Latent Herpes Viruses Reactivation In Astronauts

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<u>Introduction</u>

Space flight has many adverse effects on human physiology. Changes in multiple systems, including the cardiovascular, musculoskeletal, neurovestibular, endocrine, and immune systems have occurred (12, 32, 38, 39). Alterations in drug pharmacokinetics and pharmacodynamics (12), nutritional needs (31), renal stone formation (40), and microbial flora (2) have also been reported. Evidence suggests that the magnitude of some changes may increase with time in space.

A variety of changes in immunity have been reported during both short (≤16 days) and long (>30 days) space missions. However, it is difficult to determine the medical significance of these immunological changes in astronauts. Astronauts are in excellent health and in superb physical condition. Illnesses in astronauts during space flight are not common, are generally mild, and rarely affect mission objectives. In an attempt to clarify this issue, we identified the latent herpes viruses as medically important indicators of the effects of space flight on immunity. This chapter demonstrates that space flight leads to asymptomatic reactivation of latent herpes viruses, and proposes that this results from marked changes in neuroendocrine function and immunity caused by the inherent stressfulness of human space flight.

Astronauts experience uniquely stressful environments during space flight. Potential stressors include confinement in an unfamiliar, crowded environment, isolation, separation from family, anxiety, fear, sleep deprivation, psychosocial issues, physical exertion, noise, variable acceleration forces, increased radiation, and others. Many of these are intermittent and variable in duration and intensity, but variable gravity forces (including transitions from launch acceleration to microgravity and from microgravity to planetary gravity) and variable radiation levels are part of each mission and contribute to a stressful environment that cannot be duplicated on Earth. Radiation outside the Earth's magnetosphere is particularly worrisome because it includes ionizing radiation from cosmic galactic radiation. Increased stress levels appear even before flight, presumably from the rigors of preflight training and the anticipation of the mission (12, 32, 38, 39).

Space flight causes significant changes in human immune function (32), but the means by which these changes come about have been difficult to discern. Consistent indicators of stress associated with space flight include increased production of stress hormones, and changes in cells of the immune system. These changes include elevated white blood cell (WBC) and neutrophil counts at landing (15, 16, 35, 37). Activation of generalized stress responses before, during, and after space flight probably affects the function of the immune system. Space flight has been shown to decrease many aspects of immune function, including natural killer (NK) cell activity, interferon production, the blastogenic response of leukocytes to mitogens, cell-mediated immunity, neutrophil function and monocyte function (5, 16, 18, 21, 35-37).

REACTIVATION OF LATENT HERPES VIRUSES

Latent viruses are used as an early predictor of changes in immune system of astronauts due to space flight. Humans host 8 different herpes viruses. Following primary infection, these viruses establish a permanent presence with the host called latency. The virus may remain latent and unnoticed for years or decades. Cell mediated immunity (CMI) is the immune element most responsible for controlling latent viruses. During times of stress, latent viruses may reactivate and cause disease. Stress is processed through the Hypothermus pituitary adrenal (HPA) axis and sypamathetic adrenalmedullary axis, resulting in increased secretion of stress hormones including cortisol and catecholamines (8, 9, 11). Increased levels of stress hormones reduce the immune response, specifically the CMI resulting in proliferation of latent viruses and disease at a later stage.

Astronauts experience various stressors that may result in inhibition of their cell-mediated immunity and increased reactivation of latent viruses during space flight, potentially increasing the risk of disease among crew members. Risks associated with many infectious agents are reduced by restricting preflight contact of the flight crews with high-risk populations. However, the risk of latent virus reactivation is unaffected by such precautions. Virus reactivation could pose an important health risk for astronauts, as well as for people living and working in other extreme environments. In this paper, we present the results from our studies of reactivation of three latent herpes viruses (Epstein Barr Virus, Cytomegalovirus, Varicella Zoster Virus) in astronauts and discuss our interpretation of these results.

Epstein-Barr Virus

Epstein-Barr virus (EBV), a DNA virus is highly infectious and can be transmitted by microdroplets and by direct contact with saliva. When the acute infectious phase subsides, EBV can become latent in B lymphocytes. EBV is the causative agent of infectious mononucleosis and is associated with several malignancies, including Burkitt's lymphoma, nasopharyngeal carcinoma, and diffuse oligoclonal B-cell lymphoma (1, 13, 14, 19, 26, 30). Latent EBV may be reactivated by a range of physical and psychosocial stress factors and shed in saliva (10, 11).

EBV was used as a model for latent virus reactivation in astronauts. Approximately 95% of the adult population is infected by EBV (19, 27). This makes EBV ideal for studying virus reactivation in the relatively small astronaut population. Pierson et al., (28) collected and used a PCR assay to detect EBV DNA and demonstrate EBV reactivation in shuttle astronauts. Pierson et al. (29) demonstrated EBV DNA shed in saliva from astronauts before, during, and after space flight. EBV copies were about 10-fold higher during the flight phase than shed either before or after space flight (Figure 1). In addition, the number of EBV copies shed during space flight seemed to increase as a function of time in space. A significant increase in EBV (VCA) antibody titers before launch, at landing, and after landing was observed ($p < 0.001$) from the baseline values taken 5 to 24 months before flight. These findings are consistent with reports by Stowe et al. (34).

Cytomegalovirus

To determine if the effects of space flight observed in astronauts were limited to EBV, we studied another human herpes virus, cytomegalovirus (CMV). When CMV reactivates, it is shed in urine. CMV DNA was found in astronaut urine collected before and after flight. Figure 2 shows that 27% (15/55) of astronauts shed CMV in their urine while less than 2 per cent (1 of 61) of control subjects shed CMV (23). Plasma IgG antibodies to CMV increased significantly as compared to the controls in astronauts who shed CMV, confirming reactivation of the virus (Figure 3).

Varicella Zoster Virus

Subsequently, varicella zoater virus (VZV) was found in saliva of shuttle astronauts during and after space flight (Figure 3). This was the first report of shedding of VZV with no clinical symptoms. As expected, no shedding was found in control subjects. VZV antibodies also increased over control values substantiating occurrence of VZV reactivation. Consistent with findings from EBV and CMV studies, urinary cortisol levels increased in astronauts at landing. Further descriptions of CMV and VZV reactivation in astronauts are given by Mehta et al. (20, 23). Table 3 summarizes the data available on viral reactivation in astronauts.

All available data suggest that stress, and perhaps other factors, are processed through the HPA axis. Levels of stress hormones, including cortisol and catecholamines, in astronauts were consistently elevated at landing. Elevations in these hormones result in diminished CMI response, and decreased CMI is followed by increased reactivation and shedding of latent herpes viruses.

Having shown that either stress or environmental factors associated with space flight can trigger VZV reactivation detected as viral DNA in saliva, we refocused our research to determine if VZV reactivation constitutes a health risk to astronauts. Our approach was to demonstrate the propagation of infectious VZV from astronauts' saliva after space flight. First, however, we asked if VZV can remain infectious after being exposed to human saliva.

Saliva taken 2-6 days after landing from 3 subjects was cultured on human fetal lung cells (Figure 4). 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. Further, both antibody staining and DNA PCR demonstrated that no HSV-1 was detected in any infected culture (4).

IgG antibody against VZV was determined by enzyme immunoassay (EIA) (6, 7). Serum titers of anti-VZV IgG were determined in 5 control subjects and in 6 astronauts 10 days before flight and again 2 to 3 hours after landing (Figure 5). We found two- to three-fold greater levels of circulating anti-VZV IgG in astronauts than in control subjects. However, the combination of VZV DNA in saliva and a larger specific antibody response in serum from astronauts than in serum from control subjects further indicates sub-clinical reactivation of VZV. This study adds

VZV to the list of human herpes viruses capable of reactivation in response to acute non-surgical stress.

HSV types 1 and 2 were detected in 2 to 3% of pre- and postflight and in 8% of in-flight saliva samples collected from space shuttle crew members. HHV-6 was detected in 29% of preflight, 2% of in-flight, and 15% of postflight samples (Pierson *et al*., unpublished data).

Selected neuropeptides, including SP, CGRP, Neuropeptide Y, and VIP, were also measured in plasma from 5 astronauts before and after space flight. They were elevated immediately after landing (Figure 6). This is consistent with the decrease in cell-mediated immunity (CMI) reported earlier in astronauts (37) and Antarctic expeditioners (22, 25), and therefore is consistent with an increase in viral reactivation (28). However, until neuropeptide data can be obtained from a larger number of astronauts, the significance of changes in astronaut neuropeptide levels cannot be adequately assessed.

Medical Significance of Latent Virus Reactivation in Space Flight

The medical significance of asymptomatic viral shedding in astronauts remains unknown. During the flight phase, the mean number of EBV DNA copies shed by astronauts was 417/mL, with a maximum of 738/mL. In the saliva of acquired immunodeficiency syndrome (AIDS) patients, we found 3700 copies/mL (mean value) of EBV DNA. However, some AIDS patients had levels as low as 600 copies. Kimura (17), 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.

In renal transplant recipients with active CMV infection, Stagno *et al.* (33) found that the number of CMV genomes per mL of urine was 100-fold greater in symptomatic patients than in asymptomatic patients. The number of EBV copies found in astronaut saliva indicated that the diminishment of 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 is consistent with this scenario.

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 (3) for astronauts if their immune responses are impaired.

Recently we reported that salivary VZV DNA was associated with the severity of disease in shingle patients (24). Fifty-four zoster patients were treated with valacyclovir. On treatment day 1, 7- and 14-days later, pain was scored and saliva examined for VZV-DNA. VZV-DNA was found in every patient the day treatment was started and disappeared in 82%. There was a positive correlation between presence of VZV-DNA and pain, and between VZV-DNA copy number and pain ($P<0.0005$). VZV-DNA was present in one patient before rash, in 4 after pain resolved, and not in any of 6 subjects with

chronic pain or 14 healthy subjects. Analysis of human saliva has potential usefulness in diagnosing neurological disease produced by VZV without rash.

Acknowledgments

Figure Legends

FIGURE 1. Distribution of the number of EBV DNA 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.

FIGURE 2. Percentage of subjects (55 astronauts, 61 controls) who shed CMV DNA in urine samples collected before, during, and after short-duration space flights.

FIGURE 3. Cytomegalovirus (CMV) IgG antibody titer (mean $\log_2 \pm$ SE) in astronaut blood samples at baseline (BL), 10 days before launch (L−10), and at landing (R+3). *Significant increase from BL ($P<0.001$); †significant increase from L-10 ($P< 0.001$).

FIGURE 4. Plaque morphology as an indicator of the susceptibility to VZV infection of two types of indicator cells, primary human lung fibroblast (HLF, upper panel) and human malignant melanoma (MeWo, lower panel) cells. Sub-confluent monolayers of MeWo and HLF cells were infected with identical amounts of cell-free VZV. After 5 days of incubation, the cultures were fixed and stained to reveal VZV cytopathology (open arrows).

FIGURE 5. Antibody titer index of anti-VZV antibody in astronauts before and after space flight and in control subjects. IgG antibody against VZV was determined by enzyme immunoassay (EIA). Serum titer of anti-VZV IgG was determined in 5 control subjects and in 6 astronauts 10 days before flight (L−10) and again 2 to 3 hours after landing (R+0) $P<0.001$.

FIGURE 6. Plasma concentration (mean ± SE) of neuropeptides in blood samples from 5 astronauts at 3 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). SP, substance P; CGRP, calcitonin gene-related peptide; NY, neuropeptide Y; VIP, vasoactive intestinal peptide. From Pierson *et al.* (2005, Fig. 4, p. 239), with permission.

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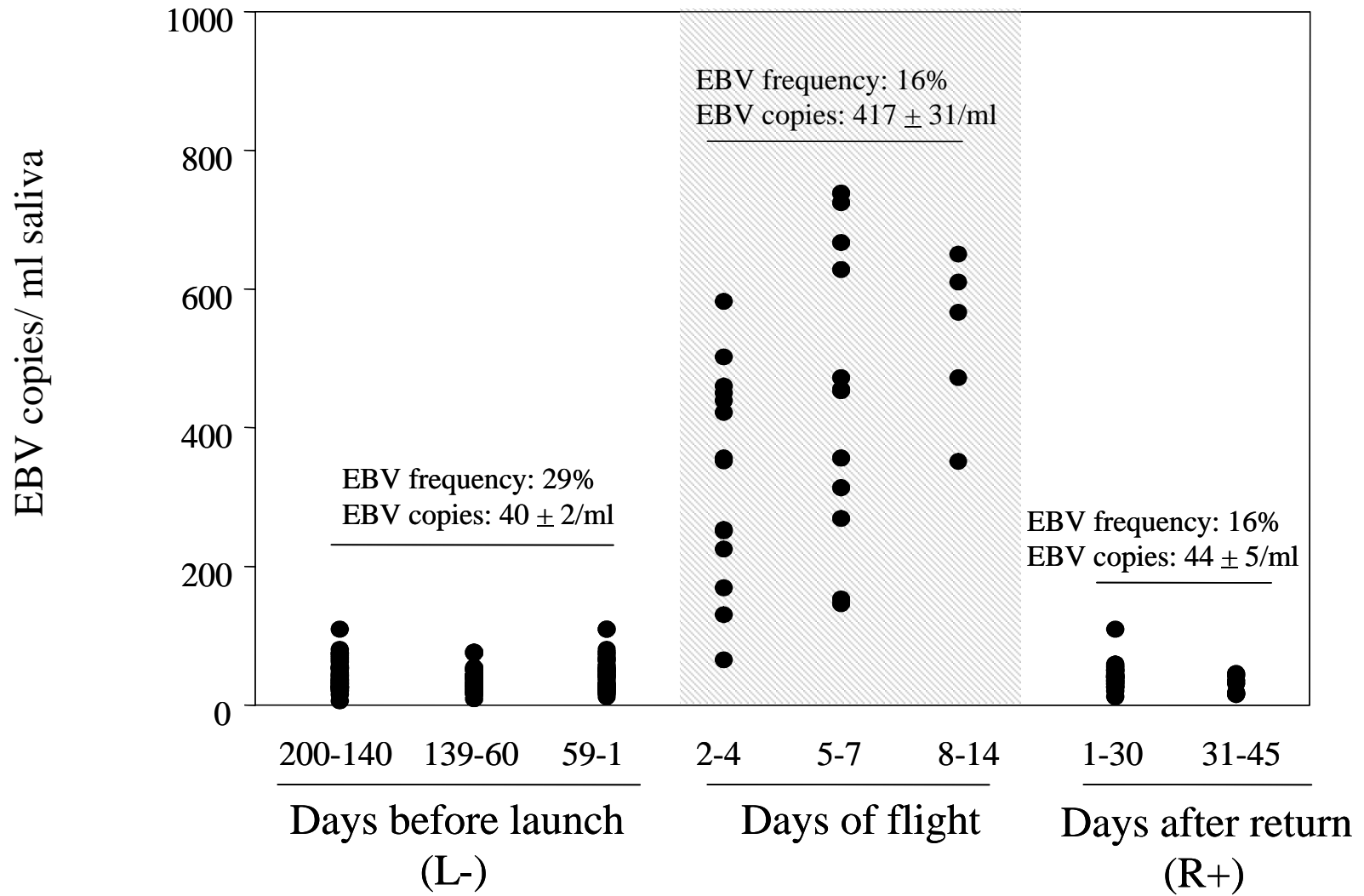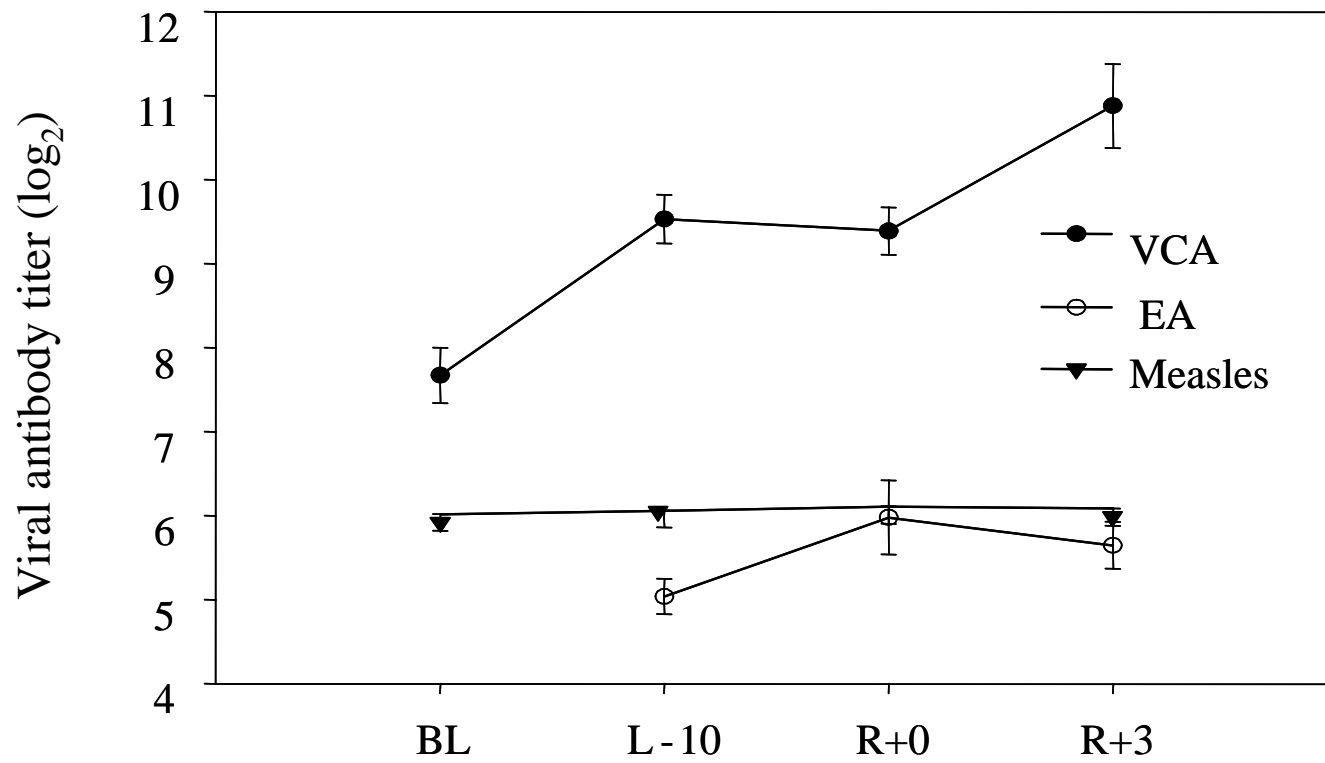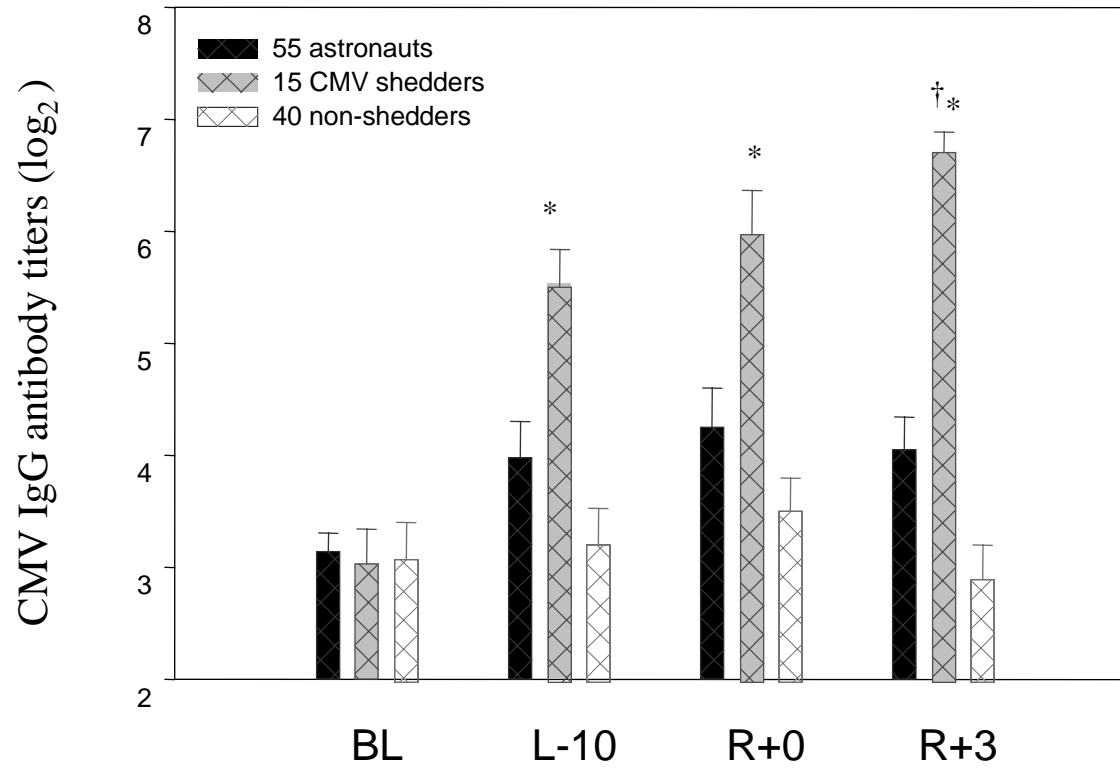40.   **Zerwekh JE**. Nutrition and renal stone disease in space. *Nutrition* 18: 857-863, 2002.
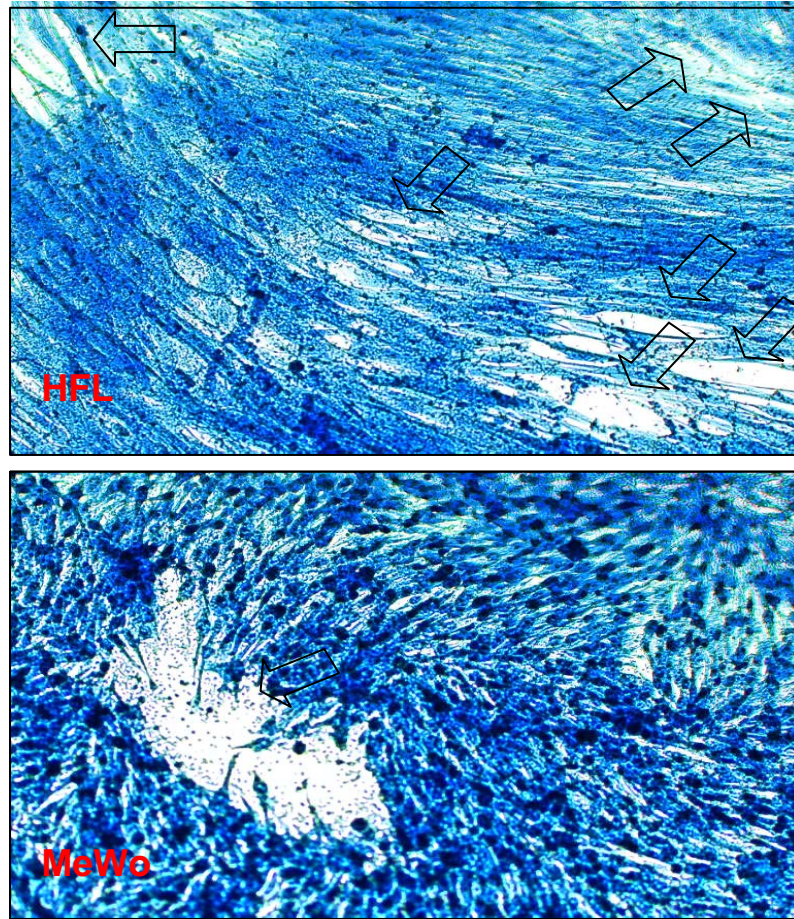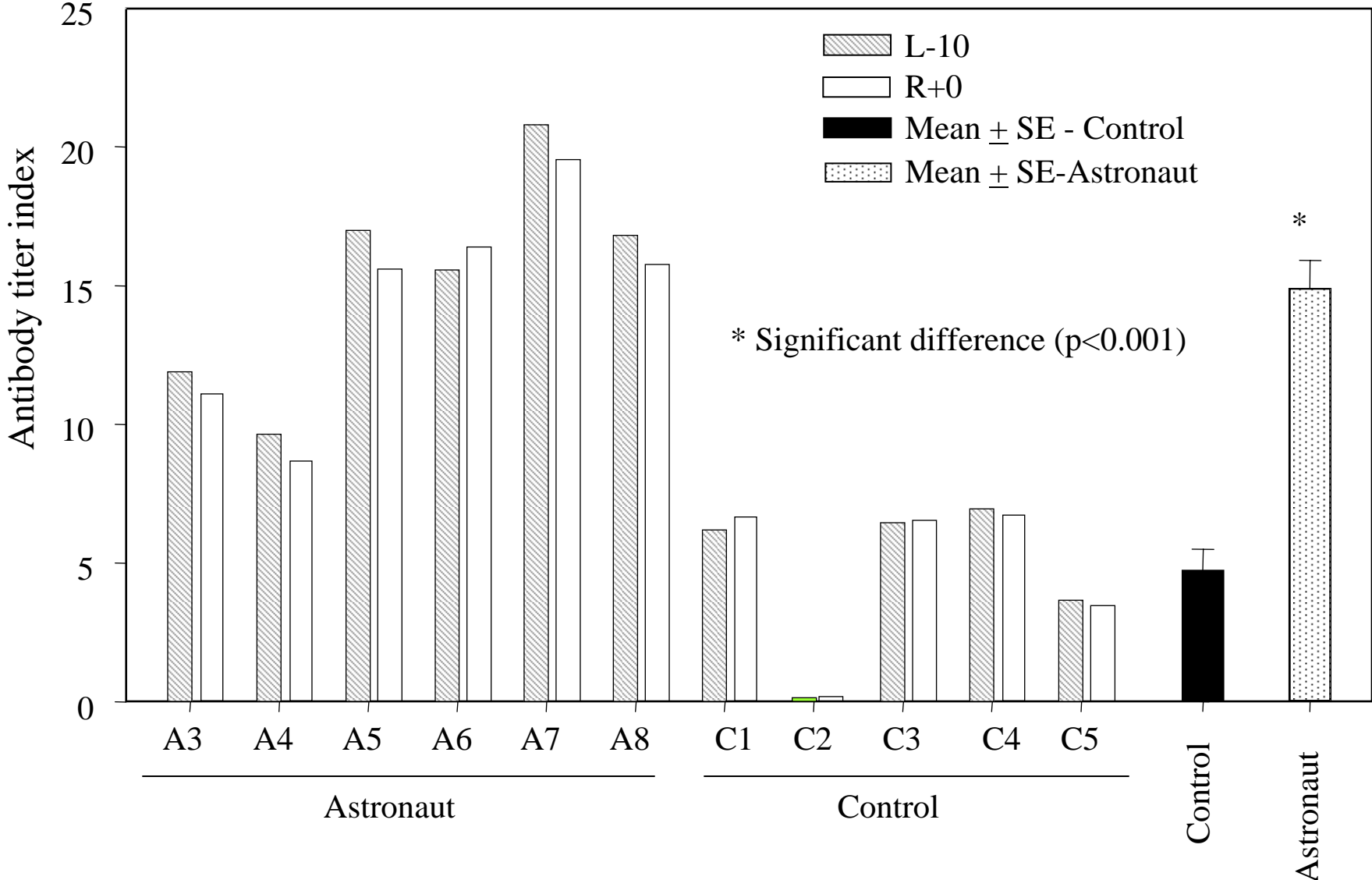
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