*Nineteenth Indian Expedition to Antarctica, Scientific Report, 2004*
*Department of Ocean Development, Technical Publication No. 17, pp 239-246*

# Effect of Cold, Isolation and Ultraviolet Radiation Stress on Human Immune System

**A.K. Singh, Vinod Kausik and T. Lazar Mathew**
Institute of Nuclear Medicine and Allied Sciences, Delhi 110 054

**Abstract**

Stressful environment conditions are a major determinant in immune reactivity. This effect is pronounced in Antarctica expeditioners where they are exposed for long to the isolation, cold and UV radiation stress. Whether these stresses alter the immune system or not, was studied in expedition members at Maitri. Various parameters like Immunoglobulin, Lymphocyte counts, Cytokine and 1, 25-hydroxy Vitamin D-3 were estimated to assess the functional status of the immune cells. Data gathered indicate Antarctic isolation is associated with alterations in the immune system due immunity. These findings have important long-term health implications.

## Introduction

Recent studies by Schmitt et al (1993) and Herbertet et al (1993) have demonstrated that environmental conditions are an important determinant of the immune response outcome. An example is the Antarctic, which is a special part of this globe, has unique conditions where members of expeditions and tourists face a challenging environment. Antarctica is a natural field station in which the environment is relatively uncomplicated and from which extrapolations might be made for other regions. The cold climate is not the only stress encountered at Antarctica but also isolation, life in small groups, physical difficulties, pressure of work and so on. The environmental stress which constitutes ultraviolet radiation (UV), polar days, high magnetic flux and sub-zero temperature throughout the year cumulatively affect the normal physiology of the person from the plains or at tropical region of plains working at Antarctica. UV radiation which is due to springtime ozone depletion over Antarctica has been known for over ten years and affects me exposed parts of the person working outside at Antarctica. UV radiation completely gets absorbed in a few micrometers of human skin, which is the main tissue at risk in such situations. The biological effectiveness of UV radiation varies with the wavelength of the

UV and the flux of the radiation shows marked variation through immune function. It is modulated by a complex set of neuroendocrine factors including sex hormones, vitamin D metabolites, Ultraviolet radiations. Antarctic expeditioners exhibit reduced cell mediated immunity. Studies have also shown a reduction in testosterone levels in wintering expeditioners and increased Vitamin D3 plasma levels suggested a correlation between levels of anxiety and reduced immune response 1,25-Dihydroxy vitamin D3, the hormonal form of vitamin D, is now believed to play a significant role in the immune responses; Ishigani (1919), both in vitro and in vivo, preventing the development of several autoimmune diseases. In Antarctica a substantial number of those working in the environment demonstrate depressed cutaneous immune response. The proposed study is undertaken to evaluate and understand the role of UV radiation in modulating the immune response directly or indirectly through vitamin D involvement and to whether the isolation and cold stress supplement this effect.

## Material and Methods

Subjects: This study was carried on 13 healthy expedition members with mean age of 35 years. Subjects volunteered for the study willingly and were explained about the study. Subjects were housed in heated huts and meals were provided three times a day ad libitum, and were performing their respective scientific/logistic activities indoors as well as outdoors.

**Blood collection:** About 20 ml of venous blood was withdrawn between 9:30am to 10:30am to minimize any effect attributable to diurnal variation on three occasions i.e. on arrival at Maitri day 1, and on day 24 at Maitri, and the day members left Maitri *i.e.* day 48, using standard methods from the vein. On each occasion enough blood was drawn from subjects for mononuclear cell separation by density gradient and serum separation and serological studies.

**Serum Separation:** Blood was allowed to coagulate at 4°C and kept for 4 hours till the clot retracted. Tubes were centrifuged at 4000 rpm for 10 minutes. Separated serum was withdrawn by dropper and frozen at -15°C till use.

**Separation of Lymphocytes:** Peripheral blood mononuclear cells were isolated by density gradient sedimentation using Histopaque 1970 (sigma, USA). Peripheral blood (10ml) collected in heparenised tubes were processed within 30 minutes of collection. The blood with anticoagulant

was diluted 2:1 in RPMI-1640 medium (sigma,USA) before layering onto 10ml Histopaque-1077. The cells were centrifuged at 400Xg for 30 min, followed by careful aspiration of the mononuclear layer. After checking the viability of the cells by trypan blue exclusion. T & B-lymphocytes were separated by affinity chromatography using the standard ready to use kits.

**Freezing of Cells:** The separated cells were washed in RPMI-1640 three times by centrifugation at 400Xg before resuspension in 2ml of a mixture of 20% FCS in RPMI-1640. Resuspended cells were cooled to 4°C and 20% dimethysulfoxide (DMSO, Sigma, USA). The solutions were decanted into an 8ml cryogenic vial and transferred to - 20°C for 4 hours and then were transferred to the liquid nitrogen tank till further use.

**Thawing of Cells:** Cells were thawed rapidly in a 37°C water bath and transferred to 10 ml centrifuge tube on ice. Deoxyribonuclease (Dnase) 0.01 % (Pancreatic Dnase, Amersham Australia) were added to minimize cell loss due to clumping and cell sample diluted drop wise 10ml RPMI-1640 solution containing sodium azide and centrifuged 200Xg at 4°C. Cells were resuspended in 10% FCS in PBS at 4°C, centrifuged at 300Xg for 10 min at 4°C and after resuspension of the cell pellet in 50ul of supernatant, l00ul of FCS and 100 ul of human immunoglobulin were added. The cell suspension was further diluted in RPMI-1640 solution containing 10% FCS and incubated for 40-60 min at 4°C. The cells were transferred to the 96 well culture plates and incubated at 37°C for growth.

Haemoglobin and Lymphocyte counts were analyzed by QBC, cell analyzer Becton and Dicknson. Interleukinl-1, Interleukin-6 and 1, 25-Hydroxy Vitamin D-3 and Immunoglobulin G were estimated by Enzyme Linked Immunosorbent assay (ELISA) using kits supplied by (R & D System Inc., Minneapolis, USA).

## Results

The pooled data of all the 13 subjects for the concentration of Haemoglobin was found Normal Fig. 1. In our study the average Hb concentration was 14.85 ±0.545 mg/ml in sample one taken on day one. The concentration of Hb increased to 15.93+0.89 mg/ml *i.e.* increase of 7.2% in sample two taken on day 24. In the sample three taken on day 48 the concentration of Hb decreased slightly to 15.63+ 0.55 mg/nl. Lymphocyte count: Total Mononuclear cell count in sample one in each subject was normal and the average count was 2022+88.49 cells / l00ul of

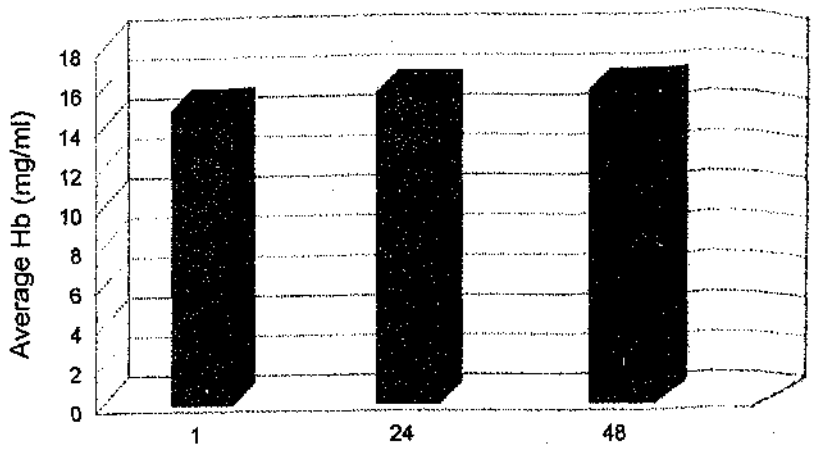


*Fig. 1; Haemoglobin Concentration in Serum*

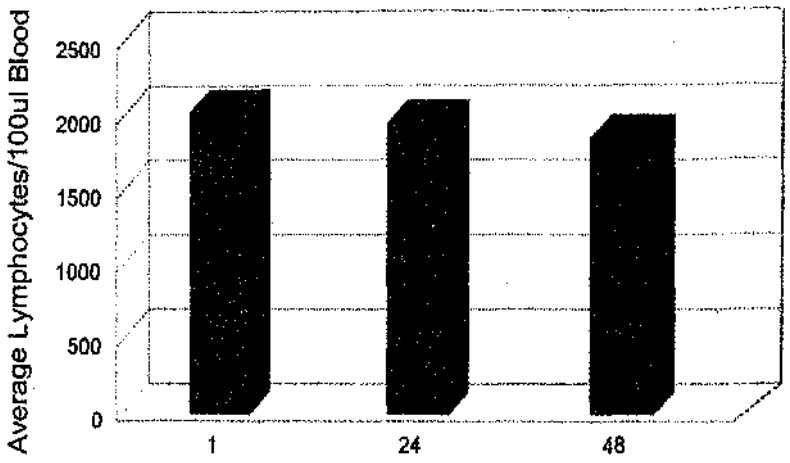


*Fig. 2: Lymphocyte Concentration in Serum*

blood and decreased to 1946.62±97.29 cells / l00ul and 1846.23 ±88.53 cells /100 ul in sample two and three respectively Fig. 2. This data shows the decrease on 3.8% and 8.75 in sample two and three respectively as compared to sample one. Immunoglobulin G concentration in sample one was found to be 35.62+5.39 mg/ml, which decreased to 31.44±9.54 mg/ml in sample two which is 11.5% decrease and there was slight increase in sample three to 31.92+4.45 mg/ml. Which is 2.58% from the sample two

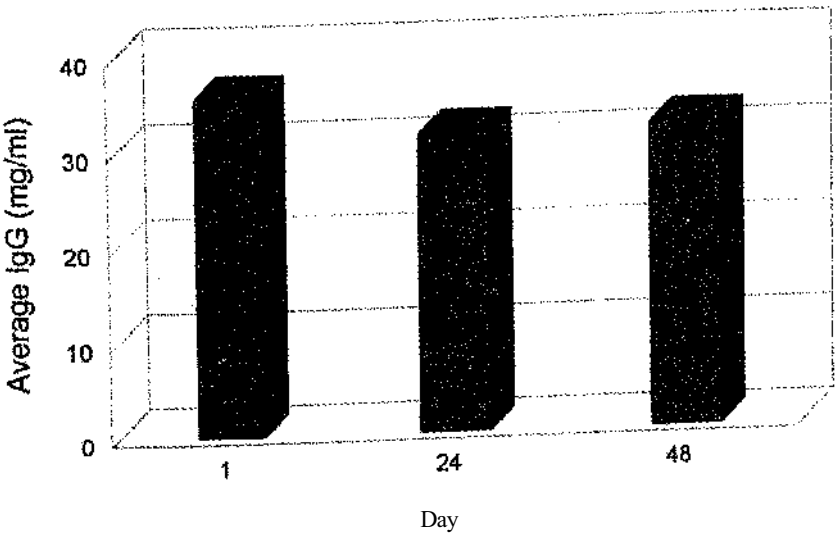


*Fig. 3; Immunoglobulin (Ig G) Concentration in Serum*

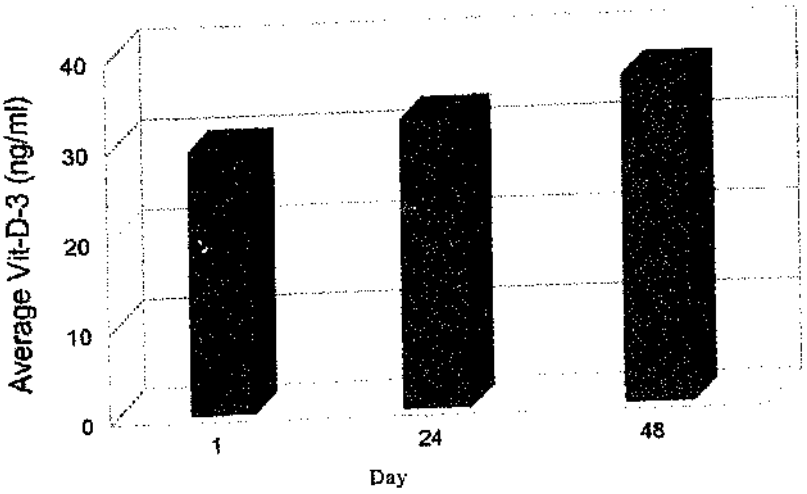


*Fig. 4:25-Hydroxy Vitamin-D-3 Concentration in Serum*

Fig. 3. 1, 25-Hydroxy Vitamin D-3 was normal in each subject and the average amount in sample one was 29.33+4.5 mg/ml which increased to 32.181±4.43mg/ml and 36.28+4.99 ng/ml in sample two and three respectively Fig. 4. It is 9.75 and 23.9 percent increase in sample two and three as compared to sample one. Cytokine i.e Interleukin 1 and Interleukin 6. The average concentration if II-l in sample one was 51.74+6.48pg/ml. It increased to 59.96+5.84 pg/ml and 61.17±6.33 pg/ml in sample two and

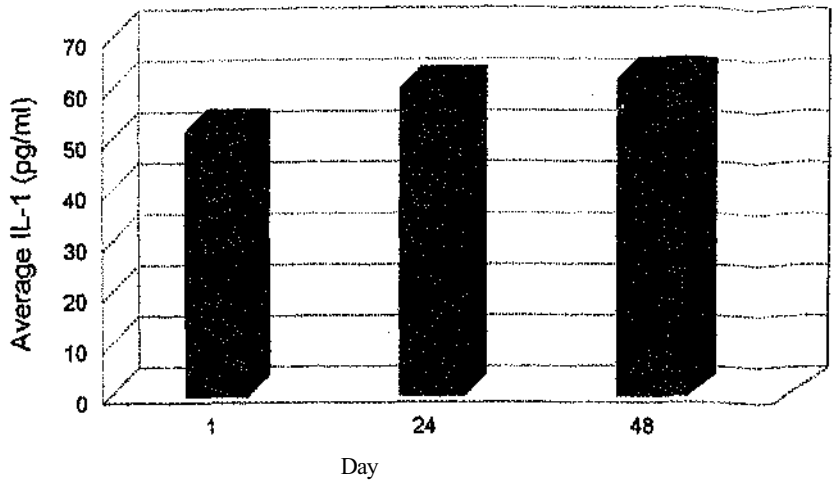


*Fig. 5: Interleukin-1 Concentration*

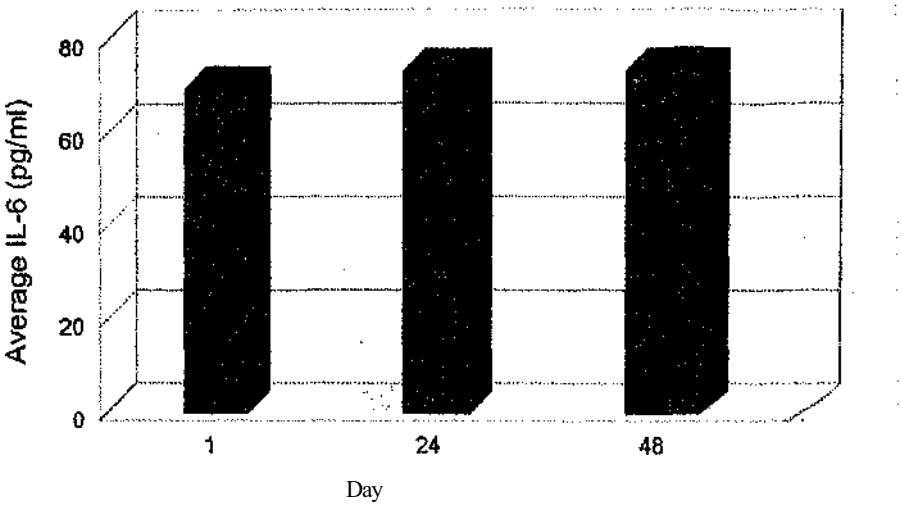


*Fig. 6: InterleuMn-6 Concentration*

three respectively. Fig. 5, which is an increase of 15.88% and 18% as compared to sample one. The pattern was similar for lbnterleukin-6. Concentration of Interleukin-6 was 69.45±6.64 pg/ml in sample one and increased to 73.11+6.96 pg/ml and 73.32±7.6-pg /ml in sample two and three Fig. 6. It is an increase of 5.78% and 6.10% as compared to sample one.

## Discussions

Stressful events are commonly believed to suppress host resistance to infections when demands imposed by the events exceeds a persons ability

to cope with stress. A stress response composed of negative cognitive and emotional state, physiological stress is elicited. Antarctica expedition members encounter this stress, which are thought to influence immune function through autonomic nervous innervating lymphoid cells Rivoler *et al* (1998). There is substantial evidence that stressful life events and perceived stress are associated with changes in immune functions. Results of this study demonstrate that the immune system of the subjects during their stay at Maitri was depressed. As regard the Haemoglobin concentration which was optimum in each subject and increased during their stay may be due to stress of cold and isolation at Maitri or an adaptive response. Increase in Heamoglobin concentration has also been reported in Antarctic expeditioners, by Rivoler *et al* (1988). Our observations support their finding. Rivoler *et al* (1998) have also reported decrease in the polymorphonuclear neutrophils and increase in lymphocyte counts. Roberts et al (1985) reported no significant change in white cell counts and absolute lymphocyte counts in each subject was normal and virtually same during their stay at Antarctic. Results of present study also show the trend of decrease in the lymphocyte counts though the decrease is very less, due to conflicting reports more work needs to be carried out.

Regarding the Immunoglobin G amount. Muchmore et al (1973) reported slight but significant decrease in the immunoglobulin G and M during winter isolation period at Antarctic. Our results indicate slight decrease in the second sample and slight increase in the third sample. The increase in the third sample is insignificant and may be an artifact. Selective rise in immunoglobulin concentration should have been associated with adaptive response and could possibly reflect non-specific changes in the levels of helper or suppressor T lymphocytes and it may be a reason that absolute lymphocyte counts has been observed to decline in our study, corroborate the report of Muchmore *et al* (1973). Studies have shown that increase in 1,25-dihydroxy vitamin D-3, the hormonal form of Vitamin D, has a correlation between levels of anxiety and reduced immune response. Seasonal variation in the 1,25-dyhydroxy vitamins D-3 with significant winter drop has also been observed. This observation shows a direct correlation of reduced UV-B exposure due to polar nights and serum levels of 1,25-dihydroxy vitamins D-3. Kowitz *et al* (1998) has shown that alpha 1,25-dihydroxy vitamin D-3 inhibits the proliferation of peripheral blood mononuclear cells. In our study we have observed the increase in the 1,25-dihydroxy vitamin D-3 and also interleukin 1 and 6. Our data is in accordance with the repot of Lemre (1995) and Saperstein (1992), where he has shown that 1,25-dihydroxy vitamin D-3 inhibits the production of cytokines. This inhibition is due to the inhibition of helper T cells. Trevors et

*al* (1997) and Sonnenfeld *et al* (1992) have also reported the similar observations of reduction in the cytokine levels and T-cell proliferation.

The present study provides clear evidence that stresses in Antarctic are associated with distinct alterations in human immune system. The alterations in immune system are the eventual results of multi-factor interactions in the body in response to various stresses, thus need more studies. In the present study base line data at Goa could not be taken due to logistic problems. It would be appropriate to extend this study on the wintering members.

## References

1. Herbert T?B, Cohen Sc. Stress and immunity in human: A meta-analytic review. Paychosom. Med. 1993 : 55 : 365-79.
2. Ishigami T, The influence of psychic acts on the progress of pulmonary tuberculosis. Am Rev Tuberculosis 1919; 2; 470-484.
3. Kowitz A, Greiner M, thieroff-Ekerdt R. Inhibitory effect of alpha- 1,25-dihydroxy vitamin D-3 on allergenic lymphocyte stimulation and langerhans cells maturation. Arch Dermatol Res. 1998, 290 ; 10: 540-46.
4. Lemire JM. Immunomodulatory action of 1,25-dihydroxy vitamin D-3. J steroid Biochem Mol Biol 1995; 53 (1-6); 599-602.
5. Rivolier J, Goldsmith R. Lugg Dj et al. Man in Antarctica., Published Tylor and Francis, London
6. Saperstein a, Brand H, Audhya T. Interleukin 1 beta mediates stress induced immunosupression via corticotropin releasing factor. Endocrinology 1992; 130: 152-58.
7. Schmitt DA, Schaffer L, Confinement and immune function. Adv Space Biol Med. 1993 ; 229-35.
8. Sonnenfield G, Measel J, Loken MR *et al.* Effect of isolation on interferon production and hematological parameters. J. Interferon Cytokine Res. 1992; 275-81.
9. Trevor RT, Desmond JL, Muller HK *et al.* Antarctic Isolation; Immune and viral studies. Immunology and Cell biology 1997; 75: 275-283.