

**N90-13942****RESPONSE OF LYMPHOCYTES TO A MITOGENIC STIMULUS DURING  
SPACEFLIGHT**

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**ABSTRACT**

Several studies have been carried out that demonstrate that immunological activities of lymphocytes can be affected by spaceflight or by models that attempt to simulate some aspects of weightlessness. Included among these are the responses of lymphocytes to external stimuli such as mitogens and viruses. When cultures of lymphocytes were flown in space, the ability of the lymphocytes to respond to mitogens was inhibited. Similar results were obtained when lymphocytes from astronauts or animals just returned from space were placed into culture immediately upon return to earth, and when models of hypogravity were used. Lymphocytes placed in culture during spaceflights produced enhanced levels of interferon compared to control cultures. When cultures of lymphocytes were prepared from cosmonauts or rodents immediately upon return to earth, interferon production was inhibited. These results suggest that space flight can have profound effects on lymphocyte function, and that effects on isolated cells may be different from that on cells in the whole organism.

**INTRODUCTION**

Over the years, it has become apparent that spaceflight can have profound effects on biological systems. Included among those systems is the immune system of mammals (Barone and Caren, 1984; Jackson and Warner, 1986). In most cases, suppression of immune responses has occurred, but there have been occasional reports of immune enhancement (Barone and Caren, 1984; Jackson and Warner, 1986). Similar results have occurred when ground-based models of weightlessness have been utilized.

The mechanism of the effects of spaceflight on immune responses remains to be established. Weightlessness, stress, and low-level radiation could all contribute to alterations in immune responses. Although studies on the effects of spaceflight on immune responses have been limited, some interesting observations have been made. In this monograph, I will review the effects of spaceflight and modeling of weightlessness on lymphocyte function as determined by the response of the lymphocytes to external stimuli such as mitogens.

**EFFECTS OF SPACEFLIGHT AND MODELING ON THE BLASTOGENIC RESPONSE OF  
LYMPHOCYTES**

Several studies have been carried out by obtaining the blood of astronauts/cosmonauts immediately after return from spaceflight. Blood was

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also obtained from astronauts and cosmonauts before flight, and in some cases, during flight, to allow for the determination of the kinetics of changes in immune responsiveness. In these experiments, white blood cells were separated from the blood and placed in tissue culture. Mitogens, such as phytohemmagglutinin or concanavalin-A were added to the cultures. Over time, lymphocytes from normal individuals would divide and incorporate  $^3\text{[H]}$ -thymidine, indicating a blastogenic response of the lymphocytes to the mitogen. The blastogenic response to lymphocytes requires interaction with another cell type, the macrophage, as well as interaction with soluble regulatory factors known as cytokines. The blastogenic response and the production of cytokines are indications of a normal functioning immune system.

Several experiments were carried out to determine the effects of spaceflight on lymphocyte blastogenesis. In most cases (Table 1), the blastogenic response of lymphocytes to mitogens was inhibited severely in cells obtained from individuals immediately after return to earth (Fischer et al., 1972; Kimzey et al., 1975 and 1976; Criswell and Cobb, 1977; Lesnyak and Tashputalov, 1981; Taylor, 1983; Taylor and Dardano, 1983; Konstantinova et al., 1985; Taylor and Neale, 1986). The duration of the flights was from several days to several months. Recent reports (Taylor, 1983; Taylor and Dardano, 1983; Taylor and Neale, 1986) have also indicated decreased levels of circulating monocytes in astronauts after spaceflight (Table 1). Since the monocyte is an important accessory cell for the blastogenic response of lymphocytes, this could have contributed to the suppression observed.

While the results described above indicate that blastogenesis of lymphocytes in response to mitogens was inhibited when the cells were taken from individuals immediately after return from space, the question still remained whether spaceflight could affect blastogenesis of lymphocytes actually held in tissue culture during spaceflight. This question was addressed by a series of experiments using simulation and actual flight studies carried out by Cogoli and his associates.

Human peripheral blood leukocytes were placed in culture in a fast-rotating clinostat. This clinostat has constantly changing gravity vectors, and has been used as a technique for simulating microgravity conditions (Cogoli et al., 1980). Lymphocyte blastogenesis was inhibited greatly when the cells were maintained in this clinostat (Table 2) (Cogoli et al., 1980).

In addition, an incubator was developed that allowed the performance of similar experiments during spaceflight. A drastic inhibition of lymphocyte blastogenesis was observed when human peripheral blood leukocytes were placed in culture and challenged with mitogen during space flight (Table 2) (Cogoli and Tschopp, 1984 and 1985; Tschopp and Cogoli, 1984). When the cells were incubated in a 1 G centrifuge during spaceflight, much of the blastogenic capacity was retained (Table 2), indicating that the microgravity conditions of spaceflight contributed to the inhibited blastogenesis that was observed during spaceflight (Cogoli and Tschopp, 1984 and 1985; Tschopp and Cogoli, 1984).

#### EFFECTS OF SPACEFLIGHT AND MODELING ON THE PRODUCTION OF INTERFERON AND OTHER CYTOKINES BY LYMPHOCYTES

Several experiments were also carried out to determine the effects of spaceflight on cytokine production by lymphocytes after mitogenic or antigenic stimulus. Cytokines are molecules that are produced by cells that are

important messengers for the development of immune responses. Without them, lymphocytes and monocytes cannot communicate effectively with each other and immune responses cannot be mounted. The cytokines that have been utilized for space studies are the interferons, important antiviral, anti-cancer and immunoregulatory molecules, and interleukin-3, an important immunoregulatory molecule.

In an Hungarian-Soviet study, blood was removed from cosmonauts and peripheral blood leukocytes were placed in culture during spaceflight (Talas et al., 1983 and 1984). When the cells were challenged with a variety of mitogens and other interferon inducers such as purified protein derivative of Mycobacterium tuberculosis, Newcastle disease virus, and polyriboinosinic-polyribocytidylic acid, interferon-alpha production was enhanced compared to ground controls (Table 3). However, when peripheral blood leukocytes were harvested from cosmonauts immediately upon return to earth after spaceflight, interferon-alpha production in response to Newcastle disease virus challenge of leukocytes was inhibited severely (Table 3) (Talas et al., 1983 and 1984). The number of replicates in this series of experiments was small, and extensive time course experiments to determine how interferon production would have varied in cell cultures from the same individuals on the ground were not carried out. Nevertheless, these experiments suggest that the in vitro response of lymphocytes to spaceflight may differ from the effects of spaceflight on lymphocytes of the intact host.

Inhibited interferon production after simulated weightlessness and spaceflight of animals was also observed. In the first set of experiments, rats and mice were maintained in an antiorthostatic, hypokinetic, hypodynamic suspension system that models some aspects of weightlessness (Morey-Holton and Wronski, 1981; Musacchia et al., 1980; Steffen et al., 1984). In this model, the rodents are suspended with a head-down tilt and no load bearing on the hind limbs. This results in simulation of some of the effects of microgravity. When the mice or rats were challenged with polyriboinosinic-polyribocytidylic acid, there was inhibited interferon-alpha/beta production in antiorthostatically suspended rodents compared to normally housed controls (Table 4) (Sonnenfeld et al., 1982; Rose et al., 1984). The inhibition was transient, as a return to normal caging after suspension resulted in recovered ability to produce interferon. Suspension in an orthostatic fashion (no-head down tilt), which does not simulate the effects of microgravity, had no effect on the capacity of mice to produce interferon-alpha/beta (Table 4) (Rose et al., 1984). It must be noted that when animals are challenged systemically with an interferon inducer such as polyriboinosinic-polyribocytidylic acid, many cell types other than lymphocytes can be induced to produce interferon-alpha/beta. Therefore, these experiments went beyond just measuring the effects of suspension on lymphocyte responses to mitogenic stimuli.

In a second series of experiments, rats were flown in Space Shuttle SL-3. Upon return to earth, spleen cells containing lymphocytes were harvested, placed in culture, and challenged with the mitogen concanavalin-A (Gould et al., 1987). After the appropriate period of incubation, the cell culture supernatant fluids were harvested and assayed for production of two cytokines, interferon-gamma and interleukin-3. Interleukin-3 is another important messenger produced by lymphocytes after mitogenic challenge, providing immunologically significant signals to cells (Gould et al., 1987). Cells from rats that had been flown for one week showed very significant inhibition of the production of interferon-gamma, but no effect on

interleukin-3 production (Table 5) (Gould et al., 1987). The results with the interferon-gamma supported previous findings in human flight and rodent suspension studies indicating that interferon-alpha/beta was inhibited. However, the lack of effect of spaceflight on interleukin-3 production indicates that all responses of lymphocytes to mitogens are not affected in the same fashion by spaceflight.

## CONCLUSIONS

The studies described above indicate that spaceflight and models that simulate microgravity can have profound effects on the response of lymphocytes to mitogens. The effects of spaceflight appear to be selective, in that all responses of lymphocytes to mitogens are not affected in a similar fashion. In addition, the effects of spaceflight on isolated lymphocytes in culture may differ from effects when lymphocytes are *in vivo* in a whole animal surrounded by other cells, soluble messengers and interact with systems other than the immune system.

The mechanism of the effects of spaceflight on immune responses remains to be established. Several possibilities exist. Among them are: 1) direct effects of microgravity on lymphocytes, 2) inability of lymphocytes to interact directly with other cell types such as monocytes/macrophages, 3) inability of lymphocytes to produce cytokines, 4) inability of lymphocytes to respond to signals from cytokines, 5) inability of antigenic or mitogenic signals to reach lymphocytes because of fluid-shifts induced during spaceflight, and 6) impaired function of lymphocytes because of faulty interaction with other non-immunological systems such as the neuroendocrine system. Other potential mechanisms surely exist. The study of these mechanisms should progress with time.

Determination of the effects of spaceflight on lymphocytes should yield other fascinating information. Since the immune system is responsible for resistance to infection, the study of lymphocytes should help to determine if long-term exposure to spaceflight conditions could compromise resistance. The ability to produce large amounts of cytokines as a result of genetic engineering probably indicates that enhanced production of cytokines as a result of spaceflight will not be an effective technique for mass production of cytokines. However, studying the response of lymphocytes to spaceflight may aid in our understanding of how the immune response is regulated and may allow the discovery of new cytokines whose actions are masked in normal ground conditions.

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TABLE 1

EFFECTS OF SPACEFLIGHT ON THE ABILITY OF SUBJECTS' CELLS TO RESPOND TO MITOGENS UPON RETURN TO EARTH

<u>Effect on Blastogenesis</u>	<u>Effect on Monocyte Number</u>	<u>Reference</u>
None	Not Tested	Fischer, 1972
Inhibited	Not Tested	Kimzey, 1975-6 Criswell, 1977 Lesnyak, 1981 Konstantinova 1985
Inhibited	Decreased	Taylor, 1983, 1983, and 1986

TABLE 2

EFFECTS OF SPACEFLIGHT ON IN VITRO BLASTOGENESIS

<u>Effect on Blastogenesis</u>	<u>Effect of Centrifugation</u>	<u>References</u>
Inhibited	Restored	Cogoli, 1984 and 1985  Tschopp, 1984
HYPOGRAVITY DUE TO CLINOSTAT ON THE GROUND		
Inhibited		Cogoli, 1980

TABLE 3

EFFECT OF SPACEFLIGHT ON HUMAN INTERFERON PRODUCTION

<u>Situation</u>	<u>Effect on Interferon-Alpha</u>	<u>Reference</u>
Leukocytes in Culture in Space	Enhanced	Talas, 1983 and 1984
Leukocytes Harvested after Return from Space	Inhibited	Talas, 1983 and 1984



TABLE 4

EFFECTS OF ANTIORTHOSTATIC SUSPENSION ON INTERFERON PRODUCTION

<u>Treatment</u>	<u>Effect on Interferon-Alpha/Beta</u>	<u>Reference</u>
Rat - 2 week	Inhibited	Sonnenfeld, 1982
Mouse - 1 week	Inhibited	Rose, 1984
Mouse - 1 week + 1 week normal cage	Recovered	Rose, 1984
Mouse - 1 week orthostatic suspension	None	Rose, 1984

TABLE 5

EFFECT OF SPACEFLIGHT ON RAT CYTOKINE PRODUCTION

<u>Duration of Flight</u>	<u>Cytokine</u>	<u>Effect</u>	<u>Reference</u>
1 week	Interferon-gamma	Inhibited	Gould, 1987
1 week	Interleukin-3	Normal	Gould, 1987

