

T_H1-biased immunity induced by exposure to Antarctic winter

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Background: Certain immune functions are known to be impaired in human beings exposed to Antarctic winter; in particular, decreased amounts of serum proinflammatory cytokines, such as TNF- α and IL-1, were noted. It is not known, however, whether this exposure has any effect on T-cell-mediated acquired immune functions.

Objectives: This study aims to investigate whether exposure to Antarctic winter has any effect on T cell-dependent immune functions.

Methods: We assessed changes in various immunologic indicators, including serum levels of various cytokines, peripheral blood V α 24V β 11 natural killer T cell numbers, and T_H1/T_H2 ratios of 40 Japanese personnel exposed to an Antarctic winter. Also, a 2-month inland traverse was executed during the isolation, and the effect on the above indicators was assessed. **Results:** All subjects were healthy during the Antarctic isolation. The levels of serum TNF- α , IL-1Ra, IL-6, and IL-1 β were dramatically reduced and remained at low levels throughout the isolation. The decrease in the levels of TNF- α and IL-1Ra was more pronounced during the inland traverse than during the rest of the isolation. The percentage of V α 24V β 11 natural killer T cells was significantly increased at the midpoint of the isolation. Most interestingly, T_H1/T_H2 ratio was increased significantly, and this T_H1 bias was most prominent at the late point of the isolation.

Conclusions: Exposure to an Antarctic winter appeared to induce T_H1-skewed immunity in human beings. (J Allergy Clin Immunol 2003;111:1353-60.)

Key words: T_H1/T_H2 cells, cytokines, allergy, inflammation

Abbreviations used

IL-1Ra: IL-1 receptor antagonist
PBMC: Peripheral blood mononuclear cell
NK: Natural killer
NKT: Natural killer T

Exposure to environmental stresses such as cold, heat, and high altitudes modify various components of immune function.¹ Severe environmental stress may have immunosuppressant effects, resulting in increased risk for immune-related diseases such as infectious diseases, allergy, cancer, and autoimmune disorders. It has been recognized that cold stress affects various aspects of both cellular and humoral immunity in experimental animals: a decrease in lymphocyte proliferation, a reduction of natural killer (NK) cell count, cytolytic activity, activation of complement, and the induction of heat shock proteins.²⁻⁵ However, it is less clear how cold stress modulates the immune system in human beings.⁶

Medical research has been performed on the Australian National Antarctic Research Expeditions for 50 years, and certain changes in immune functions have been reported.⁷ The cutaneous delayed-type hypersensitivity response was reduced with almost 50% reduction of T-cell proliferation to mitogen phytohemagglutinin. Atypical monocytosis was detected, and a striking reduction of the proinflammatory cytokine TNF- α was noted. Also, decreased serum levels of IL-10, IL-6, IL-1Ra (IL-1 receptor antagonist),⁷ and IL-1 β were detected.⁸ Very recently, the elevation of IFN- γ was reported during exposure to an Antarctic winter.⁹ It should also be noted that no significant changes in T- and B-lymphocyte subsets, immunoglobulin, or complement were reported.¹⁰ Because Antarctica is an outstanding analogue for the isolation and confinement of space missions, the same immune modulations induced by isolation in Antarctica may be induced in personnel staying in space.¹¹

The most critical players in T-cell-dependent acquired immune responses, CD4⁺ T helper cells, can be subdivided into T_H1 and T_H2 cells.¹² T_H1 cells, which produce IFN- γ and IL-2, are essential for the induction of cellular immunity, whereas T_H2 cells, which produce IL-4, IL-5, and IL-13, play a key role in humoral immunity.^{13,14} An imbalance of T_H1/T_H2 responses has been demonstrated to be associated with a relative risk for certain immune diseases.¹⁵ A bias toward T_H2 is seen in allergy and sys-

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TABLE I. Environmental features of Tokyo, Japan, Syowa station, and Relay station

	Tokyo, Japan	Syowa station	Relay station
Latitude	N 35°40'35"	S 69°00'22"	S 74°00'46"
Longitude	E 139°44'44"	E 39°35'24"	E 42°59'73"
Altitude (m)	24.4	29.2	3353
Air pressure: mean	1013.9 hPa	986.8 hPa	626.8 hPa
Temperature			
Mean	15.9°C	-10.4°C	-54.3°C
Minimum	-2.4°C	-34.2°C	-61.7°C

TABLE II. Sampling schedule

Time point of sampling		Month/year	Sample	Number
I	Before departure from Japan	October 2000	CBC/serum/PBMC	40
II	Early point of isolation	March 2001	CBC/serum	40
III	Midpoint of isolation	June 2001	CBC/serum/PBMC	40
IV	Before traverse	August 2001	CBC/serum/PBMC	9
V	Inland traverse (relay station)	September 2001	Serum	9
VI	Late point of isolation	October 2001	CBC/serum/PBMC	40
VII	After isolation	March 2002	CBC/serum	40

I-VII, Time point of sample separation; CBC, complete blood count.

temic autoimmune diseases. In contrast, immune responses in organ-specific autoimmune diseases such as type I diabetes and multiple sclerosis often manifest predominantly T_H1 phenotypes.

Natural killer T (NKT) cells were identified recently and appear to represent a novel lymphoid lineage distinct from T cells, B cells, and NK cells.^{16,17} Human NKT cells, expressing both NK receptors and $V\alpha24TCR$, have now been shown to play crucial roles in various immune responses, including anti-tumor and autoimmune responses.¹⁸ Because NKT cells produce both type-1 (IFN- γ) and type-2 (IL-4) cytokines, a role in regulating the balance of T_H1 and T_H2 cells has been suggested.¹⁹⁻²¹

In this report, we investigated changes in various immune indicators, such as serum levels of certain cytokines, percentages of $V\alpha24V\beta11$ NKT cells, and T_H1/T_H2 ratios, of 40 Japanese personnel exposed to an Antarctic winter. We found that the exposure induced dramatic decreases in serum levels of TNF- α , IL-1Ra, IL-6, and IL-1 β , a significant increase in the number of NKT cells, and progressive T_H1 -biased immune status.

METHODS

Subjects

Forty members (37 men and 3 women) of the Japanese Antarctic Research Expedition (JARE) were volunteer subjects for this study. Ages ranged from 25 to 50 years, with a mean age of 33.8 years. All had undergone predeparture clinical, psychological, and laboratory examinations to ensure a healthy population for the isolation during the Antarctic winter. Syowa Station, the mother station of JARE, was established in 1957, at 69°00'S and 39°35'E on East Ongul Island, Lutzow-Holm Bay, East Antarctica. The expedition arrived at Antarctica in December 2000 and departed from Antarctica in February 2002.

Nine members (7 men and 2 women) went on an inland traverse to Relay station for 43 days in August to October of 2001. Ages ranged from 25 to 46 years, with a mean age of 33.3 years. Relay station is located on the inland ice sheet at 74°00'S and 42°59'E (3353 m above sea level), approximately 650 km southeast of Syowa station. It is a relay point to Dome Fuji Station, and, since there is no facility, these members stayed in a snowmobile during the traverse.

The environmental features of Syowa station and Relay station are summarized in Table I. In winter (May through August), activities outside were scheduled from 9 AM to noon (3 hours) and from 1 to 5 PM (4 hours). In the summer (September through April), activities outside were scheduled from 8 AM to noon (4 hours) and 1 to 5 PM (4 hours). Subjects were informed of all procedures and possible risks associated with the study. The study was approved by the Institutional Review Board of Chiba University School of Medicine.

Sampling procedure

Venous blood was drawn between 6 and 7 AM, after an overnight fast. Blood samples were collected into plastic syringes and transferred immediately into tubes containing specific anticoagulant for lymphocytes or into vacant tubes for serum separation. For separation of peripheral blood mononuclear cells (PBMCs), blood samples were diluted with two volumes of PBS and applied to the Lymphoprep Tube (sodium diatrizoate 9.1%, polysaccharide 5.7%, AXIS-SHIELD PoC AS, Oslo, Norway). After centrifugation (800g for 30 minutes at room temperature), PBMCs in the interface were harvested and stored at -85°C in Cell Banker solution (Nippon Zenyaku Co, Fukushima, Japan). The frozen cells were subjected to flow cytometry analysis after thawing in Japan (the mean viability was approximately 58.3% in 120 samples). Sera were collected in 1-mL Cryo tubes (Nunc A/S, Roskilde, Denmark), frozen immediately, and stored at -85°C. The sampling schedule is outlined in Table II.

Blood profile and differential counts

Blood smears were prepared immediately after the specimen collection. Cell types were identified on the basis of morphologic criteria after May-Grünwald-Giemsa staining. The specimens were

analyzed in a Sysmex K4500 machine at the infirmary in Syowa station. The following indicators were assessed: leukocyte, erythrocyte and platelet counts, hemoglobin concentration, hematocrit, mean cell volume, mean cell hemoglobin, and mean corpuscular hemoglobin concentration.

Measurement of cytokine concentration in serum

Serum concentrations of cytokines were measured by sandwich ELISA kits according to the manufacturer's procedures (IL-1 β , IL-1Ra: Quantikine, R&D Systems, Minneapolis, Minn; TNF- α BioSource Europe SA, Belgium, IL-10: BioSource International, CA, IL-2: CosmoBio, Tokyo, Japan, IL-6: Fuji Rebio, Tokyo, Japan).

Flow cytometry analysis

The number of V α 24V β 11 NKT cells in PBMCs were evaluated by flow cytometry analysis, as described previously.²² Mononuclear cells were 3-color-stained with Cychrome-conjugated anti-CD3e mAb (UCHL1; Pharmingen, San Diego, Calif), FITC-conjugated anti-TCR V α 24 mAb (C15; Coulter-Immunotech, Miami, Fla), and PE-conjugated anti-TCR V β 11 mAb (C21; Coulter-Immunotech). Dead cells were gated out by propidium iodide staining, and live cells were analyzed by an EPICS-XL (Coulter) with a logarithmic amplifier.

Intracellular cytokine expression

Intracellular staining of IL-4 and IFN- γ was performed as described.²³ The cells (5×10^5) were stimulated with PMA and ionomycin for 4 hours in the presence of 2 μ mol/L monensin, which inhibited the secretion of newly produced protein. The cells then were stained with biotin-conjugated anti-CD4 for 15 minutes on ice followed by APC-conjugated avidin. After washing with PBS, cells were fixed with 4% paraformaldehyde for 10 minutes at room temperature and made permeable with 0.5% Triton X-100 (in 50 mmol/L NaCl, 5 mmol/L EDTA, 0.02% NaN₃, pH 7.5) for 10 minutes on ice. After blocking with 3% BSA in PBS for 10 minutes, cells were incubated on ice for 30 minutes with anti-IFN- γ -FITC and anti-IL-4-PE (BD Biosciences, San Jose, Calif). Flow cytometry analysis was performed on an FACS Calibur.

Measurement of total and specific IgE

Total and antigen-specific IgE concentrations in the serum were measured with the Pharmacia CAP System, IgE and RAST, FEIA (Pharmacia and Upjohn AB Diagnostics, Uppsala, Sweden). Specific IgE was measured against house dust as well as a mold mix (*Penicillium*, *Cladosporium*, *Aspergillus*, *Candida*, *helminthosporium* and *Alternaria*) and a weed mix (*Ambrosia*, *Artemisia*, *Chrysanthemum*, *Taraxacum*, and *Solidago*). The cutoff for total serum IgE was 170 U/mL and for RAST was 0.35 UA/mL. The RAST values were expressed in classes 0 through 6. A RAST class of >1 was recognized as positive.

Statistical analysis

The 1-way analysis of variance (repeated measures) was used to assess any differences among the data points. When differences existed among the means, post hoc analysis was performed with the Dunnett test. *P* values <.05 were considered to be statistically significant.

RESULTS

Complete blood count

All hematology indicators, such as leukocyte number, platelet count, mean cell hemoglobin, and mean corpuscular hemoglobin concentration, in the personnel were

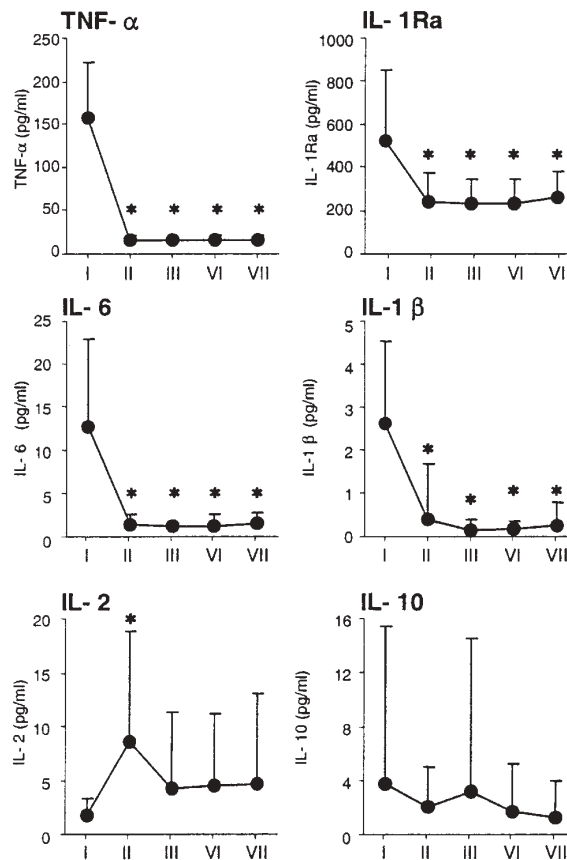


FIG 1. Changes in cytokine concentrations during Antarctic isolation. Mean values $\pm$ SD are shown (*n* = 40). I, Before departure from Japan; II, early point of isolation; III, midpoint of isolation; VI, late point of isolation; VII, after isolation. *Statistically significant within-trial differences vs I, *P* < .05.

within a normal range during the stay in Syowa station (data not shown).

Analysis of circulating cytokines

The concentrations of serum TNF- α , IL-1Ra, IL-6, IL-1 β , IL-2, and IL-10 at various time points, including before the Antarctic isolation, are shown in Fig 1. The level of TNF- α was markedly reduced at the early point of isolation (16.5 ± 5.2 pg/mL) compared with before departure from Japan (157.1 ± 65.0 pg/mL, *P* < .05) and remained at low levels during the period of isolation. The levels of IL-1Ra, IL-6, and IL-1 β were also reduced significantly during the isolation (*P* < .05). The level of IL-2 increased from 1.76 pg/mL (at point I) to 8.59 pg/mL (at point II) after arrival in Antarctica (*P* < .05). The level of IL-2 then decreased (after midpoint of isolation, point III). The level of IL-10 tended to decrease at midpoint to late point of isolation; however, this change was not statistically significant. We also examined IFN- γ , but the amounts were below detectable levels (data not shown).

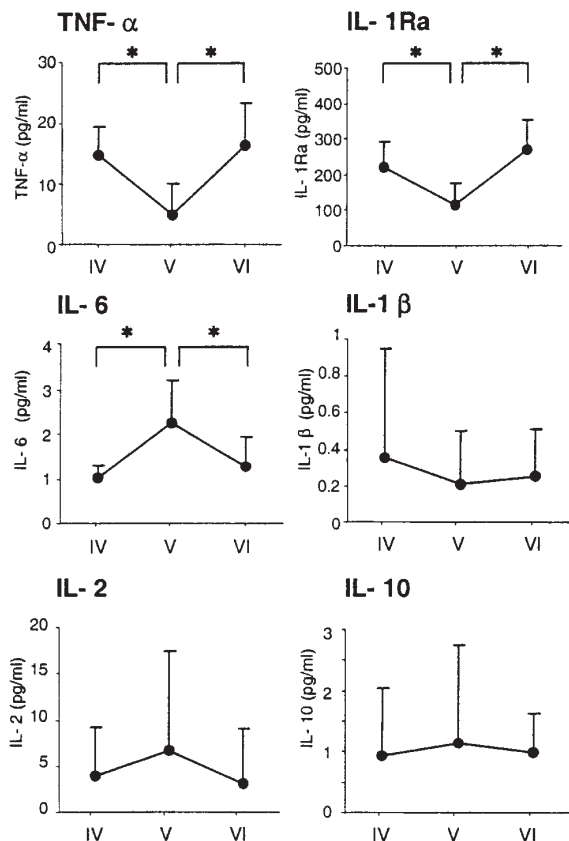


FIG 2. Changes in cytokine concentrations during inland traverse. Mean values $\pm$ SD are shown ($n = 9$). IV, Before traverse; V, during inland traverse (at Relay station); VI, after traverse. *Statistically significant within-trial differences vs V (at Relay station), $P < .05$.

Concurrently, we analyzed cytokine concentrations of blood samples of 9 members who joined the inland traverse to stay at Relay station (Fig 2). The levels of TNF- α were reduced at Relay station (time point V, 4.9 ± 5.1 pg/mL) compared with those before the traverse (time point IV, 14.8 ± 4.5 pg/mL, $P < .05$). Then, the levels returned to the levels observed before the traverse (time point VI, 16.4 ± 6.8 pg/mL). IL-1Ra was also reduced significantly and recovered after the traverse. In contrast, IL-6 was elevated at Relay station (time point V, 2.26 ± 0.92 pg/mL) compared with before traverse (time point IV, 1.02 ± 0.29 pg/mL, $P < .05$). The increased IL-6 level was also returned to the levels observed before the traverse (time point VI, 16.4 ± 6.8 pg/mL). There was no statistically significant change in the levels of IL-1 β , IL-2, and IL-10 during the inland traverse.

Percentages of V α 24V β 11 NKT cells in PBMCs

The percentages of V α 24V β 11 NKT cells in the PBMCs of 28 members who did not go on the inland traverse are shown in Fig 3. Fig 3, A, depicts mean values of percentages of V α 24V β 11 NKT cells. As can be seen, the percentages of peripheral blood NKT cells were increased at midpoint of isolation (time point III, 0.048%

$\pm 0.049\%$) compared with the frequency before departure from Japan (time point I, $0.034\% \pm 0.030\%$, $P < .05$). The levels were decreased at late point of isolation (time point VI, $0.035\% \pm 0.033\%$, $P < .05$).

Fig 3, B, shows representative flow cytometric profiles of V α 24V β 11 NKT cells of one subject. Before departure from Japan, his NKT cell frequency was 0.058% . It increased to 0.14% at midpoint of isolation. Then, at the late point of isolation he had 0.055% NKT cells, almost the same level as that of before departure from Japan. Fig 3, C, shows the individual plot of the percentages of NKT cells of 28 individuals. We also investigated the influence of the inland traverse on NKT cell frequencies of 9 members. There was no statistically significant change in the percentages of NKT cells before (time point IV, $0.039\% \pm 0.045\%$) and after (time point VI, $0.054\% \pm 0.066\%$) the inland traverse (data not shown).

Changes in T_H1/T_H2 balance

PBMCs were thawed and immediately stimulated with PMA and ionomycin for 4 hours. The production of IFN- γ and IL-4 of 16 subjects then was assessed by cytoplasmic staining of IL-4 and IFN- γ and by anti-CD4 cell surface staining. The ratio of T_H1/T_H2 (IFN- γ -producing/IL-4-producing) cells present in the CD4⁺ T cells in PBMC was determined. As shown in Fig 4, A, the T_H1/T_H2 ratio increased at the midpoint of isolation (time point III, 7.4 ± 6.5) as compared with before departure from Japan (time point I, 4.5 ± 4.2). The increased levels were more prominent at the late point of isolation (time point VI, 8.3 ± 7.9). The mean values of T_H1 of 40 personnel were slightly decreased and increased thereafter (time point I, 1.00; time point III, 0.88, and time point VI, 1.11), and those of T_H2 were decreased dramatically (time point I, 1.00, time point III, 0.49, and time point VI, 0.66). Fig 4, B shows representative IFN- γ /IL-4 profiles of one subject. T_H1/T_H2 ratio at midpoint of isolation (2.47) and late point of isolation (2.10) was higher than before departure from Japan (1.83). Fig 4, C shows individual plots of the T_H1/T_H2 ratio of 16 subjects. These results suggest that T_H1-skewed immunity was induced by exposure to an Antarctic winter.

Levels of IgE concentration

Finally, the serum levels of total IgE and antigen-specific IgE were evaluated. No symptom related to atopic or autoimmune diseases was observed in our 40 personnel during the stay in Antarctica. The concentrations of serum total IgE at all time points tested, including before the Antarctic isolation, are shown in Fig 5. The levels of serum total IgE were reduced at the early point of isolation (243.4 U/mL) compared with before departure from Japan (335.8 U/mL, $P < .05$) and remained at low levels during the period of isolation. We measured the levels of various antigen-specific IgE. Although some persons were positive for certain antigens (house dust: 21 persons; mold mix: 2 persons; and a weed mix: 3 persons), in all cases we did not detect any changes in score during the isolation. Also, there was no effect by the inland traverse. These results suggest that the levels of total IgE are decreased by exposure to an Antarctic winter.

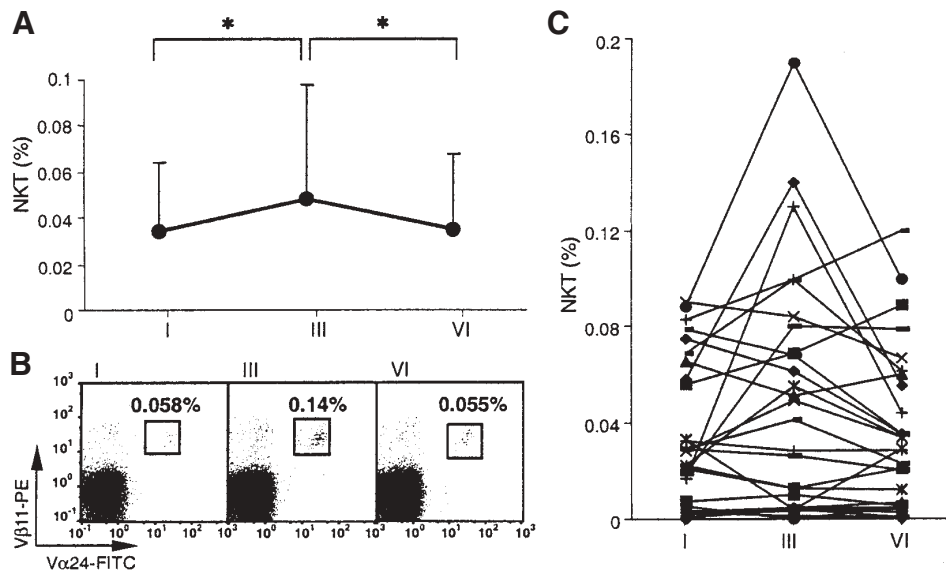


FIG 3. Frequencies of Vα24Vβ11 NKT cells in 28 personnel who did not join inland traverse. **A**, The results are depicted as mean values with standard deviation (bars). *Statistically significant within-trial differences vs III (midpoint of isolation), $P < .05$. **B**, Representative flow cytometric profiles of Vα24Vβ11 NKT cells of a subject. Percentages of Vα24Vβ11 NKT cells are shown in each panel. **C**, Changes in frequencies of NKT cells of 28 individuals.

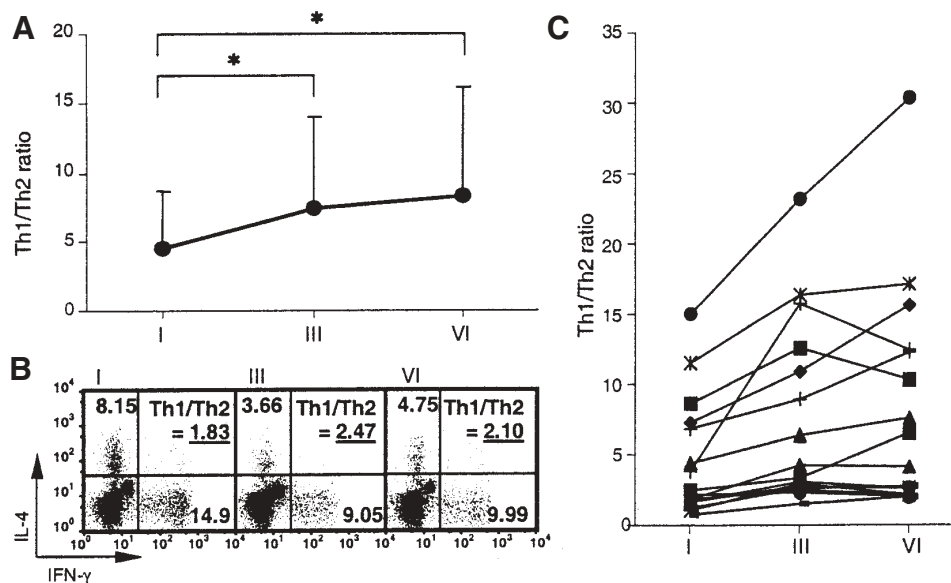


FIG 4. Change in TH1/TH2 ratio during isolation ($n = 16$). **A**, Results are depicted as mean values of TH1/TH2 ratio. Bars, standard deviations. *Statistically significant within-trial differences vs I (before departure from Japan), $P < .05$. **B**, Representative flow cytometric profiles of TH1 and TH2 cells of a subject. I, Before departure from Japan; III, midpoint of isolation; and VI, late point of isolation. Percentages of TH1 and TH2 cells and TH1/TH2 ratio (underlined) are shown. **C**, Individual plot of TH1/TH2 ratio of 16 subjects.

DISCUSSION

In this report, we studied the serum levels of TNF-α, IL-1Ra, IL-6, IL-1β, IL-2, and IL-10 cytokines, the percentages in Vα24Vβ11 NKT cells and TH1/TH2 ratios of

40 Japanese personnel exposed to an Antarctic winter. We found that the exposure to an Antarctic winter induced a dramatic decrease in the serum levels of TNF-α, IL-1Ra, IL-6, and IL-1β, a significant increase in the percentages of NKT cells, and progressive TH1-biased immune status.

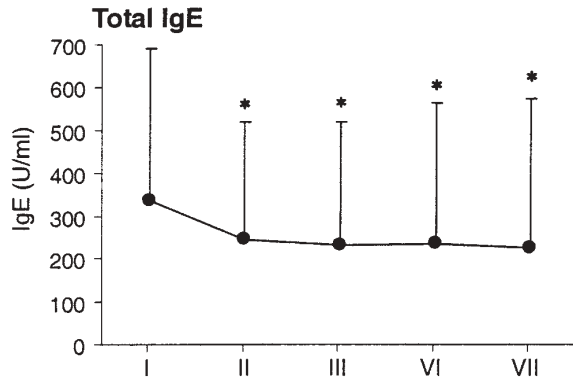


FIG 5. Changes in total IgE concentrations during Antarctic isolation. Mean values $\pm$ SD are shown ($n = 40$). *Statistically significant within-trial differences vs I (before departure from Japan), $P < .05$.

Functional bidirectional regulation exists between the endocrine and immune systems, and cytokines are thought to play a role in the cross-talk. Certain cytokines are able to modulate the hypothalamic-pituitary-adrenocortical axis response.^{24,25} Limited evidence suggests that cold exposure may also initiate changes in cytokine expression associated with a nonspecific acute phase reaction.²⁶⁻³⁰ Cold exposure stimulated monocytes to secrete proinflammatory cytokines, such as IL-1, TNF- α , and IL-6, and the serum concentrations of these cytokines were reported to be elevated. However, some reports demonstrated decreased IL-1 β and TNF- α .²⁶ The apparent discrepancy may be due to the different protocols of cold exposure and reflect direct and indirect effects of cold exposure, including adaptation.

In our study, levels of TNF- α , IL-1Ra, IL-6, and IL-1 β were markedly reduced at the early point of isolation compared with before departure from Japan and remained at low levels during the period of isolation (Fig 1). Our results in Japanese people are consistent with previous reports of Australian expeditions.^{7,8} The number of samples that we analyzed was 40, the highest in reported studies. Thus, decreased proinflammatory cytokines in the serum induced by Antarctic exposure appears to be established now. A minor discrepancy between our data and those of Australian expeditions is the recovery of TNF- α , IL-1Ra, and IL-10.⁷ We did not detect the recovery of these cytokines throughout the isolation. Also, a slight difference in the peak time point of IL-2 and IL-10 levels was noted. The discrepancy may be due to the difference in race, but further investigation is required to address this issue.

Furthermore, we assessed the effect of the inland traverse. The relay station is located on the inland ice sheet at 3353 m above sea level, approximately 650 km south-east of Syowa station. Conditions were more severe than those at Syowa Station in terms of the coldness, low oxygen concentration, and high altitude (Table I). Nine personnel stayed in a snowmobile during the traverse. We found that the levels of TNF- α and IL-1Ra were further

reduced during the traverse compared with those before the traverse (Fig 2). The levels were recovered after the traverse. In contrast, the level of IL-6 was increased significantly during the traverse. The changes in IL-6 may be a consequence of the high-altitude exposure. Exposure to high altitude is known to be sufficient to induce an increase in circulating IL-6.^{31,32} In another study, exposure to high altitude increased serum concentrations of IL-6 without changes in serum levels of IL-1 β , IL-1Ra, and TNF- α .³³ Although environmental stress during the inland traverse is more complex than that at Syowa station, such an investigation may provide a more obvious picture of the outcome of the stress of Antarctic isolation.

Although NK activity was reported to be downregulated in mice exposed to cold stress,^{3,5} the numbers of human NK cells and also NK activity were reported to be upregulated by cold exposure.^{27,34} NKT cells express both NK receptor and TCR, and thus NK cell fractions examined in the 1990s may have contained both NK cells and NKT cells. Now, the major population of human NKT cells can be identified by canonical TCR $\alpha\beta$ expression, for example, TCRV α 24V β 11.¹⁸ Various unique functions of NKT cells have emerged.^{16,35,36} We investigated the frequencies of the TCRV α 24V β 11NKT cells in the peripheral blood, and a transient increase at the midpoint of isolation was revealed (Fig 3). The levels were increased at the late point of isolation to almost the same level as that of before departure. There are several reports of increased numbers of NKT cells after restraint stress in mouse models,^{37,38} suggesting that sympathetic nerve activation and endogenous steroid hormone release control the number of NKT cells. Further studies are required to elucidate the physiological meaning of the observation that NKT cells in the human peripheral blood are increased during isolation in Antarctica.

Various types of stress have a certain effect on the T_H1/T_H2 balance.³⁹ It is well known that an imbalance in T_H1/T_H2 immunity is associated with increased risk for various immune-related diseases.^{40,41} However, it has not been established whether cold exposure induces either T_H1-biased or T_H2-biased immune status. Therefore, we were eager to study the effect of Antarctic exposure on T_H1/T_H2 balance, and an analysis at the single cell level, by cytoplasmic staining of IL-4 and IFN- γ , was executed. We found that peripheral blood CD4 T-cell profiles of personnel exposed to the Antarctic winter exhibited a bias toward T_H1 (Fig 4). The T_H1/T_H2 ratio increased at midpoint of isolation, and the increased levels were more prominent at the late point of isolation. Consistent with the T_H1 bias, total IgE levels were decreased during the Antarctic isolation (Fig 5). However, any changes in the antigen-specific IgE were detected during the isolation, suggesting that long-lived, memory-type, IgE-secreting B cells are pronounced in the body. The results described in a recent report support the T_H1-biased immune status. Isolation of human beings in Antarctica appears to shift the plasma proinflammatory/anti-inflammatory cytokine balance toward a proinflammatory profile.⁹ The level of IFN- γ was increased after Antarctic exposure, suggesting

an association with T_H1 -biased immune status. Another interesting possibility is that increased numbers of NKT cells may influence the T_H1 -biased immune status. In fact, we reported that activated mouse NKT cells produce IFN- γ to enhance T_H1 development in a certain experimental model.¹⁹ It is most likely that cold stress induced the T_H1 -biased status, but another possibility is that the T_H1 shift may be induced by the low incidence of communicable diseases during Antarctic isolation. We also observed a low incidence of communicable diseases.

Cold exposure alters immunologic and also hormonal parameters. In human beings, norepinephrine levels in the serum were reported to be increased.⁴² Cortisol is well known to affect the immune function, including neutrophil recruitment, IL-2 receptor downregulation, and apoptosis induction in T cells.⁴³ The analysis of personnel who stayed in Antarctica for the summer suggested that the levels of cortisol and growth hormones were decreased.^{10,44} We did not examine the levels of any hormones, but the numbers of NKT cells and T_H1/T_H2 balance would be influenced by changes in hormone concentrations.

The reactivation of herpes simplex virus 1 is observed more frequently in the cold and dry districts and is induced by ultraviolet exposure.⁴⁵ Thus, it is conceivable that cold exposure in the Antarctic winter may induce virus reactivation. In fact, there are some reports suggesting that the incidence of virus reactivation is increased in Antarctica.^{7,46} In either case, however, no obvious symptoms were observed. We did not observe any symptoms related to virus reactivation. The T_H1 bias may help to prevent the virus reactivation, because anti-virus immune responses in general are known to be mediated by T_H1 /IFN- γ -induced activation of cytotoxic T cells.

At this time, the molecular basis underlying the progressive T_H1 -biased immune status after exposure to an Antarctic winter is unknown. Further studies are required to identify the causes of the immune changes induced by Antarctic exposure. Such an effort may help to establish certain immunologic indicators that reflect the health conditions of personnel in future Antarctic expedition.

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