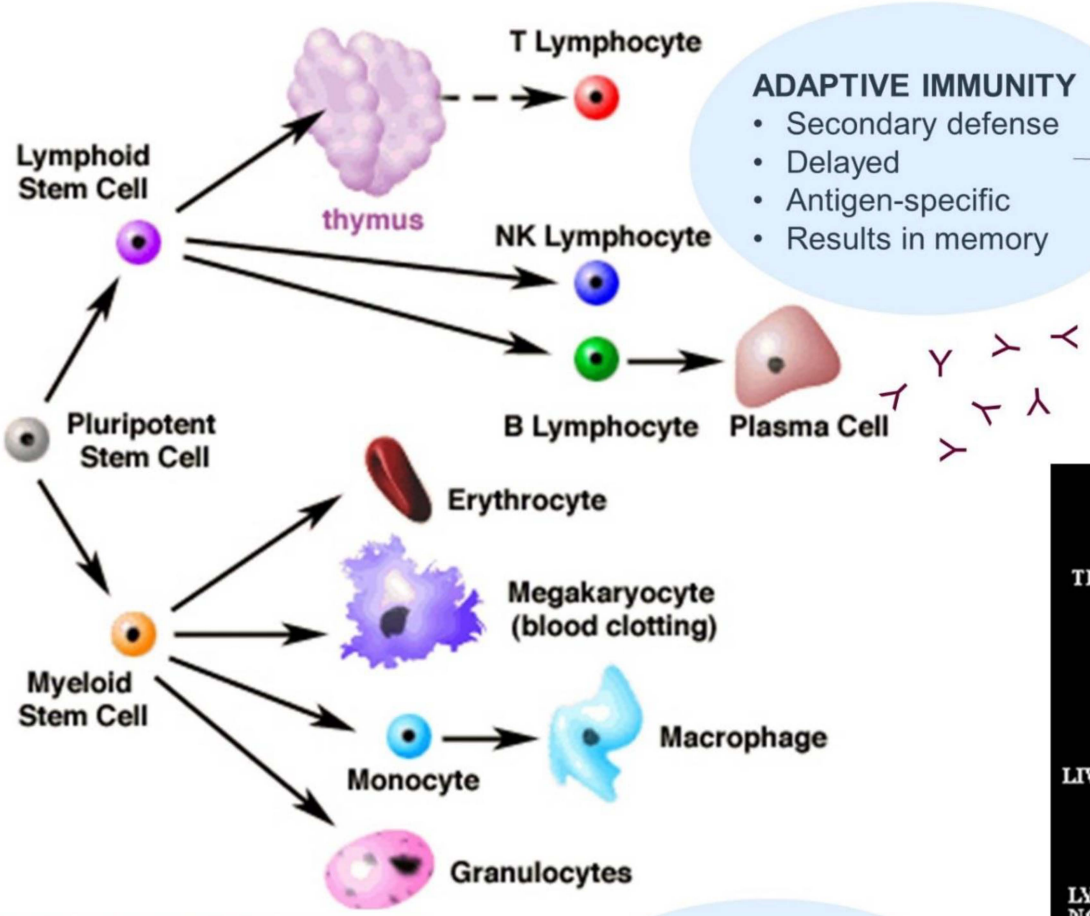
UPDATE ON SPACEFLIGHT IMMUNE SYSTEM DYSREGULATION, CLINICAL RISKS FOR DEEP SPACE MISSIONS, POTENTIAL COUNTERMEASURES

Brian Crucian, PhD, MT(ASCP)

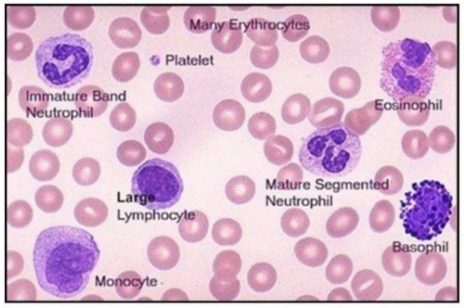
The Immune System



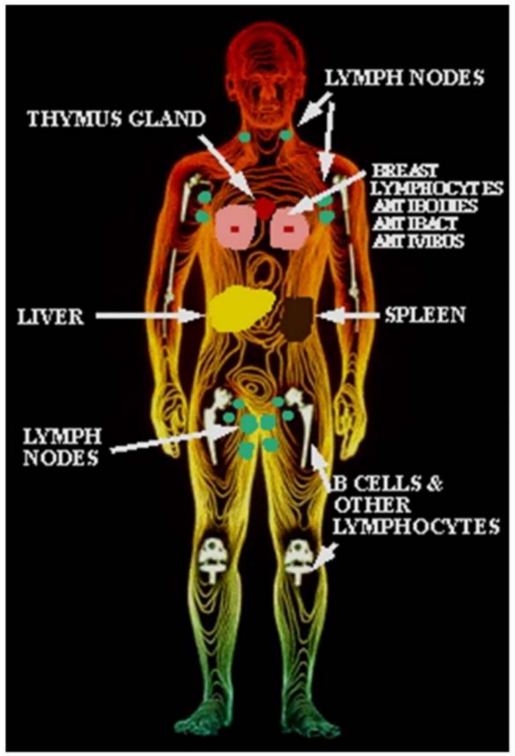
ADAPTIVE IMMUNITY
- Secondary defense
- Delayed
- Antigen-specific
- Results in memory

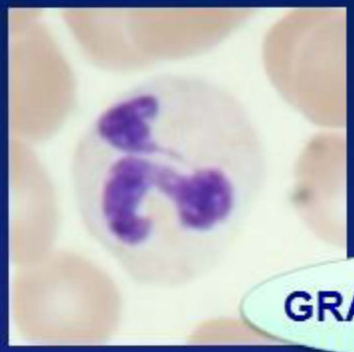
Cell mediated immunity:
Mediated by cytotoxic T lymphocytes which destroy viral infected cells, transplant cells, some tumor cells

Humoral immunity:
Mediated by B cells/ Plasmacytes. Antibodies bind specific antigens, signals other cells to engulf and remove that target from the body.

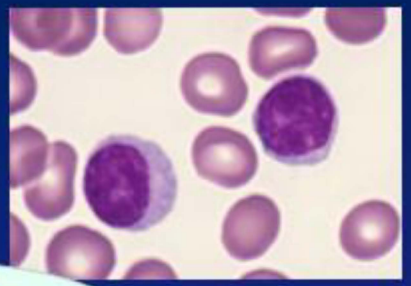


INNATE IMMUNITY
- Primary defense
- Immediate
- Non-specific
- Does not result in memory





WHITE BLOOD CELLS + RED BLOOD CELLS



GRANULOCYTES
MONOCYTES
LYMPHOCYTES

BASOPHILS
NEUTROPHILS
EOSINOPHILS

B CELLS
T CELLS
NK CELLS

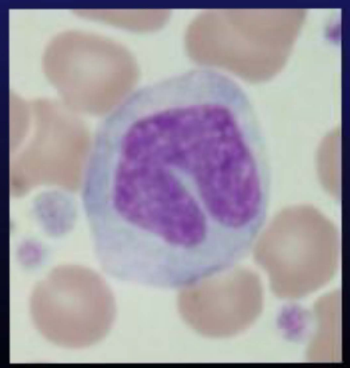
Myeloid DC
MACROPHAGES
Plasmacytoid DC

MAST CELL

CD4+ 'Helper'
CD8+ 'Cytotoxic'

Naïve B Cell
Plasma Cell
Memory B Cell

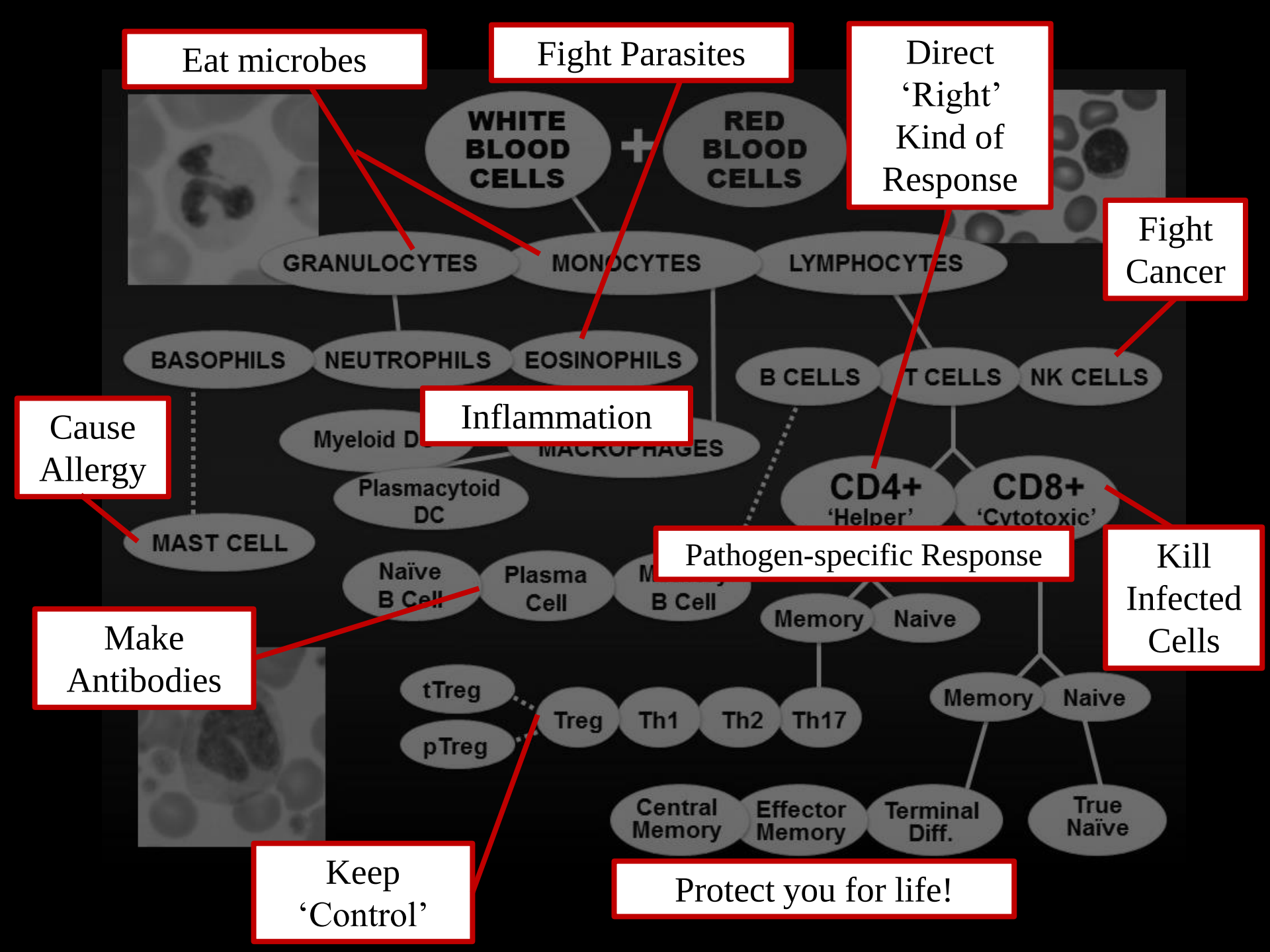
Memory
Naive



tTreg
pTreg
Treg
Th1
Th2
Th17

Memory
Naive

Central Memory
Effector Memory
Terminal Diff.
True Naïve


Eat microbes
Fight Parasites
Direct 'Right' Kind of Response
Fight Cancer
WHITE BLOOD CELLS
RED BLOOD CELLS
GRANULOCYTES
MONOCYTES
LYMPHOCYTES
BASOPHILS
NEUTROPHILS
EOSINOPHILS
B CELLS
T CELLS
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Inflammation
Cause Allergy
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MAST CELL
Pathogen-specific Response
Kill Infected Cells
Naïve B Cell
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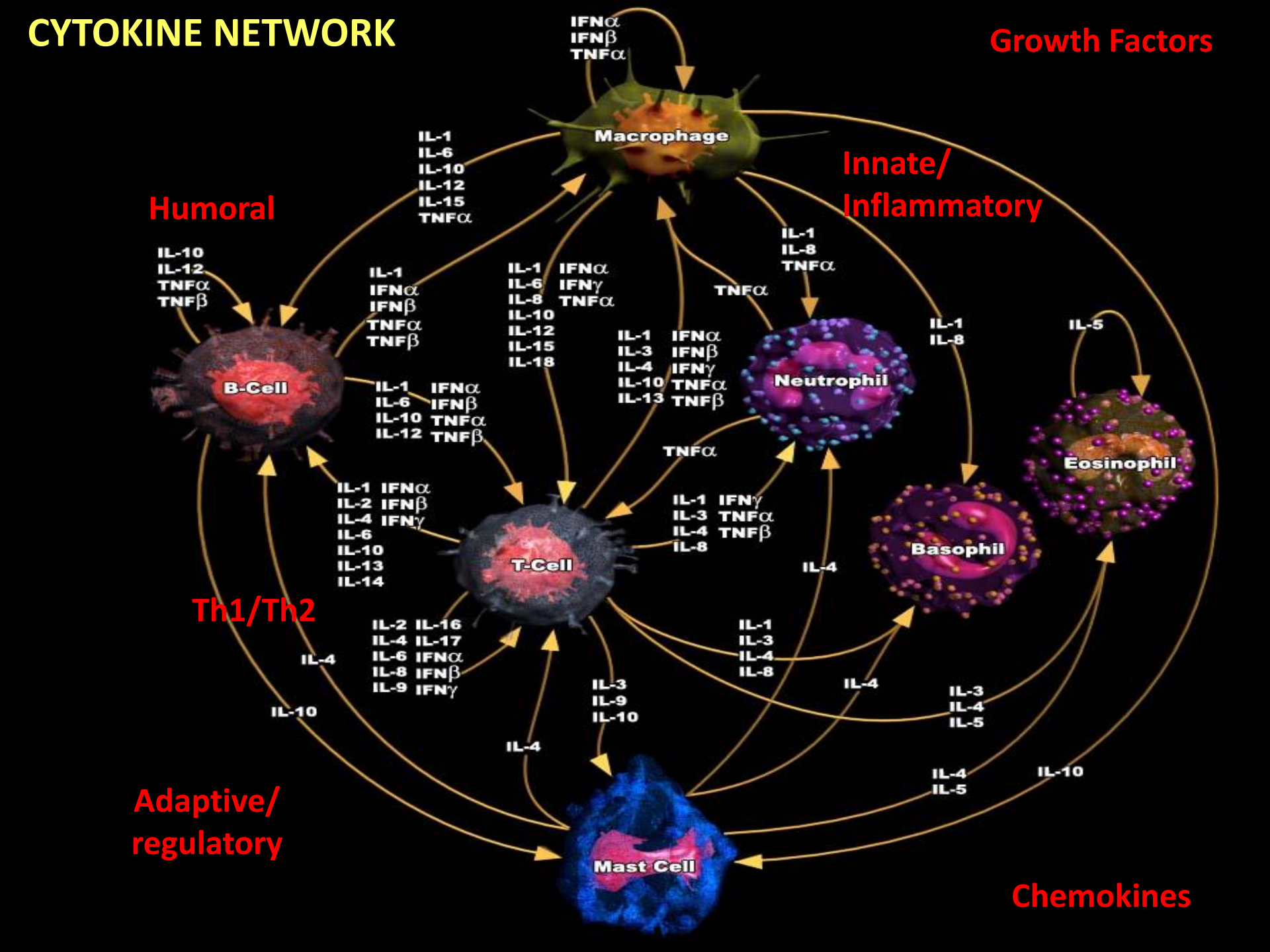
Central Memory
Effector Memory
Terminal Diff.
True Naïve
Keep 'Control'
Protect you for life!


CYTOKINE NETWORK
Growth Factors
Innate/
Inflammatory
Humoral
Th1/Th2
Adaptive/
regulatory
Chemokines
Macrophage
B-Cell
T-Cell
Neutrophil
Eosinophil
Basophil
Mast Cell
IFNα
IFNβ
TNFα
IL-1
IL-6
IL-10
IL-12
IL-15
IL-8
IL-5
IL-3
IL-4
IL-13
IL-18
IL-2
IL-16
IL-17
IL-9
IFNγ
TNFβ
TNFα

HYPOXIA/CO2

ALTERED MICROBIAL VIRULENCE

ALTERED NUTRITION

MICROGRAVITY

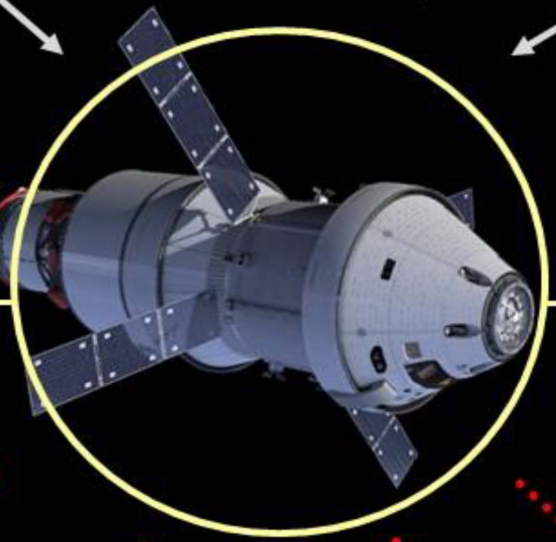
RADIATION

PHYSIOLOGICAL STRESS

ALTERED MICROBIOME

PSYCHOLOGICAL STRESS

CIRCADIAN MISALIGNMENT

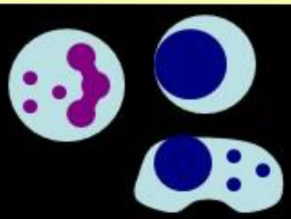


ALTERED IMMUNOCYTE DISTRIBUTION & FUNCTION

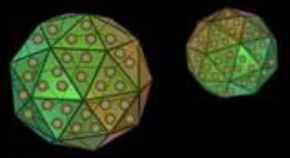
ALTERED CYTOKINE, REDOX, INFLAMMATORY BALANCE

LATENT VIRUS REACTIVATION

CLINICAL INCIDENCE



Th1/
Th2



AUTOIMMUNITY

CANCER

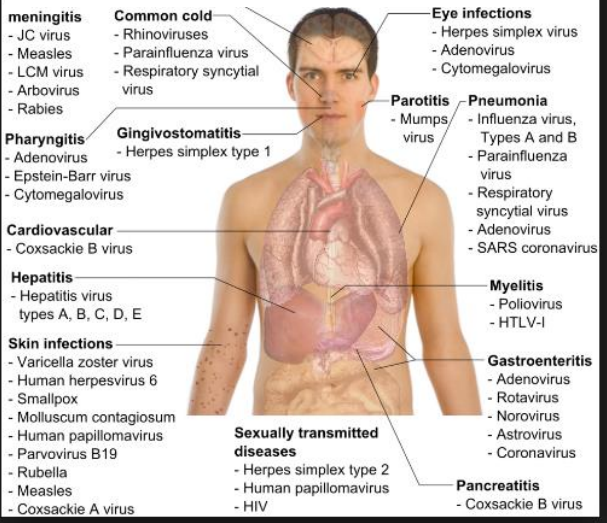
CONSEQUENCES OF PERSISTENT VIRAL REACTIVATION

CHRONIC ALLERGY/ HYPERSENSITIVITY

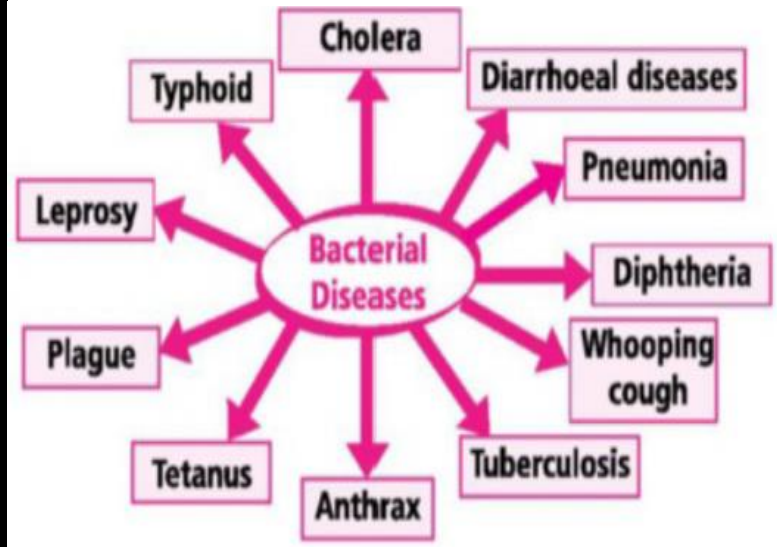
INFECTIOUS DISEASE

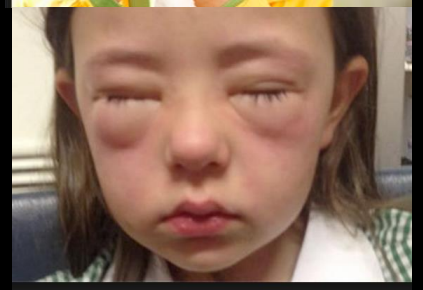
Immunity and Disease

VIRAL INFECTION

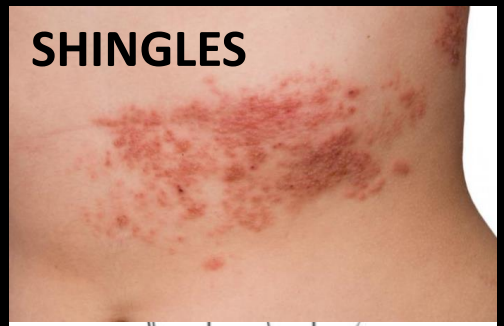


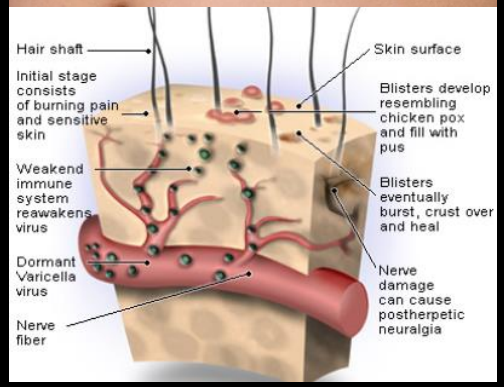
BACTERIAL INFECTION

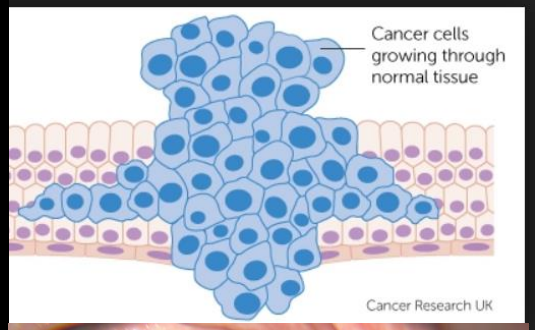


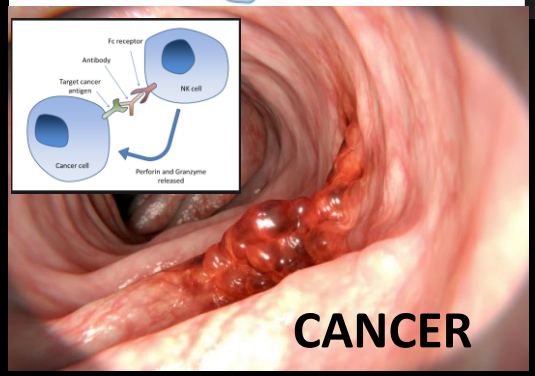


SHINGLES

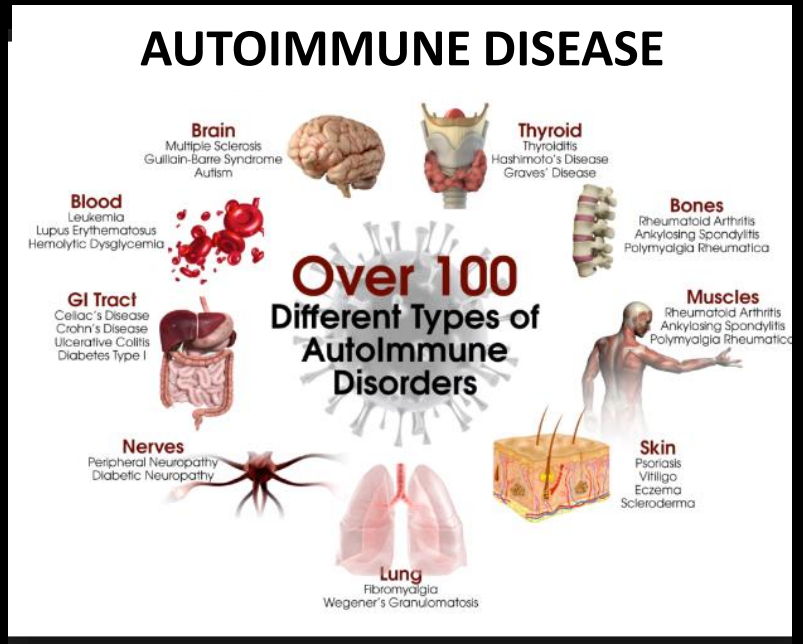




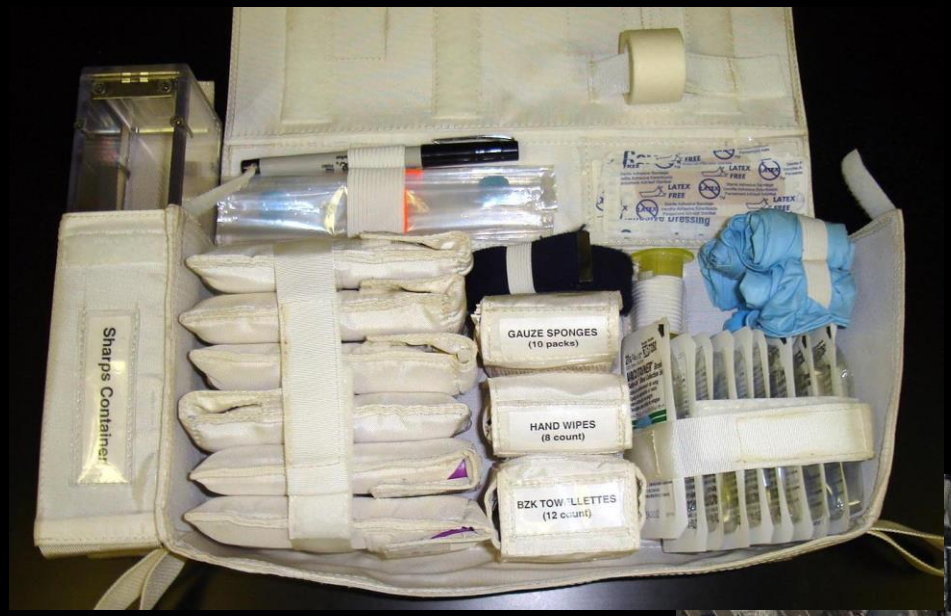




AUTOIMMUNE DISEASE



Blood and Saliva Collection- ISS



Plasma Collection - ISS

Return Ambient – 45h Delay

Late
R-1-2 days

Mid
2-4 mos

FD180

FD120

6 Months Spaceflight

Early
~2 weeks

FD60

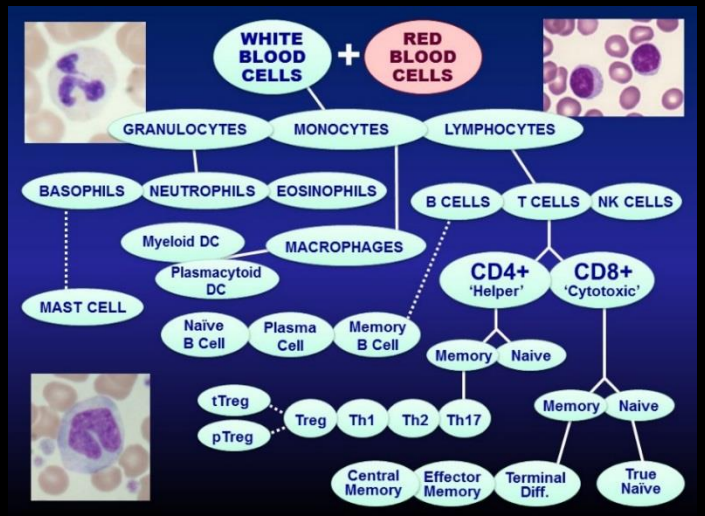
FD30

FD15

Frozen on Orbit

SPACEFLIGHT IMMUNE DYSREGULATION

- Peripheral leukocyte distribution in astronauts is relatively normal

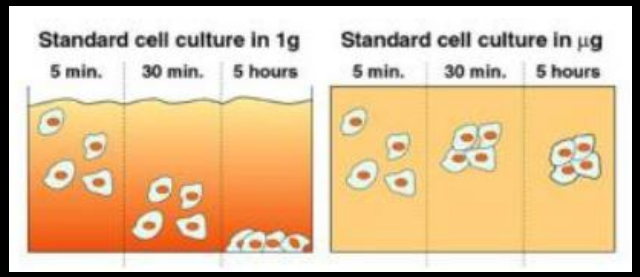


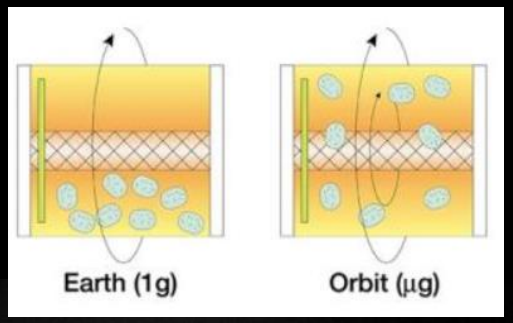
- T cell, NK cell function is inhibited by microgravity
- T cell function is reduced in astronauts; appears to be a shift in the activation threshold
- NK cells are disarmed, reduction in lytic molecule content
- B cell function in astronauts appears unaltered (limited data)

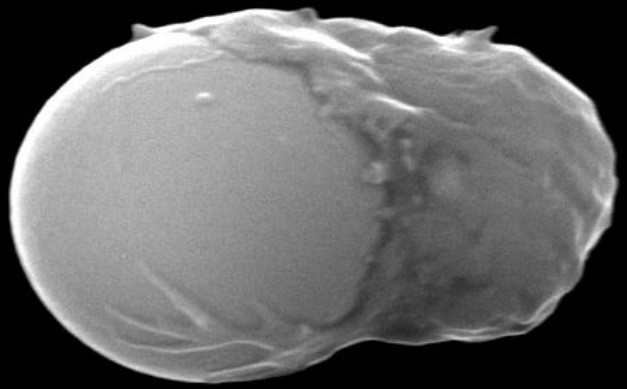
- Innate immunocyte function dysregulated during spaceflight
- Plasma cytokine concentrations are altered in astronauts
- Astronauts experience persistent reactivation of latent herpesviruses, biomarker of reduced immunity
- Astronauts demonstrate elevated stress hormones and dysregulated circadian rhythms during spaceflight

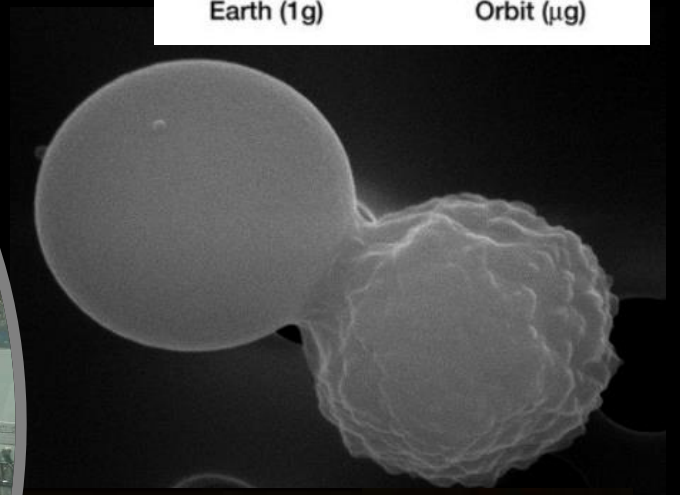
- Astronauts have some degree of clinical incidence, primarily dermatitis, allergy and infections
- Dermatitis may be associated with viral etiology
- Some crew experience persistent symptoms requiring prolonged management

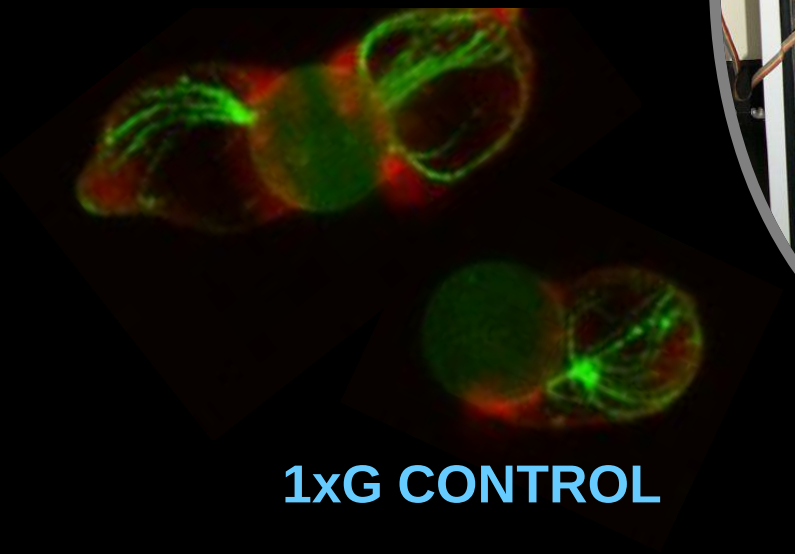
Microgravity Cell Culture

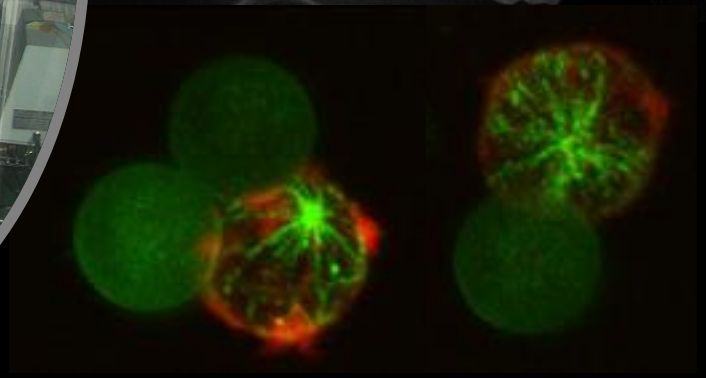












1xG CONTROL

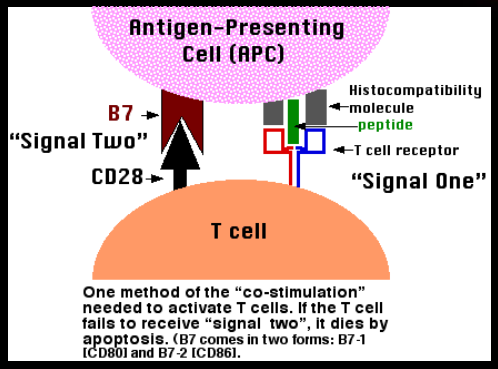
MODELED MICROGRAVITY

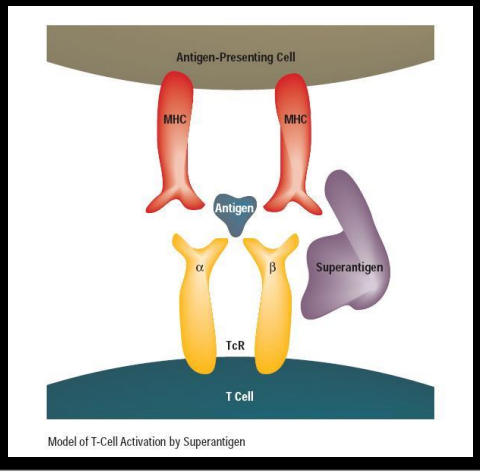
Red: Actin localization

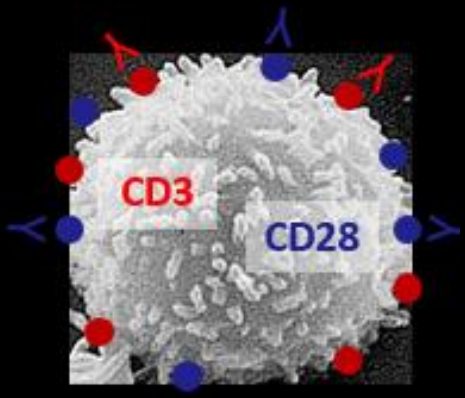
Green: Microtubules/MTOC

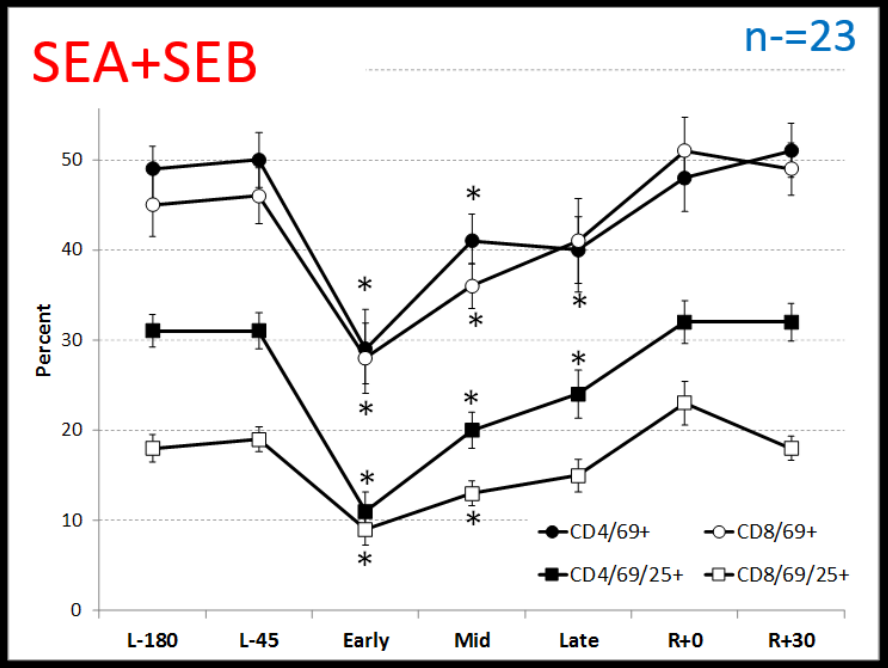
Mayra Nelman-Gonzalez

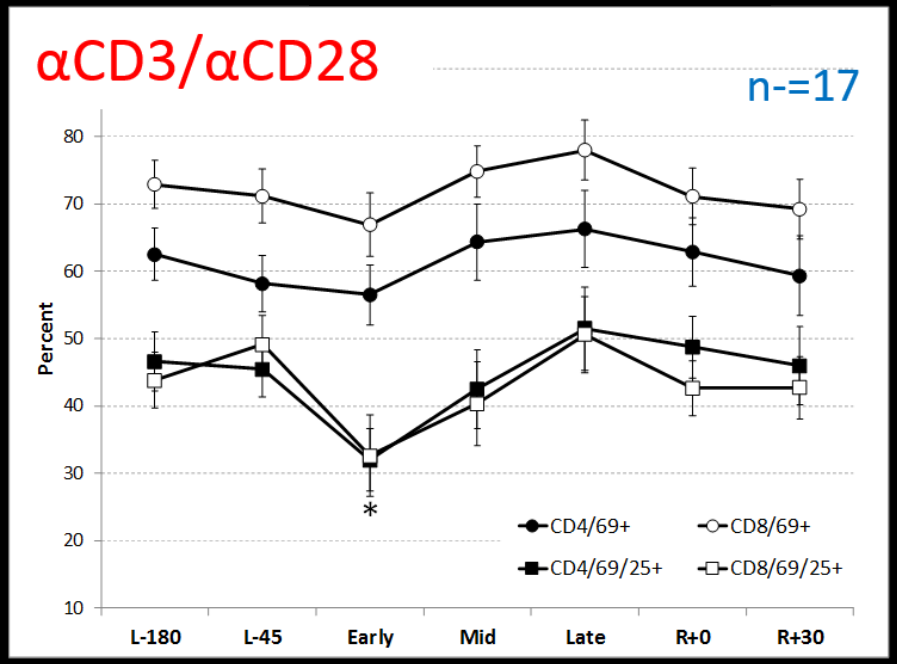
T Cell Function



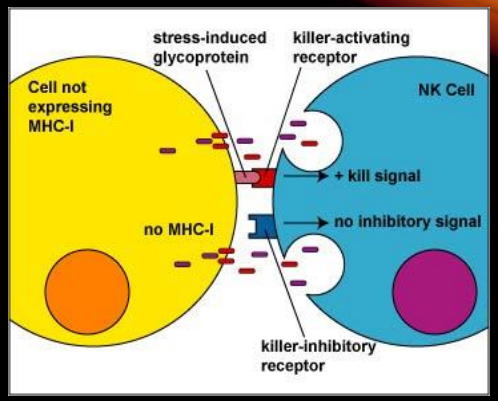


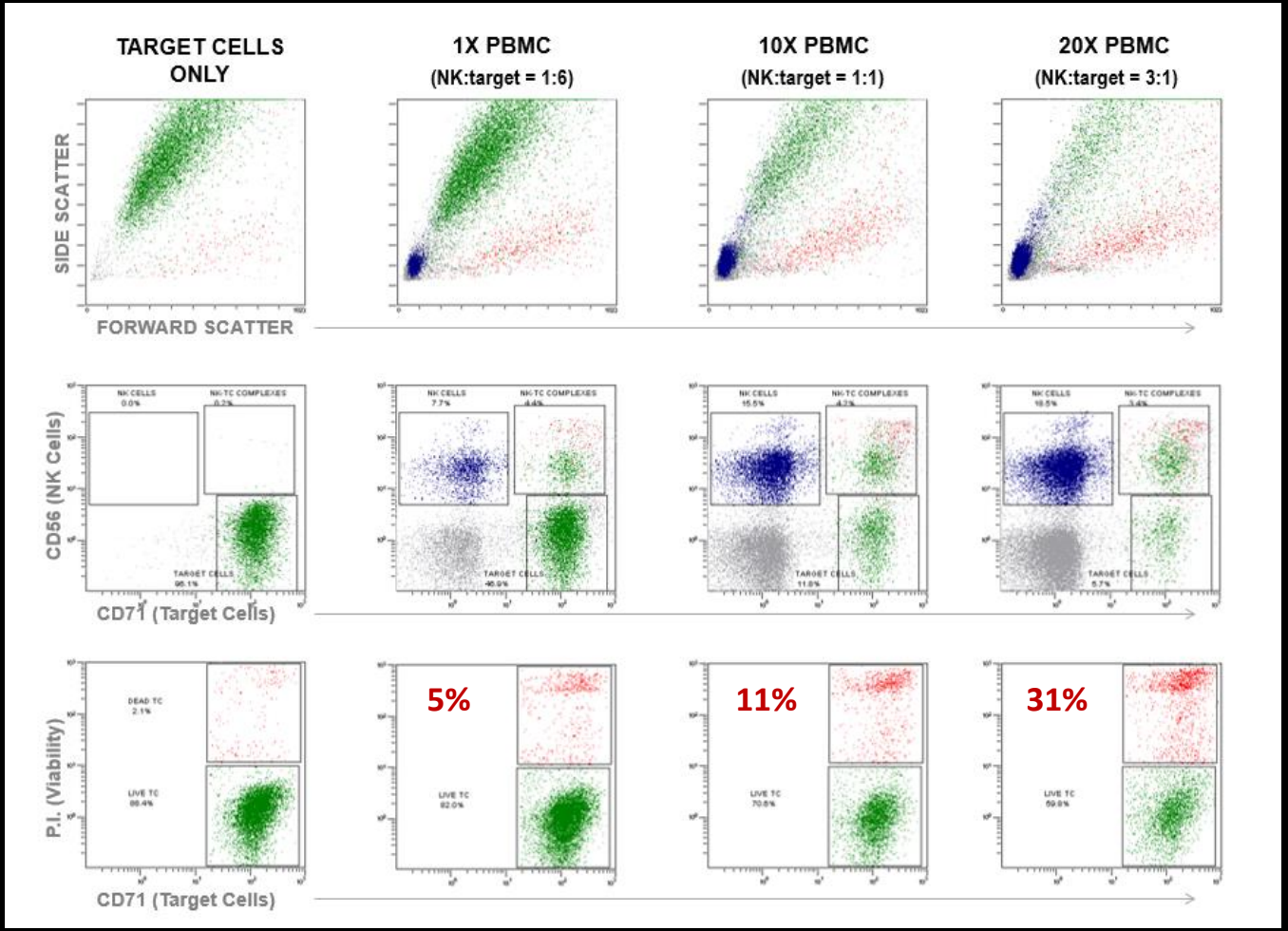






NK Cell Function





NK Cell Function

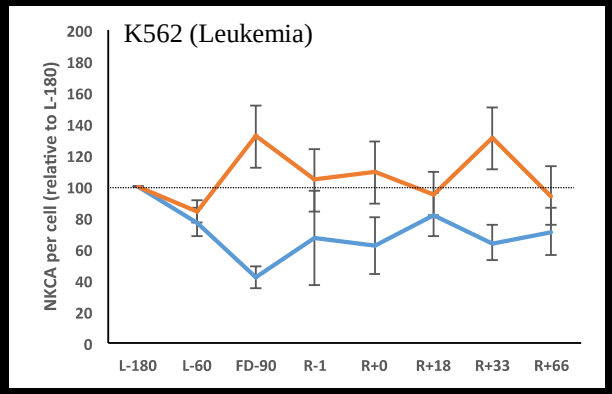
Data expressed as % change from baseline (L-180). NK-cell function did not differ between astronauts and controls at baseline

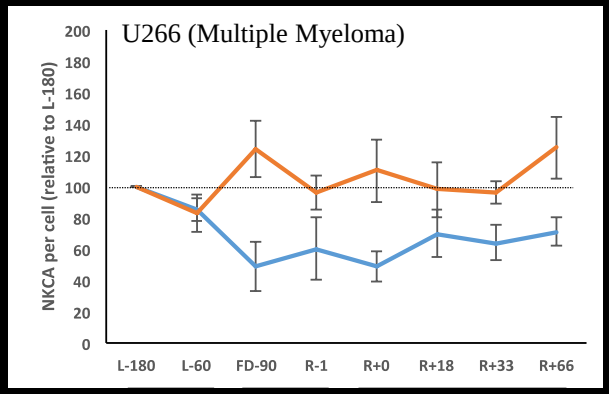
Spaceflight Reduces NK Cell Function

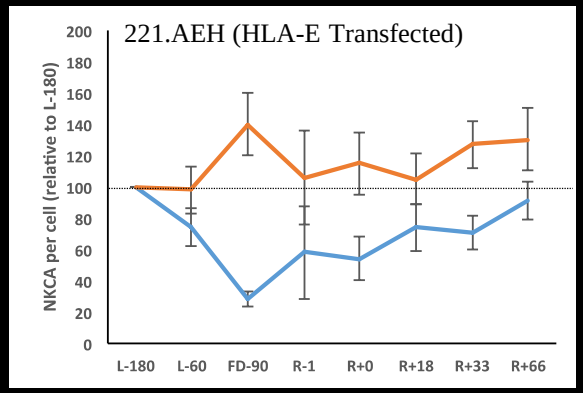
Controls (n=6)

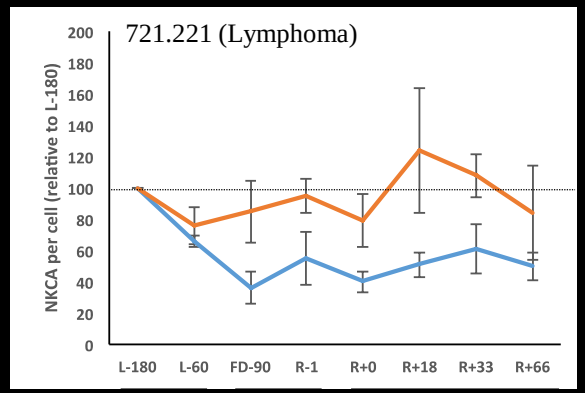
Astronauts (n=6)

Baseline NKCA









Dr. Richard Simpson

Plasma Cytokine Analysis

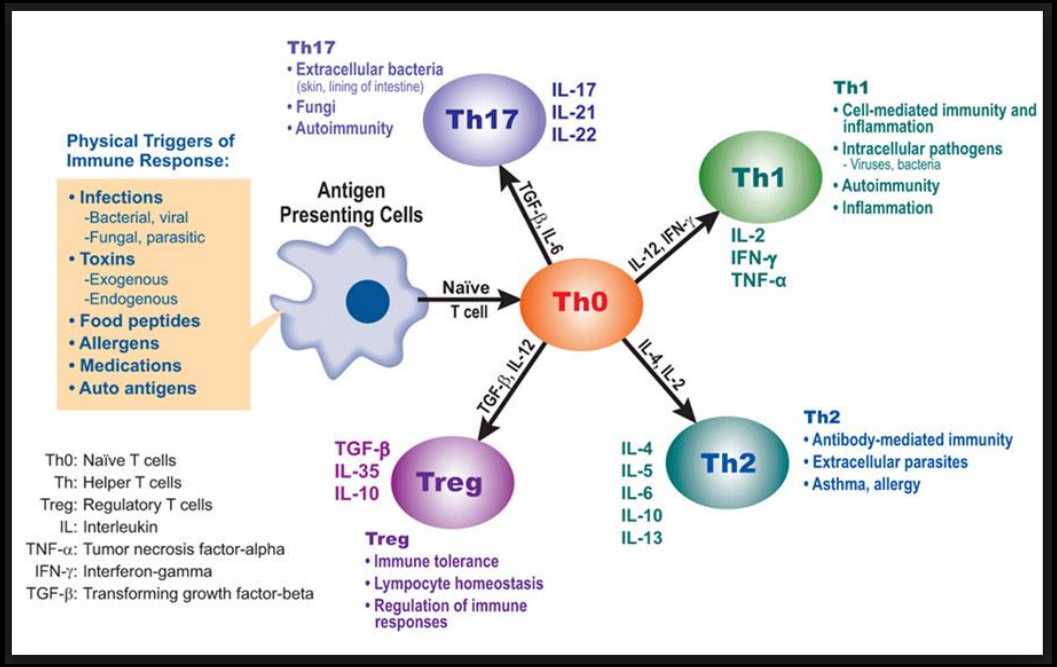


Table 1: Twenty two cytokines for analysis by category

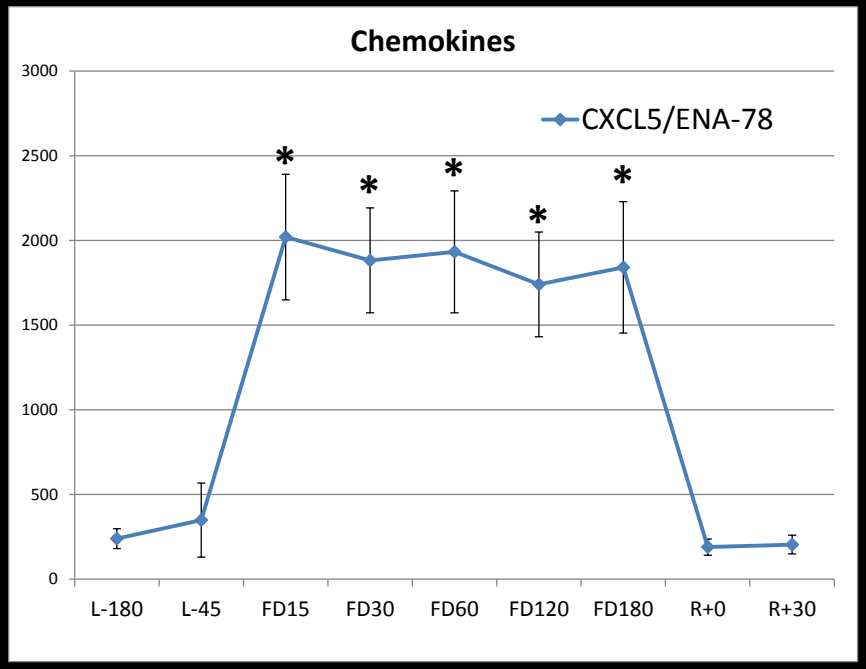
Inflammatory	Anti-Inflammatory	Adaptive/ Regulatory	Growth Factors	Chemokines
IL-1α	IL-1ra	IFNγ	G-CSF	CCL2/MCP-1
IL-1β		IL-2	GM-CSF	CCL3/MIP-1 alpha
TNFα		IL-17	FGF basic	CCL4/MIP-1 beta
IL-6		IL-4	Tpo	CCL5/RANTES
IL-8		IL-5	VEGF	CXCL5/ENA-78
		IL-10		

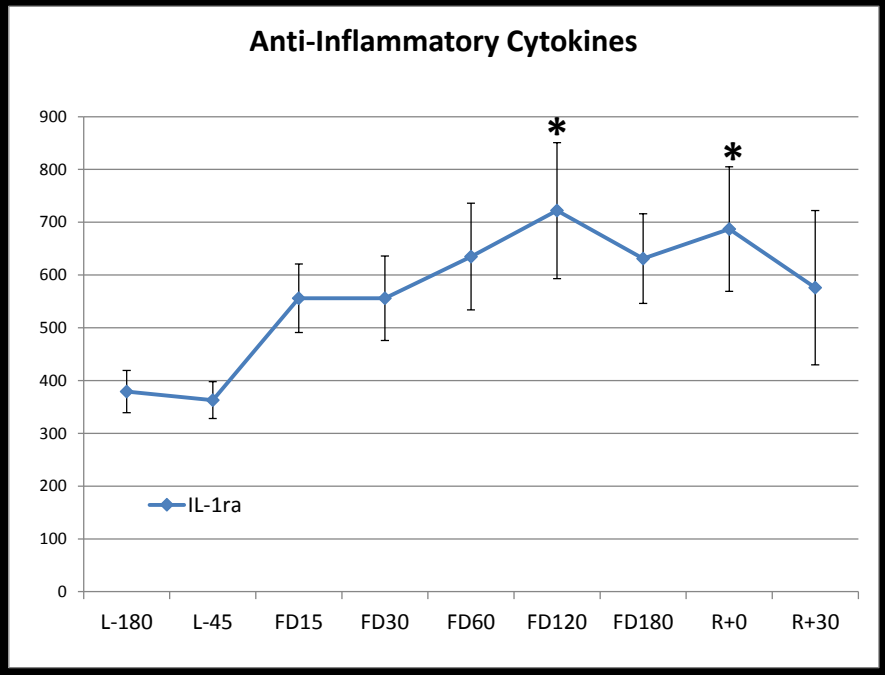
Plasma Cytokine Analysis

Table 2: Mean plasma cytokine levels for ISS astronauts before, during, and following spaceflight. Data are expressed as mean concentration pg/ml ± SEM. Bold indicates statistically significant difference p≤0.05; n=28.

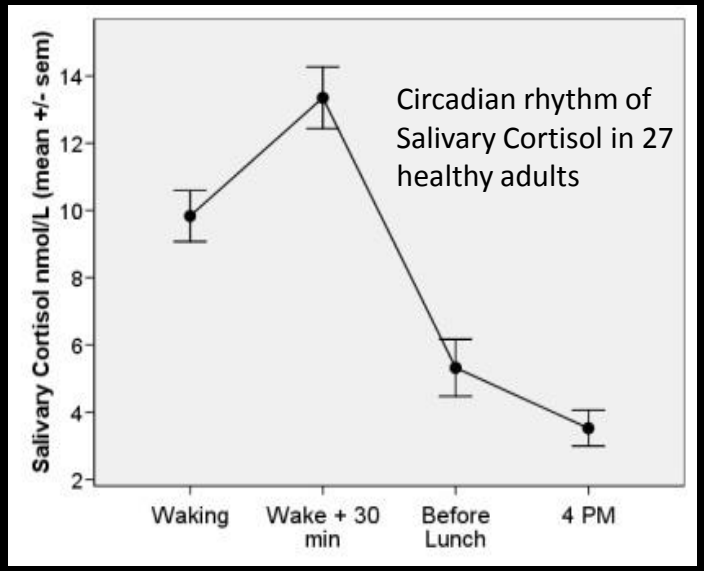
			Spaceflight						
Cytokine	**L-180**	**L-45**	**FD15**	**FD30**	**FD60**	**FD120**	**FD180**	**R+0**	**R+30**
IL-1a	0.3 ± 0.1	0.4 ± 0.3	0.9 ± 0.5	0.3 ± 0.1	2.4 ± 1.9	0.6 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
IL-1b	0.4 ± 0.1	0.7 ± 0.3	1.5 ± 1.0	0.8 ± 0.3	0.9 ± 0.5	1.3 ± 0.9	1.1 ± 0.8	0.5 ± 0.2	0.8 ± 0.3
TNFa	1.4 ± 0.1	1.4 ± 0.1	3.2 ± 1.0	**2.0*** ± 0.3	**2.1** ± 0.4	2.2 ± 0.5	2.0 ± 0.4	1.3 ± 0.1	1.7 ± 0.2
IL-6	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	**1.1*** ± 0.2	0.3 ± 0.1
IL-8	2.0 ± 0.3	2.1 ± 0.3	**8.1*** ± 2.1	**7.9*** ± 2.3	**7.7*** ± 1.7	**7.3*** ± 2.1	**6.9*** ± 2.3	2.1 ± 0.3	2.3 ± 0.4
IL-1ra	383 ± 40	370 ± 35	**567*** ± 65	**563*** ± 80	**638*** ± 101	**728*** ± 129	**661*** ± 85	**682*** ± 118	568 ± 146
IFNg	0.8 ± 0.2	0.8 ± 0.2	0.6 ± 0.1	0.7 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.3	**0.5*** ± 0.1	0.7 ± 0.2
IL-2	2.2 ± 0.6	**1.8*** ± 0.5	**1.7*** ± 0.5	2.6 ± 0.8	2.4 ± 0.7	2.5 ± 0.7	2.4 ± 0.8	2.4 ± 0.7	2.7 ± 0.9
IL-17	1.3 ± 0.3	1.1 ± 0.3	0.9 ± 0.2	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.2	0.9 ± 0.3	**0.9*** ± 0.2	0.9 ± 0.2
IL-4	0.3 ± 0.1	0.5 ± 0.3	3.2 ± 1.7	0.3 ± 0.2	1.4 ± 0.7	2.1 ± 1.5	1.6 ± 1.2	0.4 ± 0.2	0.2 ± 0.1
IL-5	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
IL-10	0.2 ± 0.0	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.2	0.2 ± 0.0	0.3 ± 0.1	0.4 ± 0.1
G-CSF	7.2 ± 1.9	7.0 ± 1.7	7.0 ± 1.8	4.5 ± 0.8	7.6 ± 2.0	14.7 ± 7.8	9.8 ± 3.2	**10.3*** ± 2.8	5.9 ± 1.4
GM-CSF	0.6 ± 0.3	0.3 ± 0.1	3.4 ± 1.9	**1.9*** ± 0.8	2.7 ± 1.3	2.8 ± 1.9	2.7 ± 1.9	0.7 ± 0.4	0.7 ± 0.4
FGFb	13.7 ± 5.4	15.4 ± 5.7	11.8 ± 3.3	21.9 ± 5.7	18.5 ± 4.9	12.1 ± 3.7	10.8 ± 2.7	11.7 ± 3.8	12.3 ± 4.3
Tpo	140 ± 16	146 ± 18	**184*** ± 18	**189*** ± 30	**191*** ± 22	**196*** ± 28	**221*** ± 24	141 ± 17	133 ± 16
VEGF	5.8 ± 0.9	6.2 ± 1.3	**10.9*** ± 1.9	**15.8*** ± 4.9	**11.3*** ± 1.7	**12.5*** ± 3.5	**11.7*** ± 1.9	5.1 ± 1.0	5.5 ± 0.9
CCL2/MCP-1	72.4 ± 6.8	78.5 ± 7.7	71.7 ± 5.4	66.0 ± 5.8	77.0 ± 7.0	84.0 ± 7.0	87.0 ± 7.7	**124*** ± 18.1	**90*** ± 7.5
CCL3/MIP-1a	20.3 ± 5.0	16.6 ± 5.0	25.9 ± 8.1	15.0 ± 4.4	19.1 ± 6.6	22.7 ± 7.4	21.7 ± 8.6	19.4 ± 6.3	18.1 ± 5.5
CCL4/MIP-1b	16.2 ± 2.2	16.7 ± 2.7	**22.3*** ± 2.9	**20.2*** ± 2.5	**22.2*** ± 2.8	24.3 ± 5.1	**21.6*** ± 3.3	17.3 ± 2.3	19.3 ± 4.0
CCL5/RANTES	3613 ± 263	3292 ± 246	3618 ± 202	3746 ± 195	3575 ± 185	3818 ± 217	4030 ± 202	3410 ± 266	3623 ± 219
CXCL5/ENA-78	231 ± 58	367 ± 219	**2065*** ± 371	**1858*** ± 310	**2015*** ± 360	**1749*** ± 309	**1860*** ± 388	190 ± 48	202 ± 55

Plasma Cytokine Analysis

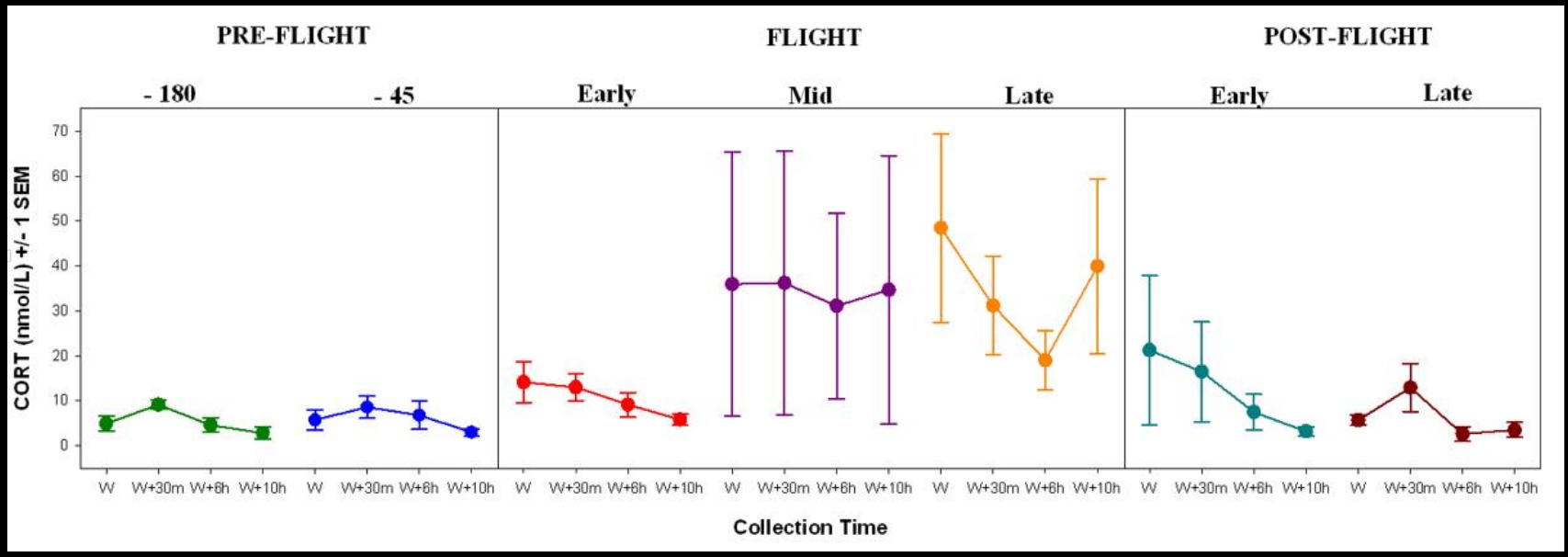




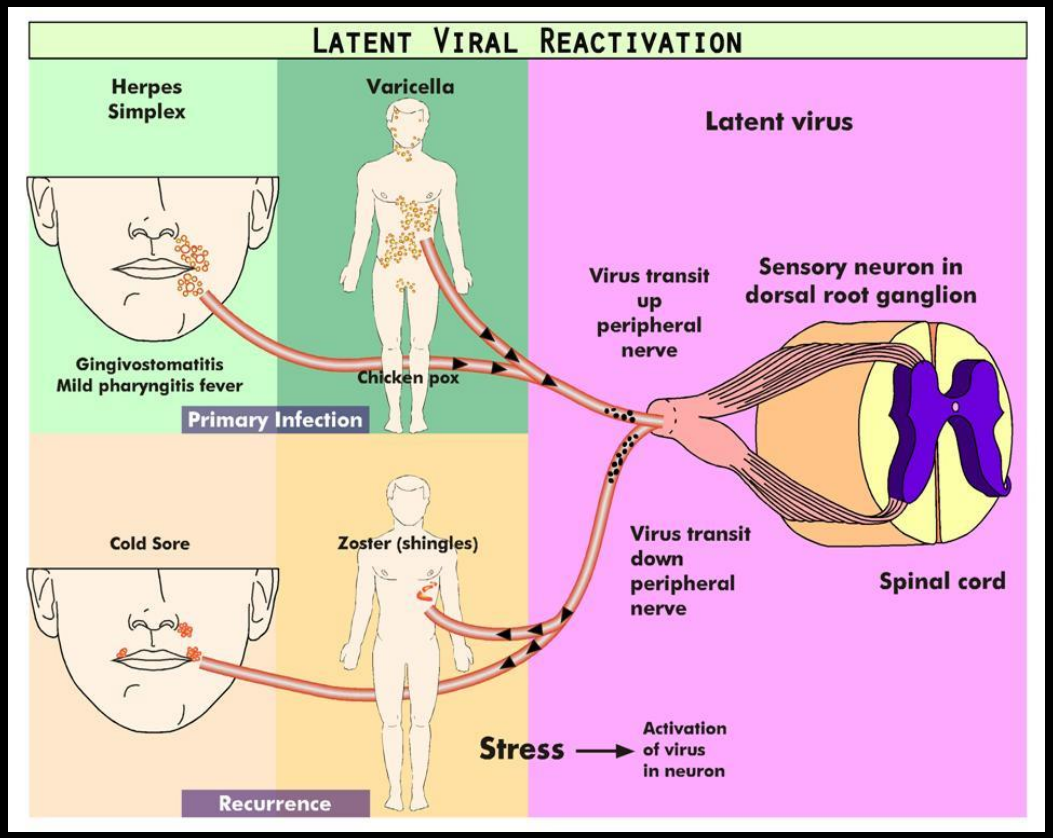
Stress Hormones/ Circadian Rhythm

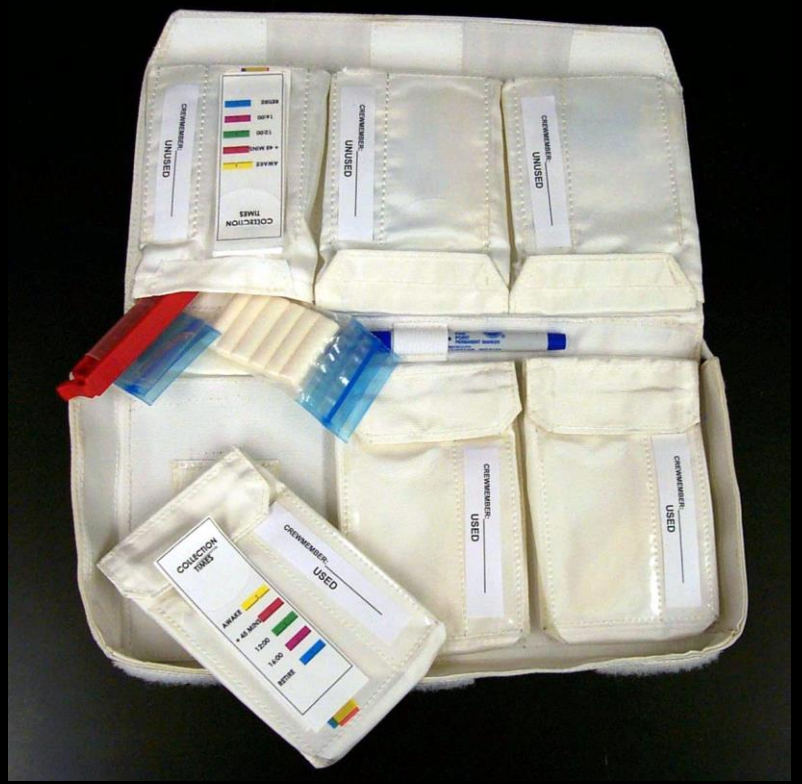


Circadian rhythm of Salivary Cortisol in 27 healthy adults

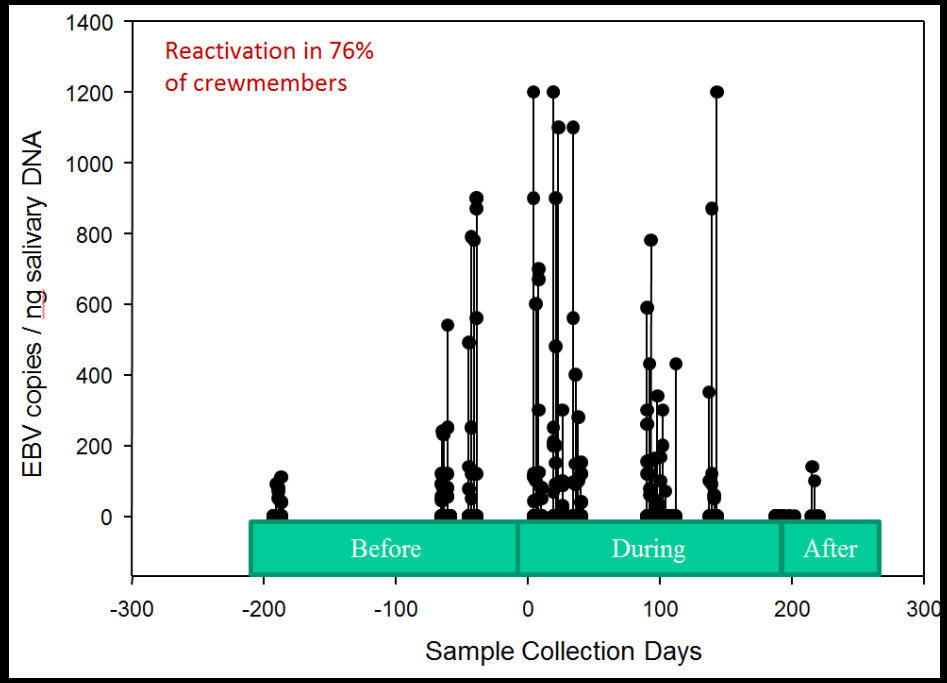


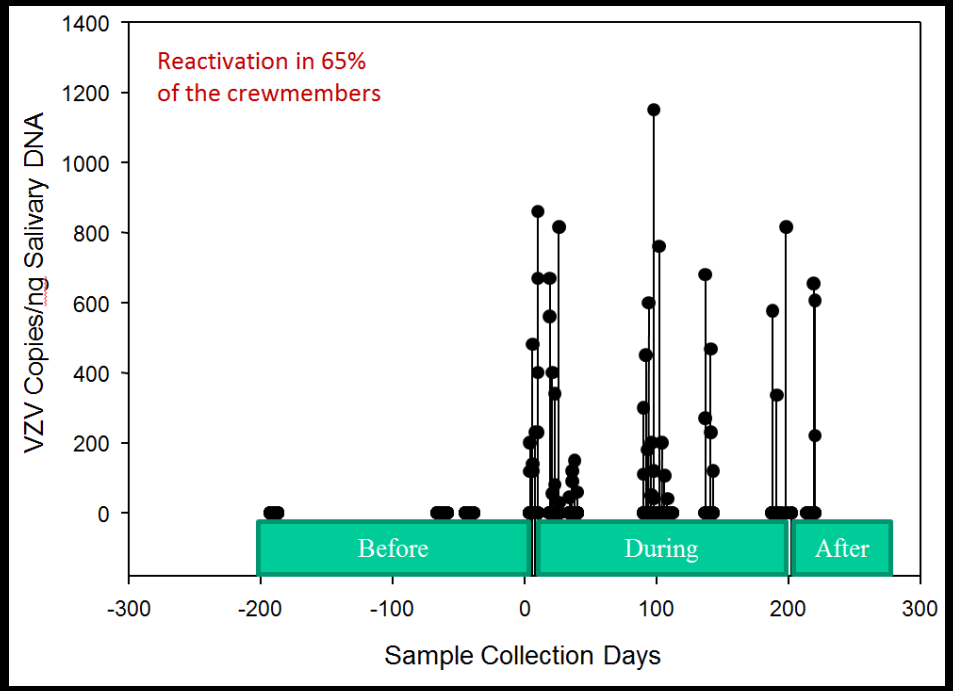
Latent Herpesvirus



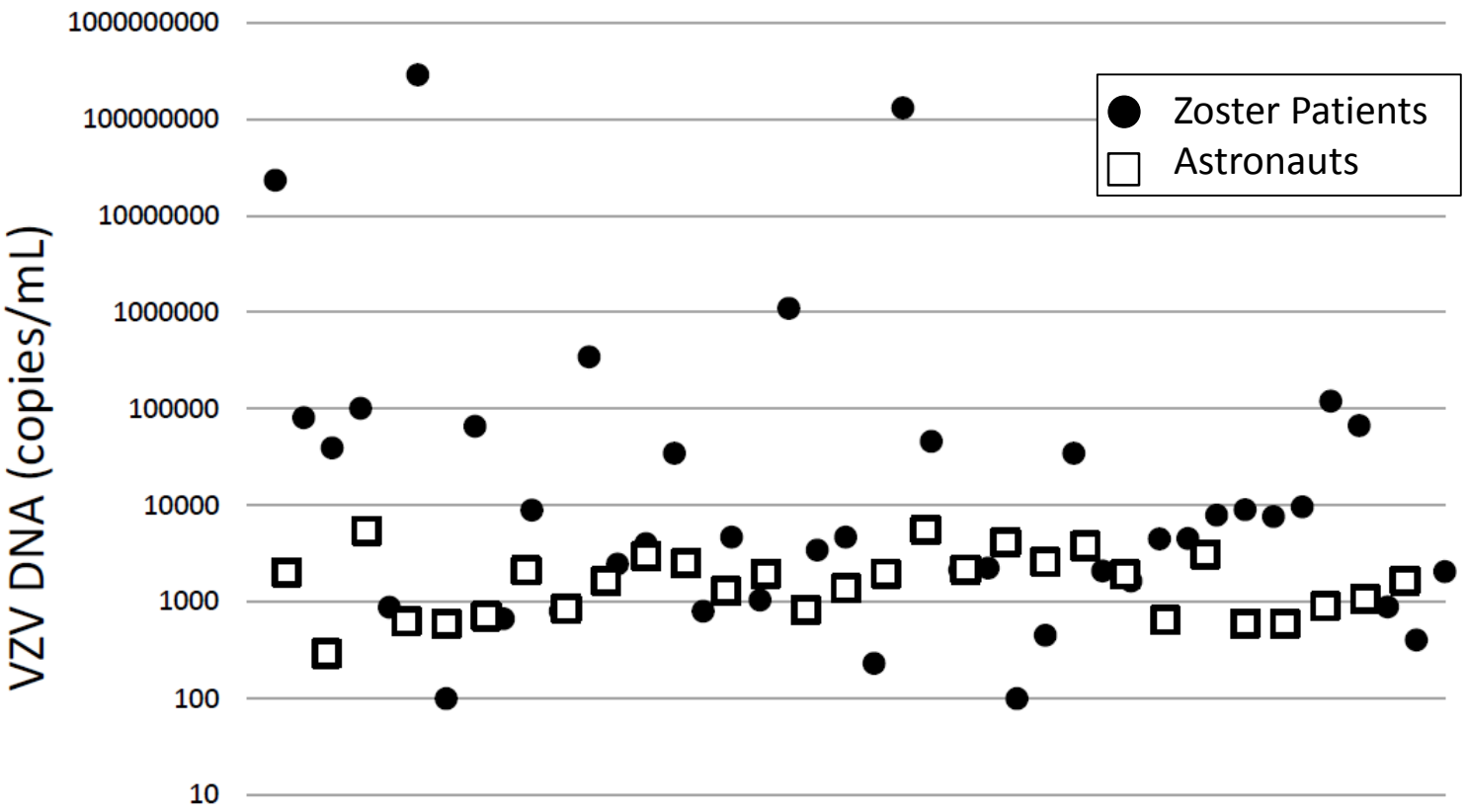


Latent Herpesvirus





Latent Herpesvirus



Zoster Patients (n=42) *100% positive*

Astronauts (n=23) *2-3 samples per crew= 59 total samples – 29/59 positive (49%)*

No VZV DNA was detected pre-flight for any crew (L-180 or L-45)

Clinical Incidence

Medical Conditions	Total events	Events/ person year
Allergic Reaction	1	0.06
Anaphylaxis	0	---
Upper Respiratory Infection (combination of rhinitis, nasal stuffiness and sneezing)	5	0.301
Eye Infection	0	---
Herpes Zoster	5	0.301
Otitis Media/Externa (ear pain, or ear stuffiness+congestion)	17	1.022
Pharyngitis (sore throat)	1	0.06
Sepsis	0	---
Sinus Infection	0	---
Skin Infection (including scalp pruritis, pus forming wounds on wrist, finger)	5	0.301
Skin Rash/Hypersensitivity (including skin conditions such as tinea versicolor, dermatitis, rosacea)	23	1.383
Urinary Tract Infection	1	0.06
Malignancies*	0	---
Autoimmunity*	0	---
Infections, Other*#	11	0.666
Total:	**69**	**4.18**

Clinical Incidence

Case Study ISS Astronaut

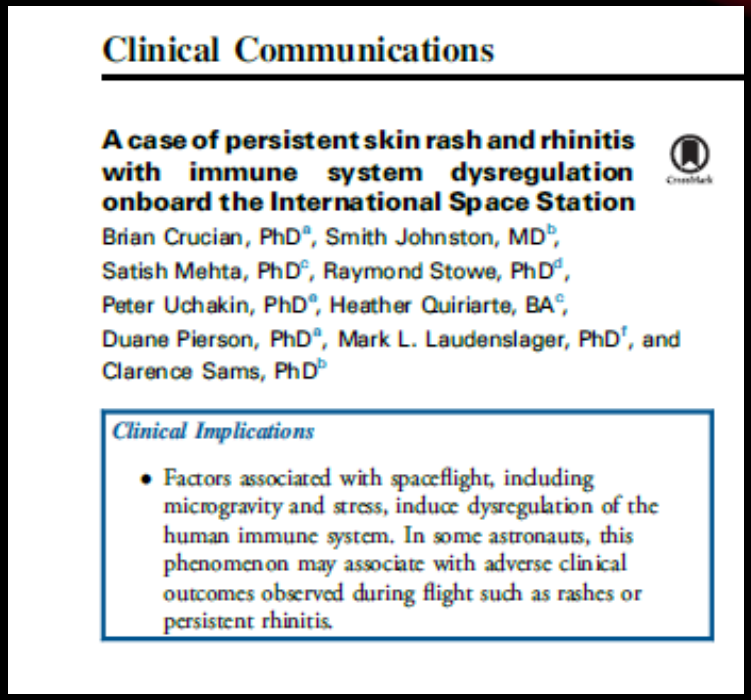
-Allergic symptoms in a non-allergic subject

-Subject developed an Atopic Dermatitis on mission day 17

-Rash was bothersome, at times severe

-A variety of treatments employed

-At times the medications of choice were exhausted



Clinical Communications

A case of persistent skin rash and rhinitis with immune system dysregulation onboard the International Space Station

Brian Crucian, PhD[a], Smith Johnston, MD[b], Satish Mehta, PhD[c], Raymond Stowe, PhD[d], Peter Uchakin, PhD[e], Heather Quiriarte, BA[c], Duane Pierson, PhD[a], Mark L. Laudenslager, PhD[f], and Clarence Sams, PhD[b]

Clinical Implications

- Factors associated with spaceflight, including microgravity and stress, induce dysregulation of the human immune system. In some astronauts, this phenomenon may associate with adverse clinical outcomes observed during flight such as rashes or persistent rhinitis.

-Rash never resolved for the duration of the mission, although it was successfully managed to a tolerable level

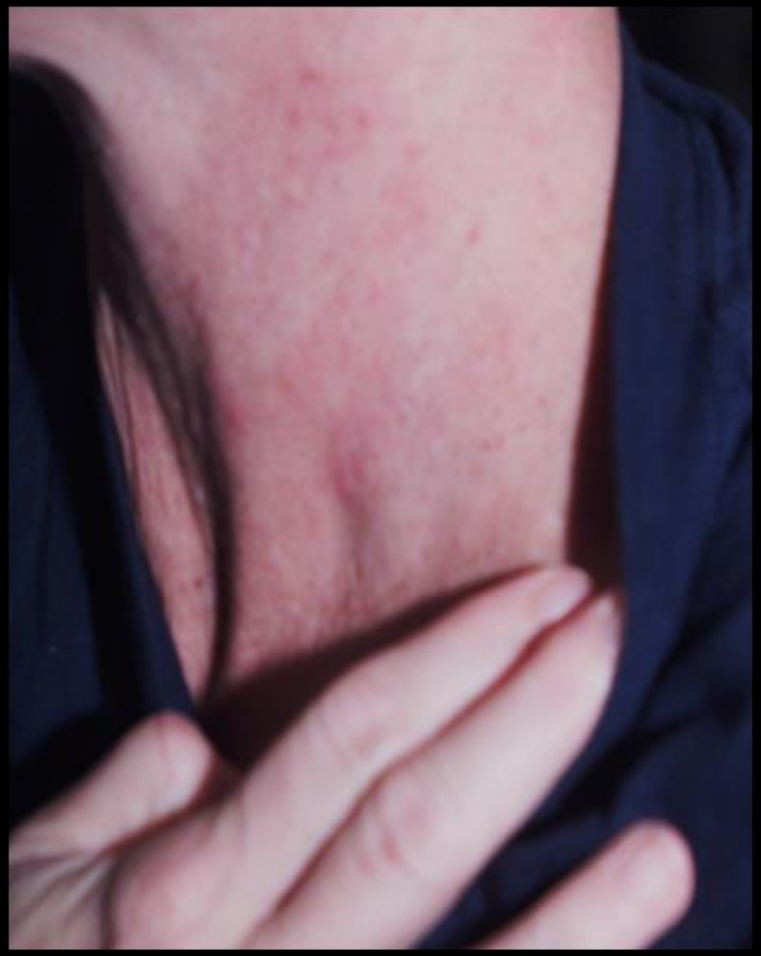
-Rash spikes generally correlated well with operational stressors

-Research findings confirm immune dysregulation persisted for the duration of the mission

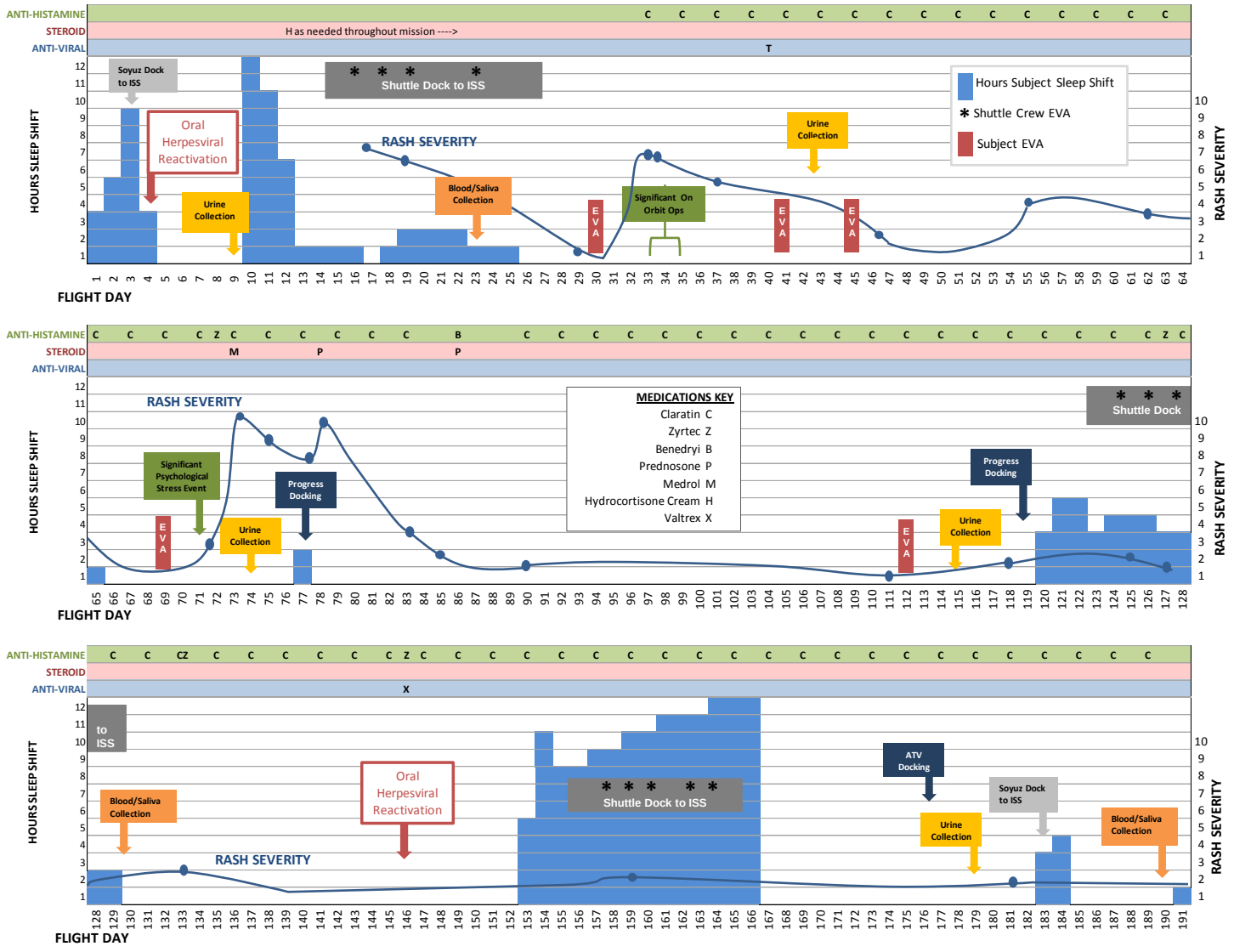
Clinical Incidence

- Rashes were observed to occur in the following locations: scalp, face, neck, chest, back, trunk, abdomen, arms and hands.

- The appearance of the rashes generally consists of bumps/nodules and/or small brown scaly patches, with or without petechiae, redness/hyperemia and itching.

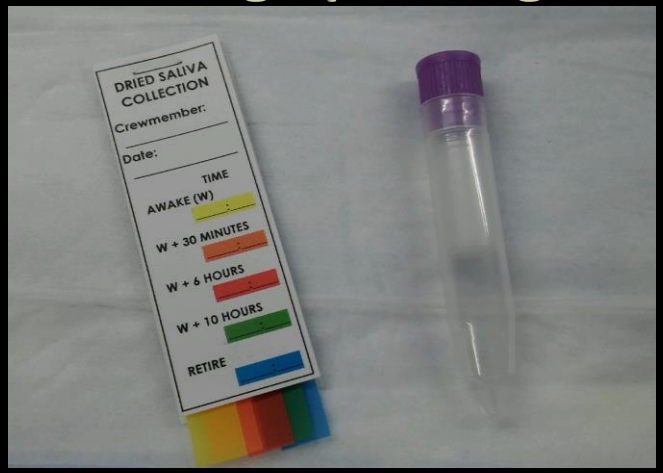


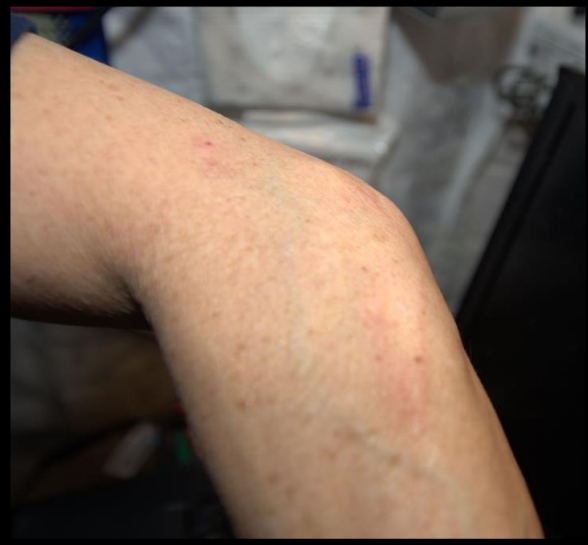
Clinical Incidence



Clinical Incidence

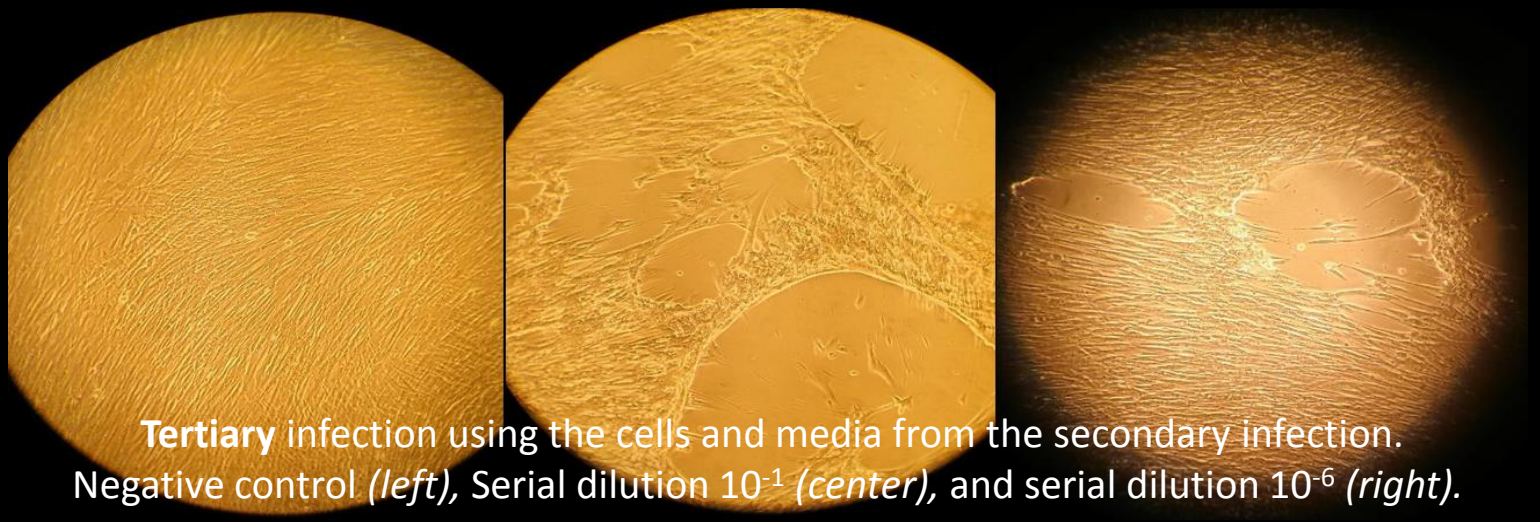
Herpes Simplex Virus type-1 reactivation associated with a case of persistent dermatitis during Spaceflight





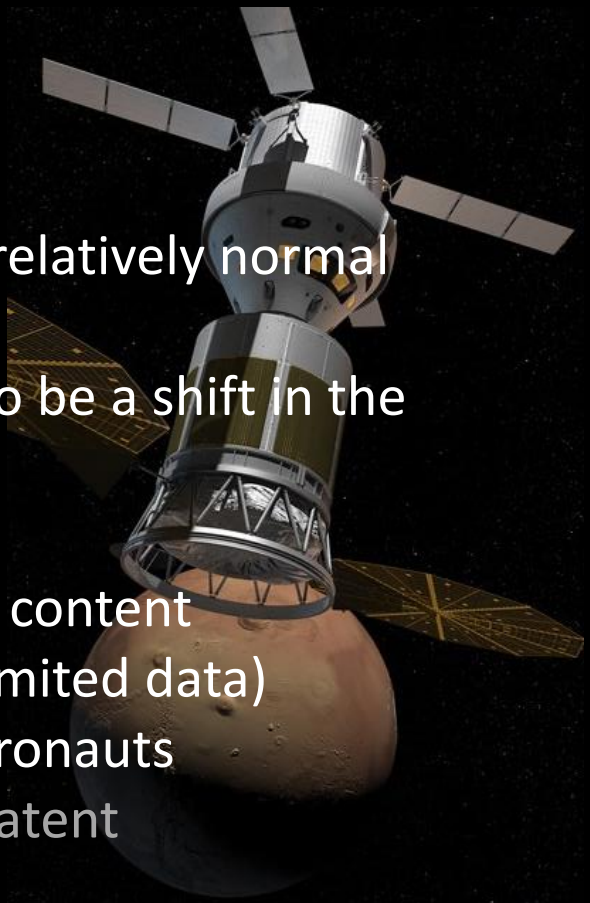
Clinical Incidence

		In-Flight	R+0	R+14
Saliva	**VZV**		Negative	Negative
	HSV1	Positive (CT-22; 5.4x10(6) copies per ng total DNA)	Positive (CT-15; 1.4x10(9) copies per ng total DNA)	Negative
Skin Lesion	**VZV**	Negative	N/A	N/A
	HSV1	Positive (CT-29; 2.4x10(4) copies per ng total DNA)	N/A	N/A



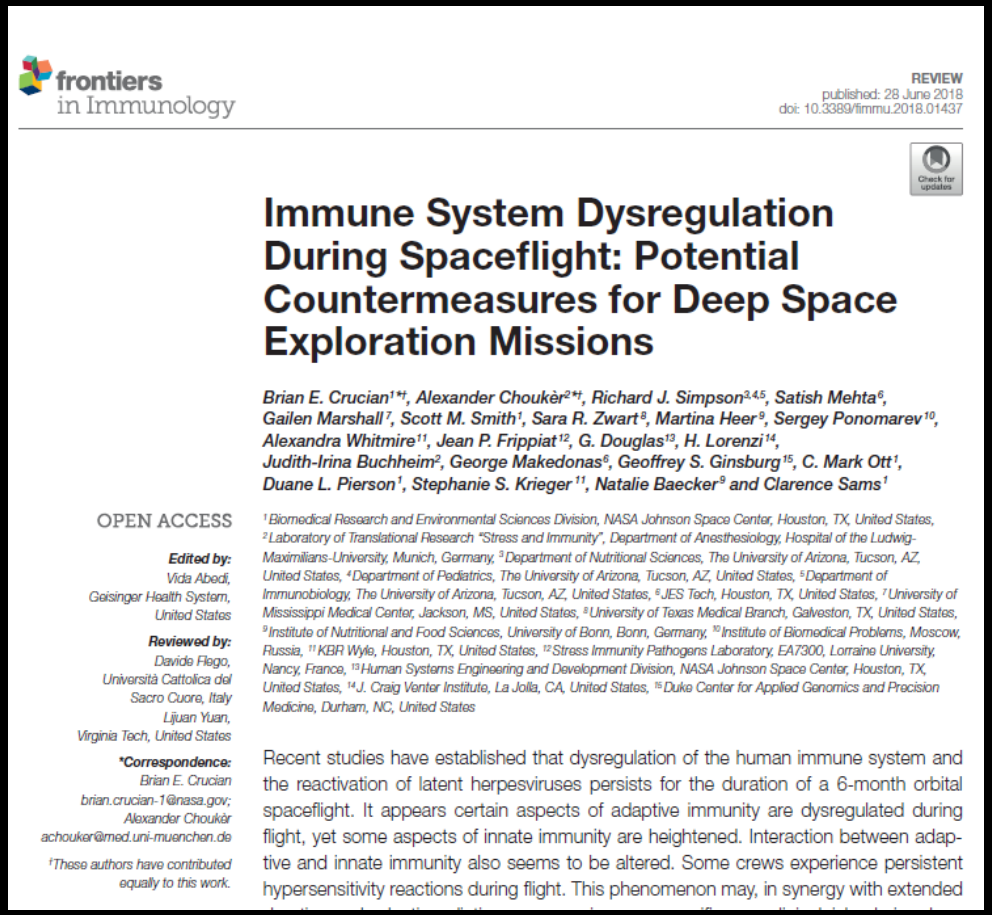
Tertiary infection using the cells and media from the secondary infection. Negative control *(left),* Serial dilution 10^{-1} *(center),* and serial dilution 10^{-6} *(right).*

Summary



- Peripheral leukocyte distribution in astronauts is relatively normal
- T cell function is inhibited by microgravity
- T cell function is reduced in astronauts; appears to be a shift in the activation threshold
- NK cell function is reduced in astronauts
- NK cells are disarmed, reduction in lytic molecule content
- B cell function in astronauts appears unaltered (limited data)
- Plasma cytokine concentrations are altered in astronauts
- Astronauts experience persistent reactivation of latent herpesviruses, biomarker of reduced immunity
- Astronauts demonstrate elevated stress hormones and dysregulated circadian rhythms during spaceflight
- Astronauts have some degree of clinical incidence, primarily dermatitis, allergy and infections
- Some crew experience persistent symptoms requiring prolonged management

Immune Countermeasures



frontiers in Immunology

REVIEW
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Immune System Dysregulation During Spaceflight: Potential Countermeasures for Deep Space Exploration Missions

Brian E. Crucian[1†], Alexander Choukèr[2*†], Richard J. Simpson[3,4,5], Satish Mehta[6], Gailen Marshall[7], Scott M. Smith[1], Sara R. Zwart[8], Martina Heer[9], Sergey Ponomarev[10], Alexandra Whitmire[11], Jean P. Frippiat[12], G. Douglas[13], H. Lorenzi[14], Judith-Irina Buchheim[2], George Makedonas[6], Geoffrey S. Ginsburg[15], C. Mark Ott[1], Duane L. Pierson[1], Stephanie S. Krieger[11], Natalie Baecker[9] and Clarence Sams[1]*

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†These authors have contributed equally to this work.

Recent studies have established that dysregulation of the human immune system and the reactivation of latent herpesviruses persists for the duration of a 6-month orbital spaceflight. It appears certain aspects of adaptive immunity are dysregulated during flight, yet some aspects of innate immunity are heightened. Interaction between adaptive and innate immunity also seems to be altered. Some crews experience persistent hypersensitivity reactions during flight. This phenomenon may, in synergy with extended

- Operational Procedures
- Functional Foods
- Nutritional Supplements
- Nutraceuticals
- Probiotics
- Pharmacological
- Exercise
- Vaccination
- Behavioral Countermeasures
- Bone Countermeasures

Personalized/Precision Medicine

Frontiers in Immunology ; June 2018; doi: 10.3389/fimmu.2018.01437

Immune Countermeasures

Potential Immunologic Countermeasures for Deep Space Missions

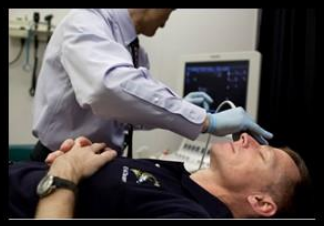
Precision Countermeasures

Pre-Mission Immunological Screen

Pre-mission immunological screen may include:
Personal history of allergy/hypersensitivity, etc.
Medication history (antihistamines, etc.)
Leukocyte distribution (NK cell subsets)
Cytokine concentration: Th1/Th2, etc.
Allergy screen, patch testing
Latent herpesvirus sero-positivity

Pathogen-Specific Mitigations

Antiviral (VZV) vaccination



General Countermeasures

Already in Place/Will be Optimized

Pre-flight medical operations screening of crewmembers
Pre-flight quarantine
Microbial screening of vehicle/payloads/foods
Environmental control
Optimized exercise equipment
Radiation shielding

Multisystem Countermeasures

Optimized exercise regimen
Adequate sleep schedules
Psychological support - family communication
Stress relieving techniques

Specific Countermeasures

Nutritional Countermeasures

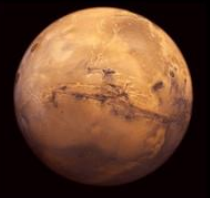
Diet optimized to reduce nutrient deficiency
Functional foods/bioactive compounds
Nutritional supplements:

- Antioxidants
- Probiotics
- Omega 3 fatty acids
- Supplemental nucleotides
- AHCC
- Pegylated-IL-2

Pharmacological Intervention

Beta blockers
Anti-cortisol
Antibiotics
Antiviral
Anti-inflammatory
Cytokine therapy

In-flight Monitoring of Immune Parameters?



PRE-FLIGHT | LAUNCH | TRANSIT PHASE | CIS-LUNAR STATION/ LUNAR SURFACE OPS | MARS FLYBY or ORBIT/ MARS SURFACE OPS

Spaceflight Immunologists

NASA JSC Immunology/Virology Laboratory