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Epstein–Barr virus shedding by astronauts during space flight☆

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Abstract

Patterns of Epstein–Barr virus (EBV) reactivation in 32 astronauts and 18 healthy age-matched control subjects were characterized by quantifying EBV shedding. Saliva samples were collected from astronauts before, during, and after 10 space shuttle missions of 5–14 days duration. At one time point or another, EBV was detected in saliva from each of the astronauts. Of 1398 saliva specimens from 32 astronauts, polymerase chain reaction analysis showed that 314 (23%) were positive for EBV DNA. Examination by flight phase showed that 29% of the saliva specimens collected from 28 astronauts before flight were positive for EBV DNA, as were 16% of those collected from 25 astronauts during flight and 16% of those collected after flight from 23 astronauts. The mean number of EBV copies from samples taken during the flights was 417 per mL, significantly greater ($p < .05$) than the number of viral copies from the preflight (40) and postflight (44) phases. In contrast, the control subjects shed EBV DNA with a frequency of 3.7% and mean number of EBV copies of 40 per mL of saliva. Ten days before flight and on landing day, titers of antibody to EBV viral capsid antigen were significantly ($p < .05$) greater than baseline levels. On landing day, urinary levels of cortisol and catecholamines were greater than their preflight values. In a limited study ($n = 5$), plasma levels of substance P and other neuropeptides were also greater on landing day. Increases in the number of viral copies and in the amount of EBV-specific antibody were consistent with EBV reactivation before, during, and after space flight.

Keywords: Antibody; DNA; Viral; Herpesvirus; Epstein–Barr virus; Polymerase chain reaction; Stress; Weightlessness

1. Introduction

Space flight is a uniquely stressful environment for humans. Astronauts experience isolation, confinement, anxiety, psychosocial stressors, sleep deprivation, increased radiation, and microgravity. These stressors may be intermittent or constant. Although other stress models (such as law enforcement officers or military personnel) experience some of these stressors, the addition of microgravity and increased radiation make space flight an environment not duplicated anywhere on Earth. Glaser et al. (1991) have shown that stress is associated with the onset, duration, and intensity of herpesvirus reactivation. Increased levels of neuropeptide Y have been found in subjects with chronic stress (Irwin et al., 1991), and an increase in neuropeptides has been associated with alterations in T-cell differentiation and the release of cytokines that lead to decreased cell-mediated immunity (Levite, 1998).

We have previously shown that shedding frequencies of Epstein–Barr virus (EBV), cytomegalovirus (CMV), and varicella-zoster virus (VZV) increase in astronauts participating in space shuttle missions (Mehta et al.,

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2000b, 2004; Payne et al., 1999). We undertook the present study to quantify EBV DNA and virus-specific antibodies before, during, and after space shuttle missions. This was done by determining the number of copies of EBV DNA shed in saliva by astronauts and control subjects, and by measuring the titer of antibodies specific to EBV antigens. The association between the number of copies of viral DNA and shedding frequency was also analyzed, and the association between viral reactivation and stress was examined by determining stress hormones in blood and urine samples. Neuropeptides were measured in a small number of blood samples to see if these hormones, which are related to stress and immune system function, were affected by space flight.

2. Methods

2.1. Subjects

All human study protocols 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. Thirty-two astronauts (24 men aged 37–57 years, mean = 44 years; 8 women aged 32–47 years, mean = 39 years) participated in the study. These astronauts were on 10 space shuttle missions of 5–14 days duration, flown during a 6-year period. All 32 randomly selected shuttle astronaut subjects were EBV seropositive. Also included in this study were 2 Russian cosmonauts participating in an 83-day mission aboard the Russian space station Mir. To see if significant changes occurred in viral DNA, antibody titers, or hormone levels of normal healthy subjects over a period of 25 days, and to compare astronaut baseline (annual medical examination) data with data from non-astronauts who had a low level of stress, a control group was included in the study. The group consisted of 18 healthy age-matched adults (14 men, 4 women), all of whom were EBV-seropositive. They were included in the study at the same time astronauts were being tested.

2.2. Sample collection

Shedding of EBV was investigated by testing saliva samples for EBV DNA. Preflight saliva specimens were collected from all astronauts every other day. Sample collection began at 6 months before launch and continued for 1–2 months. In-flight saliva samples were collected from crew members every day after their sleep period. Postflight samples were collected every day from landing day (R + 0) to as many as 45 days after landing (R + 45).

The 2 Mir cosmonauts collected saliva samples before flight (between 250 and 200 days before launch, and between 40 and 20 days before launch), several times during the 83-day flight, and once after landing, between R + 3 and R + 22.

Saliva samples were collected with Salivette kits (Sarstedt, Newton, NC), which consist of a cotton roll in a polypropylene vial. To collect a sample, a subject placed a roll in his or her mouth until it became saturated, and then returned the roll to the vial. A stability storage buffer (1.0 mL of 1% SDS, 10 mM Tris–Cl, and 1 mM EDTA) was then added to the vial and the sample was stored at room temperature during the mission.

For measurement of serum titers of antibody to EBV and plasma concentrations of selected hormones, a 10 mL EDTA blood sample was collected from each of the 32 shuttle crew members 10 days before launch (L − 10), 2–3 h after landing (R + 0), and 3 days after landing (R + 3). Baseline titers of antibodies to EBV-VCA were determined in samples archived from the annual physical examinations of the astronauts (5–24 months before flight). Plasma samples were also used for measuring neuropeptides; they were collected on days L − 10, R + 0, and R + 3) from 5 astronauts on 1 of the shuttle flights. At each of the same time points, urine was collected for 24 h from all 32 astronauts, for measurement of urinary concentrations of stress hormones.

The 25-day sample collection schedule for control subjects simulated the sample collection schedule for a 12-day space flight (day 0 = L − 10, day 22 = R + 0, and day 25 = R + 3). Samples were collected from all 18 control subjects at these three time points during the 6-year period when missions were being flown. A saliva sample, a urine sample (10 mL from a 24 h pool), and a blood sample (10 mL, EDTA) were collected at each time point.

2.3. Sample processing

Upon return of in-flight saliva samples to Earth, they were centrifuged to separate the fluid from the cotton, and the supernatant was stored frozen (−70 °C) until it was processed further. All samples collected from a given mission were analyzed simultaneously. Ground-based analysis verified that the stability buffer could preserve EBV DNA for subsequent polymerase chain reaction (PCR) analysis (Payne et al., 1999). Plasma was separated by centrifugation and stored at −70 °C until it was processed. Saliva, plasma, and urine samples from control subjects and saliva samples from Mir cosmonauts were processed in the same way as the same type of sample from shuttle crew members.

2.4. Detection of EBV DNA

Saliva samples were concentrated with a 100 K filtration unit (Filtron Technology, Northborough, MA) and

extracted by a non-organic extraction method (Qiagen, Chatsworth, CA). EBV was detected with Digene Diagnostics, Gaithersburg, MD, as described previously (Payne et al., 1999). The primers used match sequences of the EBV gene encoding the EBV DNA polymerase accessory protein, BMRF1 (Saito et al., 1989). The sequence of primer P1 was 5′,3′-GTCCAAGAGCCACC ACACCTG (Midland Certified Reagent); primer P2 was 5′,3′-biotin-CCCAGAAGTATACGTGGTGACG TAGA (Digene Diagnostics).

2.5. Quantitative estimation of EBV

The number of copies of EBV DNA was measured in EBV-positive samples by using the Viral Quant EBV quantitative PCR detection kit (Biosource International, Camarillo, CA). A known number of copies of exogenously synthesized DNA internal calibration standard (ICS) were mixed with sample DNA before it was extracted and amplified. The ICS had been constructed to contain a PCR primer-binding site identical to that of EBV DNA, and a unique capture-binding site that allowed the resulting ICS amplicon to be distinguished from the viral amplicon. EBV DNA was amplified with Viral Quant format primers, one of which is biotinylated. These primers target a conserved sequence of EBER 1. The EBER 1 gene is expressed during EBV latency as a small, non-polyadenylated RNA transcribed by RNA polymerase III. Sequences recognized by the two EBV-specific amplification primers are identical for the type 1 and type 2 strains of EBV. After PCR was performed, the amplicons were denatured and hybridized to either ICS or EBV sequence-specific capture oligonucleotides. Details are given elsewhere (Lin et al., 1993).

2.6. Measurement of EBV antibody titer

The titers of IgG antibodies to EBV viral capsid antigen (VCA) and early antigen (EA) were determined by indirect immunofluorescence assay in plasma samples. Substrate slides prepared commercially by standard methods (Lennette, 1995) and control sera were used for determining EBV IgG and measles IgG antibody titers (Bion Enterprises, Park Ridge, IL). Twofold dilutions of plasma were prepared with PBS. The endpoint titer was determined as the highest dilution of serum giving immunofluorescent cells. All specimens were batch analyzed and read blind-coded.

2.7. Stress hormones

Cortisol, adrenocorticotropic hormone (ACTH), and human growth hormone (HGH) concentrations in plasma, and cortisol, norepinephrine, and epinephrine concentrations in urine, were measured by radioimmunoassay (Foster and Dunn, 1974; Nicholson et al., 1984; Soldin et al., 1980).

2.8. Neuropeptides

Four neuropeptides—substance P, calcitonin gene-related peptide, neuropeptide Y, and vasoactive intestinal peptide—were measured in plasma by a receptor-affinity chromatographic technique coupled with immunologic detection (Phillips, 1996).

2.9. Statistical analysis

To determine if EBV activation increased significantly during flight, we first expressed the number of EBV copies in each of a subject's in-flight samples as a difference from his or her preflight values. Next, this change in EBV copies was regressed on time in flight (days) using generalized estimating equations in a general linear model setting (Zeger et al., 1988) with a normal family. In the process, standard errors were obtained using the Huber–White "sandwich" estimator (Huber, 1967; White, 1980) to account for repeated measurements from some subjects at different times during flight; for example, 1 subject's EBV count was measured on 9 different days. After fitting the regression model, we tested the null hypothesis of no increased activation any time during flight, which would be true if both the intercept and the slope of the regression line were zero. Confidence limits for the slope and intercept were also obtained.

The statistical significance of the effect of sampling time on EBV antibody titers, hormones, and neuropeptides was determined by analysis of variance with repeated measures.

3. Results

All 32 astronauts shed EBV at least once before, during, or after flight. EBV DNA was detected in 22.5% (314/1398) of all saliva samples (Table 1). This rate was significantly higher ($p < .05$) than the 3.7% EBV-positive samples from the 18 control subjects. Of the 32 astronauts, 28 shed EBV 5–6 months before flight, 25 shed during flight, and 23 shed after space flight. Of the saliva samples collected before flight, 29.1% were positive for EBV DNA. Of the samples collected during space flight, 15.7% were positive for EBV, and of those collected after flight, 15.5% of the samples were positive. Ten out of 18 control subjects shed EBV during the study and their frequencies did not vary significantly over a 25-day period (simulating a space shuttle mission).

The distribution of EBV copies found before, during, and after flight is shown in Fig. 1. Although the

Table 1
Frequency of EBV DNA in saliva samples from 32 crew members before, during, and after spaceflight

Flight phase	No. of saliva samples	No. of samples positive for EBV DNA	Percentage of samples positive for EBV DNA
Pre	716	208	29.1
In	334	52	15.7
Post	348	54	15.5
Total	1398	314	22.5

EBV DNA was detected in 3.7% (2/54) of saliva samples from 18 age- and sex-matched control subjects.

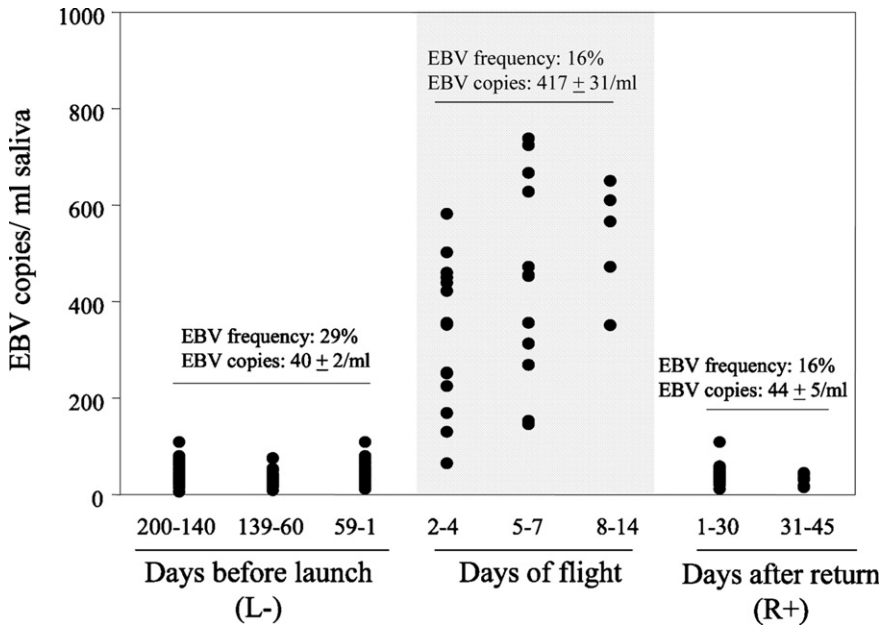

Fig. 1. Distribution of the number of EBV copies per mL of saliva in EBV-positive samples from 32 astronauts during sampling periods before, during, and after 10 space shuttle missions. Though each dot represents an EBV-positive sample, some dots overlap. Therefore, the number of dots should not be used to calculate the number of positive samples.

frequency of EBV DNA was nearly twofold greater in samples collected before flight than in those collected at other times, the number of copies of EBV DNA was significantly ($p < .05$) higher (about 10-fold) in the samples collected during flight than in those collected either before or after flight. The number of EBV copies (mean per mL saliva ± SE) was 40 ± 2 before flight (from 28 astronauts), 417 ± 31 during flight (from 25 astronauts), and 44 ± 5 after flight (from 23 astronauts). The mean number of EBV DNA copies of the 18 control subjects was 40 ± 2 per mL of saliva, similar to the number of copies in astronaut saliva samples before and after flight.

The median number of EBV copies detected in saliva samples from shuttle astronauts increased as the duration of flight increased. The estimated equation for Y (the increase in EBV count) is $Y = 24t + 215$, where t is flight time in days. The null hypothesis of no increase at any time during flight was overwhelmingly rejected ($p < .00001$). Specifically, the number of EBV copies was significantly greater during flight than before or after flight. In addition, the rate of increase in the number of EBV copies per day of flight (24 copies) was significantly greater than zero ($p = .00013$). That is, as the duration of flight increased (up to 14 days), the number of EBV copies increased significantly. The 95% confidence interval for the daily increase in EBV copies was 12 to 36.

The shuttle astronauts were on missions of 5–14 days, but 2 Russian cosmonauts participating in an 83-day mission aboard the Russian space station Mir had similar EBV shedding patterns. This included an increased number of EBV copies during the flight phase. For these cosmonauts, the mean number of EBV copies per mL of saliva was 25 ± 4 before the flight and 19 ± 7 after the flight. During the long flight, the number of EBV copies increased to 451 ± 79 (unpublished data).

Shuttle astronaut VCA antibody titers 10 days before launch (L − 10), at landing (R + 0), and 3 days after landing (R + 3) were significantly increased ($p < .001$) from the baseline values taken 5–24 months before flight (Fig. 2). At L − 10 and R + 0, titers were the same, but they increased further at R + 3 ($p < .001$). At landing, a slight increase in the titer of antibodies to EBV early antigen (EA) was observed that was not significantly different from the preflight values. EA titers were not avail-

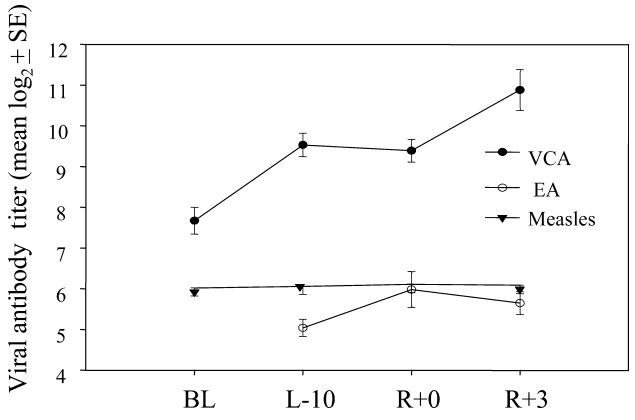

Fig. 2. Viral IgG antibody titers ($\log_2$ mean ± SE) of 32 astronauts at their annual medical examination (baseline, BL), 10 days before launch (L − 10), at landing (R + 0), and 3 days after landing (R + 3). EA titers were not available at BL.

able at the baseline. We measured measles IgG antibody titer as a control and found no change from baseline levels throughout the study interval. The VCA IgG antibody titers of 18 control subjects were similar to the astronaut baseline levels and did not change across the three sampling times (25 days).

Stress hormones were measured at three time points: 1 before launch (L − 10) and 2 after landing (R + 0 and R + 3) (Fig. 3). The levels of cortisol in plasma after landing (R + 0, 12.9 ± 1.4; R + 3, 14.7 ± 0.9) were not significantly different from the level before flight

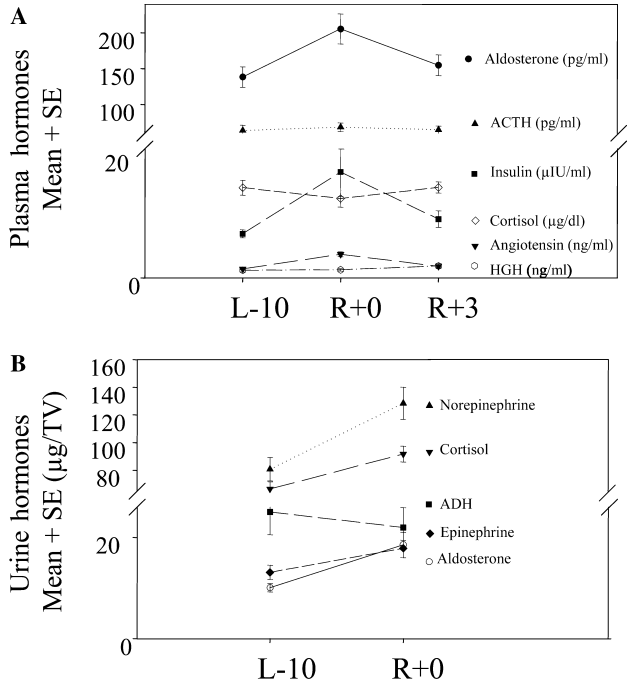

Fig. 3. Plasma (A) and urine (B) concentrations (mean ± SE) of stress hormones of 32 astronauts before and after space flights. TV, total volume (of 24 h pool).

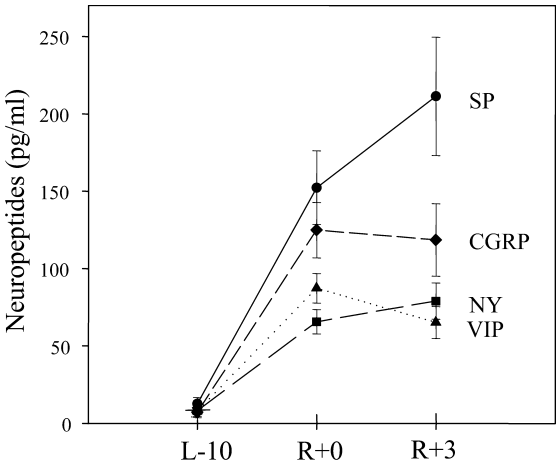

Fig. 4. Plasma concentration of neuropeptides (mean ± SE) in samples from 5 astronauts at three times before and after a 5-day mission: 10 days before launch (L − 10), at landing (R + 0), and 3 days after landing (R + 3).

(14.7 ± 1.2), but plasma levels of insulin (L − 10, 7.2 ± 0.64; R + 0, 17.2 ± 1) and aldosterone (L − 10, 138.4 ± 14.3; R + 0, 205.5 ± 20.9) increased (Fig. 3A). Plasma ACTH and HGH did not change. Urinary concentrations of cortisol (L − 10, 66.6 ± 5.2; R + 0, 91.8 ± 5.7), epinephrine (L − 10, 13.1 ± 1.4; R + 0, 17.9 ± 1.6), and norepinephrine (L − 10, 80.9 ± 8.5; R + 0, 128.4 ± 11.7) were significantly greater ($p < .05$) at R + 0 than at L − 10 (Fig. 3B). The controls had no such changes during the 25-day study period.

Neuropeptides were measured in the plasma of 5 astronauts before and after a 5-day flight and in the plasma of the control subjects. In astronaut samples, concentrations of substance P (SP), calcitonin gene-related peptide (CGRP), neuropeptide Y (NY), and vasoactive intestinal peptide (VIP) were significantly greater ($p < .05$) at R + 0 than at L − 10 (Fig. 4). No significant further changes in any of the neuropeptides measured were found 3 days after landing (R + 3). Neuropeptide levels in the control group were similar to preflight values in astronauts and did not change over the 25-day test period.

4. Discussion

Thirty-two US astronauts participated in a study of EBV reactivation during 10 space shuttle missions. Each crew member served in one of three positions on a mission: mission specialist, responsible for the conduct of science investigations; commander, with overall responsibility for the mission; and pilot, responsible for assisting the commander in controlling and operating the vehicle. These astronauts were exceptionally healthy and in excellent physical condition. All phases (before, during, and after flight) of a space shuttle mission are

demanding, and are accompanied by varying degrees of stress.

Glaser (Glaser and Kiecolt-Glaser, 1998; Glaser et al., 1985a,b) and others have found increased reactivation of EBV, as measured by increases in the titer of antibody to VCA, in several stress models. Glaser et al. (1991, 1994) also showed that reactivation of EBV varied with the type of stress. For example, physical stress associated with basic training of West Point cadets did not result in increased EBV reactivation. However, stress associated with final examinations resulted in significantly increased viral reactivation.

Astronauts are exposed to substantial levels of various types of stress, beginning well before flight. The long training period before a flight can be stressful. The differences between types of stress in different phases of a mission (including before and after the mission) may explain the differences we found in the patterns of different measures of viral reactivation. In a student stress model, titers of antibody to the EBV antigen, VCA, increased with down-regulation of the cellular immune response (Glaser et al., 1985a). We observed similar increases in anti-CMV antibodies in astronauts, beginning before the flight and continuing during the flight, and even during the first few days after landing (Mehta et al., 2000b).

Hormone profiles provided evidence of astronauts' stress exposure. Urinary concentrations of cortisol and the catecholamines were significantly higher after flight than before flight (in-flight hormone levels were not available). These data were consistent with results from the Skylab missions (Leach and Rambaut, 1977) and previous space shuttle missions (Mehta et al., 2000b, 2001; Stowe et al., 2001). Elevated levels of these stress hormones at landing may be a consequence of the stresses experienced during space flight or reentry.

Plasma concentrations of cortisol at landing were not elevated over preflight values. This is also consistent with earlier reports (Mehta et al., 2001; Stowe et al., 2000). The peak plasma concentration of cortisol, which has a circadian rhythm, may have occurred before or after the sample was collected. Plasma cortisol was collected only once before and once after space flight. Because plasma cortisol has a short (~4 h) half-life, this sampling schedule gave a "snapshot" look at plasma levels, whereas each urinary cortisol value was obtained from a pool of urine collected over a 24-h period. The "snapshot" of plasma cortisol may have missed transient but significant changes that accumulated in urine, which provided an integration of 24 h of collection (Mehta et al., 2001).

All of the neuropeptides measured (SP, CGRP, NY, and VIP) in samples from 5 astronauts were elevated immediately after landing, but until neuropeptide data can be obtained from a larger number of astronauts, the significance of changes in astronaut neuropeptide levels cannot be adequately assessed. Incubation of naive T cells with SP induced cytokine production that was associated with Th2 activity (Levite, 1998). This is consistent with the decrease in cell-mediated immunity (CMI) reported earlier in astronauts (Taylor et al., 1997) and Antarctic expeditioners (Mehta et al., 2000a; Muller et al., 1995), and hence is consistent with an increase in viral reactivation. Increased SP, CGRP, and VIP levels have been associated with decreased CMI (Ferrandez et al., 1996; Morley et al., 1987).

Studies of the immune responses of astronauts and other groups undergoing stress suggest that some aspects of immunity are impaired by the stress of space flight. We previously showed that CMI decreased in subjects who worked at Australian Antarctic science stations during the stressful winter-over period (a widely used ground analog of space flight) (Mehta et al., 2000a). Taylor et al. (1997) showed that CMI decreased in astronauts during short-duration (<10 days) space flight. Changes resulting from space flight have also been reported in white blood cell numbers, leukocyte and lymphocyte subsets, T-cell proliferation, cytokine production, natural killer cell cytotoxicity, and CMI (Konstantinova and Fuchs, 1991; Konstantinova et al., 1993; Sonnenfeld et al., 1992; Taylor, 1993a,b).

The cellular immune response plays an important role in the maintenance and replication of latent herpesviruses and re-establishment of control over virus replication after reactivation. In a group of Antarctic expeditioners, we showed that increased shedding of EBV was concomitant with decreased CMI (Mehta et al., 2000a). Changes in CMI may result in virus reactivation. This suggests that stress, working through the hypothalamus–pituitary–adrenal (HPA) axis (Glaser et al., 1985a,b), could affect immune responses and provide the mechanism of reactivation of herpesviruses in astronauts.

We have now shown EBV reactivation in astronauts by three measures: presence of virus in saliva, number of copies of viral DNA in saliva, and titer of antibodies to viral antigens. These three measures give slightly different pictures of the details of reactivation, and the effects of different stresses on astronauts may help explain these differences.

We found no correlation between shedding frequency and amount of EBV DNA in astronauts' saliva. Although the frequency of EBV shedding in saliva was highest before flight, the number of EBV copies in saliva was highest during flight, where it was 10-fold higher than before or after flight (Fig. 1). The pattern of the third measure of viral reactivation—titer of antibodies to viral antigens—was slightly different from the pattern of the other two measures. Anti-VCA titers increased before flight and continued to increase through 3 days after flight (Fig. 2).

The activities and risks experienced by shuttle crew members during the preflight period and the flight are

very different. The types of stresses, the levels of stress, the combination of stresses, and the ways individual crew members cope may be very different, resulting in substantial changes in shedding frequencies and viral numbers.

The quantitative EBV DNA data (Fig. 1) indicated that the amount (number of copies) of EBV shed in the saliva of astronauts during space flight increased as the number of days in space increased. Similar data from 2 cosmonauts aboard the Russian space station Mir showed that EBV shedding in saliva occurred throughout the nearly 3-month mission, a much longer time than the relatively short (<14 days) duration of the shuttle missions. However, the number of EBV copies shed by cosmonauts aboard Mir did not increase as a function of days in flight, as observed with astronauts on the space shuttle. The maximum number of EBV copies shed by cosmonauts on Mir was 1130 per mL of saliva, and the maximum number of copies shed by astronauts on the shorter shuttle flights was 738 per mL of saliva.

The pattern and magnitude of shedding likely responded to various events that occurred during space flight. Several stressful events occurred on Mir during this mission, including some serious failures of the environmental control system. Other difficulties resulted in postponements and rescheduling of spacewalks, resulting in additional stress. The question of the effect of time in space on viral shedding is still open. This question could be answered by following the reactivation phenomenon on longer (e.g., 6 months) missions aboard the International Space Station with suitable numbers of subjects.

The medical significance of the extent of EBV shedding in astronauts is unknown. During the flight phase, the mean number of EBV DNA copies shed by astronauts was 417 per mL, with a maximum of 738 per mL. We found 3700 copies per mL (mean value) of EBV DNA in the saliva of HIV patients. However, some HIV patients had levels as low as 600 copies. Kimura et al. (1999), who used a similar PCR assay, reported that a group of patients with infectious mononucleosis had a mean number of 158 copies of EBV DNA per mL of saliva. Stagno et al. (Stagno et al., 1975) found a 100-fold difference in the number of CMV genomes per mL of urine between symptomatic and asymptomatic renal transplant recipients with active CMV infection. EBV copy numbers indicated that the diminishment of astronauts' immune response is very mild on short shuttle flights and cannot be compared to that of patients with severely impaired immunity (such as AIDS patients). However, lengthy stays in space may result in substantial reductions in immunity, and the number of EBV copies in saliva of cosmonauts aboard the Mir space station supports this position.

Astronauts' viral shedding patterns, virus-specific antibody titer response, and increased levels of stress hormones and neuropeptides lead us to draw some conclusions about EBV reactivation associated with space flight. No correlation was observed between increased viral reactivation and age, sex, flight experience, or the nature of the crew members' duties (for example, pilot vs. non-pilot). Astronauts are exposed to multiple stressors that may reactivate EBV before, during, and after flight aboard the space shuttle. The intensity or combination of stressors during the flight may vary significantly from those stressors experienced before or after the flight. Our data indicate that more copies of EBV are shed in saliva during space flight than during the ground phases of preparing for the mission and recovering from space flight-associated stress. The increased amount of EBV DNA in saliva, coupled with the propensity of large and small saliva droplets to float in the microgravity environment of the crew compartment, may lead to increased risk of cross-infection among crew members. One would expect minimal medical effects of such events in healthy individuals, but viral reactivation is more likely to have clinical significance (Cohen, 2000) for astronauts if their immune responses are impaired.

Studies are in progress to determine if the increased reactivation of EBV in astronauts is a general effect extending to other human herpesviruses, and if it produces any health effects in astronauts on space missions longer than 14 days.

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