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Effect of extreme conditions of Antarctica on human leukocyte antigen-G in Indian expeditioners

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***Background & objectives*: Immune activation and inflammation play critical roles in the stressful environmental conditions like high altitude, extreme cold, *etc*. Human leukocyte antigen-G (HLA-G) is a non classical major histocompatiblity complex class I (MHC class- I) protein, upregulated in the context of transplantation, malignancy and inflammation. We hypothesized serum HLA-G as a possible stress biomarker and studied levels of soluble form of HLA-G (sHLA-G) in Indian Antarctic expeditioners.**

***Methods*: sHLA-G ELISA was performed in the serum of summer (n=27) and winter (n=22) Indian Antarctic expeditioners. The summer expeditioners were evaluated at three different time points, *i.e*. before leaving India, after one month ship borne journey, and after staying one month at Indian research base, Maitri in Antarctica, while winter expeditioners were evaluated at five different time points, *i.e.* before leaving India, and in the month of March, May, August and November at Antarctica.**

***Results*: One month ship borne journey did not cause any significant change in the sHLA-G level as compared to the baseline level of the summer expeditioners. sHLA-G levels were not changed significantly in the months of March, May, August and November as compared to the baseline level of the winter expeditioners.**

***Interpretation & conclusions*: Our results indicated that the extreme conditions of Antarctica did not cause any significant change in the sHLA-G level in both summer and winter expeditioners.**

Key words Antarctica - expeditioners - HLA-G - inflammation - serum - ship

Human leukocyte antigen (HLA)-G is a non classical HLA class I molecule from the major histocompatibility complex, which was initially shown to confer protection to the foetus from mother's immune system. Beyond its role in foetal-maternal tolerance, HLA-G exerts tolerogenic functions involved in transplant acceptance as well as in tumoral and viral immune escape[1]. It also has inhibitory functions in erythropoiesis and angiogenesis[2]. HLA-G occurs in both membrane-bound and soluble form. Data suggest that the soluble form of HLA-G (sHLA-G) may have a different function than its membrane-bound counterpart (mHLA-G) and that it may even counteract effects of mHLA-G[2]. sHLA-G can be generated by at least two different processes. First, because of alternative splicing, three different soluble isoforms occur that have

a stop codon in intron 4, thus preventing translation of the transmembrane domain and cytoplasmic tail. There is still a lot of debate on the expression and function of these different isoforms[3-7]. Second, sHLA-G can be produced by cleavage of the membrane-bound form from the cell surface by metalloproteinases[8,9]. At present, it is not clear as to what extent either of these processes contributes to the levels of sHLA-G found in pregnancy or under pathological conditions[8-12]. Exposure to environmental stresses such as cold, heat, and high altitudes modify various components of immune functions[13-15]. Severe environmental stress may have immunosuppressive effect, resulting in increased risk for immunity-related diseases. It has been recognized that cold stress affects various aspects of both cellular and humoral immunity[14]. The understanding of stress induced immune alterations is still not very clear. We hypothesized that the sHLA-G level in Antarctic expeditioners might be altered due to cold stress. Therefore, in the present study the level of sHLA-G were estimated in the 28th Indian Antarctic Research Expedition (InSEA-28) team members. The sHLA-G level was determined in summer expeditioners at three different stages - before leaving for expedition, on the ship and after one month stay at Indian research base Maitri, Antarctica. Winter expeditioners were evaluated at five different time points, *i.e*. before leaving India and in the month of March, May, August and November at Antarctica.

Material & Methods

This study was conducted in 2008-2010 in the Defence Institute of Physiology and Allied Sciences (DIPAS), Delhi, India, and the study protocol was approved by the ethics committee of the Institute. Written informed consent was obtained from each subject. The sample selection was done based on the number of summer and winter expeditioners.

Summer subjects: The 27 male members, age ranged from 23 to 60 yr, with a mean age of 37 ± 1.9 SE years of InSEA-28 who participated in the study, followed similar daily work routines including outdoor activities (3-8 h per day). All the subjects had the same pattern of diet throughout the journey and one month stay at Maitri, Antarctica.

Winter subjects: Twenty two male members (mean age 36 ± 1.4 SE, range 25-60 yr) of the wintering team of 28th Indian scientific expedition to Antarctica volunteered to participate in this study. All had undergone pre-departure clinical, psychological, and laboratory examinations to ensure a healthy population for the isolation during the Antarctic stay. This study was conducted at Indian research base, Maitri, Antarctica. Blood was drawn in October 2008 before leaving India to Antarctica and then in March, May, August and November in 2009. No subjects had signs or symptoms indicative of infection at the time of the study. None had used drugs that could significantly affect the immunological parameters.

Weather condition: The weather of Antarctica is influenced by its solar altitude and latitude. Antarctic summer season is considered from November to February while winter season starts in the beginning of March and lasts till October end. The daytime air temperatures from December to February frequently exceed 4°C, with the mean monthly temperature a little above 0 °C. The winter temperature at Maitri varies between -5 °C to -35 °C. Wind speed goes up to 200 kilometers per hour. Maitri is physically isolated from the civilized world from March to November each year.

Blood sampling: The blood (10 ml) collection of expeditioners was performed in the morning between 0600 and 0700 h in non heparinised vials and kept for 1 h at 4 °C. Clear serum above the blood clot was collected in fresh Eppendorf tubes and centrifuged at 300 g for 10 min. The serum was collected in small aliquots and stored at -40 °C for further analysis.

Estimation of sHLA-G levels: Estimation of sHLA-G levels in serum samples was carried out as solid phase enzyme immunoassay with ELISA kit (Cusabio Biotech, China) based on sandwich ELISA principle. The ELISA plates were coated with antibodies specific for human sHLA-G to detect the specific sHLA-G molecule present in the standard and serum samples. The anti-HLA-G antibody conjugated to biotin was added to each well followed by substrate (streptavidine/ horse radish peroxidase) addition and incubation for 20 min. Absorbance was measured by ELISA reader (Biotek, USA) at 450 nm.

Statistical analysis: Data were analyzed by repeated measure analysis with age as covariate. The analysis was conducted using SPSS 16 software (USA).

Results

sHLA-G level in summer expeditioners: The level of sHLA-G at three different time points of summer expedition group (1st -Delhi as baseline level; 2nd ship in southern ocean; and 3rd off-board at Maitri)

Table I. Repeated measure analysis of the effect of age and Antarctic environment on the sHLA-G level in Indian summer expeditioners

Effect	Measures	Value	F	Hypothesis df	Error df	*P* value
Group	Pillai's trace	0.005	0.065	2.000	24.000	0.937
	Wilks' lambda	0.995	0.065	2.000	24.000	0.937
	Hotelling's trace	0.005	0.065	2.000	24.000	0.937
	Roy's largest root	0.005	0.065	2.000	24.000	0.937
Group *age	Pillai's trace	0.050	0.635	2.000	24.000	0.539
	Wilks' lambda	0.950	0.635	2.000	24.000	0.539
	Hotelling's trace	0.053	0.635	2.000	24.000	0.539
	Roy's largest root	0.053	0.635	2.000	24.000	0.539

was measured. The baseline level of sHLA-G was 30.52 ± 5.1 ng/ml in the summer expeditioners before proceeding to Antarctica, which was considered as control. The levels increased to 50.05 ± 4.06 ng/ml after being one month on-board in Southern Ocean. The sHLA-G levels further decreased to 36.03 ± 5.3 ng/ml (Maitri) after being one month off-board at Maitri. The interaction between three different conditions and age did not show any significant difference on sHLA-G level in the summer expedition group (Table I).

sHLA-G level in winter expeditioners during stay at Maitri Antarctica: The sHLA-G level in baseline was 31.25 ± 4.15 ng/ml (Control) which was increased to 47.35 ± 5.7 ng/ml in the month of March. It was further increased to 52.43 ± 5.68 and 58.75 ± 5.02 ng/ml in the months of May and August, respectively. However, the level decreased to 41.15 ± 6.88 ng/ml in November. The difference in the sHLA-G levels was not significant. The interaction between five different conditions and age also did not show any significant difference on sHLA-G level in the winter expedition group (Table II).

Discussion

Several reports have suggested that sHLA-G affects the functions of the immune cells[2,5]. In the expectation that the sHLA-G protein may be affected by the extreme environmental conditions, we examined the level of sHLA-G protein in the serum of Indian-Antarctic summer and winter expeditioners. However, sHLA-G level did not show any significant difference as compare to the baseline level. The uniqueness of the present study lies in the data collected during the month long sea voyage in the southern ocean and also the long term stay there.

The available literature suggests the relevance of HLA-G in several inflammatory and autoimmune diseases, such as systemic lupus erythematosis (SLE). Almasood *et al*[16] reported the upregulation of HLA-G in heart failure patients and claimed as potential biomarker for heart failure cases. We have earlier reported that Antarctic expeditioners have increased proinflammatory cytokines[14]. The increased proinflammatory cytokines level could be because of increased nuclear factor-kappaB (NF-κB) expression

Table II. Repeated measure analysis of the effect of age and Antarctic environment on the sHLA-G level in Indian winter expeditioners

Effect	Measures	Value	F	Hypothesis df	Error df	*P* value
Group	Pillai's trace	0.162	0.821	4.000	17.000	0.530
	Wilks' lambda	0.838	0.821	4.000	17.000	0.530
	Hotelling's trace	0.193	0.821	4.000	17.000	0.530
	Roy's largest root	0.193	0.821	4.000	17.000	0.530
Group * age	Pillai's trace	0.229	1.263	4.000	17.000	0.323
	Wilks' lambda	0.771	1.263	4.000	17.000	0.323
	Hotelling's trace	0.297	1.263	4.000	17.000	0.323
	Roy's largest root	0.297	1.263	4.000	17.000	0.323

level. Zidi *et al*[17] showed that NF-κB induction increased HLA-G1 proteolytic shedding through the activation of metalloproteinases and it is likely that NF-κB, under stressful conditions like high altitude hypoxia, may be involved in the upregulation of blood sHLA-G1. Bourguignon *et al*[15] have reported an increased level of sHLA-G molecule in six Mount Everest expeditioners. In contrast, our data in Antarctic extremes report no significant change in the level of sHLA-G protein as compared to the baseline level in summer and winter expeditioners.

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