Shuttle-Mir Science Program Phase 1A Research Postflight Science Report

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Introduction

The Shuttle-Mir Science Program, also known as the Phase 1A program, was developed as a result of a joint agreement between the United States and the Russian Federation which initiated a cooperative human space flight program. The program consisted of two long duration missions, Mir 18 and Mir 19, and one Shuttle docking mission, Spacelab-Mir (SL-M) STS-71.

The Mir 18 mission began with the launch of the Soyuz TM21 on March 14, 1995, carrying two Russian cosmonauts, Mission Commander Lieutenant Colonel Vladimir N. Dezhurov and Flight Engineer Gennady M. Strekalov, Ph.D., and U.S. Astronaut, Mission Specialist Norman E. Thagard, M.D. The Soyuz TM21 docked with the Mir on March 16, 1995. After a 116 day stay in space, most of it on the Russian Space Station Mir, the Mir 18 crew landed at Kennedy Space Center on July 7, 1995. The STS-71 crew consisted of Commander Captain Robert L. "Hoot" Gibson, Pilot Lieutenant Colonel Charles J. Precourt, Mission Specialist Ellen S. Baker, M.D., Mission Specialist Gregory J. Harbaugh and Mission Specialist Bonnie J. Dunbar, Ph.D. The SL-M mission also provided return transportation for the Mir 18 crew and transportation for the Mir 19 crew to the Mir.

The Mir 19 mission continued the joint science program and began with the launch of U.S. Space Shuttle Atlantis carrying two Russian cosmonauts, Mission Commander Colonel Anatoly Y. Solovyev and Flight Engineer Nikolai M. Budarin, to the space station Mir. Mir 19 was concluded on September 11, 1995, with the landing of Soyuz TM21 in Russia.

The Shuttle-Mir science program used the U.S. Space Shuttle and the Russian Space Station Mir capabilities to conduct joint research activities in space. Seven research areas encompassing 28 investigations were conducted on Mir and/or the Shuttle. The overall objectives of the Shuttle-Mir missions were to obtain engineering and operational experience in conducting research on an orbital space station; to conduct specific investigations in medical support, life sciences, fundamental biology, microgravity sciences, Earth observations, and life support technology; and to characterize the environment relative to microgravity and life sciences research on Mir to better understand past and future investigations. Included in this report are the final science reports from the investigations performed on Mir 18, STS-71, and/or Mir 19.

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U.S. Principal Investigator: **CLARENCE F. SAMS, Ph.D.,** NASA/Johnson Space Center

Russian Principal Investigator: **IRINA KONSTANTINOVA, Ph.D.,** Institute of Biomedical Problems

Phenotypic and Functional Analysis of Peripheral Mononuclear Cells During Long-Duration Space Flight

U.S. Principal Investigator: **CLARENCE F. SAMS, Ph.D.,** NASA/Johnson Space Center

Russian Principal Investigator: **IRINA KONSTANTINOVA, Ph.D.,** Institute of Biomedical Problems

Co-Investigators: Richard T. Meehan, M.D. and Duane Pierson, Ph.D.

(STS-71 Final Science Report)

INTRODUCTION

THE CHANGES IN immune function which occur during space flight potentially expose the crews to an increased risk for development of illness. A thorough understanding of the immune system function during space flight is critical to the rational assessment of crew health risks. Decreased cellular immune function has been repeatedly documented after space flight and confirmed during flight by in vivo delayed-type hypersensitivity testing. Peripheral immune cells have been extensively studied pre- and postflight; however, the analysis of phenotypic and functional properties of crewmember immune cells during space flight has not yet been performed.

Objectives

Determine the phenotypic alterations in circulating immune cell subpopulations which occur during space flight and compare them to those observed immediately after flight.

Assess the functional changes in the peripheral immune cells resulting from space flight.

Determine the roles of specific cytokines (interleukin 1, interleukin 1 receptor antagonist, interleukin 2, interleukin 6, interleukin 10, tumor necrosis factor alpha, granulocyte/ macrophage colony stimulating factor) and immunoregulatory factors (prostaglandin E2) in mediating space flight-induced immune suppression.

Hypothesis

Phenotypic and functional alterations in peripheral immune cells obtained immediately after landing accurately reflect changes induced during space flight.

Space flight-induced suppression of immune function is mediated via alterations in specific cytokines and immunomodulatory factors.

Background/History

Changes in functional and phenotypic properties of circulating leukocytes have been consistently reported following space flight. A trend toward postflight lymphocytosis was reported for the 33 Apollo astronauts, though the changes were not significant due to a high degree of individual variability. During the Skylab program, a postflight increase in absolute leukocyte counts was noted coincident with a decrease in circulating T lymphocytes. Russian investigators have reported decreased numbers of T cells and NK cells following flights of highly varying duration. Leucocytosis and lymphopenia were again noted on Apollo-Soyuz and Space Shuttle missions and the leukocyte increase appeared to be primarily within the neutrophil population. Coulter Counter analysis and differential staining of blood smears from 133 Space Shuttle astronauts indicates an increase in granulocytes and a decrease in lymphocytes and eosinophils are consistent responses to short-duration orbital space flight. Further evidence of in vitro immune dysregulation includes 1) reduced mitogen-induced activation of T cells following space flight, 2) altered cytokine production, 3) reduced NK cytotoxicity, and 4) reduced delayed-type hypersensitivity responses to a panel of recall antigens applied intradermally during space flight. This demonstrates that alterations in cell-mediated immunity do occur in vivo and supports the hypothesis that the immune system is functionally altered during space flight.

METHODS/SCIENCE OPERATIONS

Functional Objectives

FO1. Collection of blood samples inflight.

FO2. Staining of cells for flow cytometry.

FO3. Activation and functional assay of lymphocytes and NK cells.

FO4. Determination of cytokine production in activated cells.

Hardware Items

HW1. Peripheral Mononuclear Cell (PMC) Staining Kit containing the Whole Blood Staining Devices (WBSDs)

HW2. Peripheral Mononuclear Cell Blood Collection Kit containing the blood collection hardware and equipment for inoculating the blood into the WBSDs of the PMC staining kit.

Method/Protocol

Blood samples were collected 150 and 35 days before flight. Anticoagulated whole blood was stained with monoclonal antibodies, processed and fixed, and the relative percentages of specific mononuclear cell subpopulations was determined by flow cytometry. Mononuclear cells were assayed for monocyte, T cell, and NK cell function. The levels of cytokines and other immunomodulatory molecules will be quantitated in serum and in culture medium after activation. Staining of cells during flight for subsequent flow cytometry was performed using the Whole Blood Staining Device (WBSD). The device consists of a 5 cm long section of plastic tubing about 1 cm in diameter. One end is sealed and the other is fitted with an injection port. Reagent chambers are separated by clamps which can be removed in sequence to mix all reagents. The samples are then analyzed on the EPICS XL Flow Cytometer. The basic immunophenotyping panel for analysis of circulating cell populations will include the following markers: CD45 (leukocytes), CD14 (monocytes), CD3 (T cells), CD16/CD56 (NK cells), CD4 (T helper/inducer), CD8 (T supressor/cytotoxic), CD19 (B cells), membrane-bound IL1a, and HLA-DR (Human Leukocyte Antigen).

Peripheral mononuclear cells were activated with mitogens and activation was assessed at 24 hours by surface marker expression. Cell supernatants were harvested, frozen, and stored at -70°C for subsequent analysis of cytokine production. The concentration of cytokines and growth factors; IL-1ra, IL-1b, IL-2, IL-6, and IL-10 in the collected medium samples will be measured by the Enzyme Linked ImmunoSorbant Assay (ELISA). The medium concentrations of PGE2 will be measured by radioimmunoassay. Transcriptional levels of the cytokines will be assayed in the RNA extracted from the flight and ground control cells.

NK cell function was assayed by the chromium release assay. This assay measures the cytotoxic activity of effector (NK) cells by quantitating the amount of radioactivity released from the killed/lysed target cells.

RESULTS

List of Pre-, In-, and Postflight Anomalies

Preflight Anomalies

L-35 blood sample was only 5.0 ml on Subject 3. Draw was short on the collection syringes due to operator error.

Inflight Anomalies

The crew reported that the 1.4 ml blood collection syringes did not draw the required volume. The crew recommended using larger volume syringes (2.7 ml minimum).

Postflight Anomalies

No major postflight anomalies. The R+7 sample collection was rescheduled to R+9 with no impact to science.

Completeness/Quality of data

There were no additional data collections for this experiment. The analysis of experimental samples is continuing. Data analyzed to date are of good quality with no apparent deficiencies in the samples.

Tables, Graphs, and Figures Index

Table 1. Data Collection Sessions/Functional Objectives

Figure 1. Relative percentage of lymphocytes in circulating peripheral mononuclear cell populations. Cells were identified by forward and side scatter charateristics using flow cytometry. Whole blood samples were used after lysis of red blood cells. Data are expressed as the percentage of total white blood cells. Samples were collected 150 and 35 days before launch (L-150 and L-35), on flight day 11 of STS 71 (FD11), on landing day (R+0), and nine days after recovery (R+9).

Figure 2. Relative percentage of monocytes in circulating peripheral mononuclear cell populations. Cells were identified by flow cytometry forward and side scatter charateristics using whole blood samples. Data are expressed as the percentage of total white blood cells. Samples were collected 150 and 35 days before launch (L-150 and L-35), on flight day 11 of STS 71 (FD11), on landing day (R+0), and nine days after recovery (R+9).

Figure 3. Relative percentage of granulocytes in circulating peripheral mononuclear cell populations. Cells were identified by forward and side scatter charateristics in lysed whole blood samples. Data are expressed as the percentage of total white blood cells. Samples were collected 150 and 35 days before launch (L-150 and L-35),

on flight day 11 of STS 71 (FD11), on landing day (R+0), and nine days after recovery (R+9).

Figure 4. Relative percentage of natural killer (NK) cells in circulating peripheral lymphocyte populations. Cells were identified as CD16/56 positive CD3 negative cells by flow cytometry. Data are expressed as the gated cell percentage of total lymphocytes. Samples were collected 150 and 35 days before launch (L-150 and L-35), on flight day 11 of STS 71 (FD11), on landing day (R+0), and nine days after recovery (R+9).

Figure 5. T cell activation response. T cells were activated with lectin and assayed for expression of cell surface activation markers at 24 hours by flow cytometry. Cells were isolated from blood collected preflight (L-35), 24 hours before landing (FD11), on landing day (R+0) and 9 days postflight (R+9). Expression of CD69 was not changed following space flight or in samples isolated during flight (top panel). Expression of IL2 receptor (CD25) was somewhat decreased postflight (bottom panel), but it was not altered in the samples taken during flight (FD11).

DISCUSSION

Status of Data Analysis

The analysis of the flow cytometry samples (preflight, inflight and postflight) has been completed. The assessment of natural killer cell phenotype and function is also complete. Peripheral mononuclear cells were activated to evaluate changes in lymphocyte function. The examination of early activation markers by flow cytometry has been performed on these samples. Cell supernatants and packed cell pellets were also collected from the activation studies. The supernatants were assayed for the levels of secreted cytokines. RNA was isolated from the packed cell pellets and mRNA levels of cytokines will be measured. The cytokine studies (mRNA and secreted) are the final samples remaining to be processed. This activity is currently in progress.

Research Findings

The circulating subpopulations of peripheral white cells were not greatly altered during flight as compared to the preflight baselines. In contrast, the samples taken within 2-3 hours after landing had the relative granulocytosis and lymphopenia observed after most space flights. A relative decrease in T cells, monocytes, B cells and NK cells was also noted after flight. These changes were not observed in the inflight sample taken during the last flight day, suggesting that they are an acute response to reentry and readaptation to unit gravity.

In vitro activation of lymphocytes isolated from crewmembers during flight or immediately after flight was not significantly different from the control periods as determined by the expression of early activation markers. Expression of CD 69 protein or IL-2 receptor (CD 25) 24 hours after mitogen activation was not altered relative to preflight control samples. All crewmembers exhibited good NK cell function on landing day, however variable decreases in cytotoxicity were noted 9 days after landing. The reasons for this effect are currently unclear, though it is consistent with previous observations during long-duration space flight on Mir.

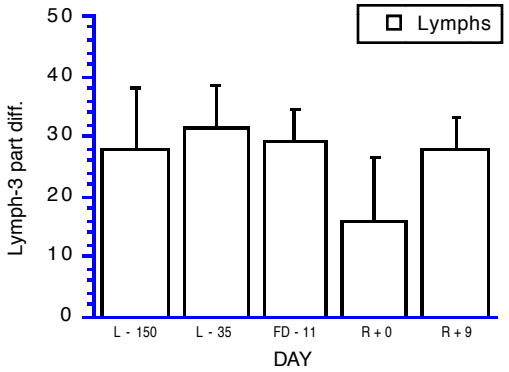
Conclusions

Statistical significance is limited by the low sample number of the study, however, some interesting trends are suggested by the current results. It appears that the major phenotypic changes which are observed after landing arise as a result of reentry and reambulation. Another interesting observation was that expression of early activation markers in cells activated in vitro immediately after flight was not substantially different from the preflight values. However, analysis of activated T cells in the radiation studies (T. Yang) indicated a delayed cell cycle entry in the activated T cells. This suggests a decoupling between early activation events and the control of cell cycle progression/proliferation in the T cells. This could have significant impact on immune regulation, as these functions are closely regulated during the coordination of an immune response. Further research will be required to determine the importance of these findings.

TABLE 1. DATA COLLECTION SESSIONS/FUNCTIONAL OBJECTIVES

Blood Collection (FO1, FO2, FO3, FO4, HW1 & HW2)

Mission	Scheduled Day	Actual Day	Actual Date	Actual Subjects	Samples/ Parameters
Mir 18	L-150	L-151	15 Oct 94	Subject1	Baseline blood
				Subject2	Baseline blood
				Subject3	Baseline blood
Mir 18	L-14	L-35	7 Feb 95	Subject1	Baseline blood
				Subject2	Baseline blood
				Subject3	Baseline blood
STS 71	FD 11	FD 4	30 Jun 95	Subject1	flight blood
				Subject2	flight blood
				Subject3	flight blood
STS 71	R+0	R+0	7 Jul 95	Subject1	postflight blood
				Subject2	postflight blood
				Subject3	postflight blood
STS 71	R+7	R+9	16 Jul 95	Subject1	postflight blood
				Subject2	postflight blood
				Subject3	postflight blood



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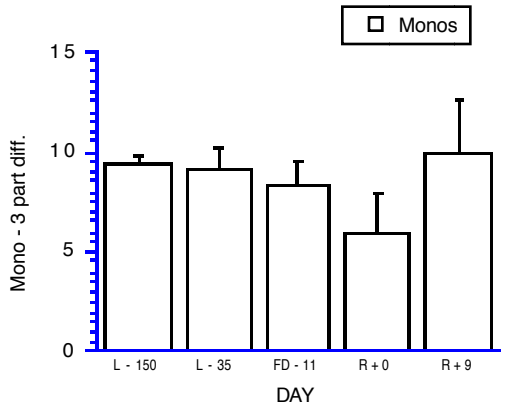

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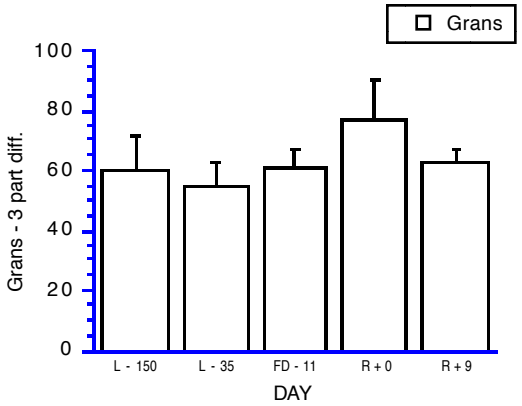
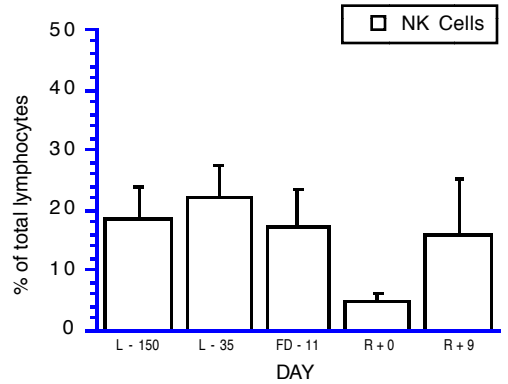


Figure 3. Relative percentage of granulocytes in circulating peripheral mononuclear cell populations. Cells were identified by forward and side scatter charateristics in lysed whole blood samples. Data are expressed as the percentage of total white blood cells. Samples were collected 150 and 35 days before launch (L-150 and L-35), on flight day 11 of STS 71 (FD11), on landing day (R+0), and nine days after recovery (R+9).



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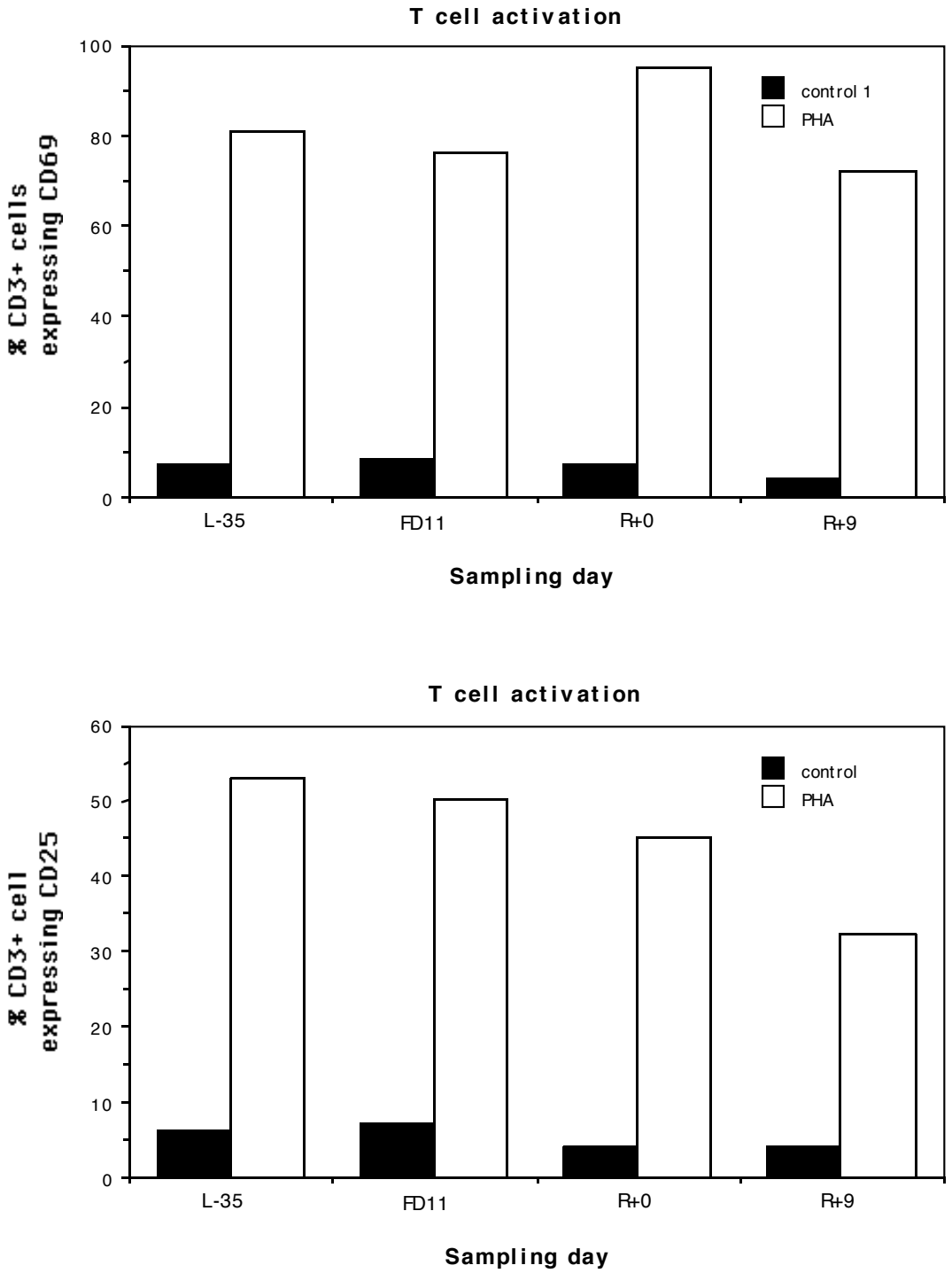

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