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Keywords: *WB-57 flight, ADS-B, suborbital radiation, payload integration, T-cells, naïve cells, airborne research.*

GJRE-D Classification: *FOR Code: 290299*

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High Altitude Science Experiments aboard NASA's WB-57 Airborne Research Platform

Pedro Llanos [α], Kristina Andrijauskaite [σ] & Sathya Gangadharan [ρ]

Abstract- Purpose: Exposure to space radiation may place astronauts at significant health risks. This is an under-investigated area of research and therefore more knowledge is needed to better plan long-term space missions. The purpose of this study was to assess the effect of radiation on murine naïve and activated T lymphocytes (T cells) and to test the effectiveness of thermal, radiation and flight tracking technology in biological scientific payloads. We cultured cells in specific cytokines known to increase their viability and exposed them to either flight or had them as ground controls. Flight cells were kept under proper environmental conditions by using an active thermal system, whereas the levels of radiation were measured by NASA's Timepix radiation sensor during ascent, cruise at 60,000 feet, and descent. In addition, an Automatic Dependent Surveillance Broadcast (ADS-B) device was utilized to track the state vector of the aircraft during flight.

Results: Radiation levels reached about 1.5 μGy during ascent, 9.5 μGy during the cruise and near 1 μGy during descent. The recorded temperature of the cells was between 21 °C to 26 °C during the cruise. Our results show that exposure to radiation at 60,000 feet increased the expression of cytotoxic T cells (CD8) but decreased the expression of T-helper cells (CD4). Thus, it modulated the cell cycle and led to the enhanced expression of the IL2 receptor CD25. Furthermore, we observed the differences in various cytokines expression patterns detected from T cell culture supernatants. During the flight, the ADS-B device unit provided partial flight data, which was concurred with the flight team during ascent and descent, but it was locked above 50,000 due to the Federal Aviation Administration (FAA) regulations, so no ADS-B data were obtained during the cruise.

Conclusions: Our results demonstrate that exposure to various radiation doses may have led to cellular alterations in both naïve and activated T cells. These cells received about six and five times more radiation levels than the ground controls during ascent and descent, respectively, and over 16 times more during the cruise. However, we suggest that treating cells with cytokines (IL-2, IL-12) may reverse some of these changes. Knowledge gained from this study could be adapted to assess the short-term effects of radiation on individuals conducting prospective suborbital missions.

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I. Introduction

Humans have made significant progress in space exploration over the last decades. However, there are still many aspects requiring attention and scientific investigation, such as exposure to radiation. Space is a hostile environment, and technological advances coupled with scientific research could accelerate the development of interventions aimed to alleviate spaceflight induced side effects. Moreover, training new generation scientists and engineers could enable to meet future research and development challenges. The Embry-Riddle High-Altitude Science and Engineering Rig (ERHASER) team flew several NanoLabs aboard NASA's WB-57 aircraft at 60,000 feet (18.29 km) on December 1, 2017to obtain scientific data and to enhance students' knowledge of Science Technology Engineering and Mathematics (STEM). The WB-57F "Long-Wing" aircraft has been used for decades for airborne research, including weather studies and aerospace technology demonstration. There were four objectives of this flight: 1) technology demonstration of an Automatic Dependent Surveillance-Broadcast (ADS-B) system, 2) characterization of flight conditions using various state-of-the-art sensors, such as temperature and Timepixradiation monitoring sensor, 3) assessing radiation effect by using in-vitro biological experiment involving naïve and activated murine T cells, and 4) testing the specific life support system.

The first aim was to study the position of the aircraft using the ADS-B device for subsonic or supersonic flights through triangulation from communication nodes along the Gulf of Mexico, which had never done before. We aimed to get insights into some challenges the Federal Administration Aviation (FAA) is facing with integrating the newly emerging era of suborbital space vehicles into the National Air Space. The second objective was to test the effects of radiation using the Timepix, a sensor that had flown on NASA's Exploration Flight Test (EFT)-1 On December 5, 2014, and that had never flown before aboard this aircraft to study the radiation levels at 60,000 ft.

Next, we wanted to assess the radiation levels on the immune cells, also called T cells. We used both

naïve and activated murine T cells, which were supplemented with cytokines IL-2 and IL-12, as well as treated with the novel supercritical CO2 extract of neem tree Azadirachtaindica (SCNE). Interleukin-2 (IL-2) is a potent T cell growth factor used for T cell expansion and treatment several types of cancer [23]. IL-12 is involved in the differentiation of naive T cells into the T helper cells. It is also known as a T cell-stimulating factor, and a promising agent in cancer immunotherapy [24]. These two cytokines facilitate their effect by targeting the immune system. We sought to investigate whether exposure to radiation and other flight stressors would have any effect on these cells, especially at the 60,000 feet, where peak galactic cosmic rays (GCR) scattering of secondary particles occur. Given the cytokines' ability to alter the cellular processes and the role of supercritical extract as the natural compound with pluripotent properties, we wanted to test whether supplementing cells with these additives would rescue the cells of radiation damage. We performed the phenotypic analysis of the cells and assessed their ability to release cytokines.

The fourth objective included the development of an Environment Control Life Support System (ECLSS) NanoLab, which could be used to host and sustain the cells at the desired temperature while other variables are being tested. Given the ambitious nature and complexity of the project, students worked closely with faculty and other collaborators from other universities to mature and make this project ready for flight in 6 months.

Space travel, including exposure to radiation, induces many challenges to the human body, especially to the immune system [6].Cosmic radiation is thought to have a significant down regulation effect on many different types of cells of the immune system short-term with B cells being the most affected, then T cells, and finally natural killer (NK) cells being the least affected. Even during the shortest trip to Mars (7-8 months), radiation levels would be of the order of >0.6 Sv, which is close or above the dose limits proposed by NASA for a single astronaut's entire career. [6]. An open question would be to understand the effects of radiation (heavy ion exposure) on various types of T cells which was the epicenter of this multidisciplinary study. Radiation effects can be related to the loss of cell functionality. Only some heavy ions contribute to GCR –these ions are delivered sequentially rather than concurrently. Very few research platforms provide the capability of exploiting the 60,000 feet region and provide insights about the radiation dose. Although several commercial research platforms (e.g., Blue Origin's New Shepard, Virgin Galactic's SpaceShipTwo) are sending unmanned missions to suborbital space (100 km), they are planning to send more frequent manned missions after 2021 This opens a window to further increase our understanding of the potential risks associated with radiation and the suborbital environment. Heavy ions that interact with molecules in the upper atmosphere generate cascades of secondary particles such as electrons, pions, and muon, and the intensity of these particles increases, which creates a peak in ionization rate around 60,000 feet. Below this altitude, there is a secondary particle flux that decreases steadily toward ground level, which is approximately 3 orders of magnitudes lower than the peak [22]. The reason we had chosen to focus on T cells is that they represent one of the main defense mechanisms to fight space travel (radiation) induced immune system alterations. This is usually achieved by reprogramming naïve cells into specialized T cells that can combat infections and other spaceflight induced damage. Naïve cells are characterized by small cell size, low proliferative rate, and low basal metabolism. For quite some time, naïve T cells were thought to be homogeneous and quiescent, however, recent studies demonstrated significant differences between naive T cells in terms of phenotype and function [8]. These new insights make them very valuable for studying immune system dysregulation and designing prevention strategies. In the human body, they are generated in the thymus and each naïve T cell recirculates from the blood through a lymph node and back to blood every 12 to 24 hours until it encounters the appropriate antigen and gets activated [9]. For the T cells to begin their immunological work, they first must be activated. Most commonly, mouse naive cells are activated by engaging the T Cell Receptor (TCR) (signal 1) and CD28 co stimulatory molecule (signal 2) with antibodies against CD3 and CD28, respectively, followed by culture with interleukin-2 (IL-2) [10]. However, recent studies suggest that naïve cells cultured in interleukin IL-2 without TCR engagement could also elicit a unique pattern of signaling associated with proliferation and up-regulation of different markers [11]. Given the significant importance, T cells play in fighting (radiation/flight) induced damage, here we provide insights into new T cell culture methods which might be used in future research for assessing cellular consequences of cosmic radiation exposure. This pilot study provides guidelines used by faculty and students from different backgrounds (Spaceflight Operations, Aerospace Engineering, and Biological Sciences) to propel their knowledge in the design, fabrication, integration, and testing of airborne research payloads [1] and allowed us to collect valuable scientific data.

II.   Material and Methods

a) Timeline Operations

The EHRASER team followed a very tight schedule to meet the Test Readiness Review (TRR) before the actual flight. TRR was conducted about 4 hours before the flight (bottom left, Figure 1). After TRR was finalized, the AOD team secured the payload onto the pallet (bottom center, Figure 1) and attached under

the belly of the aircraft (top center, Figure 1). Early daily (7:00 AM) aircraft operations started with the AOD team meets with the U2 pilots to discuss mission routing and go/no-go decision for the weather. The aircraft was then towed and loaded with 20,000 lbs of fuel at 9:00 AM. Aircrew aircraft preflight U2 suit operations (top left, Figure 1) were conducted at 9:45 AM or about 1h and 15 minutes before the flight. Aircraft engine started at 10:30 AM, and aircraft taxed at 10:50 AM. Takeoff of aircraft was at 11:00 AM (top center, Figure 1). The landing of the aircraft was at 14:30 PM. Aircraft post-landing flight operations included the AOD team delivering (top right, Figure 1) the payload to the ERHASER team at Landing + 30 min. After payload was retrieved about 1 hour after landing, the ERHASER team started immediate analysis (bottom right, Figure 1).
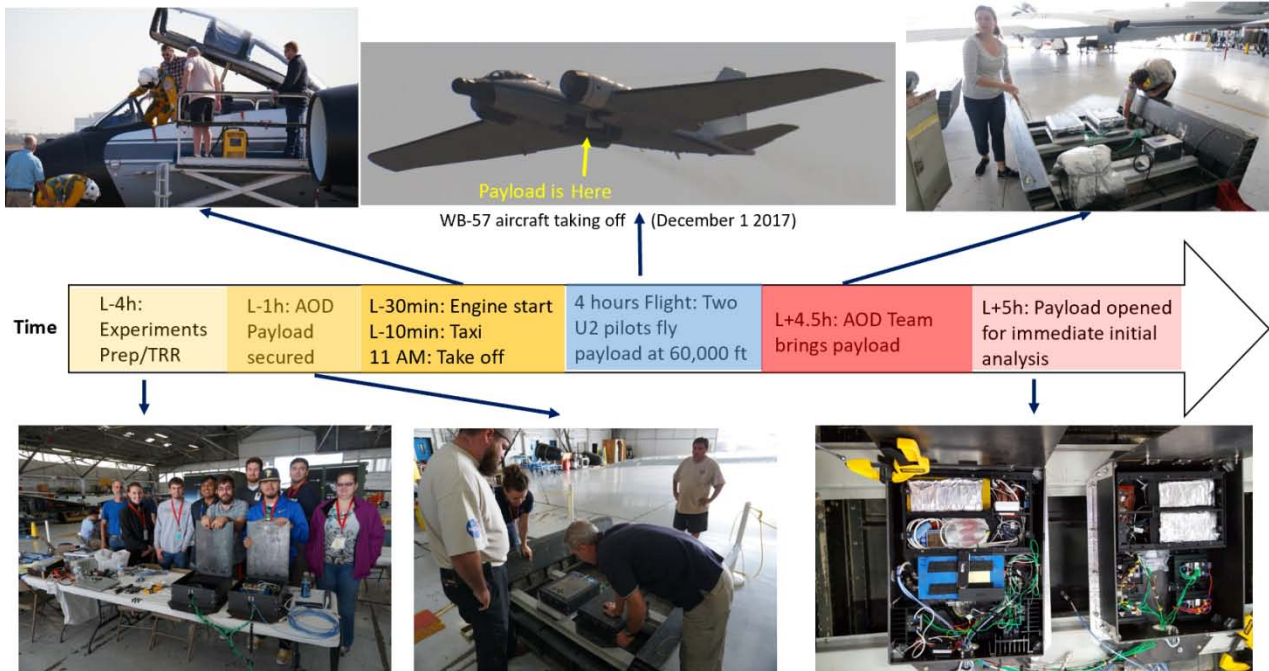

Figure 1: Timeline of the airborne research experiment

Figure 1 shows the operations' timeline for the WB-57 #927 flight including loading and unloading of the payloads by the AOD team. Two prior test flights flew on November 29 and November 30, 2017, of duration 2.2 hours and 4 hours, respectively. The actual flight (Figure 2) took place on December 1, 2017, departed at 11:00 Z and landed at 14:51 Z in KEFD, and logged 3.8 hours of flight. ERHASER's Test Readiness Review (TRR) was conducted on November 30.

b) Flight Path and Tracking with ADS-B

After several private communications with the WB-57 chief pilot Tom Parent, the ERHASER team deemed the path below in Figure 2 to be the optimal flight path for the mission. The WB-57 aircraft departed from Ellington Field Airport in Houston, where the temperature was about 15℃, and the flight reached a maximum altitude of 63,600 feet (19.39 km), right past the Armstrong line (62,000-63,500 feet), indicating that any unprotected exposure above this level causes body fluids to boil.
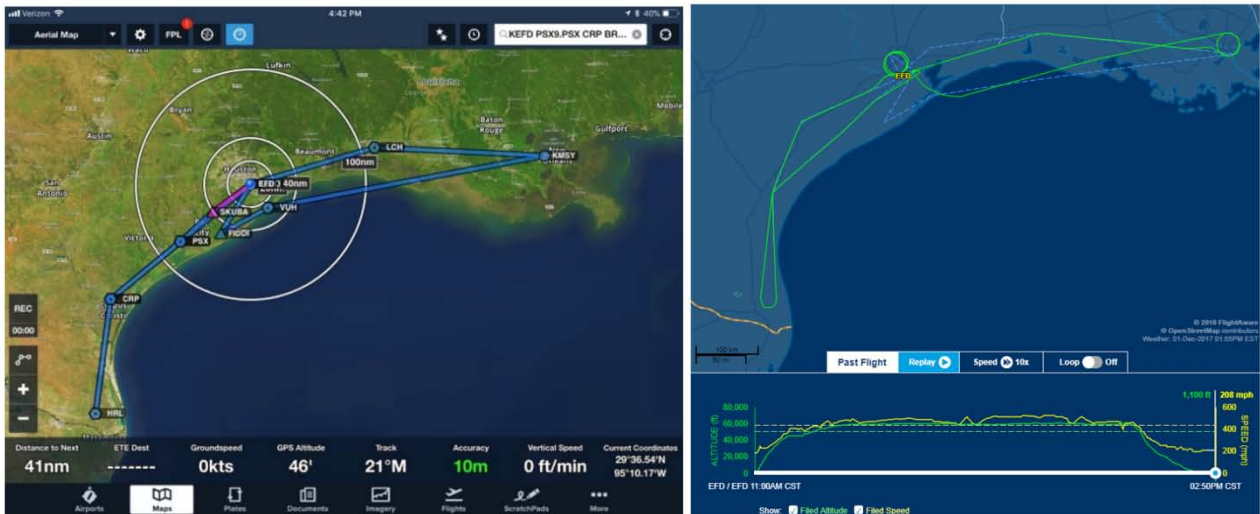
Figure 2: WB-57 flown path from Ellington field airport, Houston

The flight (Figure 2) started from Ellington field airport in Houston (KEFD), and flew over several preselected locations (airports for triangulation) in the following order: KEFD to Palacios Municipal Airport (PSX), Corpus Christi International airport (CRP), Valley International airport (HRL), back to CRP, back to PSX, back to KEFD, then Lake Charles Regional Airport (LCH), Louis Armstrong New Orleans International airport (KMSY), VUH, FIDDI, and back to KEFD.

Crewmembers wore a U2 suit (top left of Figure 1) as mandated by the FAA flight rules, weighting 14 kg.

Wearing this suit prevented crew members from any physiological deficiency while flying between 12,500 feet (3.81 km) and 50,000 feet (15.24 km). The stratosphere starts at 50,000 feet, and the WB-57 flew in the lower region of the stratosphere where the temperature is about -55 degrees Celsius with little water vapor, jet streams, and little turbulence. WB-57 pilot recorded the following temperatures during the flight: -47 ℃ at 52,200 feet, -52 ℃ at 59,000 feet, -48 ℃ at 59,200 feet, -44 ℃ at 59,000 feet, -43 ℃ at 60,400 feet, and -44 ℃ at 60,400 feet and 63,600 feet.

Figure 3: ADS-B configuration

Figure 3 accurately displays the fully assembled ADS-B system. The ADS-B has two endplates: the right plate has the UAT transmitter antenna and 1090 ES receiver antenna connections (Figure 3b); the left plate has the power and GPS connections (Figure 3c). All the antennas used on the ADS-B were the 1090 ES the UAT and the GPS receiver, attached to the WB-57 through the antenna farm. The connections used included the power cord shown in Figure 3d. The power cord shown in Figure 3d was changed by splicing directly to the power regulators because the connector from the factory was unsatisfactory in maintaining a stable connection.

c) NanoLabs Design, Contents and Active Thermal System

The ERHASER team designed and 3D printed various NanoLabs. The size of these NanoLabs was a 12 U, being a 1U a 10 cm x 10 cm x 10 cm. The

NanoLabs were designed to house the in-vitro biological experiment, and various sensors to monitor the environmental conditions, such as temperature, relative humidity, accelerations and carbon dioxide inside the payload. Since the operational thermal limits of these sensors had a various range of temperatures, a thermal system was designed for heating and cooling so that the sensors could function nominally.

Two 6U NanoLabs consisted of 30 Eppendorf 5.0 mL tubes containing immune T cells derived from mice. 20 tubes had cells that were maintained with or without cytokines IL-2 and IL-12, while the remaining 10 tubes consisted of T cells grown in the supercritical extract of Azadirachta Indica (SCNE). The liquid was contained within the sealed tubes, which were further vacuum sealed for containment against leaks. With the 6U NanoLab structure, it provided three levels of containment in the event of a leak. Tubes were sealed

using parafilm medical tape and vacuum-sealed in Ziploc bags. Ground control conditions were kept at the NASA facility.

The NanoLab sho used the edl-4S (placed in the first NanoLab), and the EL-21 CFR TP LCD (second NanoLab) sensors, which had operational thermal limits of 0 ℃ to 50 ℃, and -35 ℃ to 80 ℃, respectively. The biggest challenge was to keep the edl-4S within the operational thermal limits from -60 ℃ to a few degrees above 0 ℃, for what the team decided to use aerogel space loft 5 mm thick insulation covered with thermal tape (Figure 4). Also, the operational thermal limit of the ADS-B device was -20 ℃ to 55 ℃. This instrument was placed in a smaller 2U Nano Lab (blue) inside the main 12 NanoLab (black), and everything housed inside the metal box (Figure 4).
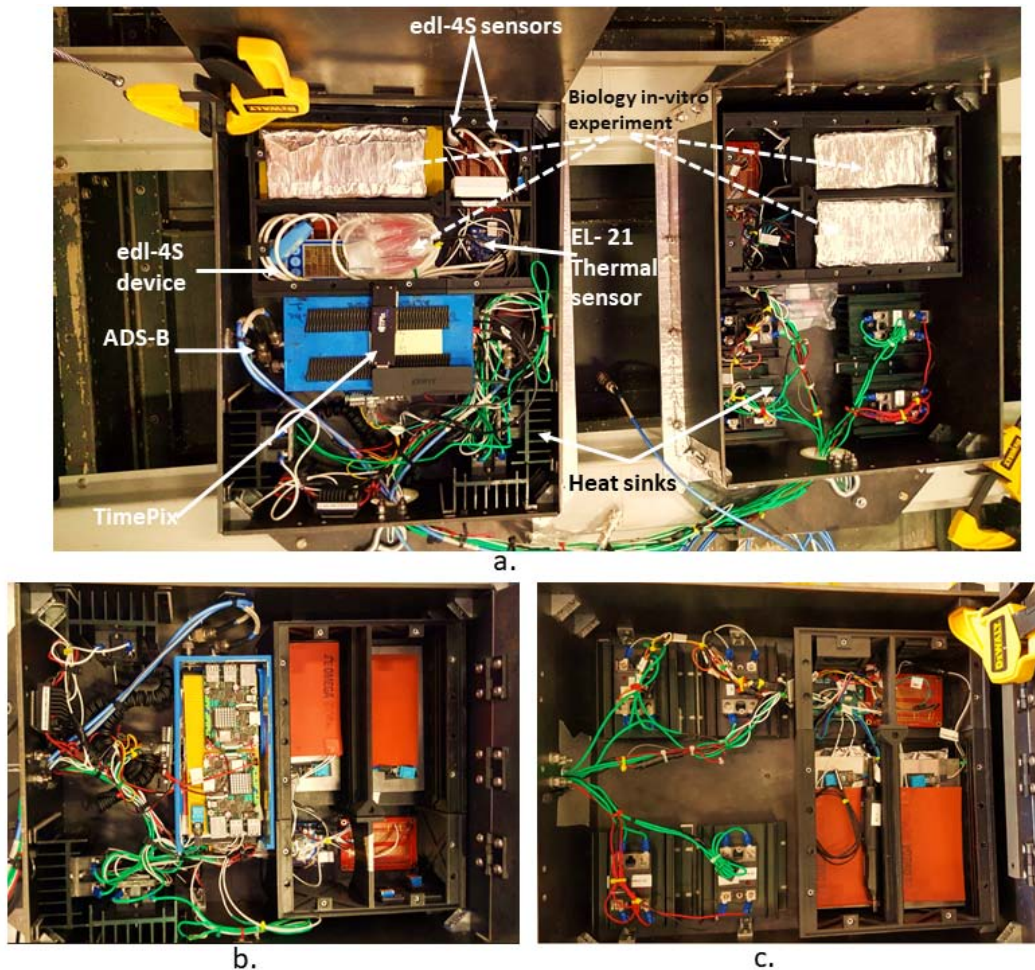
Figure 4: ERHASER payload highlighting all the subsystems. a: Payload showing both chambers with an active thermal system, ADS-B unit, Timepix radiation sensor, edl-4S unit and sensors, EL-21 thermal sensor, heat sinks, rest of avionics, and the in-vitro biological experiment. Payload was verified and approved by the AOD team at Ellington Field Airport. b: left chamber showing heating pads inside the 4U NanoLab, and inside the 2U NanoLab where the ADS-B device was located, and three heat sinks. c: right chamber showing heating pads inside the 4U NanoLab, and four solid-state relays assembled on top of the heat sinks.

The active thermal system (Figure 4) was designed to meet all the operational thermal limits of the sensors [1]. The rest of the avionics in Figure 4 included an Arduino, a pressure sensor, thermocouples and cables to provide power to the heating pads that actively controlled the temperature inside each NanoLab. The temperature inside the chambers was maintained using OMEGA® silicone heating pads mounted to the bottom of the chamber. One of the heating pads (orange) is visible on the left side chamber in Figure 4 where the edl-4S device and part of the biology experiment touches on, which was used to provide heating to both the device and the cells (Figure 4b). Another heating pad (Figure 4b) was placed inside the 2U NanoLab (yellow) where more cells were touching it, and the top was the aerogel covered with thermal tape to insulate theNanoLab from the flight conditions. Similarly, the other two heating pads (Figure 4c) were housed in the other 2U NanoLabs inside the right chamber (Figure 4c) for heating of the in-vitro biological experiment, and aerogel covered with thermal tape on top (visible in Figure 4a).

The NanoLabs needed to be designed in a manner that different containment areas maintained different temperatures while the instruments and avionics mounted inside could suitably log the data from sensors and keep the active thermal system operational for the duration of the flight.

d) *Radiation Sensor Timepix, Arduino, and OMEGA Heating Blocks*

We used the Timepix Radiation Environment Monitor to measure the dose rate and the overall dose and the high-Linear Energy Transfer (LET) on the experiment.

Radiation sensor Timepix [4] was mounted on the NanoLabs(Figure 5) and flown inside the unpressurized pallet of the WB-57.
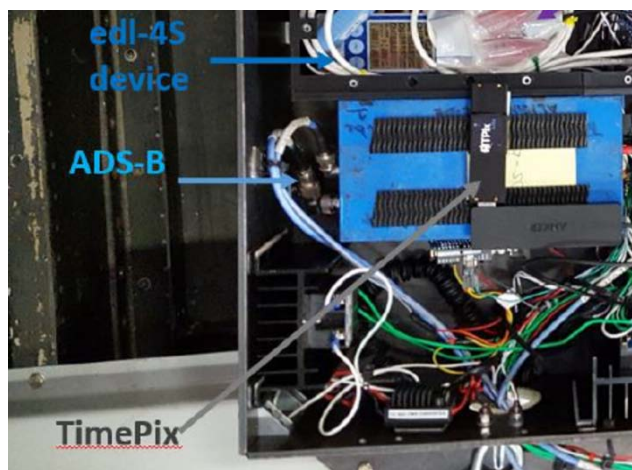
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Figure 5: Timepix radiation detector. Zoom of Figure 4a

To maintain the temperature within a few degrees Celsius in each section of the 6U NanoLab, the thermocouples were mounted in each section and their data was fed into an Arduino Uno that controlled each heater. The Arduino gathered the temperature readings from each section of the 6U NanoLab and directly controlled power given to the OMEGA® heating pads. Since the Arduino only outputs 5V, it couldn't supply the power needed to operate the heating pads (12V). Thus, solid-state relays connected to the Arduino regulated the power running to the heating pads. Once heating pads heated the tubes to the desired temperature the Arduino would turn off the power going to the heating pads using the transistors. As an added precaution, the Arduino contained a complete shutdown command for the experiment if the temperature in any of the NanoLabs reached more than 45° C.

e) *Splenocytes Isolation and T cell Activation*

T cells were generated from mice spleens which were dissected from euthanized C57BL/6 mice (2013044AR) which were bred and maintained at the UT Health Science Center specific pathogen-free facility according to the Institutional Animal Use and Care Committee (IACUC) at UT Health Science Center

standards. Animals of the female sex (n = 5) with the age of 6-8 months were used. Single cell-suspensions of lymphocytes were achieved by sterile dissociation of whole spleens with syringe plunger through a cell strainer and by lysing red blood cells with the ACK (Ammonium-Chloride-Potassium) lysing buffer (Life Technologies). Isolated splenocytes were plated in 24-well plates at 1×10^6 cells per ml concentration in 1.5 ml and activated with anti-CD3 mAb (145-2C11 clone, plate-bound, 1 μg/ml) and anti-CD28 mAb (37.51 clone, soluble, 2 μg/ml) from Bio X Cell in RPMI media containing 10 % fetal bovine serum (FBS) for 48 hours. After 48 h, cells were washed and supplemented with or without human (h) IL-2 (200 ng/ml, recombinant human interleukin-2 (rIL-2)) from NCI at Frederick Repository or mouse (m) mIL-12 (10 ng/ml) from Shenandoah Biotechnology, both or without cytokines every three days. Naïve cells were isolated as described above and supplemented with or without cytokines. Cells were also treated with 10 ug/mL of the Supercritical Extract Neem Extract, SCNE (Nisarga Ltd., Satara, Maharashta, India).

f) *Flow Cytometry*

Cells were analyzed by flow cytometry using standard procedures as previously described [12]. Briefly, cells were washed in staining buffer (PBS, 2% bovine growth serum and 0.01% sodium azide) and stained with fluorescently labeled antibodies. The fluorescently conjugated monoclonal antibodies (mAb) used in this study included: anti-mouse (m) CD4 mAb (RM4-5, dilution 1/500), anti-mCD8 mAb (53-6.7, dilution 1/500), anti-mCD25 mAb (PC61, dilution 1/250) from Biolegend. Samples were acquired on Celesta and data were analyzed using the FloJo software (Tree Star, Ashland, OR, US).

g) *Cell Cycle Analysis*

Naïve and activated T cells were washed with cold PBS and fixed overnight at 4°C in 70% ethanol. Cells were then pelleted, washed and re-suspended in 1 ml PBS with the propidium iodide (PI) at a final concentration of 20 μg/ml, ribonuclease at 20 μg/ml and EDTA at 2mM. The cells were incubated at 37°C for 30 min in the dark. PI content was assessed by flow cytometry using FL-2 channel.

h) *T cell Viability Assay*

T cell viability was assessed by using the Trypan Blue dye (Sigma) to determine the number of viable cells present in a cell suspension. Calculations were made using the hemacytometer with a light microscope.

i) *Multiplex Immunoassay*

Cell culture supernatants were collected from flown T cells and ground controls and stored at -80 °C until further processing. Cytokine secretion assay was performed using Thermo Fisher Procarta Plex Multiplex Custom-Made mouse cytokine 5-plex assay kit following

the manufacturer's instructions and analyzed with the Bio-Plex 200 Luminex-based multiplex analysis system (Bio-Rad, Hercules, CA). All samples were analyzed using duplicates. The expression of different cytokines was determined by the fluorescence intensity (FI) minus the background.

j) Statistical Analysis

Data were graphically displayed using prism 6 software. Differences in various markers expressions across different conditions were determined and compared using standard error mean (SEM) or ± standard deviation (SD). Statistical analyses are represented in Table 1.

III. RESULTS

a) Flight Path and Tracking with ADS-B

The ADS-B provided the state vector of the aircraft during the ascent and descent phases (Figure 6). There was a cut-off in the transmission over 50,000

feet due to the commercial origin of the instrument which locked above a certain altitude due to FAA regulations. The ADS-B unit provided flight information during the first 40 minutes of ascent and last 40 minutes of descent. The ADS-B returns concurred with the data provided by the Flight team. WB-57 aircraft flight path was obtained from the Flight Aware to map the proposed ADS-B ground-based transceivers along the Gulf of Mexico as shown in Figure 6. There were no returns from the open source or commercial offshore assets, which could be used to triangulate the location of the aircraft. Exploring the options and analyzing the returns led to the conclusion that other aircraft flying over the Gulf of Mexico would be better source of obtaining flight parameters and trajectory although it might heavier the computations to account for accuracy due to Doppler shift in the radio signals caused by the significant difference in velocity and altitude between the two entities.
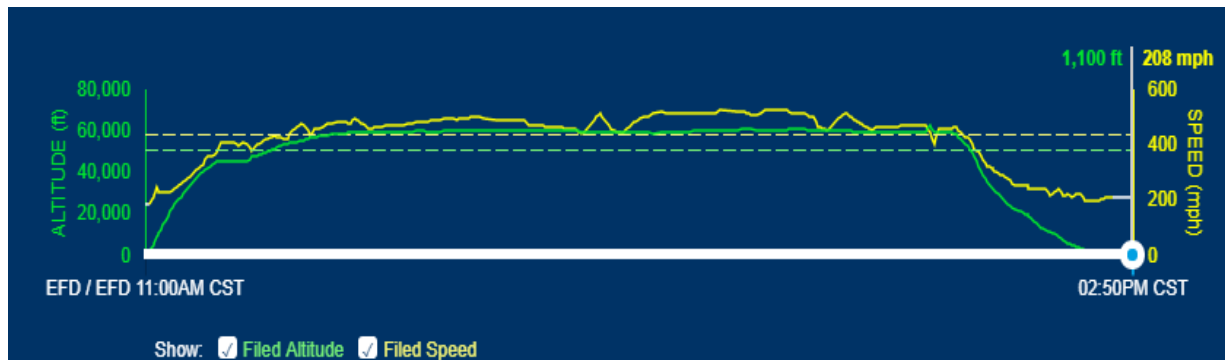

Figure 6: WB-57 flight. Yellow trajectory depicts the filed speed of the aircraft. Green trajectory displays the filed altitude of the aircraft from Flight Aware.

b) Passive and Active Thermal System

Two sensors, the edl-4S (first NanoLab) and the EL-21-CFR-TP-LCD (second NanoLab) provided information on the temperatures. Figure 7 displays the temperature data logged in the second NanoLab. The temperature was initially maintained between 25 °C and 26 °C which degraded overtime before the Arduino device instructed the heating pads to start working. This resulted in the short spikes of increasing temperature over time. Note that Figure 7 shows the Eastern time indicating one hour needed to be subtracted to compare with the Central time. The edl-4S had a temporary power interruption when pilots switched over to USB power from the battery power for testing, leading to instrument reset and loss of data. Thus, the temperature, accelerations, relative humidity and carbon-dioxide levels were not collected to avoid interferences with ground operations. The active thermal system kept the cells between 21°C and 26 °C during cruise (Figure 7), temperature decreased from about 26 °C to about 21 °C during the first 90 minutes of cruise, and then maintain it from 21 °C to near 23 °C in the last

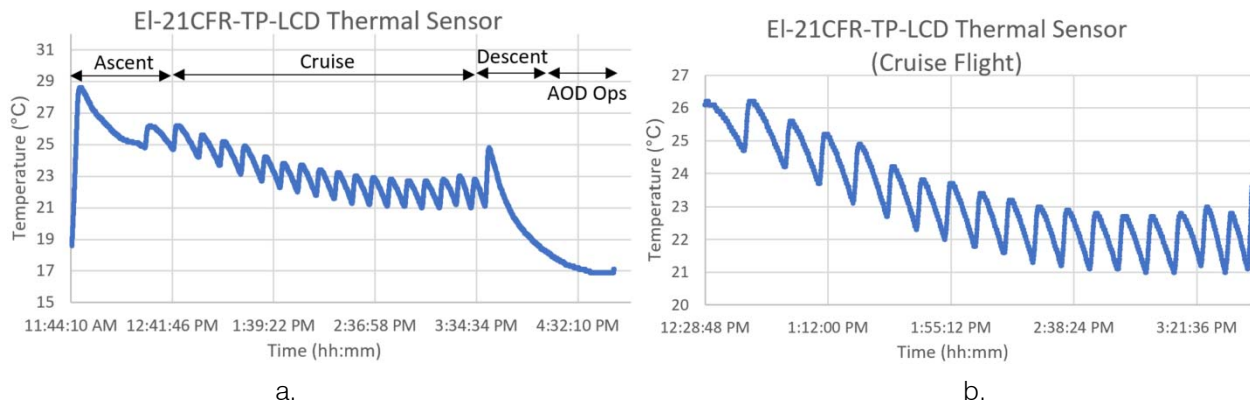90 minutes of cruise, showing a good stability of the active thermal system.

a.

b.

Figure 7: Temperature measured by the EL-21CFR-TP-LCD sensor during flight. a: temperature profile. b: zoom-in of cruise phase

c) Timepix Radiation Sensor

Throughout the flight, we observed a high trend in atmospheric radiation (Figure 8). This radiation trend behaved similarly to the total dose rate trend [3] measured by the ARMAS system during various flight scenarios (Figure 8e, 8f, 8g). This trend can be explained by the much thinner atmosphere seen at 60,000 feet altitude where the WB-57 cruised for near 2.5 hours. At this altitude, the atmospheric pressures measures close to a hundredth of what we experience around a sea level. With the lack of atmosphere, ionizing particles are free to fly until they strike whatever surface they may encounter. Most of the time this atmosphere consists of denser and lower altitude air, but sometimes these particles will strike aircraft or other objects at these altitudes. Because of the randomness of these particle strikes, the data (Figure 8) takes on a fuzzy, static appearance as there is no steady flow of particles, but a perpetual bombardment to the sensor by ionized particles once it leaves the protection of the lower atmosphere. μ

During the ascent, the average radiation dose rate measured by the TimePix sensor was about 2.22 μGy/hr. Since the ascent lasted near 40 minutes, the total amount of radiation dose was about 1.48 μGy during ascent. During the cruise, the average radiation dose rate was about 3.83μGy/hr. Since cruise lasted approximately 2.5 hours, the total amount of radiation dose that the cells received was about 9.58 μGy. Finally,

during the descent the average radiation dose rate measured was about 1.24 μGy/hr, thus, during the 40 minutes descent, the total radiation dose was about 0.83 μGy, or about twice than the radiation amount that cells received during ascent. According to the American Nuclear Society, the average yearly human dose is about 0.71μSv/hr

At sea level, the minimum measured cosmic background radiation dose [7] that reaches Earth's surface is about 0.06 μGy/hr. Cells on the ground received approximately 0.24 μGy during the 4 hours. This means that flight cells received more radiation dose levels than ground control samples. These larger radiation levels were 6 times higher during ascent, 16 times higher during the cruise, and over 5 times during descent when compared to ground levels of radiation.

a.

b.
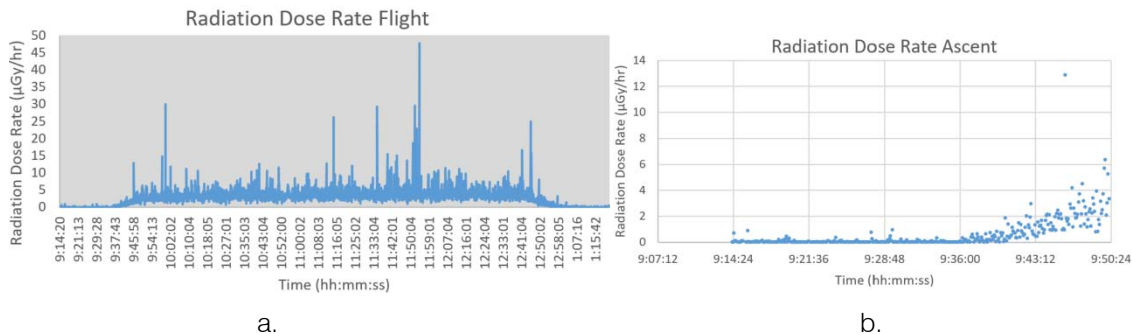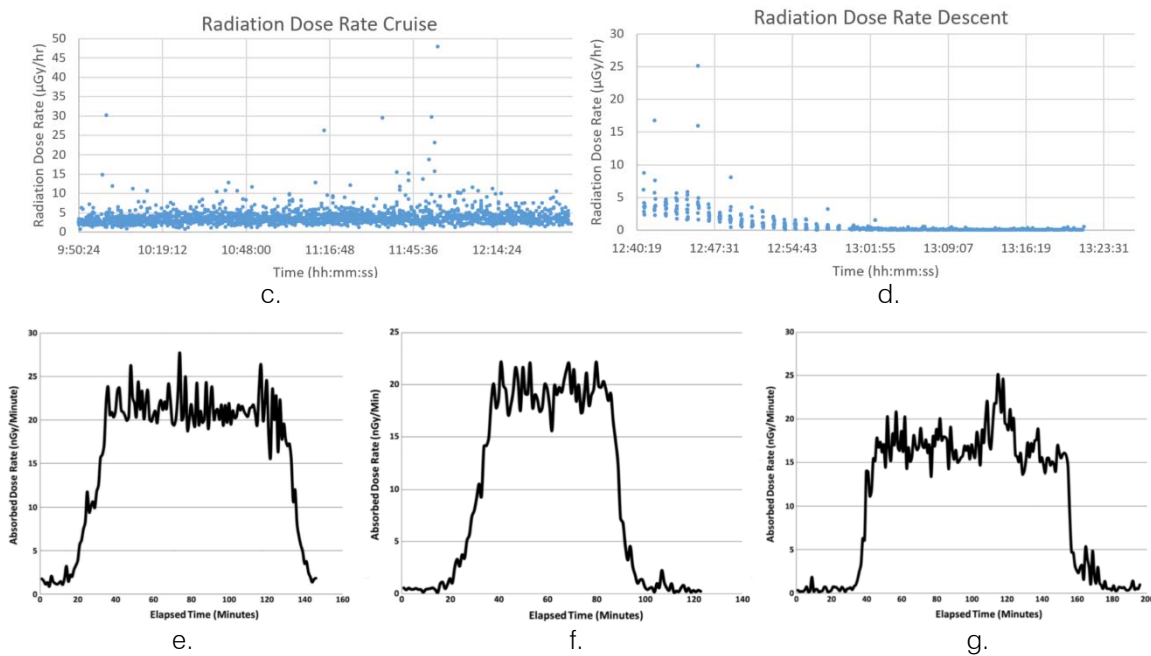
Figure 8: Total radiation dose rate. a. Flight. b. Ascent. c. Cruise. d. Descent. e. For comparison, we show the TEPC absorbed dose rate for Holbrook to Memphis as measured by the ARMAS system. f. TEPC for Holbrook to Indianapolis. g. TEPC for Houston to Los Angeles, August 2011.

There is a clear trend line at the beginning and at the tail end of the data suggesting that the strength and the frequency of particles strikes raise and lowers at a time coinciding with the WB-57 aircraft's ascent and descent phases. The maximum dose during those spike moments was recorded to be 48 μGy. This can be explained by the particles, which strike parallel to the surface and track across the length of the instrument. This results in the particle leaving a longer trace on the sensor than what would be observed if the particle was to strike the sensor head-on.

For comparison, previous studies [3] provide evidence of tissue-equivalent proportional encounters (TEPC) radiation doses of about 3.29 μSv/h (Figure 8e), 2.70 μSv/h (Figure 8f) and 1.81 μSv/h (Figure 8g), respectively during cruise at about 9.8 km (32,152 feet) for various flights in 2011 (Figure 8) using the Automated Radiation Measurements For Aerospace Safety (ARMAS) system. These radiation dose rates translate to total dose equivalent of 5.88 μSv (Figure 8e), 2.63 μSv (Figure 8f) and 3.96 μSv (Figure 8g),

respectively, or total absorbed doses of 2.34 μGy, 1.19 μGv and 2.20 μGv, respectively.

d) In-Vitro Experiment Visual Inspection

Given our biological payload consisted of sensitive cells, we inspected the cells as soon as we were given them back. On a preliminary inspection done at the Hangar, the Eppendorf tubes which had been cataloged pre-flight (Figure 9a) were laid out. It was noticed that 10 of the 32 cell mediums exhibited a change in color, which can be attributed to the temperature change. The cells which underwent temperature alterations were the ones placed in the avionics section of the NanoLabs and not directly over the heating pad. This can be attributed to the lack of heat transfer by convection due to the low density of air at the cruising altitude of the WB-57 aircraft. The two Eppendorf tubes (top right corner in Figure 9c) placed on the outside of the NanoLabs and exposed to the outside air temperature had the same indication of temperature change.
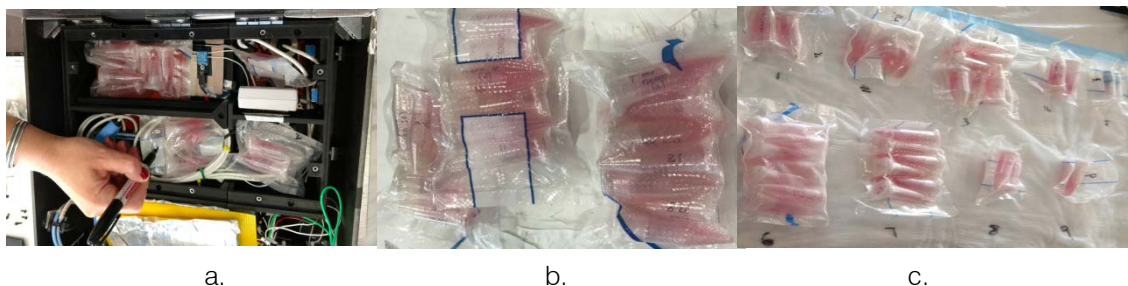

Figure 9: Cells (a) before and (b,c) after the flight after immediately taking them out of the NanoLabs

Our goal was to keep the cells at 30 °C (with the heating pads) because that is their preferential temperature. However, the actual measured temperatures ranged from about 21 °C to 26 °C. Given the tubes containing the cells were sealed and placed on top of the heating pads, it is plausible to assume that the actual temperature inside the tubes was about 3 °C to 4 °C higher.
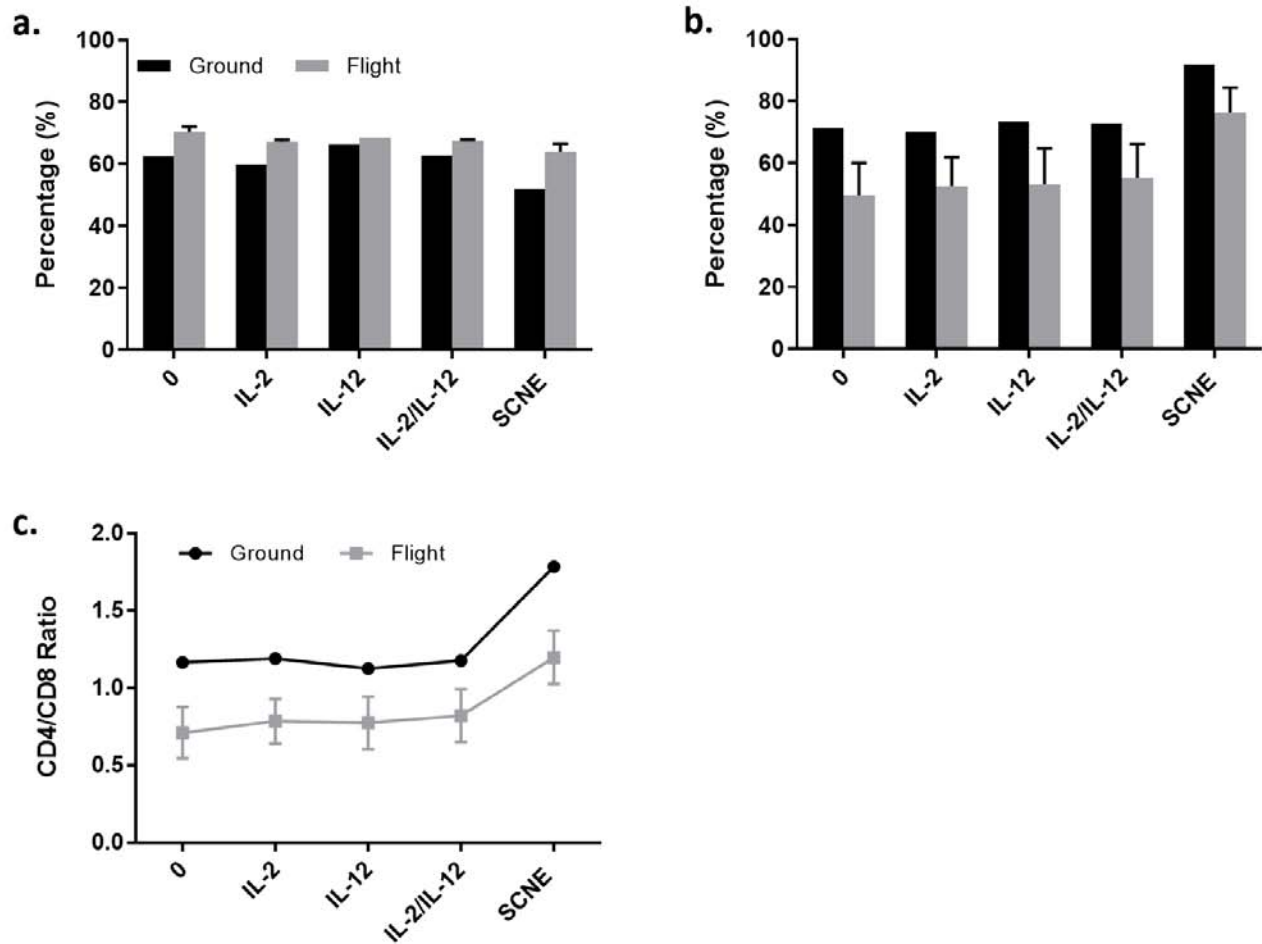
i. *Effect of Flight Stressors on the Expression of CD8 and CD4 Populations*

Exposure to flight led to an increased expression of CD8 cells in all conditions for naïve cells population with the most robust effect observed in IL-12 alone or in combination with IL-2 condition (Figure 10a).

SCNE also led to a profound increase. There was a decreased expression in the CD4 population in flown cells compared to the ground controls, except for the SCNE treatment (Figure 10b). Overall, the CD4/CD8 ratio was decreased, except for the cells treated with SCNE. A similar pattern of CD8 expression was observed in activated T cells (Figure 11a). CD4 expression was decreased in all flight conditions compared to control cells (Figure 11b). The difference of CD4/CD8 expression between flown and control cells was higher in activated cells (Figure 11c) as compared to naïve cells. Interestingly, we observed a very noticeable increase in CD4 expression in activated T cells treated with SCNE (Figure 11c).
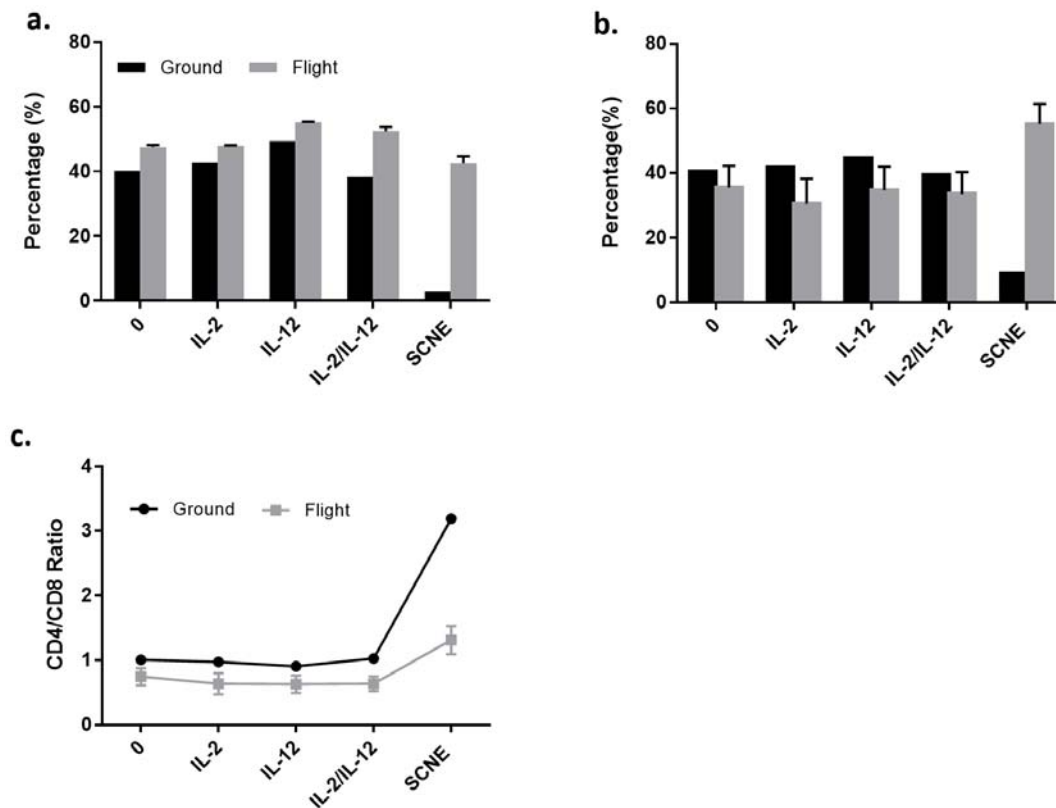

Figure 10: Assessment of naïve cells exposure to 60,000 feet flight on (top left) CD8, (top right) CD4, and (bottom) CD4/CD8 ratio expression with indicated cell culture conditions. Data represented as means +/SEM.

Figure 11: Assessment of activated T cells exposure to 60,000 feet flight on (top left) CD8, (top right) CD4, and (bottom) CD4/CD8 ratio expression with indicated cell culture conditions. Data represented as means +/SEM.

ii. *Flight Stressors Effect on the influence of the Cell Cycle*

T cell proliferation is essential for an effective adaptive immune response. A key element of proliferation is the entry of cells into the cell cycle, by transitioning from G1 into the S phase (Figure 12b), a complex process that is tightly controlled by the expression of cyclins and other enzymatic activities. There are several growth factors, known to induce T cell proliferation, such as interleukins IL-2 and IL-12. Therefore, we sought to determine, whether exposure to flight would modulate the cell cycle for T cells and whether the addition of cytokines and SCNE would have any effect on cell cycle. Usually, naïve cells stay in GO/G1 cell cycle phase. However, naïve cells treated with cytokines are known to enter other phases of the cell cycle [25]. Our data suggest that naïve cells treated with cytokines entered S and G2 phases and there was a slight difference in ground and flight conditions (Figure 12 a). Also, exposure to flight altered the cell cycle in T cells depending on the T cell culture conditions (Figure 12b).
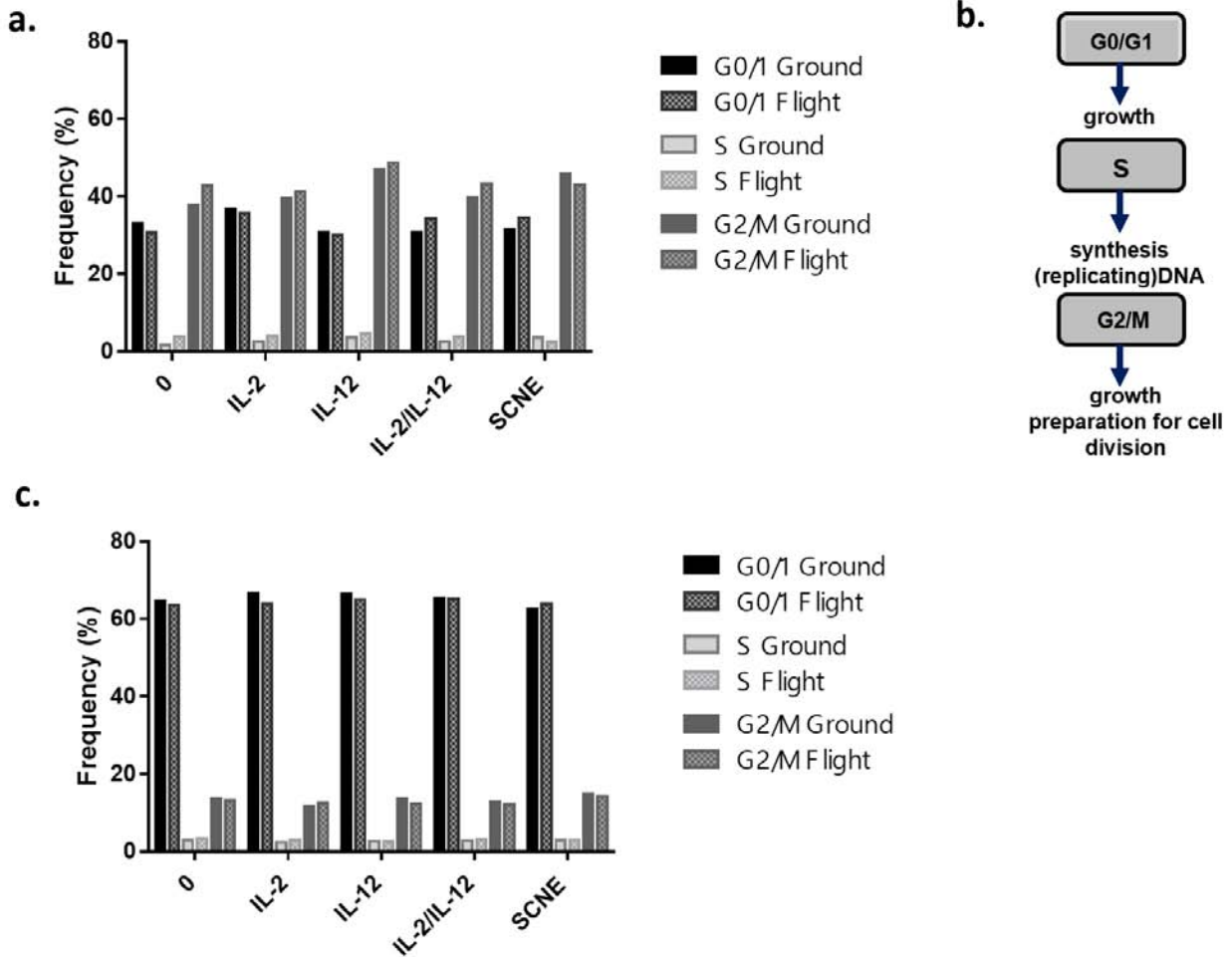
Figure 12: Cell cycle analysis of T cells exposed to 60,000 feet flight and their corresponded ground controls. Cells were harvested, fixed, stained with propidium iodide (PI) and analyzed by flow cytometry. (Top left) indicates % of frequency of naïve cells in GO/G1, S, and G2/M phases for each condition. (Top right) shows the diagram of cellular processes happening in each cell cycle phase. (Bottom) shows the distribution of activated T cells.

iii. *Flight Stressors and T Cell Proliferation Data*

We next assessed cell proliferation capabilities (Figure 13). We counted flown and ground cells post-flight. Our data indicate that adding IL-2 and IL-12 cytokines into T cell cultures leads to an enhanced T cell proliferation. We saw an uncommon decrease in cell numbers in activated ground T cells cultured in IL-2 and an increase in IL-12 (Figure 13 b). Thus, an increase in naïve ground cells cultured in SCNE (Figure 13 a). Except for the conditions mentioned above, there was not a profound difference in the ground and flown cell viability.
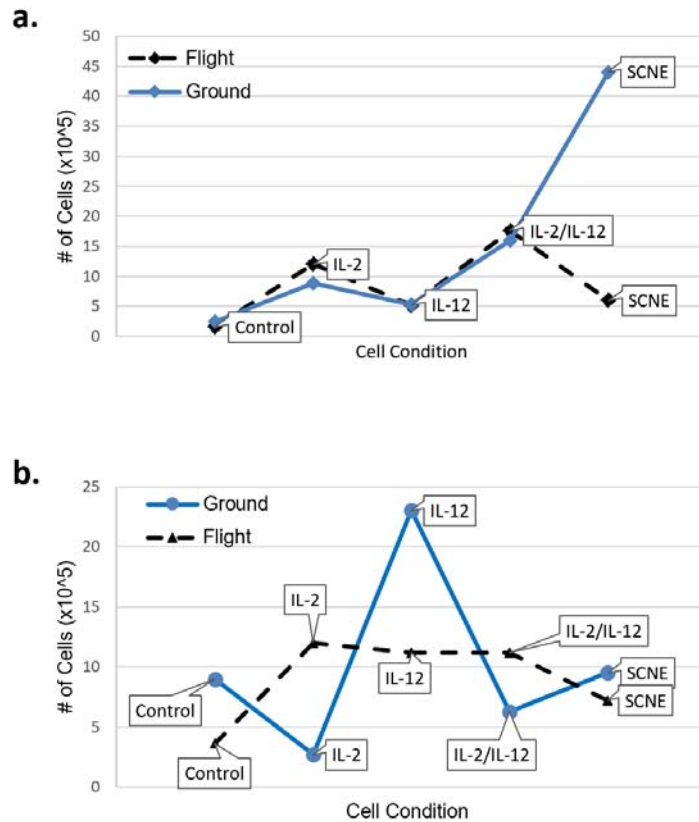
Figure 13: Assessment of (a) naïve and (b) activated T cells viability by staining cells with the trypan blue dye

iv. *Effect of Flight Stressors on the T cell Activation Marker CD25*

We next looked at the activation marker CD25. T cell activation is associated with the early synthesis of IL-2 and up regulation of IL-2 Receptor (R) CD25. Because CD25 expression requires T cell activation, IL-2R expression on resting naive T cells is limited to the low-affinity βγ IL-2R. Recent findings [11] indicated thought that IL-2Rβγ on naive CD8 T cells is functional and allows the cells to respond vigorously to moderate concentrations of IL-2 as manifested by proliferation and differentiation into effect or cells, both in vitro and in vivo. Our data on T cells show the increased expression of CD25 in all flight conditions compared to ground controls (Figure 14a). A similar pattern of expression was observed in naïve cells too (Figure 14b).
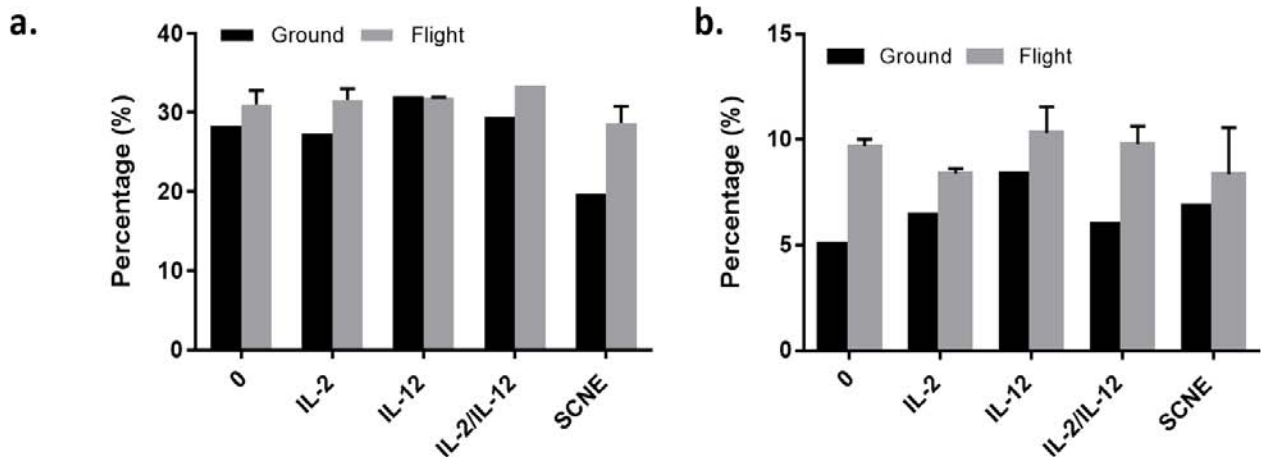

Figure 14: Assessment of CD25 expression on (left) activated T cells and (right) naïve cells for the indicated conditions. Data represented as means ± SEM

v. *Exposure to Flight Stressors Alters the Expression of Multiple Cytokines Detected from T Cell Culture Supernatants*

T cells are usually classified by the expression of glycol proteins and by the ability to produce cytokines within specific groups, such as Th1- or Th2-. Therefore, we assessed an array of different pro- and anti-inflammatory cytokines released to the cells' culture media of flown cells and their ground controls. Given IL-2 stimulated cells are unable to produce IFN-γ, we did see its expression in IL-2 conditions (Figure 15a). However, our data indicate that IFNγ expression was elevated in both naïve and activated T cells across all IL-12 culture conditions compared to ground controls. Supplementing IL-2 with IL-12 also led to strong induction of IFN-γ expression. TNFα expression was decreased in all naïve cell conditions in almost all flight conditions, except for the IL-12 condition (Figure 15b). The up-regulation of TNFα by IL-12 has been reported before [17]. IL-2 expression was elevated in nearly all activated T cell conditions, except for the IL-2/IL-12, whereas in naïve cells IL-2 was decreased except for the IL-12 and SCNE conditions (Figure 15c). As expected, IL-12 expression was elevated in IL-12 conditions (Figure 15d).
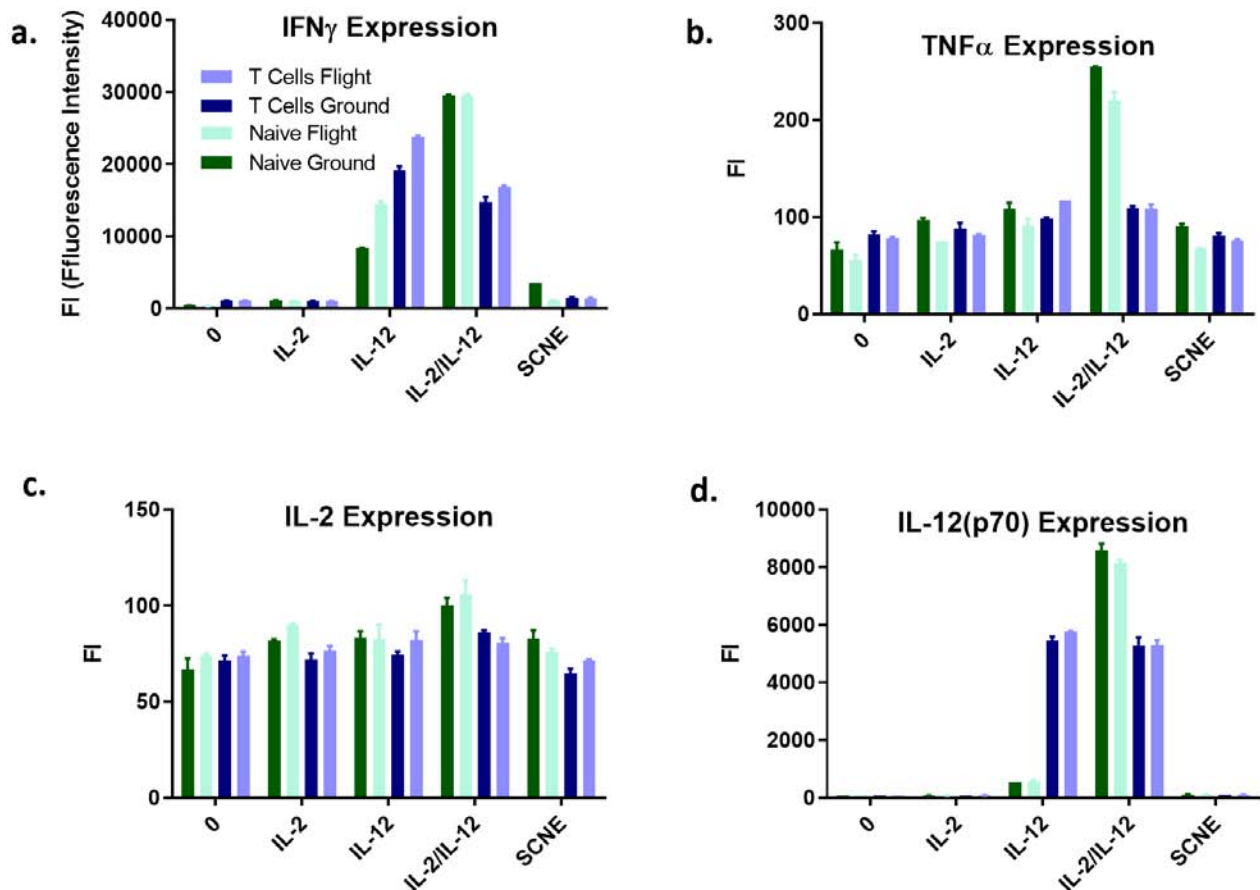
Figure 15: Th1 type cytokines expression on naïve and activated cells including (top left) IFNɣ, (top right) TNFα, (bottom left) IL-2, and (bottom right) IL-12 (p70). Expression of cytokines is displayed as the Fluorescence Intensity (FI). Data represented as means +/SEM.

The pro-inflammatory cytokine IL-6 expression was elevated in all flight conditions for both naïve and activated cells except for SCNE in naïve cells (Figure 16a). The colony-stimulating factor (G-CSF) was elevated in most flown cell conditions, apart from naïve cells not cultured with any cytokines and SCNE as well as T cells either cultured with IL-2 alone or in combination with IL-12 (Figure 16b). IL-5 expression was elevated in all flight conditions for T cells (Figure 16c) and it was the opposite effect in leukemia inhibitory factor (LIF) expression (Figure 16d).Next, we looked at the expression of IL-3, IL-4, IL-5, and IL-17 (Figures 17a,b,c,d). We saw mostly elevated expression in all of them in flown conditions, except for the IL-3 and IL-17 expression in cells cultured in IL-2 and SCNE for the latter.
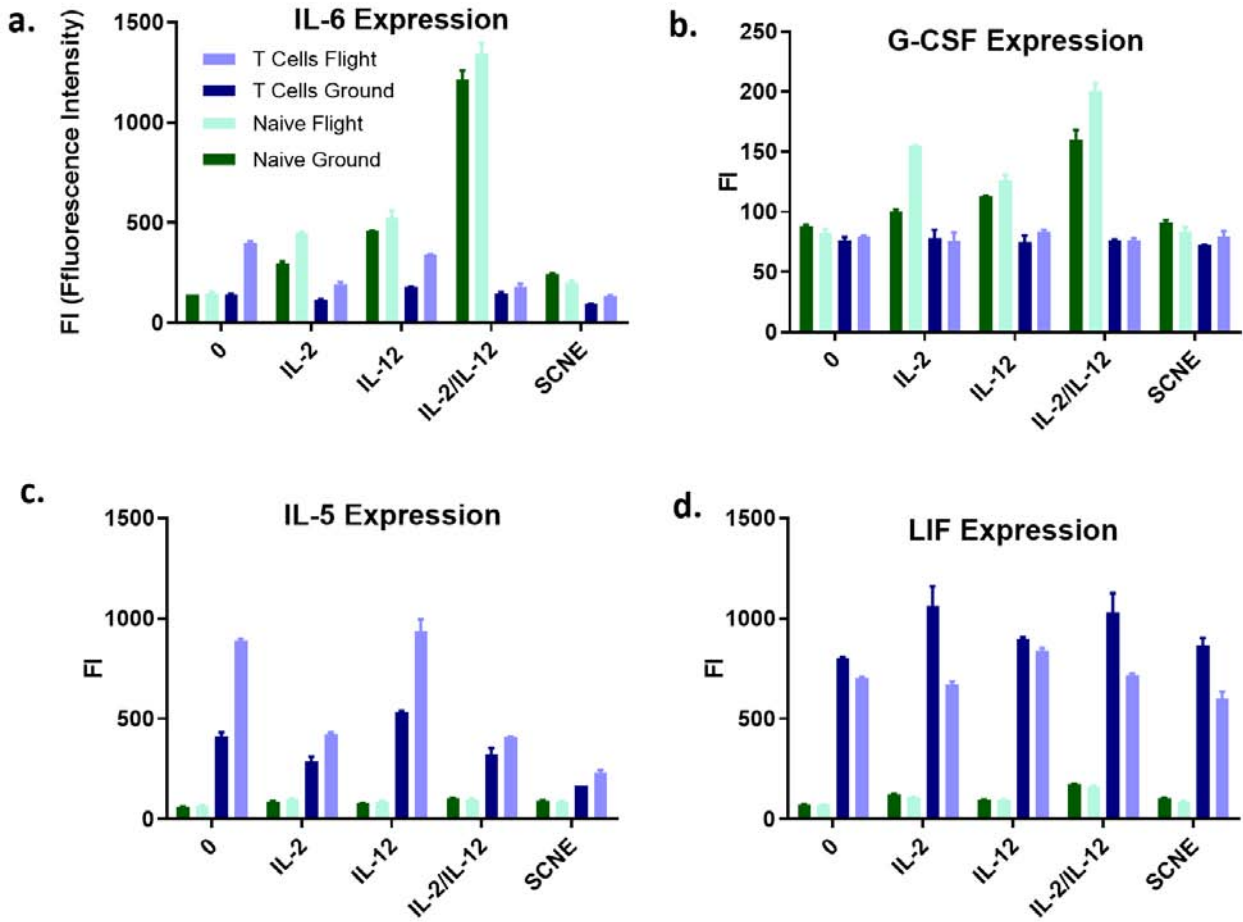
Figure 16: Cytokines expression on naïve and activated cells including (top left) IL-6, (top right) G-CSF, (bottom left) IL-10, and (bottom right) LIF. Data represented as means +/SEM
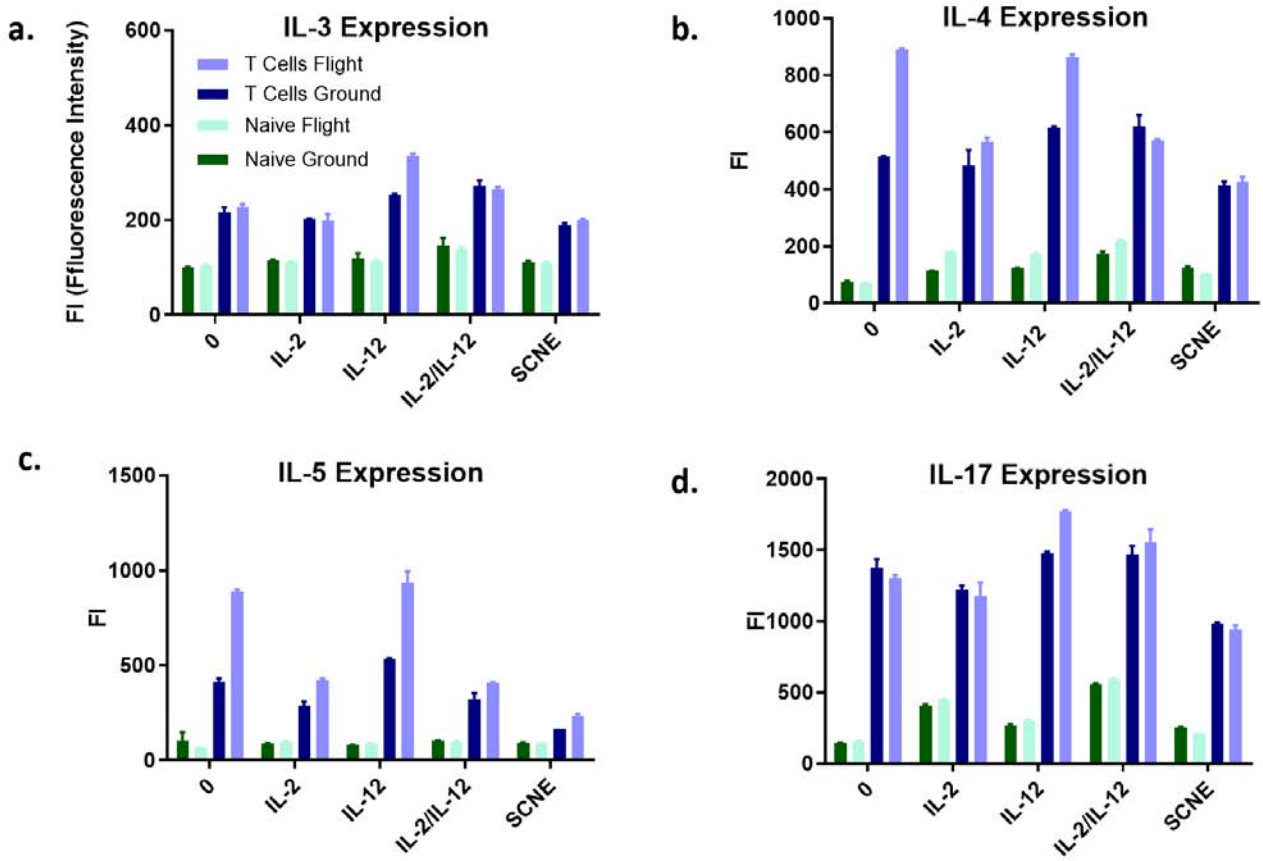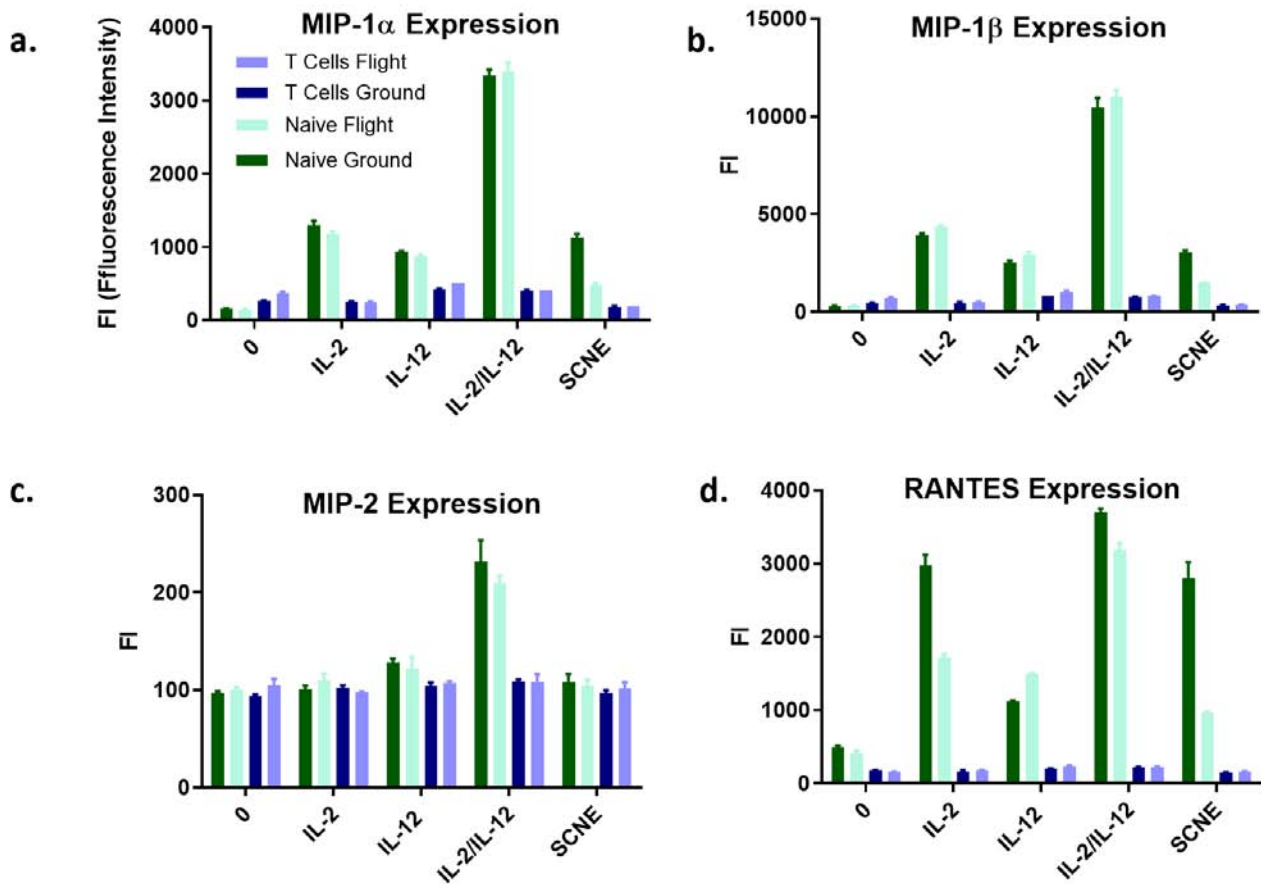
Figure 17: Cytokines expression on naïve and activated cells including (top left) IL-3, (top right) IL-4, (bottom left) IL-5, and (bottom right) IL-17. Data represented as means +/SEM

Our data show that exposure to flight leads to decreased expression of recombinant human macrophage inflammatory protein MIP-1α (Figure 18a) but decreased expression of MIP-1β (Figure 18b) in naïve cells in most conditions. Also, decreased expression of MIP-2 (Figure 18c), and RANTES (Figure 18d), except for the IL-12 condition.

Figure 18: Cytokines expression on naïve and activated cells including (top left) MIP-1α, (top right) MIP-1β, (bottom left) MIP-2, and (bottom right) RANTES. Data represented as means +/SEM

Finally, our data show the decreased expression of chemokine IP-10 in naïve cells, except for the IL-12 condition (Figure 19a). An opposite trend was observed in neutrophil chemokine (KC) expression (Figure 19b). The expression of monokine induced by interferon-gamma (MIG) was decreased in all naïve cell conditions (Figure 19c) and similar pattern observed in vascular endothelial growth factor (VEGF) expression (Figure 19d), except in activated T cells treated with IL-12. Our data highlight the differences in the responses of different cell culture conditions to exposure of flight stressors, including cosmic radiation.
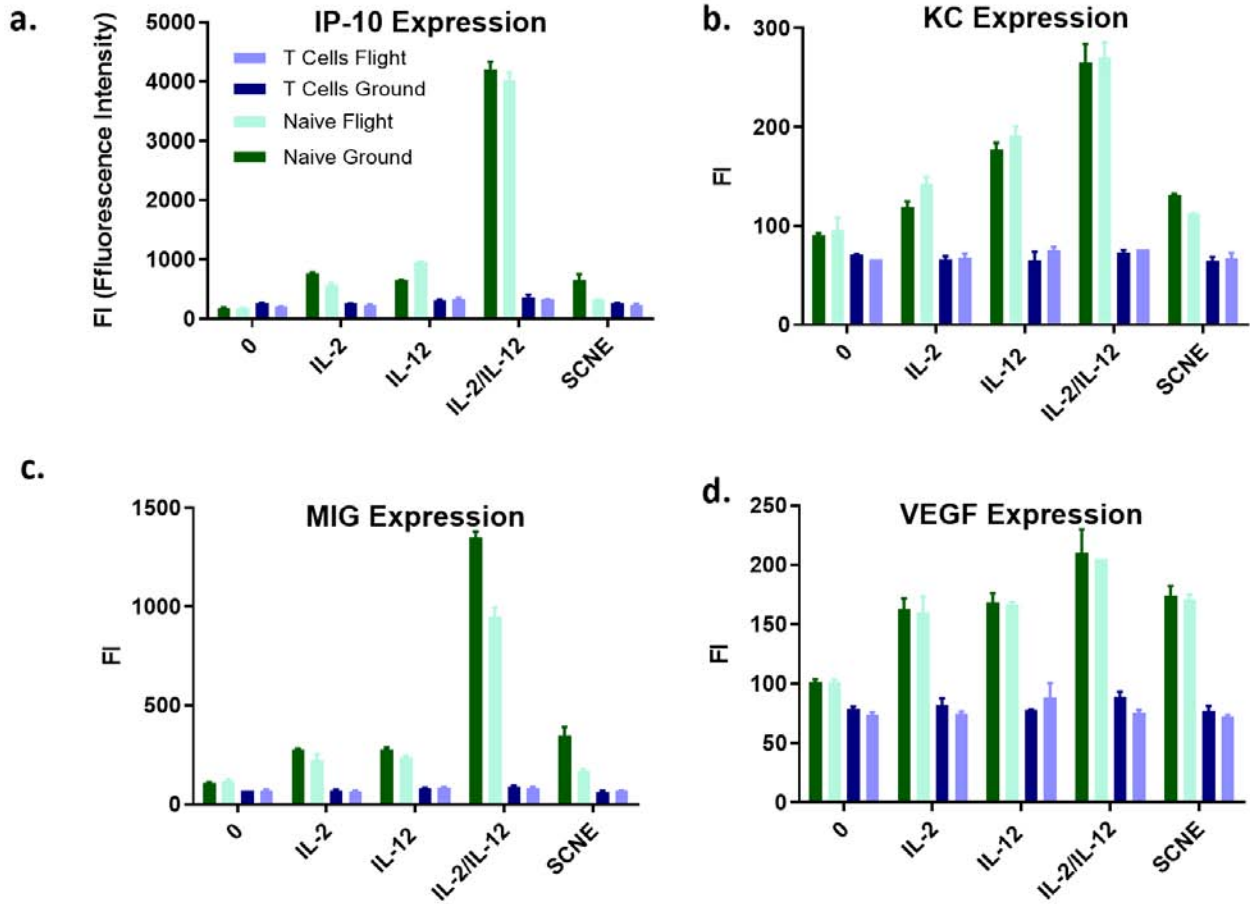
Figure 19: Cytokines expression on naïve and activated cells including (top left) IP-10, (top right) KC, (bottom left) MIG, and (bottom right) VEGF. Data represented as means +/SEM

Table 1: Statistical Analyses on Naïve and T Cells expressed as Mean ± SEM

Naïve Cells and T Cells vs. Ground Samples. Value as Mean ± SEM.					
Expression	Control	IL-2	IL-12	IL-2/IL-12	SCNE
Percentage % Naïve Cells Flight vs. Ground					
CD8	43.8 ± 3.8	45.3 ± 2.6	52.2 ± 3.8	45.4 ± 7.1	43.8 ± 3.8
CD4	38.0 ± 32.4	36.1 ± 5.6	39.7 ± 5.0	36.5 ± 2.9	32.1 ± 23.1
CD25	7.4 ± 2.3	7.4 ± 1.0	9.4 ± 1.0	7.9 ± 1.9	7.6 ± 0.8
Fluorescence Intensity Naïve Cells Flight vs. Ground					
IFN-ϒ	387.0 ± 15.5	1040.6 ± 87.9	11395.1 ± 3023.1	29556.4 ± 7.9	2254.3 ± 1200.0
IL-6	142.3 ± 2.3	372.4 ± 74.9	492.1 ± 34.6	1279.9 ± 64.4	222.3 ± 21.8
Il-17	150.4 ± 5.4	426.9 ± 22.1	284.3 ± 18.8	575.3 ± 16.3	234.0 ± 20.0
Percentage % T Cells Flight vs. Ground					
CD8	66.3 ± 3.9	63.4 ± 3.7	67.4 ± 1.1	65.0 ± 2.2	58.0 ± 6.1
CD4	60.5 ± 10.9	61.3 ± 8.7	63.2 ± 10.1	64.0 ± 8.9	64.0 ± 7.8
CD25	30.8 ± 0.5	30.9 ± 2.7	32.7 ± 1.4	34.6 ± 1.1	32.5 ± 3.6
Fluorescence Intensity T Cells Flight vs. Ground					
IFN-ϒ	1060.8 ± 16.3	992.5 ± 4.0	21385.3 ± 2315.3	15794.6 ± 1077.9	1475.3 ± 25.8
IL-6	270.6 ± 128.4	154.0 ± 40.5	259.8 ± 80.5	162.1 ± 17.4	113.4 ± 19.9
Il-17	1339.0 ± 35.5	1196.6 ± 21.4	1624.5 ± 146.0	1509.5 ± 42.0	963.4 ± 23.1

IV. Discussion

This multidisciplinary research project was a collaborative effort across different disciplines and universitiesnation wide to enhance students' experience in STEM and to obtain valuable technological and scientific data. Several space agencies including ESA, NASA, and the Chinese space agency, as well as

private sectors such as Space X and Blue Origin are pursuing human activities in deep space. Space X is currently building several spaceports in Brownsville, Texas. Therefore, the technology tested in our payloads (e.g. ADS-B) can enhance the understanding of current and probable future operational procedures of spaceflight operations in the Gulf of Mexico region and to improve surveillance over the region. Understanding suborbital requirements, procedures, and ADS-B performance are critical to better assess prospective point-to-point suborbital flights [2]. This was a valuable opportunity to observe the WB-57, due a high-altitude performance aircraft, which flies at high speeds (756 km per hour or about 0.6 Mach) and is of interest in testing the functionality of ADS-B technologies during these analog suborbital trajectories. The Sky Guard TWX Vision-Pro Plus kit was the ADS-B used for this experiment.

Numerous challenges are facing space industry today. Exposure to radiation is one of the main concerns about space travel [20, 21]. Short-term radiation effects can be originated from intense solar energetic particles (SEPs) which could induce acute radiation syndrome (ARS) or sickness, poisoning or toxicity if the body is exposed to high radiation doses at the Gy level [20]. We suggest conducting further research and expose these cells to space environment during various solar activities to further assess the effects of radiation on the immune system since varying radiation intensities may derive from different solar cycles.

There were some previously reported studies examining the effect of radiation on the T cells. For example, previous research [5] used the T cells derived from the same strain of mice as ours which were exposed to-low-dose/low-dose-rate (LDR) (57) Co γ-rays (0.01 Gy, 0.03 cGy/h), with and without acute 2 Gy proton (1 Gy/min) or γ-ray (0.9 Gy/min) irradiation; analyses of which were done on days 21 and 56 post-exposure. Our results are consistent with reported literature which showed the significant increase in CD4 expression in human T cells of HIV patients treated with neem tree derived extract [13]. Previous studies [14] investigating the effect of radiation on lymphocytes reported CD4 population being more sensitive to radiation than CD8 which is in alignment with our findings. Also, some reports [15] suggested that CD8 population might be resistant to radiation of lower doses, but very sensitive to 2 Gy. However, most past studies were conducted using whole animal radiation exposure, therefore the comparison of our results with previous studies might be not comparable. Thus, our data suggest that adding IL-12 to naïve cell cultures leads to a higher expression of CD8, whereas adding SCNE leads to a higher expression of both CD8 and CD4 in flight but not in ground conditions.

We also saw an increased expression of CD25 which is in alignment with the previous report [16] showing the increased expression of CD25 in irradiated mice using a much higher dose of radiation than in our recordings, but from the same strain mouse as ours. The difference between ground and flight conditions were more evident in naïve cells.

Finally, we looked at the expression of many cytokines released in the supernatants of T cells. T cells' ability to release cytokines plays a critical role in their functional activity, as it determines the nature and the outcome of the immune response. Thus, their ability to produce multiple cytokines has been associated with beneficial immune responses. Previous studies [19] have shown that exposure to high-LET iron ion radiation on immune T cells induces pathological changes – relevant to assess the long-term potential effects on astronauts that may experience during their long exploratory missions in space. The expression of most cytokines was shown to decrease after low doses of radiation and to increase after high doses. For example, IL-6 was reported to react at early times and IL-10 at later times, whereas IL-5 levels were consistently elevated indicating the differences in the responses to a low and high dose of radiation [15]. Our data show very high levels of IL-5 in all flight conditions.

It is important to note that most other previous studies used the whole-body radiation exposure on the immune cells in mice, therefore comparing our study findings with past studies might be complicated. In our study, we report differences in T cell subpopulations, cell cycle alterations, and cytokine release possibilities in flown cells compared to ground controls. Although unlikely, but these changes might have been also influenced by the temperature changes as we saw color changes in the cells. Also, we were surprised to see the decreased expression of cells in ground cells cultured in IL-2. This could be due to the technical error or due to the different temperature the cells were exposed at the NASA Hangar. Our previous feasibility studies [26] conducted by exposing cells to different temperatures revealed that cells are capable to survive and proliferate at the temperatures experienced during this flight. Finally, we got insights into the effect of neem tree extract on naïve and activated T cells' behavior. Given the beneficial properties of this extract [18], further research is needed to elucidate its mechanism of action.

V. Conclusions

Space travel induces numerous health hazards and technical challenges. In this study, we designed and tested the various technologies to maximize scientific findings. Our results indicate that the maximum dose of radiation was 48 μGy with ten instances of high radiation dose above 15 μGy/hr during the cruise, totaling about 9.5 μGy during the cruise, and radiation doses of about 1.2 μGy and 0.8 μGy during ascent and descent, respectively. These radiation doses are about

six times, sixteen, and five times larger than the radiation dosed experienced by the ground control samples. This radiation data was accurately measured by NASA's Timepix instrument. Thus, we obtained valuable scientific data on naïve and activated murine T cells exposed to the 60,000 feet flight and cosmic radiation. Our findings demonstrate that flown cells showed fewer CD4 positive cells, but an increased number of CD8 cells. Herein, we observed a beneficial outcome of adding cytokines IL-2 and IL-12 to the T cell cultures. This finding presents a simple strategy to enhance T cells' behavior in future studies.

T cells exposed to this flight environment expressed slightly higher than ground samples for CD8, but these flight T cells expressed approximately 20% less than the ground samples for every single condition when using CD4. However, naive cells showed a significant yield for both CD 4 and CD8. For CD4, when naïve cells were manipulated with SCNE or IL-2/IL-12 combined, flight samples showed much higher expression (near 90% higher for SCNE and near 25 % higher for IL-2/IL-12). For CD8, when naïve cells manipulated with SCNE, yield expression was about 85% higher than for ground cells, and it was nearly 10% lower for every other condition. As for CD25, a larger % expression is observed in the T cells versus the naïve cells, but relative % expression seems to be larger for the naïve cells.

Looking at the fluorescence intensity (FI), we observed that it is significantly larger for naïve cells, both flight, and ground than for T cells, especially for IL-6, G-CSF, IP-10, KC, MIG and VEGF, MIP-1α, MIP-2, and RANTES expressions when using IL-2 and IL-12 combined. Another important observation is that T cells, both flight, and ground, are significantly higher for IL-3, IL-4, IL-5, and IL-17 expressions for every single condition (no cytokine, IL-2, IL-12, IL-2/IL-12, and SCNE).

Our research findings indicate that the various levels of radiation doses may have had a significant impact on the cells, since cellular alterations of both flight naïve and activated T cells were noted. These cells received about six and five times more radiation levels than the ground controls during ascent and descent, respectively, and over 16 times more during the cruise. Temperature variations may have contributed to the cellular alterations, these variations were maintained within ± 6 °C to 7 °C of the desired 30 °C during the cruise for cells. Temperature variations during ascent and descent were within a few °C for approximately 40 minutes of flight for each segment. The temperature variation when the cells landed at Ellington Field airport, was between 17 °C to 18 °C, at that time, the AOD team was unloading our payload inside the hangar. These small temperature variations may have been associated with the fact that the payload had already been unpowered from the aircraft but because payload was inside the hangar, the temperature was relatively constant at about 14 °C to 15 °C inside the hangar. Temperature variations may have contributed to the cellular alterations, although the variation of temperature was relatively small. Although the active thermal system was proved to provide good temperature stability, especially during the cruise, our team will further work on refining the thermal system to increase its reliability to narrow down the temperature stability range within ± 2 °C for the entire flight.

As we embark for long-term human space exploration, it is critical to gain a better understanding of radiation effect on immune cells and other body systems. The results obtained from this study will be valuable for the development of further payloads against the damage of space induced radiation.

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Abbreviations: ADS-B, Automatic Dependent Surveillance-Broadcast; AOD, Aircraft Operation Division; AR, Airworthiness Review; ECLSS, Environment Control Life Support System; ERAU, Embry-Riddle Aeronautical University; ERHASER, Embry-Riddle High Altitude Science Engineering Rig; FAA, Federal Aviation Administration; LET, Linear Energy Transfer; NanoLab (1U), 10 cm × 10 cm × 10 cm; SCNE, neem tree Azadirachtaindica; SOAR, Student Opportunities in Airborne Research; STEM, Science Technology Engineering and Mathematics; TRR, Test Readiness Review; TUC, Time of Useful Consciousness; IL-2, Interleukin 2; IL-12, Interleukin 12.

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