Palmer Station, Antarctica: A Ground-Based Spaceflight Analog Suitable for Validation of Biomedical Countermeasures for Deep Space Missions

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Abstract

Astronauts are known to exhibit a variety of immunological alterations during spaceflight including changes in leukocyte distribution and plasma cytokine concentrations, a reduction in T-cell function, and subclinical reactivation of latent herpesviruses. These alterations are most likely due to mission-associated stressors including circadian misalignment, microgravity, isolation, altered nutrition, and increased exposure to cosmic radiation. Some of these stressors may also occur in terrestrial situations. This study sought to determine if crewmembers performing overwinter deployment at Palmer Station, Antarctica displayed similar immune alterations. The larger goal was to validate a ground analog suitable for the evaluation of countermeasures designed to protect astronauts during future deep space missions. For this pilot study, plasma, saliva, hair, and health surveys were collected from Palmer Station, Antarctica winterover participants at baseline, and at five overwinter timepoints. Twenty-six subjects consented to participate over the course of two seasons. Initial sample processing was performed at Palmer, and eventually stabilized samples were returned to the Johnson Space Center for analysis. A white blood cell differential was performed (real time) using a fingerstick blood sample to determine alterations in basic leukocyte subsets throughout the winterover. Plasma and saliva samples were analyzed for 30 and 13 cytokines, respectively. Saliva was analyzed for cortisol concentration and three latent herpesviruses (DNA by qPCR), EBV, HSV1, and VZV. Hair samples were analyzed for several hormones, as a measure of stress over prolonged periods of time. Voluntary surveys related to general health and adverse clinical events were distributed to participants. It is noteworthy that due to logistical constraints due to COVID-19, the baseline samples for each season were collected in Punta Arenas, Chile, after long international travel and during isolation. Therefore, the palmer pre mission samples may not reflect a true normal ‘baseline’. Minimal alterations were observed in leukocyte distribution during overwinter. The mean percentage of monocyte concentration elevated at one timepoint. Plasma G-CSF, IL1RA, MCP-1, MIP-1β, TNFα and VEGF were decreased during at least one overwinter timepoint, whereas RANTES was significantly increased. No statistically significant changes were observed in mean saliva cytokine concentrations. Salivary cortisol was substantially elevated throughout the entire winterover compared to baseline. Compared to shedding levels observed in healthy controls (23%), the percentage of participants who shed EBV was higher throughout all winterover timepoints (52-60%). Five subjects shed HSV1 during at least one timepoint throughout the season compared to no subjects shedding during pre-deployment. Finally, VZV reactivation, common in astronauts but exceptionally rare in ground-based stress analogs, was observed in one subject during pre-deployment and a different subject at WO2 and WO3. These pilot data, somewhat influenced by the COVID-19 situation, do suggest that participants at Palmer Station do undergo immunological alterations similar to, but likely in reduced magnitude, as those observed in astronauts. We suggest that overwinter at Palmer Station may be suitable test analog for spaceflight biomedical countermeasures designed to mitigate clinical risks for deep space missions.

Introduction

Astronauts are known to exhibit a variety of immunological alterations due to a diverse array of unique stressors only experienced during long-duration spaceflight. These stressors encompass a variety of factors that affect numerous systems in the body including circadian misalignment, microgravity, isolation, infrequent resupply, and increased exposure to cosmic radiation. All of these factors can contribute to immune dysregulation in humans as evidenced by numerous studies reporting altered leukocyte distribution, changes in plasma cytokine concentrations, a reduction in T-cell function, and reactivation of latent herpesviruses **(Crucian 2013; Crucian 2018; Bigley 2018; Mehta 2017; Krieger 2021)**. Immune alterations in astronauts have been positively correlated with the increased incidence of herpesvirus reactivation **(Mehta 2013)**. Additionally, clinical events such as rashes, hypersensitivity and atopic dermatitis have been reported in astronauts during long-duration spaceflight **(Crucian 2016; Crucian 2016, Mehta 2022)**. These phenomena have been reviewed thoroughly by **Krittanawong et al. 2022**.

Presently, as humans strive to extend their reach beyond low-Earth orbit through the Artemis missions, it has become imperative to evaluate countermeasures at ground-based spaceflight analogs to mitigate the negative health consequences associated with long-duration spaceflight. During missions to the Moon and Mars, the opportunity for resupply and to receive assistance during a medical emergency is unfeasible; therefore, it is necessary for astronauts to carry with them medication or devices they may need in the event of an emergency and to minimize negative health impacts from living in the deep-space environment. A deep space countermeasure protocol, compatible with operational and mission constraints, was recently developed by an international and translational team of discipline scientists **(Crucian 2018; Makedonas 2019)**.

Ground based analogs of spaceflight simulate spaceflight stressors on human physiology. **Crucian et al. (2014) evaluated** numerous potential ground-based analogs for immune dysregulation. Head down tilt bedrest, which redistributes pressure towards the upper body, has been evaluated extensively as an analog for microgravity **(Pandiarajan Frontiers in Phys)**. Published data suggest that bed rest alone is not a sufficient stressor to create immune alterations similar to those observed in astronauts **(Crucian 2009)**. Short duration deployment to the Arctic, or undersea deployment, were both found to be promising but lacking in duration and chronic stress **(Crucian 2007; Strewe 2015)**. Antarctic winterover affords the most comparable conditions to those that astronauts experience during long duration spaceflight. Participants are subjected to extreme isolation, circadian misalignment, station lifestyle, infrequent resupply, and stress. Mission duration approaches 1 year. Concordia Station, in the Antarctica interior, was evaluated for use as a potential terrestrial analog for immune dysfunction; however, due to its location at high altitude and the hypoxic environment, the alterations seen in subjects at this station were not comparable to the changes seen in astronauts during spaceflight **(Feuerecker 2014; Feuerecker 2018)**.

Recently, a review was published outlining a collection of potential spaceflight countermeasures including modified diet, stress-relieving breathing exercises, Vitamin D, and probiotic supplementation, as well as a prescribed exercise regimen **(Makedonas 2019)**. The purpose of this pilot study was to evaluate whether coastal Antarctic winterover participants exhibit immunological dysregulation similar to that observed in astronauts during spaceflight by assessing stress, immunity, and viral reactivation in these individuals. Moreover, this investigation sought to determine whether the participating subjects would exhibit immune dysregulation that could potentially be corrected

through the use of countermeasures. Once the Antarctica model is validated as an analog of immune dysregulation, the downstream goal is to evaluate NASA countermeasure protocols for deep space missions within the Antarctica winterover space analog.

This study took place over two consecutive Antarctic winterovers and included a total of 26 subjects. Saliva, blood, hair, and plasma were collected from subjects at five time points, both before and throughout the four-month winterover. A variety of immune and viral related outputs were assessed including VZV (Varicella zoster virus), EBV (Epstein-Barr virus), HSV1 (Herpes Simplex Virus 1), plasma and saliva cytokines, health surveys, stress markers in hair, and white blood cell differential.

Materials and Methods

Subjects and Sample Collections

This study consisted of individuals participating in winterover at Palmer Station, Antarctica in 2020 and 2021. Of the 26 total subjects who initially enrolled, 12 subjects completed the study in the first year and 12 subjects completed the study in the second year. One subject from the first year withdrew from the study for unspecified reasons after participating in two sample collections and no survey completions and one subject from the second year stopped participating in the surveys, completely, after the third survey.

Biological samples were collected from subjects at one timepoint before winterover deployment, designated as pre-deployment, and at four timepoints throughout the winterover approximately one month apart, designated at WO1-WO4 **(FIGURE 1)**. A baseline sampling in Denver was planned before the crew departed. However, due to the COVID-19 pandemic and isolation, in an effort to protect the Antarctica bases, crews were sent directly to Punta Arenas, Chile, where they isolated onboard the icebreaker for several weeks. It is noteworthy that the baselines occurred under stressful isolation conditions and after long international travel, as discussed below. Sample collections therefore occurred in May (pre-deployment), July, August, September, and November for the 2020 winterover. For the 2021 winterover, sample collections occurred in April (pre-deployment), June, July, August, and September. This protocol was reviewed and approved by the Institutional Review Board at the NASA Johnson Space Center, Houston, TX. Subjects were provided informed consent before data collection.

White Blood Cell Differential

To analyze basic leukocyte subsets, a Hemocue Diff system (Hemocue, Ängelholm, Sweden) was deployed to Palmer Station, Antarctica. Briefly, a fingerstick was performed, the Hemocue cuvette was placed on the blood drop and the blood was pulled into the cuvette through capillary action. The cuvette was placed into the system which performed the analysis. Outputs included WBC (10^9/L), neutrophils (10^9/L and %), lymphocytes (10^9/L and %), monocytes (10^9/L and %), eosinophils (10^9/L and %), and basophils (10^9/L and %). Data was recorded and sent to Johnson Space Center in Houston, Texas. Pre-deployment Hemocue data was only collected for the first winterover subjects due to logistical constraints in performing this analysis for the second winterover.

Plasma Cytokines

Blood was collected into 5 mL EDTA blood vacutainers with a gel separator (BD, Franklin Lakes, NJ), centrifuged at 950 x g for 10 minutes to separate plasma and frozen at -80°C on the gel separator until samples were returned to Johnson Space Center in Houston, TX for analysis.

Plasma was thawed, aliquots were made, and samples were analyzed using a MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel Premixed 30 Plex Multiplex assay (EMD Millipore, Burlington, MA) according to the manufacturer's instructions. The plasma concentrations of 30 cytokines and chemokines were measured. Data are presented as pg/mL to show cytokine concentrations present in the plasma.

Saliva Cytokines, Viruses, and Cortisol

Saliva was collected from subjects for four consecutive days surrounding the date of the blood draw at each timepoint. For diurnal consistency, samples were collected in the morning, just after waking and before eating and brushing their teeth. Passive drool was collected into saliva collection tubes (Salimetrics, State College, PA) and frozen at -80°C until they were returned to Johnson Space Center for analysis.

For cytokine analysis, after thawing, a protease inhibitor (Sigma, St. Louis, Mo) was added to the saliva samples. Samples were vortexed and centrifuged at 10,000 x g for 10 minutes at which point the supernatant was removed and diluted 1:1 with assay buffer. The diluted samples were analyzed using a MILLIPLEX MAP Human High Sensitivity T Cell Panel Premixed 13-plex multiplex assay (EMD Millipore, Burlington, MA) according to the manufacturer's instructions. The 13 cytokines analyzed are: IL-1β, TNF-α, IL-6, IL-8, IL-2, IFN-γ, IL-4, IL-5, IL-10, GM-CSF, IL-7, IL-12 (p40/p70), and IL-13. Samples were analyzed on a Luminex Magpix instrument to determine sample concentrations. Data are presented as pg/mL to show cytokine concentrations present.

Saliva analysis of EBV, HSV1, and VZV was performed as previously described **(Mehta 2014)**. Briefly, about 1mL raw saliva was thawed, vortexed, and centrifuged at 14,000 rpm for 20 minutes. About 800µL of supernatant was removed and saved for salivary cortisol analysis (see below). The remaining 200µL (cell pellet) was DNA extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany; Cat. No. 51106), per manufacturer's instructions. Viral loads (copies/mL saliva) for each virus for each sample were determined by standard curve analysis using the QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA). Viral load data is represented as an average of the 4 samples taken per timepoint. Viral copy numbers were normalized to 1mL saliva. Primers and probes for each virus have been previously published **(Mehta 2014, Mehta 2022)**.

Analysis of salivary cortisol was performed using Salimetrics Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (State College, PA; Cat. No. 1-3002) per manufacturer's instructions. In brief, 25µL of saliva supernatant from the above were applied to 96-well plates in duplicate provided by the manufacturer. Sample absorption was read at 450nm on a SpectraMax 190 (Molecular Devices, San Jose, CA) and the cortisol value (µg/dL) was determined by standard curve using a 4-parameter curve fit from standards of known concentration. Cortisol data is represented as an average of the 4 samples taken per timepoint.

Stress Markers in Hair

Approximately 3 cm of hair was collected from behind the ear of willing participants at each sample collection timepoint. The hair was placed into foil packets with a mark indicating which side was closest to the scalp. Cortisol, cortisone, Anandamide/2-AG, and testosterone were assessed in the hair samples as previously described **(Wright 2015)**.

Health Surveys

Health surveys were provided to the subjects during each of the sample collection timepoints. These surveys were entirely voluntary and consisted of questions regarding rashes and hypersensitivities, allergies, infections, and wound healing. The voluntary health survey employed for Palmer Station was nearly identical to a survey provided to ISS astronauts. The data categories tabulated are those potentially associated with persistent immune system dysregulation. For the voluntary survey of general health at Palmer Station, all subject responses are tabulated by the same Epidemiologists who also tabulate the astronaut data. This includes two subjects who withdrew from the study while ongoing during a winterover season. For any missing or incomplete first or last dates of assessments (needed for total person-year calculations), the modes of dates were used in their place.

Statistical Methods

Measures were analyzed with generalized linear mixed models to address the repeated measures within subjects. Time categories were included as a fixed effect, and subjects and winter over groups were included as the random effects. Robust standard errors were used to address non-homogenous variance across time. Expected marginal means were used to estimated mean levels as well as conduct pairwise comparisons between each winterover timepoint and baseline with Dunnett-adjusted p-values addressing the multiple testing. For models where Dunnett approximations failed, simulation adjusted p-values were used instead. Analyses were run in SAS v9.4 with the GLIMMIX procedure.

Results

All details regarding analyses including the expected marginal mean predictions and pairwise comparison testing with baseline data are detailed in the Supplemental Table.

White Blood Cell Differential

Although largely unchanged, some significant alterations were found in the basic leukocyte subsets throughout the winterover compared to baseline. Eosinophil percentage was markedly decreased at the WO4 time point, $p=0.02$ **(FIGURE 2)**. Additionally, significant increases at the WO2 and WO4 month timepoints compared to baseline in absolute monocyte counts (10^9/L) was observed ($p=0.03$, $p=0.02$, respectively), while Monocyte percentage was increased at WO1 ($p=0.04$). White blood count, lymphocytes, and neutrophils were found to be essentially unaltered. Additionally, the neutrophil to lymphocyte ratios were calculated and those were also found to be unchanged.

Plasma Cytokines

Of the 30 cytokines analyzed in plasma, five were found to be significantly altered compared to baseline, adjusted $p<0.05$ **(FIGURE 3)**. Cytokines exhibiting a downward trend throughout the winterover included G-CSF, IL1RA, MIP-1β, and TNFα. The only cytokine to show a significant

increase, p<0.01, throughout the winterover was RANTES. Additionally, although not reaching significance, IFNγ and IP-10 exhibited substantial decreases in concentration throughout the entire winterover. Similarly, VEGF showed a substantial not significant decrease.

Saliva Cytokines, Viruses, and Cortisol

Interestingly, EBV reactivation was observed in 52-60% of the subjects tested at the various timepoints throughout the winter season compared to healthy, normal controls who exhibited reactivation in 23% of subjects **(FIGURE 4a)**. HSV1 reactivation was seen in 2 subjects at WO1 and 4 subjects at WO3 while no subjects showed reactivation during the pre-deployment sample collection **(FIGURE 4b)**. Two subjects had positive VZV samples, one of which was the pre-deployment timepoint while the other had two positive timepoints during WO2 and WO3 **(FIGURE 4c)**.

Mean cortisol concentration was significantly elevated at every timepoint throughout the winterover compared to baseline, adjusted $p < 0.01$ **(FIGURE 4d)**. Mean concentration was determined to be between 35-51% higher throughout the winterover and all but one subject displayed increased salivary cortisol during at least one time point throughout the winterover compared to pre-deployment.

Of the 13 cytokines analyzed in saliva, none were found to be significantly different from baseline **(data not shown)**.

Hair Cortisol Analysis

Analysis of these samples is in progress at the European Space Agency laboratory in Munich, Germany.

Health Surveys

Each winterover crew had 5-6 subjects that reported a history of allergies (prior to staying at station). For the first winterover crew, n=15, 5 of 15 self- reported a history of allergies. The reported sensitivities included: dust, food allergies, some environmental. Two subjects reported a previous sensitivity to mold and mildew. For the second winterover crew, n=15, 6 of 15 self- reported a history of allergies. Reported sensitivities included: mold and mildew, seasonal allergies/dust.

During overwinter, incidence of dermatitis/rashes and allergic symptoms were reported to be increased **Table 1**. During winterover at Palmer Station, eleven participants had a total of 20 rash events at various assessment periods. Two noted they were having eczema flare-ups and one specified the doctor had identified their rash as "herpes virus". Allergy symptoms were self-identified in only participants who had a history of allergies. There were no new cases of allergies identified in those who had a negative history of allergy. The most common allergies were from dust, seasonal allergies, mold and mildew. Four infections were reported and treated during the two overwinter seasons, with only one event being reported during the baseline samplings. Regarding wounds and injuries, 23% (10/44) reported through the survey, were identified as being slow healing wounds/bruises by the participants.

Discussion

Immune alterations in astronauts, reduced T and NK cell function, inflammation, etc., may be considered 'subclinical', however medical events have been reported in astronauts during spaceflight. Some of these, including infections, atypical allergy, and persistent dermatitis, may be associated with diminished immunity. This association becomes more plausible, when it is considered that astronauts are subjected to a pre-flight quarantine period, and are launched to function within a quasi- isolation chamber. Clearly the myriad unique chronic and acute stress factors are impacting the immune system of astronauts.

During Antarctica winterover, most of these mission stressors are replicated to fairly high fidelity. These include long and difficult travel, persistent isolation, base/habitat lifestyle (varies amongst the different Antarctica stations…), and extreme environment. Circadian misalignment in Antarctica is profound, with most stations experience up to 3 months of 24 hour darkness. When considered en toto, Antarctica winterover seems to replicate most spaceflight stressors sans microgravity and radiation.

NASA, and its international partners, have recently formally initiated the Artemis deep space exploration program. Artemis 1, launched in late 2022, successfully completed a full mission profile to cis-lunar space and successfully landed. We anticipate naming a human crew for Artemis 2 will occur soon. Construction of a new space station ('Gateway') that will orbit the moon is underway. These missions to deep space will increase almost all mission stressors, and profoundly reduced our ability to care for the crewmembers. Clinical risks are therefore projected to be increased, whereas habitable volume, power, will be greatly reduced. the 'rapid return' option, in the event of a medical emergency, will be lost. For this reason, the development and validation of biomedical countermeasures is essential.

Previous studies demonstrated that, immunologically, overwinter in the interior stations is confounded by persistent hypobaric hypoxia. Therefore, overwinter at a normoxic coastal station, despite the comparatively reduced stressors, is likely the best option to test NASA countermeasures. The current study, essentially a pilot, was designed to cost effectively assess the effect of overwinter at Palmer station on human immunity by returning simple biosamples for analysis.

Given the reduction in stressors compared to spaceflight and winterover in the antarctica interior, and the lack of pervasive/endemic illness, it is unsurprising that the bulk leukocyte distribution is relatively unaltered **(Figure 2)**. This does not mean that immunity is unaltered. In fact, spaceflight studies have confirmed a profound reduction in T and NK cell function in astronauts, when both the bulk and fine leukocyte distribution is minimally impacted **(Crucian 2015)**. The desired outcome is a stress-associated impact to immune function, without widespread clinical pathology.

While cellular function could not be assessed during this pilot study, plasma cytokines have shown to be an excellent indicator of compromised immunity in astronauts **(Crucian 2014, Krieger 2021)**. Astronauts display a pattern of immune/cytokine changes that is similar to that observed in zoster patients **(Makedonas 2019)**. Somewhat surprisingly, given the virus data detailed below, there were minimal plasma cytokine alterations observed in the Palmer subjects **(Figure 3)**. However, the observed alterations in inflammatory cytokines (IL-1Ra, TNF), growth factors (G-CSF), Th2 (IL-4), as well as VEGF and RANTES, do indicated an in-vivo hormonal dysregulation of immunity.

Previous data has revealed that the latent herpesviruses have a different 'stress threshold' sufficient to result in virus reactivation to detectable levels **(Cohrs 2009)**. EBV has a lower threshold, and in

astronauts even simple pre mission stress is sufficient to elevate EBV levels in astronauts **(Mehta 2017)**. Conversely, VZV has a much higher stress threshold, and has never been observed preflight in any astronaut, even though VZV reactivation has been commonly observed in astronauts during spaceflight **(Mehta 2017)**. We have suggested previously that a 'continuum' of stress exists among spaceflight, ground analogs, terrestrial situations, etc., such that measurable stress may be detected absent immune alterations. Increasing stress will further perturb immunity, and severe stress will eventually result in clinical manifestations **(Krieger 2019)**.

For the Palmer subjects, reactivation of EBV at baseline, and during winterover, appeared to be elevated compared to our database of other healthy subject conditions. It seems clear that the elevation in EBV at baseline resulted from the compromised stress conditions of travel and isolation, as well as having literally been sampled *during* transit to Palmer. This is absolutely not the definition of a pre mission baseline sample… however due to COVID-19 it was the only baseline sample available. COVID effects on NSF support of Antarctica bases was very similar to the impact on NASA supporting astronauts returning from ISS **(Makedonas 2020)**. We have therefore presented the virus reactivation findings as also compared to our database 'normal range'. When examined compared to 'normal', both incidence and shedding levels, of EBV are elevated during the winterover period **(Figure 4)**. Further, we have observed HSV reactivation in 3 subjects during the overwinter, and VZV reactivation (which is rarely observed in healthy normal (Kennedy 2015)) in two overwinter subjects.

The observed increase in saliva cortisol, especially considering that the baseline sampling occurred during stressful conditions (travel/isolation) confirms overwinter stress among the subjects. This is noteworthy and fits the paradigm of stress leading to immune dysregulation, leading to latent virus reactivation.

The results from the clinical survey are very revealing. Rashes/dermatitis, allergies, and even infection are elevated compared to baseline, and what would be expected for isolated individuals. Dermatitis is a consistently-reported issue for some astronauts during spaceflight **(Crucian 2016a; Crucian 2016b)**. Recently, in a single astronaut case study HSV reactivation was correlated with dermatitis during spaceflight **(Mehta 2022)**. This suggests that in astronauts, at least some dermatitis events may have an immune/viral etiology. That incidence of skin rash in Palmer subjects was elevated (2x-3x among the four reporting periods) reveals the possibility of a similar clinical model. Alternatively, there may be something unique in the Palmer environment, or skin care regimen, that may be influencing dermatitis events. For at least one subject, the base physician identified the skin rash as 'herpes virus'.

The authors were surprised to see the overwinter incidence of reported 'allergy' symptoms. Atypical allergy is also reported in some astronauts during flight, also in an environment suggestive that allergy events should be reduced (less pollen, etc.). However, coastal bases may experience more humidity than bases at altitude in central Antarctica. There was at least 1 statement by one of the participants that indicated mold and mildew are not absent, and suggested those locations may be associated with coughing, itchy skin.

In summary, the data in concern are suggestive that overwinter at Palmer station, Antarctica, is a terrestrial analog for spaceflight associated immune system dysregulation. Certainly, the stressors, and resulting measurable outcomes, are milder than spaceflight. The pattern of alterations, particularly the elevation in cortisol and latent virus reactivation, is strongly suggestive that the basic paradigm of

stress>immune dysregulation>virus reactivation/clinical event indeed occurs at Palmer. Therefore, Palmer should be appropriate, and 'stressful enough' to serve as a platform to validate planned biomedical countermeasures for deep space missions. The follow up countermeasure study has been identified and selected by NASA, and will deploy during the 2023, 2024 winterover seasons. If successful, an ISS validation is possible… leading to an operation immune countermeasure designed to protect astronauts at the moon, or on the way to Mars.

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Figures/Tables

Table 1: Frequency Distribution Among Immune Type Events by Self-Reported Survey Assessment Period with Calculated Incidence Rates

	Pre-Deploy	WO1	WO2	WO3	WO4	Total Frequency	Person-Years	Incidence/Year
Rashes	2	4	4	4	6	20	10.6	1.8868
Allergies	2	5	5	5	4	21	10.6	1.9811
Infection	1	1	0	2	1	5	10.6	0.4717
Slower Wound Healing	1	4	2	2	1	10	10.6	0.9434
Total	6	14	11	13	12	56	10.6	5.283

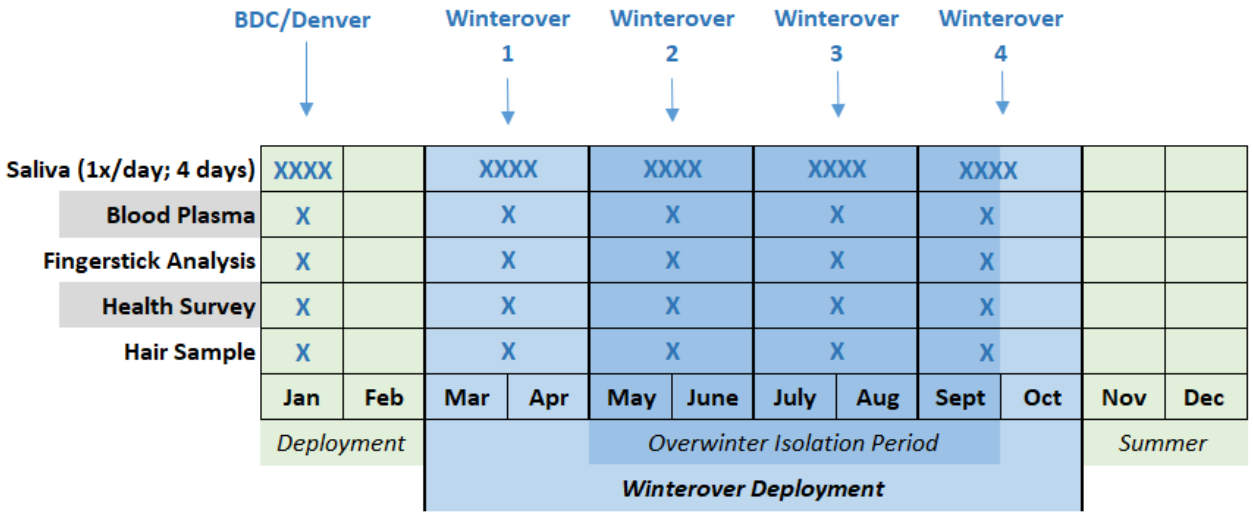

Figure 1: Sampling matrix versus mission phase/date for the Palmer Antarctica pilot study.

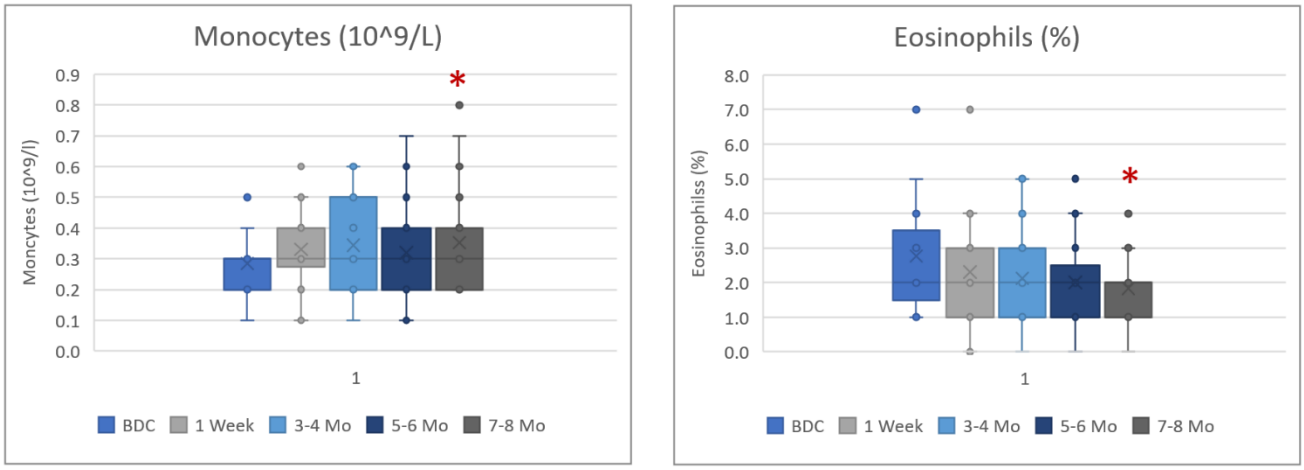

Figure 2: Concentration and relative percentage of A) monocytes and B) eosinophils, respectively, before deployment and at four time points throughout the winterover along with overlayed boxplots. Significance was evaluated via a mixed effects model comparing other time points to the pre-deployment baseline time point. Significant differences (adjusted $P \leq 0.05$) are indicated (*). All estimates and pairwise comparison details can be found in the Supplemental Table.

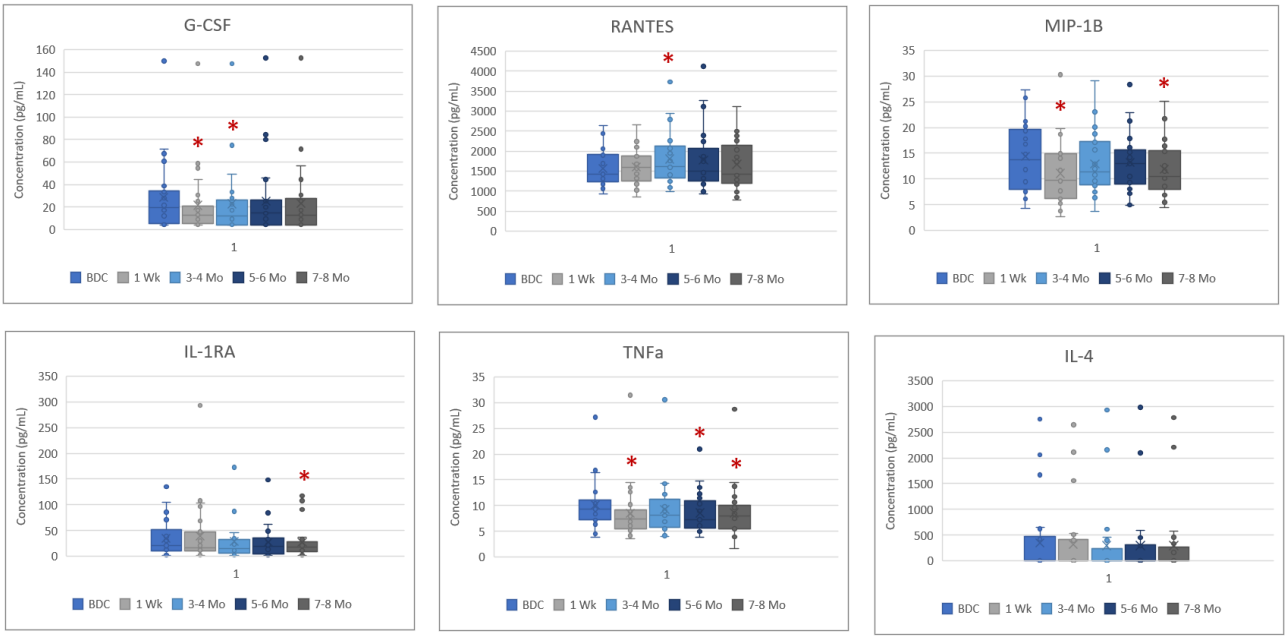

Figure 3: Concentrations of plasma A) G-CSF, B) VEGF, C) IL-1RA, D) TNFα, E) IL-4, and F) RANTES before deployment and at four time points throughout the winterover along with overlayed boxplots. Significance was evaluated via a mixed effects model comparing other time points to the pre-deployment baseline time point. Significant differences (adjusted P≤ 0.05) are indicated (*). All estimates and pairwise comparison details can be found in the Supplemental Table.

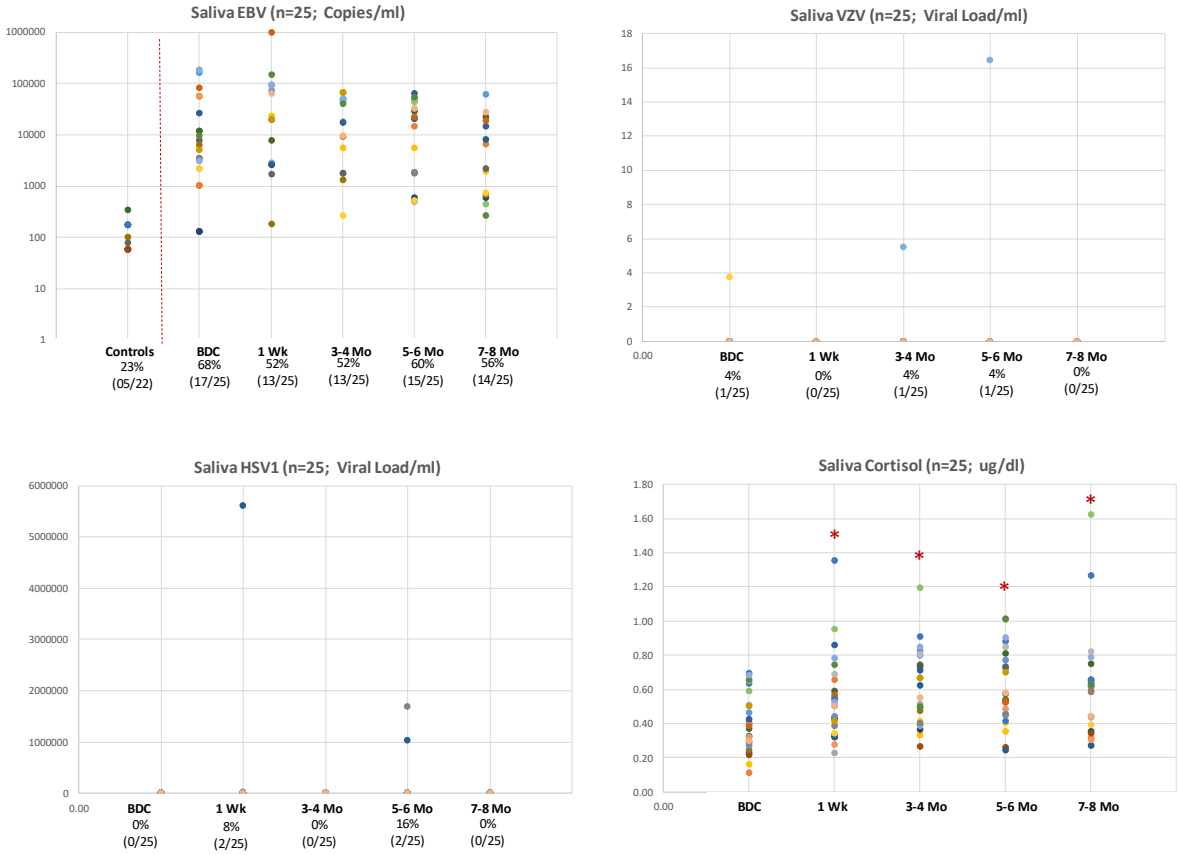

Figure 4: Concentration of salivary A) EBV, B) HSV1, C) VZV, and D) Cortisol before deployment and at four time points throughout the winterover. Saliva EBV graph also contains normal, healthy controls.