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# COSMOS 2229 IMMUNOLOGY STUDY (Experiment K-8-07)

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## SUMMARY

The purpose of the Cosmos 2229 11 and 1/2 day mission was to begin experiments to determine the suitability of the rhesus monkey as a surrogate for humans in space research. In this study, experiments examining the effects of space flight on immunological responses of rhesus monkeys were performed to gain insight into the effect of space flight on resistance to infection. Experiments were performed on tissue samples taken from the monkeys before and immediately after flight. Additional samples were obtained approximately one month after flight for a post-flight restraint study. Two types of experiments were carried out throughout this study. The first experiment examined the responsiveness of rhesus bone marrow cells to recombinant human granulocyte/macrophage colony stimulating factor (GM-CSF). In the second experiment, monkey peripheral blood and bone marrow cells were stained using a variety of antibodies directed against cell surface antigenic markers. Human reagents that cross-reacted with monkey tissue were utilized for the bulk of the studies. Results from both studies indicated that there were changes in immunological function attributable to space flight. Bone marrow cells from flight monkeys showed a significant decrease in their response to CSF-GM when compared to the response of bone marrow cells from non-flight control monkeys. Antibody staining of both blood and bone marrow cells from flight monkeys showed alterations in leukocyte subset distributions when compared to antibody staining patterns of non-flight controls. These results suggest that the rhesus monkey will be a useful surrogate for humans in future studies which examine the effect of space flight on immune response, particularly when conditions do not readily permit human study.

## INTRODUCTION

Data from studies reported over the past several years have indicated that various alterations in immunological parameters occur after space flight (Barone and Caren, 1984; Cogoli, 1981 and 1984; Durnova *et al.*, 1978; Gould *et al.*, 1987a; Konstantinova *et al.*, 1985; Lesnyak and Tashputalov, 1981; Mandel and Balish, 1977; Sonnenfeld *et al*, 1990; Talas *et al.*, 1983 and 1984; Taylor *et al.*, 1983 and 1984). Immunological changes similar to those observed after space flight have been reported in various ground base studies, including antiorthostatic suspension of rats (Caren *et al.*, 1980; Gould and Sonnenfeld, 1987b; Rose *et al.*, 1984; Sonnenfeld, *et al..*, 1982). These changes involve alterations in lymphoid organ size (Durnova *et al.*, 1976), alterations in the production of interferons (Talas *et al.*, 1983 and 1984; Gould *et al.*, 1987a), and alterations in lymphocyte activation (Cogoli *et al.*, 1981 and 1984).

The 11.5 day Cosmos 2229 space flight attempted to further explore space flight effects on immune response. Immunological parameters similar to those parameters affected in rats after similar flights, including Cosmos 2044, were chosen for study in Cosmos 2229. Results from the Cosmos 2044 study had shown that space flight inhibited the ability of GM-CSF to stimulate colony formation in rat bone marrow cells, and altered various leukocyte subset population distributions, such as the CD4+ and CD8+ cell subsets (Sonnenfeld *et al.*, 1992). These studies and recent ground-based studies establishing immunological techniques for handling rhesus monkey cells (Sonnenfeld *et al.*, 1993) made possible testing of the hypothesis that the rhesus monkey could be used as a surrogate for humans in future studies. This could be of great advantage, since rhesus monkey and human immune systems have been shown to be closely related. The purpose of the current study was to further validate use of the rhesus monkey as a model for humans in future space flight testing.

The areas of immunological importance examined in the Cosmos 2229 flight were represented by two sets of studies. The first set of studies determined the effect of space flight on the ability of bone marrow cells to respond to granulocyte/monocyte colony stimulating factor (GM-CSF). GM-CSF is an important regulator in the differentiation of bone marrow cells of both

monocyte/macrophage and granulocyte lineages and any change in the ability of these cells to respond to GM-CSF can result in altered immune function (Waheed and Shadduck, 1979).

A second set of studies determined space flight effects on the expression of cell surface markers on both spleen and bone marrow cells. Immune cell markers included in this study were those for T-cell, B-cell , natural killer cell, and interleukin-2 populations. Variations from a normal cell population percentage, as represented by these markers, can be correlated with alterations in immunological function (Jackson and Warner, 1986). Cells were stained with fluorescein-labelled antibodies directed against the appropriate antigens, and then analyzed using a flow cytometer.

## MATERIALS AND METHODS

Two juvenile male rhesus monkeys (*Macacca mulatta*) were born at the Soviet Primate Center in Sukhumi, Georgia and sustained throughout the duration of studies reported in this paper at the Institute of Biomedical Problems in Moscow, Russia. The monkeys were flown under chair restraint for 11.5 days during the Cosmos 2229 (Bion 10) flight from December 19, 1992 to January 10, 1993. Details concerning flight conditions, condition of monkeys pre- and post-flight, as well as maintenance procedures for all animals used in this study, are described in the *Mission Description* section of this technical memorandum.

Tissue sampling from the monkeys for pre-flight studies occurred approximately 1.5 months prior to flight. Tissue sampling was also done at various time periods after flight, from 1 to 12 days post-flight recovery. Two types of tissue samples were obtained: peripheral blood and bone marrow. Additional samples were obtained from monkeys for a post-flight study examining the possible role of restraint in those effects observed after space flight. The restraint study was carried out 40 days post-recovery for the same duration of time as the flight. Other controls involved the testing of bone marrow and blood obtained from flight-pool monkeys as well as the testing of bone marrow and blood samples from two standard vivarium control monkeys (#s 85 and 3224) at regular intervals throughout each experimental period. Results from the testing of standard vivarium control monkeys indicated when changes in test values were due to a failure of experimental procedure. The sampling schedule is given in the results section of this report.

Bone marrow samples were obtained through needle biopsy of the posterior of the head of the humerus (left or right) of monkeys under Ketamine/Xylazine anesthesia. Each bone marrow sample was transferred to a 15 ml polypropylene centrifuge tube containing McCoy's medium (Gibco BRL, Grand Island, NY) supplemented with antibiotics, sodium bicarbonate, hepes buffer, L-glutamine, and fungizone. Bone marrow cells were centrifuged and resuspended in supplemented McCoy's medium with 10% FBS (as a washing step). Cell counts were obtained on a hemocytometer, using trypan blue dye exclusion for determination of viability. One x $10^5$ bone marrow cells/mL were resuspended in a 2% methylcellulose solution prepared in

supplemented McCoy's media containing 30% FBS (Shadduck and Nagabhushnam, 1971). Medium for experimental group cultures contained a concentration of 40 ng/ml recombinant human GM-CSF (a gift of Dr. Steven Gillis, Immunex Research and Development Corp, Seattle, WA). The GM-CSF was from lot 620-028-5, and had a specific activity of at least 5 x $10^7$ units/mg protein. For each animal tested, five 35 mm tissue culture dishes, each containing 1 ml of the bone marrow cell suspension, were set up for control ( - CSF) and for experimental ( + CSF) groups. Dishes containing suspended cells were incubated in a 37°C incubator with 5% $CO_2$ (Shadduck and Nagabhushanam, 1971). After 7 days of incubation, 10 microscope fields from each petri dish were evaluated for the number of colonies formed, a colony represented by aggregates of 50 or more cells (Sonnenfeld, *et al.*, 1990). The GM-CSF data was analyzed using a pooled estimate of variance and linear contrast analysis.

The following procedure was implemented to evaluate cell surface antigenic markers on bone marrow cells (Jackson and Warner, 1986). One x $10^6$ bone marrow cells, suspended in supplemented McCoy's media with 10% FBS, was allocated to each microcentrifuge tube. Cell suspensions were centrifuged for 1.5 min at 1,000 x g, supernatant removed, and 5 µl of the appropriate antibody added to each microcentrifuge tube. Background values were taken into account by including a microcentrifuge tube containing cells, but no antibody, for each animal tested. Cells and antibody (or no antibody) were allowed to incubate at 4°C for 25 min. Antibodies used in this study were obtained from Becton-Dickinson Immunocytochemistry Systems, San Jose, CA, except as noted below:

1. Leu 2a (CD-8, cytotoxic T lymphocyte)
2. Leu 3a (CD-4, helper T lymphocyte)
3. Leu 4 (CD-3 signal transducer for T lymphocyte)
4. Leu 11 a (CD-16, Natural killer cell/monocyte)
5. Anti-human IgM (B cell - purchased from Sigma Chemical Co., St. Louis, MO)
6. Anti-monkey IgG (B cell - purchased from Organon-Teknika Corp. W. Chester, PA)
7. Anti-monkey IgG F(ab)' (B cell - purchased from Organon-Teknika Corp.)

9 Goat anti-rabbit IgG (Purchased from Accurate Chemical Co., Westbury, NY)

3 No antibody added.

After the 25 minute incubation, red blood cells were lysed for 6 minutes at room temperature with one ml of lysing solution/microcentrifuge tube (8.26 g ammonium chloride, 1.00 g potassium bicarbonate, 37 mg of tetrasodium EDTA brought to 1 L with distilled water, pH 7.4). Cell suspensions were then centrifuged for 1.5 min at 1,000 x g, and resuspended in FTA buffer (BBL Microbiology Systems, Cockeysville, MD), pH 7.4, containing 0.1% sodium azide. Cell suspensions were again centrifuged for 1.5 min at 1,000 x g, supernatant removed, and cells fixed by resuspension in 0.5 ml of 1% paraformaldehyde prepared in FTA buffer. The staining procedure for peripheral blood was exactly the same as for bone marrow except that 20 µl of heparin-treated blood was placed into each microcentrifuge tube and then stained.

Fixed cells from blood and bone marrow staining were maintained at 4°C, flown to the United States at this temperature, and later analyzed at the University of Louisville to determine the presence of antigenic markers using a Profile II flow cytometer (Coulter Electronics, Hialeah, FL). Lymphocytic and myelogenous regions were gated on three-part differentials using forward vs. side scatter plots.

Statistical analysis of bone marrow cell response to GM-CSF was accomplished by using a pooled estimate of variance in the hypothesis testing of differences between two group means. Flow cytometry results from antibody stained peripheral blood and bone marrow cells were analyzed using linear contrast and factorial anova. Alpha was set *a priori* at $p \leq 0.05$

# RESULTS

## Effect of space flight on the response of bone marrow cells to GM-CSF

Bone marrow cells from some monkeys within the pool of flight animals showed a lower than normal response to human GM-CSF prior to flight (Table 1). Bone marrow from monkeys exposed to space flight showed decreases in the ability to form colonies in response to GM-CSF when compared bone marrow cells from standard vivarium control monkeys (Table 2). As time progressed, recovery towards the normal GM-CSF developed, but suppression of colony formation occurred again at 12 days post landing (Table 2). There was also significant variability in the response of bone marrow from standard vivarium control monkeys to GM-CSF across the pre- and post-flight testing periods (figures 1, 2, and 3). Except in the post-flight restraint study (Tables 1, 2, and 3), there was always an increase in colony formation if GM-CSF was present in the cultures when compared to control cultures ( - CSF) from the same animal.

## Effect of space flight on the percentage of cells expressing cell surface antigenic markers

Results from antibody staining of peripheral blood and bone marrow were very similar in the pattern of response. Data from anti-monkey IgG only rather than data from both this antibody and the other positive control antibody used in the staining study, anti-monkey IgG $F(ab')_2$, is included since the data were very similar. All given cell population percentages have had background values subtracted through gating against unstained cell populations. Prior to flight, several of the flight pool animals showed significant differences in stained cell population percentages for the different surface antigens (Tables 4-10). For all leukocyte surface markers tested, a decreased expression of the surface antigens occurred immediately after flight (recovery + 1 day, and recovery + 2 days), followed by a shift toward more normal values at recovery + 3 days. A return to suppression occurred at recovery + 12 days (Tables 11-17). There was also variability in the flow cytometry data from standard vivarium control monkeys within and across pre- and post-flight testing periods (Tables 11-17).

**Effect of postflight restraint on the percentage of cells expressing cell surface antigenic markers**

Flow cytometry data from the antibody staining of both peripheral blood leukocyte and bone marrow have been included in the tables section because of differences in the response of these two cells types. Restraint of the flight animals resulted in decreases in the percentage of peripheral blood leukocytes carrying the CD-8 marker and in the percentage of bone marrow cells carrying the HLA-DR marker (Tables 18-31). These were the only changes observed in the response of flight animals to restraint, but restrained controls showed other alterations (Tables 18-31).

## DISCUSSION

Results of the current study suggest that space flight affects immunological parameters of the rhesus monkey. This study suggests that the bone marrow cell response to colony stimulating factor, as well as leukocyte subset cell population distributions of both peripheral blood and bone marrow leukocytes, were altered after space flight. It is worth noting that most immunological parameters examined in Cosmos 2229 were suppressed for some period of time after flight. Immunological results from Cosmos 2229 differed from previous Cosmos flights involving rats in that the number of bone marrow colonies formed in response to colony stimulating factor was depressed, but leukocyte population percentages found from antibody staining showed both increases and decreases after flight (Sonnenfeld *et al.*, 1992). A number of possible explanations can be given to account for comparative discrepancies between the results of Cosmos 2229 and previous flights. Among these explanations are species difference and differences in flight conditions which, regardless of species difference, may have had some immunological impact. Further experimentation is required to answer these questions.

All immune parameters tested in the study of flight monkeys appeared to return towards a more normal level by 3 days post- landing; however, by 12 days post-landing, these responses were again suppressed. This second drop occurring after recovery could have been due to stress on the monkeys due to increases in scientific testing and handling of the animals. There were differences in the level, but not the pattern, of immune response observed in each of the two flight monkeys. These differences may be explained by the dehydration and reduced food intake experienced by one of the flight monkeys both during and immediately after flight.

There were experimental difficulties observed in both pre- and post- flight experiments. First, there were unusual responses of bone marrow to GM-CSF and unusual leukocyte phenotyping in some flight pool animals monkeys prior to flight support pre-flight levels of immunosuppression (Sonnenfeld *et al.*, 1993). Explanations for the unusual pre-flight immunological responses of the flight pool animals could include the nutritional status of these monkeys, or immunosuppression caused by stresses from increased handling. Additionally,

prenatal conditions and nurturing of the monkeys could have had important influences on pre- and post-flight results. Second, there were differences across testing periods in the response of standard vivarium control monkey bone marrow cells to GM-CSF as well as differences in the results of cell population staining percentage. This variation from testing time to testing time has not observed in previous studies (Sonnenfeld *et al.*, 1993). Results from standard vivarium control monkey samples were included as baseline values to insure that those changes observed after flight were directly due to space flight and not to possible problems with experimental procedure. Despite the changes in observed in these vivarium control values across testing times, when differences between results from standard vivarium control and flight animals occurred, they were of similar proportions. This allowed for interpretation of the flight data, and reinforced the need for this type of standard vivarium control in future flight experiments.

After the flight, both flight animals were placed in flight chairs in an attempt to determine the effect of restraint on immunological parameters measured in this study. Data from the study of the response of bone marrow from restrained flight animals to GM-CSF were uninterpretable because bone marrow taken from standard vivarium control monkeys at corresponding time periods in the restraint study showed no response to GM-CSF. However, it appeared that restraint of the flight animals resulted in only some of the immunological changes in leukocyte phenotypes that were altered by space flight. Some of the restrained animals had changes in immune parameters that were different from those observed after space flight. Therefore, restraint probably played some small role in those immunological changes observed after space flight, but, certainly, was not responsible for the majority of the changes which occurred after flight.

## SUMMARY AND CONCLUSIONS

The current study indicates that exposure of rhesus monkeys to space flight resulted in inhibition of the response of bone marrow cells to GM-CSF and depression of the percentage of peripheral blood and bone marrow leukocyte antibody markers. B cells, bearing surface immunoglobulin, appeared to be less affected than were T cells. As time progressed following

landing, the flight monkeys appeared to recover their immune responses; however, post-flight testing possibly contributed to a second drop in immunological response. Restraint appeared to play some role in the effect of space flight on immune response, but restraint alone was not responsible for all of the immunological changes observed after flight .

Despite experimental difficulties, immunological results from the Cosmos 2229 space flight provide interesting new data suggesting that space flight indeed effects some immune responses of rhesus monkeys. These results indicate there may be an effect of species difference when comparing the immunological impact of space flight on monkeys and rats, as well as possible effects of stress and microgravity. The importance of these and other factors having possible immunological implications should be determined and taken into consideration for the design of future studies involving space flight. Further experimentation is required to establish the degree to which these and other factors are involved in changes resulting from space flight, mechanisms for these changes, and possible measures which can be used to abrogate or mediate such influences.

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TABLE 1

Preflight Response of Bone Marrow Cells to GM-CSF

| Animal # | Condition During Flight | # Colonies - CSF | # Colonies + CSF |
|---|---|---|---|
| 85 | Standard Vivarium Control | 0 | 8 |
| 3224 | | 0 | 6 |
| 151 | Flight | 5 | 25 |
| 906 | | 3 | 2 |
| 476 | Flight Pool Control | 0 | 0 |
| 775 | | 0 | 2 |
| 856 | | 2 | 14 |
| 907 | | 0 | 43 |

Statistical considerations:

Significance, $p < 0.05$, determined using a pooled estimate of variance for a two-tailed t-test.

Standard vivarium control monkeys (#s 85 and 3224)* vs standard vivarium control monkeys (#s 85 and 3224)**: $9.902 \times 10^{-3} < 0.05$.

Flight monkey (#151)* vs flight monkey (#151)**: $14.142 < 4.303$

Flight pool control monkey (# 856)* vs flight pool control monkey (# 856)**: $8.49 > 4.303$

Flight pool control monkey (#907)* vs flight pool control monkey (#907)**: $330.41 > 4.303$

*: - GM-CSF

**: + GM-CSF

TABLE 2

Postflight Response of Bone Marrow Cells to GM-CSF

| Animal # | Condition During Flight | # Colonies - CSF | # Colonies + CSF |
|---|---|---|---|
| 85 | Standard Vivarium Control at R + 3 | 15 | 37 |
| 3224 | | 19 | 41 |
| 151 | Flight at R + 3 | 22 | 16 |
| 906 | | 17 | 17 |
| 775 | Flight Pool Control at R + 3 | 11 | 19 |
| 476 | Flight Pool Control at R + 5 | 7 | 31 |
| 775 | | 19 | 25 |
| 838 | Flight Pool Control at R +10 | 11 | 14 |
| 1324 | | 10 | 11 |
| 85 | Standard Vivarium Control at R + 12 | 11 | 16 |
| 3224 | | 5 | 13 |
| 151 | Flight at R + 12 | 8 | 22 |
| 906 | | 6 | 10 |

Stattistical considerations:

Significance, $p < 0.05$, determined using a pooled estimate of variance for a two-tailed t-test.

TABLE 2 - Continued

Standard vivarium control monkeys at R + 3 (#s 85 and 3224)* vs standard vivarium control monkeys at R + 3 (#s 85 and 3224)**: $8.07 \times 10^{-3} < 0.05$.

Standard vivarium control monkeys at R + 3 (#s 85 and 3224)* vs standard vivarium control monkeys at R + 12 (#s 85 and 3224)**: $5.13 \times 10^{-3} < 0.05$.

Standard vivarium control monkeys at R + 3 (#s 85 and 3224)** vs flight pool control monkeys at R + 10 (#s 85 and 1324)**: $4.39 \times 10^{-3} < 0.05$.

Standard vivarium control monkeys at R + 3 (#s 85 and 3224)** vs flight pool control monkeys at R + 5 (#s 775 and 476)**: $0.0464 < 0.05$.

Standard vivarium control monkeys at R + 12 (#s 85 and 3224)** vs flight pool control monkeys at R + 5 (#s 775 and 476)**: $0.0283 < 0.05$.

Standard vivarium control monkeys at R + 3 (#s 85 and 3224)* vs flight pool control monkeys at R + 10 (#s 85 and 1324)*: $0.0438 < 0.05$.

Standard vivarium control monkeys at R + 3 (#s 85 and 3224)* vs flight monkeys at R + 12 (#s 151 and 906)*: $0.0233 < 0.05$.

Standard vivarium control monkeys at R + 3 (#s 85 and 3224)** vs flight monkeys at R + 12 (#s 151 and 906)**: $0.034 < 0.05$.

Standard vivarium control monkeys at R + 12 (#s 85 and 3224)* vs flight monkeys at R + 3 (#s 151 and 906)*: $0.0493 < 0.05$.

Standard vivarium control monkeys at R + 3 (#s 85 and 3224)** vs flight monkeys at R + 3 (#s 151 and 906)**: $4.15 \times 10^{-3} < 0.05$.

Flight monkeys at R + 12 (#s 151 and 906)* vs flight monkeys at R + 3 (#s 151 and 906)*: $0.0217 < 0.05$.

Flight pool control monkeys at R + 5 (#s 476 and 775)** vs flight pool control monkeys at R + 10 (#s 476 and 775)**: $0.0219 < 0.05$.

Flight pool control monkeys at R + 10 (#s 838 and 1324)* vs flight monkeys at R + 12 (#s 151

and 906)*: 0.0443 < 0.05.

TABLE 2 - Continued

Flight pool control monkeys at R + 10 (#s 838 and 1324)* vs flight monkeys at R + 3 (#s 151 and 906)*: 0.0359 < 0.05.

Flight pool control monkeys at R + 5 (#s 476 and 775)** vs flight monkeys at R + 3 (#s 151 and 906)**: 0.0317 < 0.05.

*: - GM-CSF

**: + GM-CSF

TABLE 3

Effect of Postflight Restraint on the Response of Bone Marrow Cells to GM-CSF

| Animal # | Condition | # Colonies - CSF | # Colonies + CSF |
|---|---|---|---|
| 85 | Standard Vivarium Control | 5 | 7 |
| 3224 | | 4 | 5 |
| 151 | Flight + Restraint | 2 | 2 |
| 906 | | 4 | 8 |
| 803 | Flight Pool Control + Restraint | 8 | 5 |
| 907 | | 8 | 10 |
| 588 | Vivarium Control | 12 | 24 |
| 1417 | | 6 | 7 |

*Significance, $p < 0.05$, compared to standard vivarium control group ( - GM-CSF)

**Significance, $p < 0.05$, compared to standard vivarium control group ( + GM-CSF)

There was no significance, $p < 0.05$, within groups comparing - GM-CSF to + GM-CSF.

TABLE 4

Preflight Staining of Peripheral Blood with Anti-HLA-DR

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 28.1 |
| 3224 | | 14.3 |
| 151 | Flight | 9.6 |
| 906 | | 0 |
| 476 | Flight Control Pool | 7.2 |
| 775 | | 4.7 |
| 803 | | 0 |
| 856 | | 0 |
| 858 | | 0.1 |
| 892 | | 0 |
| 907 | | 2.5 |
| 1404 | | 0.2 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and individual flight monkeys

TABLE 5

Preflight Staining of Peripheral Blood with Leu-2a (CD 8)

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 3.2 |
| 3224 | | 8.8 |
| 151 | Flight | 14.3 |
| 906 | | 0 |
| 476 | Flight Control Pool | 2.3 |
| 775 | | 1.0 |
| 803 | | 0 |
| 856 | | 0.6 |
| 858 | | 0 |
| 892 | | 0 |
| 907 | | 0.1 |
| 1404 | | 0.3 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and individual flight monkeys

TABLE 6

Preflight Staining of Peripheral Blood with Leu-3a (CD 4)

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 1.4 |
| 3224 | | 0.8 |
| 151 | Flight | 5.8 |
| 906 | | 0 |
| 476 | Flight Control Pool | 0.6 |
| 775 | | 0.8 |
| 803 | | 0 |
| 856 | | 1.0 |
| 858 | | 1.8 |
| 892 | | 0 |
| 907 | | 0.2 |
| 1404 | | 0 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and individual flight monkeys

TABLE 7

Preflight Staining of Peripheral Blood with Leu-4 (CD 3)

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 0.6 |
| 3224 | | 5.9 |
| 151 | Flight | 3.6 |
| 906 | | 0 |
| 476 | Flight Control Pool | 1.2 |
| 775 | | 0 |
| 803 | | 0 |
| 856 | | 3.1 |
| 858 | | 1.6 |
| 892 | | 0 |
| 907 | | 0 |
| 1404 | | 0.5 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and individual flight monkeys

TABLE 8

Preflight Staining of Peripheral Blood with Leu-11a (CD 16)

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 1.5 |
| 3224 | | 3.6 |
| 151 | Flight | 9.2 |
| 906 | | 0 |
| 476 | Flight Control Pool | 1.8 |
| 775 | | 1.4 |
| 803 | | 0 |
| 856 | | 0.4 |
| 858 | | 0.4 |
| 892 | | 1.1 |
| 907 | | 0.4 |
| 1404 | | 2.0 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and individual flight monkeys

TABLE 9

Preflight Staining of Peripheral Blood with Anti-Human IgM

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 50.1 |
| 3224 | | 40.2 |
| 151 | Flight | 32.1 |
| 906 | | 39.9 |
| 476 | Flight Control Pool | 56.6 |
| 775 | | 64.3 |
| 803 | | 56.6 |
| 856 | | 38.7 |
| 858 | | 81.4 |
| 892 | | 51.9 |
| 907 | | 63.3 |
| 1404 | | 77.2 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and individual flight monkeys

TABLE 10

Preflight Staining of Peripheral Blood with Anti-Monkey IgG

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 48.4 |
| 3224 | | 69.8 |
| 151 | Flight | 63.5 |
| 906 | | 32.4 |
| 476 | Flight Control Pool | 49.4 |
| 775 | | 32.0 |
| 803 | | 84.4 |
| 856 | | 69.1 |
| 858 | | 89.9 |
| 892 | | 87.9 |
| 907 | | 19.3 |
| 1404 | | 71.8 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and individual flight monkeys

TABLE 11

Postflight Staining of Peripheral Blood with Anti-HLA-DR

| Animal # | Condition During Flight | | % Lymphoid Cells Stained |
|---|---|---|---|
| 85 | Standard Vivarium Control | R + 3 | 0 |
| 85 | | R +12 | 3.1 |
| 3224 | | R + 3 | 2.0 |
| 3224 | | R + 12 | 0.2 |
| 151 | Flight | R + 2* | 0 |
| 151 | | R + 3** | 20.0 |
| 151 | | R + 12 | 0.2 |
| 906 | | R + 1 | 0 |
| 906 | | R + 2* | 3.0 |
| 906 | | R + 3** | 5.0 |
| 906 | | R + 12 | 0.2 |
| 476 | Flight Control Pool | R + 5*** | 0 |
| 775 | | R + 5*** | 11.0 |
| 838 | | R + 10 | 40.0 |
| 1324 | | R + 10 | 8.0 |

*Significance, $p < 0.05$, between standard vivarium control monkeys at R + 3 (#s 85 and 3224) and R + 2 flight monkeys (#s 906 and 151)

**Significance, $p < 0.05$, between standard vivarium control monkeys at R + 3 (#s 85 and 3224) and R + 3 flight monkeys (#s 906 and 151)

***Significance, $p < 0.05$, between standard vivarium control monkeys at R + 3 (#s 85 and 3224) and R + 3 flight monkeys (#s 476 and 775)

TABLE 12

Postflight Staining of Peripheral Blood with Leu-2a (CD 8)

| Animal # | Condition During Flight | | % Lymphoid Cells Stained |
|---|---|---|---|
| 85 | Standard Vivarium Control | R + 3 | 13.7 |
| 85 | | R +12 | 4.1 |
| 3224 | | R + 3 | 0 |
| 3224 | | R + 12 | 0 |
| 151 | Flight | R + 2 | 0 |
| 151 | | R + 3 | 19.6 |
| 151 | | R + 12 | 0 |
| 906 | | R + 1 | 0.3 |
| 906 | | R + 2 | 1.2 |
| 906 | | R + 3 | 1.1 |
| 906 | | R + 12 | 0 |
| 476 | Flight Control Pool | R + 5 | 0 |
| 775 | | R + 5 | 5.6 |
| 838 | | R + 10 | 1.1 |
| 1324 | | R + 10 | 22.3 |

*Significance, $p < 0.05$, between standard vivarium control monkeys at R + 3 (#s 85 and 3224) and flight monkey groups

**Significance, $p < 0.05$, between standard vivarium control monkeys at R + 12 (#s 85 and 3224) and flight monkey groups

TABLE 13

Postflight Staining of Peripheral Blood with Leu -3a (CD 4)

| Animal # | Condition During Flight | | % Lymphoid Cells Stained |
|---|---|---|---|
| 85 | Standard Vivarium Control | R + 3 | 9.5 |
| 85 | | R +12 | 1.3 |
| 3224 | | R + 3 | 4.1 |
| 3224 | | R + 12 | 0.2 |
| 151 | Flight | R + 2 | 5.2 |
| 151 | | R + 3 | 15.6 |
| 151 | | R + 12 | 0 |
| 906 | | R + 1 | 0.3 |
| 906 | | R + 2 | 0.8 |
| 906 | | R + 3 | 4.1 |
| 906 | | R + 12 | 0 |
| 476 | Flight Control Pool | R + 5 | 0.4 |
| 775 | | R + 5 | 2.8 |
| 838 | | R + 10 | 2.4 |
| 1324 | | R + 10 | 8.3 |

*Significance, $p < 0.05$, between standard vivarium control monkeys at R + 3 (#s 85 and 3224) and flight monkey groups

**Significance, $p < 0.05$, between standard vivarium control monkeys at R + 12 (#s 85 and 3224) and flight monkey groups

TABLE 14

Postflight Staining of Peripheral Blood with Leu-4 (CD 3)

| Animal # | Condition During Flight | | % Lymphoid Cells Stained |
|---|---|---|---|
| 85 | Standard Vivarium Control | R + 3 | 11.3 |
| 85 | | R +12* | 1.3 |
| 3224 | | R + 3 | 0 |
| 3224 | | R + 12* | 0.2 |
| 151 | Flight | R + 2** | 0 |
| 151 | | R + 3* | 22.7 |
| 151 | | R + 12* | 0 |
| 906 | | R + 1* | 0 |
| 906 | | R + 2** | 2.3 |
| 906 | | R + 3* | 10.1 |
| 906 | | R + 12* | 0 |
| 476 | Flight Control Pool | R + 5** | 0.6 |
| 775 | | R + 5** | 0 |
| 838 | | R + 10 | 5 |
| 1324 | | R + 10 | 14.6 |

*Significance, $p < 0.05$, between standard vivarium control monkeys at R + 3 (#s 85 and 3224) and flight monkey groups

**Significance, $p < 0.05$, between standard vivarium control monkeys at R + 12 (#s 85 and 3224) and flight monkey groups

TABLE 15

Postflight Staining of Peripheral Blood with Leu-11a (CD 16)

| Animal # | Condition During Flight | | % Lymphoid Cells Stained |
|---|---|---|---|
| 85 | Standard Vivarium Control | R + 3 | 10.1 |
| 85 | | R +12 | 2.3 |
| 3224 | | R + 3 | 0 |
| 3224 | | R + 12 | 0.2 |
| 151 | Flight | R + 2 | 0 |
| 151 | | R + 3 | 7.2 |
| 151 | | R + 12 | 0 |
| 906 | | R + 1 | 0.8 |
| 906 | | R + 2 | 4.0 |
| 906 | | R + 3 | 3.5 |
| 906 | | R + 12 | 0.8 |
| 476 | Flight Control Pool | R + 5 | 0 |
| 775 | | R + 5 | 7.0 |
| 838 | | R + 10 | 0.8 |
| 1324 | | R + 10 | 12.7 |

*Significance, $p < 0.05$, between standard vivarium control monkeys at R + 3 (#s 85 and 3224) and flight monkey groups

**Significance, $p < 0.05$, between standard vivarium control monkeys at R + 12 (#s 85 and 3224) and flight monkey groups

TABLE 16

Postflight Staining of Peripheral Blood with Anti-Human IgM

| Animal # | Condition During Flight | | % Lymphoid Cells Stained |
|---|---|---|---|
| 85 | Standard Vivarium Control | R + 3 | 54.4 |
| 85 | | R +12 | 33.3 |
| 3224 | | R + 3 | 47.3 |
| 3224 | | R + 12 | 14.7 |
| | | | |
| 151 | Flight | R + 2 | 26.6 |
| 151 | | R + 3 | 72.3 |
| 151 | | R + 12 | 16.5 |
| 906 | | R + 1 | 4.0 |
| 906 | | R + 2 | 25.7 |
| 906 | | R + 3 | 30.0 |
| 906 | | R + 12 | 13.3 |
| | | | |
| 476 | Flight Control Pool | R + 5 | 19.0 |
| 775 | | R + 5 | 49.6 |
| 838 | | R + 10 | 45.2 |
| 1324 | | R + 10 | 24.4 |

*Significance, $p < 0.05$, between standard vivarium control monkeys at R + 3 (#s 85 and 3224) and flight monkey groups

**Significance, $p < 0.05$, between standard vivarium control monkeys at R + 12 (#s 85 and 3224) and flight monkey groups

TABLE 17

Postflight Staining of Peripheral Blood with Anti-Monkey IgG

| Animal # | Condition During Flight | | % Lymphoid Cells Stained |
|---|---|---|---|
| 85 | Standard Vivarium Control | R + 3 | 78.5 |
| 85 | | R +12 | 49.3 |
| 3224 | | R + 3 | 71.2 |
| 3224 | | R + 12 | 56.8 |
| 151 | Flight | R + 2* | 56.3 |
| 151 | | R + 3 | 83.0 |
| 151 | | R + 12 | 58.0 |
| 906 | | R + 1 | 47.8 |
| 906 | | R + 2* | 60.3 |
| 906 | | R + 3 | 65.8 |
| 906 | | R + 12 | 60.5 |
| 476 | Flight Control Pool | R + 5 | 59.8 |
| 775 | | R + 5 | 45.5 |
| 838 | | R + 10 | 37.3 |
| 1324 | | R + 10 | 46 |

*Significance, $p < 0.05$, between standard vivarium control monkeys at R + 3 (#s 85 and 3224) and flight monkey groups

**Significance, $p < 0.05$, between standard vivarium control monkeys at R + 12 (#s 85 and 3224) and flight monkey groups

TABLE 18

Effect of Restraint on Staining of Peripheral Blood with Anti-HLA-DR

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 27.5 |
| 3224 | | 0.8 |
| 151 | Flight + Restraint | 8.6 |
| 906 | | 25.2 |
| 803 | Flight Pool + Restraint | 13.4 |
| 907 | | 2.9 |
| 588 | Vivarium Control | 16.7 |
| 1417 | | 4.6 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 19

Effect of Restraint on Staining of Bone Marrow with Anti-HLA-DR

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control* | 15.4 |
| 3224 | | 16.6 |
| 151 | Flight + Restraint* | 3.8 |
| 906 | | 4.0 |
| 803 | Flight Pool + Restraint* | 12.6 |
| 907 | | 9.0 |
| 588 | Vivarium Control | 40.6 |
| 1417 | | 39.3 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 20

Effect of Restraint on Staining of Peripheral Blood with Leu-2a (CD -8)

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 11.7 |
| 3224 | | 0.9 |
| 151 | Flight + Restraint | 4.8 |
| 906 | | 11.1 |
| 803 | Flight Pool + Restraint | 21.8 |
| 907 | | 0.8 |
| 588 | Vivarium Control | 1.6 |
| 1417 | | 2.2 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 21

Effect of Restraint on Staining of Bone Marrow with Leu-2a (CD -8)

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 3.8 |
| 3224 | | 10.1 |
| 151 | Flight + Restraint | 0 |
| 906 | | 0.8 |
| 803 | Flight Pool + Restraint | 0 |
| 907 | | 2.8 |
| 588 | Vivarium Control | 4.4 |
| 1417 | | 0 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 22

Effect of Restraint on Staining of Peripheral Blood with Leu-3a (CD -4)

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 6.2 |
| 3224 | | 0 |
| 151 | Flight + Restraint | 0 |
| 906 | | 7.6 |
| 803 | Flight Pool + Restraint | 0 |
| 907 | | 0 |
| 588 | Vivarium Control | 0 |
| 1417 | | 1.3 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 23

Effect of Restraint on Staining of Bone Marrow with Leu-3a (CD -4)

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 1.5 |
| 3224 | | 0 |
| 151 | Flight + Restraint | 4.4 |
| 906 | | 0.6 |
| 803 | Flight Pool + Restraint | 0 |
| 907 | | 5.7 |
| 588 | Vivarium Control | 0 |
| 1417 | | 0 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 24

Effect of Restraint on Staining of Peripheral Blood with Leu-4 (CD -3)

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 14.6 |
| 3224 | | 0 |
| 151 | Flight + Restraint | 11.4 |
| 906 | | 23.4 |
| 803 | Flight Pool + Restraint | 3.0 |
| 907 | | 0 |
| 588 | Vivarium Control | 10.6 |
| 1417 | | 26.8 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 25

Effect of Restraint on Staining of Bone Marrow with Leu-4 (CD -3)

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 21.4 |
| 3224 | | 10.3 |
| 151 | Flight + Restraint | 39.9 |
| 906 | | 13.7 |
| 803 | Flight Pool + Restraint | 17.6 |
| 907 | | 18.5 |
| 588 | Vivarium Control | 13.5 |
| 1417 | | 45.4 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 26

Effect of Restraint on Staining of Peripheral Blood with Leu-11a (CD -16)

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 15.8 |
| 3224 | | 0 |
| 151 | Flight + Restraint | 10.2 |
| 906 | | 14.2 |
| 803 | Flight Pool + Restraint | 1.6 |
| 907 | | 0 |
| 588 | Vivarium Control | 1.6 |
| 1417 | | 2.6 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 27

Effect of Restraint on Staining of Bone Marrow with Leu-11a (CD -16)

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 6.6 |
| 3224 | | 25.9 |
| 151 | Flight + Restraint | 8.9 |
| 906 | | 30.9 |
| 803 | Flight Pool + Restraint | 10.0 |
| 907 | | 16.3 |
| 588 | Vivarium Control | 13.0 |
| 1417 | | 26.6 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 28

Effect of Restraint on Staining of Peripheral Blood with Anti-Human IgM

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 63.6 |
| 3224 | | 74.6 |
| 151 | Flight + Restraint | 40.5 |
| 906 | | 82.6 |
| 803 | Flight Pool + Restraint | 51.5 |
| 907 | | 72.8 |
| 588 | Vivarium Control | 54.8 |
| 1417 | | 62.7 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 29

Effect of Restraint on Staining of Bone Marrow with Anti-Human IgM

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 53.3 |
| 3224 | | 71.7 |
| 151 | Flight + Restraint | 84.5 |
| 906 | | 86.0 |
| 803 | Flight Pool + Restraint | 58.9 |
| 907 | | 84.8 |
| 588 | Vivarium Control | 46.9 |
| 1417 | | 83.9 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 30

Effect of Restraint on Staining of Peripheral Blood with Anti-Monkey IgG

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 85.7 |
| 3224 | | 79.2 |
| 151 | Flight + Restraint | 81.8 |
| 906 | | 87.1 |
| 803 | Flight Pool + Restraint* | 68.5 |
| 907 | | 72.9 |
| 588 | Vivarium Control | 58.5 |
| 1417 | | 74.0 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys

TABLE 31

Effect of Restraint on Staining of Bone Marrow with Anti-Human IgM

| Animal # | Condition During Flight | % Lymphoid Cells Stained |
|---|---|---|
| 85 | Standard Vivarium Control | 70.7 |
| 3224 | | 70.6 |
| 151 | Flight + Restraint | 76.1 |
| 906 | | 51.2 |
| 803 | Flight Pool + Restraint | 36.8 |
| 907 | | 74.4 |
| 588 | Vivarium Control | 78.5 |
| 1417 | | 42.3 |

*Significance, $p < 0.05$, between standard vivarium control monkeys (#s 85 and 3224) and restraint monkey groups or vivarium control group monkeys