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CYTOKINES AND MACROPHAGE FUNCTION IN HUMANS - ROLE OF STRESS

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*The NASA Technical Monitor for this grant is:

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INTRODUCTION

We have begun this study to commence the determination of the role of mild chronic stress in the effects of space flight on macrophage/monocyte function, a component of the immune response. Medical students undergoing regular periods of stress and relaxation have been shown to be an excellent model for determining the effects of stress on immune responses (1). We have begun using this model using the macrophage/monocyte as a model leukocyte. The monocyte/ macrophage plays a central role in immunoregulation (2). The studies to be included in this three year project are the effects of stress on: 1) interactions of monocytes with microbes, 2) monocyte production of cytokines, 3) monocyte phagocytosis and activity' and 4) monocyte expression of cell surface antigens important in immune responses. Stress hormone levels will also be carried out to determine if there is a correlation between stress effects on immune responses and hormonal levels.

Psychological testing to insure subjects are actually stressed or relaxed at the time of testing will also be carried out. The results obtained from the proposed studies should be comparable with space flight studies with whole animals and isolated cell cultures. When complete this study should allow the commencement of the establishment of the role of stress as one compartment of the induction of immune alterations by space flight.

METHODS, RESULTS AND DISCUSSION

The first year of this study was designed to allow the establishment of techniques to be used and the establishment of a first group of subjects. A group of 5 medical students and three control subjects has been established for these early studies. The group has been tested at several time intervals to date; at times of examination stress and pending vacation. The examination sampling times should be times of stress for the medical students and the pending examination time should be a time of minimal stress.

The results obtained to date suggest the following:

- 1) As a result of the psychological testing, two of the medical student subjects consistently had values in the range of clinically anxious men and women. One control (an expectant father) was also in that range at one test point. The other three medical students and remaining controls were never in the range of clinical anxiety. Additional medical students who were added later in the study are currently being evaluated for anxiety level.
- 2) The level of tumor necrosis factor- α produced by the medical student subjects who were in the clinical anxiety range was decreased substantially compared to medical students who were not clinically anxious and controls. There was good correlation between level of anxiety and decrease in tumor necrosis factor- α production. This was true in the early part of the study, but not as the study advanced. Anxiety score correlations are under analysis for the later part of the study.
- 3) The levels of serum albumin were equivalent in all subjects at all times, indicating that immunological changes were not induced by alterations in diets of the clinically anxious level subjects.
- 4) No difference was detected in the cortisol levels (26.0mg/dL vs 22.9, $p=0.7$).

5) ACTH levels were increased during the examination period compared to the non-examination period ($p < 0.01$).

6) Regarding leukocyte subset distribution, no differences were found in the levels of cell surface markers for CD14 ($p = 0.3$) and HLA-DR ($p = 0.7$) between both groups. CD15 levels were lower during the examination periods compared to the non-examination period (55% vs 62%, $p = 0.001$).

8) TNF-beta levels increased in the academically stressed student group.

These results suggest that stress can alter macrophage function. The changes were not due to changes in diet, and were related to the level of anxiety induced in the subjects. Final analysis of the data is currently in progress. It appears that we will be successful in showing a correlation between stress and alterations in macrophage function. After completion of all of the data analyses, the work will be prepared for publication.

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PUBLICATIONS

Fowler, N.A., Fernandez-Botran, R., Hakenwerth, A.M., Borden, J., and Sonnenfeld, G. Tumor necrosis factor beta levels are increased in cultured white blood cells of academically stressed medical students. *American Society for Gravitational and Space Biology Bulletin*, 9:31, 1995.

MEASUREMENT OF IMMUNE PARAMETERS IN MEDICAL STUDENTS DURING ACADEMIC EXAMINATIONS

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Stress is a potential component of space flight. In order to study the possible role of stress in the effects of space flight on immune responses we used chronic mild stress, the academic stress model for medical students. In this study, 18 first and second year medical students enrolled in the Univ. of Louisville School of Medicine, were followed over one academic year. Blood was drawn from all students simultaneously at four different timepoints corresponding to two different periods (2 timepoints per period). The examination periods was compared to a non-examination periods for all students. Albumin, cortisol and adrenocorticotrophic hormone (ACTH) levels were measured in the plasma. Peripheral blood leukocytes were analyzed by flow cytometry using markers for monocytes (CD14), granulocytes (CD15) and major histocompatibility complex class II antigens (HLA-DR). Paired t-tests and Wilcoxon Signed Rank tests were used to compare the mean of the 2 "examination" values to the mean of the 2 "non-examination" values. Albumin levels were similar during the two study periods ($p=0.1$). No difference was detected in the cortisol levels ($26.0\mu\text{g/dL}$ vs 22.9 , $p=0.7$). ACTH levels were increased during the examination period compared to the non-examination period ($p<0.01$). Regarding the cell staining, no differences were found in the levels of cell surface markers for CD14 ($p=0.3$) and HLA-DR ($p=0.7$) between both groups. CD15 levels were lower during the examination periods compared to the non-examination period (55% vs 62% , $p=0.001$). These results indicate that academic stress in medical students can induce immune changes similar to some of those observed during space flights. Funded by NASA grant NAG9-728.