Immune responses in adult female volunteers during the bed-rest model of spaceflight: Antibodies and cytokines

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Background: It is unknown whether a prolonged period of bed rest will affect human immune responses, particularly in female subjects.
Objective: We sought to measure immune responses in adult female subjects exposed to prolonged bed rest.
Methods: Adult (25-40 years) female volunteers (n = 24) were maintained in a supine (6° tilt) head-down bed-rest (HDBR) position for 60 days: 8 with HDBR only, 8 with HDBR and regular muscular exercise, and 8 with HDBR and dietary protein supplementation. Subjects were immunized with bacteriophage φX-174. Antibody production and plasma cytokine levels were determined.
Results: The rate of primary antibody production of the HDBR plus exercise group increased faster ($P = .01$) and to a higher level versus that of the HDBR-only group ($P = .03$) and that of the HDBR plus diet group (trend $P = .08$). The rates of secondary antibody production between the 3 groups were similar, but the level of antibody in the HDBR plus exercise group remained higher versus that in the HDBR-only group ($P = .03$). Both the HDBR ($P = .001$) and HDBR plus diet ($P = .02$) groups had time-related progressive increases in TNF-α receptor levels, but the HDBR plus exercise group remained at baseline. The HDBR plus exercise group experienced an acute increase in IL-1 receptor antagonist levels versus the HDBR ($P = .02$) and the HDBR plus diet ($P = .02$) groups, with similar increases in RANTES levels.
Conclusions: The exercise countermeasure accelerated primary antibody production and increased antibody levels to bacteriophage φX-174 and also opposed the potentially harmful effects of increased TNF-α levels caused by prolonged bed rest, possibly by activating the anti-inflammatory cytokine IL-1 receptor antagonist and the chemotactic factor RANTES.
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Key words: *Head-down tilt, bed rest, φX-174 immunization, IgG class switching, spaceflight model, proinflammatory and anti-inflammatory cytokines, female study subjects*

One of the most crucial concerns about using human subjects in space travel is the effects of extreme environments and conditions that might weaken immunity primed for life on earth and lead to chronic infection, autoimmune disease, and the development of malignancy.[1,2] In addition to space radiation,[1-4] weightlessness in space caused by microgravity is a major risk of long-duration spaceflight that might lead to serious health hazards.[5,6] In trying to design model systems that mimic the effects of microgravity, the supine head-down (6°) tilt with bed-rest (HDBR) model has been developed. This model results in a loss of load bearing on lower extremities with loss of muscle mass and bone changes and a shift of fluid to the head, findings discovered in space travelers.[7] Almost all previous evaluations of immunity in the HDBR model have been performed in male subjects, leaving a vacuum of information on female subjects.[8] Studies in human medicine demonstrate a profound effect of sex hormones on antibody responses, as seen in the 3:1 to 4:1 ratio of female to male subjects experiencing abnormal and pathologic antibody responses in autoimmune diseases.[9] Several countermeasures have been developed to correct the negative effects of the spaceflight environment on the musculoskeletal system, such as aerobic and load-bearing exercises and addition of branched-chain amino acids to the diet.[10]

Immunization with the T cell–dependent neoantigen bacteriophage φX-174 (φX-174)[11] has been used to measure primary and secondary antibody responses in human subjects in the Antarctic spaceflight model to evaluate the risks that spaceflight poses for human subjects involved in space exploration.[12] Studies of

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Abbreviations used
GEE: General estimating equations
HDBR: Head-down bed rest
IL-1RA: IL-1 receptor antagonist
Kv: K value
φX-174: Bacteriophage φX-174
sTNFR1: Soluble TNF receptor 1

plasma cytokine balances in these and other study subjects revealed evidence of T-cell activation events, possibly in response to shedding of Epstein-Barr virus (EBV).[12-15]

In the current investigation we assessed the ability of adult female subjects to respond appropriately to immunization with φX-174 and concomitantly measured their plasma cytokine levels.

METHODS

Study population

Twenty-four healthy female volunteers, 25 to 40 years old and nonsmoking, participated in this experiment (Women's International Space Stimulation for Space Exploration-500 60-day HDBR study).[7] They were distributed in 3 groups: 8 control subjects, 8 subjects assigned to regular muscular exercise, and 8 subjects receiving dietary protein supplementation. The Institutional Review Boards of the Centre National d'Etdudes Spaticles, the European Space Agency, the National Aeronautics and Space Administration of the United States, the Canadian Space Agency, Baylor College of Medicine, the University of Texas MD Anderson Cancer Center, and Binghamton University approved the protocol and consent forms, and all participants signed an informed consent form. The experiments were performed at the Hopital Rangueil, Universite Paul Sabatier, Institut de Médecine et Physiologie Spatiale, Toulouse, France, during 2005. The experiment lasted 100 days: 20 days for baseline data collection before the day (T0) of randomization to groups and confinement, 60 days of absolute HDBR, and 20 days (assume normal position) for recovery.

Nutritional supplementation

The energy intake provided to the study subjects during the HDBR period for all groups was limited to 110% of the resting metabolic rate, as determined by means of indirect calorimetry. The protein intake of the control and exercise groups was limited to 1.0 g of dietary protein per kilogram of body weight per day, whereas that of the nutritional supplementation group was increased to 1.45 g of dietary protein per kilogram of body weight per day in an attempt to counteract muscle atrophy.[7] This increased protein was supplied in the form of 3.6 g of free leucine, 1.8 g of free isoleucine, and 1.8 g of valine at meals. The total daily maximum energy was achieved by decreasing proportionally carbohydrate and fat intake.

Exercise (supine position) program

By using a special back-support waist seal, leg-suspension pulleys, and a horizontal treadmill lower body negative pressure chamber, rigorous exercise (resistance and aerobic) was examined as a variable in the 60-day HDBR position.[7,16-18] Exercise subjects performed 40 minutes of exercise, followed by 10 minutes of rest. During the course of the 60-day bed-rest period, 29 exercise sessions (3-4 sessions per week) were prescribed for each subject.

Plasma samples

Periodic blood specimens during the entire 100-day experiment were drawn from the subjects on waking between 7 and 7:30 AM and placed in anticoagulation tubes (Na–ethylenediamine tetra-acetic acid, 1 mg/mL of blood, and 300 kallikrein-inhibiting units/mL aprotinin). These plasma specimens were centrifuged and immediately frozen at −70°C and shipped on sufficient dry ice to Houston (cytokine assays) and Seattle (φX-174 assay).

φX-174 immunization

Study subjects were given a modified (3-week shortened) immunization protocol plan (3 weeks between primary and secondary antigen inoculations instead of the standard 6-week interval) because of the need for other life scientists to collect samples and the 60-day limitation of the HDBR position. Accordingly, subjects were immunized with the standard preparation (1×10^{11} plaque-forming units/mL) of φX-174 at a dose of 0.02 mL/kg body weight. This dose of vaccine was given 21 days into the HDBR position and again at 42 days (T21 and T42, respectively). Blood was obtained for assessing circulating φX-174 levels at 15 minutes and 1 week after the first immunization and for φX-174–specific neutralizing antibodies before and at 1 and 3 weeks after primary immunization in contrast to the routine 1, 2, 4, and 6 weeks after primary immunization and at 2, 4, and 5.4 weeks after secondary immunization (instead of 1, 2, and 4 weeks).[11]

Assay of immune responses to φX-174

φX-174 clearance was determined by using a plaque assay.[11,12] Total φX-174–specific antibody levels in each plasma sample were determined by using a φX-174 neutralization assay, and the antibody level was expressed as the rate of φX-174 inactivation, or K value (Kv), as derived from a standard formula.[11] Specific antibody titers were plotted as $\log_{10}$ Kv against time. Twenty-one male and 31 female subjects, ages 18 to 45 years, formed the normal historical control cohort. Plasma specimens collected at 2 weeks after secondary immunization (T56) were treated with 2-mercaptoethanol to estimate the subjects' ability to switch from IgM to IgG antibody.[11]

Plasma cytokine and chemokine levels

Plasma samples were analyzed for cytokines, soluble cytokine receptors, and chemokines by using Luminex multiplexed fluorescent bead immunoassays (Luminex Corp, Austin, Tex) that were blinded to experimental conditions.[14] The minimum detectable levels of analytes with the above-mentioned kits were as follows: RANTES, less than 15 pg/mL; IL-1 receptor antagonist (IL-1RA), 2.06 pg/mL; soluble TNF receptor 1 (sTNFR1), less than 15 pg/mL; IL-10, 0.13 pg/mL; and IL-12p40, less than 15 pg/mL. The interassay coefficient of variation was 3% to 11.6%, and the intra-assay coefficient of variation was 4.3% to 18.7%.

Statistical analysis

All analyses were performed with the SAS DATA, MEANS, TTEST, and GENMOD procedures. All statistical tests were conducted by using a significance level of .05 for declaring statistical significance.

Antibody responses to bacteriophage φX-174. In an effort to anchor the analysis with the best starting point for each group's regression trajectory, it was assumed that each group-specific trajectory was flat between time 14 days (T14) and the first φX-174 injection point (T21). From T21, a knotted linear spline with knots at T28, T42, and T60 was fit to the data using the general estimating equations (GEE) methods of Liang and Zeger,[19] which allow for correlation among repeat observations within individuals. Statistical inferences were performed by comparing the different slopes by using the estimated β coefficients from the GEE analysis and by comparing the point estimates at 21, 28, 42, 60, and 80 days. The choice of knots corresponds to times when significant changes were made to each subject's condition or when important measurements were made.

Data are presented graphically as the point estimates of the raw geometric means and the estimated linear spline obtained from the GEE analysis data with 95% confidence limits for the spline at the knots of each curve.

The IgG percentage was calculated by dividing the IgG count from one sample by the averaged antibody counts (IgG and IgM combined) from 2 samples collected at T56.[11] The SAS GENMOD procedure was used to detect the difference of IgG percentage among the 3 groups at T56.