

Changes in Cytokines at Extreme Surroundings in Antarctica

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We evaluated the impact of the extreme environmental conditions at high altitudes in Antarctica on health from the viewpoint of cytokines. The subjects were 7 men who joined an inland research party participating in the 40th Japanese Antarctic Research Expedition. They underwent serial hematological examinations during the expedition for over 100 days at high altitudes (the highest point was 3810 m). The serum concentration of erythropoietin (EPO) increased promptly, indicating its usefulness for altitude adaptation. The serum concentration of interleukin-6 (IL-6) increased at high altitudes, and showed 2 peaks. Changes in IL-6 levels did not correlate with arterial oxygen tension, serum C-reactive protein or EPO levels. Some psychological stress and various factors may be related to IL-6 levels. Since the subject who suffered bone fractures had a very high concentration of IL-6 and his symptoms of mountain sickness were severer than the others, elevation of IL-6 may be related with mountain sickness. All subjects remained in relatively good health for 3 months. It is thought that the human body can protect itself against extreme surroundings in Antarctica.

Key words: Antarctica; cytokine; high altitude; interleukin-6; mountain sickness

The average altitude of Antarctica is 2450 m; therefore the Antarctic inland is an extremely cold place and the air is rarefied. Confinement to Antarctica for certain periods and isolation from the outside world produce various sources of stress (Palinkas, 1990). Such extreme environmental conditions cause hypoxia, mountain sickness, frostbite, depression and so on, but it is not easy to obtain data on basic biochemical parameters.

Hypoxia stimulates vascular endothelial cells, leukocytes and macrophages in vitro to release pro-inflammatory cytokines (Yan et al., 1995; Naldini et al., 1997). Interleukin-6 (IL-6) is a mediator of the inflammatory response and the production of acute phase proteins (Bendtsen, 1991; Herrmann et

al., 1994; Adamik et al., 1997). Although several studies examined cytokines at high altitudes, their studies examined rapid changes in IL-6 in a short period (Klausen et al., 1997; Hartmann et al., 2000).

We evaluated the influence of such conditions (high altitudes, hypoxia, cold, mental stress and so on) in the long term on health from the viewpoint of cytokines in an Antarctic inland research party.

Subjects and Methods

The subjects were 7 members of an inland research party participating in the 40th Japanese Antarctic Research Expedition which lasted from 1998 to

Abbreviations: CRP, C-reactive protein; EPO, erythropoietin; IL-6, interleukin-6; PaO₂, arterial oxygen tension; RBC, red blood cell

2000. All subjects were healthy Japanese men, and at the beginning of this study, their mean age was 33.7 ± 5.0 years (ranges 27 to 40). Although one of them (Subject X) broke his right 2nd, 3rd and 4th metatarsal bones after the 1st blood sampling, these fractures healed under conservative therapy, and he could join this group. The subjects gave informed consent, and the experimental protocol was approved by the Headquarters of Japanese Antarctic Research Expedition and National Institute of Polar Research, Japan.

The subjects split up and set off in 4 large snow vehicles measuring 3.5×7 m. The party left Showa Station ($69^{\circ}00'S$, $39^{\circ}35'E$, 29 m above sea level), the Japanese Antarctic base, on November 1, 1999, and took 4 weeks to Dome Fuji Station ($77^{\circ}19'S$, $39^{\circ}42'E$, 3810 m), the Japanese Antarctic inland base. They began to survey after 2 weeks of their stay at Dome Fuji Station, and returned to Showa Station on February 11, 2000. In these areas the sun does not set in December and January because of the high southern latitude. The following was their daily work schedule. The subjects got up at 0600, had a 3 regular balanced meals a day and went to bed at 2200. They drove snow vehicles about 50 km in 6 h and did outdoor work for short periods, 10 to 30 min, several times a day. The normal temperature inside the vehicles was about $20^{\circ}C$, but only $-10^{\circ}C$ in early morning since their vehicles had not been warmed up yet.

Arterial blood was sampled indoors or within the snow vehicles ($20^{\circ}C$) at the following points: A, Showa Station 1 month before departure; B, at a 3032-m altitude 15 days after the start of the expedition; C, Dome Fuji station on Day 34 (Day 1, the departure day); D, at 2960 m on Day 53; E, at 2077 m on Day 65; F, Showa Station 5 days after their return. The atmospheric pressure and outside temperature at each blood sampling point are shown in Table 1.

We measured the levels of Hb, red blood cell (RBC) count and serum erythropoietin (EPO) as the indexes of hematological adaptation to hypoxia. Serum levels of C-reactive protein (CRP) and IL-6 were measured as the indexes of inflammation and stress, respectively. We measured arterial oxygen

tension (PaO_2), Hb and RBC count by a portable clinical analyzer, i-STAT (i-STAT Co., East Windsor, NJ), and a microscope, as soon as the blood was sampled. Serum samples were then immediately frozen at $-20^{\circ}C$ and stored at $-80^{\circ}C$ after the expedition until analysis. The serum CRP level was measured by latex agglutination immunoassay, serum EPO level by radioimmunoassay and serum IL-6 level by chemiluminescent enzyme immunoassay. By use of a modified van Beaumont formula (Dill and Costill, 1975), calculations were made of the % change in plasma volume from the initial point to each point.

Moreover, the occurrence of mountain sickness during the expedition was investigated. Symptoms of mountain sickness observed were headache, gastrointestinal symptoms (anorexia, nausea or vomiting), fatigue or weakness, dizziness or lightheadedness and difficulty in sleeping, which were quantified on the basis of the Lake Louise consensus on the definition of altitude illness (Roach et al., 1993). In counting the severity of each symptom, 1 point was given for mild involvement, 2 points for moderate, 3 points for severe and 4 points for extreme involvement. The mountain sickness score was taken as the sum of the points.

Intergroup differences were examined by analysis of variance. Relationships among variables were ascertained by Spearman's correlation coefficient by the rank sum test. Differences were considered statistically significant at $P < 0.05$.

Results

The PaO_2 decreased significantly as the atmospheric pressure gradually decreased, showing a positive correlation ($r = 0.92$, $P < 0.001$). At the start of the expedition, the serum concentration of EPO increased significantly (48.1 ± 25.0 mU/mL, normal level 8 to 36 mU/mL), while the RBC count increased later, after the peak of EPO. The Hb levels peaked at Point E, after the peak of the RBC count. There was no significant change in serum CRP during this period (Table 1).

Table 1. Climate and clinical parameters at different determination points

	Blood-sampling point					
	A	B	C	D	E	F
Time after departure (day)	–	15	34	53	65	108
Altitude above sea level (m)	29	3,032	3,810	2,960	2,077	29
Atmospheric pressure (hPa)	974.9	657.9	585.1	666.1	732.7	982.5
Air temperature (°C)	–14.6	–34.2	–41.7	–25.3	–19.2	–2.3
PaO ₂ (mmHg)	86.1 ± 13.7	49.9 ± 3.8	44.3 ± 5.3	57.3 ± 5.1	62.6 ± 2.6	93.3 ± 8.0
EPO (mU/mL)	20.3 ± 7.7	48.1 ± 25.0	38.5 ± 23.1	16.2 ± 5.3	16.5 ± 4.5	14.5 ± 6.5
RBC (× 10 ⁴ /μL)	463 ± 52	484 ± 66	567 ± 75	551 ± 65	513 ± 48	497 ± 51
Hb (mg/dL)	14.5 ± 1.6	15.6 ± 0.5	16.9 ± 0.9	17.9 ± 1.5	18.0 ± 1.0	16.3 ± 1.2
CRP (mg/dL)	0.1 ± 0.1	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mountain sickness score	–	1.6 ± 1.3	0.7 ± 0.8	0.1 ± 0.4	0.0 ± 0.0	–

Number of subjects, 7.

All results are shown as mean ± SD.

A–F, see the text.

CRP, C-reactive protein; EPO, erythropoietin; PaO₂, arterial oxygen tension; RBC, red blood cell.

* *P* < 0.05.

** *P* < 0.01.

The time changes in serum concentration of IL-6 are illustrated in Table 2. Except for Subject X, serum concentrations of IL-6 were within normal ranges (< 4.0 pg/mL) 1 month before departure (Point A). The levels then rose significantly higher than the initial Point-A level (0.58 ± 0.15 pg/mL) at Point B (1.28 ± 0.45 pg/mL, *P* < 0.05), and returned to nearly the initial level at Point F (0.57 ± 0.27 pg/

mL). The highest serum IL-6 level was found at Point E (1.38 ± 0.89 pg/mL), which was significantly higher than at Points A and F (*P* < 0.05 and *P* < 0.01). The plasma volumes decreased by an average of 9%, 22%, 32%, 34% and 19% at Points B, C, D, E and F, respectively. Thereby the mean serum IL-6 at each point was overestimated by 9%, 28%, 43%, 48% and 21%, respectively. However,

Table 2. Changes in the serum concentration of IL-6

		Blood-sampling point						
		A	B	C	D	E	F	
IL-6	(pg/mL)	0.58 ± 0.15	1.28 ± 0.45	1.03 ± 0.54	0.95 ± 0.45	1.38 ± 0.89	0.57 ± 0.27	
		* —		* —			* —	
				* —			** —	
Corrected IL-6 [†]	(pg/mL)	0.58 ± 0.15	1.18 ± 0.50	0.81 ± 0.47	0.67 ± 0.33	0.94 ± 0.65	0.47 ± 0.23	
		* —		* —			* —	
				* —			** —	

Number of subjects, 6, excepting for one who suffered the bone fractures.

All results are shown as mean ± SD.

[†] The level of IL-6 after correction for hemoconcentration.

A–F, see the text.

IL-6, interleukin-6.

* $P < 0.05$.

** $P < 0.01$.

even with corrections for hemoconcentration, the IL-6 level at Point B would be higher than those at Points A, D and F ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively). The value of IL-6 at Point E would still be higher than at Point F ($P < 0.05$). Subject X had a very high concentration of IL-6 at every determination point; the values at Points A to F were 36.2, 103.0, 56.8, 44.6, 45.4 and 36.7 pg/mL, respectively.

Mild mountain sickness was observed in 5 of the men 2 weeks after departure. Although 4 of them showed symptoms of exposure to cold, such as headache, nasal congestion and fatigue, they recovered within 2 to 10 days. However, Subject X showed unusual headache and general fatigue persisted for 6 weeks.

The concentration of IL-6 did not correlate with the PaO₂ ($r = -0.28$, $P = 0.07$), EPO level ($r = 0.04$, $P = 0.79$) and mountain sickness score ($r = 0.28$, $P = 0.08$).

Discussion

Cold, low pressure and low oxygen are known to affect human physiology at high altitudes. Among these factors, low oxygen is considered to be the most important. In this study, we observed that the

serum concentration of EPO promptly increased: this reaction, we thought, was necessary for humans to adapt to a high altitude. Since the number of erythrocytes peaked within 6 weeks, one may conclude that hematological adaptation to a high altitude is completed in several weeks (Wilkerson, 1975).

The concentrations of IL-6 at Points B and E were higher than those at a low altitude. It is known that hypoxia increases serum IL-6 (Klausen et al., 1997; Hartmann et al., 2000). Klausen et al. (1997) reported that the values of serum IL-6 were related to arterial blood oxygen saturation and EPO values, with rapidly reacting changes in a short period. We could not examine early changes in cytokines because of other various inland research and a strict work schedule. In our long-term observation, the serum concentration of IL-6 was not related to serum EPO and hypoxemia. Since the concentration of IL-6 did not correlate with the PaO₂ in this study, factors other than low oxygen may also contribute to this change. Changes in serum CRP were very slight, and therefore, inflammation might have no association with changes in IL-6.

Cold itself was determined to have had little influence on results because the subjects spent most of their time within the snow vehicles, which were heated. We thought that cold in this study was a

temporary stimulus, and one of psychological stressors. Several studies examined that psychological stress is associated with an increase in serum concentration of IL-6 (Maes et al., 1999; Song et al., 1999). It has been reported that the restrain stress increased the plasma concentrations of IL-6 in mice (Nukina et al., 1988). Lantis (1968) observed that the social environment of polar regions would comprise a more potent source of stress than the physical environment. In our study, the serum concentration of IL-6 increased again after hematological adaptation to the altitude was assumed to be completed. Although we could not estimate the mental conditions of the subjects objectively, psychological stress under such conditions may also have contributed to the increase of IL-6.

The changes in IL-6 were slight in general; in Subject X, however, IL-6 remarkably increased during the trip. The fractures he suffered before departure and a kind of posttraumatic stress disorder (Maes et al., 1999) in addition to hypoxia were thought to be the reasons for this change. On the other hand, since his symptoms of mountain sickness were severer than in the others, elevation of IL-6 may be related to mountain sickness.

We were unable to identify any background factor that might explain why the serum concentration of IL-6 changed in this research. However, none of the subjects suffered from severe mountain sickness like pulmonary and brain edema because of the slow rise in altitude. All subjects remained in relatively good health for 3 months: the human body is proof that humans can survive in extreme environmental conditions in Antarctica and acclimating to such surroundings is possible.

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