

SPACE FLIGHT-INDUCED REACTIVATION OF LATENT EPSTEIN-BARR VIRUS

Raymond P. Stowe¹, Ph.D., Duane L. Pierson², Ph.D., Alan D.T. Barrett¹, Ph.D.

¹Department of Pathology, University of Texas Medical Branch, Galveston, TX, and
²Life Science Research Laboratories, NASA-Johnson Space Center, Houston, TX

Abstract

The majority of humans are infected with Epstein-Barr virus (EBV) early in life and thereafter carry the virus in a latent form. Reactivation of latent EBV may be an important threat to crew health during extended space missions. EBV is the causative agent of infectious mononucleosis as well as nasopharyngeal carcinoma, Burkitt's lymphoma, and different kinds of B-lymphocyte lymphomas in immunosuppressed individuals. Control of replication *in vivo* is restricted primarily by cytotoxic T-lymphocytes, and severe clinical symptoms have been associated with reactivation of latent viruses in patients with defective cellular immunity. Decreased cellular immune function has been reported both during and after space flight. Preliminary studies have demonstrated increased EBV shedding in saliva as well as increased antibody titers to EBV lytic proteins. We hypothesize that the combined effects of microgravity along with associated physical and psychological stress will decrease EBV-specific T-cell immunity and reactivate latent EBV in infected B-lymphocytes. If increased virus production and clonal expansion of infected B-lymphocytes is detected, then pharmacological measures can be developed and instituted

prior to onset of overt clinical disease. More importantly, we will begin to understand the basic mechanisms involved in stress-induced reactivation of EBV in circulating B-lymphocytes.

Introduction

The first elements of the international space station were recently carried into space, and it is anticipated that the station will be completed as early as the year 2006. Operational activities in and on the international space station will add commercial activities in addition to current scientific and flight test activities. These activities bring with them the potential for the usual types of accidents, including trauma, burns, and infections that may be complicated by the physiologic changes associated with microgravity.¹ Thus, these factors may significantly impact crew productivity and their recovery from industrial accidents and disease.

A number of studies have shown that immune function in astronauts is altered during and after space flight. Decreased cytokine production, altered distribution of leukocyte and lymphocyte subsets, and decreased delayed-type hypersensitivity have been observed.²⁻⁴ We have previously shown that neutrophil function was altered after short-term space flights.⁵ In addition, urinary cortisol and catecholamines were also elevated after flight, and these immunosuppressive agents are known to alter the trafficking and/or function of neutrophils, eosinophils, and endothelial cells.^{6,7}

Glucocorticoids and catecholamines have also been shown to inhibit interleukin (IL)-12 production and increase IL-10 production by monocytes which drives the immune response towards a Th2 cytokine profile (IL-4, IL-5, and IL-10) and away from a Th1 profile (IL-

¹ Copyright © 2001 by Raymond P. Stowe. Published by the American Institute of Aeronautics and Astronautics, Inc. with permission.

12, IL-2, interferon-gamma (IFN- γ).⁸⁻¹⁰ One mechanism by which glucocorticoids achieve this Th1 \rightarrow Th2 switch is by increasing the production of I- κ B, which binds to the transcription factor NF- κ B and inhibits translocation to the nucleus.^{11,12} NF- κ B is a ubiquitous multiprotein complex involved in the transcriptional activation of several host defense genes including the cytokines IL-2, IL-8, and IL-12.¹³ Importantly, the modulation of cytokine gene expression may predispose individuals to viral infections by decreasing cell-mediated immunity.^{14,15}

Infections Associated with Space Flight

The risk of infectious disease during space flights has become of increasing concern to the U.S. space program as flight durations are extended. The unique conditions associated with space flight (e.g., physical and psychological stress, microgravity, and crowded living conditions) may predispose crewmembers to infectious diseases. Before implementation of a preflight quarantine period during the Apollo program, numerous infections were documented in the crews, including influenza, viral gastroenteritis, upper respiratory tract infections, pharyngitis, and other in-flight disease events of presumably viral origin.¹⁶⁻¹⁸ Establishment of the preflight quarantine period reduced the incidence of illnesses associated with most bacterial, fungal, viral, and parasitic pathogens.

Reactivation of latent viruses, however, is not mitigated by preflight quarantine and may pose a significant health risk to crewmembers during space flight. The herpesvirus family, which includes herpes simplex type-1 and type-2, cytomegalovirus, Epstein-Barr virus (EBV), varicella-zoster virus, human herpesvirus-6, -7, and -8 are all double-stranded DNA viruses that may establish a lifelong latent infection in the host.¹⁹ Several

preflight episodes of herpes-simplex-induced rhinitis and pharyngitis have been identified. Herpes simplex was isolated from an Apollo-15 crewmember's throat before launch;²⁰ an in-flight episode of herpetic lip lesions in an Apollo-17 astronaut yielded herpes simplex after landing.¹⁸ Herpesvirus infections are characterized by an acute phase, generally associated with minor morbidity and mortality, followed by a chronic latent phase that reflects a balance between viral replication and the host immune response. Impaired cellular immunity has been associated with both mild and severe clinical symptoms due to reactivation of herpesvirus, as well as an increase in recurrent disease.^{21,22}

Recurrent herpesvirus infections in astronauts may be partly caused by stress, possible enhancement in microgravity, or a combination of both. A common finding of stress studies on earth is reactivation of EBV in association with decreased cellular immunity. EBV is the most widely studied member of the herpesvirus family because of its clinical and oncogenic importance in causing a number of diseases.²³ Surprisingly, very little is known about EBV infections in astronauts which, if unchecked, may cause a variety of diseases. The lack of information regarding latent virus infections provides fertile ground for investigation of both ground-based and flight research in order to determine the disease risks associated with space flight.

EBV Pathogenesis

EBV is a herpesvirus that infects all human populations (reviewed in refs. 24 and 25). Typically, greater than 95% of the adult population carries the virus, which is normally acquired asymptotically in early childhood. When primary infection is delayed until adolescence or adulthood, approximately half the cases result in the clinical illness called

infectious mononucleosis. EBV infections are usually self-limiting and are characterized by fever, pharyngitis, and cervical lymphadenopathy. Once acquired, the virus persists in the host throughout life. It is carried in "latent" form in peripheral blood B-lymphocytes and is shed in the form of infectious virus particles in saliva by healthy individuals which serve as a reservoir for reinfection.

Primary EBV infection elicits a cascade of antibodies directed at several structural and nonstructural viral proteins. IgM antibodies to EBV virus capsid antigen (VCA) appear quickly, persist only for a short time, and do not reappear. Thus, their presence is presumptive evidence of recent primary infection. Anti-early antigen (EA) antibodies appear next along with anti-VCA IgG antibodies. Subsequently, antibodies to EBV-nuclear antigen (EBNA) appear during resolution of symptoms. In previously infected individuals, anti-VCA and anti-EBNA IgG antibodies are always present. A dysfunction of the immune surveillance system against EBV infection permits productive cycles of viral replication, which lead to the production of EA and VCA IgG antibodies. Reciprocal titers to EA > 80 are believed to be indicative of active disease (either primary, chronic, or reactivated).

Virus-specific memory T-cells also have a critical role in controlling EBV reactivation and restricting proliferation of EBV-infected B-cells during latent infection. The basis for this evidence was *in vitro* observations, which revealed EBV-specific T-cells through their capacity to control virus-induced transformation of infected B-cells. Thus, when adult donor lymphocytes are exposed to virus and placed in culture, the proliferation of virus-infected B-cells (which occurs within the first 2 weeks postinfection) is followed by a complete regression of growth brought about

by cytotoxic T-lymphocytes (CTLs) reactivated *in vitro*. These CTLs do not kill autologous mitogen-activated blasts, but only MHC-matched EBV-infected B-lymphocytes (a classic CD8+, class I restricted response). Accordingly, these T-cells are not present in EBV-seronegative persons.

The importance of EBV-specific T-cells in controlling outgrowth of EBV-infected cells is also demonstrated clinically by the fact that severe T-cell immunodeficiency in humans, such as occurs in solid transplant recipients and in patients with AIDS, is associated with lympho-proliferative syndromes involving B-lymphocytes naturally infected with EBV. Notably, regression of polyclonal lymphomas has been reported after relaxation of immunosuppressive therapy in posttransplant patients.

Experiment Objectives

Recent studies have shown decreased immune function and alterations in leukocyte subpopulations during and after space flight. Stress-related changes (i.e., increased cortisol, epinephrine, and norepinephrine) appear to be significant factors involved in these immune changes. Related research has demonstrated that stress increases susceptibility to disease, with emphasis on decreased cellular immunity. Impaired cellular immunity, such as in AIDS and posttransplant patients, results in increased frequency and severity of herpesvirus infections (e.g., EBV). These observations led to the hypothesis that space flight and associated stressors will decrease EBV-specific T-lymphocyte immunity and reactivate latent EBV.

There are three main objectives associated with this experiment: (1) characterize EBV reactivation using serological and molecular techniques; (2) determine virus-specific T-

lymphocyte immunocompetence; and (3) measure stress hormones in plasma and urine.

As previously stated, decreased herpesvirus-specific T-cell immunity permits productive cycles of viral replication, which leads to increased production of anti-viral antibodies. One hypothesis to be tested in this experiment will be that stress will result in reactivation of EBV. Antibody titers to EBV lytic antigens will be measured just prior to space flight and compared to values obtained at baseline. Another hypothesis is that acute replication of EBV will occur as a result of space flight. Finally, viral load in peripheral blood will be quantitated along with molecular detection of EBV lytic transcripts.

Measurement of antigen (virus)-specific T-cell function will also be determined in this experiment. During acute EBV infection, there is a marked lymphocytosis comprised primarily of T-cells expressing CD45RO, a memory T-cell marker.²⁵ The CTL response, which includes both CD4⁺ and CD8⁺ T-cells, appears directed against a few epitopes that are mainly derived from EBNA 3, 4, and 6. This CTL response is also necessary for release of EBNA from the nuclear membrane of EBV-infected B-cell, since the anti-EBNA antibody is noticeably absent or low in immunologically compromised individuals. Thus, measurement of EBV-specific T-cells will be important to correlate with viral load and in assessing viral reactivation.

Stress hormones will also be measured in order to correlate stress levels with EBV reactivation. Importantly, additional stress may be associated with long-duration missions including crew rotations on Space Station, year-long lunar excursions, or Mars expeditions taking >1 year. Most of the environmental stressors have already been demonstrated in controlled studies to suppress immune function including sleep deprivation,

disruption of circadian rhythms, separation from family, vigorous exercise (e.g., multiple space walks), confinement, and sensory deprivation.

In summary, EBV reactivation, potentially exacerbated by the additional hazards of spaceflight, may have important health consequences during long-term space flights. This experiment will address fundamental questions on space flight and virus-specific immunity. Elevated stress hormone levels observed during space flight may decrease immune function that is required to fight infections and prevent tumor cell outgrowths. Thus, one potential concern is the development of a virally associated disease or lymphoma within the infected individual. In addition, reinfection or transmission to a previously uninfected individual (resulting in primary infection) may be another concern. Thus, space flight may result in an increased frequency and/or severity of both primary and reactivated disease. If increased reactivation and expansion of EBV-infected B-lymphocytes results, then pharmacological measures should be instituted for long-term missions. In addition, the study of astronauts (under stress and during space flight) may provide important information about the fundamental mechanisms underlying stress-associated immunological changes that may lead to a better understanding of latent herpesvirus reactivation in humans living on Earth.

References

1. Johnson PC. (1982) Medical operations and life science activities on space station. NASA technical memorandum 58248, National Aeronautics and Space Administration, Houston, Texas.
2. Gmunder FK, Konstantinova I, Cogoli A, *et al.* (1994) Cellular immunity in cosmonauts during long duration spaceflight on board the orbital MIR station. *Aviat. Space Environ. Med.* 65, 419-423.

3. Meehan RT, Whitson P, Sams CF. (1993) The role of psychoneuroendocrine factors on spaceflight-induced immunological alterations. *J. Leukoc. Biol.* 54, 236-244.
4. Taylor GR, Konstantinova I, Sonnenfeld G, Jennings R. (1997) Changes in the immune system during and after spaceflight. *Adv. Space Biol. Med.* 6, 1-32.
5. Stowe RP, Sams CF, Mehta SK, *et al.* (1999) Leukocyte subsets and neutrophil function after short-term spaceflight. *J. Leukoc. Biol.* 65, 179-186.
6. Fauci AS, Dale DC, Balow JE. (1976) Glucocorticosteroid therapy: Mechanisms of action and clinical considerations. *Ann. Inter. Med.* 84, 304-315.
7. Schleimer RP, Sterbinsky SA, Kaiser J, *et al.* (1992) IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium: association with expression of VCAM-1. *J. Immunol.* 148, 1086-1092.
8. Blotta MH, DeKruyff RH, Umetsu DT. (1997) Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ lymphocytes. *J. Immunol.* 158, 5589-5595.
9. DeKruyff RH, Fang Y, Umetsu DT. (1998) Corticosteroids enhance the capacity of macrophages to induce Th2 cytokine synthesis in CD4+ lymphocytes by inhibiting IL-12 production. *J. Immunol.* 160, 2231-2237.
10. Elenkov IJ, Papanicolaou DA, Wilder RA, Chrousos GP. (1996) Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: Clinical implications. *Proc. Amer. Assoc. Physicians* 108, 374-381.
11. Auphan N, DiDonato JA, Rosette C, Helmsberg A, Karin M. (1995) Immunosuppression by glucocorticoids: inhibition of NF- κ B activity through induction of I kappa B synthesis. *Science* 270, 286-290.
12. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS. (1995). Role of transcriptional activation of I κ B α in mediation of immunosuppression by glucocorticoids. *Science* 270, 283-286.
13. Blackwell TS, Christman JW. (1997) The role of nuclear factor- κ B in cytokine gene regulation. *Am. J. Respir. Cell Mol. Biol.* 17, 3-9.
14. Glaser R, Kiecolt-Glaser JK, Bonneau RH, *et al.* (1992) Stress-induced modulation of the immune response to recombinant hepatitis B vaccine. *Psychosom. Med.* 54, 22-29.
15. Sheridan JF, Feng N, Bonneau RH, *et al.* (1991) Restraint stress differentially affects anti-viral cellular and humoral responses in mice. *J. Neuroimmunol.* 31, 245-255.
16. Berry CA. (1969) Apollo 7 to 11: Medical concerns and results (NASA TMX-5). Washington DC, NASA.
17. Berry CA. (1970) Summary of medical experience in the Apollo 7 to 11 manned spaceflight. *Aerospace Med.* 41, 500-519.
18. Hawkins WR, Ziegleschmid JF. (1975) Clinical aspects of crew health. In: RL Johnston *et al.* (Eds), *Biomedical Results of Apollo (NASA SP 368)*, Washington DC, NASA, pp. 43-81.
19. Pagano JS, Lemon SM. (1986) The herpesviruses. In: A Braude *et al.* (Eds), *Infectious Diseases and Medical Microbiology*, Philadelphia, Saunders, pp. 470-477.
20. Ferguson JK, Taylor GR, Mieszkuc BJ. (1975) Microbial investigations. In: RL Johnston *et al.* (Eds), *Biomedical Results of Apollo (NASA SP 368)*, Washington DC, NASA, pp. 83-103.
21. Korsager B, Spencer ES, Mordorst C, Anderson HK. (1975) Herpesvirus hominis in renal transplant recipients. *Scand. J. Infect. Dis.* 7, 11-19.
22. Donnenberg AD, Chaikof E, Aurelian L. (1980) Immunity to herpes simplex virus type 2: Cell-mediated immunity in latently infected guinea pigs. *Infect. Immun.* 30, 99-109.
23. Khanna R, Burrows SR, Moss DJ. (1995) Immune regulation in Epstein-Barr virus-associated diseases. *Microbiol. Rev.* 59, 387-405.
24. Rickinson AB. (1990) *The Epstein-Barr Virus: Recent Advances*. New York, Wiley Medical Publications.
25. Kieff E, Liebowitz D. (1990) *Virology*. New York, Raven Press.