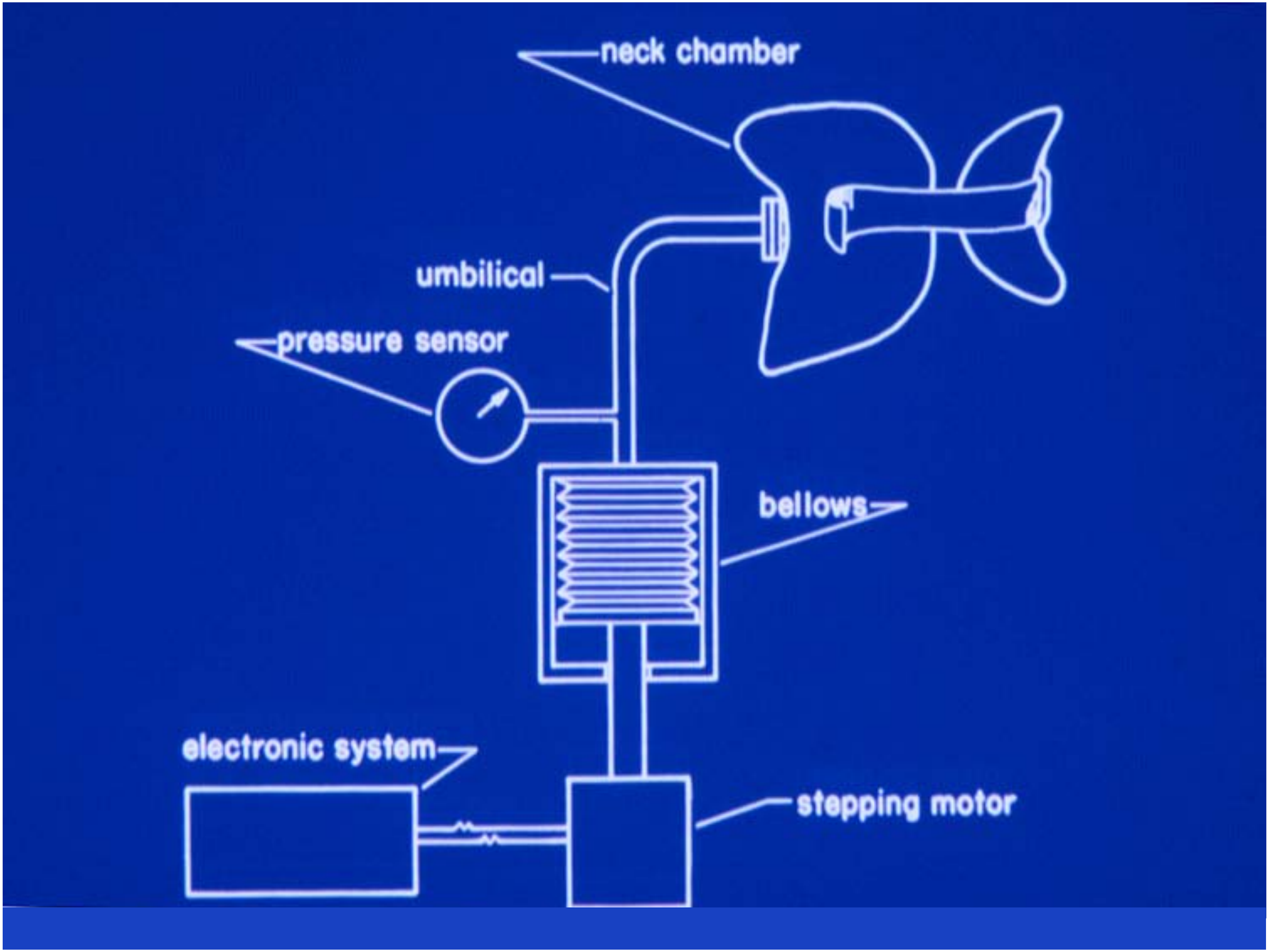
- Space Adaptation Syndrome
- Orthostatic Intolerance
- Cardiovascular Deconditioning
- Space Anemia
- Space Osteoporosis
- Loss of Immune Response

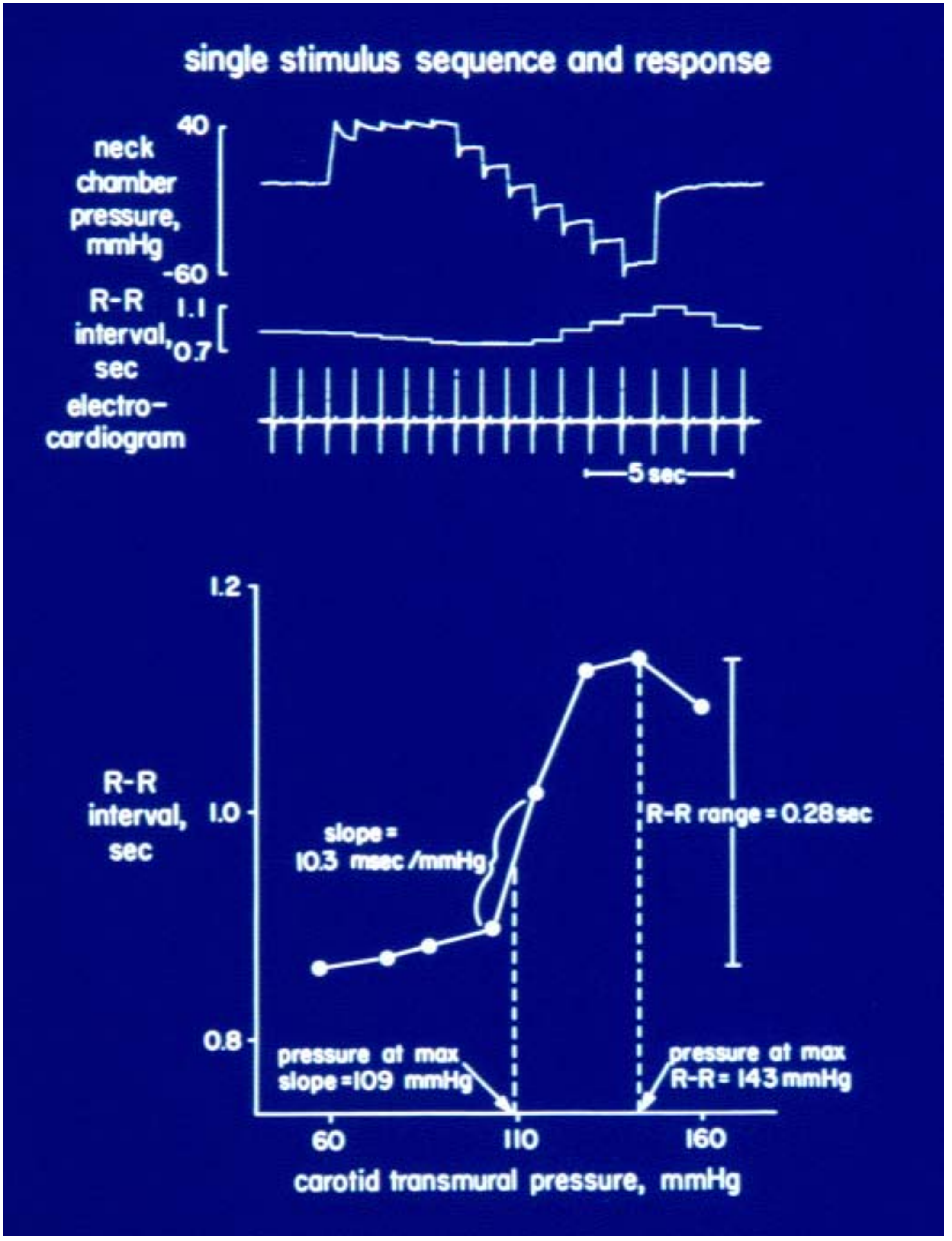

❶
❷
❸
❹
❶ On Earth, blood tends to pool in the lower body.
❷ Promptly upon entering weightlessness, fluids shift toward the head.
❸ After a time, the body adapts to weightlessness. The kidneys reduce the volume of fluid, relieving pressure in the head and chest.
❹ The body reacts immediately upon reentering Earth's gravity; fluids are shifted from the head toward the feet.

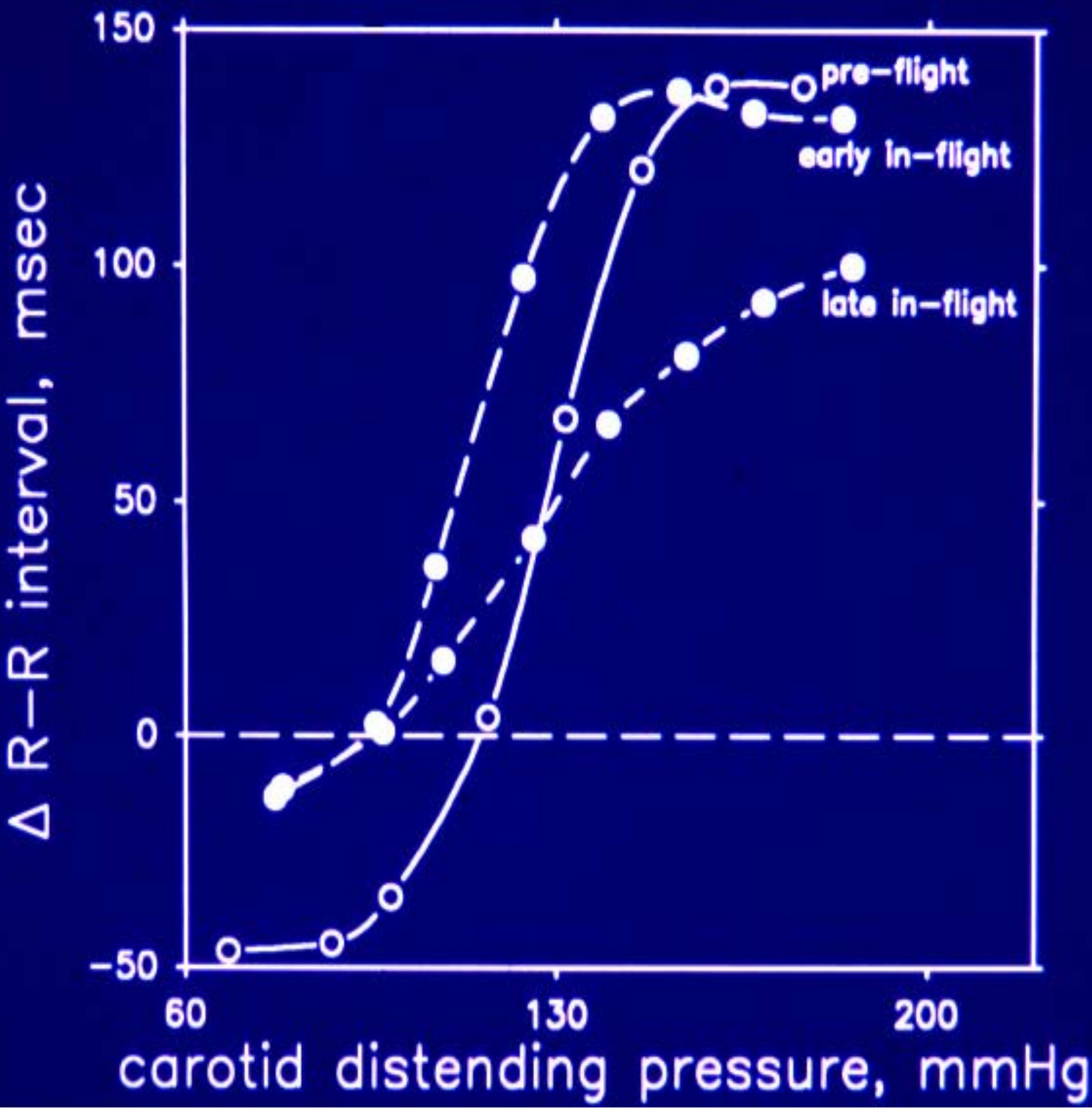

STANFORD
AFRES
CALIFORNIA
Colorado
State



N. auriculotemporalis, A. maxillaris
Chorda tympani, A. tympanica ant.
Truncus n. facialis
Rr. communicantes
R. digastricus
N. hypoglossus
N. vagus
Lig. stylohyoideum
Ganglion cervicale sup.
A. occipitalis, A. carotis ext.
N. laryngeus sup.
N. cardiacus cervicalis sup.
A. carotis communis
R. cardiacus sup. n. vagi
R. cardiacus medius
A. vertebralis
R. hyoideus a. lingualis
N. laryngeus sup. (R. ext.)
A. thyreoidea sup. (R. ant.)
R. cricothyreoideus
R. marginalis sup. gld. thyr.
N. recurrens, A. thyreoidea inf.
A. thyreoidea inf. (R. marginalis inf.)
Trigonum brachiocephalicum

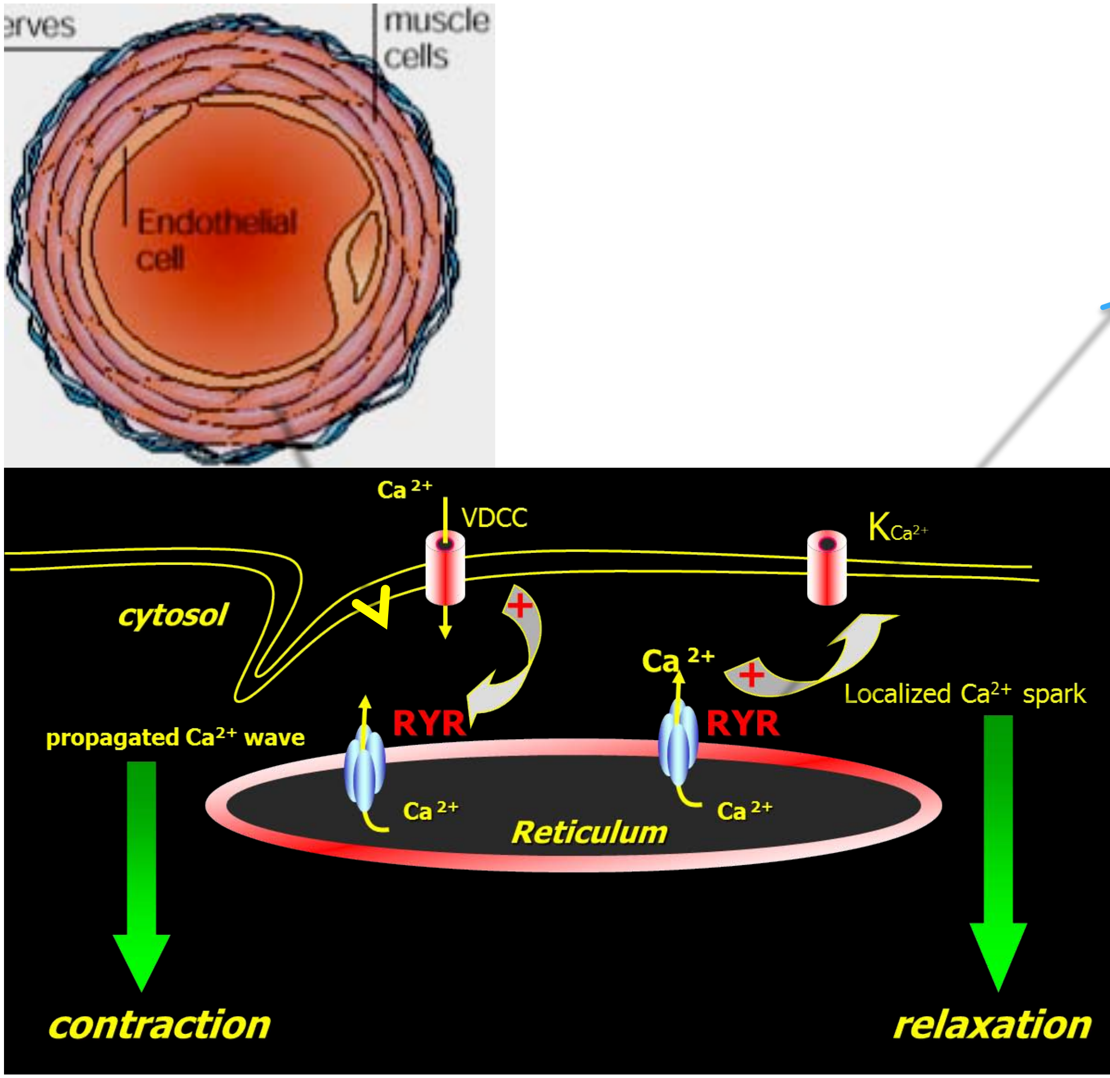

neck chamber
umbilical
pressure sensor
bellows
electronic system
stepping motor


single stimulus sequence and response
neck chamber pressure, mmHg
40
-60
R-R interval, sec
1.1
0.7
electro-cardiogram
5 sec
1.2
1.0
0.8
R-R interval, sec
slope = 10.3 msec/mmHg
R-R range = 0.28 sec
pressure at max slope = 109 mmHg
pressure at max R-R = 143 mmHg
60
110
160
carotid transmural pressure, mmHg



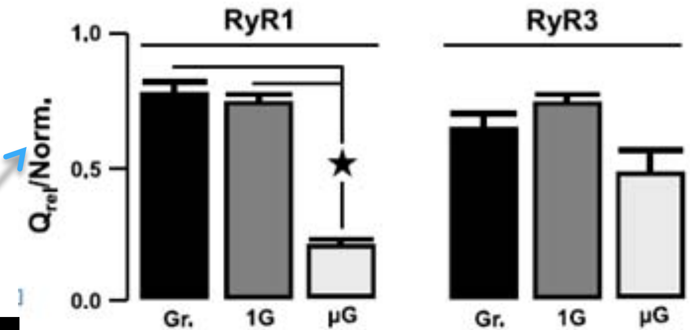
150
100
50
0
-50
60
130
200
Δ R-R interval, msec
carotid distending pressure, mmHg
pre-flight
early in-flight
late in-flight

Changes in vascular smooth muscle cell contraction regulating calcium channels – relevance to orthostatic intolerence

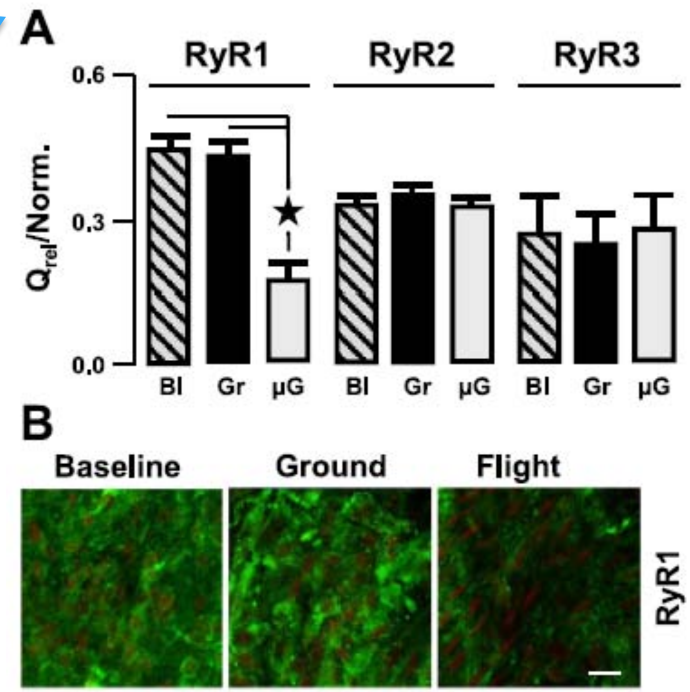


Spaceflight regulates ryanodine receptor subtype 1 in portal vein myocytes in the opposite way of hypertension Fabrice Dabertrand, F Yves Porte, Nathalie Macrez and Jean-Luc Morel *J Appl Physiol* **112**:471-480, (2012)

ISS KUBIK MYOCYTE
Portal Vein Myocyte Cultures



STS-118 Mouse Portal Vein



Bone Loss on Apollo Missions approximately 2% in less than two weeks

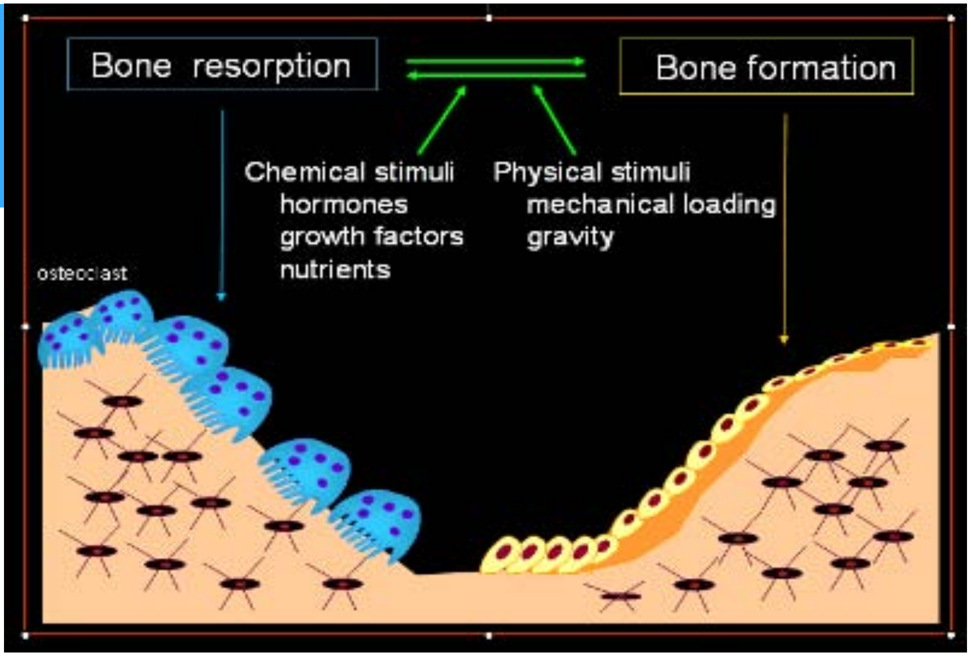
Loss of OS Calcis Bone

3
2
1
Skylab 2
0
Skylab 3
%Δ -1
-2
Apollo 14, 16, 18
-3
-4
Skylab 4
-5
- 6

10 20 30 40 50 60 70 80 90

DAYS

Biology spaceflight research achievements



Microgravity changes in bone cells have implications for finding the cause of space osteoporosis.

ISS experiments have shown that:

• Bone marrow stromal cells (stem cell) had inhibited differentiation into osteoblasts.

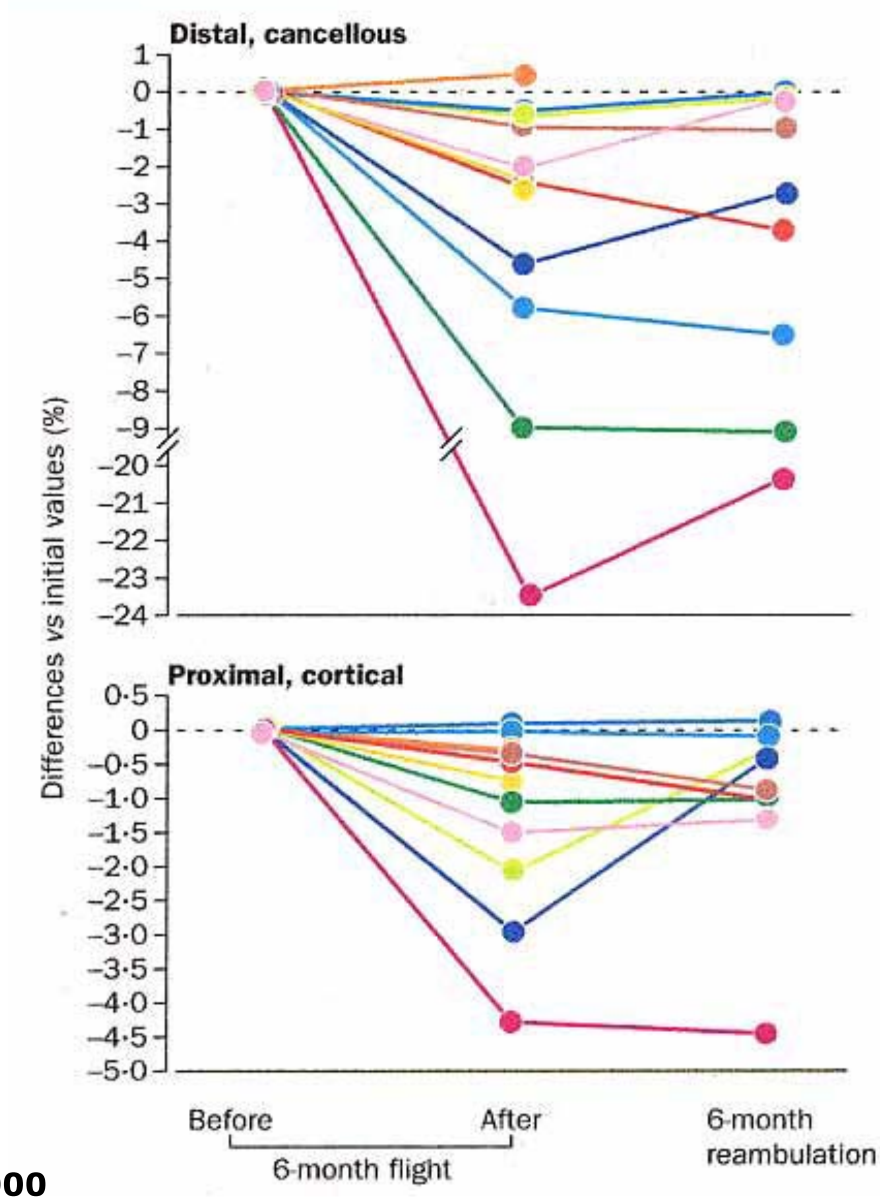
• Osteoblasts had reduced growth in spaceflight and had reduced gene expression of key bone growth factors.

• Several other studies have shown that osteoblasts have reduced growth in microgravity and altered gene expression.

•Osteoclast maturation and activity has been observed to be increased.

•The results of these experiments show that different cell types involved in bone maintenance have different responses to the unloaded microgravity environment. Therefore, this provides several potential cell types and molecular pathways which may be targeted for development of countermeasures against bone loss.

BMD changes in tibia



Vico et al, Lancet 2000

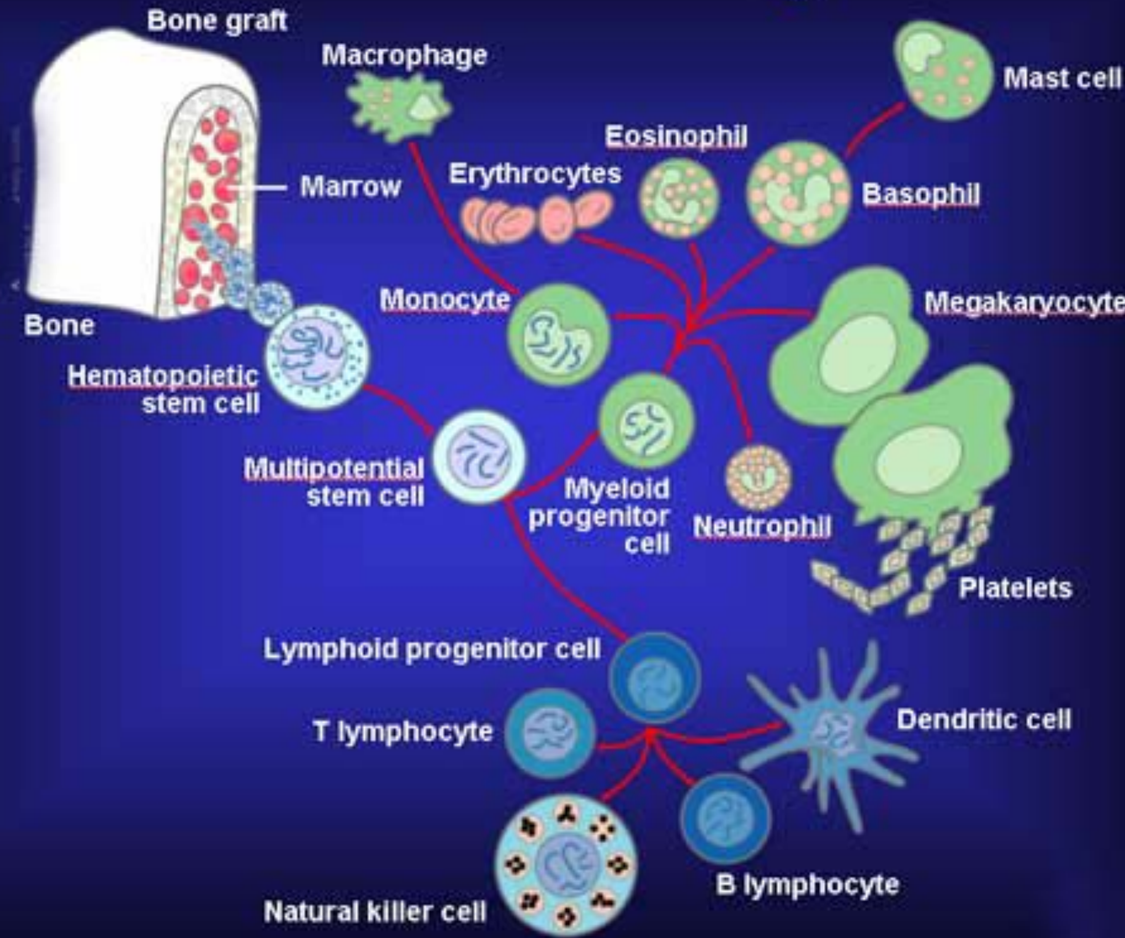
Immune response was inhibited in returning Apollo astronauts

Infection in Spaceflight

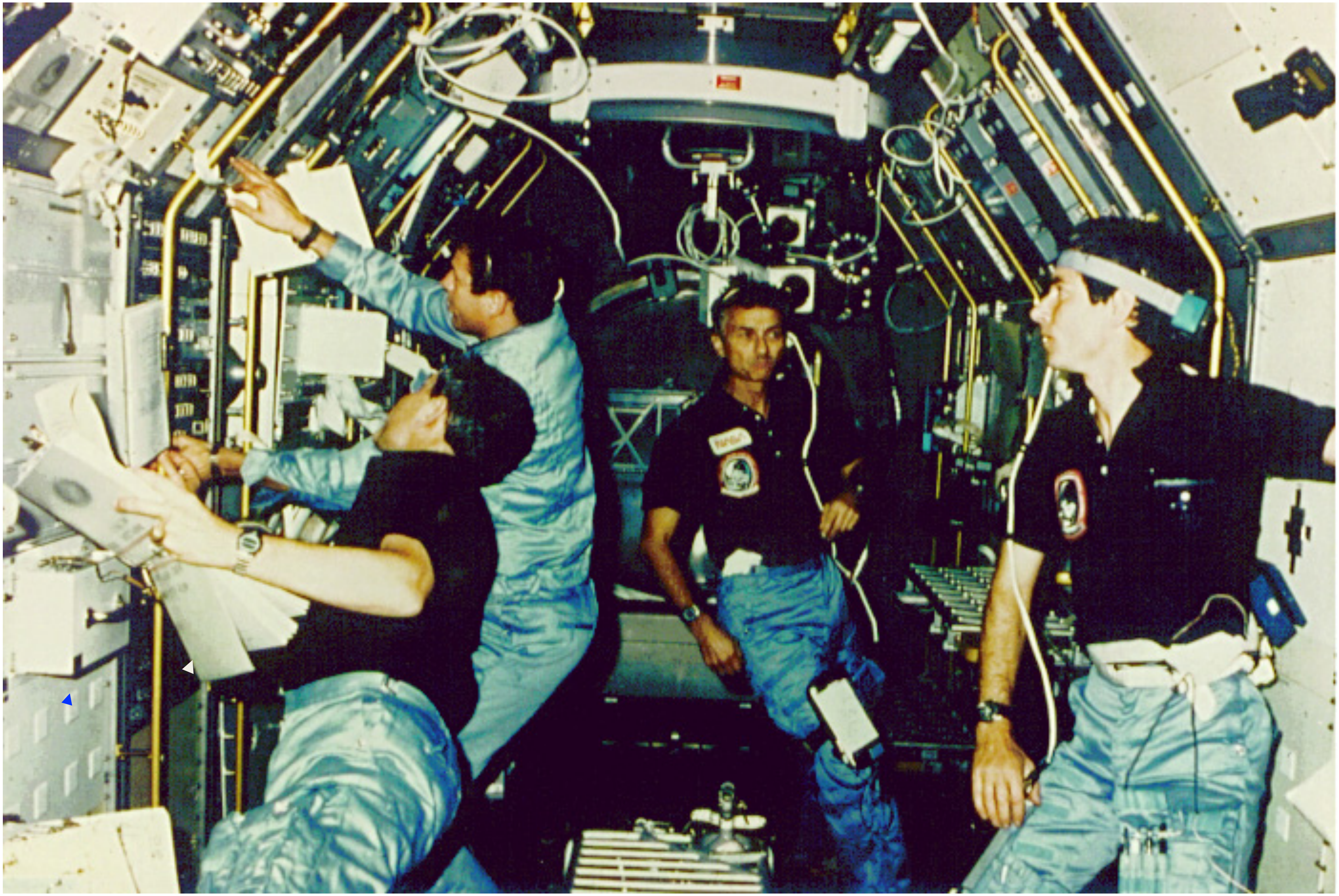
- Apollo 7 all three crew members had a cold.
- Apollo 13, one of the crew members became ill with a common bacteria which usually does not affect people with normal immune systems.
- In all, 15 of the 29 Apollo astronauts had an infection either during flight or within one week after flight.

(Biomedical Results of Apollo, Johnston, Dietlein, and Berry, NASA Washington, D.C. 1975

Cells of the Immune System



Spacelab 1, November 1983



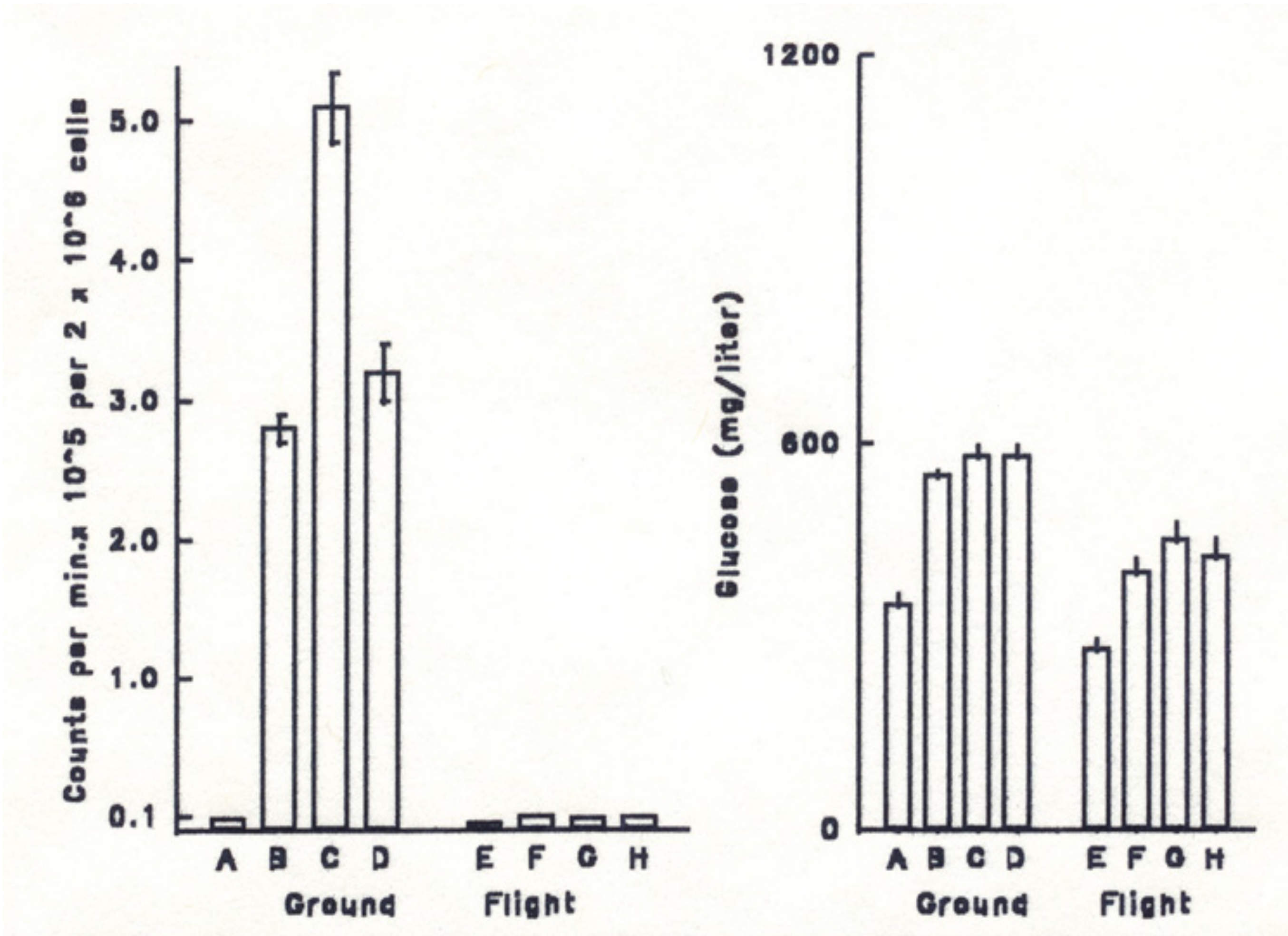
Science

Cell Sensitivity to Gravity

Abstract. *Cultures of human lymphocytes exposed in microgravity to the mitogen concanavalin A showed less than 3 percent of the activation of ground controls. This result supports the hypothesis, based on simulations at low* g *and experiments at high* g, *that microgravity depresses whereas high gravity enhances cell proliferation rates. The effects of gravity are particularly strong in cells undergoing differentiation.*

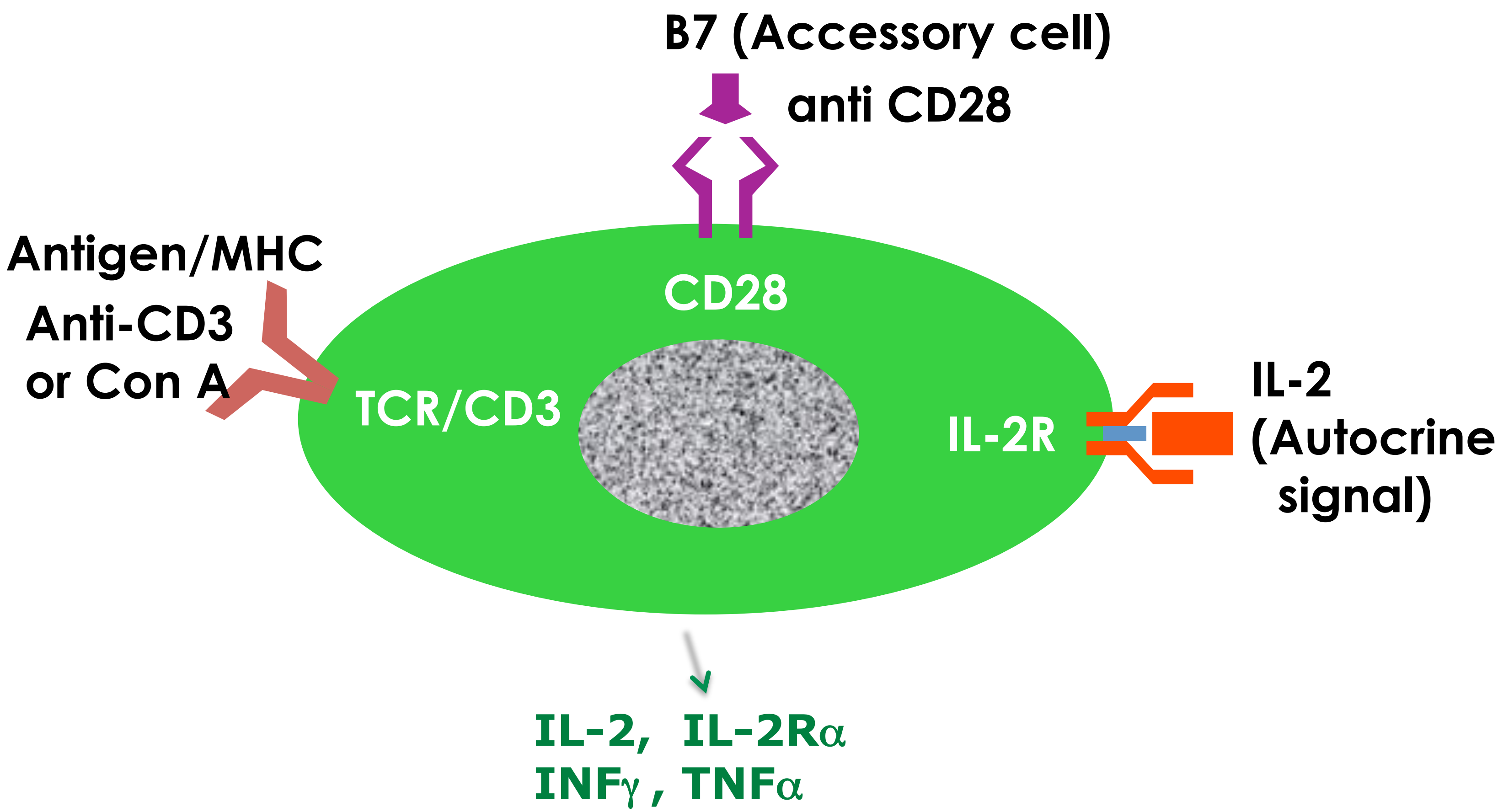
An answer to the question of whether cells are sensitive to gravity was one of the objectives of experiment 1ES031, on the effect of weightlessness on lymphocyte proliferation, performed on board Spacelab 1. Another objective was to establish, by exposing cultures of human lymphocytes to a mitogen during spaceflight, whether functional changes occurred in the cells responsible for the immune response. Several investigators duction was recently observed in cultures of human lymphocytes flown on the Soviet space station Salyut 6 (*3*). One of the few experiments dedicated so far (*4*) to the study of the effects of microgravity on cells was performed on Skylab with a strain of human embryonic lung cells, WI-38 (*5*). Although there were no significant changes in cell proliferation and motility, glucose consumption was significantly lower in the cells

Con-A Activation of Lymphocytes in Microgravity

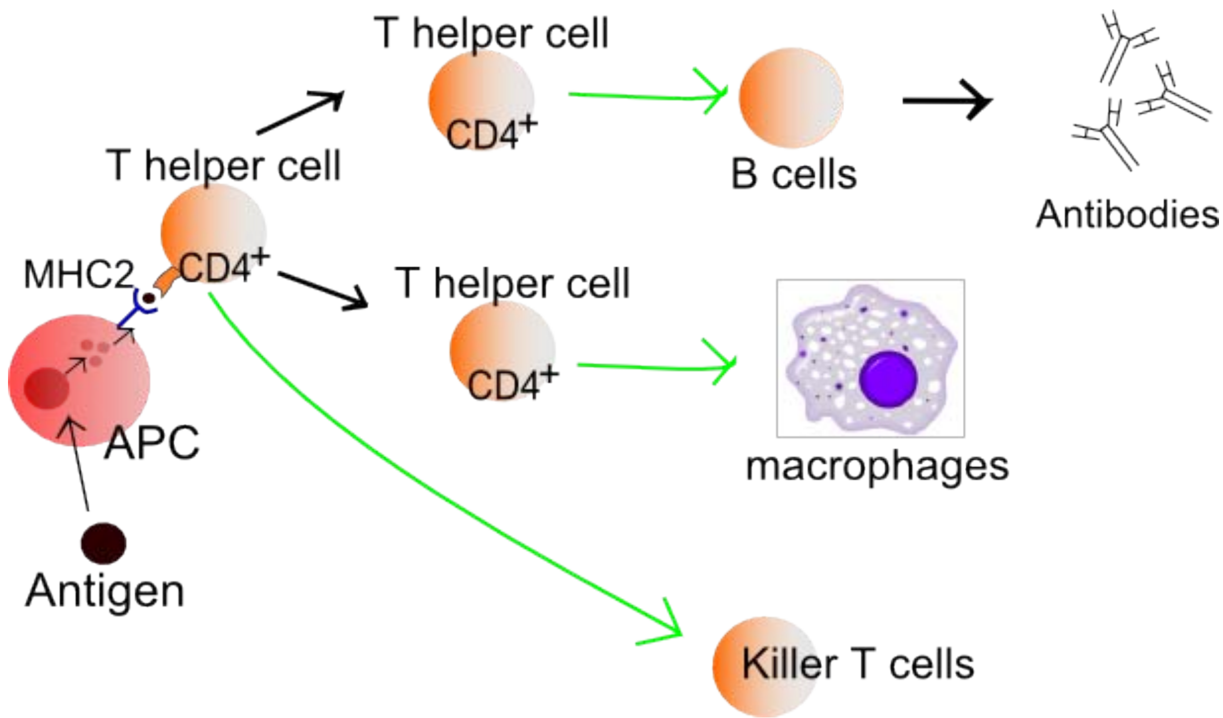


Cogoli et al 2000

The 3 signals required for full T cell activation



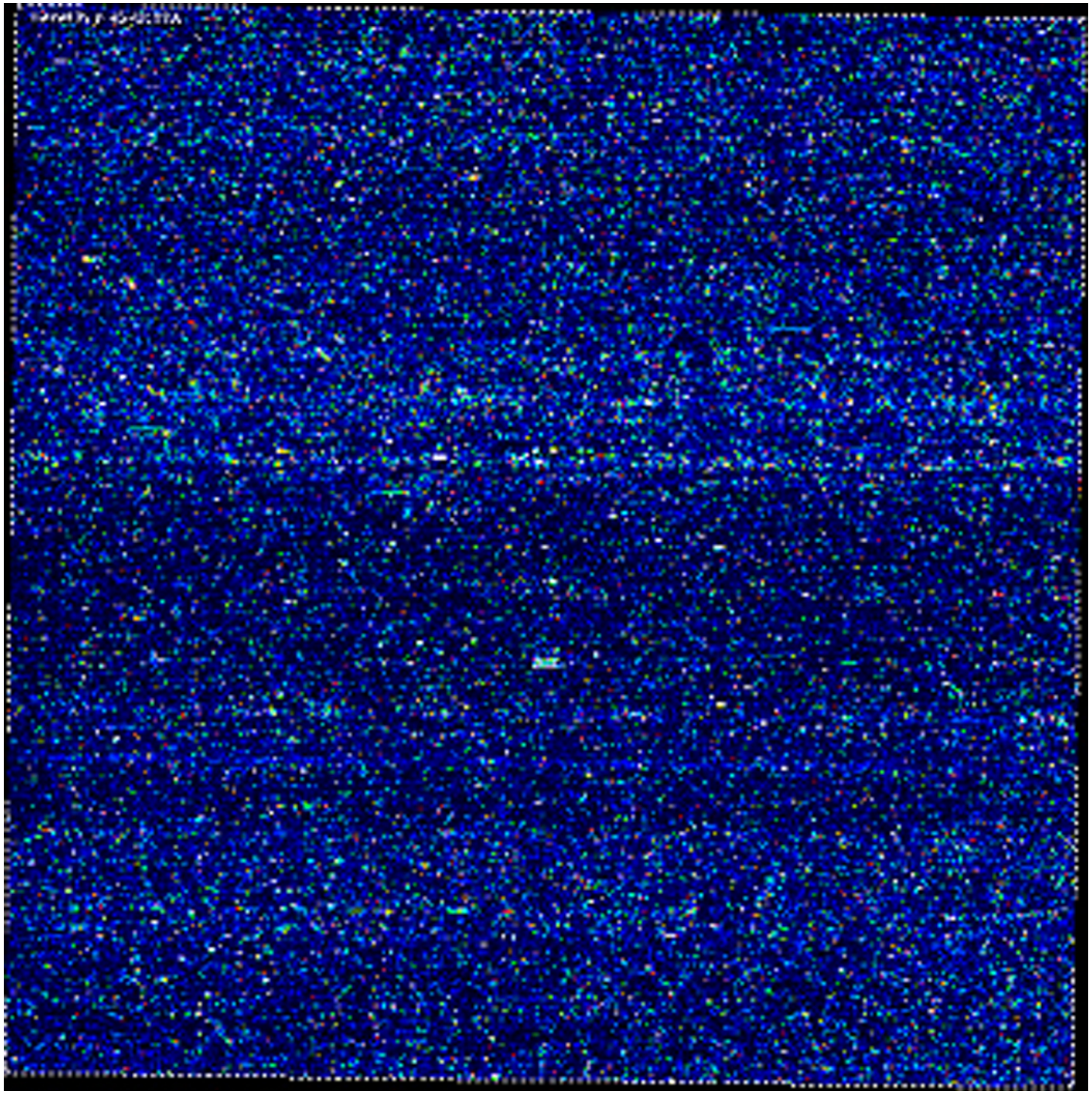
Activation of T cells in the Immune System



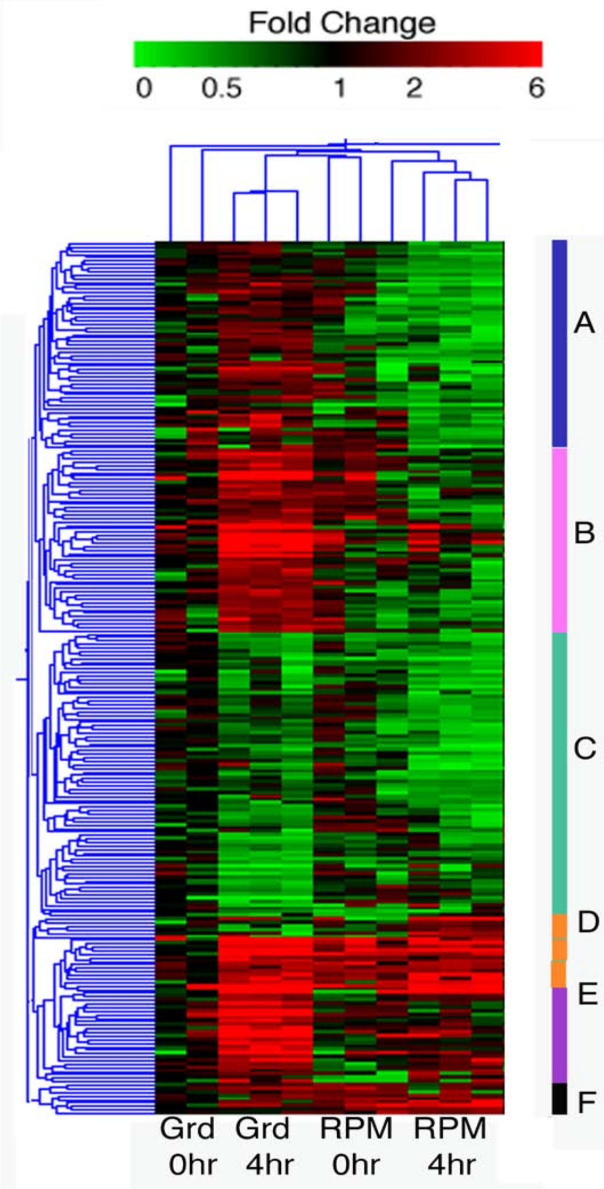
Random Positioning Machine

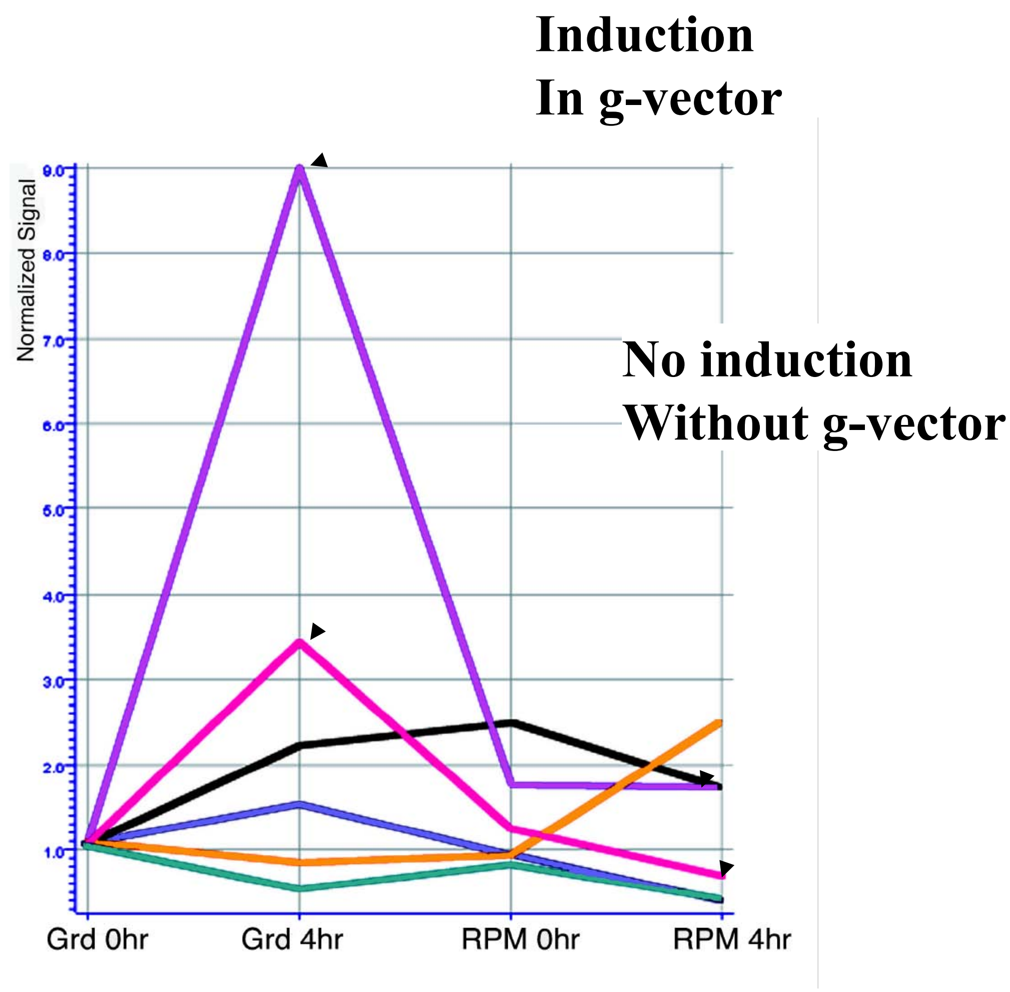

GeneChip®
Human Genome U133A Array
P/N: 510681
Lot #: 3001322
Exp. Date: 03/08/04
For Research Use Only

Visualization of gene array chip



Hierarchical clustering of gene expression profiles in T-cells activated on ground or on the RPM





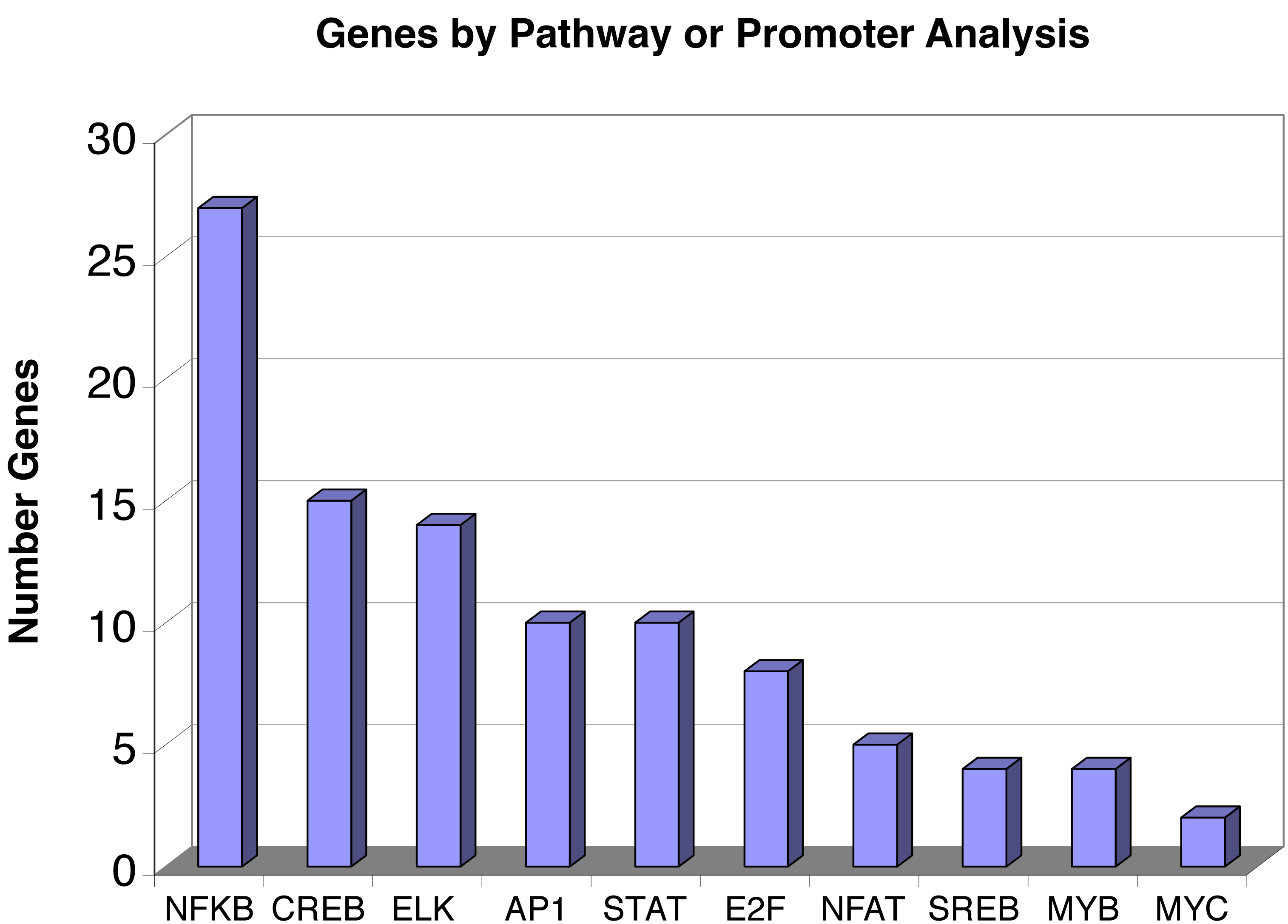
Boonyaratanakornkit et al FASEB J

Key genes and transcription factors

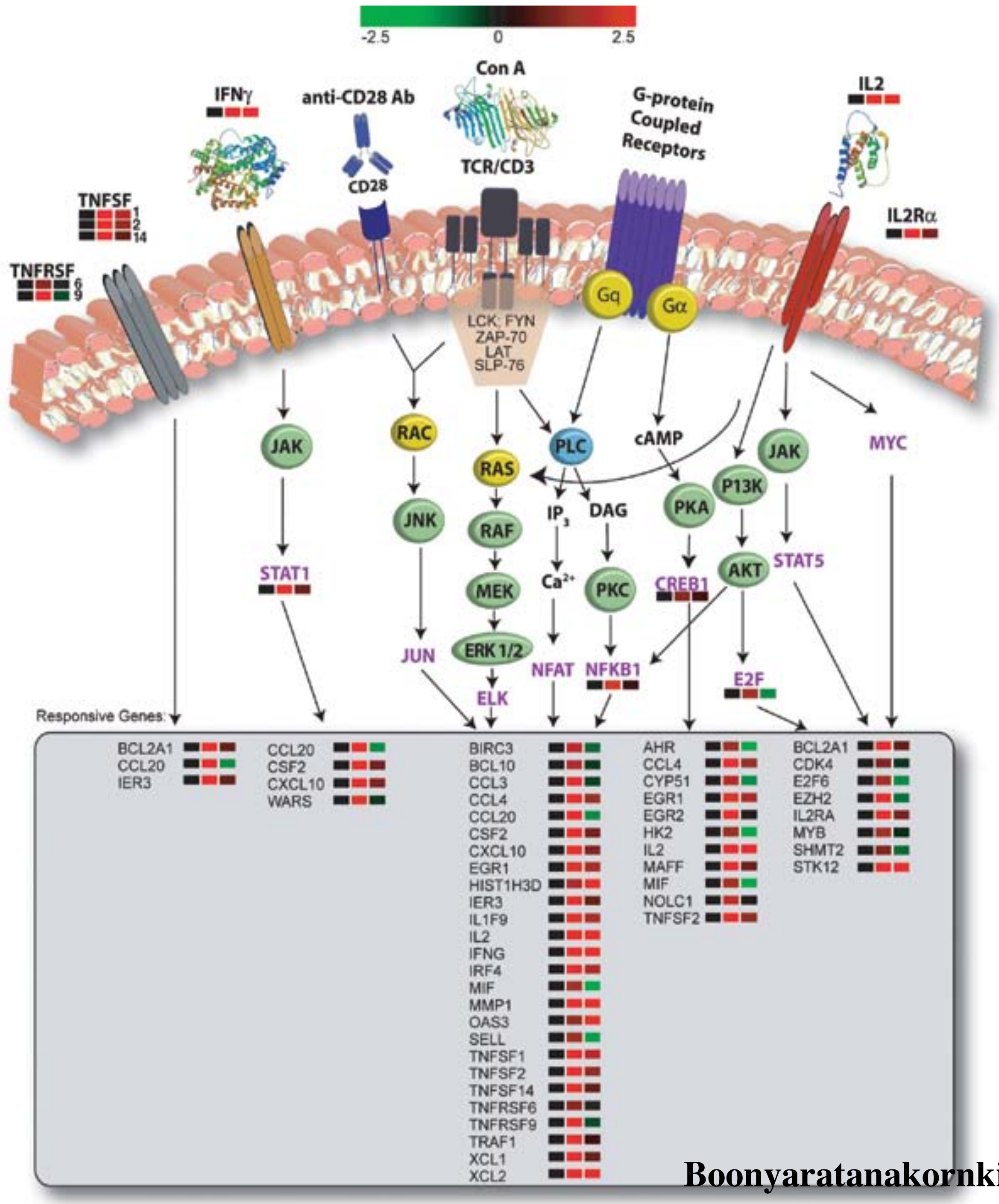
Gene Symbol	1g 0 hr	1g 4 hr	vg 4 hr	Pathways Involved
	Expression Ratios			
IFNG	1.1	**2147.2**	38.6	NFKB (AKT, PKC); STAT (JAK)
XCL2	0.9	**300.4**	**13.7**	NFAT (Ca^{2+})
IL2RA	0.9	**52.5**	**2.4**	STAT (JAK)
IL2	0.9	**12.3**	1.1	AP1 (JNK); CREB (MEK, PKA, PKC); NFAT (Ca^{2+}); NFKB (AKT, PKC); STAT (JAK)
CSF2	1.0	**6.4**	**3.0**	NFKB (AKT, PKC); STAT (JAK)
STAT1	1.1	**3.6**	1.6	STAT (JAK)
LTA	0.8	**3.1**	**1.5**	NFKB (AKT, PKC)
TNFA	0.9	**2.7**	1.4	AP1 (JNK); CREB (MEK, PKA, PKC); NFAT (Ca^{2+}); NFKB (PKC, AKT)
MIF	0.5	**2.5**	0.5	AP1 (JNK); CREB (MEK, PKA, PKC)
NFKB1	0.8	**2.2**	0.9	NFKB (AKT, PKC)

Boonyaratanakornkit et al FASEB J

92 reasons for a failure of T-cell activation in simulated μg



Boonyaratanakornkit et al FASEB J



Boonyaratanakornkit et al FASEB J

LEUKIN experiment, Launch Site, Baikonur, Kazakhstan


TU-134A-3
KOSMOS


РКК „ЭНЕРГИЯ"
им. С.П. КОРОЛЕВА

LUKOIL DRAG RACING
LUKOIL DRAG RACING
х634х
РКК Энергия
им. С.П.Королева
х634хх


LEUKIN 21
lysing 2
LEUKIN 17
activator


A2
A3
B6
B5
B4
B3
B1
C1
C2
C3
C5
A7

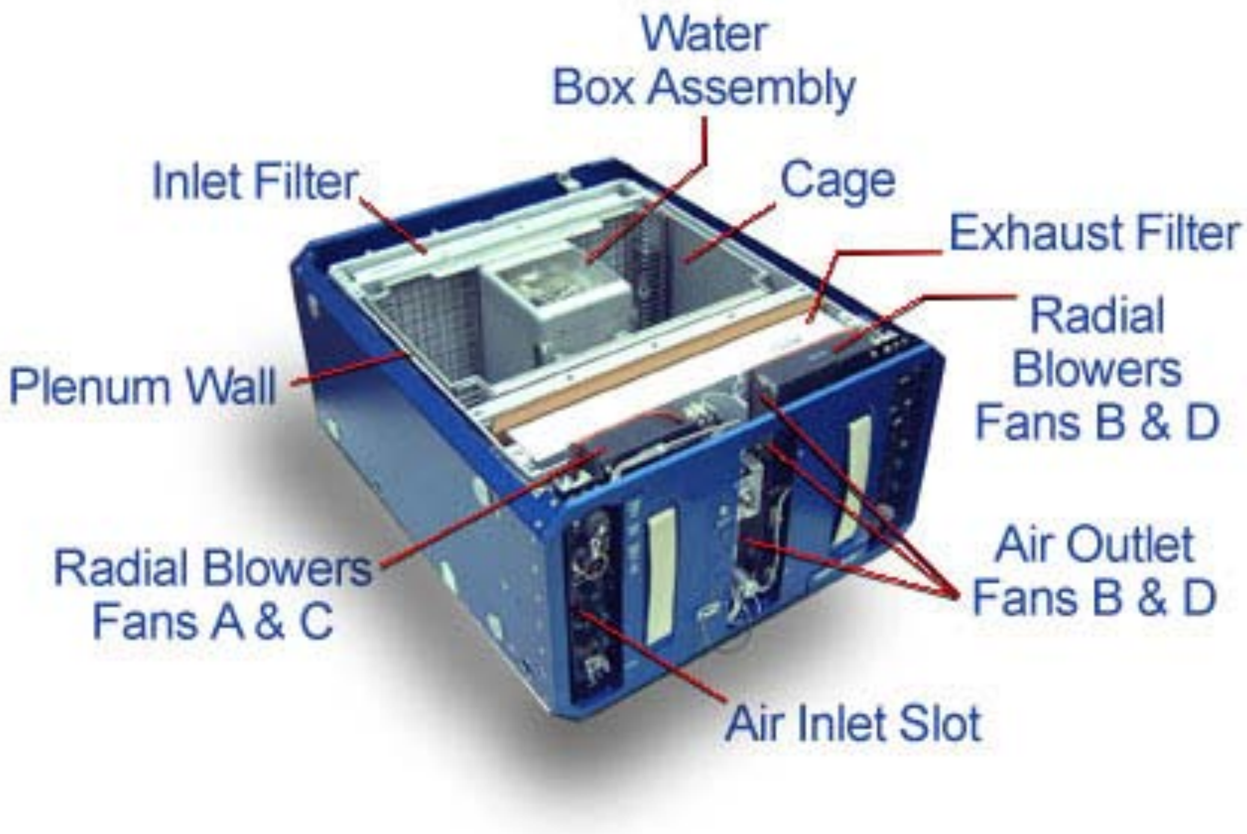
Heat Map form Activation of T cells in Spaceflight

Heat map showed common gene expression as seen
In the RPM ground data. Figure not shown since it
Is under review.

Are T cells in other mammals activated normally in spaceflight?

Test the effects of microgravity *in vivo* in spaceflight in the mouse model on STS-131.

ANIMAL ENCLOSURE MODULE



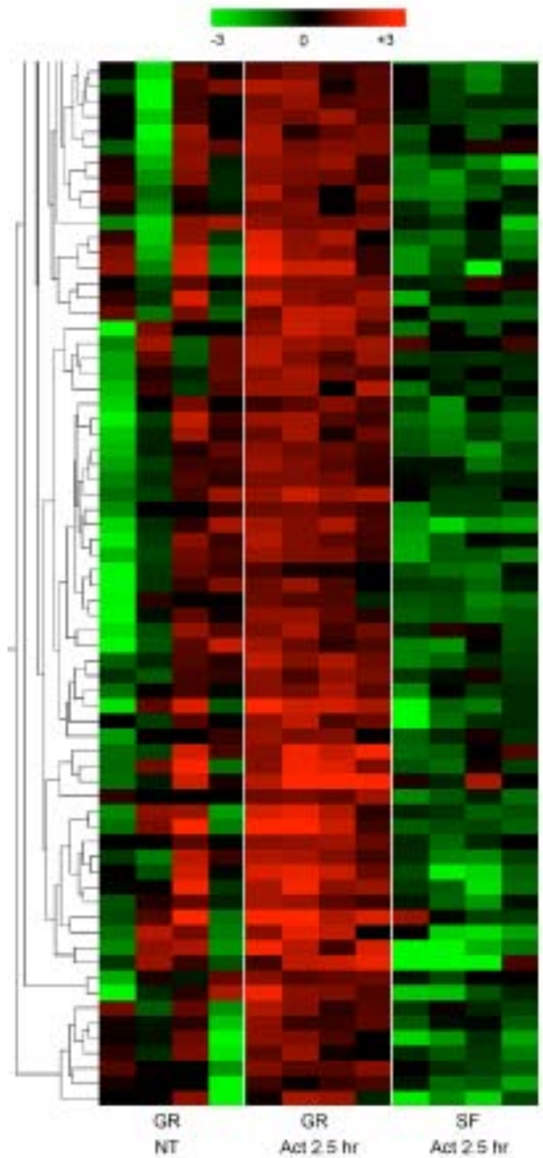
Experimental Design for C57BL/6 mouse STS-131 T cell activation Experiment

STS-131 T-Cell-Prime

Experimental Read-outs

Parameter	Method
T cell maturation *in vivo*	flow cytometry
T cell cytokine production	*in vitro* stimulation, culture supernatant ELISA
T-cell global gene expression	*in vitro* restimulation, RNA isolation, microarray analysis and QRTPCR

Gene Array Heat Map analysis of Mouse T-cells



Conclusions

- Taken together, these data suggest a physiological dependence of the mouse immune system on gravity.

- These data also suggest that at least two mammalian immune systems that developed on this planet are dependent on gravity for full function.

Chemokines and cytokines reduced in space

- The standard chemokines and cytokines that are upregulated in T cell activation were severely and significantly reduced in spaceflown mice.

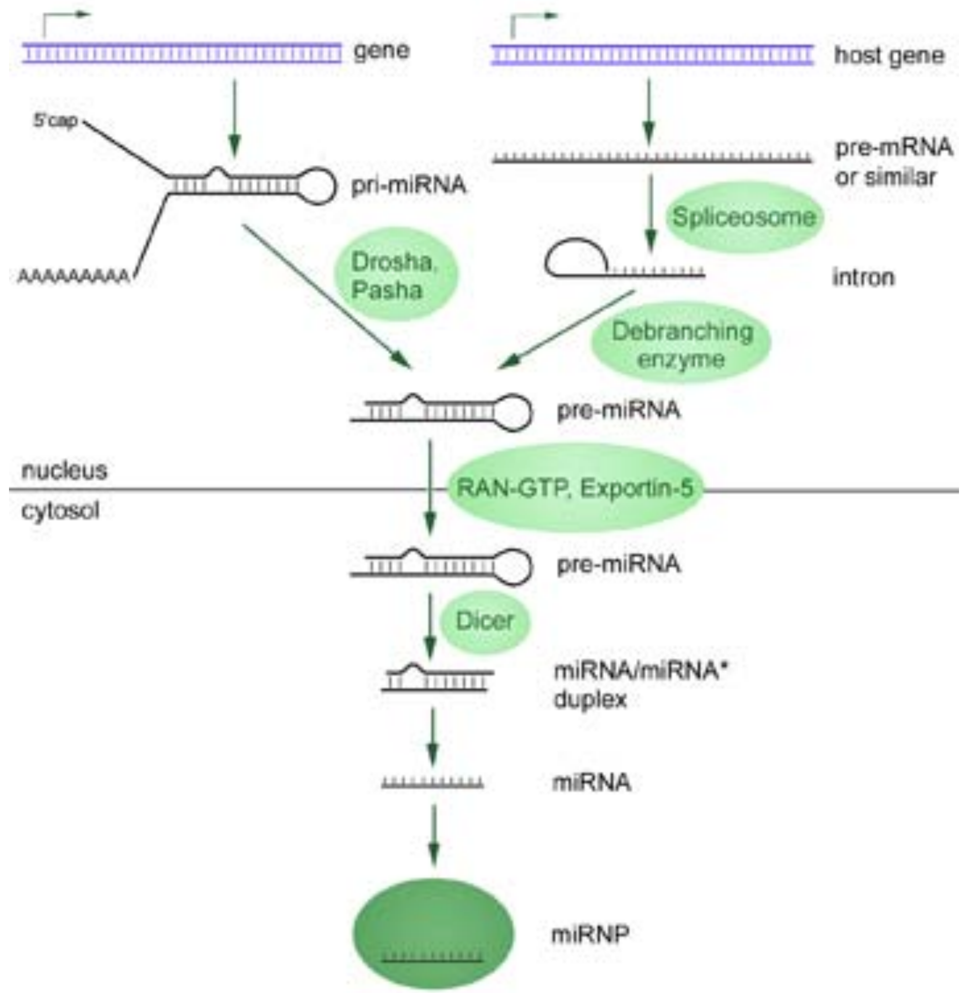
Chemokines and cytokines reduced in space

- The standard chemokines and cytokines that are upregulated in T cell activation were severely and significantly reduced in spaceflown mice.

Newly discovered miRNA regulates protein synthesis

- MicroRNA (miRNA) are small non-coding RNAs about 20 nucleotides in length
- Only recently scientists have found that miRNAs regulate mammalian protein regulation.
- miRNAs act as cell rheostats to regulate cell growth and function.

Synthesis of miRNA

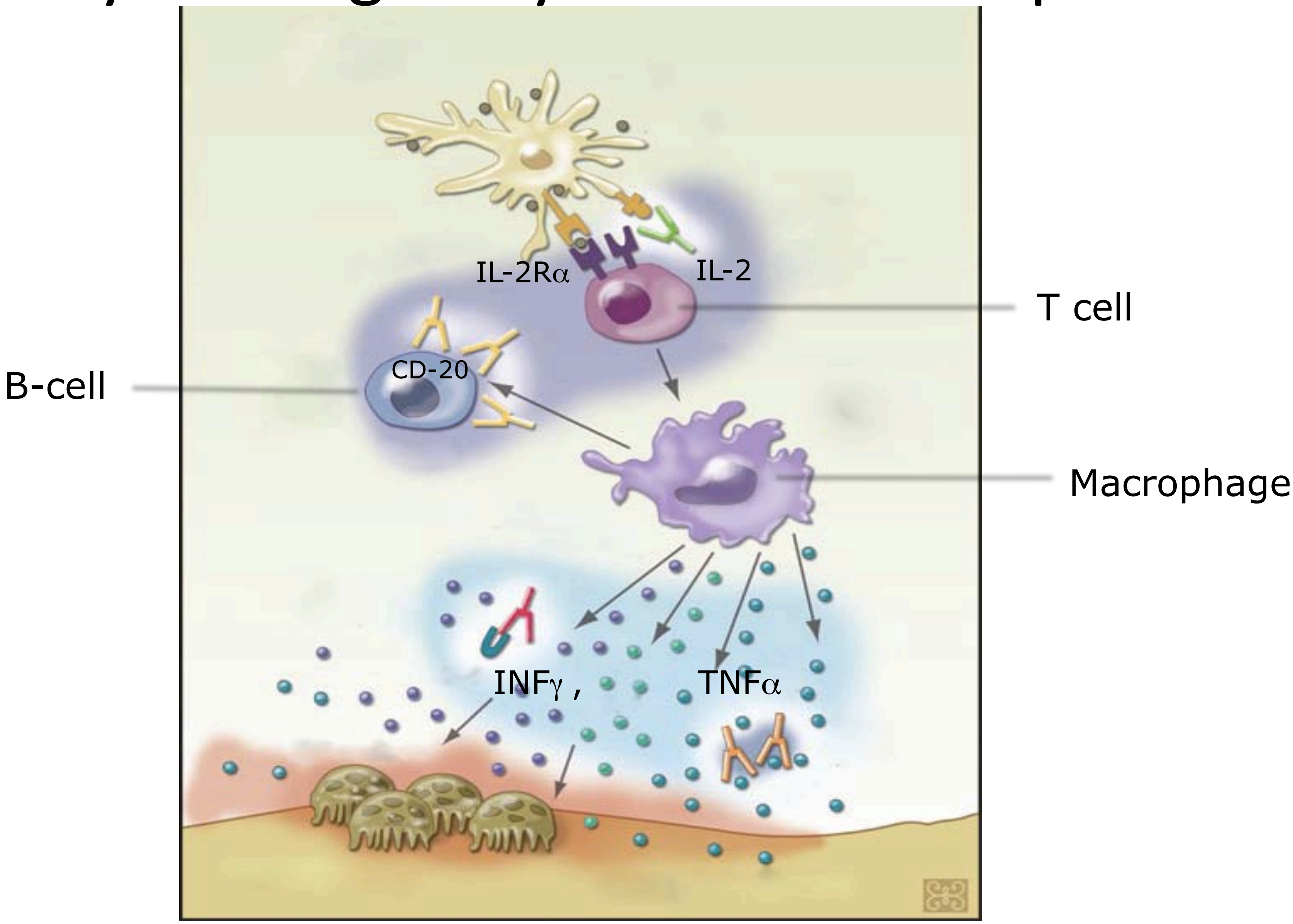


Is miRNA Regulated in the Human Immune System?

- The Leukin experiment was designed to analyze RNA expression, and majority of the miRNAs were lost during extraction.

- Analysis showed a dysregulation of miR-21 synthesis.

Biological Drugs Act on the Immune System by Binding to Cytokines or receptors



miRNA working as a rheostat may become a more finely-tuned new class of drug

- Treatment of rheumatoid arthritis
- Autoimmune disease
- Prevention of rejection of organ transplants
- Treatment of leukemias

Upcoming NIH Studies of the Immune Function in Spaceflight and Aging on ISS

- Due to comorbidies found in the aging population, it is difficult to understand the specific cause of immune loss in the elderly.
- Many of the same hallmarks of immune loss in spaceflight are the same as in aging. e.g. lower IL-2Rα expression, the inability to properly activate the T cell and respond to infection.
- In our upcoming studies on ISS, we will investigate the changes in T cell activation in spaceflight.


esa
2
Comat

- We will examine the regulation of immune activation of human cells in spaceflight using micro RNA arrays and miRNA arrays. Develop best model for regulation.
- We will then analyze the immune function in an aging population vs T cells in controls that are younger than 55 and space-flown T cells. We will find and test new pathways of miRNA regulation of the immune system.

Areas of Studies for Biological Discoveries of New Drug Targets

- Human physiological changes-
  - Orthostatic intolerance
  - Space anemia
  - Cardiovascular deconditioning
  - Muscle loss
  - Immune response of memory cells, B-cells and macrophages
  - Osteoporosis

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Chai Fei Li, DVM
Jim Boonyaratanakornkit, Ph.D , MD
Tammy Chang, MD
Marlene Grenon, MD
Sandra Spurlock
Tara Candelario

Joe Messier
Jesus Aguago

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Millie.Hughes-Fulford@ucsf.edu