Subclinical shed of infectious varicella zoster virus in astronauts.

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Aerosol borne varicella zoster virus (VZV) enters the nasopharynx and replicates in tonsillar T-cells, resulting in viremia and varicella (chickenpox). Virus then becomes latent in cranial nerve, dorsal root and autonomic nervous system ganglia along the entire neuraxis (1). Decades later, as cell-mediated immunity to VZV declines (4), latent VZV can reactivate to produce zoster (shingles). Infectious VZV is present in patients with varicella or zoster, but shed of infectious virus in the absence of disease has not been shown. We previously detected VZV DNA in saliva of astronauts during and shortly after spaceflight, suggesting stress induced subclinical virus reactivation (3). We show here that VZV DNA as well as infectious virus in present in astronaut saliva.

VZV DNA was detected in saliva during and after a 13-day spaceflight in 2 of 3 astronauts (Fig. panel A). Ten days before liftoff, there was a rise in serum anti-VZV antibody in subjects 1 and 2, consistent with virus reactivation. In subject 3, VZV DNA was not detected in saliva, and there was no rise in anti-VZV antibody titer. Subject 3 may have been protected from virus reactivation by having zoster <10 years ago, which provides a boost in cell-medicated immunity to VZV (2). No VZV DNA was detected in astronaut saliva months before spaceflight, or in saliva of 10 age/sex-matched healthy control subjects sampled on alternate days for 3 weeks (88 saliva samples).

Saliva taken 2-6 days after landing from all 3 subjects was cultured on human fetal lung cells (Fig. panel B). Infectious VZV was recovered from saliva of subjects 1 and 2 on the second day after landing. Virus specificity was confirmed by antibody staining and DNA analysis which showed it to be VZV of European descent, common in the US (5). Further, both antibody staining and DNA PCR demonstrated that no HSV-1 was detected in any infected culture.

This is the first report of infectious VZV shedding in the absence of clinical disease. Spaceflight presents a uniquely stressful environment which includes physical isolation and confinement, anxiety, sleep deprivation, as well as exposure to increased radiation and microgravity. It is interesting that in our study, VZV and not HSV-1 reactivation was detected, since stress-induced HSV-1 reactivation has been reported (6). Future studies are needed to determine the specific inducer of VZV reactivation.

Figure legend. Subclinical shed of infectious VZV in astronaut saliva. **Panel A**: DNA was extracted from saliva samples collected 109-133 days before liftoff (preflight), days 2-13 during flight (inflight, yellow area) and for 14 days after landing (post flight). The VZV DNA copy number per ml saliva is shown in blue boxes. VZV DNA was detected in 2 of 3 astronauts during and shortly after spaceflight. A rise in titer of circulating anti-VZV-specific antibody was also detected in the two subjects whose saliva was positive for VZV DNA. **Panel B**: Infectious virus was obtained from the saliva samples obtained on day 2 post flight from subjects 1 and 2, but not from subject 3. Pictures in row 1 show VZV-specific staining in cells that had been inoculated with saliva of subjects one and two; no staining was seen in cells that had been inoculated with saliva of subject three. Row 2 is an enlargement of the stained monolayers showing multiple VZV-specific plaques (magnification bar = 2.0 mm). Row 3 is a further enlargement showing a single VZV plaque (magnification bar = 0.2 mm). Material and methods are presented in the accompanying supplemental data.

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Supplemental data.

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.

Saliva samples. Saliva was collected with Salivette kits (Sarstedt, NC), concentrated by ultrafiltration, and DNA extracted by affinity chromatography on silica-matrix as previously described (5).

Real-Time PCR. TaqMan primers and probes for cellular GAPdH and VZV gene 63 were used to determine virus genome copy number by fluorescence-based simultaneous amplification and product detection (5).

Virus detection. HFL cell cultures were inoculated with saliva samples and passed twice before fixation in 4% paraformaldehyde for immunostaining with rabbit anti-VZV-IE63 or anti-HSV-1-ICP22 antibody (4).

Plasma antibody testing. The endpoint antibody titer to VZV was determined as the highest dilution of plasma giving positive immunofluorescent staining of VZV-infected cells. All specimens were batch analyzed and read blind-coded (6).

VZV genotype analysis. PCR-based diagnostic assays were performed on extracted DNA (2). Single nucleotide polymorphisms in ORF38, ORF54 and ORF62 (positions 106262 and 107252) were determined using fluorescent resonance energy transfer-based PCR performed on LightCycler (3). Sequence variation within ORF21 (positions 33725-33728) and ORF22 (positions 37837-38264) was determined to accurately identify virus genotype. Nucleotide locations are based on the sequence of Dumas strain (1).

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sample	Flight Days	VZV copies / ml saliva			VZV antibody titer		
		subject 1	subject 2	subject 3	subject 1	subject 2	subject 3
AME			ns	ns	1:80	1:80	1:80
Preflight	-133	0	0	0			
Preflight	-131	0	0	0			
Preflight	-129	0	0	0			
Preflight	-127	0	0	0			
Preflight	-125	0	0	0			
Preflight	-123	0	0	0			
Preflight	-121	0	0	0			
Preflight	-119	0	0	0			
Preflight	-125	0	0	0			
Preflight	-117	0	0	0			
Preflight	-115	0	0	0			
Preflight	-113	0	0	0			
Preflight	-111	0	0	0			
Preflight	-109	0	0	0			
Preflight	-10	ns	ns	ns	1:320	1:640	1:80
Inflight	1	ns	ns	ns			
Inflight	2	224	18	0			
Inflight	3	0	247	0			
Inflight	4	0	0	0			
Inflight	5	128	0	0			
Inflight	6	0	0	0			
Inflight	7	200	0	0			
Inflight	8	0	0	0			
Inflight	9	2500	650	0			
Inflight	10	0	75	0			
Inflight	11	450	0	0			
Inflight	12	0	0	0			
Inflight	13	120	23	0			
postflight	14	ns	ns	ns	1:320	1:320	1:80
postflight	15	1250	560	0			
postflight	16	45	0	0			
postflight	17	0	340	0			
postflight	18	110	45	0			
postflight	19	0	23	0			
postflight	20	0	0	0			
postflight	21	0	0	0			
postflight	22	0	0	0			
postflight	23	0	0	0			
postflight	24	0	0	0			
postflight	25	0	0	0			
postflight	26	0	0	0			
postflight	27	0	0	0	1:80	1:80	1:80
postflight	28	0	0	0			

AME; annual medical exam (1-3 months prior to liftoff)

B