Acta Astronautica 146 (2018) 111–116

Contents lists available at ScienceDirect

Acta Astronautica

journal homepage: www.elsevier.com/locate/actaastro

Latent viral reactivation is associated with changes in plasma antimicrobial protein concentrations during long-duration spaceflight

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ARTICLE INFO

Keywords:
Innate immunity during spaceflight
Antimicrobial proteins
Lysozyme
LL-37
HNP1-3
Herpes viral reactivation

ABSTRACT

Long duration spaceflights are associated with profound dysregulation of the immune system and latent viral reactivations. However, little is known on the impact of long duration spaceflight on innate immunity which raises concerns on crewmembers' ability to fight infections during a mission. The aim of this study was to determine the effects of spaceflight on plasma antimicrobial proteins (AMPs) and how these changes impact latent herpesvirus reactivations. Plasma, saliva and urine samples were obtained from 23 crewmembers before, during and after a 6-month mission on the International Space Station (ISS). Plasma AMP concentrations were determined by ELISA, and saliva Epstein-Barr virus (EBV) and varicella zoster virus (VZV) and urine cytomegalovirus (CMV) DNA levels were quantified by Real-Time PCR. There was a non-significant increase in plasma HNP1-3 and LL-37 during the early and middle stages of the missions, which was significantly associated with changes in viral DNA during and after spaceflight. Plasma HNP1-3 and Lysozyme increased at the late mission stages in astronauts who had exhibited EBV and VZV reactivations during the early flight stages. Following return to Earth and during recovery, HNP1-3 and lysozyme concentrations were associated with EBV and VZV viral DNA levels, reducing the magnitude of viral reactivation. Reductions in plasma LL-37 upon return were associated with greater CMV reactivation. This study shows that biomarkers of innate immunity appeared to be partially restored after 6-months in space and suggests that following adaptation to the space environment, plasma HNP1-3 and lysozyme facilitate the control of EBV and VZV reactivation rate and magnitude in space and upon return on earth. However, the landing-associated decline in plasma LL-37 may enhance the rate of CMV reactivation in astronauts following spaceflight, potentially compromising crewmember health after landing.

1. Introduction

Astronauts are exposed to myriad of stressful stimuli during long duration spaceflight missions, including microgravity, cosmic rays, sleep deprivation, circadian rhythm disruption, and the psychological stressors associated with isolation, confinement and endangerment [1,2]. Prolonged exposure to these stressors has been purported to cause immune system dysregulation and latent viral reactivation [3], which could jeopardize mission objectives and compromise crew safety [4]. Unfortunately, comprehensive studies on immune system responses *during* long-duration spaceflight missions are lacking, due mostly to technical and logistical constraints associated with obtaining biological samples on orbit and returning them to Earth for analysis. While several studies have examined immune system changes before and after spaceflight, interpretation of these data are confounded by the transient changes in immune function that occur due to the acute biological stress response that accompanies landing and re-adaptation to the 1G environment. In addition, most of the spaceflight immunology research has focused on adaptive immunity [5–8], but very few studies have made a comprehensive attempt to characterize innate immune responses during or following long duration missions.

Antimicrobial proteins (AMPs) are highly conserved polypeptides

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https://doi.org/10.1016/j.actaastro.2018.02.039
Received 28 October 2017; Received in revised form 4 February 2018; Accepted 23 February 2018
Available online 2 March 2018

with antibacterial, antifungal and/or antiviral properties and play a central role in innate immune responses. AMPs are essentially secreted by macrophages and neutrophils [9] as one of the first lines of defense against a variety of microorganism and pathogens. In particular, lysozyme and the cathelicidin LL-37 exhibit a broad spectrum of antimicrobial activity against bacteria, fungi, and in the case of LL-37 microbicidal activity against viral pathogens [10]. Other AMPs appear to have a narrower antimicrobial action, with the alpha-defensins HNP1-3 essentially protecting the host against enveloped viruses [11]. In addition, AMPs have been shown to exert strong chemotactic effects on adaptive immune cells [12], further amplifying and optimizing the immune response to pathogenic challenges. AMPs are constitutively expressed in healthy individuals, but are rapidly secreted in response to pro-inflammatory stimuli and viral challenge [9]. In this context, AMPs have been shown to possess strong antiviral properties against herpesviruses such CMV [13,14] and EBV [15], viruses known to reactivate during and after long duration spaceflight [16–18]. A recent study indicates that all astronauts who flew in the International Space Station (ISS) have been infected with EBV [19], and that a majority of them exhibited live viral shedding at some point during their missions [19]. Although most herpesvirus reactivations remain asymptomatic in immunocompetent hosts [20], they can rapidly develop into debilitating and even life-threatening conditions in individuals with various degrees of immunosuppression [21]. Impaired adaptive immune function during long duration spaceflight has been hypothesized to be responsible for the enhanced latent viral reactivation observed in astronauts [3,7,22], however little is known on the effect of sustained psychological and physiological stressors on plasma LL-37, HNP1-3 and lysozyme concentrations, along with their impact on the rate and magnitude of latent viral reactivation. Indeed, increased LL-37 secretion on earth has been shown to reduce the risk of viral infections [23,24], and consequently it can be postulated that reductions in plasma AMPs would expose astronauts to increased risks of infections and viral reactivations, which may in turn endanger mission objectives and crew safety.

The aim of this study was to determine, for the first time, the impact of a long duration spaceflight mission on the concentration of the AMPs LL-37, lysozyme, and HNP1-3 in plasma. We also examined the relationship between plasma AMPs and previously analyzed CMV, EBV and VZV DNA levels [19], indicative of latent viral reactivations. We hypothesized that spaceflight would be associated with reductions in plasma AMPs, which in turn would be associated with increased herpesvirus reactivations.

2. Methods

2.1. Participants

Twenty-three astronauts (18 M, 5 F) ranging in age from 43.1 to 59.8 years old (mean = 52.1 ± 3.8 yrs) participated in this study. The astronauts were affiliated with NASA, ESA, JAXA or CSA and participated in a 6-month mission to the ISS. Data were collected over 12 separate ISS missions. The Committees for the Protection of Human Subjects (CPHS) at the University of Houston and at NASA JSC approved the study, and informed consent was obtained from all subjects.

2.2. Sample collection

Intravenous blood samples were collected from an antecubital vein into a 10 ml Vacutainer® (containing a solution of Acid Citrate Dextrose (ACD)) before launch (180 and 45 days before launch), in flight (Early flight: Flight Day 10; Middle flight: Flight Day 90; Late flight: 1 Day before return on earth), immediately upon return to earth (Landing) and finally 30 days following return from the ISS (Recovery) (Fig. 1.). Upon arrival to the laboratory, the plasma was separated using centrifugation and stored at −80 °C until analysis. Pre and post-flight samples were processed immediately following collection. Although plasma AMPs and Albumin concentrations were found to be stable at ambient temperature up to 96 h (data not shown), all samples collected on the ISS were returned to the laboratory at ambient temperature and processed within 36–48 h of collection.

The plasma AMP concentrations in the two samples collected 180 days and 45 days before launch from each astronaut were averaged and combined under a single pre-flight measure to create a more robust baseline value.

Urine and saliva samples were collected at the same time as the blood samples to determine CMV (urine), EBV (saliva), and VZV (saliva) viral load and previously published [19]. In brief, aliquots of urine were sampled from 24-h urine pooled at each timepoint, and fasting saliva samples were collected using sterile Salivette cotton rolls (Sardstedt, Newton, NC) immediately upon awakening. The urine samples were frozen until return to Earth, while the salivettes were stored in stability buffer (0.5% SDS, 10 mM Tris-Cl and 1 mM EDTA) at room temperature for up to 2 weeks before return to Earth for subsequent analysis [18]. Viral DNA was extracted and quantified by Dr. Mehta at NASA JSC by polymerase chain reaction as previously described [19,25].

2.3. Serologic testing for AMPs and albumin concentrations

Plasma samples were analyzed in duplicate for LL-37, HNP1-3 and lysozyme by ELISA in accordance with the manufacturer's instructions (Hycult Biotech, Uden, The Netherlands and Abcam, MA, USA). Due to the unique technical challenges associated with the microgravity environment, anecdotic reports from previous spaceflight experiments have highlighted great variabilities in blood volumes drawn using commercially available vacutainers. As a result, blood samples are at risk of being inconsistently diluted by the Acid citrate dextrose solution present in the collection tube, leading to artificial changes in AMP concentrations. The potential for such inaccuracies to occur was controlled using plasma albumin concentration. Indeed plasma albumin concentration is known to be stable during and after spaceflight [26,27], and was consequently measured to adjust for differences in plasma AMPs dilution by ELISA determination (Abcam, MA, USA). Samples were read on a

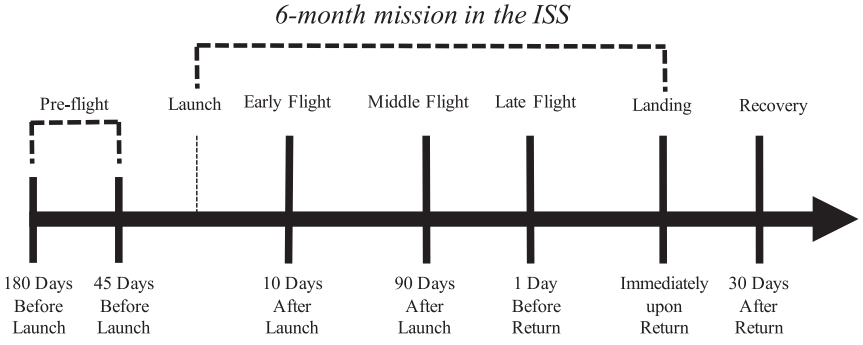

Fig. 1. Plasma and Saliva samples were collected during the study at 7 different occasions throughout the mission. Baseline values (Pre-flight) were calculated by averaging plasma AMPs concentration and viral DNA levels at 180 days and 45 days before launch. In-flight samples were drawn 10 days after launch (Early), 90 days after launch (Middle flight) and 1 day prior to return on Earth (Late flight). Finally return samples were taken immediately upon return and 30 days after return on earth (Recovery).