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FINAL REPORT

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SPACEFLIGHT AND IMMUNE RESPONSES OF RHESUS MONKEYS

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INTRODUCTION

Evidence from both human and rodent studies has indicated that alterations in immunological parameters occur after space flight [1]. The number of flight experiments has been small, and the full breadth of immunological alterations occurring after space flight remains to be established. Among the major effects on immune responses after space flight that have been reported are: alterations in lymphocyte blastogenesis and natural killer cell activity [1-3], alterations in production of cytokines [4,5], changes in leukocyte sub-population distribution [6], and decreases in the ability of bone marrow cells to respond to colony stimulating factors [6]. Changes have been reported in immunological parameters of both humans and rodents [1]. The significance of these alterations in relation to resistance to infection remains to be established.

The objective of the studies contained in this project was to determine the effects of space flight on immune responses of Rhesus monkeys. The hypothesis was that space flight and the attendant period of microgravity will result in alteration of immunological parameters. The parameters tested included: production of cytokines, composition of leukocyte subpopulations, functional activities of immunologically significant cells, and differences in effects on cells from primary and secondary lymphoid tissues. The significance of the work was a determination of the range of immunological functions of the Rhesus monkey, a primate similar in many ways to man, affected by space flight. Changes in immune responses that could yield alterations in resistance to infection could have been determined. The duration of alterations in immune responses might also have been determined. This could have yielded useful information for planning studies that could contribute to crew health. Additional information on the nature of cellular interactions for the generation of immune responses could also have been obtained.

In after a review in late 1995, a decision was reached not to include the immunology experiments of monkey in the Bion flight experiments. For this reason, the project was terminated in 1996. Results available include establishment of the assay systems for immunology in monkeys, the ARRT experiment to determine the effects of restraint on immune parameters, and support studies of stress effects on immune responses in murine systems.

METHODS

The methods used in the study have involved the determination of leukocyte subpopulations and the production of cytokines. For the leukocyte subpopulation analysis, whole blood was taken using EDTA as the anticoagulant or lymph node cells were harvested. Samples of whole blood or lymph node cells were stained with the following monoclonal antibodies obtained from the Becton-Dickinson Monoclonal Center, Mountain View, CA: Leu 2a, Leu 3a, Leu4, Leu 11a, Leu 12, Leu-M3, HLA-DR, IgG, and IL-2 receptor. Additional cells were stained with monkey anti-IgM and Monkey anti-IgM (Fab')₂. Control cells were either stained with a monoclonal antibody directed against an irrelevant species or were unstained [7]. Cells were then analyzed for fluorescence using a Becton-Dickinson Facscan or Facscalibur flow cytometer [7].

For the cytokine production, samples of whole blood were placed on ficoll-hypaque density gradients to purify mononuclear cells. The mononuclear cells or the lymph node cells were treated with either phytohemagglutinin-P or polyribosinic-polyribocytidylic acid. After the appropriate incubation period at 37°C, the culture supernatant fluids were harvested and stored at -70°C [8]. The supernatant fluids were assayed by biological assay using vesicular stomatitis virus and Hep-2 cells for interferon-alpha and -gamma production [8].

RESULTS AND DISCUSSION

In the grant period, we perfected techniques for determination of interleukin production and leukocyte subset analysis of rhesus monkeys. These results are outlined in detail in publication number 2, appended to this report.

Additionally, we participated in the ARRT restraint test to determine if restraint conditions for flight in the Space Shuttle could contribute to any effects of space flight on immune responses. All immunological parameters listed in the methods section were tested. Evaluation of the data suggests that the restraint conditions had minimal effects on the results observed, but handling of the monkeys could have had some effect. These results are outlined in detail in manuscript number 3, appended to this report.

Additionally, to help us develop our rhesus monkey immunology studies, we carried out preliminary studies in mice to determine the effects of stressors on immunological parameters. We were able to show that there were gender-based differences in the response of immunological parameters to a stressor. These results are outlined in detail in manuscript number 4, appended to this report.

PUBLICATIONS (appended to this report)

1) Morton, D.S., Swiggett, J.P., Hakenewerth, A.M., Fowler, N.A., and Sonnenfeld, G. Effects of movement limitation on immunological parameters. *American Society for Gravitational and Space Biology Bulletin* 9:31, 1995.

This was presented at the annual meeting of the American Society for Gravitational and Space Biology, October, 1995.

2) Schmitt, D.A., Sonnenfeld, G., Husson, D., Tkaczuk, J., Andre, E., and Schaffar, L. *In vitro* interleukin-1 and -2 production and interleukin-2 receptor expression in the rhesus monkey. *Life Sciences* 11:931, 1997.

3) Sonnenfeld, G., Morton, D.S., Swiggett, J.P., Hakenewerth, A.M., and Fowler, N. Movement limitation and immune responses of rhesus monkeys. In Press, NASA technical memorandum, 1997.

4) Dréau, D. Morton, D.S., Foster, M., Swiggett, J.P., and Sonnenfeld, G. Immune alterations in male and female mice after 2-deoxy-D-glucose administration. Submitted for publication, 1997.

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