

Suppression of human anti-inflammatory plasma cytokines IL-10 and IL-1RA with elevation of proinflammatory cytokine IFN- γ during the isolation of the Antarctic winter

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Cellular immune function has been shown to be decreased and latent virus shedding to be increased in human beings isolated during the Antarctic winter, a model used for assessing some effects of space flight. However, the balance of proinflammatory (IFN- γ) and anti-inflammatory (IL-10 and IL-1RA) cytokines has not previously been evaluated. We therefore sought to determine whether isolation during the Antarctic winter would alter the proinflammatory and anti-inflammatory cytokine balance. Cytokine levels were measured with ELISA in monthly plasma samples from January through September 1999 in 21 study subjects in the Antarctic and 7 control subjects on Macquarie Island. There was a significant time-dependent increase in plasma IFN- γ ($P = .039$) as well as decreases in IL-10 ($P = .042$) and IL-1RA ($P = .053$) in the study subjects compared with the control subjects. The study subjects also had significantly increased plasma IFN- γ levels ($P \leq .045$) but decreased IL-10 and IL-1RA levels ($P \leq .036$) at individual time points of isolation. Isolation of human beings in the Antarctic appears to shift the plasma cytokine balance toward a proinflammatory profile. These observations are consistent with T-cell activation that might be due to activation of latent viruses, and they could hold importance for determining the risks of space flight. (*J Allergy Clin Immunol* 2002;109:854-7.)

Key words: Antarctic winter-over, model of isolation, effect of space flight, proinflammatory vs anti-inflammatory cytokine balance, latent virus infection, T-cell activation

Abbreviations used

IL-1RA: IL-1 receptor antagonist
TNF- α R1: TNF- α receptor 1

Although lacking the effects of radiation and microgravity, the Antarctic winter-over model of space flight features the common conditions of isolation, containment, stress, and possible microbial contamination. We and others have used this model in an attempt to determine whether these conditions would affect different components of the human immune responses. For example, Muller et al¹ have demonstrated diminished delayed-type hypersensitivity to recall antigens in more than 250 study subjects in Antarctica compared with control subjects stationed on Macquarie Island, where a harsh winter climate exists but access to the mainland is possible.¹ Moreover, increased Epstein-Barr virus shedding was detected in a smaller cohort of these study subjects in comparison with control subjects.² Another model of space flight, sleep deprivation, has been shown to alter the homeostatic balance of sleep regulation, which is controlled by the neuroendocrine-immune system messenger molecules TNF- α receptor 1 (TNF- α R1) and IL-6.³ To test the hypothesis that Antarctic winter isolation would adversely affect the balances of proinflammatory⁴ and anti-inflammatory cytokines,⁵ we measured the plasma levels of IFN- γ , IL-12, IL-10, and IL-1 receptor antagonist (IL-1RA).

METHODS

Study population

The study group of Casey, Antarctica ($n = 11$), and the control group of Macquarie Island ($n = 7$) have been described previously in a bacteriophage study.⁶ In addition, another study group, in Davis, Antarctica ($n = 10$), participated in the present study. The Davis sub-

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Supported by the National Aeronautics and Space Administration, Cooperative Agreement NCC-958 with the National Space Biomedical Research Institute, and the Australian Antarctic Division.

Received for publication December 19, 2001; revised February 7, 2002; accepted for publication February 8, 2002.

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0091-6749/2002 \$35.00 + 0 1/84/123873

doi:10.1067/mai.2002.123873

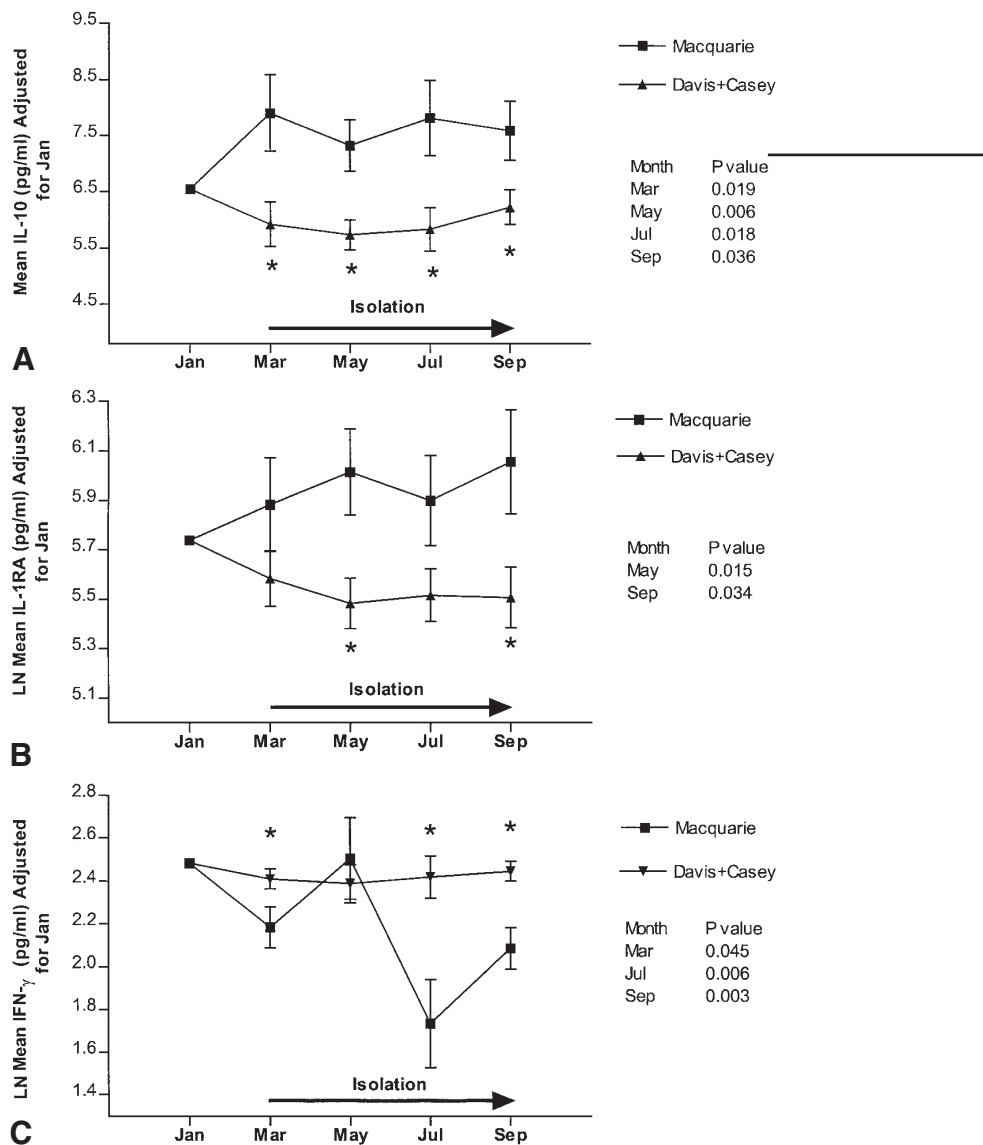


FIG 1. Sequential bimonthly plasma cytokine levels in young healthy men in Antarctica (Casey, $n = 11$; Davis, $n = 10$; total study group, $n = 21$) and on Macquarie Island (control group, $n = 7$) during the winter of 1999. **A**, IL-10; **B**, IL-1RA; **C**, IFN- γ . The January 1999 data points of groups were adjusted to a single value, and the subsequent data points (March through September) were given as single data points with adjustment of values to the overall mean in January. The monthly means represent the average of plasma levels at 2 time points during each bimonthly period (ie, February/March, April/May, June/July, August/September). IFN- γ and IL-1RA values are expressed as natural log values. SEMs are indicated by brackets around the means. The laboratory control ($n \geq 20$) ranges were as follows: IL-10, 5.10 to 8.5 pg/mL; IL-1RA, 3.87 to 7.06 ln pg/mL; and IFN- γ , 2.17 to 3.01 ln pg/mL.

jects were similar to those at Casey and were part of the same 1999 Australian National Antarctic Research Expedition, which left Australia in October 1998, arrived at the Antarctic destinations in December 1998, and were permanently isolated (no return to mainland possible) for the Antarctic winter in late March 1999.

This investigation 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.

Assay of cytokines and cytokine receptor antagonist

The frozen plasma samples were rapidly thawed and brought to room temperature. Next, the samples were assayed for IFN- γ , IL-10, IL-12, and IL-1RA through use of Quantikine ELISA kits (R & D Laboratories, Minneapolis, Minn) and as previously described.³ Serial dilutions of known concentrations of IFN- γ , IL-10, IL-12, and IL-1RA were used to construct a standard curve for each of the

analytes. The concentrations of IFN- γ , IL-10, IL-12, and IL-1RA in samples were interpolated from their respective standard curves. The minimum detectable levels of IFN- γ , IL-10, IL-12, and IL-1RA with the Quantikine ELISA kits were 8.0 pg/mL, 3.9 pg/mL, 5.0 pg/mL, and 22.0 pg/mL, respectively.

Statistical analysis

For statistical analysis of data, analysis of covariance for repeated measures was used to assess (1) the effects of time across months (January through September 1999) and (2) the interaction of time and control (Macquarie Island) versus isolation (Davis, Casey) conditions for each of the immune measures. January measures were treated as covariates in the analysis, so all groups started at the same mean value. For the last 8 months of observation, 2-month values were averaged to produce a single bimonthly value. Thus, 4 time points of data were analyzed (February/March, April/May, June/July, August/September) in addition to the initial measurement in January. A natural log transformation was applied to the IFN- γ and IL-1RA data before analysis because of the lack of normality.

RESULTS

IL-10

The repeated-measures analysis revealed that there was a significant time-dependent decrease ($P = .042$) in the plasma levels of IL-10 in Antarctic winter-over study subjects (Davis and Casey, Antarctica) during the period of isolation in comparison with the control subjects stationed on Macquarie Island (Fig 1, A). Bimonthly comparisons between study and control subjects revealed significant differences from March through September ($P \leq .036$). For example, the adjusted value (mean $\pm$ SEM) in September for the study subjects (6.22 ± 0.31 pg/mL) was 18% lower than that for the control subjects (7.59 ± 0.53 pg/mL).

IL-1RA

Study subjects exhibited a time-dependent decrease in plasma IL-1RA levels over the course of the Antarctic winter in comparison with control subjects ($P = .053$; Fig 1, B). Bimonthly comparisons of study and control subject values revealed statistically significant differences in the isolation period ($P \leq .034$). With antilog conversion, the adjusted value (mean $\pm$ SEM) in September for the study subjects (24.7 ± 1.01 pg/mL) was 42% lower than that for the control subjects (42.8 ± 1.06 pg/mL).

IFN- γ

During the period of isolation, there was a significant time-dependent difference in the plasma level of IFN- γ between the study subjects and control subjects stationed on Macquarie Island ($P = .039$; Fig 1, C). Bimonthly comparisons of study and control subject values revealed statistically significant differences in the isolation period ($P \leq .045$). With antilog conversion, the adjusted value (mean $\pm$ SEM) in September for the study subjects (11.6 ± 1.05 pg/mL) was 43% higher than that for the control subjects (8.09 ± 1.10 pg/mL).

IL-12

The time-dependent differences of IL-12 were not statistically significant, and neither were the month-by-month comparisons of the groups (data not shown). However, the trend of bimonthly IL-12 production over the isolation period was similar to that for IFN- γ .

DISCUSSION

Our previous work with cytokines and soluble cytokine receptors concerned the sleep-deprivation model of space flight. In that model, we learned that acute sleep deprivation of 4 days elevated the plasma levels of the sleep-regulating factors TNF- α RI and IL-6.³ Here we demonstrate that the long-term effects of the severe Antarctic winter with isolation, stress, containment, and possible microbial contamination caused time-dependent decreases in the anti-inflammatory cytokine IL-10 (secreted by T_H2 cells) and IL-1RA (secreted by mononuclear phagocytes) as well as a time-dependent increase in plasma levels of the proinflammatory cytokine IFN- γ , a substance secreted by T_H1 cells and natural killer cells that activates cytotoxic T cells. These data extend the preliminary cytokine studies of study subjects (no controls) in Antarctic isolation by Tingate et al² and Donovan et al (personal communication, December 14, 2001), and they relate to the alterations of various cytokine levels in animal and human conditions involving stress and isolation.⁷⁻⁹

Although these studies of cytotoxic cytokines in Antarctic winter-over subjects suggest an alteration in T-cell activation response, our previously published study of the specific IgG antibody responses to the T-cell-dependent neoantigen ϕ -X174 did not reveal deficiencies.⁶ Our evidence of altered plasma cytokine levels in study subjects in comparison with control subjects suggest that an immune activation event has taken place in the study subjects' response to some antigenic challenge—possibly viral antigen, such as might occur in reactivation of a latent virus.¹⁰ In keeping with the information showing weakened cellular immunity and an increase in Epstein-Barr virus shedding in the same model,¹⁻³ it is reasonable to suspect that the T-cell system reacts less well under the conditions of the Antarctic winter-over but sufficiently well to produce help for IgG class switching.

IL-10 and IL-1RA are potent anti-inflammatory cytokines that are capable of shifting the balance of proinflammatory and anti-inflammatory cytokines, likely to be important in survival mechanisms. We conclude that the Antarctic winter-over model of space flight has proved to be valuable in this study, in that it demonstrated a tipping of the cytokine balance toward a proinflammatory profile—findings consistent with latent virus activation of T cells.¹⁰

We thank the Antarctic and Macquarie Island volunteers for their participation in the study and Dr John Lednický for specimen handling.

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