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Reactivation of latent viruses is associated with increased plasma cytokines in astronauts

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ABSTRACT

Success of long duration space missions will depend upon robust immunity. Decreased immunity has been observed in astronauts during short duration missions, as evident by the reactivation of latent herpes viruses. Seventeen astronauts were studied for reactivation and shedding of latent herpes viruses before, during, and after 9–14 days of 8 spaceflights. Blood, urine, and saliva samples were collected 10 days before the flight (L-10), during the flight (saliva only), 2–3 h after landing (R + 0), 3 days after landing (R + 3), and 120 days after landing (R + 120). Values at R + 120 were used as baseline levels. No shedding of viruses occurred before flight, but 9 of the 17 (designated "virus shedders") shed at least one or more viruses during and after flight. The remaining 8 astronauts did not shed any of the 3 target viruses (non-virus shedders). Virus-shedders showed elevations in 10 plasma cytokines (IL-1α, IL-6, IL-8, IFNγ, IL-4, IL-10, IL-12, IL-13, eotaxin, and IP-10) at R + 0 over baseline values. Only IL-4 and IP-10 were elevated in plasma of non-virus shedders. In virus shedders, plasma IL-4 (a Th2 cytokine) was elevated 21-fold at R + 0, whereas IFNγ (a Th1 cytokine) was elevated only 2-fold indicating a Th2 shift. The inflammatory cytokine IL-6 was elevated 33-fold at R + 0. In non-shedding astronauts at R + 0, only IL-4 and IP-10 levels were elevated over baseline values. Elevated cytokines began returning to normal by R + 3, and by R + 120 all except IL-4 had returned to baseline values. These data show an association between elevated plasma cytokines and increased viral reactivation in astronauts.

1. Introduction

Spaceflight is a uniquely stressful environment [1]. Stressors that astronauts experience may be constant or intermittent and may include some or all of the following: isolation, confinement, variable gravitational forces, increased radiation, psychosocial stressors, sleep deprivation, noise, physical exertion, deconditioning, anxiety, and others, all of which may contribute to diminished immunity during spaceflight. Although immune system dysregulation has been documented during and after spaceflight [2–5], it is not known if these changes increase infection susceptibility or pose a significant health risk to astronauts. However, it is important to identify potential clinical risks due to altered immunity in space so that appropriate countermeasures can be put in place to ensure crew safety during exploration-class missions (i.e. to Mars) where medical capabilities will be limited and casualty return options unlikely or even impossible.

The logistical constraints of spaceflight have hampered comprehensive immune studies of astronauts during the flight phase. For many years, conclusions of immune status have been largely based on measurements taken before and after spaceflight and not during the flight phase itself, thus making it difficult to ascertain the effects of spaceflight independently from the stressors associated with landing and re-adaptation to the 1G environment. More recently, saliva sampling on orbit has made it possible to document latent viral reactivation as marker of systemic immune function during the flight phase [6,7]. Maintenance of latency is dependent upon a robust immune system, and during periods of reduced immunity, latent herpes viruses reactivate and shed in body fluids. Epstein-Barr virus (EBV), varicella-zoster virus (VZV) [8], and some other herpes viruses are released into saliva upon reactivation, while cytomegalovirus (CMV) is shed in urine following reactivation [9]. Reactivation of EBV, CMV, and VZV has been documented in astronauts during spaceflight [6–10]. Reactivation of these viruses has been largely asymptomatic, but clinical symptoms have resulted from VZV and herpes simplex virus type 1 reactivation. Increases in viral reactivation that are similar to spaceflight but smaller in magnitude have also been described in Antarctic expeditioners, aquanauts in an undersea habitat, and in other

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ground-based spaceflight analogs [11,12]. Moreover, because latent viral reactivation has been shown to occur prior to spaceflight, it is likely that increased hypothalamic–pituitary–adrenocortical (HPA)-axis and the sympathetic nervous system (SNS) activity, and the resulting release of catecholamines and cortisol, compromise immune elements responsible for maintaining herpes viruses in a latent state [6].

Cytokines are cell-signaling protein molecules that are critical to the development of both pro- and anti-inflammatory immune responses following infection or injury. Th1 immune responses involve the action of cytokines such as IL-2, IFNγ, IL-12, and IL-17 that mostly stimulate cellular immunity and the involvement of CD4+ and CD8+ T-cells. Cytokines such as IL-4, IL-5, IL-10, and IL-13 are mostly responsible for stimulating Th2 humoral immune responses and the activation of antibody producing effector B-cells. Blood T-cells stimulated after spaceflight were found to have a lower IFNγ:IL10 secretion ratio compared to pre-flight samples [13] indicating that spaceflight is associated with a Th2 cytokine shift. However, it is not known if this cytokine shift compromises astronaut immunity or if it is associated with latent viral reactivation during flight.

The aim of this study was to determine if plasma cytokine shifts in response to spaceflight are associated with shedding of one or more latent herpes viruses (EBV, CMV or VZV) in astronauts. A multiplex assay that simultaneously examined up to 21 cytokines was used to measure plasma cytokine levels in astronauts before and after spaceflight. Reactivation of three latent viruses was determined by the polymerase chain reaction in saliva collected before, during and after flight or in urine collected before and after flight. Plasma cytokine levels in non-virus-shedding astronauts were compared to astronauts shedding reactivated latent viruses. We hypothesized that plasma cytokine responses to spaceflight would be associated with latent viral reactivation in astronauts.

2. Methods

2.1. Subjects

Seventeen astronauts (14 M, 3F) ranging in age from 36–59 and 10 age-matched healthy control subjects (6 M, 4F) were included in the study. The astronauts participated on a Space Shuttle mission of 9–14 days' duration. Eight different shuttle missions were utilized to acquire the 17 participants. Ten healthy, age- and gender-matched subjects from the NASA Test Subject Facility were selected for "controls." All human study protocols used were approved by the Committee for the Protection of Human Subjects of the Johnson Space Center, Houston, TX, and informed consent was obtained from all subjects. All subjects included in the study were seropositive for EBV, VZV, and CMV.

2.2. Saliva

Saliva samples were collected from each astronaut, before, during, and after spaceflight, using cotton rolls (Salivette kits, Sarstedt Inc., Newton, NC). Preflight samples were collected on arising every other day for 1 month, beginning about 3–4 months before launch. Daily in-flight saliva samples were collected throughout the mission. Post flight samples were collected on landing day and then daily for 15 days, and a sample was also collected 120 days after flight. Saliva samples were collected every other day for 4 weeks from 10 healthy control subjects. Salivary DNA was extracted and amplified for EBV or VZV using specific probes and primers [8,9,12].

Urine samples were collected from astronauts 10 days before flight, 2–3 h after landing, and 3 days after landing. Three urine samples were also included from each of the 10 healthy controls identical to the astronauts and matching with their blood draws as explained below. CMV was measured in these samples as described by Mehta et al. [9].

2.3. Blood

For astronauts, a 10 ml blood sample was collected in an ethylenediaminetetraacetic acid Vacutainer® 10 days before the flight (L-10), 2–3 h after landing (R + 0), 3 days after landing (R + 3), and 120 days after flight (R + 120). The R + 120 time point was used to establish their baseline plasma cytokine levels. Blood samples from control subjects were obtained identically to those from astronauts, 3 times during simulated 12 day spaceflights (day 0 = L-10, day 22 = R + 0, and day 25 = R + 3). These subjects participated throughout the 2-year period when actual missions were being flown. After blood collection, plasma was separated by centrifugation and stored at −70 °C until it was processed. All samples collected from actual or simulated missions were analyzed within 90 days after the end of the mission.

2.4. Cytokine measurements

Twenty-one cytokines were measured using the human cytokine premixed LINCOplex Kit (HYCTO-60K-PMX) and the Luminex 100TM using the Luminex microbead technology. The kit contains 21 distinct fluorescent bead populations that all fluoresce differently along two color-channels. The instrument is able to resolve the 21 bead populations based on their distinct fluorescence patterns. Each bead population is coated with non-fluorescent 'capture' antibodies to the cytokines, one bead group per cytokine. Following sample incubation, detection is realized by a fluorescently labeled cytokine-specific 'detection' antibody. The detection antibodies all commonly fluoresce along a third color wavelength. Therefore, cytokine concentration for all individual cytokines is plotted along a single third color and derived as mean fluorescence intensity. Standards for each cytokine, and sera for controls, as well as positive and negative control samples are supplied by the manufacturer. A 96-well format was used with all standards run in duplicate and all samples in triplicate, following the kit instructions. Sample fluorescence was noted as the median fluorescence per sample with a minimum of 100 beads per sample, following flow cytometric methods. The data were analyzed using the MasterPlex QT Quantitation software from MiraiBio Inc., CA.

2.5. Statistical analysis

Astronauts were classified as "virus shedders" ($N = 9$) or "non-virus shedders" ($N = 8$) using criteria described in the Results section. Using these same criteria, all 10 healthy controls were classified as "non-shedders." In addition, we compared non-shedding astronauts with healthy controls (all non-shedding). Statistical analysis was performed using SigmaStat 2.03 (Systat Software Inc., San Jose, CA). The Student's t test was used for paired (intra-group) data. Statistical significance was determined by analyzing changes over time compared with the control group. A difference between means was considered significant if $p < .05$, which is indicated by asterisks on each data figure.

3. Results

3.1. Latent virus reactivation

Nine of 17 astronauts ("virus shedders") shed one or more of the target viruses EBV, VZV, or CMV in their saliva (EBV and VZV) or urine (CMV) samples during, or after spaceflight (Table 1). In