CONSEQUENCES OF CONTAMINATION OF THE SPACECRAFT ENVIRONMENT: IMMUNOLOGIC CONSEQUENCES

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ABSTRACT

Long-term space voyages pose numerous known and unknown health hazards, to the human immune system. Well-studied clinical examples of secondary immunodeficiencies created on Earth, lead one to predict that the conditions of prolonged space flight would weaken the human immune responses that normally hold infection and cancer in check. From evidence gathered from humans flown for prolonged periods in space and from human models of space flight studied on Earth, it is reasonable to suspect that space travelers to the planet Mars would experience a weakening of immunity. Subtle defects of immune system cell structure and function have been observed in astronauts, such as weakening of specific T-lymphocyte recall of specific antigens. Ground-based models also have demonstrated alterations of immune function, such as the elevation of neuroendocrine immune system messengers, interleukin-6, and soluble tumor necrosis factor-alpha receptor in sleep deprivation. Since severe immune compromise the clinical consequences of reactivation of latent virus infections and the development of cancer, has yet to be seen in space flight or in the Earth models, it is extremely important to begin to quantify early changes in immunity to predict the development of immune system collapse with poor clinical outcomes. This approach is designed to validate a number of surrogate markers that will predict trouble ahead. Inherent in this research is the development of countermeasures to reduce the risks of infection and cancer in the first humans going to Mars.

INTRODUCTION

The remarkable equilibrium by which man and animals maintain their integrity and ward off the threats of microbial infections, from without or within themselves, and the unregulated growth of transformed malignant cells is mediated by complex host defense systems comprised of specialized cells and proteins produced by memory-specific recall and by innate immune cells and proteins that require no previous sensitization. This host defense system, consisting of: antibodies, T-cells, phagocytes, and complement, all of which derive from the pleuripotent stem cell of the bone marrow (humans). The ontogeny of these components involves complex differentiation pathways that have been discovered to be susceptible to either genetic lesions or non-genetic lesions due to external conditions. Immune disorders that result from genetic lesions are called primary immunodeficiencies, and those that result from non-genetic lesions are called secondary immunodeficiencies. The best example of primary immunodeficiency is severe combined immunodeficiency disease (SCID), a rare (1:100,000 live births) disorder. David the "Bubble Boy", who survived for 12 years in a sterile bubble built by space engineers, is an example of a child with primary immunodeficiency that results from a mutation in the gamma chain of interleukin (IL)-2, 4, 7, 9, and 15 receptors on T-cells. The best example of secondary immunodeficiency is a person infected by the human immunodeficiency virus (HIV), who has the acquired immunodeficiency disease (AIDS). HIV has a predilection for the most important T-cell in the immune repertory, the helper T-cell (also known as the $CD4^+$ T-cell).

For the past 40 years, immunologists have been trying to gather definitive information on the possible development of a secondary immunodeficiency due to space travel (Shearer & Sonnenfeld, in press) From past and current research investigations, we know that all the conditions humans face in long-term space flight have an impact upon the immune system. The easiest to imagine is radiation, that for 100 years has been known to induce changes in host resistance to the point that infections and malignant cells gain the advantage and conquer the host. The science of space immunology, therefore, is founded upon well-received principles and assumptions that prescribe a need for well-planned and executed experiments capable of predicting the risks to the human immune system in long-term space travel. Inherent in these research projects is the need to consider a countermeasures program to make the risks to the immune system acceptable for astronauts in long-term space travel. To ignore the risks of secondary immunodeficiency as only theoretical concerns is to repeat the tragic experiences of the early practitioners of x-ray imaging in medicine, who took no special precautions for shielding themselves or their patients. Interplanetary space travel is a daunting challenge not just to engineers, but also to biomedical researchers, particularly immunologists, who want human astronauts to be able to return to Earth and enjoy both a normal life span and an excellent quality of life.

SPACE-INDUCED CHANGES IN HUMAN IMMUNITY

Immune cells

It is fairly certain that $CD4^+$ T-cells are affected by space travel, because lymphocyte proliferation to mitogens and antigens is decreased by the microgravity of space travel (Gmünder, et al., 1990). There is debate as to whether this decrease in lymphocyte proliferation is due to some inherent space flight induction or whether the lymphocytes, mitogens, and antigens do not attach to their appropriate cell surface receptors due to lack of gravity although in-flight centrifugation producing 1-*g* gravity

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still yielded lymphocyte cultures with reduced proliferation (Cogoli, 1993). Cytotoxic ($CD8^+$) T-cell killing of target cells and the function of macrophage as antigen-presenting cells have been shown to be decreased in human blood samples obtained in space flight (Gmünder, et al., 1994) and in the Jurkat human T-cell line (Limouse, et al., 1991). Production of certain cytokines, such as IL-1, IL-2, and tumor necrosis factor-alpha (TNF-α), by cultures of human peripheral blood leukocytes has been affected by the effects of space travel (Chapes, et al., 1992; Konstantinova, et al., 1993). Many of these observations were made upon cell cultures brought back to Earth, adding the confounding variables of storage, landing in multiple gravity stresses, and readaptation to Earth. In studying changes in immune cells and components in astronauts brought back to Earth, the following deviations have been recorded in their peripheral blood cells: decreases in lymphocyte numbers despite an increase in total leukocyte numbers, increases in the number of B-cells and $CD4^+$ T-cells, and decreases in the number of $CD8^+$ T-cells (Meehan, et al., 1992; Taylor, 1993). Delayed type hypersensitivity (DTH) skin test reactivity to recall antigens has been noted to be diminished in short- and long-term space flight (Taylor and Janney, 1992; Gmünder, et al., 1994). In all of these studies there is variability in reporting, and inconsistency of results is common. In general, very short space flights of less than 2 weeks duration do not produce alterations in these immune components.

Immunoglobulins

Specific human antibody function has not been studied in human space travelers. Instead, the indirect assessment of antibody function (serum immunoglobulin concentration) has been relied upon because of the difficulties in performing immunization during space flight. In long-term space voyages, an increase in serum IgA and IgM levels has been reported (Konstantinova and Fuchs, 1991), whereas in short-term space travel no increases in serum immunoglobulin concentration were noted (Voss, 1984).

Innate immunity

Natural killer (NK) cells require no prior sensitization to attack virus-infected cells, and they are thought to represent an early cell type for protection in the development of immune systems. Decreases in the capacity of NK cells to kill target cells in tissue culture has been recorded in peripheral blood cells obtained by short- and long-term space travelers (Woods and Chapes, 1994).

GROUND-BASED MODELS OF HUMAN SPACE FLIGHT: IMMUNE CHANGES

Stress, exercise, high altitudes, bed rest/head down tilt

Because stress is almost certainly a constant condition of human space flight, investigators have sought to establish a ground-based stress model where the same type of stress could be experienced by a large number of subjects at the same time. The most useful model turned out to be a large class of medical students at examination time. In this model, numerous subtle changes in peripheral blood immune cell numbers, lymphocyte proliferation, and cytokine production were observed (Kiecolt-Glaser, et al., 1986). After examination time, all of these alterations in immune function were reversed.

Moderate to extreme exercise causes an increase in neutrophil counts but a decrease in the ability of these neutrophils to lyse bacteria. Decreases in NK cell function, IL-2 production, lymphocyte proliferation, and $CD8^+$ T-cell numbers have also been observed (Nehlsen-Cannarella, et al., 1991).

Exposure to high altitude poses similar conditions (lowered air pressure, isolation, stress) that astronauts experience, and investigations of humans placed in this position showed similar changes in immune function to those recorded in astronauts after space flight (Meehan, et al., 1988).

The bed rest/head down tilt models (up to 6 months duration) mimic some conditions (muscle disuse, lack of load bearing on muscles and bones, and cephalad fluid shift) of space travel. Similarly, changes recorded in cytokine production, peripheral blood lymphocyte subset distribution, and neutrophil function resemble those occurring in astronauts during space flight (Hargens, et al., 1983). Unfortunately, the very small number of humans who are willing to participate in this model and the difficulty of assuming participants severely limits its usefulness.

Caves, chambers, Antarctic winter-over

Human enclosure in caves, chambers and during the Antarctic winter-over results in isolation that is thought to replicate the isolation astronauts experience during comparable time intervals in space. Accordingly, investigators have documented changes in cytokine production and peripheral blood lymphocyte subset distribution, but in early research conducted in the isolation model of the Antarctic winter-over, no increase in common respiratory virus infections was found (Williams, et al., 1986).

In collective studies over a 10-year period, researchers of the Australian National Antarctic Research Expedition (ANARE) were able to demonstrate in over 240 subjects a decrease in DTH skin test reactions to recall antigens (Muller, et al., 1995). During the 1993 winter-over, the Australian investigators correlated this depression of DTH skin test reactivity with an in vitro decreased mitogen proliferation response (Pitson, et al.,

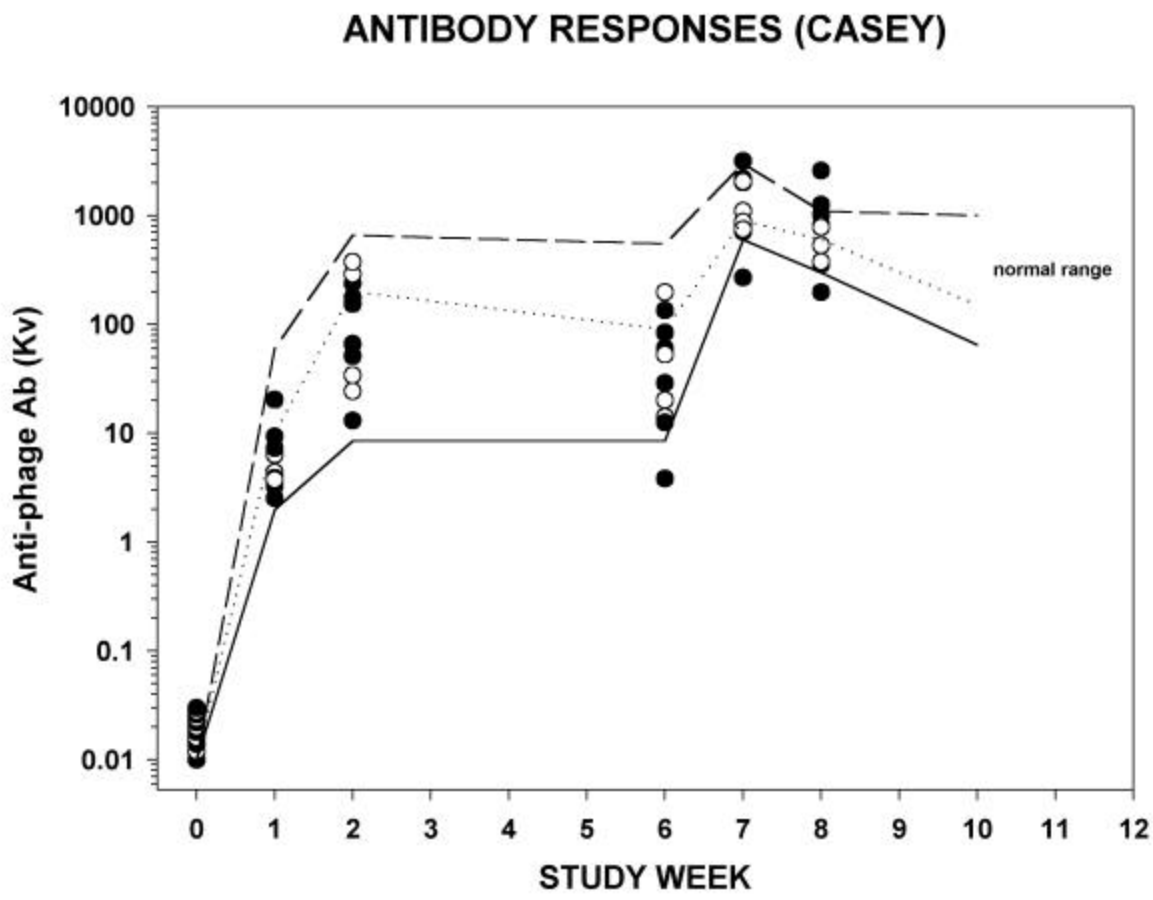

Figure 1. Subjects began the ANARE winter-over at the Antarctic Casey Outpost on March 1, 1999. *The 1st immunization with the bacteriophage X-174 was given on July 13, 1999 (study week 0); the 2nd immunization was given on August 24, 1999 (study week 6). Blood samples for antibody assays were obtained at the times indicated prior to and after each immunization. Each symbol represents the total antibody titer for an individual at the indicated time point. The normal range represents maximum, median, and minimum values, respectively, for normal subjects in Dr. Ochs' clinic and laboratory. Subjects on Macquarie Island had similar results (data not shown). Taken with permission from Shearer WT, et al. J Allergy Clin Immunol, 2001.*

1996). Moreover, alteration of cytokine production by the peripheral blood immune cells of these subjects was documented, as well as the important observations of induction of atypical infectious mononucleosis, increased shedding of herpesvirus, and expansion of the Epstein-Barr virus (EBV) lymphocyte population (Tingate, et al., 1997; Mehta, et al., 2000). Finally, the important control population of similar subjects stationed on Macquarie Island (harsh winter, but access to the mainland) did not display these changes

In collaborative prospective studies, investigators of the NSBRI Immunology, Infection, and Hematology Team and ANARE investigated the ability of subjects in the 1999 winter-over to respond to the neoantigen bacteriophage ϕX-174. Bacteriophage ϕX-174 is a virus that infects *E. coli.* It does not reproduce in man, does not cause illness, and engenders T-cell dependent antibody responses when given as a vaccine (Ochs, et al., 1971). It has been used to detect specific antibody responses to an antigen never experienced by humans under normal conditions. As such, over the past 3 decades it has proved invaluable in diagnosing states of primary and secondary immunodeficiency in infants, children, and adults (Wedgwood, et al., 1975; Pyun, et al., 1989; Fogelman, et al., 2000). Three months into the 8-month Antarctic winter-over, the primary immunization with bacteriophage, and 6 weeks later the secondary immunization, was given to test subjects in the Antarctic and to control subjects on Macquarie Island. Periodic plasma specimens were obtained, frozen at -70° C, stored until the Antarctic summer, and shipped on dry ice to the testing laboratory. Using well-established laboratory methods (Shearer, et al., 2001a), these researchers demonstrated that both test and control subjects had normal clearance of the bacteriophage from the blood (by 7 days), normal primary and secondary antibody responses, normal IgM to IgG switching, and normal percentages of IgG antibody (Fig. 1). Thus, the isolation of the Antarctic winter-over in the 1999 expedition did not demonstrate deficiencies of specific antibody response to the neoantigen bacteriophage ϕX-174. There is no reason to think that the 1999 expedition would be

different than earlier expeditions, although the leadership and crew were different. In so far as the Antarctic winter-over is a replica of some of the conditions of long-term space flight, it must be remembered that it lacks two important risk factors: microgravity and solar radiation. Therefore, using this model to assume that astronauts would not develop deficiencies of antibody responses is not warranted. Additional research on specific antibody production using other ground-based model systems will be necessary to make that prediction.

Sleep deprivation

There has been renewed interest in the importance of sleep in preserving normal host immune defense mechanisms (Moldofsky, et al., 1989; Dinges, et al., 1994; Born, et al., 1997; Krueger, et al., 1998). Several clinical conditions involving disturbance of the neuroendocrine immune system balance have suggested a causal relationship between lack of proper sleep and development or accentuation of pathological conditions (Ganguli, et al., 1993; Weitzman, et al., 1994; Born, et al., 1995). The stress of sleep deprivation and sleep disruption is known to be a normal component of life aboard spaceships or space stations (Santy, et al., 1988; Takasaki, et al., 1993; Gundel, et al., 1993; Gundel, et al., 1997; Monk, et al., 1998). In addition to denying sleep, the sleep deprivation model also contains the stress of rigid adherence to a set schedule of motor-cognitive tasks. Astronauts in their daily routines experience the latter stress. The scientific background of these human experiences has been provided by animal experiments that have documented a linkage of common cytokine messenger molecules that connect the components of the neuroendocrine immune system. Interleukin-1β, IL-6, and TNF-α instill sleep when placed into the cerebral cortex of small animals (Krueger, et al., 1998). These effects of sleepiness are known to be mediated through activation of the transcription factor NFkB (Chen, et al., 1999), which causes upregulation of receptors for these cytokines, such as the receptor for TNF-αRI which regulates sleep (Fang, et al., 1997). The sleep deprivation model has demonstrated that humans deprived of sleep experience increases in IL-1β, IL-6, leukocytes, NK cells, and IL-6 (Dinges, et al., 1995; Dinges and Chugh, 1997; Vgontzas, et al., 1999; Späth-Schwalbe, et al., 1998). Administration of endotoxin to humans raises blood levels of TNF-α and IL-1β and induces sleep (Herman, et al., 1998; Korth C, et al., 1996).

In the context of these discoveries of sleep deprivation and altered immune responses, investigators of the NSBRI have utilized plasma samples obtained from human subjects enrolled in an Air Force Office of Research study to examine whether levels of cytokines and their receptors are altered in human subjects prevented from sleeping for 88 hours in two cohorts: one, with total sleep deprivation (TSD) and the other with two 2-hour naps permitted at 12 AM and 12 PM on days 2, 3, and 4 of the sleep deprivation period (Shearer, et al., 2001b). The TSD group produced increased plasma soluble TNF-αI (Fig. 2) and IL-6 (Fig. 3) levels compared to the PSD group, indicating that even in the relatively short time of 4 days, lack of sleep altered production of a sleep regulating cytokines and cytokine receptor. These results are supported by the observations of other investigators that link TNF-αRI and IL-6 to sleepiness. Although the total sleep deprivation in this experimental system is unlikely to be endured by astronauts in a given period of time, chronic sleep restriction (such as only 4 hours per night for 14 days) results in escalating homeostatic sleep drive to levels equivalent to total sleep deprivation (Kuo, et al., 1998). It is known that chronic elevation of blood cytokines leads to upregulation of viral receptors on target cells, such as is seen in HIV infection where high levels of IL-1β, TNF-α, IL-6, and IL-10 increase the target cell expression of chemokine receptors that facilitates entry of the HIV virion (Belec, et al., 1994). Latent EBV is known to become reactivated by alterations of immune response that can lead to chronic infections and development of virus-driven lymphomas (Shearer, et al., 1985; Aguilar, et al., 1999). Thus, this model system of sleep deprivation may be suggestive of possible grave consequences of immune system aberration in long-term space travelers deprived of sufficient sleep.

COUNTERMEASURES

The National Aeronautics and Space Administration and NSBRI Critical Pathway Risks have established levels of countermeasure preparedness. Since the risks posed for astronauts on long voyages are suspected and not proven, the level of countermeasure readiness is, expectedly, at a research level. Nevertheless, because of the 4-decade-long experiences with humans with congenital immunodeficiencies and the nearly 20-year history of coping with the best understood secondary immunodeficiency, AIDS, considerable progress has been made in restoring immunity and preventing it from being damaged. Restoration of humoral immunity can be accomplished with immunoglobulin treatments, and bone marrow stem cells can replace defective or damaged stem cells and their immune system descendants. For decades vaccines have prevented human infection, and simple measures such as adequate nutrition and proper sleep have enabled humans to avoid serious complications of intercurrent Infections. Application of these measures to restore immunity or even prevent immune damage in space needs to have a high priority.

SURROGATE MARKERS TO MEASURE IMMUNITY IN SPACE

Using data gathered from human models of immunosuppression in therapeutic radiation, malnutrition, and AIDS, it is clear that a system for detecting alterations in the immune responses of space travelers is

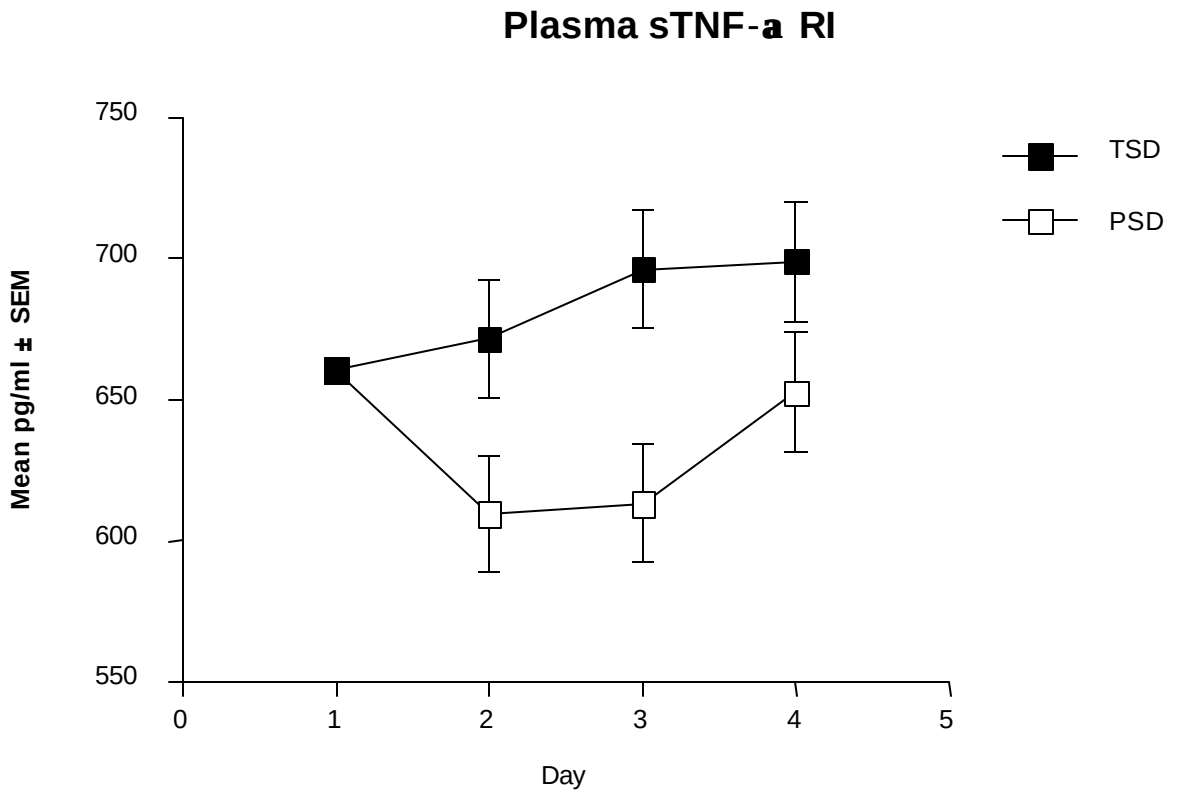

Figure 2. *Sequential daily plasma sTNF-αRI levels from n=21 healthy young adult males undergoing 88-hours of total sleep deprivation (TSD) from the morning of Day 1 through Day 4; and from n=21 healthy adult males undergoing 88-hours of partial sleep deprivation (PSD) on Days 1-4, which consisted of two 2-hour nap opportunities per day (i.e., every 12 hours, at 2:45 p.m. to 4:45 p.m. and 2:45 a.m. to 4:45 a.m. Since the TSD and PSD conditions were not different until Day 2 (naps began), the Day 1 data points were averaged to a single value and the subsequent data points (Days 2-4) were given as single data points (either TSD or PSD), with adjustment of values for the normalization of data in Day 1. Daily means (SEMs) represent the average of plasma levels at four time points each day (9:00 p.m., 3:00 a.m., 9:00 a.m., 3:00 p.m). See text of results of statistical analyses. Taken with permission from Shearer WT, et al. J Allergy Clin Immunol, 2001.*

needed. The limitations of testing equipment and laboratory reagents, plus the lack of time and expertise in performing complex laboratory analysis in space, make it imperative that surrogate markers of immune failing be established and validated. Currently, the most critical measurement of specific immunity is the CD4+ T-cell count, which has been illustrated by the AIDS model. Although this classification scheme for degree of immunosuppression was discovered for a specific viral infection, the application can be made to other conditions of immunosuppression, such as cancer chemotherapy. $CD4^+$ T-cell counts falling into less than 200 cells/μl of blood predict that the individual will become susceptible to opportunistic infections. The ability to use this surrogate marker in astronauts in long-term space travel will prove invaluable in determining impairment in immunity. This and other surrogate markers of protective immunity need to be adapted for long-term space flights, such as that of the journey to Mars.

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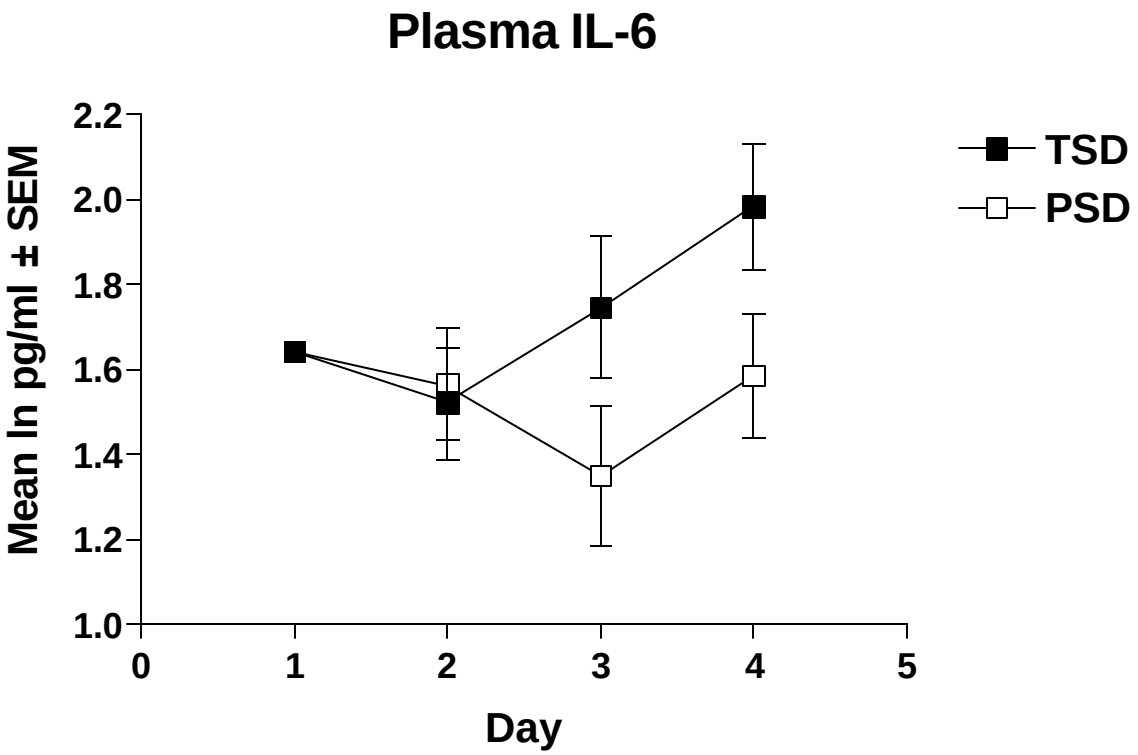

Figure 3. *Sequential daily plasma IL-6 levels from n=19 healthy young adult males undergoing 88 hours TSD from the morning of Day 1 through Day 4; and from n=21 healthy adult males undergoing 88-hours PSD on Days 1-4, which consisted of two 2-hour nap opportunities per day (see Methods). IL-6 data were normalized using a natural log (ln) transformation. Day 1 data points were further normalized to a single value and the subsequent data points (Days 2-4) were given as single data points (either TSD or PSD), with adjustment of values for the normalization of data in Day 1. Daily means (SEMs) represent the average of plasma levels at four time points each day (9:00 p.m., 3:00 a.m., 9:00 a.m., 3:00 p.m.). See text for results of statistical analyses. Taken with permission from Shearer WT, et al. J Allergy Clin Immunol, 2001.*

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