EXPERIMENT K-6-23

EFFECT OF SPACEFLIGHT ON LEVELS AND FUNCTION OF IMMUNE CELLS

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SUMMARY

Two different immunology experiments were performed on samples received from rats flown on Cosmos 1887. In the first experiment, rat bone marrow cells were examined in Moscow for their response to colony stimulating factor-M. In the second experiment, rat spleen and bone marrow cells were stained in Moscow with a variety of antibodies directed against cell surface antigenic markers. These cells were preserved and shipped to the United States where they were subjected to analysis on a flow cytometer. The results of the studies indicate that bone marrow cells from flown rats showed a decreased response to colony stimulating factor than did bone marrow cells from control rats. There was a higher percentage of spleen cells from flown rats staining positively for pan-T-cell, suppressor-T-cell and innate interleukin-2 receptor antigens than from control animals. In addition, a higher percentage of cells that appeared to be part of the myelogenous population of bone marrow cells from flown rats stained positively for surface immunoglobulin than did equivalent cells from control rats.

INTRODUCTION

Many alterations in immune responses after space flight (Barone and Caren, 1984; Cogoli, 1981 and 1984, Durnova et al., 1976; Gould et al., 1987a; Konstantinova et al., 1985; Lesnyak and Tashputalov, 1981; Talas et al., 1983 and 1984; Taylor et al., 1983 and 1986) or antiorthostatic suspension (Caren et al., 1980; Gould and Sonnenfeld, 1987b; Rose et al., 1984; Sonnenfeld et al., 1982) have been reported. These changes have ranged from alterations in lymphoid organ size (Durnova et al., 1976) to alterations in lymphocyte activities (Cogoli et al., 1981 and 1984) to alterations in interferon production (Talas et al., 1983 and 1984; Gould et al., 1987a). The full range of these changes and the significance of these changes with regard to resistance to infection has not been established.

The purpose of the immunology studies flown on Cosmos 1987 was to begin a systematic attempt to define the range of immunological parameters affected by space flight. Two different areas were chosen for study. The first involved a determination of the effect of space flight on the ability of cells to respond to an external immunological stimulus. For this purpose, bone marrow cells from flown rats were exposed to colony stimulating factor-monocyte/macrophage (CSF-M). CSF-M is an important regulator of the differentiation of bone marrow cells of the monocyte/macrophage lineage, and an alteration in the ability of cells to respond to CSF-M could result in altered immune function (Waheed and Shadduck, 1979). The cells were observed for their responses to CSF-M.

The second set of studies involved a determination of the effect of space flight on the expression of cell surface markers of spleen cells and bone marrow cells. These markers represent various immunologically important cell populations, and an alteration in the percentage of cells expressing the markers could result in an alteration of immunological function (Barone and Caren, 1984; Jackson and Warner, 1986). The markers that were tested included T-cell markers, B-cell markers, natural killer cells markers, and interleukin-2 receptors. The studies were carried out by staining the cell populations with fluorescein-labelled antibodies directed against the appropriate antigens. The stained cell populations were then analyzed utilizing a flow cytometer, and compared with stained cell populations from control rats for changes in percentages of cells expressing the markers.

MATERIALS AND METHODS

Ten male specific pathogen free rats of Czechoslovakian-Wistar origin (Institute of Endocrinology, Bratislava, Czechoslovakia) were flown on the Cosmos 1887 Biosputnik flight for 12 and on-half days. Flight, housing, feeding and recovery conditions were as described in the Mission

Description section of this technical report. Tissue from rats number 6-10 were used in our project. After sacrifice of the rats, bone marrow cells were extruded with a needle and syringe from the left femur of the rats using RPMI-1640 medium (MA Bioproducts, Walkersville, MD) supplemented with 10% fetal bovine serum, antibiotics, glutamine and 2-mercaptoethanol. One quarter of the spleen of each of the five rats was dissociated into individual cells and placed into supplemented RPMI-1640 medium. All of the sample were placed into transporter vials, held and 4 °C and transported to Moscow. Samples reached the laboratory and analytical work began 30-32 hrs after removal of the tissue from the rats.

Control and vivarium rats were treated as described in the Mission Description section of this technical report. Tissues were removed and treated as described for the flight tissue.

Upon arrival at the laboratory in Moscow, the cells were centrifuged and washed. Cells were washed in phosphate buffered saline, and counted in an haemocytomer using trypan blue dye for determination of viability. For colony stimulating factor, 10^5 bone marrow cells were suspended in 1 ml of McCoy's 5A medium (MA Bioproducts, Walkersville, MD) supplemented with 10% fetal bovine serum and antibiotics and containing 0.3% agar (Shadduck and Nagabhushanam, 1971). Included in the medium was 0.1 ml of colony stimulating factor-M (a gift of Dr. Robert N. Moore, University of Tennessee). The experiment was carried out in duplicate. The suspended cells were then placed in 35 mm tissue culture dishes and then incubated in a 37 °C incubator with 5% CO_2 (Shadduck and Nagabhushanam, 1971). After the appropriate incubation period (5-6 days), 5-10 microscope fields on each slide were evaluated for the number of colonies (aggregates of 50 cells or more) formed.

For the cell surface antigenic markers, the following procedure was carried out (Jackson and Warner, 1986). Approximately 1 x 10^5 bone marrow or spleen cells were placed in a microcentrifuge tube. The cells were resuspended in FTA buffer (7.65 g sodium chloride, 1.27 g disodium phosphate, 0.10 g monosodium phosphate, and 0.21 g monopotassium phosphate brought to 1 liter volume with distilled water at pH 7.4), and centrifuged for 1.5 min at 1,000 x g. Supernatants were removed and cells were resuspended. 5 µl of the appropriate antibody was added to the cells. All antibodies were obtained from Accurate Chemical and Scientific, Westbury, NY, except for anti-asialo GM-1 (obtained from WAKO Fine Chemicals, Dallas TX) and anti-rabbit IgG (Sigma Chemical, St. Louis, MO). The cells were allowed to incubate at 4 °C for 25 min. The antibodies used were as follows:

1. Anti-asialo GM-1 (anti-natural killer cells)
2. 0X-39 (anti-interleukin-2 receptor)
3. OX-1 (anti-pan-leukocyte marker)
4. W3/25 (anti-helper T-cell)
5. OX-8 (anti-suppressor T-cell)
6. OX-12 (anti-rat IgG Fab')
7. W3/13 (anti-pan T cell)
8. OX-4 (anti-polymorphic Ia)
9. Anti-rabbit IgG
10. No antibody added.

All antibodies were fluorescein tagged except for anti-asialo GM-1 and 0X-39. For these two antibodies the following special additional procedure was carried out. Cells stained with these antibodies were resuspended in 1 ml of FTA buffer and centrifuged for 1.5 min at 1,000 x g. The cells were resuspended in residual buffer and 5 ul of a second, fluorescein-conjugated anti-antibody and 25 µl of fetal bovine serum were added. The second antibody for anti-asialo GM-1 was anti-rabbit IgG and for OX-39 was anti-mouse IgG. Incubation for these samples was at 4 °C for 25 min.

At this point, the following procedure was carried out for all cells stained with all of the antibodies. One ml of lysing solution (8.26 g ammonium chloride, 1.00 g potassium bicarbonate, 37 mg of tetrasodium EDTA, brought to 1 l with distilled water at pH 7.4) was added to each sample and the cells were allowed to incubate at room temperature for 6 min to lyse erythrocytes. The cells were then centrifuged at 1,000 x g for 1.5 min, and then resuspended in 1 ml of FTA buffer. Cells were then centrifuged again and fixed by resuspending in 0.5 ml of 1% paraformaldehyde. The cells were then placed at 4 °C and transported to the United States. The cells were analyzed for fluorescence, an indicator of presence of the antigen, using an autofluorograph IIs interfaced with a 2151 Data Handler computer system (Ortho Diagnostics, Westwood, MA). For this analysis, lymphocytes and myelogenous cells (neutrophils and monocytes) were first differentiated by forward vs. right angle scatter. The green, that is, fluorescein, fluorescence of each "gated" population was then plotted on a 1,000 channel histogram from which the percentage of positive-stained cells was determined. The lower threshold for this determination was set to exclude 95-98% of the cells in tube #10 to which no antibody had been added. The individual background from tube #10 for each rat was subtracted from each of the stained populations.

RESULTS

Effect of spaceflight on the response of bone marrow cells to CSF-M.

Due to insufficient cells and CSF-M, experiments with CSF-M were carried out in duplicate only on flight and synchronous control rats. On flight samples, the results were read after 6 days of incubation. On synchronous control samples, the results were read after 5 days of incubation due to concern for dehydration of the cultures. The results of the assays show a sizeable reduction in the number of colonies from cells from flown rats exposed to CSF-M compared to cells from synchronous control rats exposed to CSF-M (Table 1).

Effect of spaceflight on the percentage of cells expressing cell surface markers.

For spleen cells, profound changes in the percentage of lymphocytes expressing various cell surface antigenic markers were observed. The results are summarized in Table 2. These results indicate that there appears to be a higher percentage of cells expressing pan T-cell antigens (W3/13), suppressor T-cell antigens (0X-8), and expressing endogenous interleukin-2 receptor markers (OX-39) (Table 2). No other changes in the percentage of spleen cells expressing a marker were observed. Measurable numbers of myelogenous cells were not detected in any of the specimens.

Because of a shortage of bone marrow cells, these cells were stained only with anti-pan leukocyte (OX-1) and anti-rat IgG fab' (OX-12). Lymphocytes and myelogenous cells were analyzed differentially as described in Materials and Methods. For the lymphoid cells, there was an equivalent increase in the percentage of cells expressing pan-leukocyte markers and IgG in both flight and synchronous control cells (Table 3). For the myelogenous cells, there was also an increase in cells expressing pan-leukocyte marker in both flight and sychronous control cells; however, there was a large increase in the percentage of cells from flight animals expressing surface IgG compared to both synchronous and vivarium control cells (Table 3).

DISCUSSION

The results of this study suggest that several of the immunological markers examined were affected profoundly by spaceflight. This is in agreement with earlier studies that indicated that other immunological parameters could be affected by spaceflight (Barone and Caren, 1984, Konstantinova et al., 1985; Taylor et al., 1983 and 1986).

In this case, it appears that the ability of bone marrow cells to respond to CSF-M was impaired after spaceflight. This could have yielded impaired production of monocytes, which could result in compromised ability to present antigens and to phagocytose potential pathogens.

In addition, there appears to be an increase in the percentage of T-cells, and suppressor T-cells in particular, in the spleens of flown rats. This increase in suppressor cells which extends previous observations, could, in part, account for immunosuppression observed after spaceflight (Konstantinova et al., 1985; Taylor et al, 1983 and 1986).

Another finding of interest is the increase in the percentage of cells with receptors for interleukin-2. Since the spleen cells were not stimulated with mitogens to increase expression of interleukin-2 receptors, these are representative of endogenous receptors on resting T cells. The increase in interleukin-2 receptors could indicate an increased immune activity, possibly induced by flight conditions, that is held in check by the increased number of suppressor cells.

Also, increases in percentages in T cells were observed in lymphoid bone marrow cells from flown and synchronous control rats compared to vivarium control rats. Since the increases were similar in both flown and synchronous control rats, it is likely that conditions of handling were responsible for the changes. The situation with expression of surface IgG on bone marrow cells is different. For the lymphoid population, an equivalent increase occurs in cells from both flown and synchronous control rats; however, for the myelogenous population, there is a large increase in cells bearing surface IgG from the flown rats only. This could indicate that spaceflight may induce a non-specific blastogenesis of the bone marrow cells that is down-regulated by increased suppressor T-cell activity.

Two unexpected results were also observed. First was the staining of spleen cells with anti-rabbit-IgG. This occurred on spleen cells from all flight and control rats. We would have not expected this to occur, and the handling, storage and shipment required could have contributed to this unusual result. Second, the percentage of spleen cells from rats in both synchronous and vivarium groups staining with anti-suppressor T-cell was higher than expected. However, since a much higher proportion of spleen cells from the flight animals stained with anti-suppressor T-cell than from either control group, we feel it is valid to conclude that spaceflight resulted in increased levels of suppressor T-cells.

CONCLUSIONS

The current study presents additional data to indicate that spaceflight, even of a relatively short duration, affects certain parameters of the immune system. Through this study, we have been able to demonstrate some specific cell populations that appear to be affected by spaceflight. These results suggest possible future directions for research in this area.

It is recognized that a number of factors associated with this flight should be considered in assessing its significance. Several areas will require further research. First, the current study was very small using only five animals.The study needs to be repeated to confirm the data and to allow a thorough statistical analysis. Second, due to limitations in the number of cells available, all desirable parameters, particularly with regard to monoclonal antibody fluorescein staining of bone marrow cells, could not be carried out. Monoclonal antibody staining of lymph node and thymus cell, if possible, might yield additional useful information. It would be desirable to expand the study to allow that additional work. Third, the functional significance of the immunological changes observed needs to be established. Fourth, a determination of the role of various factors in spaceflight, for example stress, microgravity, etc., in inducing immunological changes remains to be carried out.

Finally, the technical difficulties that resulted in a two-day delay in sacrifice and tissue sampling needs to be taken into account. This two-day maintenance of the flown animals at normal gravity could have affected the outcome of this study.

In any case, the results of the current study suggest that several profound alterations in parameters that play important roles in regulation of immune responses occur as a result of spaceflight. This is an interesting finding that supports previous findings, and the results suggest the experiments warrant repetition and expansion.

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TABLE 1

EFFECT OF SPACEFLIGHT ON THE RESPONSE OF BONE MARROW CELLS TO CSF-M

Rat Number	Number of Cells in 5 Microscope Fields	
	Flight Rats	Synchronous Control Rats
6	2, 1*	15, 10
7	1, 0	1, 5
8	2, 1	14, 10
9	2. 1	4, 9
10	1, 3	5, 5

* Results are of two replicate cultures for each rat.

TABLE 2

EFFECT OF SPACEFLIGHT ON THE PERCENTAGE OF SPLEEN CELLS EXPRESSING CELL SURFACE MARKERS

Rat#	BKG*	GM-1	OX-39	W3/25	OX-8	OX-12	W3/13	OX-4	Rbt Ig
Flight Animals									
6	2.2	66.2	75.5	38.7	62.5	35.6	56.3	34.5	88.3
7	2.2§	53.9	94.6	55.4	95.1	26.2	91.2	69.6	94.1
8	2.2	43.1	93.6	35.4	85.8	45.0	77.3	75.0	95.9
9	2.3	47.1	91.8	39.1	75.6	29.5	51.2	43.0	92.7
10	1.2	89.9	96.2	46.9	96.2	43.8	57.5	68.7	94.4
Vivarium Control Animals									
6	2.7	39.0	50.0	39.0	51.0	30.1	41.8	36.1	20.6
7	3.3	60.7	85.2	31.0	53.3	34.7	32.0	38.3	58.3
8	1.6	46.7	82.0	32.3	47.9	42.1	43.7	49.0	75.2
9	2.1	70.7	64.1	35.6	48.0	33.1	36.5	39.9	77.9
10	2.5	57.8	61.4	32.5	59.9	42.5	43.1	34.8	89.2
Synchronous Control Animals									
6	2.3	39.6	74.9	33.9	40.3	43.7	42.1	50.1	72.4
7	2.0	44.6	69.7	-†	39.5	44.0	30.6	49.4	42.9
8	2.0	59.6	84.7	35.0	57.2	39.3	49.5	45.0	30.9
9	2.9	51.3	63.5	32.0	49.2	34.4	33.6	38.1	76.1
10	2.6	43.8	76.3	37.1	38.5	41.8	38.5	43.5	26.4

*Abbreviations used: BKG = Background (unstained no-antibody control); GM-1 = anti-natural killer cells; OX-39 = anti-interleukin-2 receptor; OX-1 = anti-pan-leukocyte marker; W3/25 = anti-helper T-cell; OX-8 = anti-suppressor T-cell; OX-12 = anti-rat IgG Fab'; W3/13 = anti-pan T cell; OX-4 = anti-poylmorphic Ia; Rbt Ig = anti-rabbit IgG.

§Insufficient cells in control sample, control used from rat number 6

†Apparently no antibody applied

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TABLE 3

EFFECT OF SPACEFLIGHT ON THE PERCENTAGE OF BONE MARROW CELLS EXPRESSING CELL SURFACE MARKERS

Rat #	No Antibody (Background)		Anti-pan Leukocyte		Anti-rat IgG Fab	
	Lymph*	Myelog	Lymph	Myelog	Lymph	Myelog
Flight Animals						
6			63.2	97.2	37.5	55.6
7			52.9	95.7	58.9	28.7
8			30.8	92.0	26.2	19.2
9			57.5	96.1	62.9	32.8
10			59.7	96.5	41.0	21.0
Vivarium Control Animals						
6	4.1	1.6	23.4	69.4	24.9	4.9
7	0.4	1.4	17.9	72.6	13.5	7.4
8	1.3	2.4	17.9	74.1	11.5	5.2
9	1.5	2.3	17.1	79.3	13.0	6.3
10	0.2	1.7	14.3	72.1	9.4	3.7
Synchronous Control Animals						
6			40.0	94.2	28.0	8.3
7			60.2	90.9	59.7	17.4
8			48.4	96.8	28.3	8.1
9			40.2	77.2	21.9	9.9
10			44.3	88.7	43.6	13.6

*Abbreviations used: lymph = lymphocytic cell population; myelog = myelogenous cell population

Due to insufficient cells available, the no antibody control was carried out only on vivarium controls. All other values are calculated from those background values.