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Immune suppression of human lymphoid tissues and cells in rotating suspension culture and onboard the International Space Station

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Abstract

The immune responses of human lymphoid tissue explants or cells isolated from this tissue were studied quantitatively under normal gravity and microgravity. Microgravity was either modeled by solid body suspension in a rotating, oxygenated culture vessel or was actually achieved on the International Space Station (ISS). Our experiments demonstrate that tissues or cells challenged by recall antigen or by polyclonal activator in modeled microgravity lose all their ability to produce antibodies and cytokines and to increase their metabolic activity. In contrast, if the cells were challenged before being exposed to modeled microgravity suspension culture, they maintained their responses. Similarly, in microgravity in the ISS, lymphoid cells did not respond to antigenic or polyclonal challenge, whereas cells challenged prior to the space flight maintained their

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antibody and cytokine responses in space. Thus, immune activation of cells of lymphoid tissue is severely blunted both in modeled and true microgravity. This suggests that suspension culture via solid body rotation is sufficient to induce the changes in cellular physiology seen in true microgravity. This phenomenon may reflect immune dysfunction observed in astronauts during space flights. If so, the ex vivo system described above can be used to understand cellular and molecular mechanisms of this dysfunction.

Keywords

Immune response; Space flight; Microgravity

Introduction

The ability of astronauts to develop and maintain appropriate humoral and cellular immune responses in the microgravity of space flight is critical for their mission, especially in prolonged flight. Indeed, developing primary immune responses to new immunogens (e.g., new pathogens accidentally taken from the ground or immune surveillance to emerging cancer cells) and secondary immune responses to reintroduced immunogens (e.g., endogenous pathogens released from latency or taken from the ground) is paramount to human health and, therefore, to human space exploration. Astronauts of long- and short-duration space flights show decreased mitogenic responses (Cogoli and Tschopp 1985; Taylor et al. 1986), loss of cytokine production or decreased responsiveness to cytokines (Sonnenfeld et al. 1990), major changes in peripheral or splenic immune cell populations (Taylor et al. 1986; Sonnenfeld et al. 1990), alterations of natural killer cell activity (Sonnenfeld et al. 1990), and decreased delayed type hypersensitivity responses (Taylor and Janney 1992). Blunting of the cellular immune response was also evidenced by the very high incidence of in-flight infectious disease in the early days of the US space program and by curtailment of a long-duration Russian space flight due to infectious disease (Nicogossian et al. 1993). Space flight-related immune dysfunction occurs in test animals as well (Konstantinova et al. 1993). Although these impairments of immune functions are reversible after return to normal gravity, they may pose problems in the future with much longer flights to distant planets and they give a physiological reality to this once purely academic problem. Currently, it is not clear whether these various alterations of the immune system are due directly to microgravity experienced in space or to other factors such as stress and cosmic radiation to which astronauts and test animals are exposed during space flight. Therefore, to understand whether there is a causative relationship between microgravity and immune dysfunction and to separate the effects of microgravity from ones caused by other factors, we cultured explants of human lymphoid tissue and isolated cells from human lymphoid tissue both in the rotating wall vessel (RWV), which provides a low-shear environment that models microgravity (Schwarz et al. 1992; Tsao et al. 1992; Goodwin et al. 1993; reviewed in Unsworth and Lelkes 1998), and in true microgravity onboard the International Space Station (ISS).

In this study, we report that, in RWV, both lymphoid tissue explants and isolated cells demonstrated impaired responses to polyclonal activators, including decreases in antibody and cytokines secretion. Similar defects were observed in lymphoid cells cultured in true microgravity onboard the ISS. Our results suggest that environmental factors stemming from microgravity directly affect lymphoid tissue.

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Materials and Methods

Culture of human lymphoid tissues and lymphocytes

Tonsils were received as surgical excess from routine tonsillectomies and dissected into 2-mm blocks. Tissue blocks were cultured as air–liquid interface cultures on Gelfoam sponges (Upjohn, Kalamazoo, MI) as previously described (nine blocks per gel in a well with 3 mL of culture medium, three wells per condition; Glushakova et al. 1995) or in 10-mL disposable RWVs (30 blocks/10 mL vessel; high aspect ratio vessels [HARVs]; Synthecon, Houston, TX) rotated at 25 rpm (Margolis et al. 1997). Transfers of tissue between culture systems included transfer of tissue blocks as well as any cells that migrated out of the tissues. Medium was sampled and changed every 4 d. Lymphoid cells were collected by mechanical dissociation of tissue and purified with lymphocyte separation medium (ICN/Cappel, Costa Mesa, CA). T and B cells were enriched by negative selection on a MACS magnetic sorter (Miltenyi Biotec, Auburn, CA) or using nylon wool columns (Accurate Chemical, Westbury, NY); cell purity was verified by flow cytometry using appropriate antibodies. Cells were cultured at a density of $1–2.0\times10^6$ cells per milliliter in tissue culture flasks or RWVs rotating at 10 rpm. Nonrotating RWV cultures were established by maintaining oxygenation to the cultures but disconnecting the belt for rotation to create static conditions.

Challenge with antigens and activators

Recall antigens, tetanus toxoid (TT) and diphtheria toxoid (DT; Accurate Chemical), were used at 0.1 μg/mL. The polyclonal activators used were pokeweed mitogen (PWM; Sigma Chemical, St. Louis, MO) at 2.5 μg/mL, phytohemagglutinin (PHA; Sigma Chemical) at 5 μg/mL with 20 U/mL of IL-2 (Invitrogen, Carlsbad, CA), and Staphylococcus aureus Cowan I strain (SAC; Calbiochem, La Jolla, CA) used at 0.01% (v/v) with 20 U/mL of IL-2.

Measurement of antibody responses

Antigens and activators were added to the culture medium of static and RWV cultures; control cultures without challenge were run in parallel. Medium was changed and sampled every 3 to 4 d. Antibodies to TT and DT in the culture medium were measured by capture enzyme-linked immunosorbent assay. Plates were coated with 5 μg/mL toxoid. Hyper-Tet, human tetanus immune globulin (Miles, Elkhart, IN), and diphtheria antitoxin, equine concentrated globulin (Connaught Laboratories, Swiftwater, PA), were used as standards. Peroxidase-labeled goat secondary antibodies (ICN/Cappel) were used at 1 μg/mL. Immunoglobulin (Ig) levels were measured in a similar manner using Fc-specific antibodies and purified human IgG, IgM, and IgA (ICN/Cappel) as standards. Plates were developed with TMB substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) and read. Values were calculated using DeltaSoft software (Biometallics, Princeton, NJ) and four-parameter regression. Anti-TT IgG and anti-DT IgG were calculated in antitoxin units (in microunits per milliliter), and IgG, IgM, and IgA were calculated in nanograms per milliliter. In nonchallenged tissues, anti-TT IgG and anti-DT production were negligible with only low levels seen occasionally at days 3–4. Baseline production of IgG, IgM, and IgA by nonchallenged tissues was subtracted from the production by challenged cultures.

Measurement of proliferation responses

PWM, PHA, or SAC were added to the culture medium for 3 d; control cultures without activators were run in parallel. Cell viability was checked on all cultures by trypan blue (Invitrogen) dye exclusion or by propidium iodide (Boehringer Mannheim, Indianapolis, IN) staining by flow cytometry. For proliferation assays, cells were transferred to 96-well plates,

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pulsed for 18 h with 0.5 μCi of ^{3}H thymidine (20–30 Ci/mmol; Amersham, Arlington Heights, IL), harvested, and counted.

Measurement of glucose

Medium samples collected from cultures were assayed for glucose levels using the Beckman Glucose Analyzer II (Beckman, Fullerton, CA). Glucose use was determined by subtracting glucose levels in medium samples from glucose levels in starting culture medium.

Measurement of cytokine production

Antibodies to 16 cytokines/chemokines (IFN-γ, IL-2, IL-4, MIP-1α, MIP-1β, SDF-1β, TNF-α, IP-10, IL-1α, IL-1β, IL-6, IL-8, IL-15, RANTES, IL-12, and IL-16; R&D Systems, Minneapolis, MN) were covalently bound to predetermined microsphere sets (Luminex, Austin TX) according to the manufacturer's instructions. Samples and standards were incubated with antibody-coupled microsphere sets (1,250 beads per well) in 96-well filter plates (Millipore, Bedford, MA) at 4°C overnight. Then, the bound beads were washed twice. A mixture of detection antibodies (0.1–1 mg/mL) was added to the wells, mixed, and incubated at 37°C for 1 h. Following two washes, streptavidin–phycoerythrin (Molecular Probes) was added at 16 μg/mL and incubated at 37°C for 20 min. The plates were placed for 1–2 min on a microplate shaker and 120 μL of the sample or standard was analyzed on the Luminex 100 (Luminex). Data analysis was performed by DeltaSoft software on the median fluorescence intensity recorded for each bead set.

ISS culture of human lymphoid cells

Lymphoid tissue cell suspensions were generated by mild mechanical disruption of tonsils followed by passage through a 40-μm filter. Cells from each tonsil donor were frozen in multiple aliquots either after preparation (nonactivated) or after activation with PWM, TT, and DT for 3 d. Freezing was conducted in fetal bovine serum (Gemini BioProducts, CA) with 7.5% dimethyl sulfoxide at a cooling rate of 1°C/min to –85°C. Frozen cells were stored in liquid nitrogen. Cells from five different donors were thawed and injected into Teflon culture bags (American Fluoroseal, Gaithersburg, MD) with or without activators and cultured in a 37°C 5% CO_2 incubator on the ground or in the ISS at 2.0×10^6 cells per milliliter, giving three experimental conditions: nonactivated, activated (cells thawed in activator containing culture bags), and preactivated (challenged with activators for 3 d in normal gravity prior to freezing). Similar experiments with cell suspensions from different donors were performed on the ground in RWV (10 mL HARVs at 10 rpm) and static (six-well plates) cultures). Medium samples were withdrawn every 3 d over a 12-d period. At day 12 of culture, cells were fixed with formalin for preservation and stored at 4°C. The activated ISS cell sample for one donor was lost prior to inoculation, and another donor did not respond to our activators. This resulted in three donors for analysis of the activated condition and four donors for the preactivated condition, rather than five donors.

Examination of medium samples from the ISS experiment showed that a large number of cells were withdrawn from the culture bags on the first sampling at day 3, probably because they were collected in the injection neck of the culture bag. These trapped cells were removed with the first medium sample and could not make any significant contribution to the events measured in the bulk of the medium over the entire time course of the experiment. Cell counts on the medium samples showed that an average of 43.4±3.8% of cells were lost in ISS at day 3 compared to 5.3±1.5% in ground controls; cell counts on medium samples from later time points showed much less disparity. To compensate for this cell loss at day 3, total values measured for various parameters were normalized for the amount of cell loss for both ISS and ground samples.

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Statistical analysis

All data analyses were performed with Prism 5.0a (GraphPad Software, San Diego, CA; http://www.graphpad.com). Because the data distributions failed the D'Agostino and Pearson omnibus normality test, one-way analyses of variance were performed using the nonparametric Kruskal–Wallis test with Dunn's multiple comparison post test. When applicable (n>16) and normally distributed data, paired Student's t test was used.

Results

Modeled microgravity immunosuppresses human lymphoid tissue ex vivo

As described earlier (Glushakova et al. 1998; Fitzgerald et al. 2004) and confirmed in this study, tissue blocks in static culture mounted secondary immune responses to recall antigen by secreting antigen-specific antibodies into the culture medium, and they increased their Ig production in response to PWM. In this study, we compared the responses of blocks of human lymphoid tissue to recall antigen (TT and DT) challenge and/or polyclonal (PWM) activation in normal gravity in static cultures and in modeled microgravity in RWVs. We have previously reported that both static cultures of tissue blocks on collagen rafts at the air–liquid interface and dynamic cultures of tissue blocks in RWVs maintain the cytoarchitecture and the metabolic activity of human lymphoid tissue for 2 to 3 wk (Margolis et al. 1997). Responses to the recall antigens, TT and DT, were measured by the release of anti-TT IgG and anti-DT IgG into the culture medium. Results for TT and DT were similar for all experiments performed; therefore, only TT responses are shown. Responses to polyclonal activation by PWM were measured by tissue release of IgG, IgM, and IgA into the culture medium. While all three isotypes are increased with PWM challenge, IgM is the most significantly upregulated; therefore, below we show data on IgM responses only.

Typically, anti-TT IgG was detectable in static cultures by day 8 after antigenic challenge and continued to be produced for the duration of the experiment. In contrast to static cultures, when blocks of tissue were cultured in RWVs, the secretion of anti-TT IgG was dramatically reduced (Fig. 1*a*). On average, experiments with tissues from six donors reached only 18±8.0% of that of matched static cultures (p<0.001). The same suppression was observed for IgM secretion in response to polyclonal activation, IgM production in static cultures increased substantially after 4 d, while in RWV IgM production was weak (Fig. 1*b*). In experiments with tissues from nine donors, on average, IgM production in RWV reached only 22±10% of that of static cultures (n=9, p<0.001).

To understand if the decrease in antibody production by human lymphoid tissues in RWVs was due to a failure to initiate or to maintain humoral immune responses, we transferred tissue blocks from static culture to RWV culture and vice versa and monitored their responses to PWM or to recall antigen. Initiation of responses was evaluated by challenging tissue blocks in RWVs with TT, DT, and PWM, followed by transfer to static culture after 3 or 6 d of culture in RWVs. When these tissue blocks were transferred to static culture at day 3, they produced less antibodies than the tissue blocks that remained in static cultures, and when transferred at day 6, antibody production was further decreased. This was true for both specific antibody production in response to TT challenge (Fig. 1*c*) and for polyclonal IgM production in response to PWM stimulation (Fig. 1*d*). On average, anti-TT IgG and IgM production of tissues cultured in RWV for 3 d and then transferred to static cultures were, respectively, 56±18% (n=6, p=0.05) and 31±11% (n=6, p=0.001) that of tissue that remained in static culture. Transfer of tissues at day 6 resulted in further decrease of antibody production, which, after transfer, reached, on average, 7±5% (n=4, p<0.001) and 2±1% (n=4, p<0.001) that of static cultures for anti-TT IgG and IgM, respectively. This

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decrease of the immune response was similar to that for tissue that remained all the time in static culture but challenged on day 6. These results indicate that immune responses are initiated in RWVs, but the extent of response decreases with the length of culture in modeled microgravity.

We next examined the maintenance of immune responses. Tissue blocks were challenged with TT, DT, and PWM in static culture and transferred at day 8 to RWVs. Culture in modeled microgravity only moderately affected the ability of human lymphoid tissue to maintain immune responses primed in static cultures. In tissues from nine donors, anti-TT IgG was, on average, 93±51% that of static cultures (n=6, p=0.89) and IgM secretion was 46±15% that of tissue blocks remaining in static culture (n=9, p=0.007). Thus, human lymphoid tissues challenged in modeled microgravity appear to initiate immune responses, but these responses diminish or do not mature with increased exposure to this environment. Modeled microgravity has a less subtle affect on established immune responses with polyclonal responses being more influenced than antigen-specific responses.

Modeled microgravity immunosuppresses human lymphocytes ex vivo

In order to characterize which cellular compartment was affected by culture in modeled microgravity, we studied the responsiveness of total lymphocytes, T cells, and B cells isolated from human lymphoid tissue to polyclonal activators.

Total lymphocytes were activated with PWM, T cells with PHA and IL-2, and B cells with SAC and IL-2 for 3 d in static culture or in RWVs and their proliferation was measured by incorporation of ^{3}H thymidine. Lymphocytes in static cultures responded to cell type-specific polyclonal activators with average stimulation indexes (counts per min in challenged/counts per min in nonchallenged cells) of 21±12 (n=21) for total lymphocytes, 36±10 (n=6) for T cells, and 70±20 (n=7) for B cells. The proliferative response of RWV-cultured cells varied from donor to donor. To account of this variability, we pooled data obtained in experiments with cells from different donors by normalizing the stimulation indexes by those for matched tissues in static cultures. In general, when cultured in RWVs, total lymphocytes, T cells, and B cells were unresponsive to these activators: The responses reached 5±3% (n=21, p<0.001), 2±1% (n=6, p<0.001), and 2±1% (n=7, p<0.001) that of their static counterparts, respectively, whereas lymphocyte viability was 78±6% that of static cultures (n=3, p=0.01). Thus, lymphocytes in RWVs remain largely viable but do not proliferate in response to polyclonal activation. This inhibition of proliferative responses in the RWV requires rotation of the vessel and, therefore, the modeling of microgravity since, in nonrotating RWVs, these responses were largely restored: proliferation was, on average, 69±15% (n=5, p=0.04) that of static cultures for total lymphocytes, 74±14% (n=4, p=0.05) for T cells, and 60±13% (n=5, p=0.01) for B cells.

To investigate whether the mitogenic responses of isolated lymphocytes cultured in RWV recovered with return to static culture, we activated lymphocytes in RWVs for 3 d then transferred them to static culture and measured their proliferation 3 d later. These lymphocytes showed almost no proliferation after transfer to static culture, the average proliferation was 6±5% (n=12, p<0.001), 3±1% (n=4, p<0.001), and 1±0.5% (n=4, p<0.001) that of lymphocytes kept in static culture for total lymphocytes, T cells, and B cells, respectively. To analyze how quickly isolated lymphocytes lose responsiveness to mitogens, lymphocytes activated in RWV were transferred to static cultures after 1, 2, and 3 d of culture. Lymphocytes transferred after 1 d of culture in RWV proliferated to 66±27% that of matched counterparts activated and cultured in normal gravity (n=9, p=0.08). With increasing exposure to modeled microgravity, lymphocytes lose their ability to proliferate in response to PWM (Fig. 2).

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Thus, under model microgravity conditions, explants of human lymphoid tissues and isolated lymphoid cells responded neither to antigenic nor to polyclonal stimulation, suggesting that microgravity might be involved in this phenomenon. To determine the extent to which true microgravity alters immune responses, we performed similar experiments onboard the ISS.

Microgravity in space suppresses human lymphocytes ex vivo

To accommodate experimental constraints inherent to working in the ISS, we slightly modified the experimental protocol developed for RWV experiments. We prepared ground-frozen suspensions of cells isolated from lymphoid tissue, which were delivered to the ISS by Shuttle Endeavour (Orbital Vehicle-105). Trained astronauts thawed the cells in space, inoculated, and maintained them in Teflon culture bags. Ground controls for ISS experiments consisted of aliquots of the cell suspensions isolated from matched tissues, frozen and thawed at the same time, inoculated in Teflon bags, and cultured in equipment identical to that used onboard the ISS. To compare the effect of true microgravity (ISS) to that of modeled microgravity (RWV), we performed parallel experiments in RWV and static cultures. We monitored cell metabolism and responses to PWM by measuring glucose consumption, antibody production, and cytokine/chemokine secretion as described below.

To monitor cell metabolic activity, we measured the level of glucose usage by cells in normal gravity, RWV, or in ISS. On average, under normal gravity conditions, cell activation by PWM resulted in increased glucose consumption from 35.8±4.0 mg/dL in nonactivated cells to 101.0±8.2 and 131.7±9.6 mg/dL for activated and preactivated cells, respectively ($p<0.001$, $n=16$). In contrast, in RWV, glucose consumption by activated cells, 26.8±3.9 mg/dL, was not significantly different from that of control resting cells, 21.9±2.9 mg/dL ($p=0.06$, $n=16$). Preactivated cells, however, significantly increased their glucose consumption to 90.6±12.5 mg/dL ($p<0.001$, $n=16$; Fig. 3*a*).

Under microgravity at the ISS, glucose consumption by activated cells was not different from that of control resting cells (16.6±6.0 versus 17.6±2.3 mg/dL, $p=0.65$, $n=3–5$), whereas preactivated cells significantly increased their glucose consumption (61.6±2.3 mg/dL, $p=0.003$, $n=3–5$; Fig. 3*b*). The observed effects of microgravity cannot be accounted for by the method of culture in Teflon bags required for ISS experiments. Indeed, in matched ground control experiments in Teflon bags, cell activation on average increased glucose consumption from 19.7±2.6 mg/dL in nonactivated cells to 100.7±7.6 and 107.0±13.1 mg/dL for activated and preactivated cells, respectively ($0.005<p<0.02$, $n=3–5$). Thus, in all culture conditions, cells were metabolically active. Cells activated with PWM either in RWV or in true microgravity did not increase cell metabolism, whereas preactivated cells did.

Unlike cells in tissue, the cell suspension responses to the recall antigen TT were low regardless of culture conditions; thus, antibody production analysis was limited to Ig increases in response to PWM. As expected, under normal gravity conditions, activated and preactivated cells significantly increased their antibody production compared to their nonactivated counterparts. On average, IgM production increased 24.5±11.3-fold and 15.6±6.0-fold for activated and preactivated conditions, respectively ($0.008<p<0.003$, $n=16$; Fig. 4*a*). Activated cells in RWV did not significantly increase antibody production ($p=0.17$) compared to their nonactivated counterparts (1.8±0.5-fold increase) and, on average, IgM production by activated cells was 0.8±1% of that for activated cells under normal gravity conditions ($p<0.001$). Preactivated cells in RWV did increase their antibody production by 9.8±2.9-fold ($p=0.008$) compared to their nonactivated counterparts, and IgM production was 85±27% that of normal gravity conditions for preactivated cells ($p=0.61$; Fig. 4*a*).

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Both activated and preactivated cells cultured in Teflon bags under normal gravity significantly increased their antibody production (p=0.008, n=3–5): IgM production increased 34.2±9.7-fold and 3.1±0.3-fold for activated and preactivated cells, respectively (Fig. 4*b*). Cells activated in ISS did not significantly increase antibody production compared to their nonactivated counterparts (0.99±0.16 fold, p=0.51), the average IgM production was 0.2±0.1% that of activated cells in normal gravity conditions (p<0.001, n=3). Preactivated cells, however, significantly increase antibody production in ISS (2.4±0.6 p=0.03), but the average IgM production was 44±21% that of preactivated cells in normal gravity (p=0.05, n=4; Fig. 4*b*). Collectively, these results show that cells cultured either in RWV or on the ISS respond to activation similarly: cells activated in microgravity did not produce antibodies, whereas preactivated cells did. However, preactivated cells in ISS were more impaired than preactivated cells in RWV.

We also assessed cell response to PWM by measuring the levels of cytokines and chemokines in the culture medium using a multiplexed bead assay. Medium samples were assayed for IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-12, IL-15 IL-16, IP-10, MIP-1α, MIP-1β, RANTES, SDF-1β, and TNF-α. Production of IL-1β, IL-4, IL-12, IL-15, and IP-10 were below the detection threshold of our assay. Compared to nonactivated cells cultured under normal gravity (static), IL-6 and IL-8 production decreased, on average, to 44±8% (p=0.03) and 42±7% (p=0.003) of control ones (n=16), respectively; however, the production of all other detected cytokines was significantly increased in cells activated under static condition. Preactivated cells, however, significantly increased all but the production of IL-2 and MIP-1β under normal gravity (Fig. 5*a*). On the contrary, cells activated in RWV did not significantly increase the production of any measured cytokine (Fig. 5*a*), and in the case of SDF-1b, activated cells in RWVs significantly produced less cytokine than nonactivated cells. In contrast, preactivated cells significantly increased the production of all cytokines in RWV, although to a lesser extent than cells activated in static control conditions.

Compared to their nonactivated flight controls, cells activated and cultured in Teflon bags in microgravity onboard the ISS did not increase any cytokine production. However, in the ISS, preactivated cells significantly increased the secretion of TNF-α and SDF-1β (Fig. 5*b*). Cells activated and cultured in Teflon bags on ground (ground controls) significantly increased the production of INF-γ, TNF-α, IL-16, MIP-1α, MIP-1β, and SDF-1β, while preactivated cells significantly increased their secretion of IL-1α and RANTES (Fig. 5*b*). The large biological variability, often observed in experiments using lymphoid tissue in measuring other parameters such as viral replication (Karlsson et al. 2005), as well as the limited number of samples allowed for experimentation in the ISS, undermine the nature of the effects observed in true microgravity. With the exception of an increase in the secretion of TNF-α and SDF-1β by cells preactivated on ground and cultured in space, none of the effects observed onboard the ISS reached statistical significance. Yet, collectively, these results show that cells cultured either in RWVs or on the ISS respond similarly to activation: cells did not increase cytokines when activated in true or simulated microgravity, whereas preactivated cells did.

Discussion

In this study, (1) blocks of human lymphoid tissue, isolated tissue lymphoid cells, and their individual subsets cultured in RWVs lose their ability to respond to recall antigen challenge or to polyclonal activation as measured by cell proliferation and antibody and cytokine secretion; (2) both tissues and isolated cells have the ability to initiate such responses in the RWV but lose this ability over time; (3) tissues or isolated cells challenged prior to RWV culture maintain partial responses when transferred to RWVs; and (4) isolated cells in the

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ISS responded in a similar manner: they are unresponsive to recall antigen challenge in microgravity, but developed their response in microgravity if challenged prior to microgravity exposure. Under all the above-listed conditions, both tissues and isolated cells apparently remained viable since, at least in the RWV, the immunosuppression was reversible and lymphocytes could be subsequently induced to proliferate (Sundaresan et al. 2002). Also, glucose utilization was similar for ground and flight for preactivated and unstimulated cells.

Technical, safety, and constraints of conducting experiments at the ISS did not allow the monitoring of all the parameters we recorded in our ground experiments, such as challenging cells after microgravity exposure and proliferation measurements. Nevertheless, the results of ground studies using RWVs and microgravity studies in the ISS are in striking agreement and allow us to conclude that microgravity interferes with immune responses of tissue lymphocytes. Analysis of glucose utilization by cells in RWV and the ISS showed that all cells were metabolically active and that cells activated in microgravity did not upregulate glucose consumption as did their control counterparts under normal gravity, whereas preactivated cells did. Cytokine/chemokine analysis showed the same general trends: cells activated in microgravity did not upregulate cytokines/chemokines as did their normal gravity controls, whereas preactivated cells did. Analysis of antibody production also demonstrated similar results: tissues or isolated cells activated in microgravity did not produce antibodies in contrast to their normal gravity controls, but preactivated tissues/cells did. While the overall effect of culture in RWVs and in the ISS is similar on tissues or isolated tissue cells, there are small differences between cells and tissues in RWVs cultures as well as differences for cells cultured in RWV and in the ISS.

Tissue blocks challenged in RWVs were able to secrete slightly more antibody than isolated cells in suspensions; they produced more than 20% of the IgM of static cultures whereas suspended cells produced only 1% that of static cultures. Tissues were able to initiate responses in RWVs and recover/maintain immune responses when returned to normal gravity, whereas isolated cells lost these abilities more quickly. Tissues challenged in RWVs for 3 d and then transferred to normal gravity retained approximately 30% of IgM responses of their static counterparts and resident tissue lymphocytes were proliferative (data not shown). Cells challenged in RWVs for 3 d showed little or no proliferation and did not recover their responsiveness with return to static culture. These results suggest a protective role of the tissue on immune responses. If these results are extrapolated to the situation in vivo, during spaceflight, plasma cells that migrate from lymphoid tissue into the blood on their way to the bone marrow (Benner et al. 1977) may be more sensitive to microgravity-triggered impairment.

Preactivated tissue cells are not affected to the same extent in RWV and ISS. IgM production of cells cultured in RWV reached 85% of that of their static counterparts, while cells culture in the ISS reached only 44%. Thus, it appears that true microgravity interferes with the function of preactivated cells to a greater extent than modeled microgravity. However, this difference might also be attributed to the loss of cells at the first sampling in the ISS cultures. What stage of immune response is sensitive to the microgravity? Our experiments indicate that microgravity impairs predominantly early events in cell activation since lymphocytes returned from RWV to static cultures do not recover this response. In contrast, lymphocytes treated with polyclonal activators in static culture remain activated when transferred to RWVs.

The impairment of immune activation under microgravity was also evident in the lack of cytokine secretion upon activation in both modeled and true microgravity. Also, earlier microgravity-related changes in morphology and function of isolated cells were reported

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(Montgomery et al. 1978), including changes in cytoskeleton organization (Hughes-Fulford and Lewis 1996), which is believed to be a gravity sensor (Ingber 1997), and modification of the intracellular distribution of PKC (Schmitt et al. 1996), which have been implicated in altered capping and locomotion of lymphocytes under microgravity (Cooper and Pellis 1998), may contribute to the observed immuno-suppression. Although, unlike some plant cells, mammalian cells do not possess gravisensing organelles, our data together with earlier published results indicate that cell and tissue functions are affected by microgravity.

Whichever the mechanism of the microgravity-induced immunosuppression, our results allow differentiation between systemic factors such as loss of sleep, psychological and physical stress, lack of load bearing, and confinement and microgravity itself in studying the space flight-related immune dysfunction (Cogoli 1993). Our data demonstrate that the effect of microgravity on tissue cells per se may cause immunodeficiency and this effect can now be studied under controlled laboratory conditions via solid body rotation. This result strengthens the potential relevance of other studies using modeled microgravity (e.g., Tsao et al. 1992). This ex vivo phenomenon may reflect immune dysfunction observed in astronauts during space flights. If so, the ex vivo system described above can be used to understand cellular and molecular mechanisms of this dysfunction. Further understanding of these mechanisms may reveal general causes of various human immunodeficiencies.

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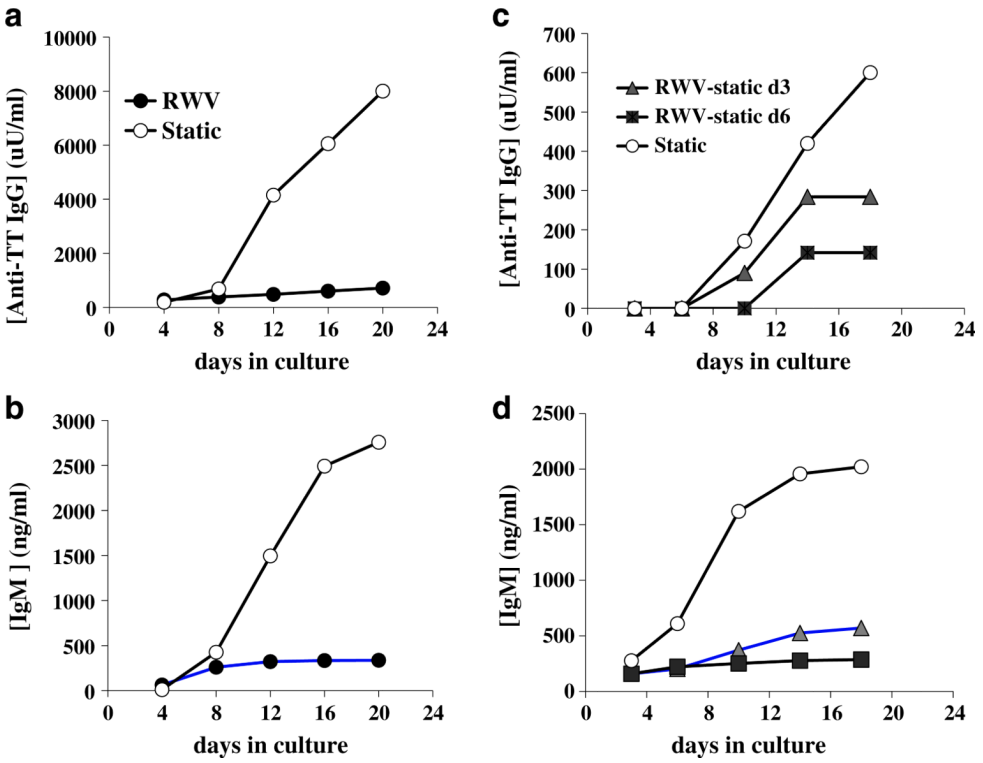

Figure 1.

Humoral immune responses of lymphoid tissue blocks. Tissue blocks in static and RWV culture were challenged ex vivo for 3–4 d. Medium was changed and sampled for antibody production every 3–4 d. Cumulative amounts of Ig production over culture length are shown for each graph. (*a*) Anti-TT IgG production by tissue challenged with TT in static (*filled squares*) or RWV (*filled diamonds*) cultures. The graph represents one typical experiment out of six. (*b*) IgM production by tissue challenged with PWM in static (*open circles*) or RWV (*filled circles*) cultures. The graph represents one typical experiment out of nine. (*c*) Anti-TT IgG production by tissue challenged with TT in static (*open circles*) or in RWV cultures for 3 d (*filled triangles*) or 6 d (*filled squares*) then transferred to static conditions. The graph represents one typical experiment out of four. (*d*) IgM production by tissue challenged with PWM in static (*open circles*) or in RWV cultures for 3 d (*filled triangles*) or 6 d (*filled squares*) then transferred to static conditions. The graph represents one typical experiment out of four.

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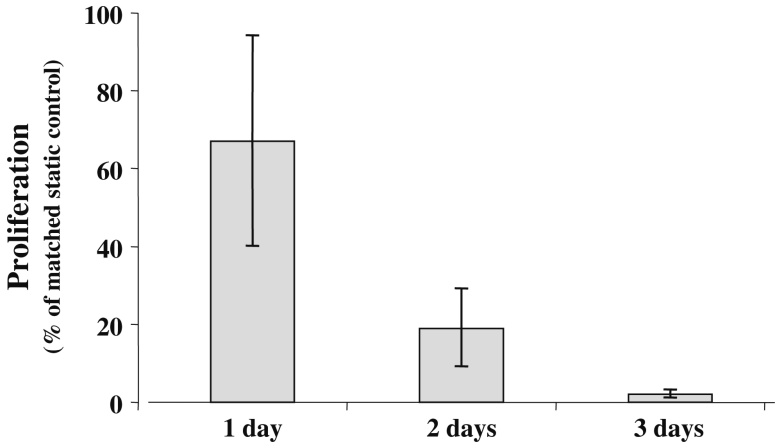

Figure 2.

Activation of isolated lymphocytes in RWVs. Total lymphocytes were activated with PWM for 1, 2, or 3 d in static or RWV culture and proliferation was measured by ^{3}H thymidine incorporation. Proliferation (in counts per min) by lymphocytes in the RWV is expressed as percentage of counts per minute by cells in static culture for the same length of time (mean ± SEM, n=7–9 for each condition).

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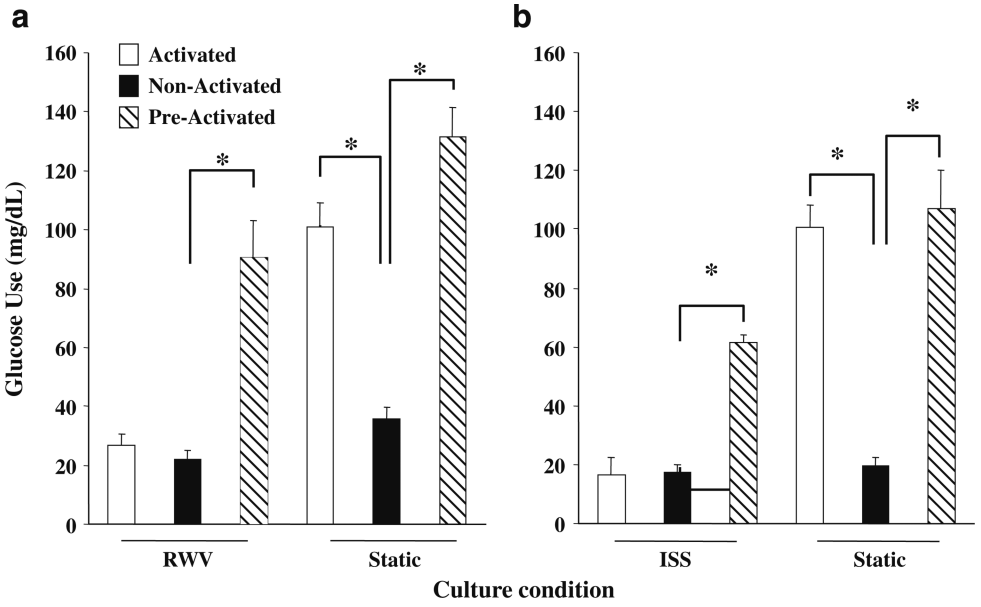

Figure 3.

Glucose use by lymphoid tissue cells. Tonsil cell suspensions (nonactivated, activated with PWM, or previously activated with PWM) were cultured in static and altered gravity culture for 12 d. Glucose use was calculated for the 12-d period. (*a*) Glucose use by cells in RWV or static culture (mean ± SEM, n=16). (*b*) Glucose use by cells in ISS or static culture (mean ± SEM, n=3–5).

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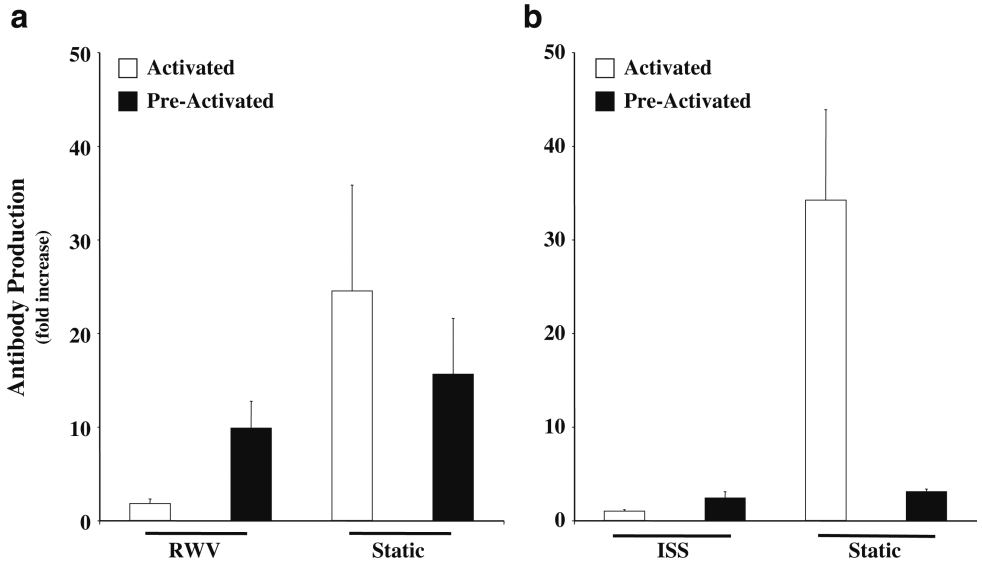
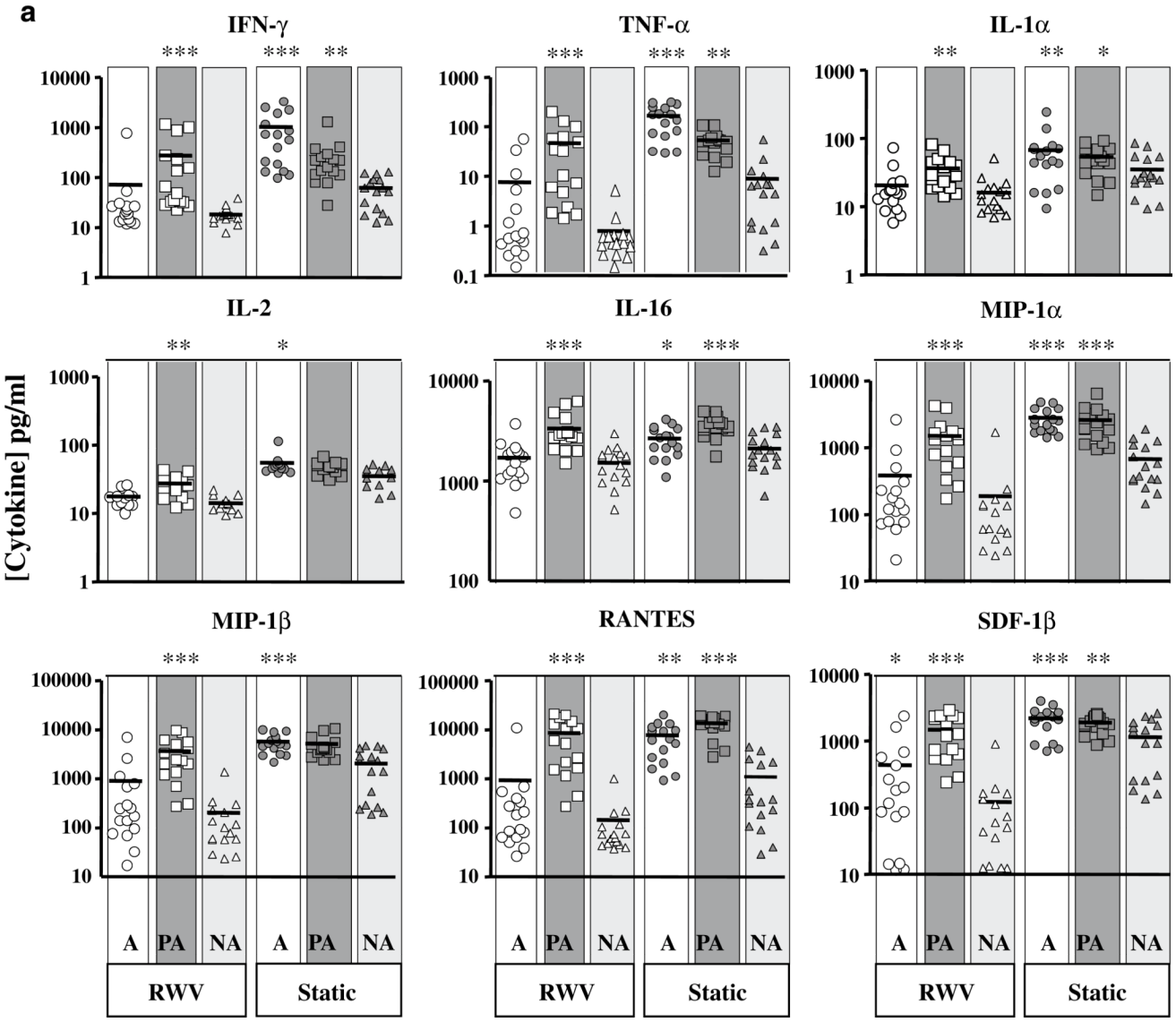


Figure 4.

Humoral immune responses of lymphoid tissue cells. Tonsil cell suspensions (nonactivated, activated with PWM, or previously activated with PWM) were cultured in static and altered gravity culture for 12 d. Antibody production was measured on medium samples taken at day 12. (*a*) IgM production by cells in RWV or static culture. Antibody production was calculated and expressed as a fold increase over that of nonactivated control cultures (mean ± SEM, n=16). (*b*) Ig production by cells in ISS or static culture. Antibody production was calculated and expressed as a fold increase over that of nonactivated control cultures (mean ± SEM, n=3–5).



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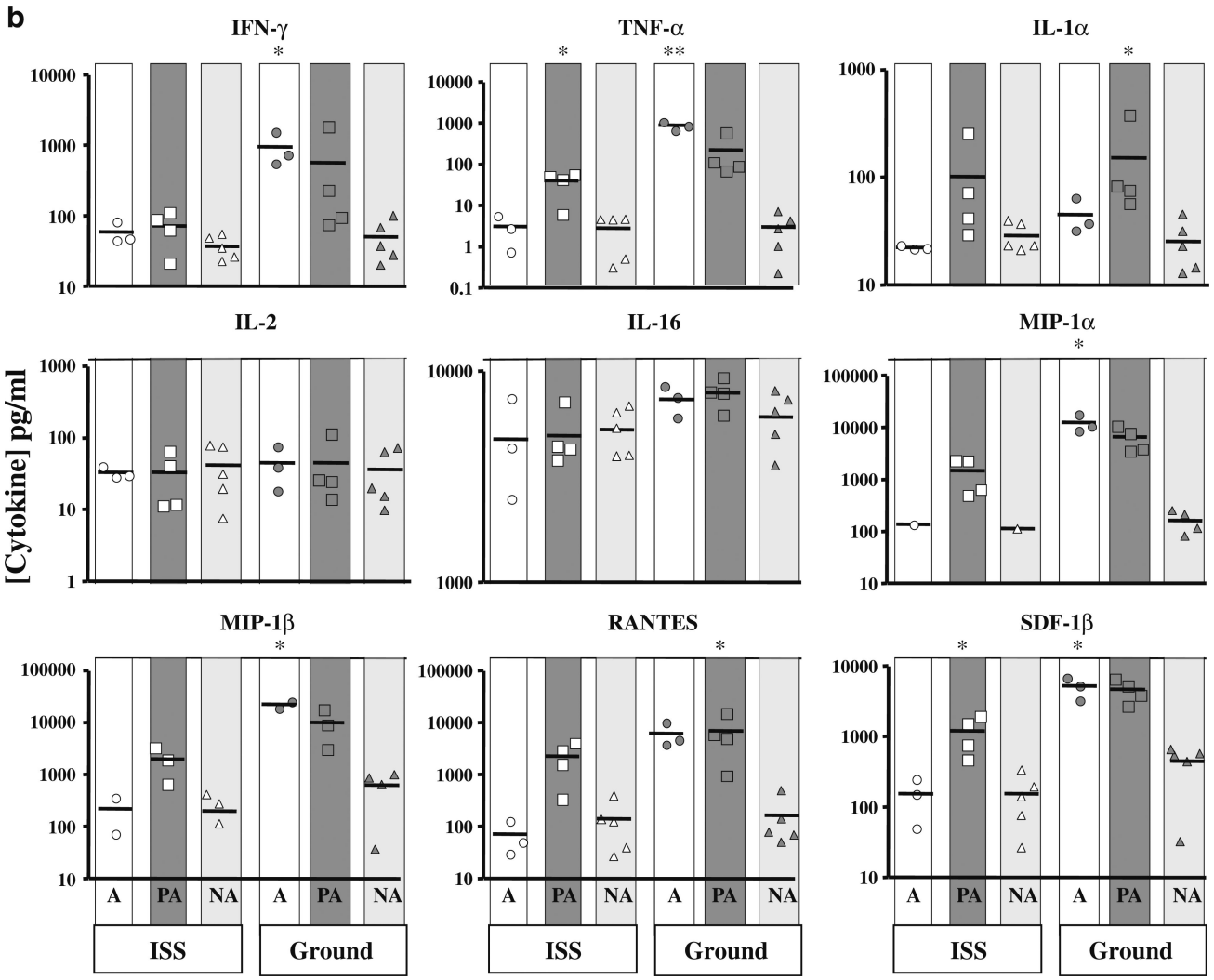

Figure 5.

Cytokine/chemokine production by lymphoid tissue cells. Tonsil cell suspensions (nonactivated [*NA*], activated [*A*], or previously activated [*PA*] with PWM) were cultured under normal gravity and under microgravity conditions in RWV or onboard the ISS. Concentrations of cytokine/chemokine accumulated in the culture medium at day 12 were measured. The levels of significance of cytokine production in activated tissue compared to the nonactivated counterpart are as follows: *$p<0.05$, **$p<0.01$, and ***$p<0.001$. (*a*). Production of cytokines by activated and preactivated cultures in RWV and static conditions. (*b*). Production of cytokines by activated and preactivated cultures in Teflon bags onboard ISS and on the ground.