

Antibody responses to bacteriophage ϕ X-174 in human subjects exposed to the Antarctic winter-over model of spaceflight

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Background: It has been proposed that exposure to long-term spaceflight conditions (stress, isolation, sleep disruption, containment, microbial contamination, and solar radiation) or to ground-based models of spaceflight will alter human immune responses, but specific antibody responses have not been fully evaluated.

Objective: We sought to determine whether exposure to the 8-month Antarctic winter-over model of spaceflight would alter human antibody responses.

Methods: During the 1999 Australian National Antarctic Research Expeditions, 11 adult study subjects at Casey, Antarctica, and 7 control subjects at Macquarie Island, sub-Antarctica, received primary and secondary immunizations with the T cell-dependent neoantigen bacteriophage ϕ X-174. Periodic plasma samples were analyzed for specific antibody function.

Results: All of the subjects from Casey, Antarctica, cleared bacteriophage ϕ X-174 normally by 1 week after primary immunization, and all had normal primary and secondary antibody responses, including immunologic memory amplification and switch from IgM to IgG antibody production. One subject showed a high normal pattern, and one subject had a low normal pattern. The control subjects from Macquarie Island also had normal immune responses to bacteriophage ϕ X-174.

Conclusions: These data do not support the hypothesis that de novo specific antibody responses of subjects become defective during the conditions of the Antarctic winter-over. Because the Antarctic winter-over model of spaceflight lacks the important factors of microgravity and solar radiation, caution must be used in interpreting these data to anticipate normal antibody responses in long-term spaceflight. (*J Allergy Clin Immunol* 2001;107:160-4.)

Key words: Spaceflight model, Antarctic winter, ϕ X-174 antibody responses

Abbreviations used

ANARE: Australian National Antarctic Research Expeditions
DTH: Delayed-type hypersensitivity

For several decades, investigators in space exploration have debated about whether the human immune system would be adversely affected by the conditions of long-term space travel (ie, stress, isolation, sleep disruption, containment, microbial contamination, and radiation).¹ Evidence obtained from space travelers has demonstrated diminished or altered PBMC reactivity to mitogens,²⁻⁶ T-cell killing of target cells,⁴⁻⁶ production of cytokines,⁷⁻¹⁰ distribution of PBMC subsets,^{11,12} natural killer cell activity,¹⁰ and delayed-type hypersensitivity (DTH) skin tests to recall antigens.^{13,14} B-cell immunity has been tested by measurements of serum Ig levels, which show transient increased values in long-term spaceflight up to several months in one study¹⁵ but no increase in short-term spaceflight up to several weeks in another study.¹⁶ It must be acknowledged that changes in serum Ig concentrations do not equate to changes in serum antibody concentrations. Almost all of these reports suffer from the fact that the analyses were performed on very small numbers of subjects with blood specimens brought back to Earth, with all the possible confounding variables incurred because of storage and delay. Numerous ground-based model systems have been used in attempts to reproduce various conditions of space travel that may alter human immune responses.¹ Stress models include academic stress models,^{17,18} sleep disruption models,¹⁹⁻²¹ strenuous exercise,²² high altitudes,²³ and the bed-rest/head-down tilt model.²⁴ Isolation, both in the form of placing individuals in isolation chambers or caves, as well as the Antarctic winter-over model, has been used to demonstrate the effects of spaceflight on immune responses.²⁵⁻²⁹ These studies in caves and isolation chambers have yielded changes in immune responses similar to those observed during spaceflight, including decreased cytokine production and alterations in leukocyte subset distribution. Several studies involving over 240 subjects (in several winter experiments) have been performed by the Australian National Antarctic Research Expedition (ANARE), which demonstrated a statistically

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significant decrease in DTH skin tests to recall antigen on numerous occasions compared with an appropriate control cohort.³⁰⁻³³ These *in vivo* tests of immune suppression were correlated in a cohort of 17 subjects with their *in vitro* counterparts during the 1993 ANARE winter-over study.³⁴ This report confirmed the depression of cellular mediated immunity by the DTH test and demonstrated a correlation with a 50% decreased PHA proliferation test result, altered production of inflammatory cytokines, induction of atypical mononucleosis, increased herpesvirus shedding, and expansion of the EBV-infected B-lymphocyte population.^{34,35} These abnormal immune responses were seen in ANARE missions that involved total isolation and confinement in harsh winter conditions for 8 months. It must be acknowledged that in all of these ground-based models of the effects of spaceflight, at least 2 very important factors (microgravity and solar radiation) are missing. Nevertheless, short of performing these experiments in spaceflight, almost a virtual impossibility because of insufficient sample sizes and lack of sophisticated immunologic laboratories aboard spaceships, it is important to proceed with these model systems on Earth to be able to predict the possible harmful consequences of long-term spaceflight on the human immune system.

Therefore to determine specific antibody responses to a neoantigen in human subjects exposed to the Antarctic winter-over model of spaceflight, we immunized test and control subjects with a T cell-dependent neoantigen, bacteriophage ϕ X-174, during the 1999 ANARE winter-over study and measured their viral clearance and primary and secondary antibody responses.

METHODS

Web site video

For an illustration of actual spaceflight and ground model conditions and experiments, go to the Journal's home page at <http://www.mosby.com/jaci>.

Study population and informed consent

Subjects were members of 2 ANARE groups. The first consisted of 11 men (ages 24-55 years; mean, 39 years) who overwintered at Casey, located in East Antarctica (66° 17'S, 110° 32'E). This expedition left Australia by ship in October 1998, arrived at Casey in November, and was totally physically isolated between early April 1999 and late November of that year. The members of the expedition returned to Australia by ship in December 1999. The second group of 7 men (ages 26-47 years; mean, 36 years) overwintered on Macquarie Island (54° 29'S, 158° 57'E) in the sub-Antarctic. They left Australia and arrived at Macquarie Island in November 1998 and were isolated between mid-April and early September 1999 but could have been retrieved by ship during this period because the island is not subject to sea ice. The subjects returned to Australia in December 1999.

Although the climate at Macquarie Island is wet and cold and requires different outer clothing as that for the dry and cold conditions in Casey, living conditions, food, and scientific programs are similar. Macquarie Island is a World Heritage Island and has prolific wildlife. No air access is available to either station during the austral winter. In view of the possibility of reaching Macquarie Island by ship throughout the whole year, the second group was chosen as a control group.

All subjects were volunteers and had undergone a stringent pre-departure medical examination, which included clinical, psychological, and laboratory examinations. They were vaccinated against tetanus, hepatitis A and B, poliomyelitis, and tuberculosis. The study was approved by the Australian Antarctic Division Ethics Committee (Human Experimentation), and informed consent was obtained from each subject after the nature and possible consequences of the study were fully explained.

Bacteriophage ϕ X-174 immunization

The host strain for bacteriophage ϕ X-174 is *Escherichia coli*. The bacteriophage does not replicate or cause illness in human subjects, and it induces helper T cell-dependent antibody responses when used as an immunogen.³⁶ Over the past 3 decades, it has proved invaluable in identifying abnormalities in the primary (IgM) and secondary (IgG) antibody responses in immunodeficient and immunosuppressed infants, children, and adults.³⁶⁻⁴² ANARE subjects were immunized intravenously at the start of the third month of total physical isolation (July 1999, primary injection) of the Antarctic winter-over and 6 weeks later (booster injection) with doses of 0.02 mL/kg body weight with a standard preparation containing 1×10^{11} plaque-forming units/mL. The immunizations were well tolerated. Blood was obtained at 15 minutes, 1 week, 2 weeks, and 6 weeks after each immunization, and the plasma was frozen at -70°C. During the Antarctic summer (December 1999-January 2000), the frozen plasma specimens were delivered (on dry ice) to the testing laboratory in Seattle.

Assay of immune responses to bacteriophage ϕ X-174

Bacteriophage clearance was determined by using a previously described plaque assay.³⁷ Plasma obtained at 15 minutes after primary immunization was compared with the 1-week postimmunization sample for each subject studied.

Total ϕ X-174-specific antibody in each plasma sample was determined by using a standardized phage neutralization assay³⁷ and was expressed as the rate of phage inactivation or K value (Kv) as derived from a standard formula. Specific antibody levels were plotted as log Kv against time.

Plasma specimens collected at 2 weeks after secondary immunization were selected to estimate the subjects' ability to switch from IgM to IgG antibody. The relative percentage of IgG phage-specific antibody was assessed by comparing Kv values for 2-mercaptoethanol-treated samples with untreated samples from the same plasma specimen. Antibody resistant to 2-mercaptoethanol was considered to be IgG.³⁷ This method of differentiation of IgG specific for ϕ X-174 bacteriophage has been validated by an enzyme-linked immunoassay by using normal control subjects.³⁸ In this validation study there was an orderly switch from IgM to IgG3/IgG1 and, after multiple (tertiary) immunizations, to IgG2/IgG4, but IgG1 was still more than 50%, followed by IgG2 and IgG3. Several months after the tertiary (long-term) immunization, 80% was IgG1, 10% was IgG2, and IgG3 and IgG4 were less than 1%. During the primary response, there was a very small amount of IgG1 and IgG3 (only at 6 weeks, just before the secondary immunization is given). During the secondary response, over 80% was IgG1 and IgG3, 18% was IgG2, and <1% was IgG4. All of these antibody responses were against phage-associated antigens.³⁸

Statistical analysis

Antiphage antibody titers for subjects were compared with normal values obtained for a group of historical male control subjects in the laboratory of Dr Hans Ochs. Titers at specified time points for

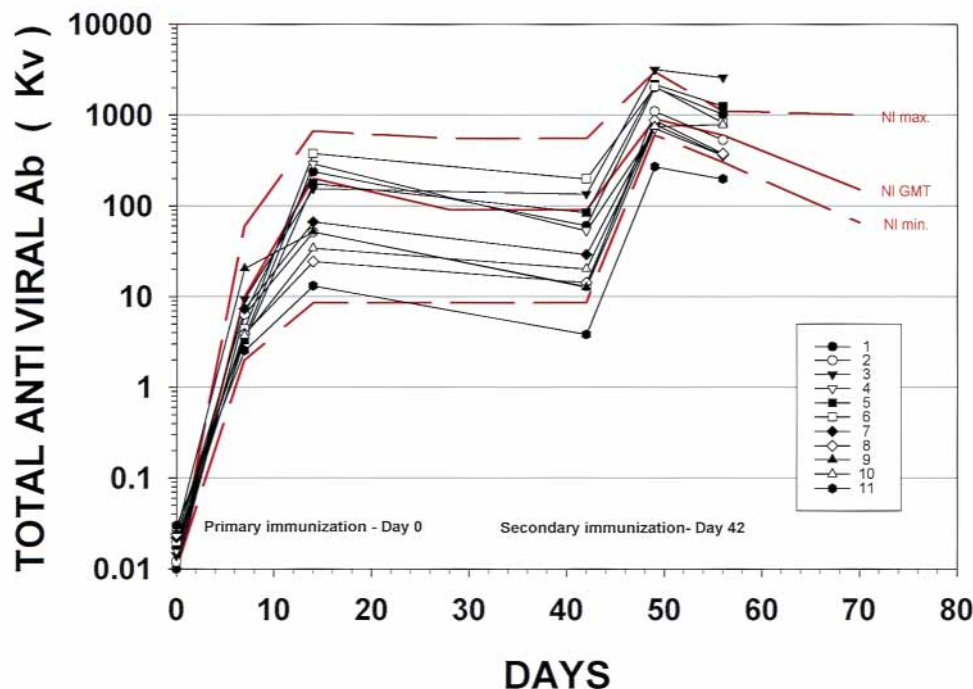


FIG 1. Antibody responses to the neoantigen bacteriophage ϕ X-174 in human study subjects in the Antarctic winter-over model of spaceflight in Casey, Antarctica. Study subjects ($n = 11$) began the ANARE winter-over isolation at Casey, Antarctica, on April 7, 1999. The first immunization with bacteriophage ϕ X-174 was given on July 13, 1999 (study week 0); the second immunization was given on August 24, 1999 (study week 6). Blood samples for antibody assays were obtained at the times indicated before and after each immunization, and each symbol represents the total antibody titer for an individual at the indicated time point; subjects are numbered 1 to 11. The normal (NI) range represents maximum (*max*) and minimum (*min*) values, respectively, for normal subjects. GMT, Geometric mean titer.

TABLE I. Summary of bacteriophage ϕ X-174 responses in study subjects at Casey, Antarctica ($n = 11$) and control subjects on Macquarie Island, sub-Antarctica ($n = 8$)

	Casey	Macquarie Island	Normal men* ($n = 20$)
Phage clearance by day 7	11/11 (100%)	8/8 (100%)	100%
Primary response† (Kv) at day 14	83.4 (14-371)	117 (28-810)	84 (8-697)
Secondary response† (Kv) at day 56	650 (206-2526)	471 (118-1687)	303 (126-784)
IgM to IgG switch	11/11 (100%)	8/8 (100%)	100%
% IgG‡	45 (18-85)	42 (19-82)	47 (22-76)

*Values are from 20 normal male control subjects (see Ochs et al³⁶ and Wedgewood et al³⁷).

†Values represent geometric means and 95% confidence intervals.

‡Values represent arithmetic means and 95% confidence intervals.

the control group (Macquarie Island) and the experimental group (Casey station) were compared by using Mann-Whitney rank sum analysis for nonnormally distributed data and a 2-tailed *t* test for normally distributed data.

RESULTS

Bacteriophage ϕ X-174 clearance was completed by 7 days in all 11 subjects, which was similar to that found in normal control subjects. The primary and secondary antibody responses to bacteriophage ϕ X-174 immunizations for the Casey, Antarctica, study subjects are presented in Fig 1. All of the study subjects' specific antibody responses during primary immunization fell within the normal range, except for that of study subject 11, whose

6-week postprimary (presecondary) Kv was slightly below the normal range. During the secondary response, all subjects demonstrated amplification, immunologic memory, and switch from IgM to IgG antibody (note the logarithmic scale). One subject (study subject 3) had values slightly above and one (study subject 11) had values slightly below the normal range. These responses of the study subject cohort and those of the control cohort on Macquarie Island are summarized in Table I.

DISCUSSION

These results demonstrate that during the 1999 ANARE Antarctic winter-over study, human subjects immunized

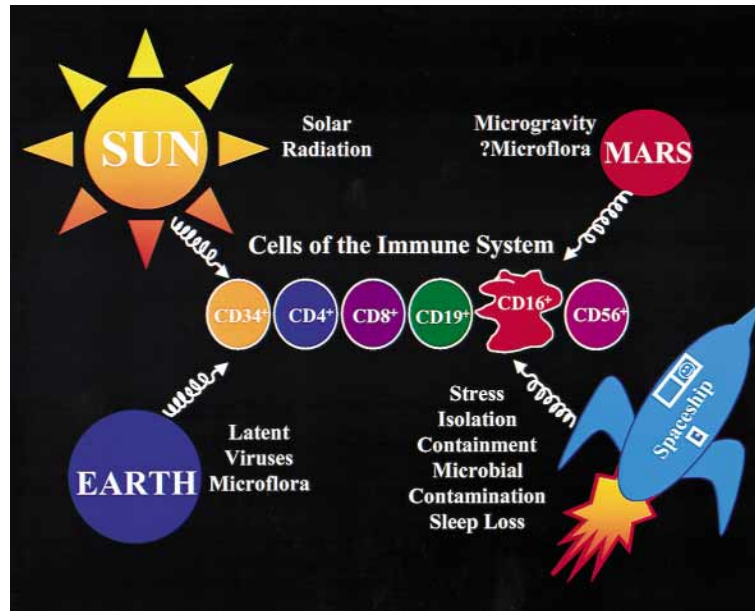


FIG 2. Conditions of long-term spaceflight that may weaken cells of the immune system. The voyage to and from the planet Mars is estimated to consume 3 years. During that time, human space travelers will be exposed to stress, microgravity, isolation, containment, sleep disruption, microbial contamination, and solar radiation (up to 2 Gy of proton and gamma radiation). All of these conditions are known or suspected causes of immunosuppression, which is possibly sufficient to lead to reactivation of latent viral infections and malignancy. The immune cells that may be susceptible to these causes of immunosuppression include the bone marrow stem cell (CD34⁺), helper T cell (CD4⁺), cytotoxic T cell (CD8⁺), B cell (CD19⁺), monocyte-macrophage (CD56⁺), and natural killer cell (CD16⁺). Because of the inherent difficulties of assessing these risks in spaceflight, ground-based models that incorporate some of the conditions of long-term spaceflight offer the best hope of adequately predicting the harm that may occur to the human immune system in interplanetary travel.

with the T cell–dependent antigen bacteriophage ϕ X-174 exhibited normal primary and secondary immune responses. Because CD4⁺ T cells are required to provide costimulatory signals to B cells through the CD40 ligand/CD40 signaling pathway and through the production of cytokines to achieve isotype switching and immunologic memory, it appears that the CD4⁺ T-cell function was not affected by the Antarctic exposure. The production of predominantly IgM during the primary and IgG during the secondary antibody responses was of equal quality and quantity in study and control subjects, and antibody-dependent clearance of bacteriophage ϕ X-174 virus was intact. These results differ from those of this 1993 ANARE study,³¹ but different techniques with assessment of immune function were used (antibody assay in 1999 vs DTH skin test responses in 1993). The 1993 ANARE group and the 1999 ANARE group all had the same living conditions, food, clothing, and work programs but had different leadership and a different mix of participants. This might account for a less stressed group in 1999 than in 1993, although many ANARE groups over a 10-year period had altered immune effects.

Thus to the degree that the Antarctic winter-over experience mimics the conditions of space travel, the present experiment does not predict that humoral anti-

body deficiency will develop to a T cell–dependent neoantigen, such as bacteriophage ϕ X-174. However, compared with the anticipated 3-year duration of the Mars Mission, the 8-month duration of the Antarctic exposure may not have been sufficient to cause antibody deficiency. Also, in addition to the stress, isolation, and containment of the Antarctic winter-over study, long-term space travel poses the additional hazards of microgravity, microbial contamination (recycling of water), and solar radiation with protons, neutrons, electrons, x-rays, and heavy metal ions (Fig 2). The damage of solar-equivalent proton radiation in a murine model has been emphasized by acute and severe depression of T cells, B cells, natural killer cells, and specific antibody formation to the neoantigen sheep red blood cells.^{43,44} In this context of severe immunosuppression, it is likely that reactivation of latent viral infections, such as EBV and polyomavirus, might be expected, as well as virally driven lymphomas.^{45,46} Although this Antarctic winter-over model of spaceflight did not produce antibody deficiency, additional research is needed on antibody and cellular immune responses in this and other model systems to predict safe passage of astronauts in interplanetary space travel.

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