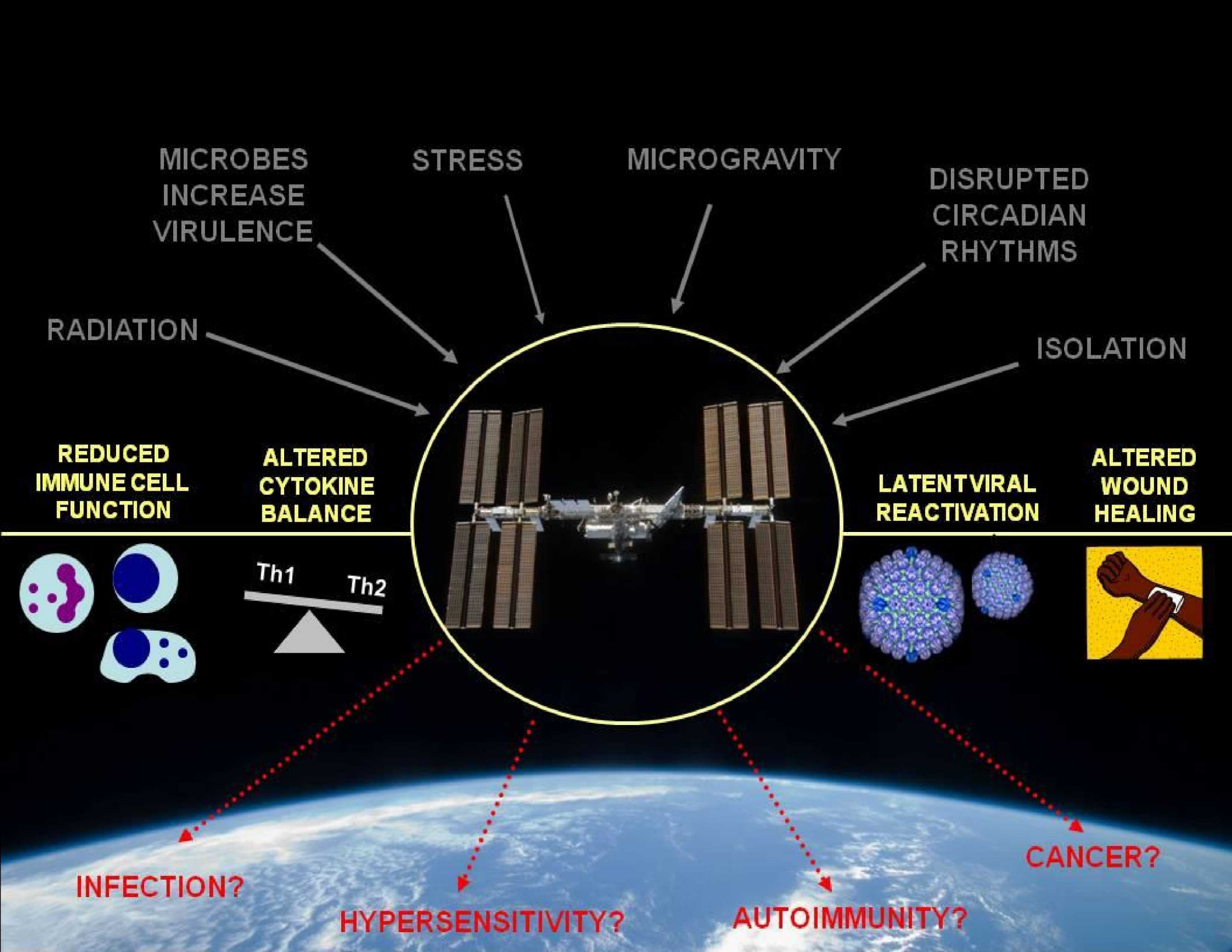
Immune System Dysregulation, Viral Reactivation and Stress *During* Short-Duration Space Flight

European Space Agency (ESA)
International Society for Gravitational Physiology (ISGP)
ISSBB Symposium
European Low Gravity Research Association (ELGRA)

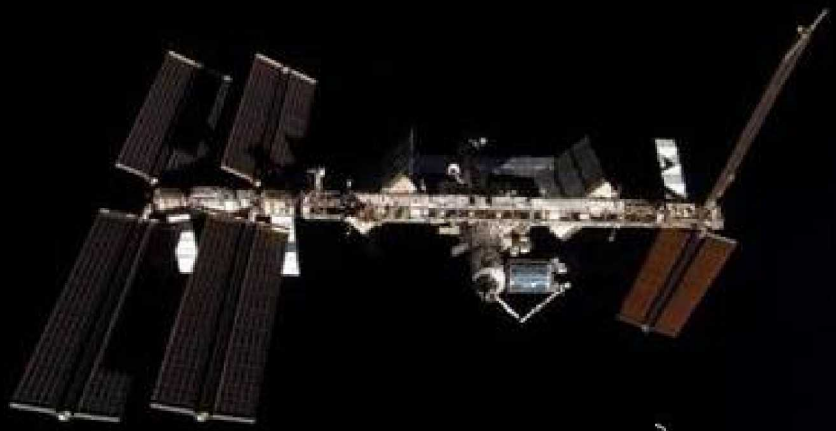
'Life in Space for Life on Earth'
13-18 June 2010
Trieste – Italy

Brian Crucian, Satish Mehta, Raymond Stowe, Peter Uchakin,
Heather Quiriarte, Duane Pierson and Clarence Sams


MICROBES INCREASE VIRULENCE
STRESS
MICROGRAVITY
DISRUPTED CIRCADIAN RHYTHMS
RADIATION
ISOLATION
REDUCED IMMUNE CELL FUNCTION
ALTERED CYTOKINE BALANCE
Th1
Th2
LATENT VIRAL REACTIVATION
ALTERED WOUND HEALING
INFECTION?
HYPERSENSITIVITY?
AUTOIMMUNITY?
CANCER?

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

Long 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.

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)

JLB

Review

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 1961, Yuri Gagarin became the first human to leave the confines of Earth. Since then, over 450 people have traveled into space, but so far, only 24 astronauts (those of the Apollo missions) have traveled beyond the first 400–500 km of the low-Earth orbit, in which the magnetic field of the Earth deflects a significant fraction of radiation. Beyond the Van Allen radiation belt, where charged particles are trapped in the magnetic field of the Earth, astronauts are exposed to solar and cosmic radiation.

On July 20, 1969, Neil Armstrong and Edwin Aldrin became the first humans to land on the moon. This summer, we celebrated 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 H. Obama's 2010 budget request, released on February 26, 2009, confirmed that NASA will stay on track to return to the moon by 2020. A mission to Mars and back will take a minimum of 520 days, of which roughly 1 month will be spent on the martian surface, and the rest will be spent in transit. At its furthest, the crew will be some 360 million km away from home. Consequently, astronauts will have to exercise an unprecedented level of autonomy and teamwork [2]. During the mission, they will experience 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 distance 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 attenuate 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 demineralization, skeletal muscle atrophy, and immune system suppression (**Fig. 1**), but also from common diseases that might cause specific problems under these circumstances. Another risk may be the development of pathogens in a closed environment, where air, food, waste, and water are recycled. Confinement 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 martian surface, such as dust or chemicals that 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 astronaut Eugene Cernan weary and filthy with rock dust. A trip to Mars will certainly multiply the hazards of space travel.

Humans are ready to accept great risks to go where no one has gone before, but do we have sufficient and sound biologi-

Abbreviations: AHCC=active hexose correlated compound, CNES=French National Space Center, ESA=European Space Agency, Ets=E26 transformation specific, HDBR=head-down bed-rest, IML-2=International Microgravity Laboratory 2, ISS=International Space Station, PKA/PKC=protein kinase A/C, respectively, PMN=polymorphonuclear neutrophil, ROS=reactive oxygen species, SLS-1=Spacelab Life Sciences 1

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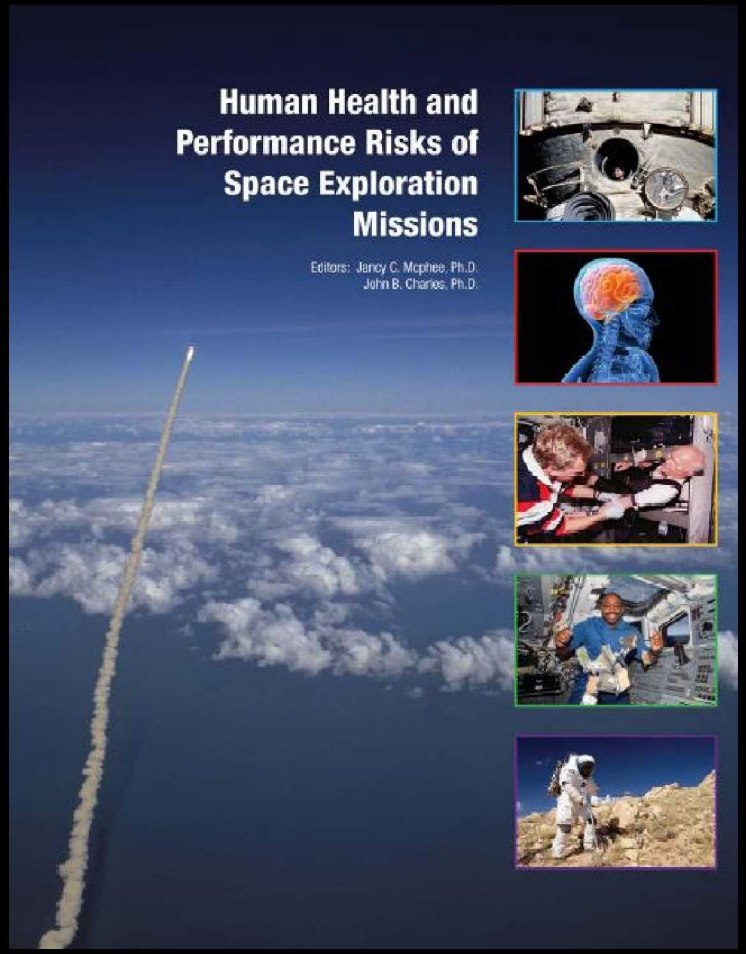
J Leukoc Biol. 2009 Nov;86(5):1027-38.



Human Research Program
Human Health Countermeasures Element

Evidence Book

Risk of Crew Adverse Health Event Due to Altered Immune Response

June 2009

National Aeronautics and Space Administration
Lyndon B. Johnson Space Center
Houston, Texas

http://humanresearch.jsc.nasa.gov/elements/smo/hrp_evidence_book.asp

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.

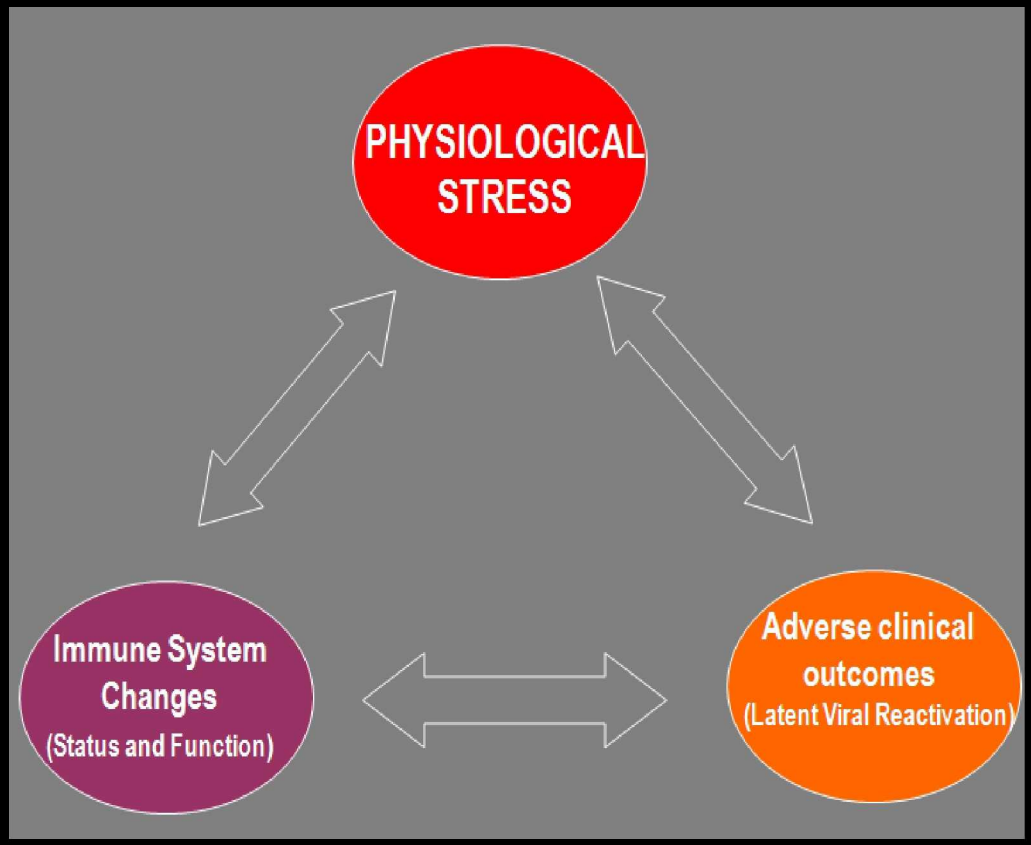
Assays

JSC Immunology Laboratory
- Leukocyte subsets
- T cell function
- Intracellular/secreted cytokine profiles

Mercer University
- Plasma cytokine balance
- Leukocyte cytokine RNA

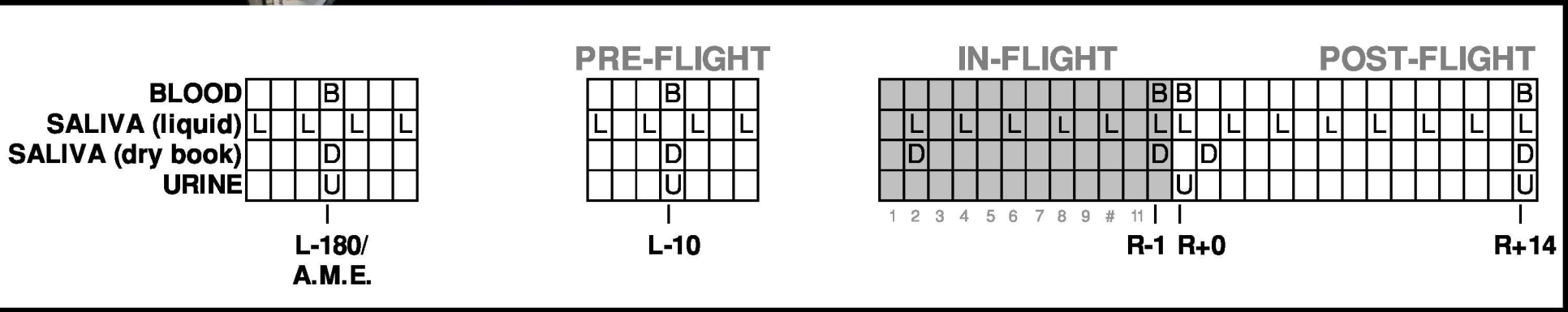
Microgen Laboratories
- Virus specific T cell number
- Virus specific T cell function
- Plasma stress hormones

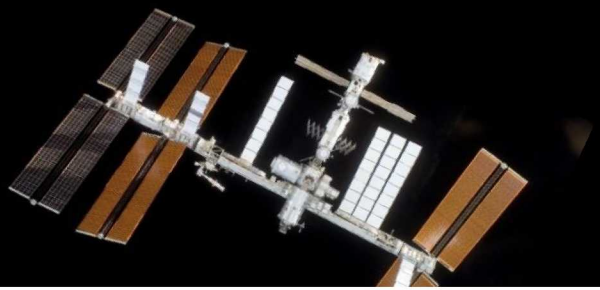
JSC Microbiology Laboratory
- Latent herpesvirus reactivation (saliva/urine)
- Saliva/urine stress hormones
- Circadian rhythm analysis



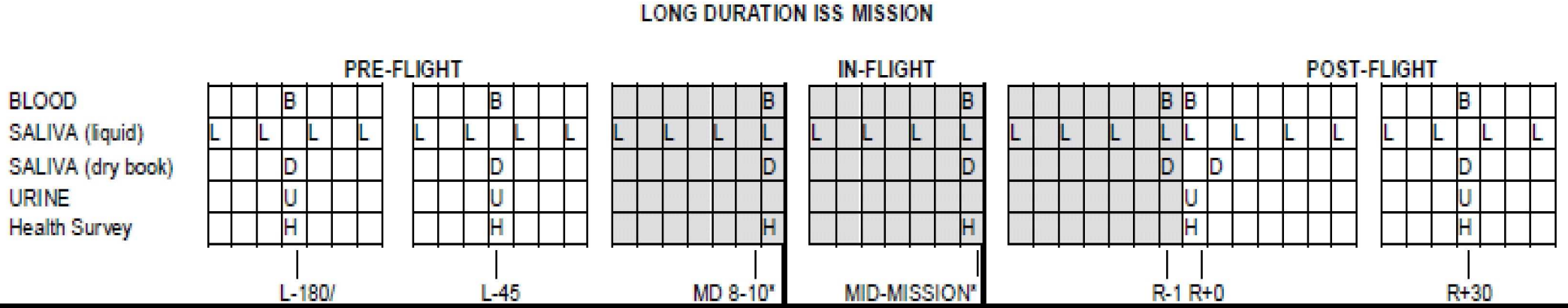
Samples - Timepoints

Short Duration

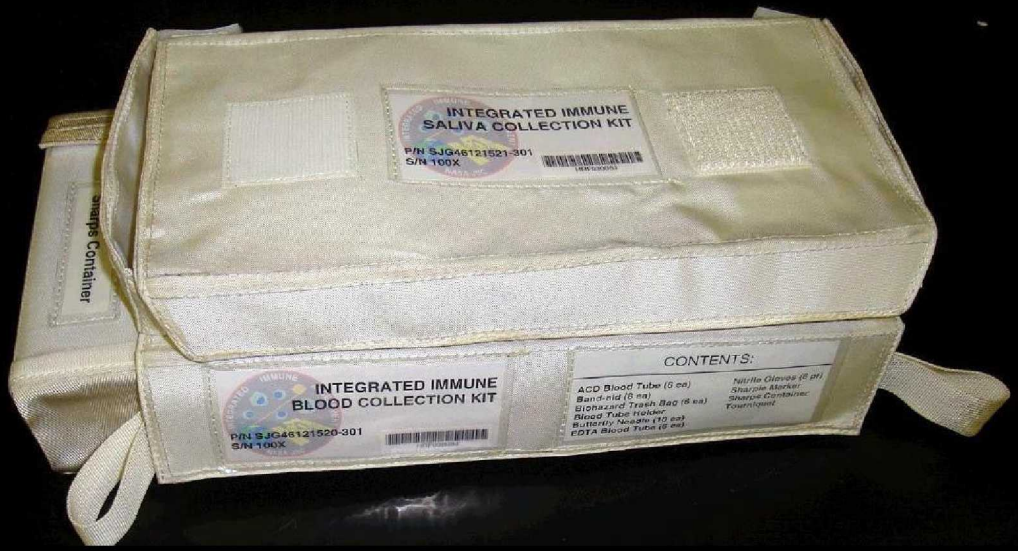


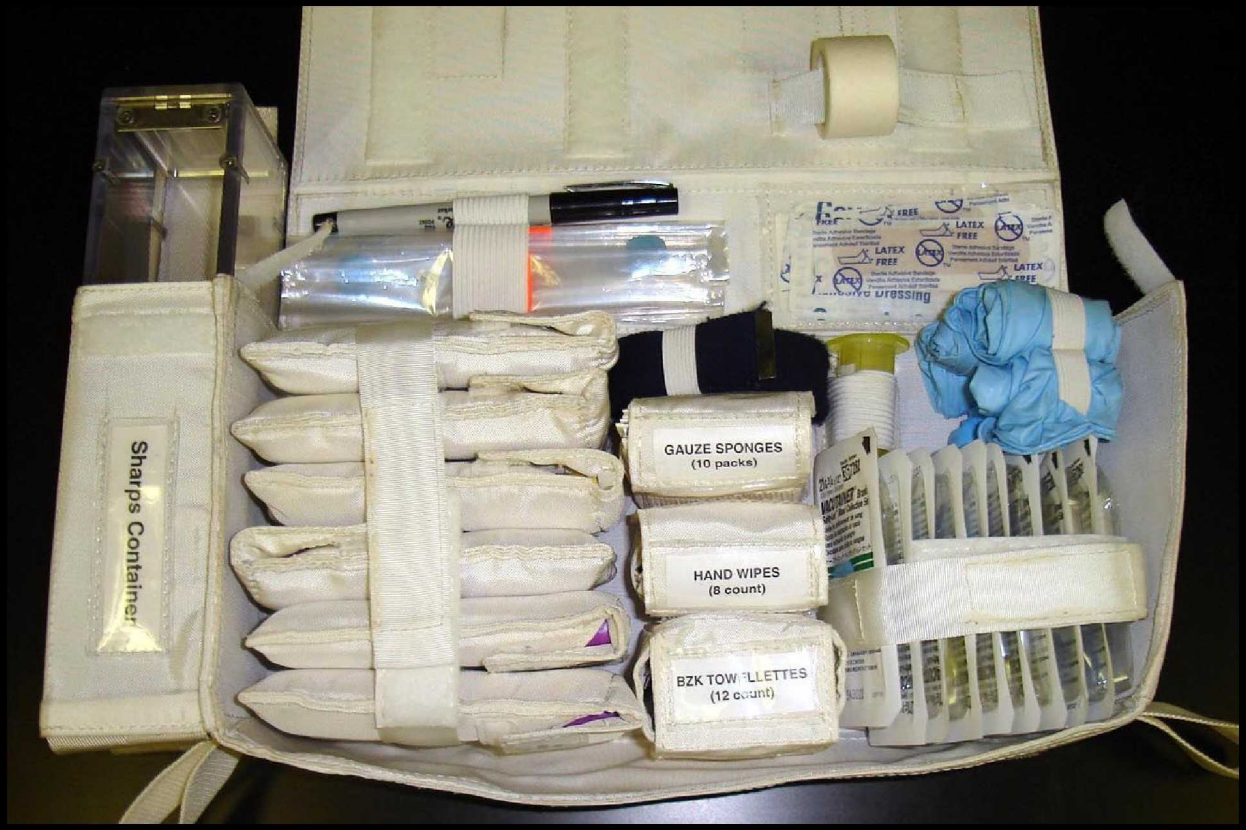


Long Duration



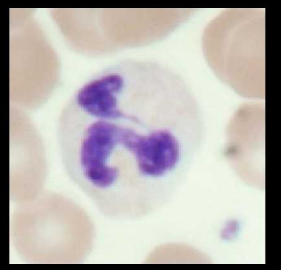
Flight Hardware





DISTRIBUTION OF IMMUNE CELLS

WHITE BLOOD CELLS + RED BLOOD CELLS



GRANULOCYTES
MONOCYTES
LYMPHOCYTES

BASOPHILS
NEUTROPHILS
EOSINOPHILS

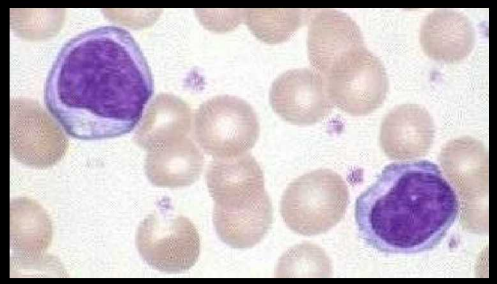
MAST CELLS

B CELLS
T CELLS
NK CELLS

MACROPHAGE
DENDRITIC CELL

CD4+ 'Helper'
CD8+ 'Cytotoxic'

PLASMA CELLS

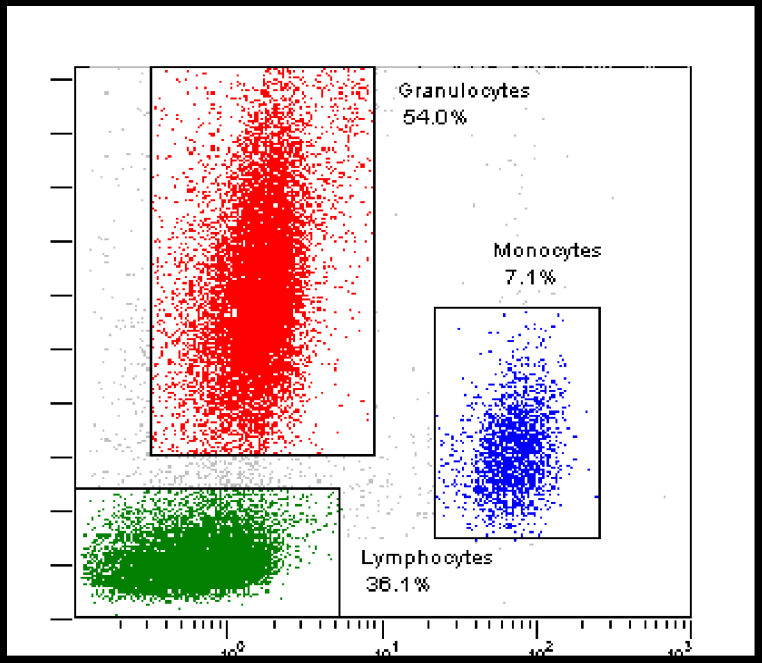


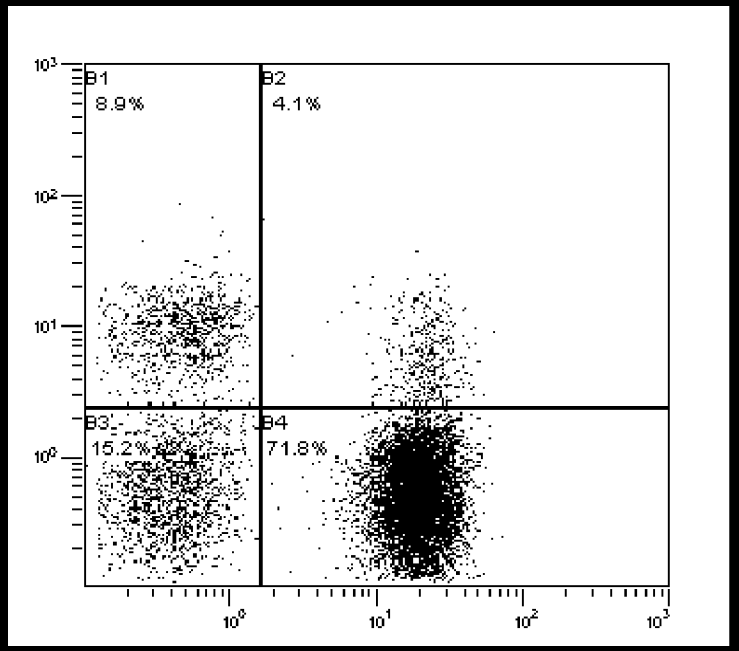
Memory
Naive
Memory
Naive

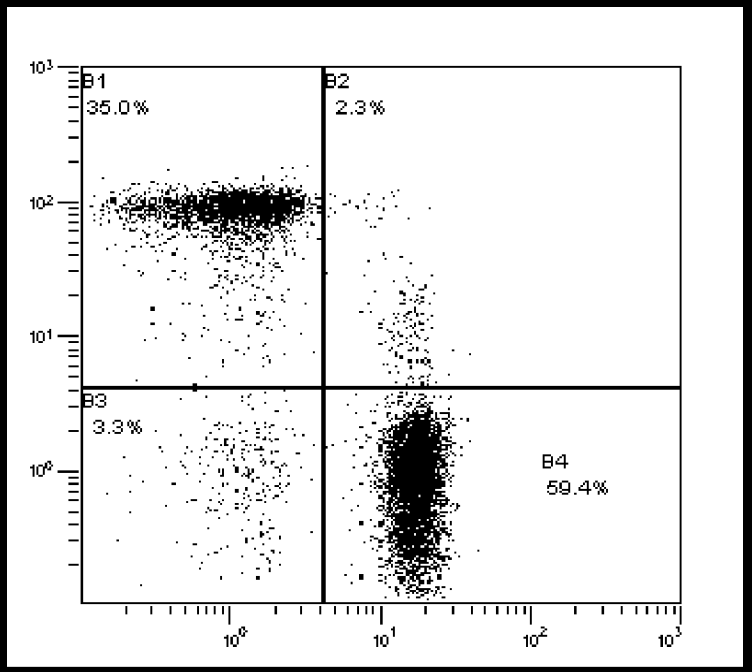
Th1
Th2
Th17
Treg

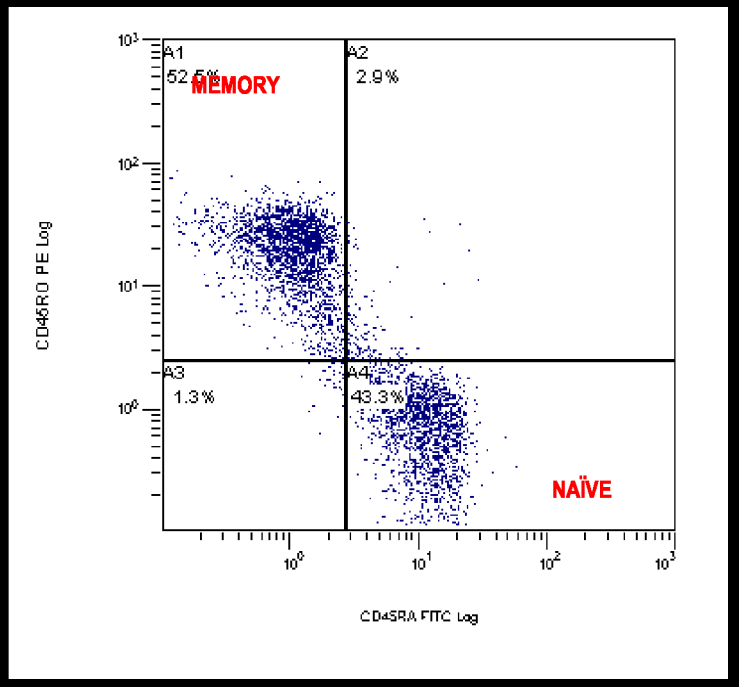
True Naive
Central Memory
Effector Memory
Terminal Diff.

Peripheral Leukocyte Distribution

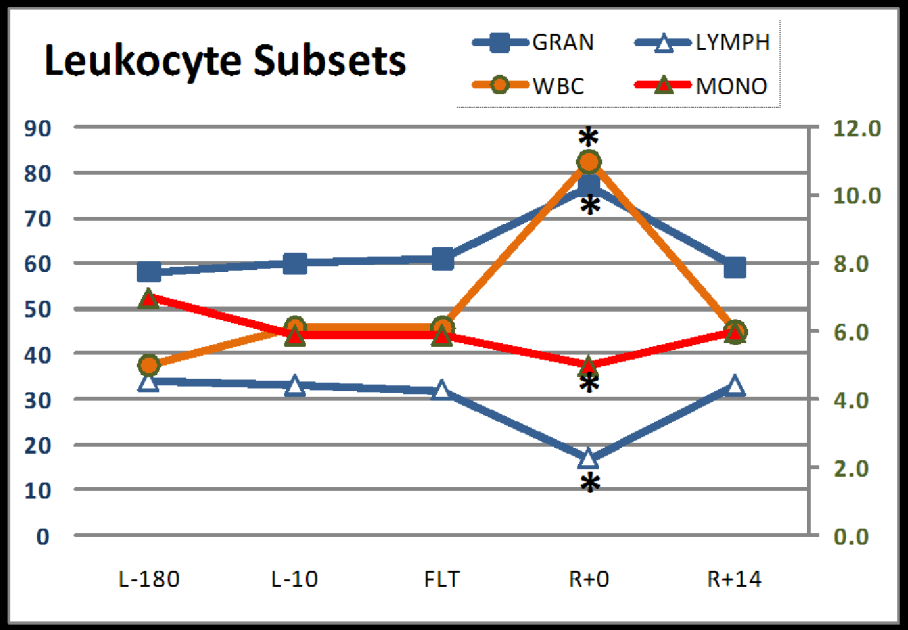


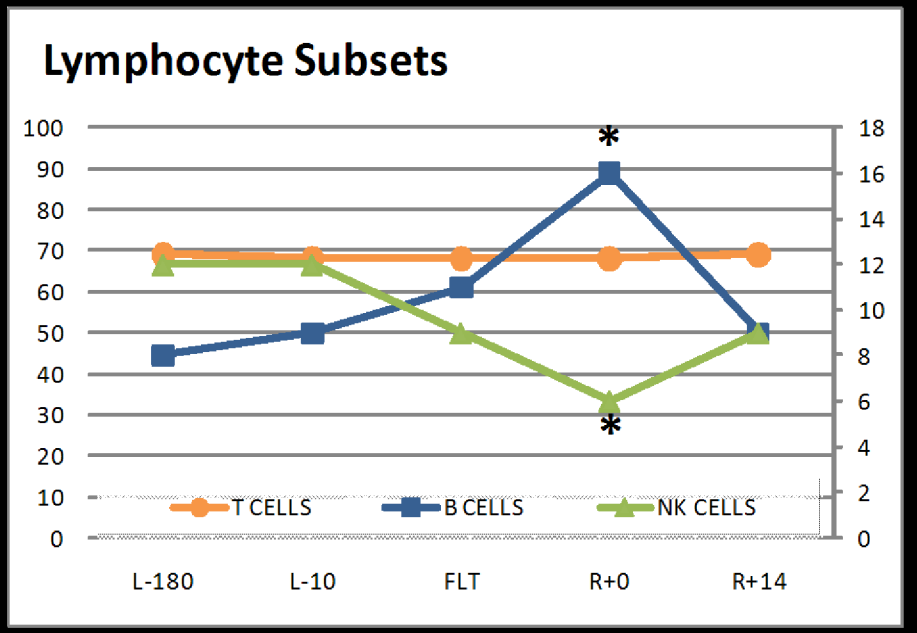


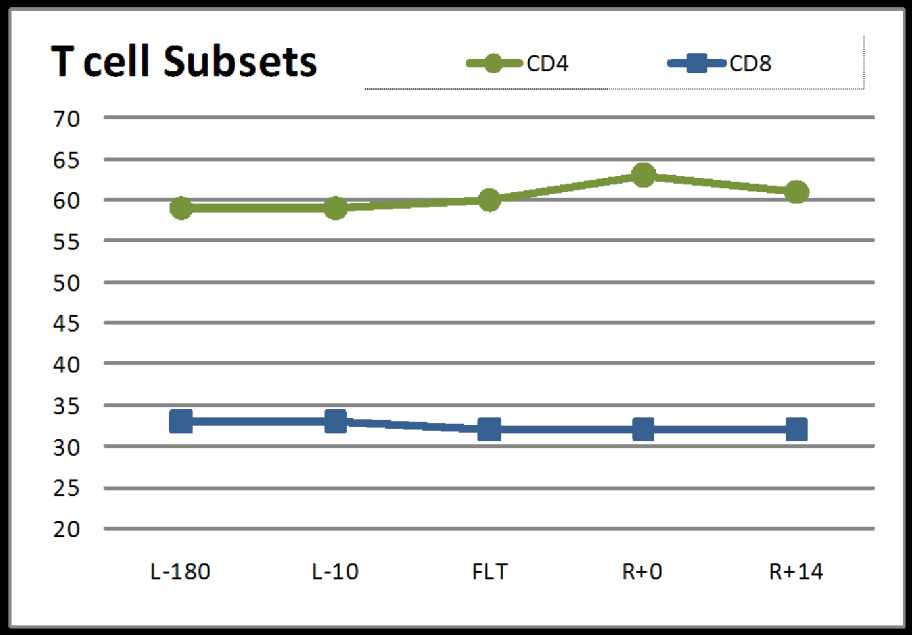


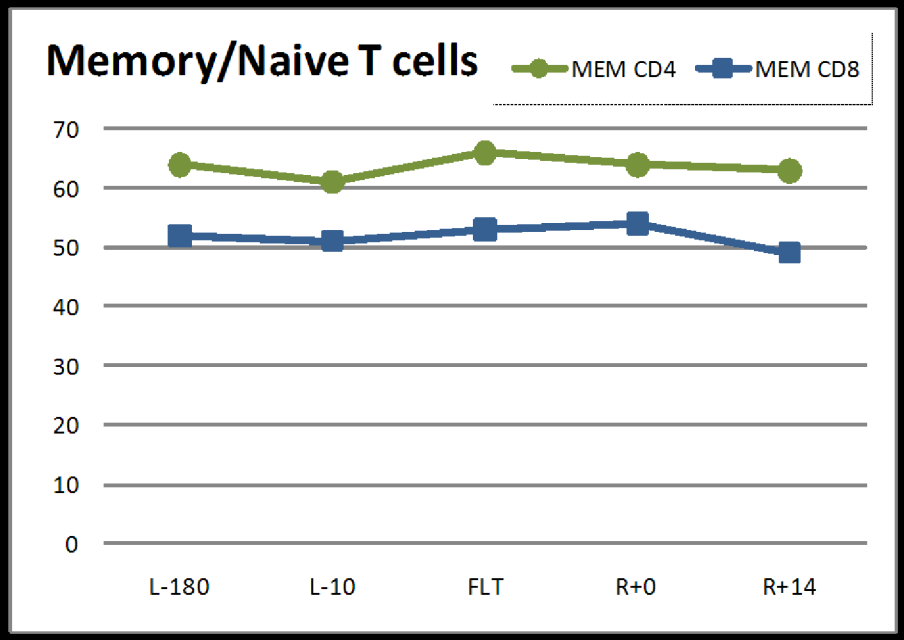


Peripheral Leukocyte Distribution

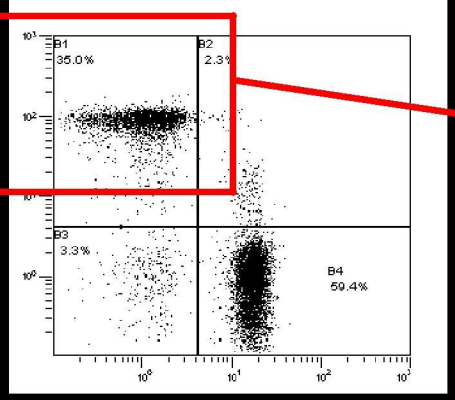


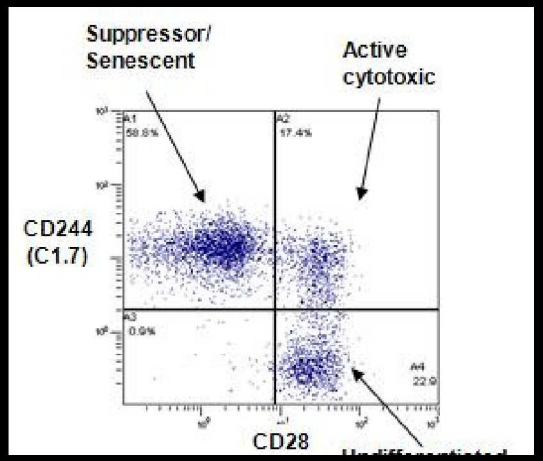


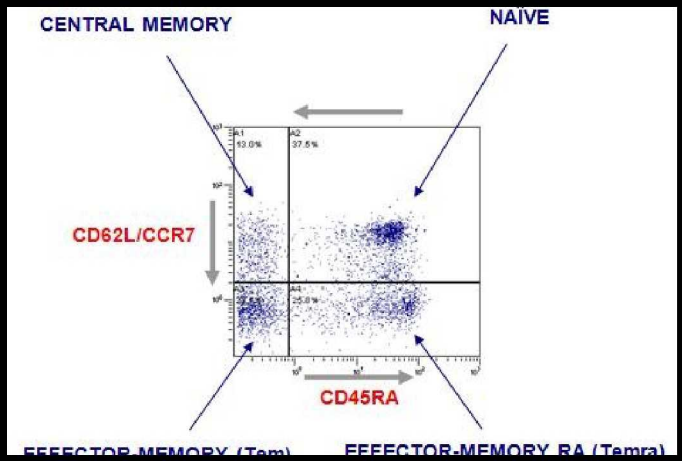




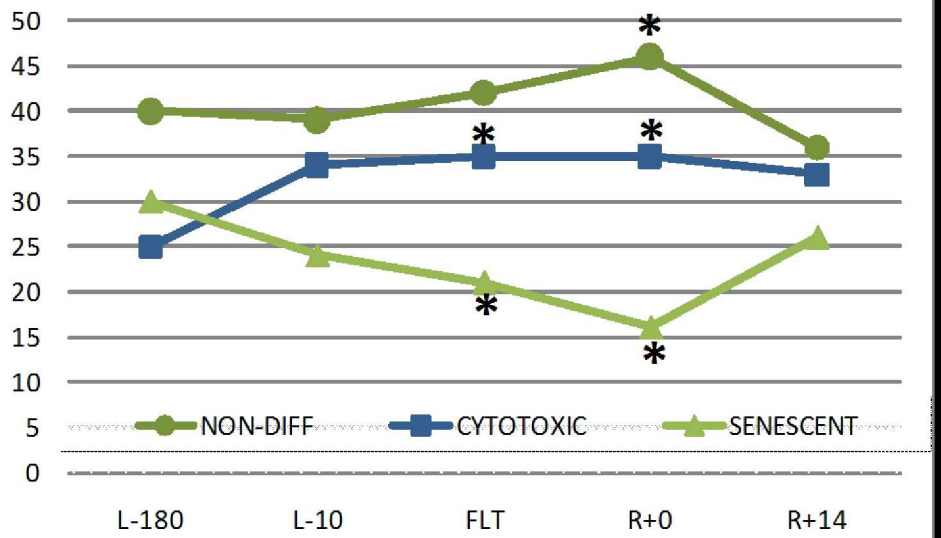
CD8+ T cell Subsets



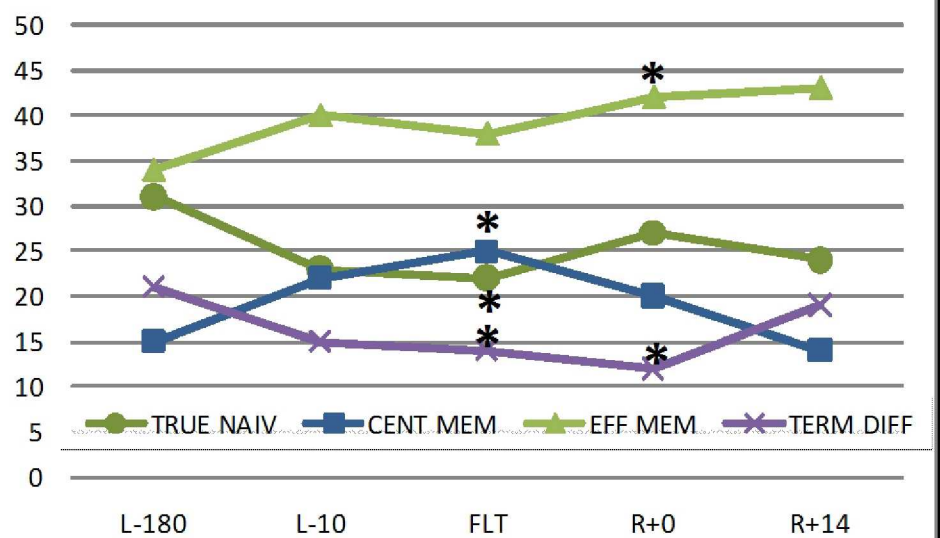




CD8+ Differentiation State

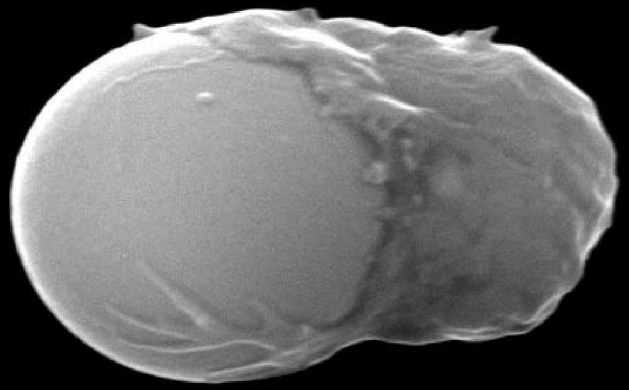


CD8+ Central/Memory

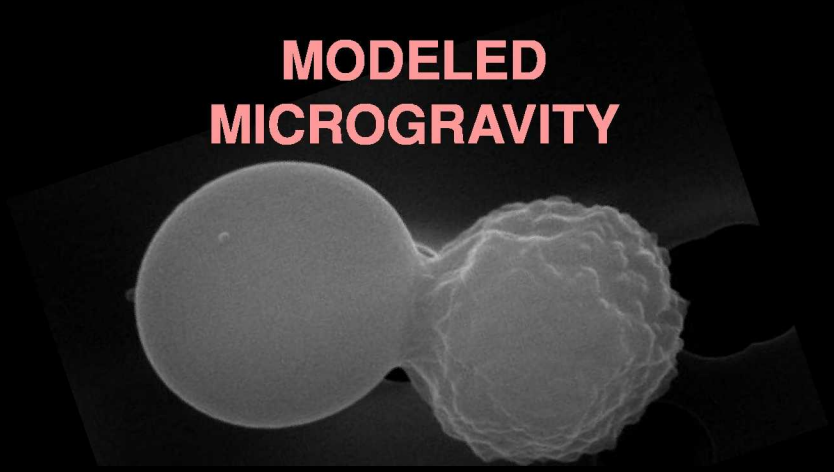


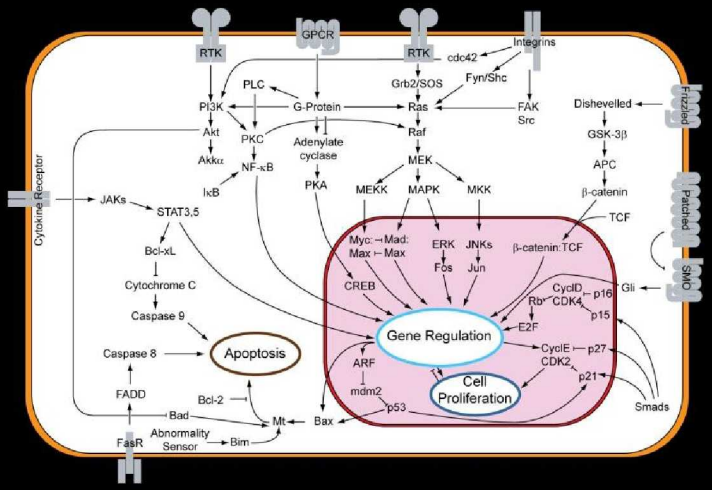
T Cell Activation

1xG CONTROL



MODELED MICROGRAVITY

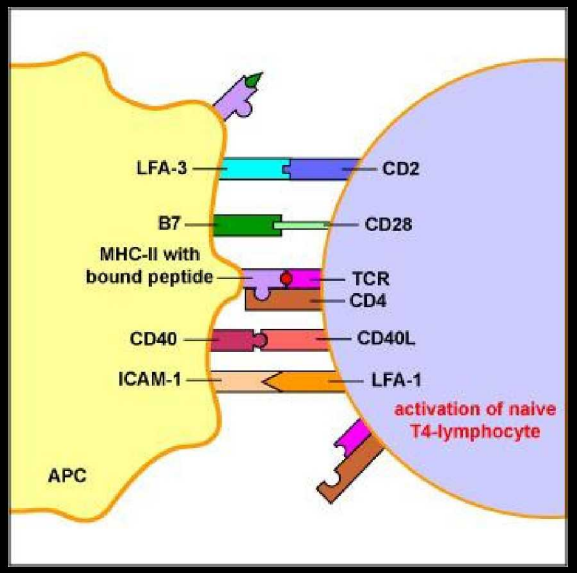


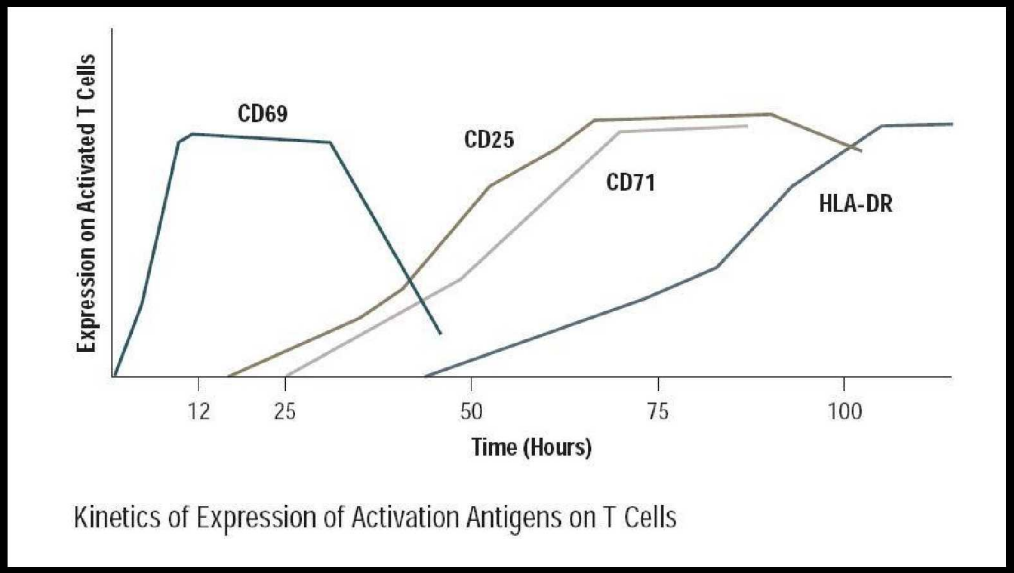


KINETICS OF T CELL ACTIVATION

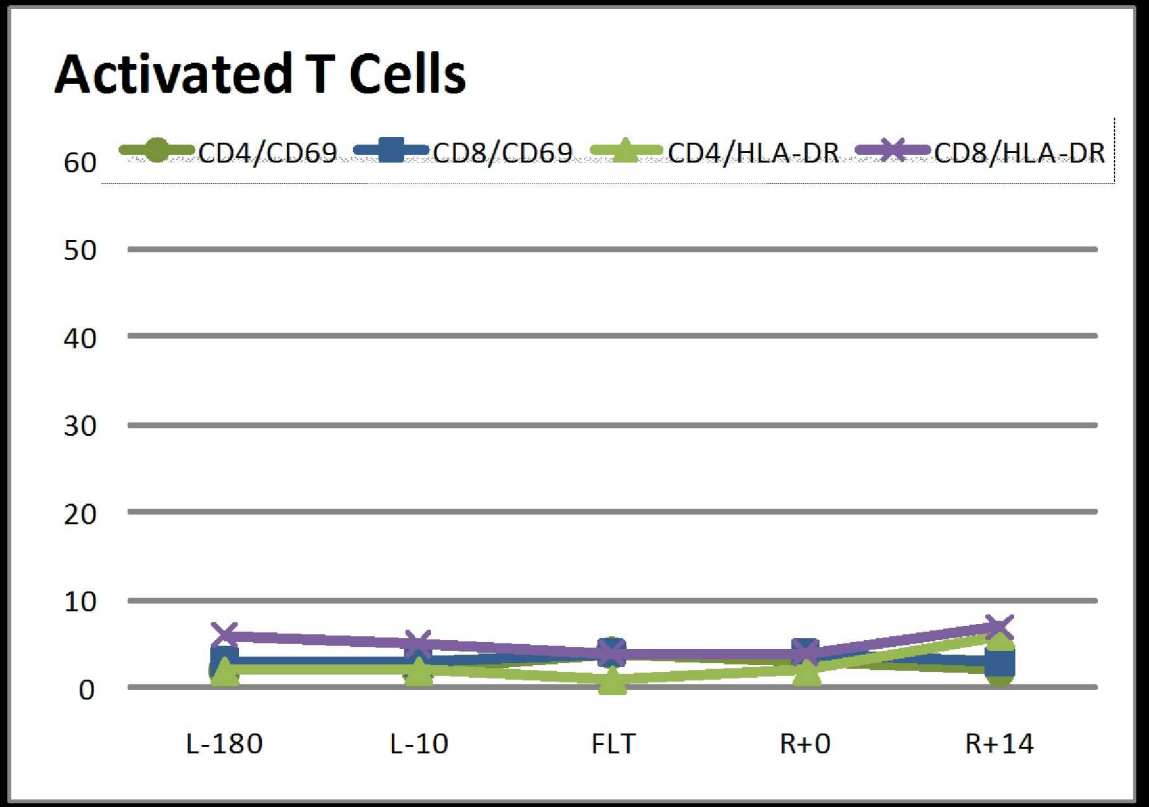
T 0:00	Ligand-receptor binding
0-5 sec	Membranes increase permeablity to ions Shifts in ions from one intracellular compartment to another Changes in membrane potential Changes in intracellular pH
0-5 min	Changes of state in membrane lipids and proteins Activation of adenylate cyclase, ATPase, and other membrane-associated enzymes Changes in cyclic nucleotide concentractions Changes in receptor distribution and mobity occur Adhension molecule conformational changes
T +30 min	Coalescence of patched receptors into cap at one pole of the cell (dependant on contractioin of cytoskeletal microfilaments, ATP energy source)
T +6-12hr	Expression of CD69 on T cell surface
T +24 hr	Secretion of IL-2, cell surface epxression 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 Epression 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

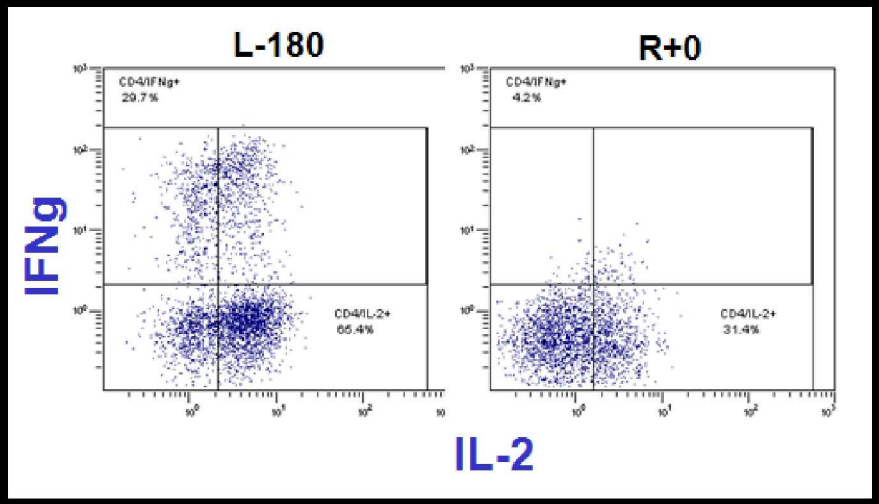


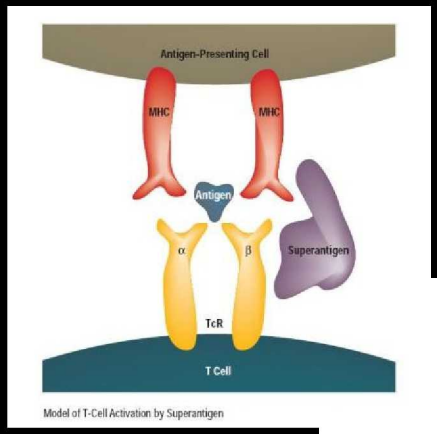


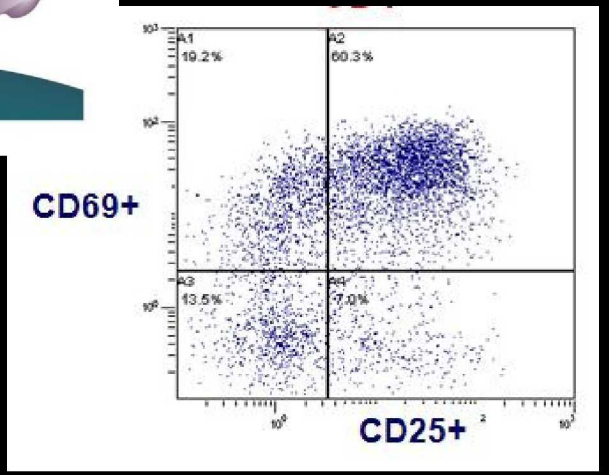
Kinetics of Expression of Activation Antigens on T Cells

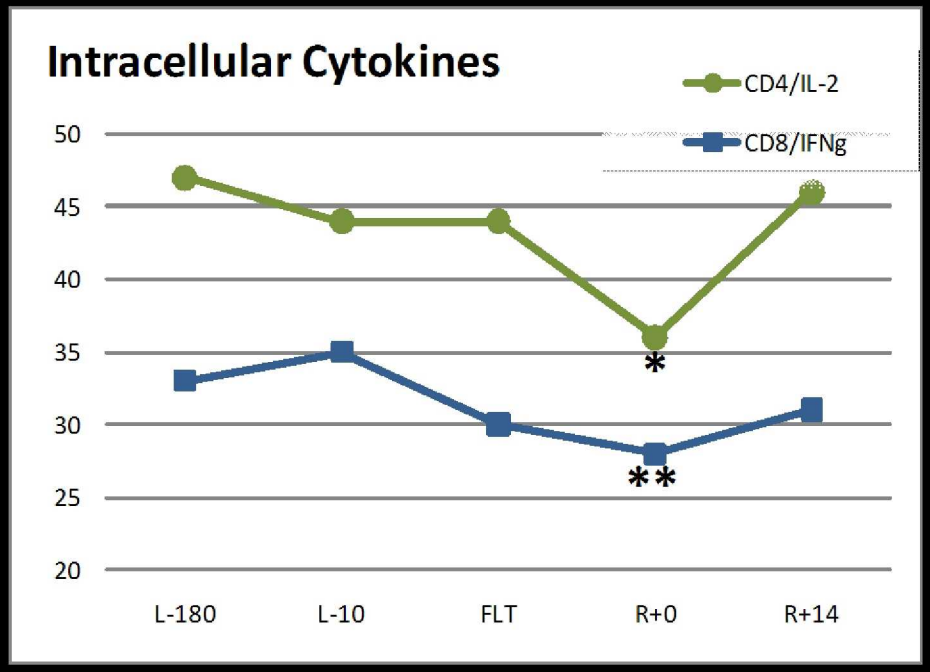


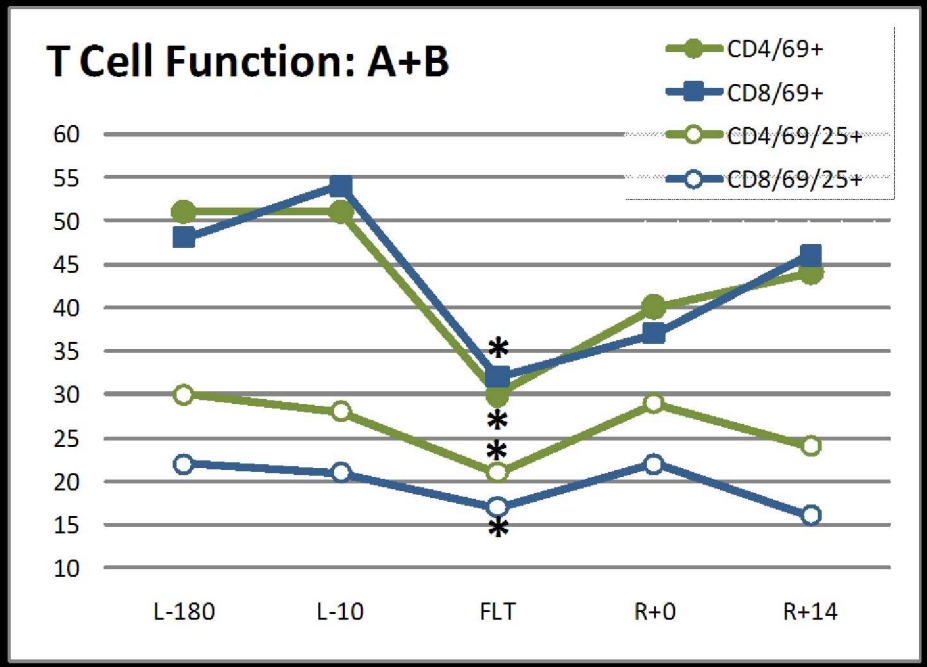
T Cell Function: Intracellular Cytokine, Early Blastogenesis











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

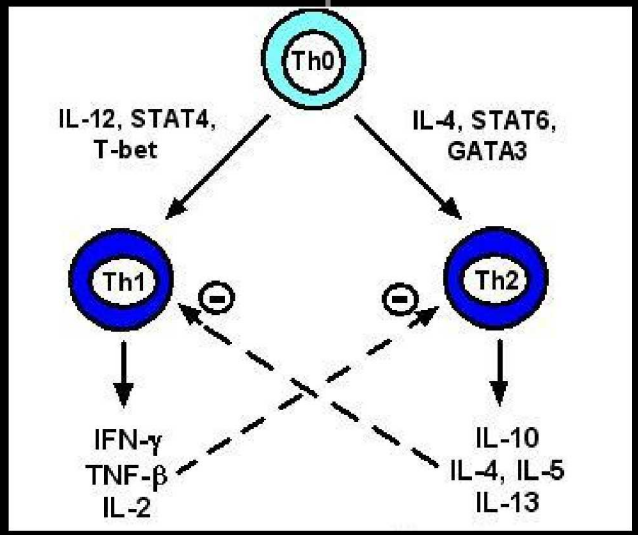
Th2 - Antibody response to extracellular pathogens, parasites

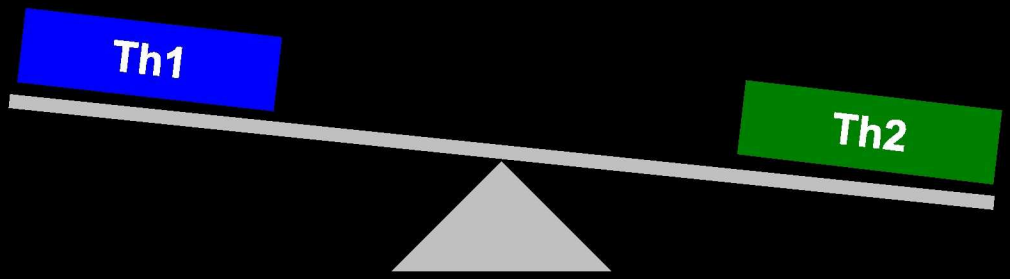
Normal Function

- Humoral (Antibody) Responses
- 'Anti-Inflammatory Response

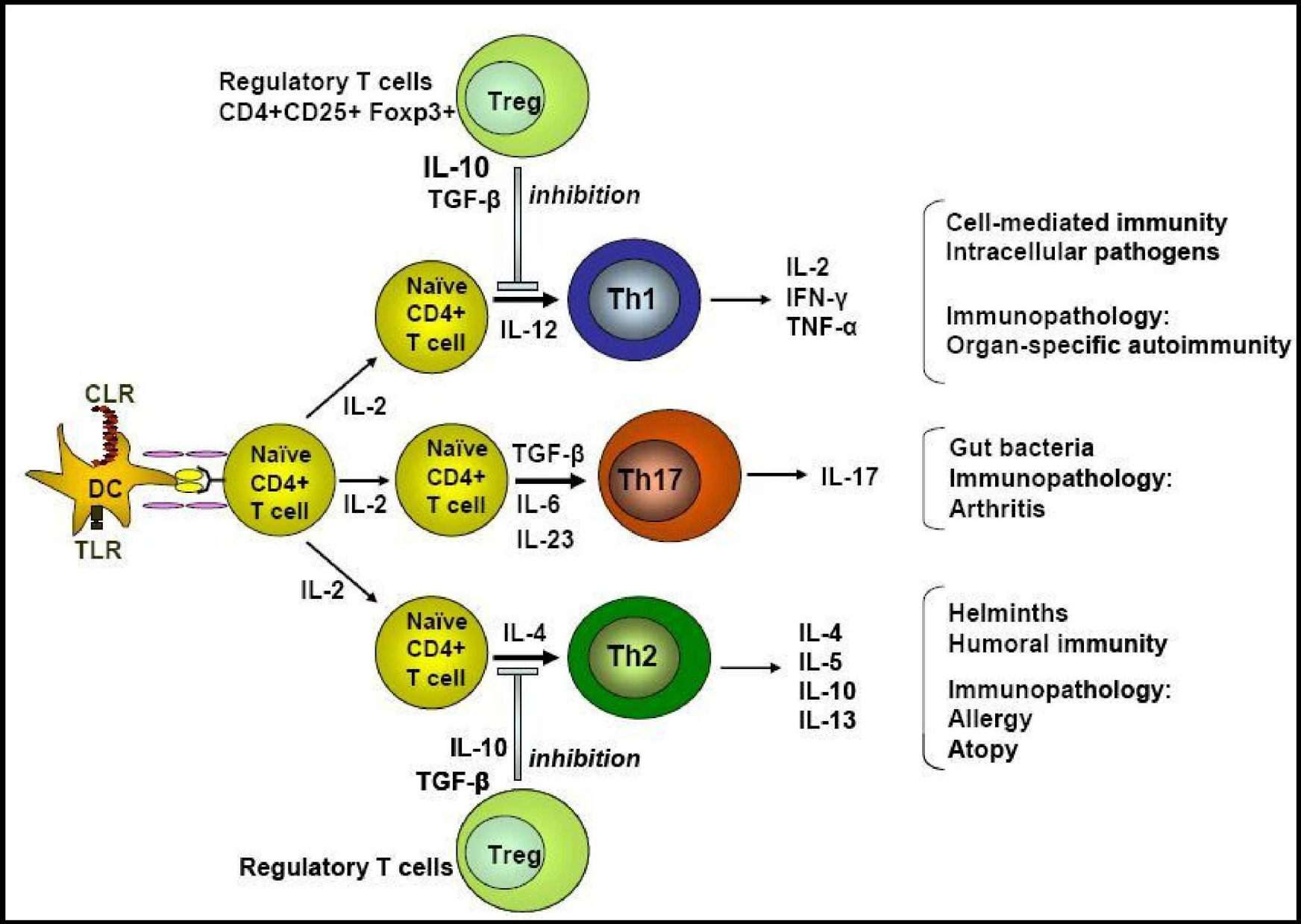
Disease correlations:

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

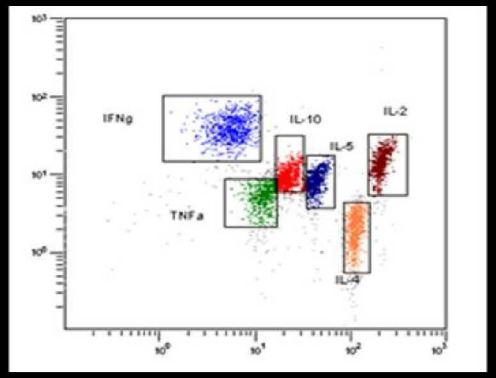


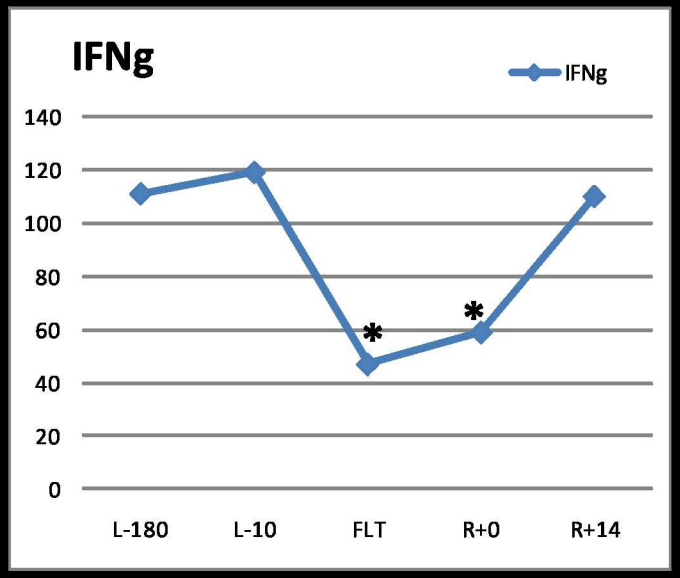


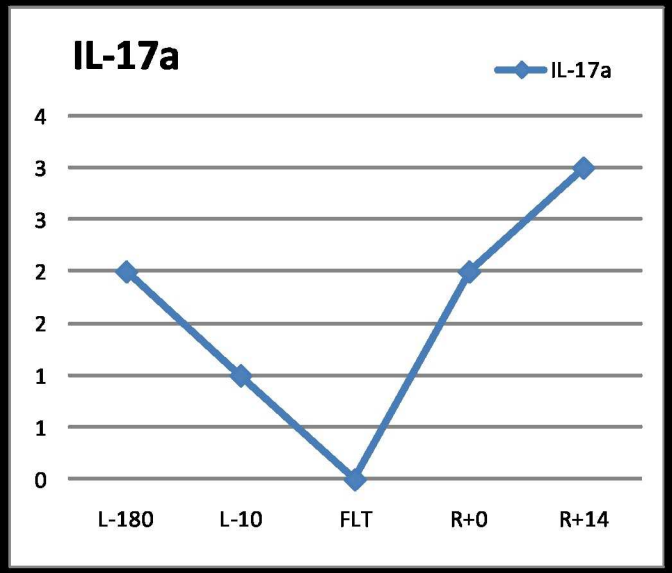
Cytokines: Th1/Th2/Th17

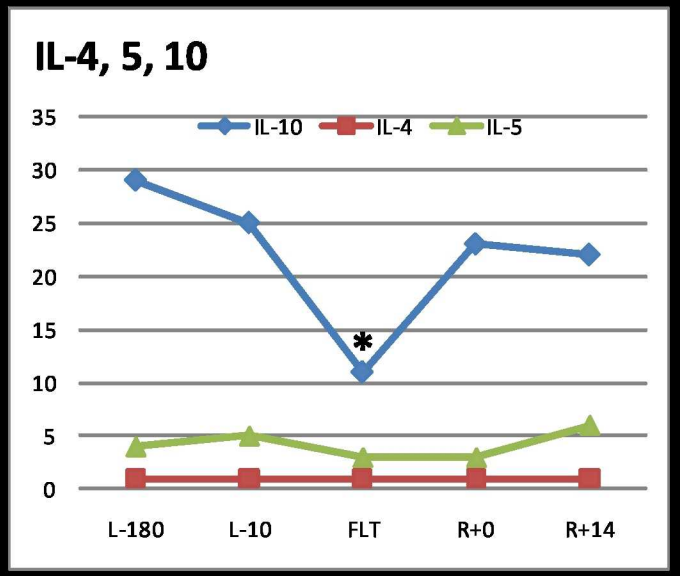


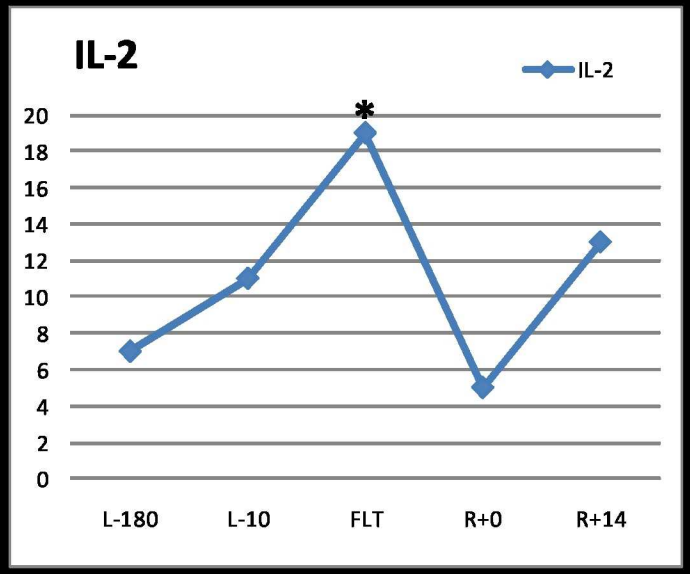
Secreted Cytokine Profiles (T cell stimulation)

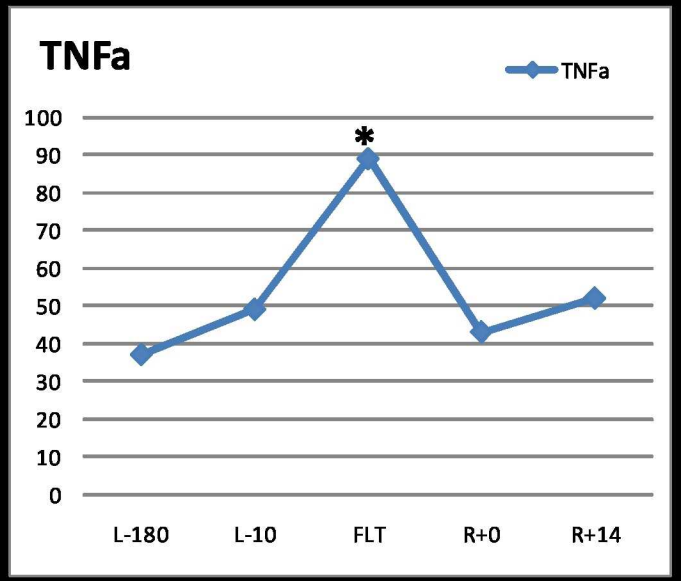


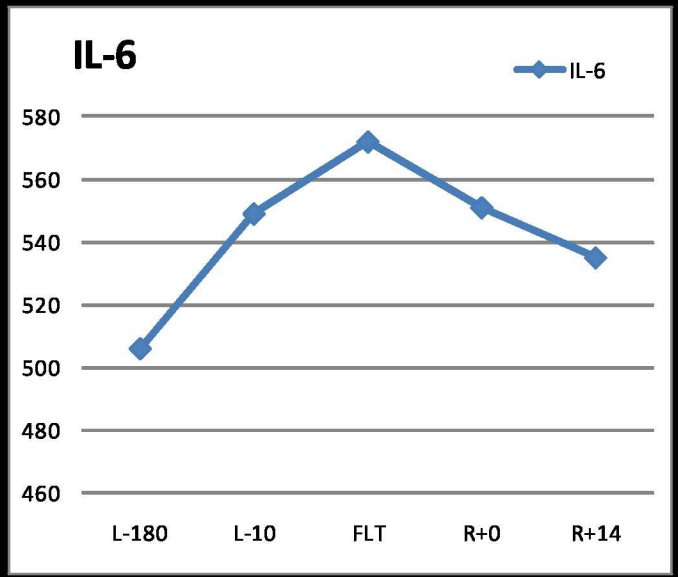




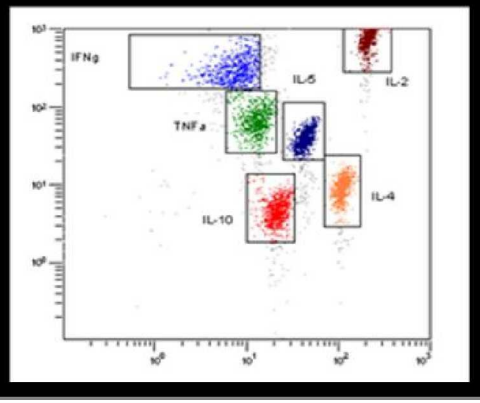


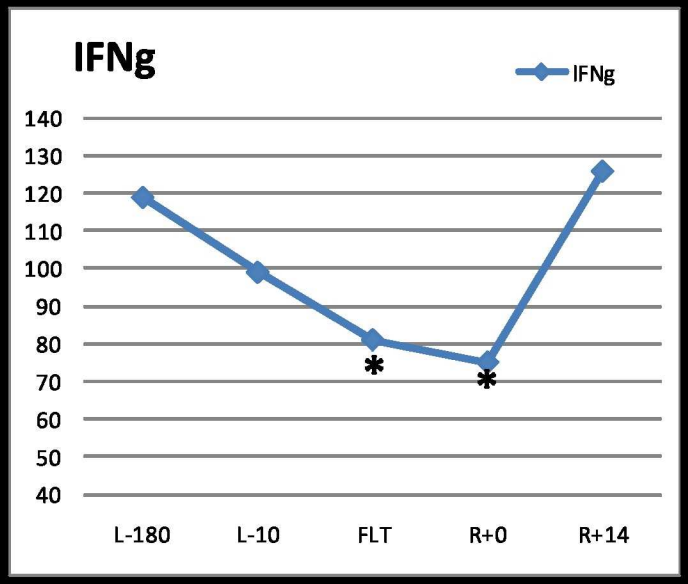


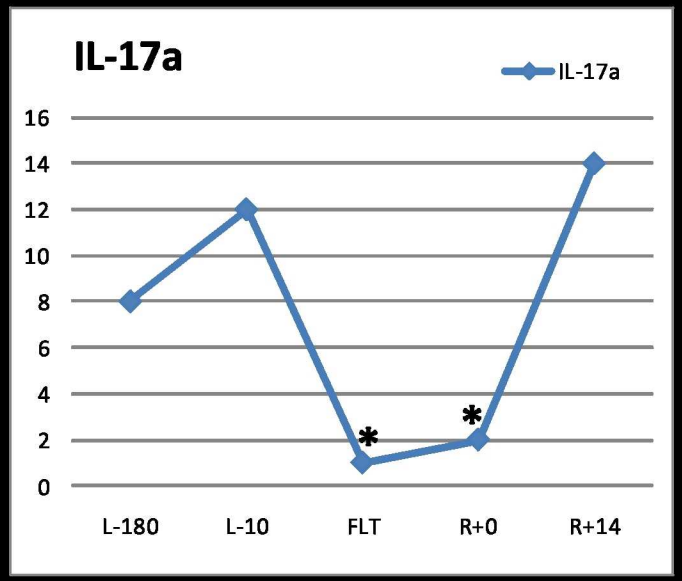


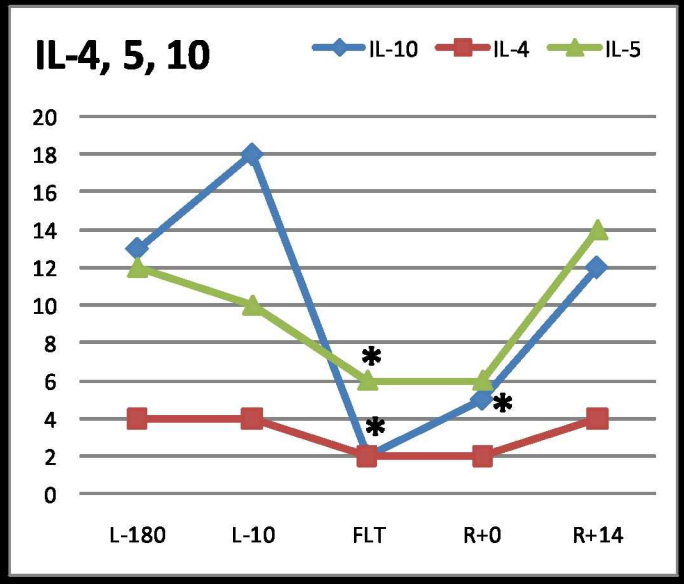


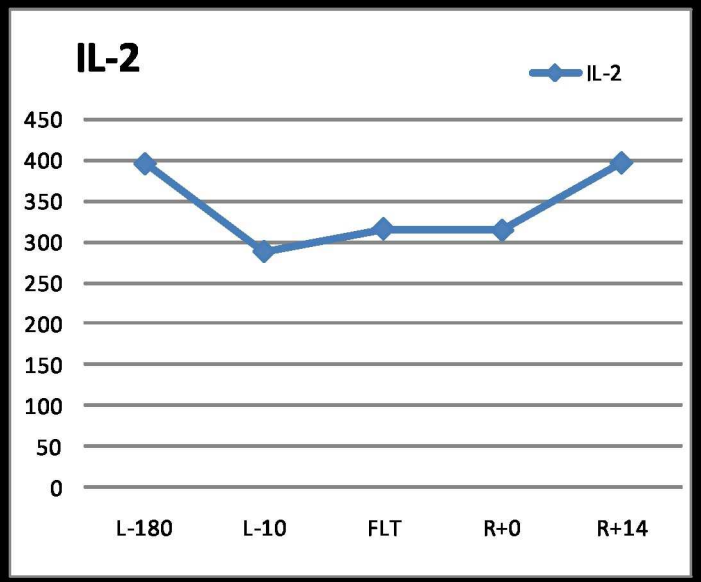
Secreted Cytokine Profiles (PMA-I stimulation)

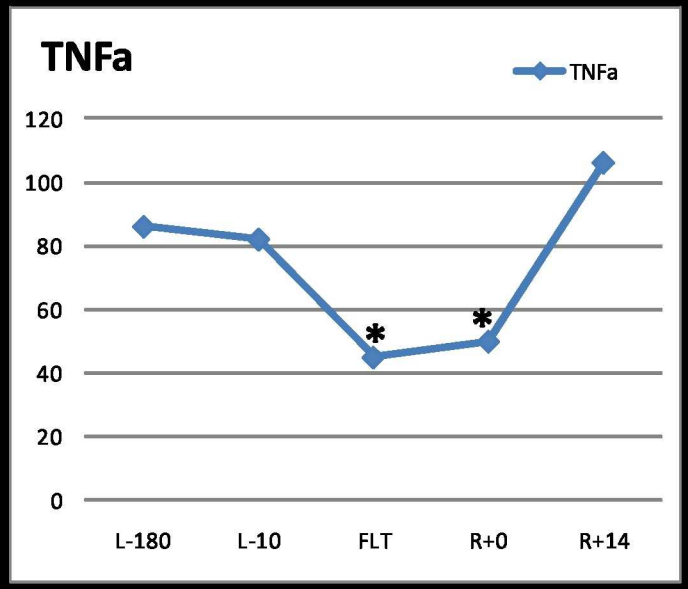


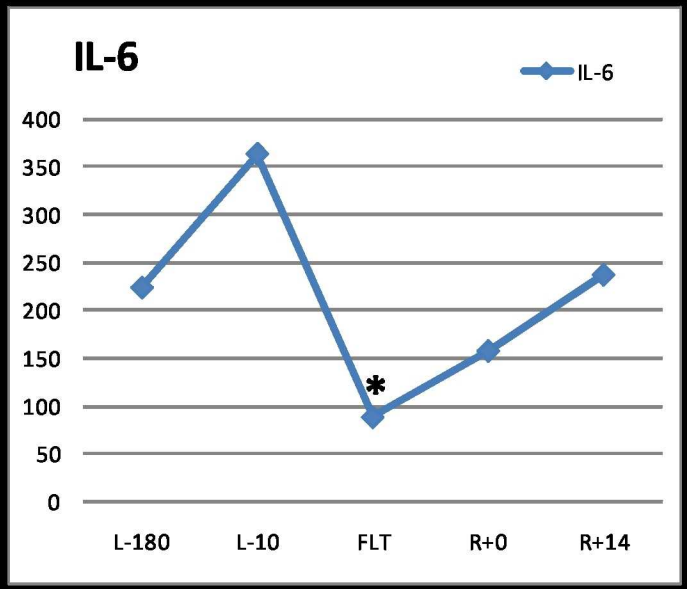




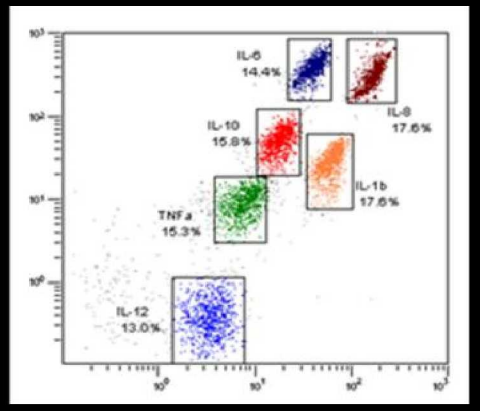


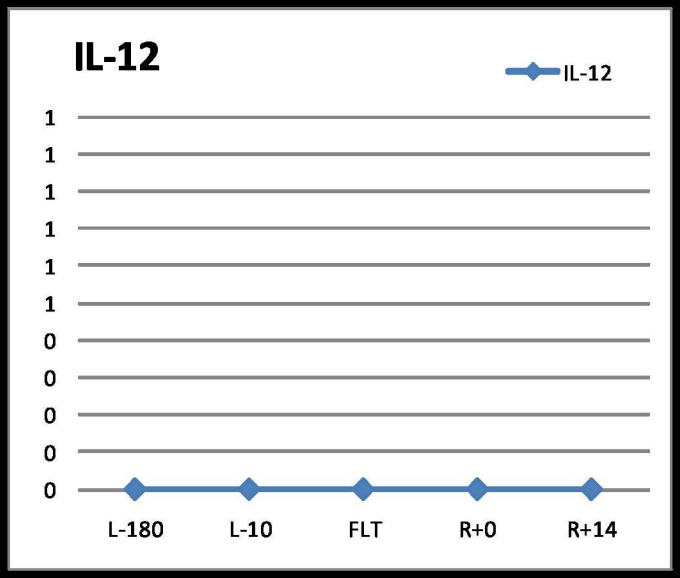


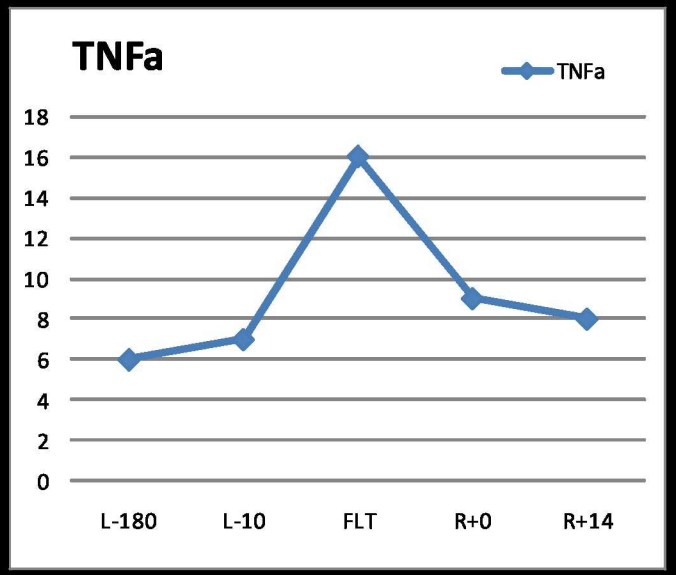


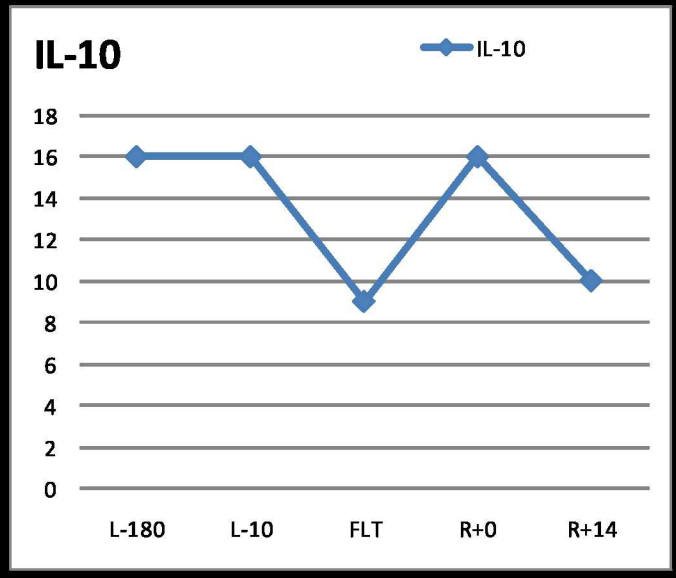


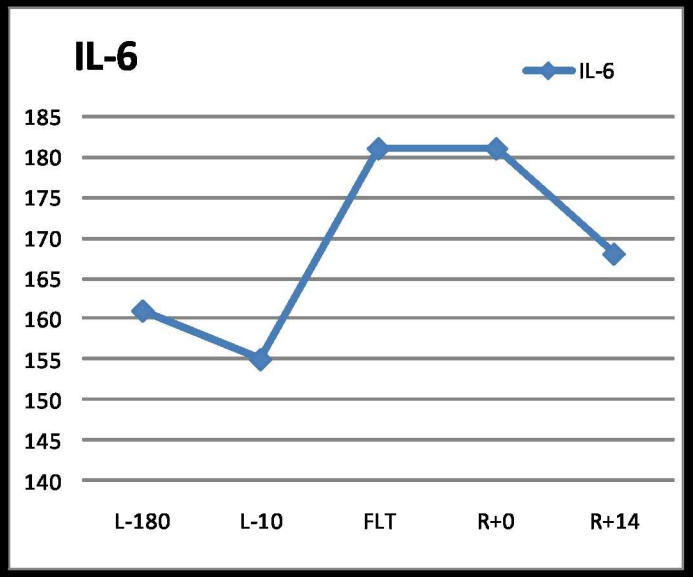
Secreted Cytokine Profiles (monocyte stimulation)

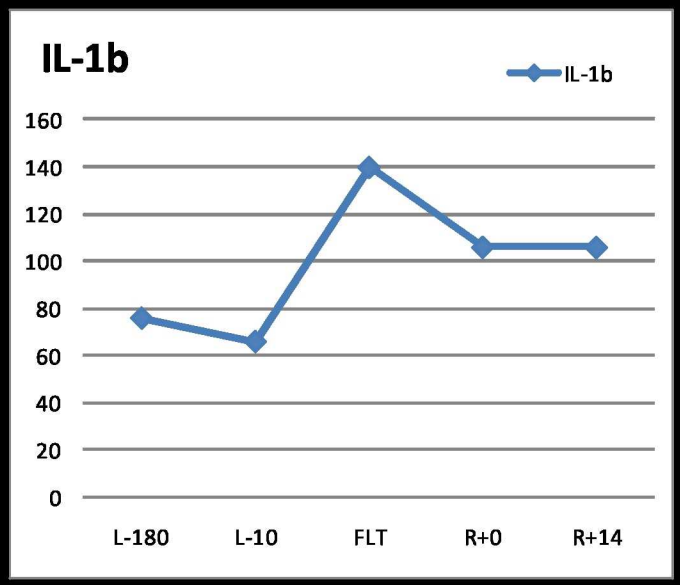


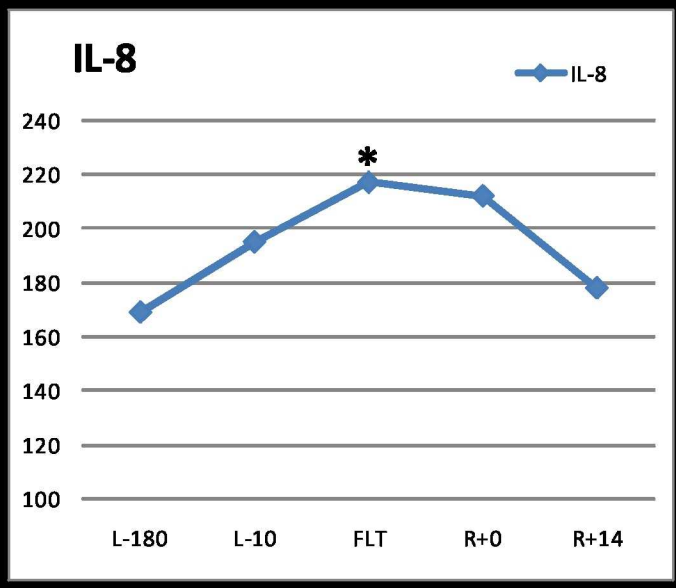












In-flight Secreted Cytokine Summary (short-duration)

T cells (CD3/CD28)

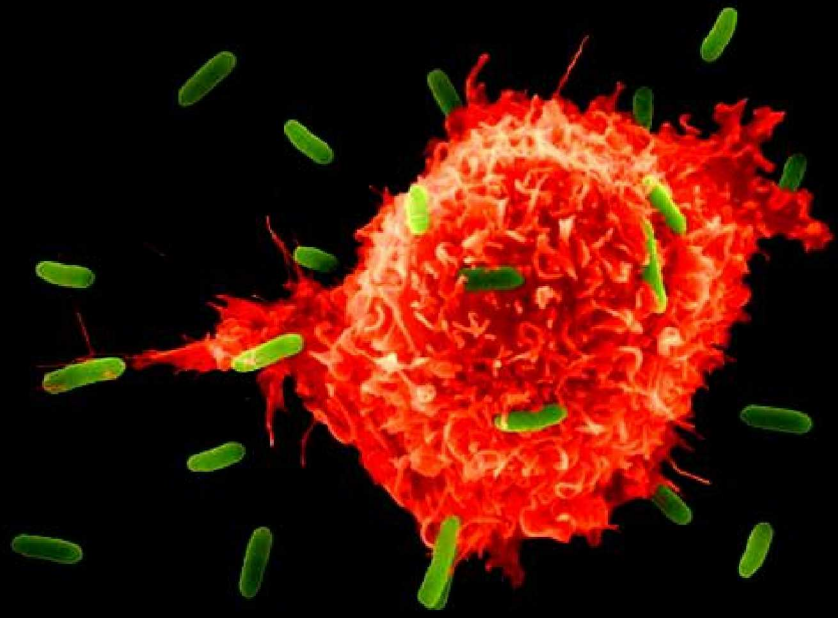
Adaptive immunity:	IFNg	↓
	IL-2	↑
	IL-4	--
	IL-5	--
	IL-10	↓
	IL-17	↓
Innate/Inflammatory:	IL-6	nc
	TNFa	↑

Monocytes (LPS)

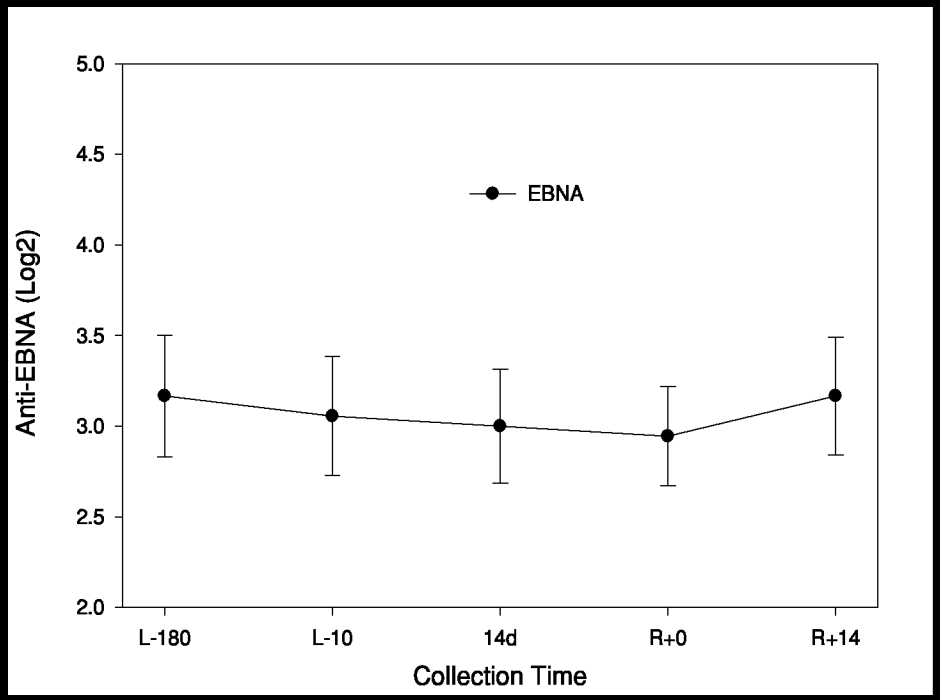
Innate/Inflammatory:	IL-1b	nc
	TNFa	nc
	IL-6	nc
	IL-8	↑
	IL-10	↓

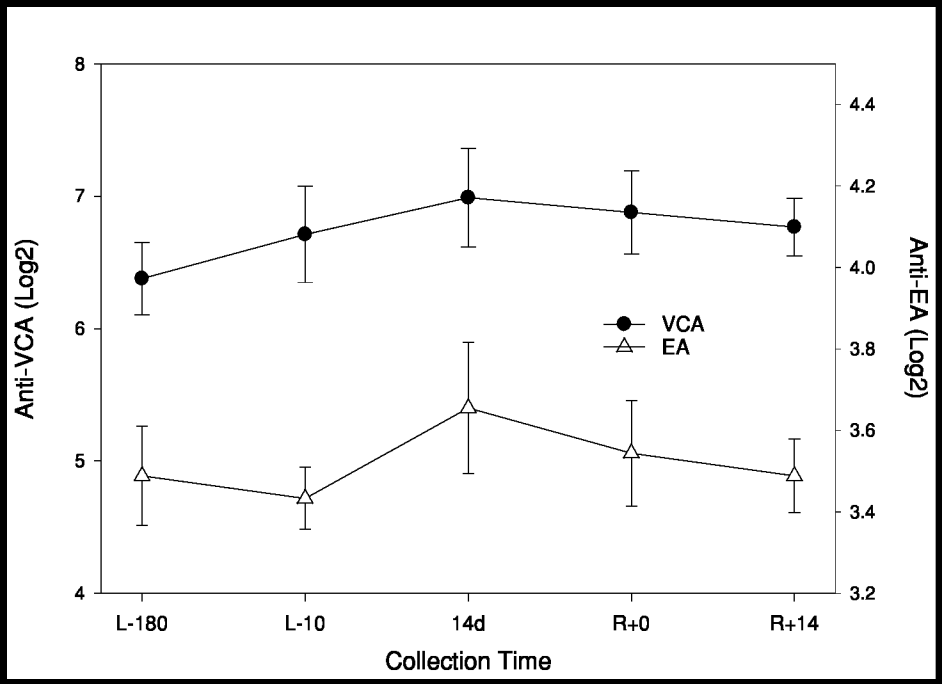
All Cells (PMA+ion)

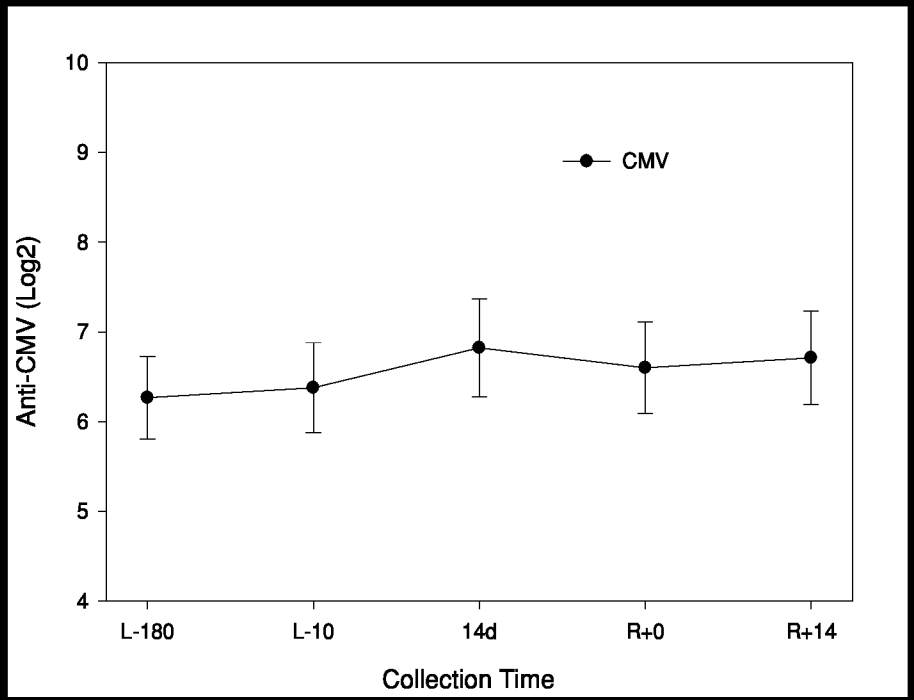
Adaptive immunity:	IFNg	↓
	IL-2	nc
	IL-4	↓
	IL-5	↓
	IL-10	↓
	IL-17	var
Innate/Inflammatory:	IL-6	↓
	TNFa	↓

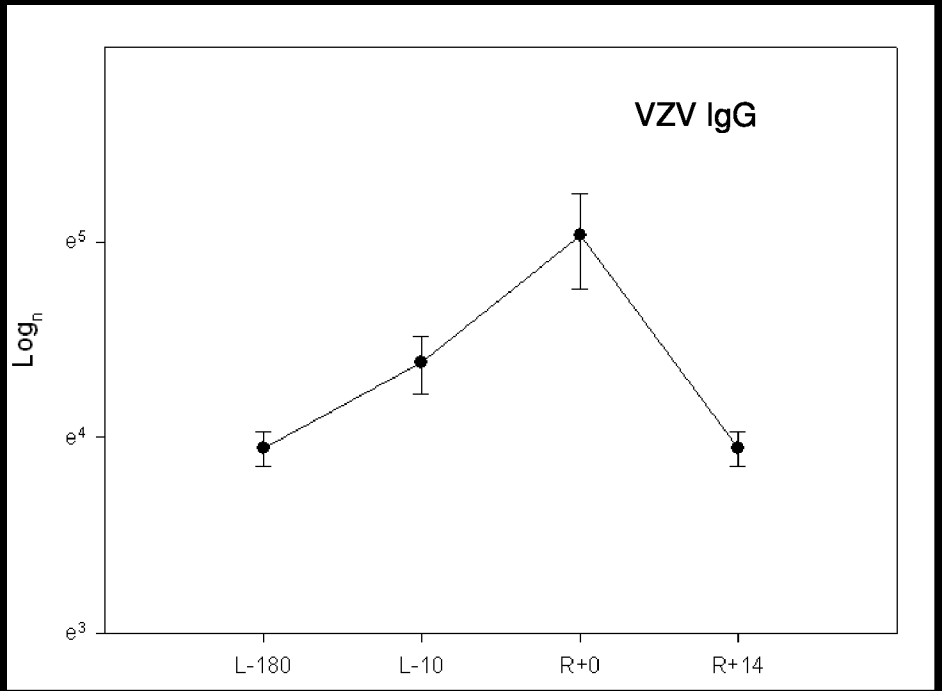


Viral Antibody Titers



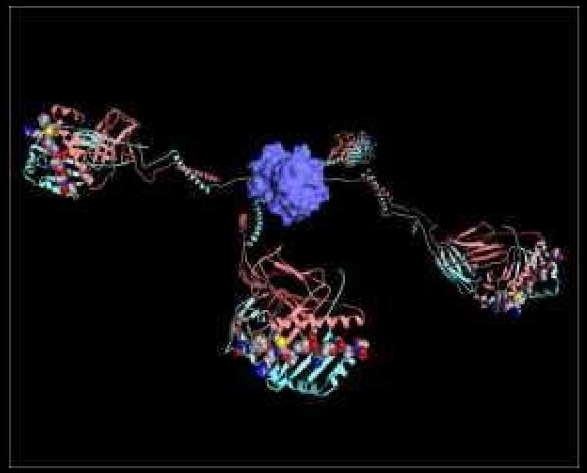


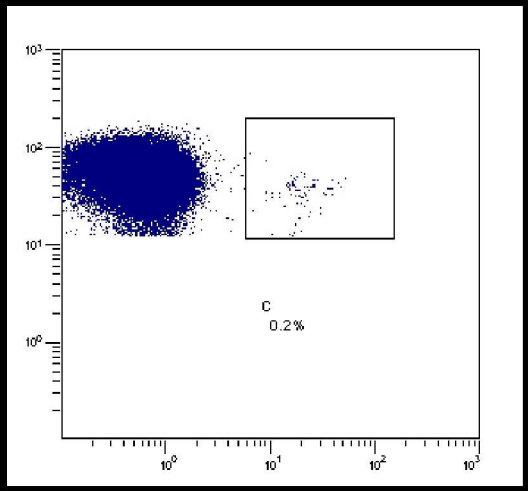




Virus-specific T cell Number/Function

Tetramer Assay





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

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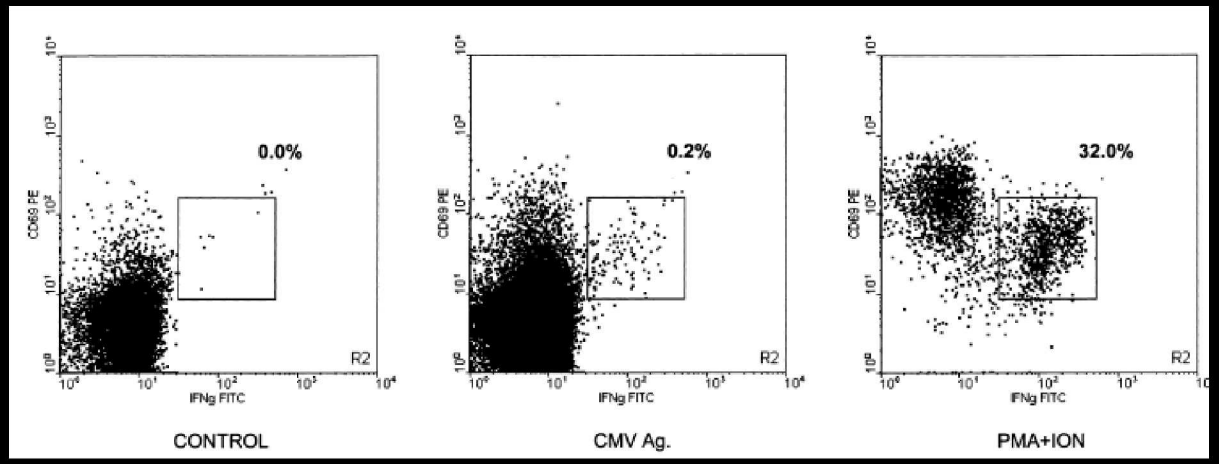
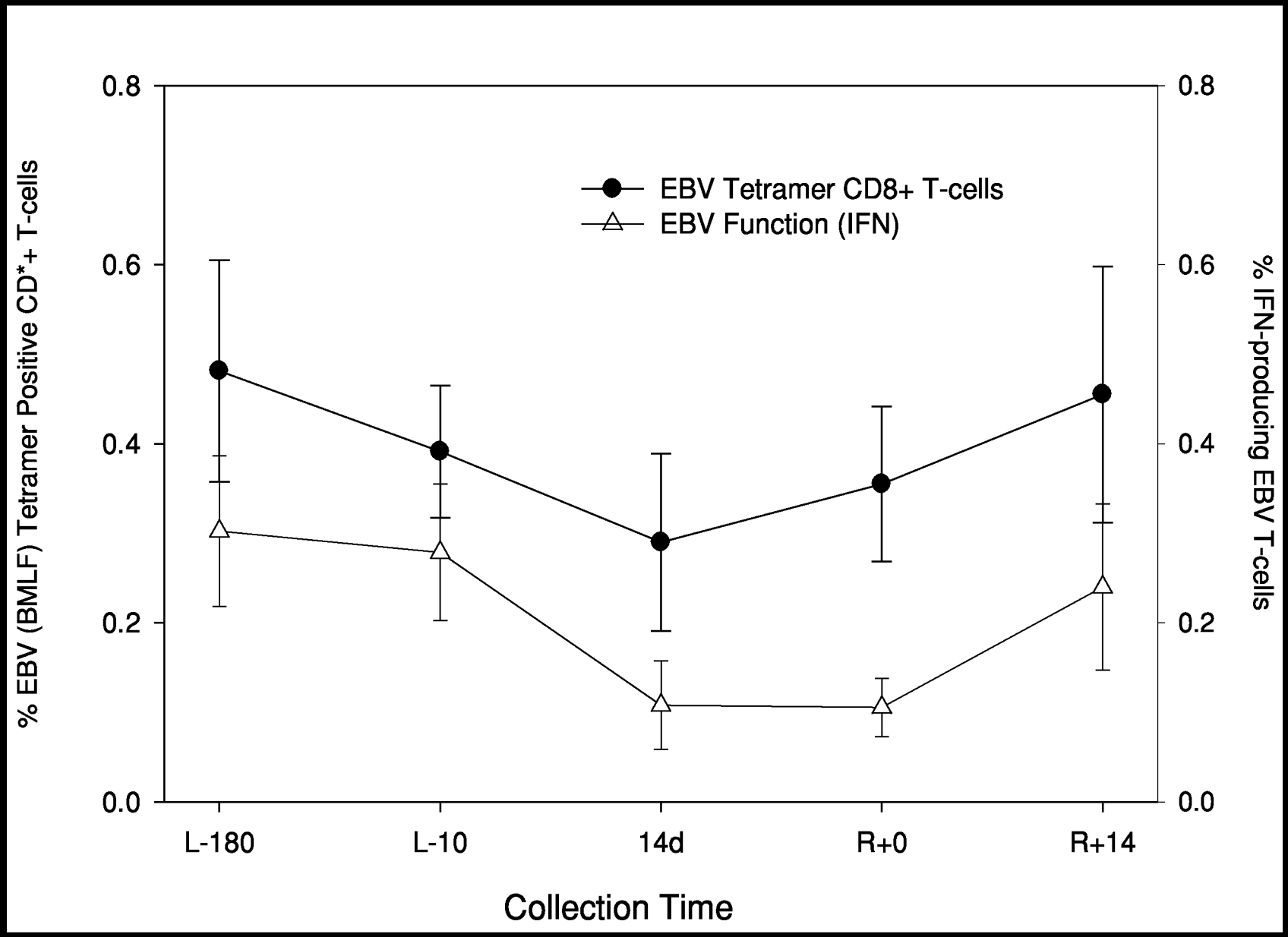
Abstract

The ability to detect cytomegalovirus-specific T-cells ($CD4^+$) in the peripheral blood by flow cytometry has been recently described by Picker et al. In this method, cells are incubated with viral antigen and responding (cytokine producing) 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 primarily due to the superantigen/mitogenic properties of the virus which non-specifically activate T-cells. By modifying culture conditions under which the antigens are presented, we have overcome this limitation and developed an assay to detect and quantitate EBV-specific T-cells. The detection of cytokine producing 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 uninhibited. Using this method, EBV-specific T-cells may be legitimately detected using flow cytometry. No reduction in the numbers of antigen-specific T-cells was observed by the early removal of target antigen when verified using cytomegalovirus antigen (a virus with no non-specific T-cell activation properties). In EBV-seropositive individuals, the phenotype of the EBV-specific cytokine producing T-cells was evaluated using four-color flow cytometry and found to be $CD45^+$, $CD3^+$, $CD4^+$, $CD45RA^-$, $CD69^+$, $CD25^-$. This phenotype indicates the stimulation of circulating previously unactivated memory T-cells. No cytokine 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 (CD45, CD3, CD69, IFNγ/IL-2) allows the total quantitative assessment of EBV-specific T-cells while monitoring the interference of EBV non-specific mitogenic activity. This method may have significant utility for the monitoring of the immune response to latent virus infection/reactivation.

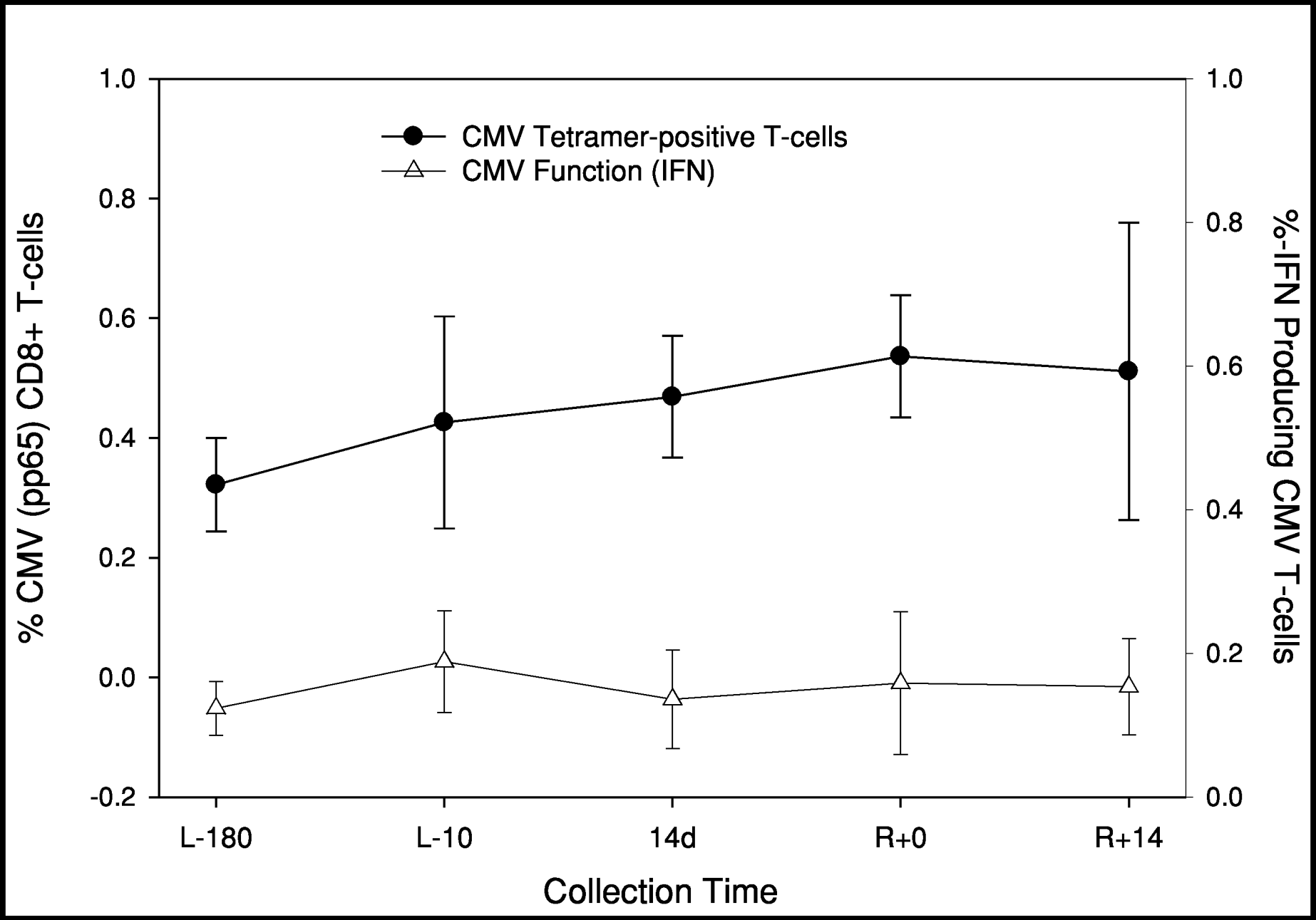
Peptide Stimulation Assay



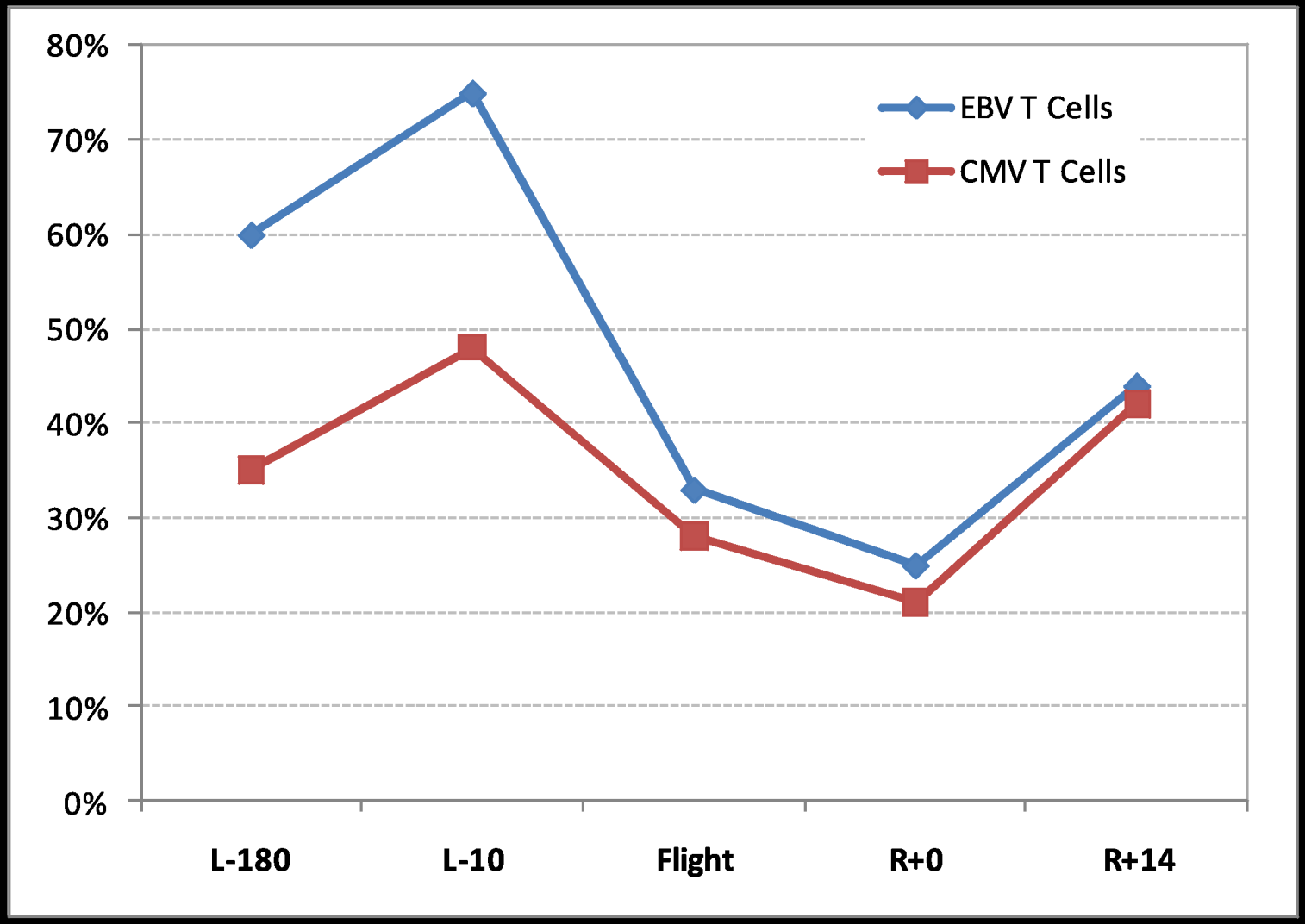
Virus-specific T cell Number/Function



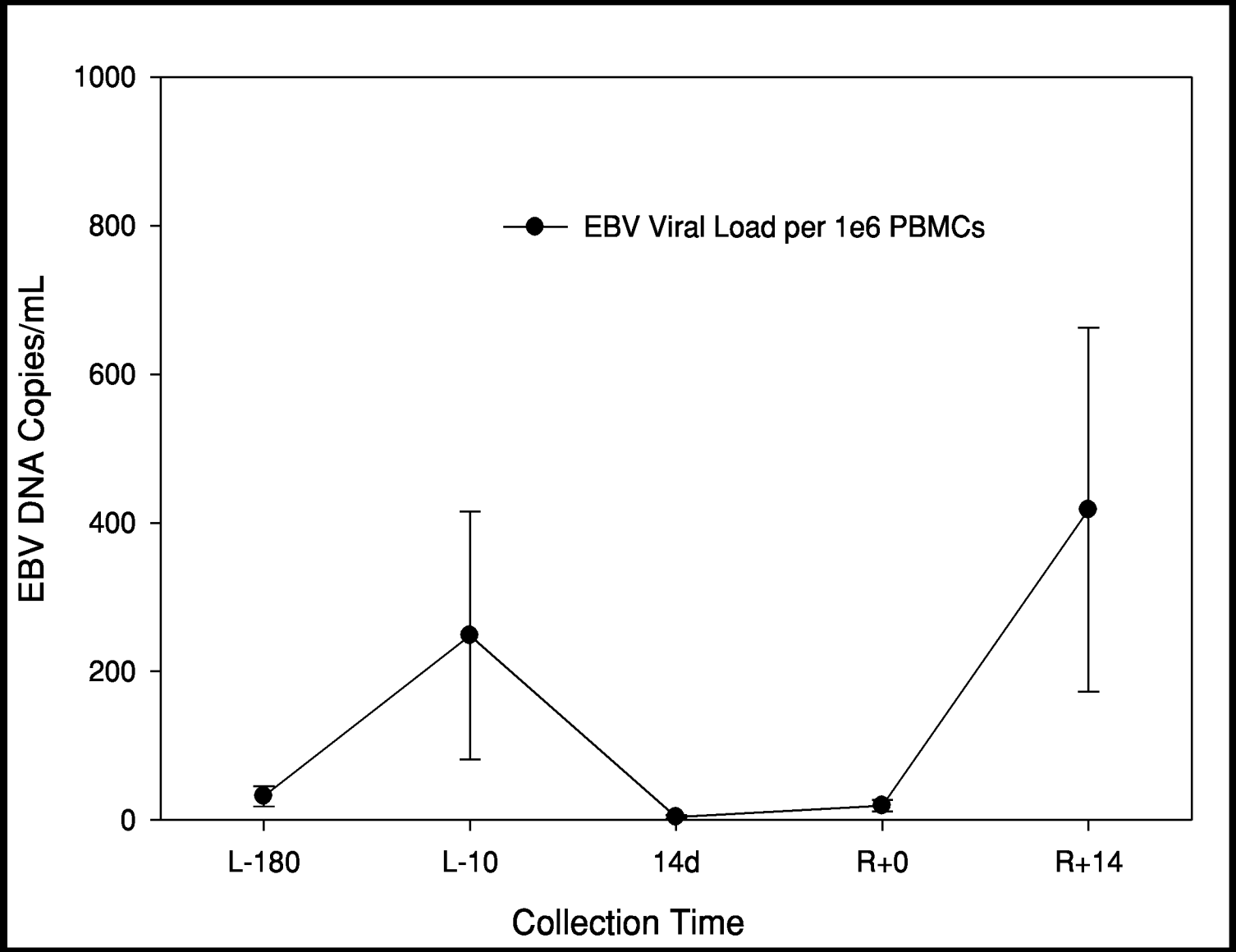
Virus-specific T cell Number/Function



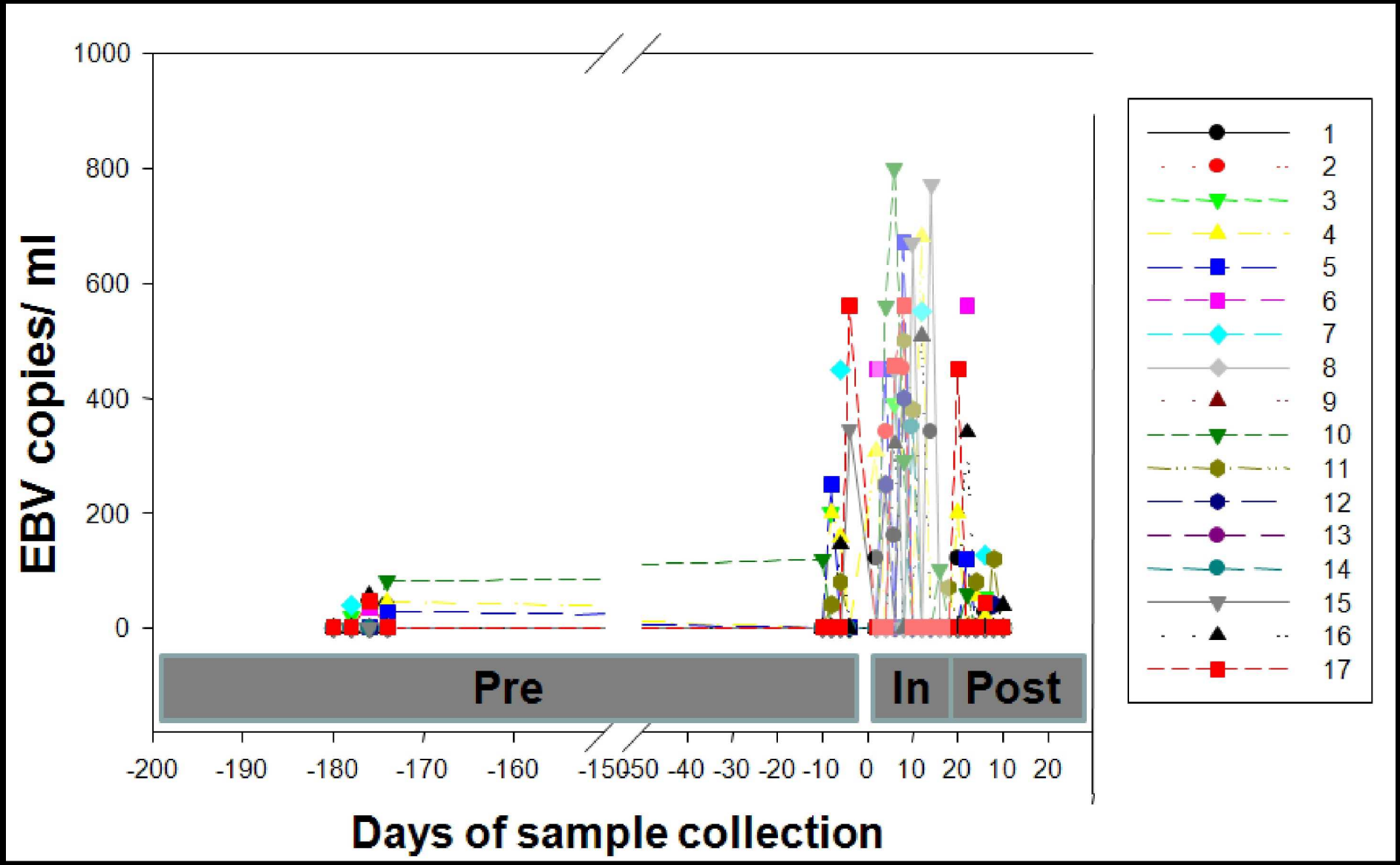
Virus Specific T Cells – Functional Percentage



EBV Viral load



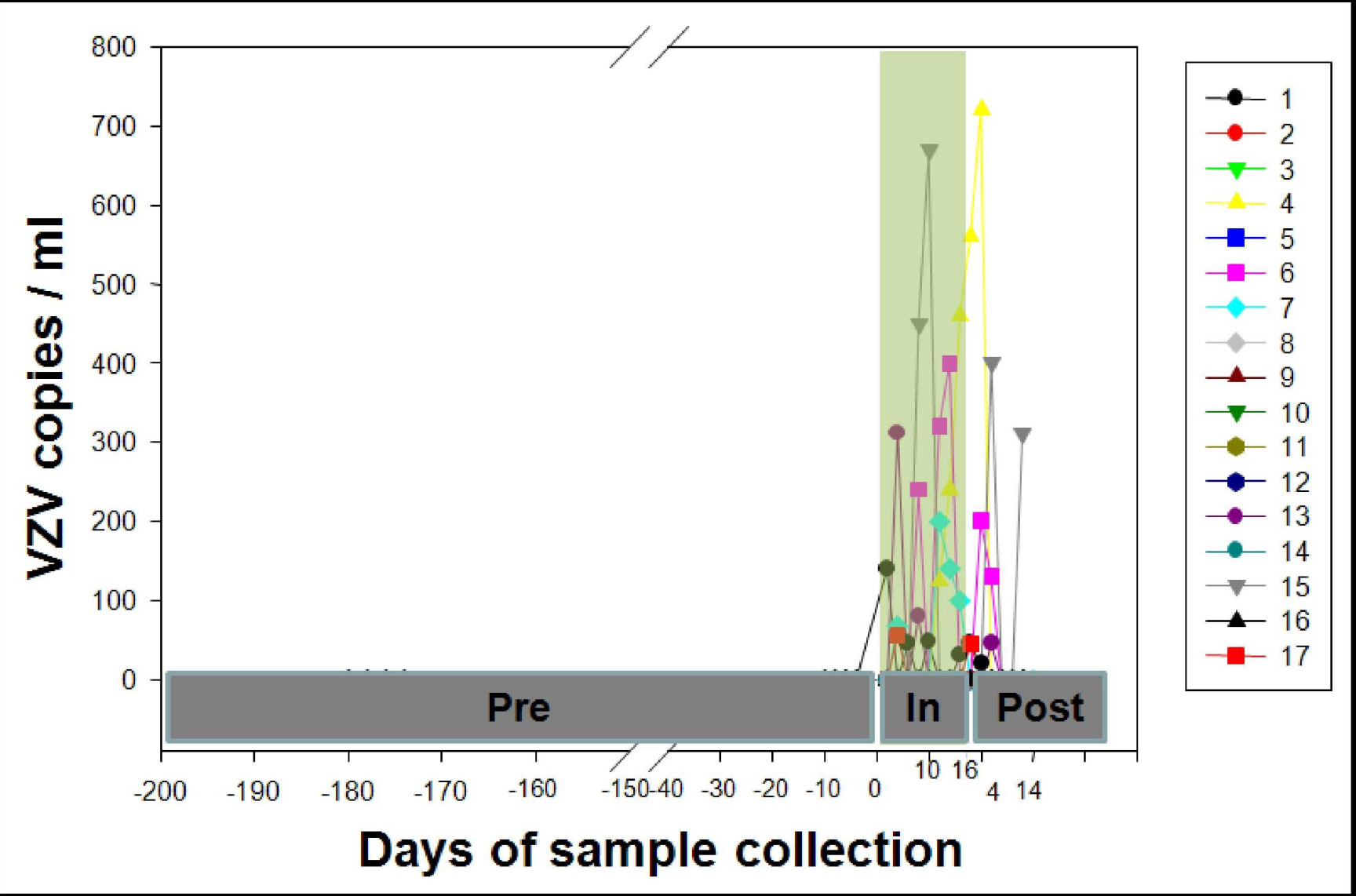
Viral Reactivation: Saliva EBV DNA



EBV shed in 82% of crewmembers

Samples positive for virus - Pre: 16.2%, During: 23.4%, Post: 23.1%

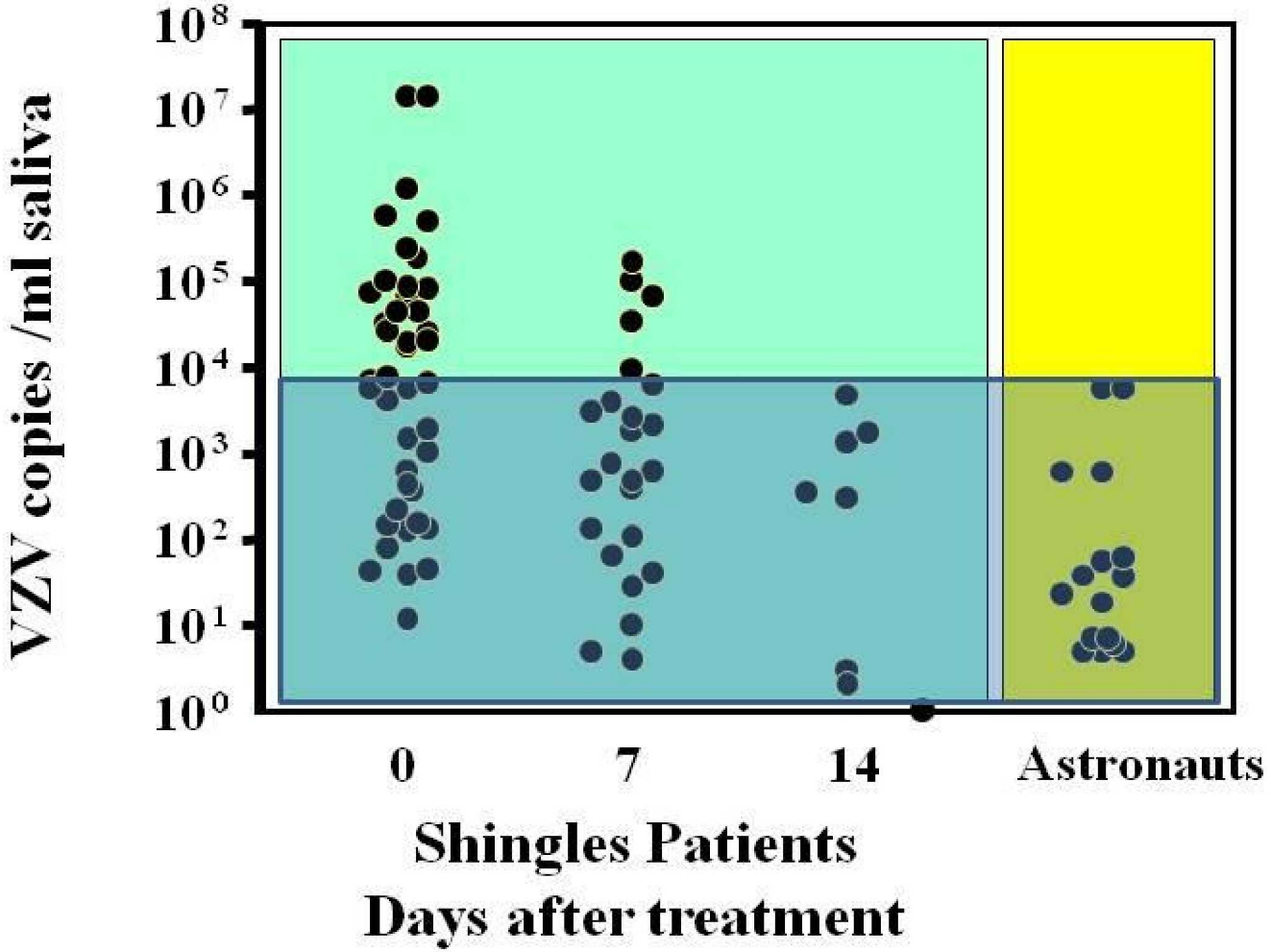
Viral Reactivation: Saliva VZV DNA



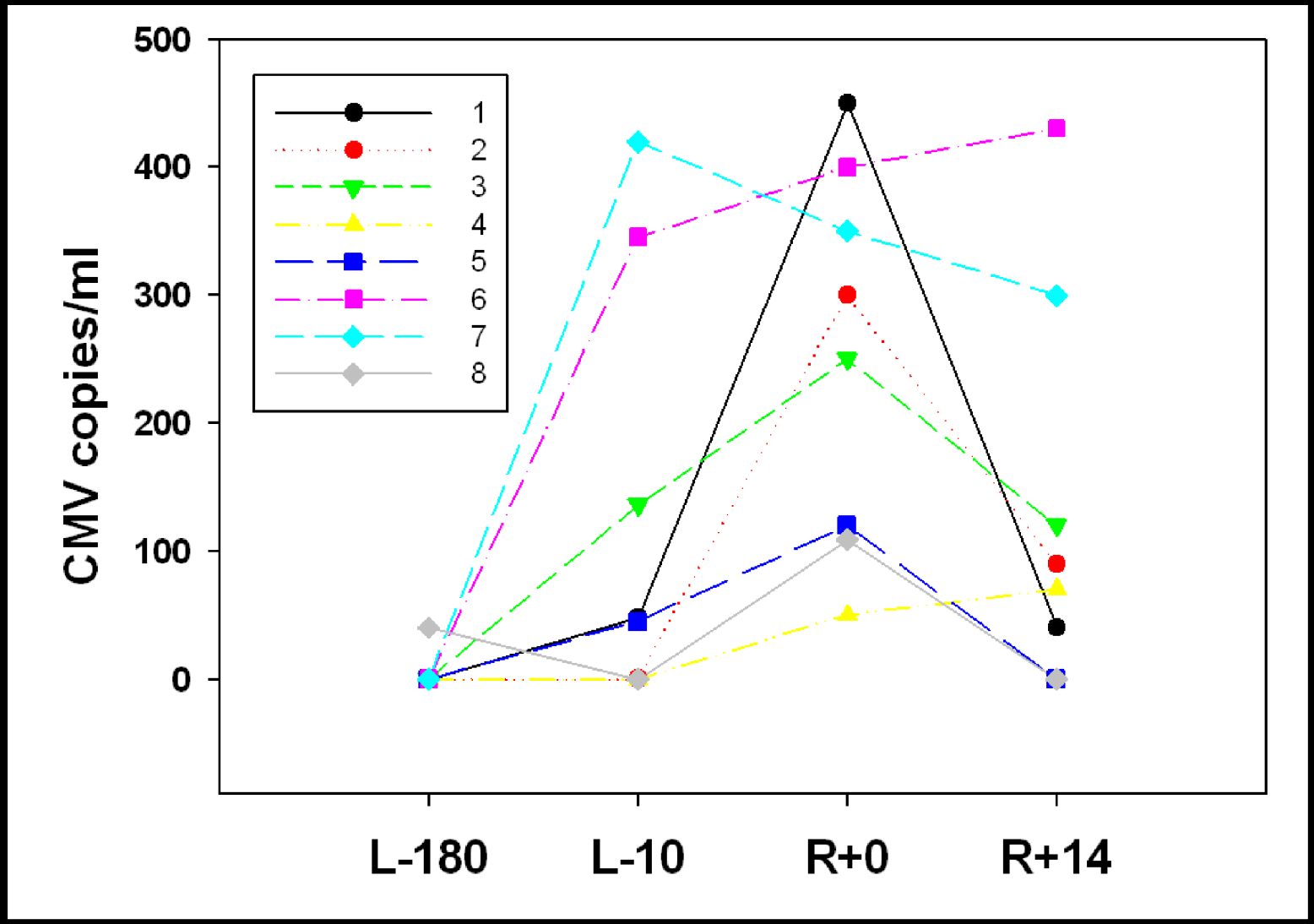
VZV shed in 41% of crewmembers

Samples positive for virus - Pre: 0.0%, During: 16.0%, Post: 7.7%

Salivary VZV in Shingles patients & Astronauts



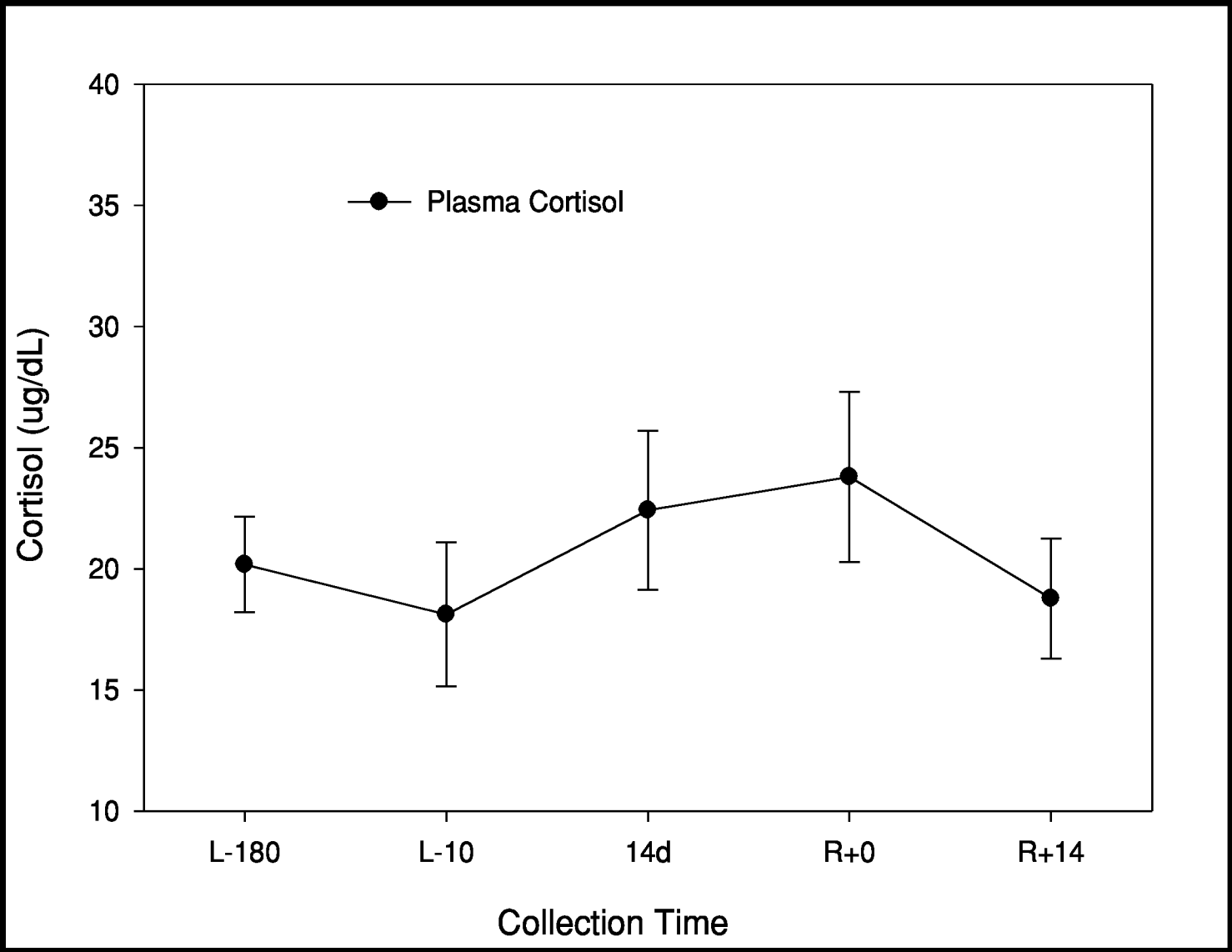
Viral Reactivation: Urine CMV DNA



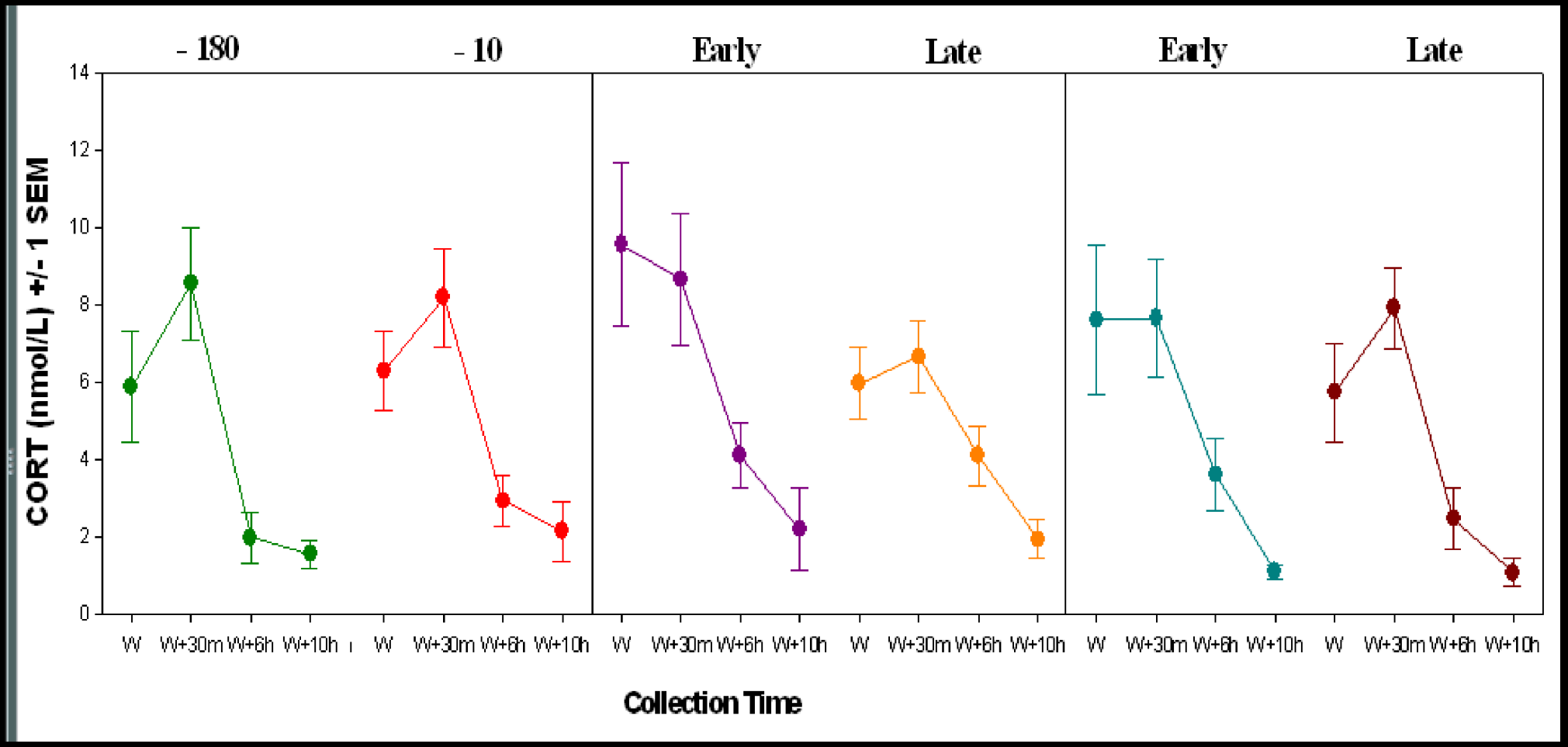
CMV shed in 47% of crewmembers

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

Stress Hormone Levels



Stress Hormone Levels – Circadian Rhythms



Conclusions

•Some measures of immune dysregulation are not merely related to landing stress/re-adaptation to gravity, but are present during flight.

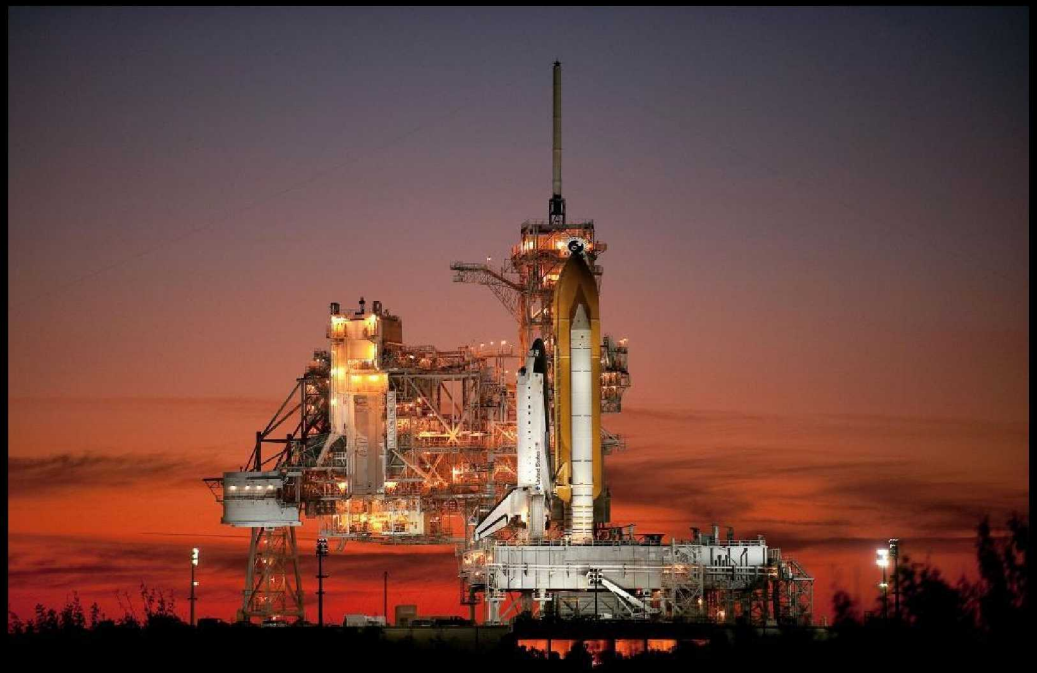
•Bulk leukocyte subsets largely unaltered during flight. Some alteration within CD8+ T cell subsets occurs during short duration flight.

•Diminished T cell function, alterations in various cytokine production profiles (secreted, mRNA) occurs during short duration flight.

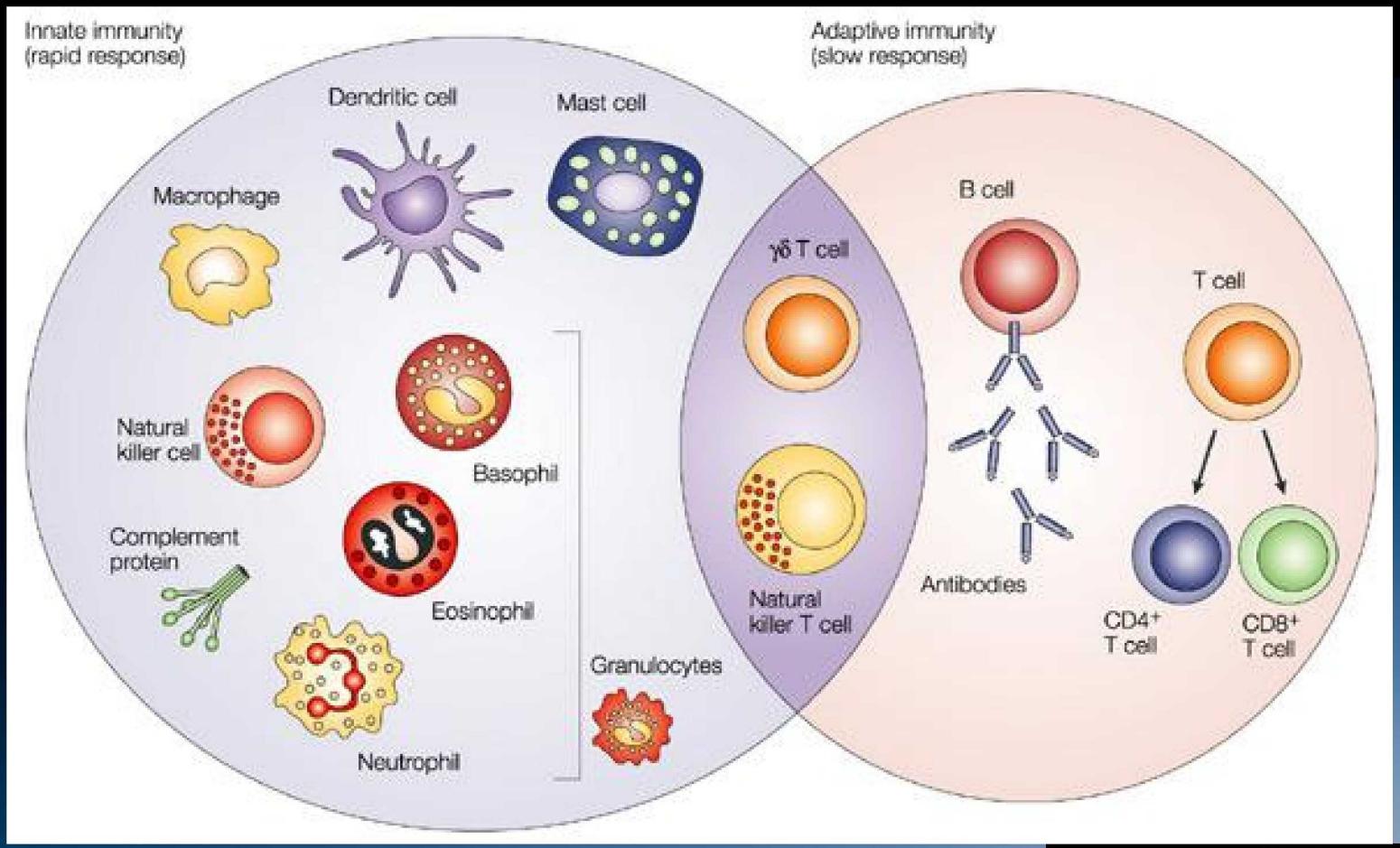
•CMV, EBV viral antibody titers trended to elevation during flight. EBV specific T cell number and function reduced during flight (correlated with EBNA), CMV specific T cells elevated, function unchanged.

•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.



ESA/NASA Immunology Flight Studies



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Questions?