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CHANGES IN GRAVITY INHIBIT LYMPHOCYTE LOCOMOTION THROUGH TYPE I COLLAGEN

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SUMMARY

Immunity relies on the circulation of lymphocytes through many different tissues including blood vessels, lymphatic channels, and lymphoid organs. The ability of lymphocytes to traverse the interstitium in both nonlymphoid and lymphoid tissues can be determined *in vitro* by assaying their capacity to locomote through Type I collagen. In an attempt to characterize potential causes of microgravity-induced immunosuppression, we investigated the effects of simulated microgravity on human lymphocyte function *in vitro* using a specialized rotating-wall vessel culture system developed at the Johnson Space Center. This very low shear culture system randomizes gravitational vectors and provides an *in vitro* approximation of microgravity. In the randomized gravity of the rotating-wall vessel culture system, peripheral blood lymphocytes did not locomote through Type I collagen, whereas static cultures supported normal movement. Although cells remained viable during the entire culture period, peripheral blood lymphocytes transferred to unit gravity (static culture) after 6 h in the rotating-wall vessel culture system were slow to recover and locomote into collagen matrix. After 72 h in the rotating-wall vessel culture system and an additional 72 h in static culture, peripheral blood lymphocytes did not recover their ability to locomote. Loss of locomotory activity in rotating-wall vessel cultures appears to be related to changes in the activation state of the lymphocytes and the expression of adhesion molecules. Culture in the rotating-wall vessel system blunted the ability of peripheral blood lymphocytes to respond to polyclonal activation with phytohemagglutinin. Locomotory response remained intact when peripheral blood lymphocytes were activated by anti-CD3 antibody and interleukin-2 prior to introduction into the rotating-wall vessel culture system. Thus, in addition to the systemic stress factors that may affect immunity, isolated lymphocytes respond to gravitational changes by ceasing locomotion through model interstitium. These *in vitro* investigations suggest that microgravity induces non-stress-related changes in cell function that may be critical to immunity. Preliminary analysis of locomotion in true microgravity revealed a substantial inhibition of cellular movement in Type I collagen. Thus, the rotating-wall vessel culture system provides a model for analyzing the microgravity-induced inhibition of lymphocyte locomotion and the investigation of the mechanisms related to lymphocyte movement.

Key words: immunity; microgravity; space travel; inflammatory cells; interstitium; matrix.

INTRODUCTION

The ability of lymphocytes to locomote through the interstitium is critical to afferent and efferent immune responses. As lymphocytes undergo the processes of differentiation, maturation, maintenance, and renewal, they must adhere to specific vessels and cross intercellular matrices (35). In the effector phase, inflammatory reactions require that lymphocytes traverse the interstitium to manifest reactions such as delayed hypersensitivity and tumor or allograft rejection. Analysis of lymphocyte locomotion through Type I collagen has been performed using an adaptation of the method of Ratner and Heppner (28), wherein lymphocytes are introduced between two layers of polymerized collagen containing complete tissue culture medium (1,17,29). A proportion of the lymphocyte population locomotes randomly and omni-directionally through the collagen. Lymphocytes may locomote from 500 to 1500 μm from the originating site of introduction to the matrix (1,17).

In both short- and long-term space travel there is a delayed, yet significant, decline in specific parameters of immune function (2,7,9–11,16,21–23,31,32,37–42). Synthesis of cytokines such as interferon gamma (IFN-γ), interleukin-2 (IL-2), and possibly interleukin-1 (IL-1) may be affected; upon return to unit gravity, these conditions resolve (3). Some of the effects are stress related and parallel previously documented physical stress-induced changes in immunity (12) and, to a lesser extent, emotional stress (25). Approximation of microgravitational conditions at ground level has been achieved in a specialized cell culture system using the rotating-wall vessel (RWV) bioreactor developed at NASA-Johnson Space Center

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and commercially available from Synthecon, Inc. (Friendswood, TX). Simulation of microgravity is achieved in the RWV by randomizing gravitational vectors such that the net gravitational vector integrated over time approaches zero (13,34,43). The RWV has been used to provide a reduced shear environment for the propagation of mammalian cells (4,14,15,20,33). This reduced shear environment enables propagation of cells resulting in differentiating cellular aggregates. Successful propagation and differentiation in the RWV has been observed in colon cancer cell cultures (20) and in cultures containing small intestine epithelium with mesenchymal cells (15). Using the RWV culture system, we tested the *in vitro* effects of approximated microgravity on lymphocyte locomotion. Here, we present a model for the analysis of lymphocyte locomotion under simulated microgravitational conditions that may be useful in the investigation of the mechanism of cellular locomotion and its inhibition in space.

Materials and Methods

Peripheral blood lymphocyte cell (PBL) isolation. Buffy coat source leukocytes from normal human donors were obtained from the Gulf Coast Regional Blood Center, Houston, Texas. Buffy coats were diluted in Hanks' balanced salt solution (HBSS), layered over Ficoll-Paque (Pharmacia LKB, Piscataway, PA), and centrifuged at 600 ×g for 20 min. Isolated PBLs were removed from the interface and washed three times in HBSS before being resuspended in RPMI-1640. Cell counts were determined manually with a hemacytometer.

Stock collagen preparation. Type I collagen was isolated from frozen rat tail tendons (Pel-Freeze, Rogers, AK) according to previously described methods (27). In brief, tendons were carefully removed, rolled into small pellets, weighed, and sterilized overnight in 70% ethanol. The ethanol was poured off and the collagen pellets rinsed three times with sterile tissue culture grade water. The pellets were dissolved in sterile 0.1% glacial acetic acid (also prepared in tissue culture grade water) at a ratio of 300 ml acid per gram of collagen. The solution was cooled to 4° C and stirred for 48 to 72 h, and then centrifuged at 12 000 ×g for 30 min at 4° C and the supernatant carefully removed. This concentrated collagen solution was determined to contain approximately 1.5 to 1.8 mg Type I collagen per ml acid by hydroxyproline assay (44) and was stored in sterile bottles at 4° C until needed.

Media. HBSS with calcium and magnesium salts and RPMI-1640 were prepared by the Tenneco Core Facility at the University of Texas M.D. Anderson Cancer Center (Houston, TX). Complete medium (c-RPMI) was prepared by supplementing RPMI-1640 with 10% fetal bovine serum (FBS, GIBCO, Grand Island, NY) and 100 µg/ml penicillin/streptomycin (GIBCO). A concentrated RPMI-based media solution (Solution A) was prepared for the dilution and polymerization of stock collagen. Solution A was prepared by mixing 20 ml 10× RPMI-1640 (GIBCO), 20 ml FBS, 10 ml 7.5% $NaHCO_3$, 2.5 ml 100× penicillin/streptomycin solution (GIBCO). Solution A was kept at 4° C until needed.

Reagents. Acetic acid (0.1%) and 0.34 *N* NaOH solution were prepared in tissue culture grade, double distilled water and filter sterilized (0.22 µm filter) before use. A 7.5% $NaHCO_3$ solution was obtained commercially prepared (Sigma Chemical Co., St. Louis, MO).

Collagen microcarrier lymphocyte locomotory assay. Lymphocyte locomotion was determined using a microassay derived from a previously described mini-assay (17). On ice, 900 µl of concentrated Type I rat tail collagen was combined with 100 µl of 0.1% acetic acid, 167 µl Solution A, 50 µl 7.5% $NaHCO_3$, and 42 µl 0.34 *N* NaOH to make a working collagen solution. For single-phase microcarrier experiments, 25 µl of a working collagen solution was added to Terasaki tray wells (Nunc, Inc., Naperville, IL) and the trays incubated at 37° C for 5 min to polymerize the collagen. For dual-phase microcarrier experiments, 10 µl of a working collagen solution was added to Terasaki tray wells and the tray incubated at 37° C for 3 min to polymerize. To each well, 5×10^5 PBLs in approximately 1 µl of a working collagen solution were dropped carefully onto the polymerized collagen surface and the tray was incubated again at 37° C for 3 min to polymerize the PBL-collagen layer. Once this cell layer was polymerized, 15 µl of a working collagen solution was carefully overlayered on top of the PBL-collagen layer. The trays were then incubated at 37° C for 3 min to polymerize the collagen. To facilitate removal of collagen microcarriers from Terasaki trays, trays were flooded with cold c-RPMI and polymerized collagen beads were gently lifted from trays with the aid of a 23-gauge hypodermic needle. Collagen beads were then transferred into centrifuge tubes with c-RPMI and refrigerated until needed.

In single-phase microcarrier experiments, microcarriers were transferred to RWVs (Synthecon, Houston, TX) at a concentration of 200 microcarriers/10^7 PBLs/100 ml c-RPMI culture volume. Controls were incubated at the same density in 24-well culture trays in stationary mode. In dual-phase microcarrier experiments, microcarriers were placed in RWVs at a concentration of 200 microcarriers/100 ml culture volume. Control cultures contained the same microcarrier-to-media density as their RWV counterparts. In a humidified, 7% CO_2 incubator at 37° C, RWV cultures were rotated at 14 rpm, whereas control cultures were incubated in 24-well culture trays in stationary mode. At 24-h intervals, four to six microcarriers were removed from RWV and from control cultures and transferred to tubes containing 1% paraformaldehyde fixative, and the locomotion distance was measured.

Measurement of cell locomotion. Cell locomotion was defined as the distance between the microcarrier surface (single-phase microcarrier) or cell/collagen interface (dual-phase microcarrier) and the three leading-edge cells. This measurement was determined by fine focus microscopy using an inverted microscope with phase contrast (Nikon Diaphot, Japan). Using a predetermined calibration factor, this measurement was converted from fine focus units to locomotory distance in micrometers (µm). Data from locomotion experiments are expressed as mean locomotory distance (µm) ± standard error of the mean (SEM).

Flow cytometric analysis of cell surface markers. Lymphocytes were examined after 48 h in RWV and in stationary conditions. Expression of cell surface molecules was analyzed by flow cytometry (EPICS C; Coulter, Hialeah, FL). Direct staining with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (mAb) was accomplished by incubating up to 1×10^6 cells for 40 min with selected mouse anti-human mAb. FITC-conjugated irrelevant isotype-specific mouse monoclonal IgG_1 or IgG_{2a} were used as control mAb. Indirect staining was performed using unconjugated mouse anti-human mAb followed by an additional 30 min incubation with FITC-conjugated goat anti-mouse secondary antibody. Control for indirect staining included incubation with FITC-conjugated secondary antibody alone. Stained cells were washed twice in cold HBSS with 2% FBS. All incubations were carried out at 4° C. In both protocols, saturated concentrations of mAb were used as suggested by the manufacturer. We used the following antibodies for surface markers: P1E6 (Telios, San Diego, CA) for detecting CD49b (VLA-2 α chain); monoclonal antibody L25 (B. W. McIntyre) for detecting CD49d (VLA-4 α chain); 18D3 and 33B6 antibodies (B. W. McIntyre) for detecting nonoverlapping epitopes of CD29 (Integrin β1 chain); Ta1 (Coulter) and AC7 (B. W. McIntyre) for detecting CD26 (Dipeptidyl Peptidase IV); and anti-CD43 and anti-CD44 mAb purchased from Becton Dickinson Co. (Mountain View, CA). Percent positive cells and mean channel fluorescence were calculated by subtracting the appropriate control histograms from the test histograms by using a cumulative subtraction routine (Oversub) within the Elite Immuno-4 software (Coulter). To reveal the nature and magnitude of changes induced by the RWV, stationary values were subtracted from the RWV values.

Phytohemagglutinin (PHA) response. Briefly, following culture in RWV and stationary conditions for 48 h, PBLs (1×10^5 in 100 µl c-RPMI) were placed in wells of a 96-well, round-bottomed tray (Corning, Corning, NY). To each well, PHA-m (Sigma) was added so that the final concentration of PHA ranged from 0–5 µg/ml. All samples were prepared in quadruplicate. Trays were incubated at 37° C for 24 h. To each well, 0.5 µCi [*methyl*-^{3}H]-thymidine (^{3}H-TdR, 2 Ci/mmol specific activity, Amersham Corp., Arlington Heights, IL) was added. Wells were incubated at 37° C for 4 h, harvested on glass fiber mats, and analyzed by standard liquid scintillation. Results are reported as mean stimulation index (SI) where SI = cpm of stimulated cells/cpm of spontaneous uptake control.

MTT activity. The tetrazolium salt conversion method for indirect measurement of cell growth was performed using the modification by Hansen et al. (18). Following RWV or stationary cultures, PBLs (1×10^5 in 100 µl c-RPMI) were placed in wells of a 96-well, flat bottomed tray (Corning). Twenty-five microliters of a sterile 5 mg/ml solution of MTT [3,(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide, Sigma] in phosphate-buffered saline (PBS) was added to each well, and the tray was incubated for 2 h at 37° C. An extraction buffer was prepared by combining 20% wt/vol sodium dodecyl sulfate (SDS), 50% N,N-dimethyl formamide (DMF), 2.5% of an 80% acetic acid, and 2.5% 1 *N* HCl (all Sigma). After incubation, 100 µl of extraction

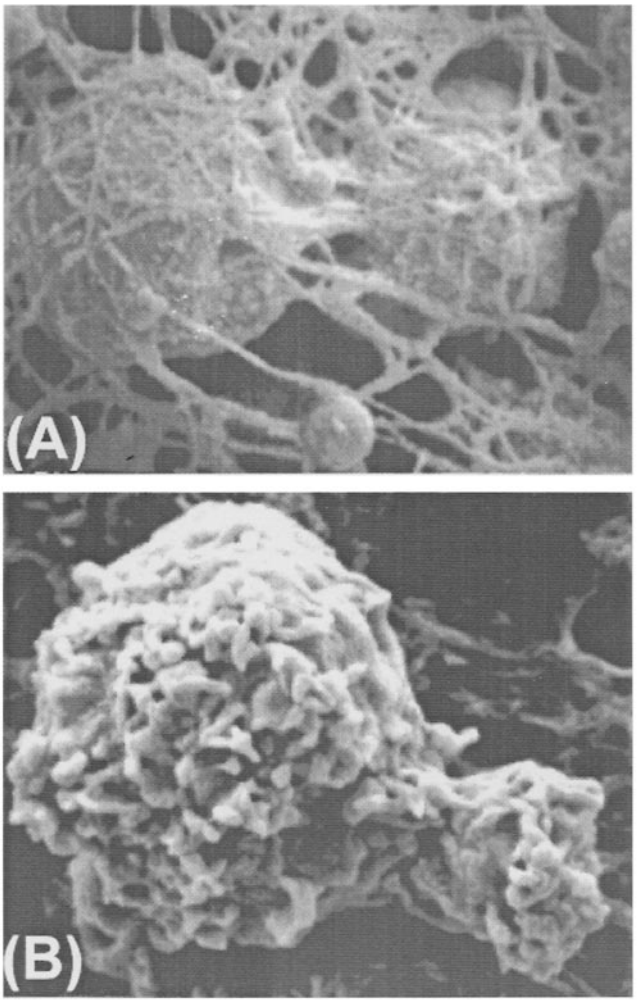

FIG. 1. Scanning electron micrograph of lymphocytes in Type I polymerized rat tail collagen microcarrier. Nonmotile cells (*A*) remain rounded and attached to the matrix while motile cells (*B*) display a constriction that results in polarity.

buffer was added and the tray was incubated overnight at 37° C. Absorbance at 570 nm was then determined using a MR700 ELISA reader (Dynatech Laboratories, Chantilly, VA).

Cell activation. PBLs were cultured in either c-RPMI alone or c-RPMI media with 1 μg/ml anti-CD3 and 200 U/ml IL-2 for 10 d at 37° C. Cells were then inoculated into RWV (14 rpm rotational speed) and/or static flask cultures and then incubated for 48 h. PBL locomotion was then assayed using the standard mini-locomotion assay (17) in 8-chamber slides (Nunc). The leading front locomotion distance was estimated after 20 h of incubation.

Statistics. Analysis of variance (ANOVA) [including Fisher's least significant difference (LSD), Student-Newman Keuls, and Dunnett's tests] and the Student's *t*-test were performed with StatView 512 + (Abacus Concepts, Calabasas, CA) and InStat (GraphPAD Software for Science, San Diego, CA) for the Apple Macintosh SE computer. Comparisons were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

The ability of lymphocytes to traverse the interstitium is critical to normal immune function. Biologically arrested spontaneous locomotion has been observed in tumor-infiltrating lymphocytes (1,17) and in lymphocytes from hosts treated with immunosuppressive agents (30). The decline in immune parameters associated with space flight is documented in both human and animal models (2,7,9–11,16,21–23,31,32,37–42). However, due to the demanding nature of space flight, immune response decline may be influenced by psychoneuroendocrine effects. *In vitro* studies in true microgravity are limited due to the frequency of staffed missions. The development of the RWV culture system has provided investigators the ability to approximate microgravity *in vitro* in a conventional laboratory setting. In these studies, we used the RWV culture system to investigate the effects of microgravity on lymphocyte locomotion. Locomoting cells are readily distinguished from stationary cells based on their shape (Fig. 1). Prerequisite to locomotion is the polarization of cells, as shown in Fig. 1 *B*.

Collagen beads were prepared in a single-phase microcarrier system and lymphocytes were added to the culture medium and allowed to attach to the beads in stationary culture and in the RWV. Attachment to the collagen bead surface was demonstrated in both culture systems. In stationary culture, locomotion of approximately 300 to 800 μm into the collagen bead was observed after 24–120 h (Fig. 2). Despite retaining their ability to attach to the collagen microcarriers, locomotion into the collagen matrix was arrested in RWV cultures at all time points (Fig. 2).

In the dual-phase microcarrier system, lymphocytes at the initiation of the culture are localized at the interface of the two collagen layers (Fig. 3). The two-layer analysis with lymphocytes at the interface allays the need for the lymphocyte to attach to the collagen matrix while suspended in the RWV. Furthermore, the density of the inoculum at the interface maximizes cell-cell contact such that the randomization of *g* forces cannot affect the attachment of cells to the matrix. By 24 h, 2 to 5% of the population locomotes from the origin to a distance that may be as much as 1200 μm from the origin. Measurement of the migration distance as a function of time affords an estimate of the locomotory capacity of the lymphocyte population

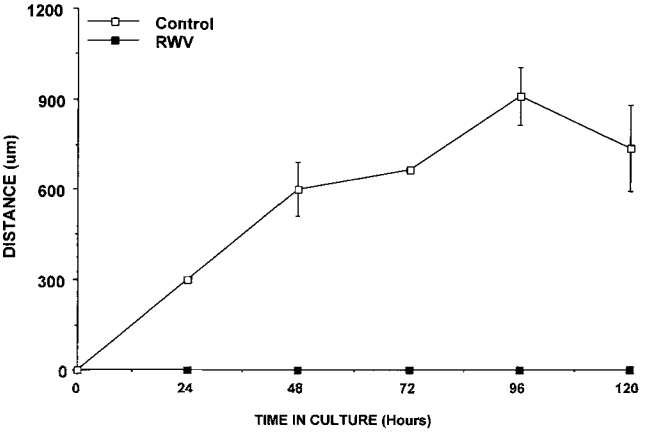

FIG. 2. Rotating-wall vessel (RWV) conditions permit attachment of lymphocytes to single-phase collagen beads but do not support locomotion. Single-phase collagen microcarriers (200 microcarriers/100 ml culture) were incubated with 10^7 per ml lymphocyte suspension in the RWV. Controls were incubated at the same density in 24-well culture trays in stationary mode. At 24-h intervals, samples were fixed and analyzed for depth of locomotion into the microcarrier. Data are presented as mean locomotory distance (μm) ± standard error of the mean (SEM).

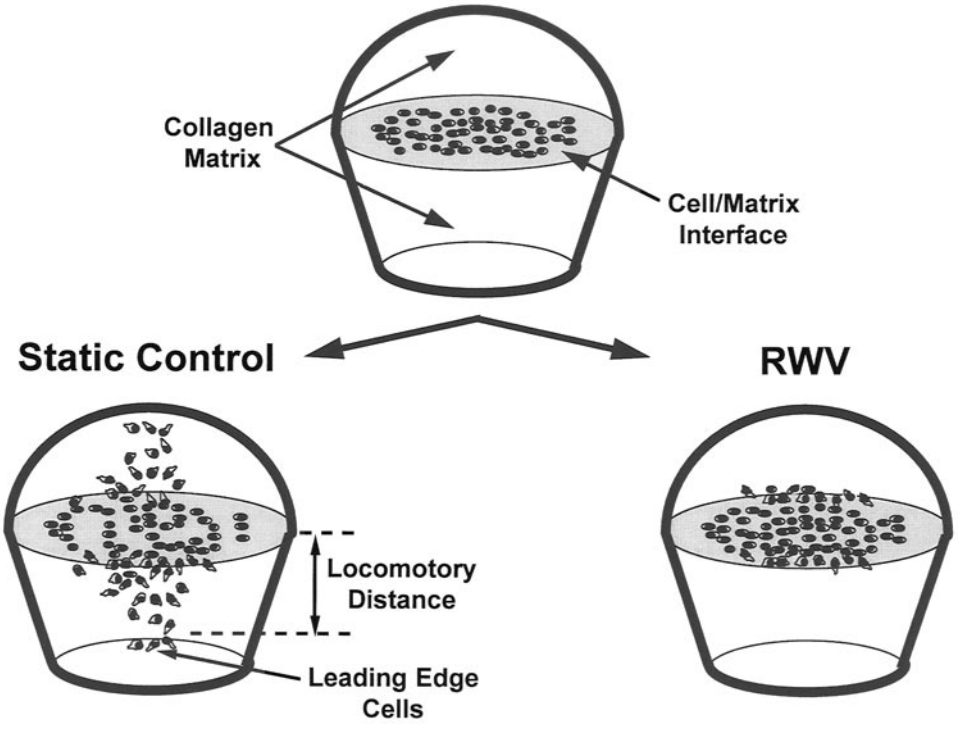

FIG. 3. Lymphocyte locomotory activity in biphasic microcarriers: schematic depiction of photomicroscopy. In randomized and uniform gravity, 5×10^5 lymphocytes were introduced at the interface of biphasic microcarriers. At 24 h, lymphocytes at uniform g have locomoted through the matrix and are visible at more than 500 μm from the interface. In contrast, the carriers obtained from the RWV showed little evidence of locomotion.

subjected to randomized g vectors. Comparison of the locomotion through microcarriers maintained in stationary culture and in the RWV revealed that randomization of the gravitational vectors inhibited locomotion (Fig. 4). Over a period of 5 d, stationary microcarriers in two separate cultures afforded locomotion distances of better than 590 μm, while in the paired RWV cultures, no substantial movement was observed (Day 5, Control 1 597.68 ± 36.6 μm, RWV 1 0.0 ± 0.0; Control 2 606.05 ± 59.40 μm, RWV 2 75.95 ± 17.11 μm; $P < 0.01$).

The prospect that lymphocyte viability affects loss of locomotion in the RWV was addressed in two ways. Cultures of lymphocytes were established in c-RPMI medium in stationary and in RWV conditions and the total surviving cell population estimated daily for 9 d. Fig. 5 shows that in both systems there is an initial decline in viability that continues in the stationary cultures. In contrast, the RWV-maintained culture contained four to five times more viable cells and has retained nearly 70% of the originating population. Secondly, dual-phase microcarriers cultured in the RWV for 0, 6, 24, 48, and 72 h were transferred to stationary culture and allowed to locomote in normal gravity for an additional 72 h (Fig. 6). Lymphocytes recovered from the arrested state when harvested after 6 h in the RWV and locomotion distances were slightly less, but not significantly different, from the 0 h timepoint control. However, by 24 h in the RWV, recovery was severely diminished as compared to the 0 h timepoint [RWV1 0 h = 484.34 ± 67.95 μm 24 h = 256.24 ± 43.77 μm; ($P < 0.05$); RWV2 0 h = 449.5 + 52.95 μm 24 h = 111.60 + 74.96 μm; ($P < 0.05$)] and essentially nonrecoverable after 72 h in the RWV [RWV1 0 h = 484.34 ± 67.95 μm 72 h =

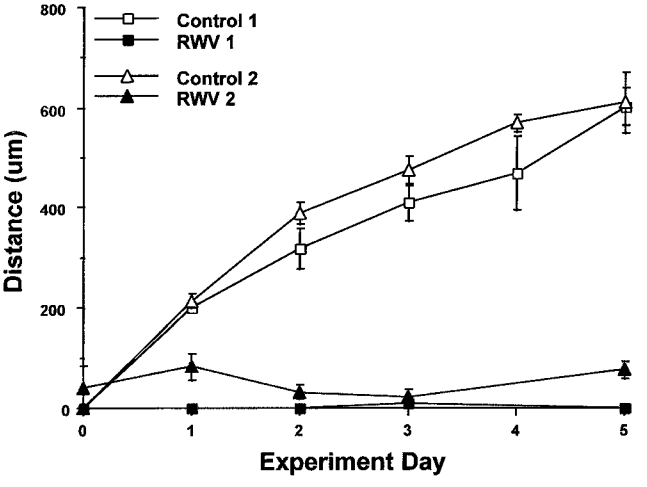

FIG. 4. Arrested locomotion in randomized gravity. Dual-phase collagen microcarriers were prepared as shown in Fig. 3. Two hundred microcarriers suspended in 100 ml of medium in the rotating-wall vessel (RWV) were incubated at 37° C for 5 d. Control microcarriers were incubated under the same conditions but in stationary culture in 24-well culture trays. At 24-h intervals, four to six microcarriers were removed and transferred to 1% paraformaldehyde fixative and the locomotory distance estimated as described (17). Data are presented as mean locomotory distance (μm) ± standard error of the mean (SEM).

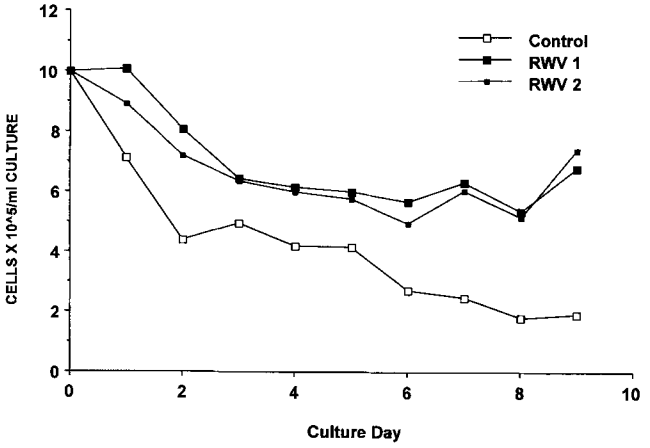

FIG. 5. Survival of lymphocytes in the rotating-wall vessel (RWV). The RWV was set up with 1×10^6 lymphocytes per ml and then incubated for 9 d. Control cultures at the same density were prepared in 24-well culture trays. Daily samples were obtained and cell counts and Trypan Blue exclusion assays performed. Data are presented as number of viable cells/ml of culture.

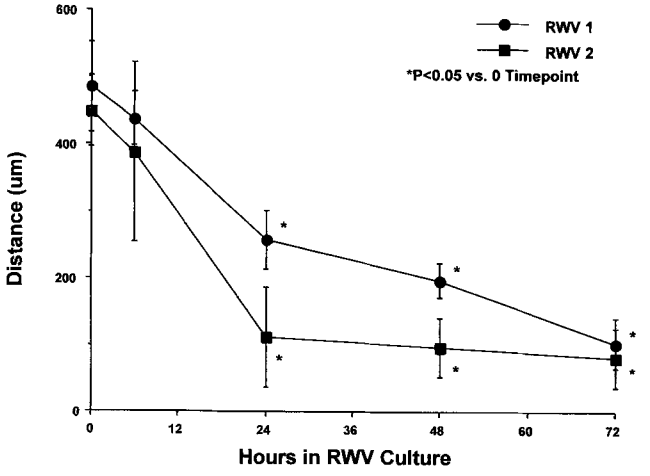

FIG. 6. Locomotion of peripheral blood lymphocytes (PBL) recovered from the rotating-wall vessel (RWV). Dual-phase microcarriers containing lymphocytes were incubated in the RWV for a total of 72 h. At 0, 6, 24, 48, and 72 h, eight microcarriers were removed; four microcarriers were fixed; and the remaining four transferred to stationary culture for an additional 72 h of incubation. Locomotory activity was then measured and data presented as mean locomotory distance (μm) ± standard error of the mean (SEM).

101.62 ± 37.39 μm; ($P < 0.05$); RWV2 0 h = 449.5 + 52.95 μm 72 h = 80.6 + 43.4 μm; ($P < 0.05$)]. Thus, although there is a modest loss in viability in the RWV, the inhibition of locomotion proceeds, indicating an intrinsic change in lymphocyte function.

Recently, cell-surface adhesion molecules were proposed to have a role in T-lymphocyte locomotion (24). Integrins and other cell-surface proteins form a network of adhesion molecules with overlapping binding specificities for extracellular matrix constituents (19,36). The locomotion of lymphocytes through Type I collagen may involve molecules such as the integrins VLA-2 and VLA-4. The former binds Type I collagen, while the latter has predilection for fibronectin. In addition, VLA-4 mediates homotypic aggregation of T-lymphocytes (5,6,8), possibly a signal essential to locomotion. CD26 is the enzyme dipeptidylpeptidase 4, an amino peptidase that has specificity for penultimate proline residues. Furthermore, CD26 may bind collagen or fibronectin (26) at a site other than the catalytic site. Table 1 shows the flow cytometric analysis of the expression of selected adhesion molecules on the surface of PBL harvested after 48 h of culture in conventional flasks and in the RWV. CD26 expression was analyzed with two nonoverlapping antibodies that bind to different epitopes of CD26, Ta1, and AC7. Although the proportion of cells expressing CD26 is not changed substantially in the RWV, there is a 20% increase on a per cell basis in the epitope recognized by AC7; under normal stationary culture conditions, expression of the AC7 epitope occurs in lymphocytes that locomote (data not shown). In contrast, the number of cells expressing CD49b (VLA-2 α-chain) is increased from 10 to 17% with a concomitant increase in the number of molecules per cell. A more modest change occurred in the α-chain of VLA-4 (CD49d). Many of the VLAs are β1 integrins and, therefore, the expression of β1 was investigated using two antibodies that resolve nonoverlapping epitopes. While the CD29 (VLA-β_1) epitope defined by 18D3 remained unchanged, there was decreased expression of the 33B6 epitope in the PBL from the RWV. Two other adhesion molecules, CD43 and CD44 (HCAM), were unaffected. These results are suggestive of potential conformational changes in VLA-2 that may result from the randomization of gravitational vectors.

In addition to adhesion to extracellular matrices, some of these cell-surface markers identify specific stages of lymphocyte activation. The effect of randomized gravitational vectors on lymphocyte activation was investigated in cells maintained in the RWV for 48 h. The results suggest that response to the polyclonal T-cell activator PHA was significantly diminished (data not shown) when tested through a range of concentrations. Thus, the capacity to respond is significantly impaired. Metabolically, the lymphocytes show diminished capacity in the reduction of MTT activity (Fig. 7). Responses of lymphocytes may be evaluated as the proportion of cells exiting

TABLE 1

FLOW CYTOMETRIC ANALYSIS OF THE EXPRESSION OF CELL SURFACE ADHESION MOLECULES ON LYMPHOCYTES MAINTAINED IN STATIONARY AND ROTATING-WALL VESSEL (RWV) CULTURES FOR 48 H

	Culture Conditions	
Cell Surface Molecule[a]	Stationary	RWV
CD26 (DPP4)		
TA1	38.6[b] (1.51)[c]	40.5 (1.63)
AC7	47.4 (2.99)	49.4 (3.61)
CD49		
b (VLA-2)	10.4 (1.68)	17.0 (2.08)
d (VLA-4)	83.3 (3.45)	81.6 (3.68)
CD29 (VLA-β1)		
18D3	54.7 (1.63)	50.5 (1.64)
33B6	74.9 (3.07)	62.8 (2.47)
CD43	94.9 (11.21)	95.2 (11.60)
CD44	83.0 (23.30)	79.8 (21.90)

[a]Selected adhesion molecules were investigated by flow cytometry using direct and indirect immunofluorescence.

[b]Percent cells designated positive.

[c]Mean channel fluorescence.

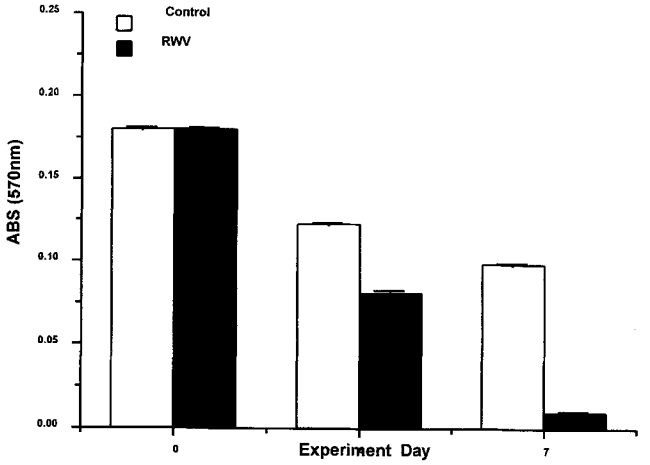

FIG. 7. Response of rotating-wall vessel (RWV) cultured peripheral blood lymphocytes (PBL) to (MTT). After 0, 4, or 7 d in static control or RWV culture, the ability of PBLs (1×10^5/well) to convert MTT was measured by spectrophotometric absorbance at 570 nm.

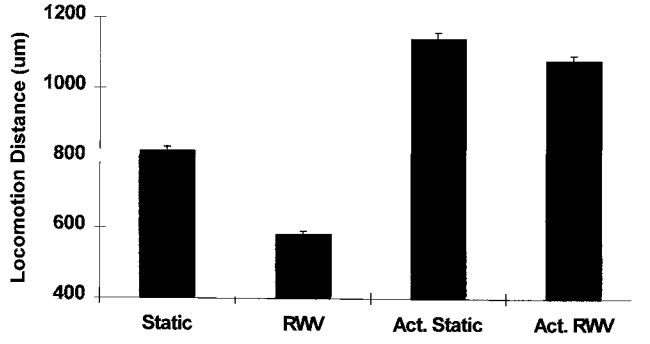

FIG. 8. Effect of cell activation prior to rotating-wall vessel (RWV) culture on peripheral blood lymphocyte (PBL) locomotion. PBL were cultured in c-RPMI with 1 μg/ml anti-CD3 and 200 U/ml interleukin-2 for 10 d at 37° C. Cells were then cultured in static flasks or RWVs for an additional 48 h. PBL locomotion assayed thereafter and compared with locomotion of non-activated lymphocytes. Data are presented as mean locomotory distance (μm) ± standard error of the mean (SEM).

G_0 and entering G_1, since locomotion is associated with cells in the G_1 phase of the cell cycle. Therefore, the failure to locomote may be a consequence of the diminished proliferative response.

Diminished activation may be a critical link in the failure of lymphocytes to locomote in the simulated microgravity of the RWV. To test this hypothesis, normal and activated PBL were assessed for locomotion in collagen microcarriers in the RWV. PBL were incubated for 10 d with c-RPMI in the presence of anti-CD3 antibody and IL-2 and then introduced into the RWV for 48 h. Thereafter, the lymphocytes were transferred to layers of collagen in eight-chamber slides (17) and the locomotion estimated after 20 h of incubation in Type I collagen matrix and compared with locomotion of non-activated lymphocytes (Fig. 8). Unactivated PBL locomotion was significantly inhibited in the RWV, while the activated PBL not only locomoted at greater distances in stationary culture, they were only marginally affected by the RWV. Thus, activation may ameliorate the arrest in locomotion observed in randomized *g*.

Preliminary analysis of the response to true microgravity indicates that locomotion is significantly impaired in human peripheral blood leukocytes. Fig. 9 shows the results from four different donor PBL preparations on the STS-56 shuttle mission. These results are confirmatory of the initial observation on STS-54. There was significant inhibition of locomotion in all four specimens. Flight specimens evidenced some locomotory activity in the early phase. This may be due to *g* forces resulting from manipulation of the spacecraft.

Thus, the possible effects that randomization of *g* vectors may have on the locomotory process are numerous, but the present observations suggest that some conditions for initiating locomotion have been simulated, with the exception of polarization of the cells. The originating interface in the stationary cultures evidences morphologic polarization, while the same interface in the RWV displays rounded cells that have no indication of movement. Comparative morphologic analysis suggests that polarization occurred in the populations undergoing significant translocation through the Type I collagen matrix (data not shown). Thus, lymphocyte locomotion may be thwarted by: (a) not allowing the cells to polarize; (b) rendering contractile vectors unordered so that there is no net directional translocation; and (c) altering the synthetic apparatus of the cell such that production of locomotion essential factors does not occur. Polarization is an essential prelude to locomotion and some investigations show that the polar morphology indicates those lymphocytes in the population that are poised for locomotion (30). The proportion of polarized cells in RWV-maintained collagen microcarriers is substantially different from stationary collagen microcarrier cultures. Finally, lymphocyte locomotion through Type I collagen requires the presence of cell surface proteins, removal of which ceases locomotion until resynthesis occurs. Since synthesis of cytokines is impaired in microgravity (7), it is conceivable that in the RWV renewal of components essential to locomotion may also be impaired.

Ground-based modeling of microgravity using a randomized *g* vector system induces a potentially severe impairment in the ability of lymphocytes to traverse a three-dimensional collagen matrix. Such an impairment may result in diminished response to neoantigens and an inability of lymphocytes to participate in inflammatory responses essential to immunity in infection and neoplasia. The relationship of the observations in the RWV to the events occurring in true microgravity are best established in space flight-based experiments. A parallel series of experiments in an initial shuttle mission supports this contention. Failure of lymphocytes to locomote in true microgravity establishes the RWV as a ground-based model for the analysis of inflammatory cell movement in long-term space travel.

The RWV has been used to provide a reduced shear environment for the propagation of mammalian cells (4,14,15,20,33). This communication is the first report to indicate that the RWV provides an environment for modeling inhibition of lymphocyte movement. The hypothesis that randomized *g* is a high fidelity simulation of true microgravity and both environments abate lymphocyte locomotion was tested in space shuttle missions STS-54 and STS-56 and preliminary results suggest that locomotion is substantially impaired in microgravity. The observation that randomization of gravitational vectors inhibits lymphocyte locomotion is a significant advancement of our understanding of the physical factors that may impact upon immune function at the cellular level. Furthermore, the RWV may be a useful model in investigating immune cell function in microgravity.

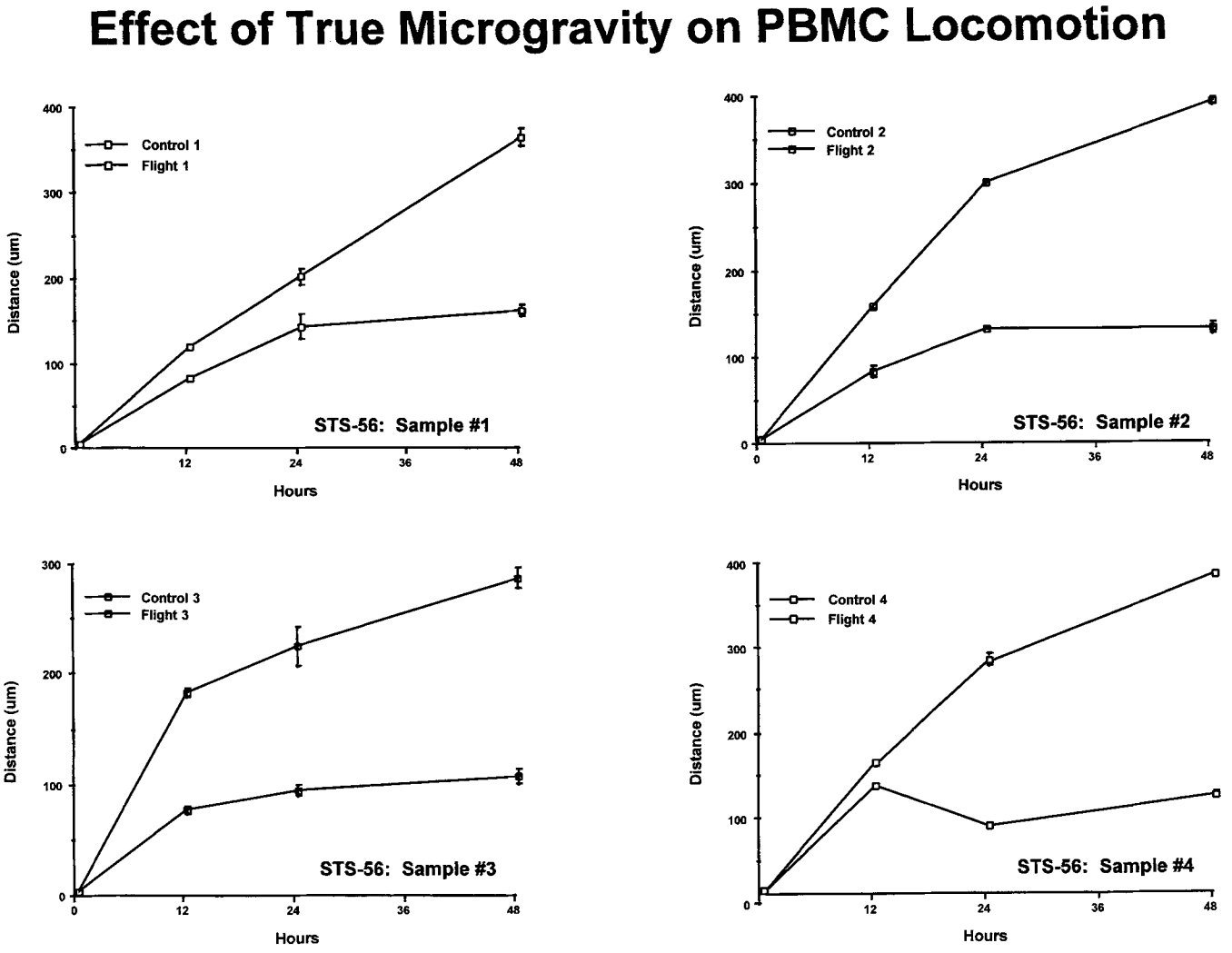

FIG. 9. Impaired locomotion in microgravity. Peripheral blood lymphocytes (PBL) were obtained from four different donors and introduced to the interface in a dual-phase gelled collagen system in quadruplicate. The specimens were transferred to the NASA Biotechnology Specimen Temperature Controller (BSTC) and maintained at 8° C to retard locomotion. The payload was transferred to the space shuttle and flown on mission STS-56. Upon achieving orbit, the specimens were exposed to 37° C for 12, 24, and 48 h. Thereafter, all specimens were returned to 8° C until postflight analysis. Each panel displays the locomotion observed in flight and ground-based control specimens.

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