

EFFECT OF MICROGRAVITY ON MAMMALIAN LYMPHOCYTES

H. Banerjee, M. Blackshear, K. Mahaffey, A.A. Khan
Department of Biology
Elizabethcity State University
Elizabethcity, NC 27909

L. Delucas
CBSE, University of Alabama at Birmingham
Birmingham, AL 35294

ABSTRACT

The effect of microgravity on mammalian system is an important and interesting topic for scientific investigation, since NASA's objective is to send manned flights to planets like Mars and eventual human colonization. The Astronauts will be exposed to microgravity environment for a long duration of time during these flights. Our objective of research is to conduct in vitro studies for the effect of microgravity on mammalian immune system and nervous system. We did our preliminary investigations by exposing mammalian lymphocytes and astrocyte cells to a microgravity simulator cell bioreactor designed by NASA and manufactured at Synthecon, Inc. (USA). Our initial results showed no significant change in cytokine expression in these cells up to a time period of 120 hours exposure. Our future experiments will involve exposure for a longer period of time.

INTRODUCTION

During space flight the function of the immune system changes significantly. Several papers reported that postflight the number and the proportion of circulating lymphocytes in astronauts are modified (Uchakin et al 2002), the in vitro mitogen induced T cell activation is depressed (Cogoli et al. 2002 Konstantinova et al. 1993) and there are detectable differences in cytokine production. Lymphocytes as well (Chapes et al. 1992). One of the possible modifying forces is the microgravity condition itself. Our aim was to analyze mechanisms responsible for changing lymphocyte functions in low gravity environment. For terrestrial simulation of microgravity we used a Rotary Cell Culture System (RCCS) developed by NASA.

In these experiments we exposed mouse B Lymphocyte cells and human Astrocytoma cells to microgravity conditions and then analyzed the cells for cytokine expression. We exposed the cells to different time periods, however, our initial results failed to show any significant changes in cytokine expression under microgravity conditions.

MATERIALS AND METHODS

Mouse B Lymphocyte cells and Human Astrocytoma cells were purchased from ATCC, VA, USA and cultured in L-15 medium at 37 °C in a cell culture incubator. Cells were exposed to microgravity conditions in a Rotary Cell Culture System (RCCS) developed by NASA for 24 hours, 48 hours and 72 hours with proper control. After exposure cells were collected, lysed by antigen lysis buffer and cytokine expression (TGF-beta1 and IL-6) was determined by standard ELISA technique according to manufacturers instructions.

RESULTS

No significant changes in any cytokine expression tested was found during that particular exposure period in any of the cell lines tested.

DISCUSSION

Several attempts have been made to investigate the effects of microgravity on the growth and function of animal cells. Cellular activation of immune T lymphocytes is greatly affected by microgravity. On the other hand, little is known about the effects of microgravity on B lymphocytes. Thus, we attempted to study the effect of microgravity simulation on mouse B lymphocytes.

Our current experiment did not show any significant change in cytokine expression in microgravity exposed immune cells. We look forward to do experiments with longer exposure time, since astronauts are exposed to microgravity conditions for months and years. Also for shorter time periods of exposure, we did not see any significant change in Human brain cells like Astrocytes on the specific cytokine expression that were tested. Several researchers have reported alteration of the immune system due to microgravity conditions (Uchakin et al 2002).

However, we did not notice any significant change in cytokine expression in any of our experiments done for shorter intervals of time i.e., 24 hours, 48 hours, and 72 and 120 hours.

ACKNOWLEDGEMENT

This research is supported by NIH-EARDA Grant, NASA/ONR grant NAG5-1254 and NSF-EPSCOR University of Alabama subcontract# DTD42501. We are very grateful to Mr. James Harrington of NASA for his continuous support.

BIBLIOGRAPHY

- 1) Meloni MA, Galleri G, Carta S, Negri R, Costanzo G, De Sanctis V, Cogoli A, Pippia P. Preliminary study of gene expression levels in human T-cells exposed to cosmic radiations. *J Gravity Physiology*. 2002 Jul;9(1):P291-2.
- 2) Chaps SK, Sims SJ, Forman AD, Bateman TA, Zimmerman RJ. Effects of space flight and IGF-1 on immune function. *Adv Space Res*. 1999;23(12):1955-64.
- 3) Cogoli A. From cell biology to biotechnology in space. *Korean J Biol Sci*. 2000 Sep;4(3):195-200.
- 4) Konstantinova NA, Matveeva NA, Sirko IV, Firsov NN. The influence of cryoglobulins on the temperature-dependent erythrocyte backscattering nephelometry. *Clin Hemorheol Microcirc*. 2004;30(1):25-32.
- 5) Uchakin PN, Tobin BW, Morukov BV, Larina IV, Cabbage ML. Type 1 vs. type 2 cytokine secretion in vitro and its regulation by hydrocortisone in humans subjected to 120-day anti-orthostatic bed-rest regime. *J Gravity Physiology*. 2002 Dec;9(2):71-82.

Effect of 48 hours of microgravity simulation on IL-6 expression from mammalian lymphocytes

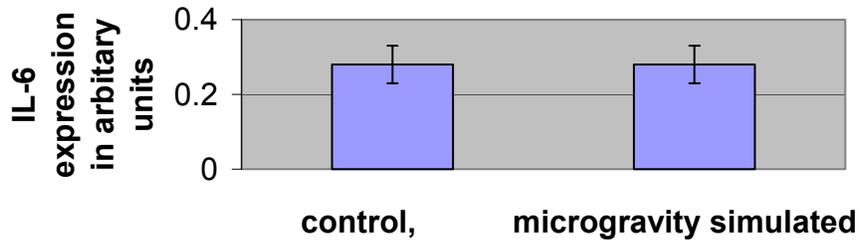


Figure 1

Effect of 72 hours of microgravity simulation on TGF-B1 in mammalian lymphocytes

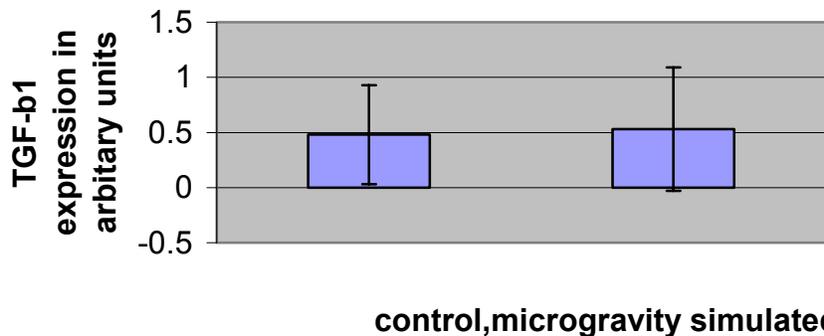


Figure 2

Effect of 72 hours of microgravity simulation on TGF-B1 in mammalian lymphocytes

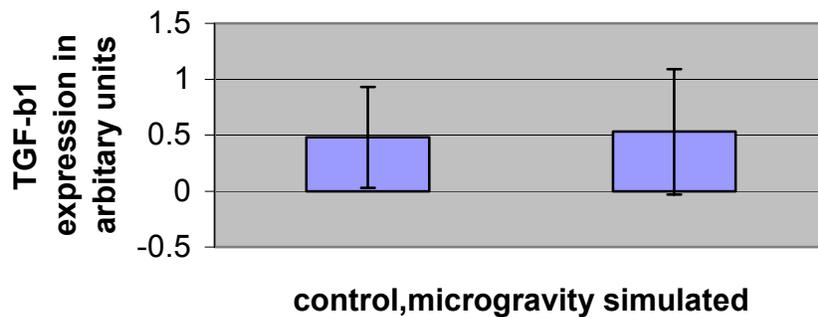
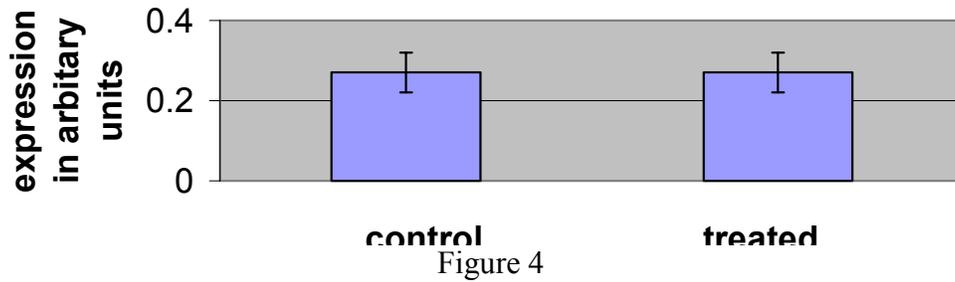
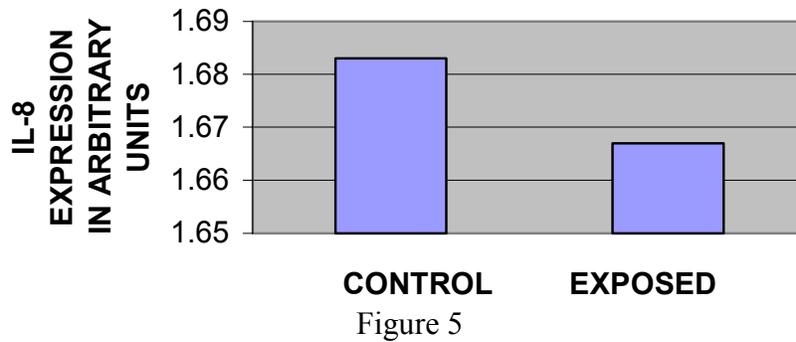


Figure 3

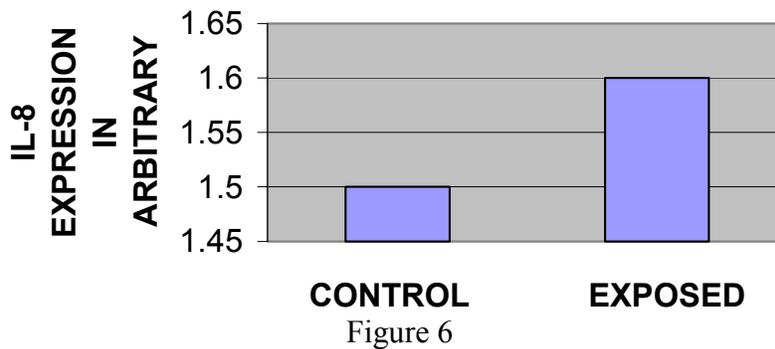
Effect of 48 hours of microgravity simulation on TGF-b1 expression from mammalian lymphocytes.



IL-8 EXPRESSION IN ASTROCYTOMA CELLS AFTER 24HR EXPOSURE



IL-8 EXPRESSION IN ASTROCYTOMA CELLS AFTER 120HR EXPOSURE TO MICROGRAVITY



VCAM EXPRESSION IN ASTROCYTOMA CELLS AFTER 24HR EXPOSURE TO MICROGRAVITY SIMULATION

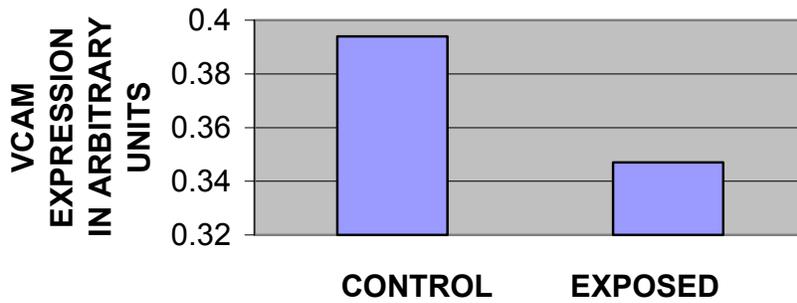


Figure 7

VCAM EXPRESSION IN ASTROCYTOMA CELLS AFTER 120HR EXPOSURE TO MICROGRAVITY

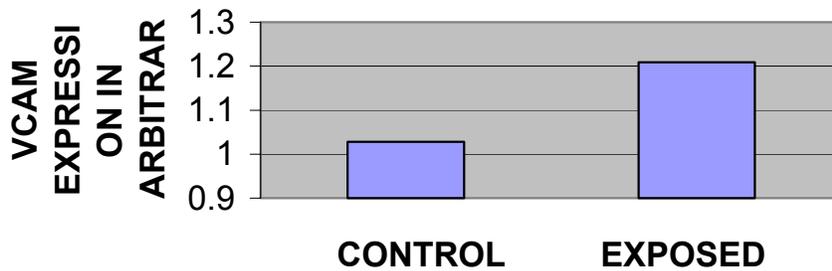


Figure 8