MODELED MICROGRAVITY INHIBITS APOPTOSIS IN PERIPHERAL BLOOD LYMPHOCYTES

DIANA RISIN¹ AND NEAL R. PELLIS

Biotechnology Program, Wyle Laboratories — Life Sciences, Systems and Services (D. R.)
and the Cellular Biotechnology Program, NASA-Johnson Space Center (N. R. P),
Houston, TX 77058

Running Title: Apoptosis in microgravity

¹To whom correspondence should be addressed at The Biotechnology Program (BT/37), Wyle Laboratories — Life Sciences, Systems & Services, 1290 Hercules Drive, Suite 120, Houston, TX 77058. Tel: (281) 483-7650; Fax: (281) 483-0402; e-mail: drisin@ems.jsc.nasa.gov

SUMMARY

Microgravity interferes with numerous lymphocyte functions (expression of cell surface molecules, locomotion, polyclonal and antigen-specific activation, and the protein kinase C activity in signal transduction). The latter suggests that gravity may also affect programmed cell death (PCD) in lymphocyte populations. To test this hypothesis, we investigated spontaneous, activation- and radiation-induced PCD in peripheral blood mononuclear cells (PBMC) exposed to modeled microgravity using a rotating cell culture system. The results showed significant inhibition of radiation- and activation-induced apoptosis in modeled microgravity and provide insights into the potential mechanisms of this phenomenon.

Keywords: microgravity, apoptosis, immunity
INTRODUCTION

Impairment of the immunity in humans even in short term space flights is a recognized risk of undetermined magnitude (Taylor, 1993). Long term orbital space missions and the anticipated interplanetary flights increase the concern for more pronounced effects on the immune system and the ensuing potential clinical consequences. Impairment of the immunity in space may be due to numerous physiological changes caused by space-related factors, which in turn affect the immune system, or alternatively, it may be due to direct effects of microgravity and the space environment on lymphoid cells and their interactions.

Indeed, in modeled microgravity (MMG) experiments on Earth we and others (Cogoli et al., 1980, 1990; Cogoli, 1993; Risin et al., 1995; Pellis et al., 1997, 1999; Cooper and Pellis, 1998; Walthier et al., 1998) showed that microgravity directly affects multiple lymphocyte functions. It interferes with expression of cell surface molecules (Cooper and Pellis, 1998), causes inhibition of lymphocyte locomotion (Pellis et al., 1997), suppresses polyclonal and antigen-specific lymphocyte activation (Cogoli et al., 1980, 1990; Cogoli, 1993; Risin et al., 1995; Cooper and Pellis, 1998; Pellis et al., 1999), and selectively inhibits protein kinase C (PKC) isoforms (Pellis et al., 1999). Some of these effects were also confirmed in cell culture experiments in real space conditions (Cogoli et al., 1984, 1988, 1993; Cogoli and Tschopp, 1985; Pellis et al., 1997) during Spacelab, Biokosmos and Shuttle Missions. The results of these studies strongly indicated that microgravity interferes with fundamental biological processes associated with functional and structural changes in cell surface membranes, cell surface molecules and in their interaction. Based on the data and on their interpretation, we hypothesized that microgravity also affects programmed cell death (PCD) in lymphocyte populations and that
this mechanism could contribute to the impairment of the immunity. This is supported by the following considerations. First, the immune response critically depends on permanent cell renewal and differentiation. Second, apoptosis plays a pivotal role in the process of cell renewal and in sustaining tissue homeostasis, especially in such dynamic cell populations as the immune system. Third, apoptosis is known to be regulated through the surface-related interactions, which may be affected by changes in gravity.

The objective of this study was to test the hypothesis whether modeled microgravity affects programmed cell death in lymphocyte cell populations and through this mechanism interferes with the immune functions.

Studies on apoptosis in lymphoid cells in microgravity are limited to a few observations (Congdon et al., 1996; Lewis et al., 1998). However, it is difficult to relate the results of this studies to the analysis of the impairment of apoptosis and the immune response in space since they were performed either on immortalized permanently proliferating cells (Jurkat) or on lymphoid cells isolated from lymphoid organs of experimental animals flown in space, respectively. Our experiments were conducted on human PBMC and activated T cells and microgravity was modeled using rotating wall vessel culture system that provides three essential conditions: 1) randomization of the gravity vector through the cell, 2) a state of continuous fall, and 3) maintaining a quiescent, low shear environment (Schwarz et al., 1992; Tsao et al., 1992; Goodwin et al., 1993; Jessup et al., 1993). Furthermore, the investigation of isolated cell populations in modeled microgravity affords assessment of physical effects in the absence of potentially confounding physiological factors, i.e., stress.
MATERIALS AND METHODS

Peripheral blood mononuclear cells (PBMC). Peripheral blood mononuclear cells were isolated from healthy donors using a Ficoll Hypaque gradient (Ficoll-Paque, Pharmacia Biotech AB, Uppsala, Sweden) by centrifugation at 700 g for 20 min at room temperature. The cells from the interface were washed three times with Hanks balanced salt solution (HBSS) and resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS).

Activation of T cells. Activation of T cells was achieved by PBMC stimulation by anti-CD3 antibodies (generous gift of Dr. Bradley W. McIntyre, University of Texas M. D. Anderson Cancer Center) at a final concentration 100 ng/ml and human recombinant IL-2 (100 U/ml, Life Technologies, Grand Island, NY) for 3-4 days. The cells were replenished with IL-2 (100 U/ml) every 3-4 days. They were used in the study following at least 11 days of activation.

Induction of apoptosis. Effects of microgravity were investigated in spontaneous and induced apoptosis models. Apoptosis was induced either by gamma irradiation of PBMC or by T cell receptor reengagement in activated T cells (so called activation-induced PCD).

PBMC were γ-irradiated using $^{137}$Cs source at a dose rate of 15 Gy/min in irradiator with a rotating platform. To induce PCD in T lymphocytes previously activated cells were restimulated by PHA-M (final concentration 5µg/ml) or by PMA+ionomycin (final concentrations 5 ng/ml and 500 ng/ml, respectively). All reagents are from Sigma Chemical Co., St. Louis, MO.

In several experiments to induce apoptosis in activated T cells we used recombinant human soluble Fas ligand (rhs APO-1/Fas ligand, Alexis Biochemicals, San Diego, CA) at 50-80 ng/ml with 1 µg/ml of an enhancer protein.
Modeled microgravity and control cell cultures. To approximate MG conditions on Earth, we used specialized rotating-wall vessel culture system developed at the NASA-Johnson Space Center and commercially available from Synthecon, Inc. (Friendswood, TX). This very low shear culture system randomizes gravitational vectors and approximates the microgravity environment by sustaining cells in continues free fall (Schwarz et al., 1992; Tsao et al., 1992; Goodwin et al., 1993; Jessup et al., 1993). This culture system was successfully used before for the analysis of the effects of microgravity on PBMC locomotion in parallel studies on Earth and in Space performed during space flight missions STS-54 and STS-56 (Pellis et al., 1997).

Controls were cultured in stationary conditions in plastic tissue culture flasks. All cultures were maintained at 37° C in atmosphere of 95% air and 5% CO₂.

Transmission electron microscopy (TEM). Cells were processed according to the standard procedure. Briefly, the pellets were fixed in a solution containing 3% glutaraldehyde, 2% paraformaldehyde, and 0.1 M cacodylate buffer, pH 7.4 for 1 h. After fixation, the samples were washed and treated with 0.1% tannic acid in 1% glutaraldehyde for 20 min, postfixed with 1% buffered osmium tetroxide for 1 h, and stained with 1% aqueous uranyl acetate for 1 h (Muller et al., 1994). They were further dehydrated in increasing concentrations of ethanol and then infiltrated and embedded in Spurr’s low viscosity medium. The blocks were polymerized in a 60° C oven overnight. Thin sections were cut, stained with uranyl acetate and lead citrate, and examined in a JEOL 1200-EX transmission electron microscope.

Assessment of apoptotic death. Assessment of apoptotic death by flow cytometry was performed using an approach, described by Nicoletti and coworkers (Nicoletti et al., 1991). After washing in PBS, the cells were gently resuspended in hypotonic PI solution (PI 50µg/ml in
0.1% sodium citrate plus 0.1% Triton X-100 (Sigma Chemical Co., St. Louis, MO), and maintained at 4°C in the dark until the flow cytometric analysis. The PI fluorescence of the nuclei was measured using a Coulter EPICS flow cytometer (Hialeah, FL). The percentage of apoptotic nuclei was estimated by assessment of the hypodiploid DNA peak in the DNA fluorescence histogram.

**Cell surface Fas/CD95 and Fas Ligand/CD95L expression.** Cell surface Fas/CD95 and Fas ligand/CD95L expression was examined by flow cytometry (EPICS C; Coulter, Hialeah, FL). Staining for CD95 was accomplished by incubating of 1x10⁶ cells with saturating concentration of anti-human Fas mAb (clone 7C11, Coulter Corporation, Miami, FL) directly conjugated with RD1-phycoerythrin derivative for 40 min. RD1-conjugated mouse IgM Ab were used as a control. Stained cells were washed twice in cold HBSS with 2% FBS. All incubations were at 4°C. Staining for Fas ligand (FasL) was performed using biotin conjugated mouse anti-human Fas ligand monoclonal antibody, clone NOK-1 followed by streptavidin-phycoerythrin conjugate. Biotin-conjugated mouse IgG₁κ Ab were used as a control. All reagents from PharMingen, San Diego, CA. Cell surface Fas ligand expression was also analyzed using directly FITC-conjugated monoclonal rat anti-human Fas ligand Ab (clone H11, Alexis Biochemicals, San Diego, CA). Percentage of positive cells and mean channel fluorescence were calculated by subtracting the control histogram from the test histogram using a cumulative subtraction routine ( Oversub) within the Elite Immuno-4 software (Coulter Corp., Hialeah, FL).

**Bcl-2 and Bax protein expression.** For immunodetection of the intracellular bcl-2 protein, we used FITC-conjugated monoclonal mouse Ab against human bcl-2 oncprotein (Accurate Chemical & Scientific Corporation, Westbury, NY) and followed the manufacturer’s protocol.
Briefly, the cells were washed with PBS, permeabilized and fixed in 0.25% paraformaldehyde and 70% methanol. After that, the monoclonal Ab (10 µl per 1×10⁵ cells) were added and the cells were incubated for 30 min at 4°C. The cells were stained in DNA-staining medium which contained 50 µg/ml PI and 100 units/ml ribonuclease A. Mouse FITC-conjugated IgG1 Ab were used as a negative control.

For immunodetection of bax protein monoclonal mouse Ab against human bax (Immunotech, a Coulter Co., Miami, FL) were used. Following cell permeabilization procedure, performed by using IntraPrep™ Permeabilization Reagent (Immunotech, a Coulter Co., Miami, FL) according the manufacture’s protocol, the cells were stained by the unconjugated monoclonal mouse Ab against human Bax (Immunotech, a Coulter Co., Miami, FL), 2 µg per 5×10⁵ cells for 15 min at room temperature. After washing in PBS, they were additionally incubated for 15 min with FITC-conjugated goat anti-mouse secondary antibody (Becton Dickinson, San Jose, CA) and further analyzed by flow cytometry (EPICS C; Coulter Corp., Healeah, FL). Controls for indirect staining included incubation with FITC-conjugated secondary antibody alone.

**Statistical analysis.** Statistical analysis was performed using GraphPad Prism 2.1 Software (San Diego, CA). Significance was determined using the Student’s t-test. P<0.05 was considered statistically significant.

**RESULTS**

**Spontaneous and radiation-induced PCD in MMG and stationary conditions.** The level of spontaneous apoptosis in stationary conditions in non-activated PBMC, as well as in activated T cells, was under 6%. It was practically the same in cultures from RWV (Table 1). Radiation
caused a substantial increase in PCD (Fig. 1). Consistent with other investigations (Delic et al., 1993; Seki et al., 1994), we have found that the effect of radiation on PCD is dose dependent. The highest level of apoptotic death in stationary culture conditions was observed at 20 Gy (Fig. 1). We have also observed that the sensitivity of PBMC to radiation-induced PCD varied among the individual donors.

Maintaining the PBMC after radiation in rotating wall vessel caused substantial reduction in the number of cells undergoing programmed cell death (Fig. 1). The inhibitory effect was most prominent in those cell samples that demonstrated the highest levels of radiation-induced PCD in stationary conditions.

**Activation-induced PCD in MMG.** To examine whether microgravity interferes with mechanisms involved in triggering of apoptosis in PBL during immune response, we analyzed the effect of modeled microgravity on activation-induced PCD. For this purpose, we used isolated T cells, activated by Anti-CD3 antibodies and IL-2 and restimulated by exposure to PHA-M or to PMA+ionomycin as described in Materials and Methods. Reengagement of T cell receptor in this model by PHA induced PCD in the range of 42.9% to 64.7% (49.2±1.51%). The level of PCD trigged by adding PMA and ionomycin was in the range of 23.5% to 42.3% (32.5±2.31%). Again, exposure to modeled MG significantly reduced PCD (Fig. 2).

Apoptotic death of the cells was confirmed by transmission electron microscopy, which revealed characteristic cytoplasmic and nuclear changes (data not shown).

**Receptor-ligand interaction in MMG.** To approach the possible mechanisms of the observed rescue effect of MMG, we investigated the conceivable impact of microgravity on receptor-ligand interaction (T cell receptor - PHA). For this purpose, we examined the
possibility to abrogate the MG-induced inhibition of apoptosis by stimulation of the activated T cells with PHA in stationary conditions prior to their transfer to the RWV. Regardless of the sequence of the events - whether PHA was added prior or during exposure to microgravity - no substantial difference in the extent of apoptosis was observed (Fig. 3). These results strongly suggest that the interaction between PHA and cell surface receptor molecules remains intact in modeled MG and does not contribute to the observed inhibitory effect. This conclusion was also supported by direct measurements of binding of FITC-labeled PHA to lymphocytes in stationary and microgravity conditions, which showed that the level of binding was practically the same in stationary and MMG conditions (Cooper and Pellis, 1998).

**The effect of MG on Fas and Fas ligand expression and interaction.** Another possibility examined in this study was the interference of modeled MG with Fas and FasL expression and interaction. No difference in expression of membrane-bound Fas Ag (Fig. 4) and Fas ligand in simulated MG vs stationary conditions in either activated or activated and restimulated by PHA T cells was observed. The level of Fas ligand expression on the surface of these cells both in MG and stationary conditions was very low, almost marginal.

At the same time, adding of exogenous recombinant soluble Fas ligand into the media before cell placement in MG totally abrogated the inhibitory effects (Fig. 5).

**The effect of MG on the expression of Bcl-2 family proteins.** No difference was found in the expression of Bcl-2 protein or bax antigen between the activated T cells maintained either in stationary or in RWV conditions. This was also confirmed in experiments in which PCD in preliminary activated T cells was triggered by PHA (Figs. 6 and 7).
DISCUSSION

The results demonstrate that modeled microgravity inhibits PCD in lymphocyte populations. Inhibition occurred in two types of experiments: 1) when PCD was induced by gamma-radiation of PBMC, and 2) when PCD in activated T cells was triggered by PHA-M or PMA+ionomycin restimulation. The results obtained in both systems demonstrate a significant decrease in apoptosis when human lymphocytes are transitioned to modeled microgravity. The model, in which PCD is induced in activated T cells through the mechanism of reengagement of T cell receptor, is particularly important. It represents in vitro the main mechanism involved in triggering apoptotic death in lymphocytes during physiological immune response and thus, relates the findings to the immune response in vivo.

The observed rescue effect indicates that MG might interfere with the major physiological mechanisms involved in control of PCD in lymphoid cells. To elucidate the possible MG-sensitive mechanisms of apoptosis several assumptions were investigated. First, whether microgravity interferes with receptor-ligand interaction, in our case - PHA and PHA-receptor. Thus we attempted to abrogate the effects of MG by engagement of the PHA receptors by the ligand molecules in stationary conditions prior to placing the cells in the RWV. There was no substantial difference in the extent of apoptosis regardless of whether PHA was added prior or during exposure to microgravity (Fig. 3), suggesting that the interaction between PHA and cell surface receptor molecules remained intact in modeled MG.

The second possibility is the interference of MG with the expression and with the interaction of Fas and Fas ligand, which play a key role in triggering activation-induced apoptosis, including
PCD triggered by PHA and PMA+ionomycin (Brunner et al., 1996). This mechanism is documented in non-transformed T cells, T cell hybridomas, and in alloreactive T cell clones (Singer and Abbas, 1994; Alderson et al., 1995; Brunner et al., 1995; Dhein et al., 1995; Ju et al., 1995; Van Parijs et al., 1996). T cell receptor triggering upregulates Fas and induces expression of the Fas ligand on cell surface. Further interaction of Fas and Fas ligand on the surface of the same or neighboring cells initiates the necessary steps for the suicide program. Fas-FasL interactions are also involved in PCD induced by ionizing radiation in malignant and normal lymphocytes (Belka et al., 1998). Based on these and other data it was anticipated that this mechanism may be affected by MMG. However, we did not find any difference in the expression of Fas antigen and membrane-bound FasL on the surface of activated T cells cultured either in stationary or in modeled microgravity conditions. The expression of Fas ligand on the surface of cultured cells was marginal, which is consistent with other observations on the level of FasL expression (Dhein et al., 1995). Alone, these results may indicate that the role of Fas-FasL mechanism in modeled microgravity-induced inhibition of apoptosis is minimal. However, the inhibition of apoptosis in MG was totally abrogated by exogenous soluble Fas ligand. Thus, microgravity most likely affects not the expression, but the interaction of membrane-bound Fas and Fas ligand on the surface of the same or neighboring cells. When soluble FasL becomes available in the media the apoptotic program is fully activated. Without added FasL, shedding of the membrane bound FasL into the media is extremely low and not sufficient to trigger the process and thereby overcome the inhibitory effects of MG.
The third assumption is the involvement of the Bcl-2 family proteins. Members of the Bcl-2 family regulate apoptosis in mammals and may act as inhibitors or promoters of apoptosis. The most prominent regulators are Bcl-2, Bcl-xL and Bax (Reed, 1994, 1998; Chao and Korsmeyer, 1998; Zamzami et al., 1998). The Bcl-2 family members dimerize, with one monomer antagonizing or enhancing the function of the other. For example pro-apoptotic proteins such as Bax dimerize with anti-apoptotic family member Bcl-2 (Oltvai et al., 1993; Yin et al., 1994; Sedlak et al., 1995). In this way, the ratio of inhibitors to activators in a cell determines the outcome, namely, whether the cell will undergo apoptosis, or this process will be abolished (Korsmeyer et al., 1993; Oltvai et al., 1994). This issue lately became more uncertain, when it was demonstrated that heterodimerization is not required for pro-survival activity (Cheng et al., 1996). Also, certain death signals, including CD95-mediated apoptosis in peripheral T cells, bypass control by Bcl-2 (Krammer, 1999). We did not find any difference in the expression of Bcl-2 protein or bax antigen in cells maintained in stationary conditions and in RWV with or without PHA triggering (Fig. 6 and 7). This indicates that inhibition of apoptosis in activated T lymphocytes in modeled MG may not be related to changes in expression of bcl-2 or bax antigen.

In summary, our findings document inhibition of apoptotic cell death in human lymphocytes in modeled microgravity conditions. The exact mechanisms of this phenomenon are still unknown. However, it is unlikely that changes in the expression of Fas antigen, Fas ligand, bcl-2 or bax protein are involved. The fact that inhibition of apoptosis is abrogated by exogenous soluble FasL implies that the MMG impairs membrane-bound Fas and FasL interaction. MMG may inhibit apoptosis by 1) altering receptor affinity for the ligand, 2) interfering with receptor distribution, or 3) minimizing the tangential area of cell-cell interaction and thereby preventing the threshold interaction level necessary for signal transduction.
In general we consider that MG interferes with certain, yet unknown conditions important for cellular interactions involved in PCD. The observations may be related to consequential changes in the cell shape, in the cytoskeleton, in cell movement, or in any other vector-dependent molecular rearrangements.

ACKNOWLEDGMENTS

We thank Dr. C. D. Bucana for her expert help in performing the electron microscopy studies and interpreting their results. We are also grateful to Ms. M. Young for her assistance in manuscript preparation. This work was supported by NRA OLMSA-02 and NSCORT NAG5-4072 grants.
REFERENCES


FIGURE LEGENDS

FIG. 1. Spontaneous and radiation-induced PCD in human PBMC maintained in stationary and microgravity conditions. PBMC were γ-irradiated at 7.5; 20 and 40 Gy and then cultured in RWV or in stationary conditions. PCD was assessed 18 hrs after restimulation. Data are presented as mean ± standard error of the mean.

FIG. 2. Effect of modeled microgravity on spontaneous, PHA, and PMA+ionomycin induced programmed cell death in activated peripheral blood T cells. Activated T cells were restimulated by PHA-M or by PMA+ionomycin and then cultured in RWV or stationary conditions. PCD was assessed 18 hrs after restimulation. Data are presented as mean ± standard error of the mean.

FIG. 3. PHA-induced PCD in activated T cells exposed to microgravity at different time points. PHA was added to activated T cells 2 hrs prior (-2 time point), immediately before (0 time point) or 24 hrs after (24 time point) cell placement into RWV. PCD was assessed 18 hrs after restimulation and compared with PCD in cells restimulated and kept for the same time in stationary conditions. Data are presented as mean ± standard error of the mean.
FIG. 4. Fas antigen expression in activated T cells restimulated with PHA. Activated T cells were cultured in RWV or in stationary conditions. The cells were sampled after 24 hrs in culture without restimulation and 2, 6 or 18 hrs after restimulation by PHA. Fas antigen expression was assessed at all time points and the data are presented as mean ± standard error of the mean.

FIG. 5. Effect of modeled microgravity on FasL induced programmed cell death in activated peripheral blood T cells. Soluble FasL was added to activated T cells cultured in RWV or in stationary conditions and PCD was assessed 18 hrs after. Data are presented as mean ± standard error of the mean.

FIG. 6. Bcl-2 expression in activated T cells restimulated with PHA. Activated T cells were cultured in RWV or in stationary conditions. The cells were sampled after 24 hrs in culture without restimulation and 2, 6 or 18 hrs after restimulation by PHA. Bcl-2 expression was assessed at all time points and the data are presented as mean ± standard error of the mean.

FIG. 7. Bax expression in activated T cells. Activated T cells were cultured in RWV or in stationary conditions. The cells were sampled after 24 hrs in culture. Bax expression was assessed and the data are presented as mean ± standard error of the mean. Panel A - Percent positive cells, Panel B - Mean fluorescence intensity.
TABLE I

SPONTANEOUS APOPTOSIS IN PBMC AND ACTIVATED T CELLS IN STATIONARY AND MODELED MG CONDITIONS

<table>
<thead>
<tr>
<th></th>
<th>Static</th>
<th>RWV</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC</td>
<td>5.95 ± 1.01</td>
<td>5.1 ± 0.98</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>(n=4)</td>
<td>(n=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activated T-cells</td>
<td>5.32 ± 0.40</td>
<td>6.79 ± 0.67</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>(n=12)</td>
<td>(n=12)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Human PBMC and activated T cells were cultured in RWV or in stationary conditions for 18-24 hrs. Cells were harvested and apoptosis assessed by Flow cytometry. Data are presented as mean ± standard error of the mean.
Apoptotic Cells (%) vs Dose (Gy)

- Control
- 7.5 GY
- 20 GY
- 40 GY

- Static
- RWV

Significantly different at P=0.02
Apoptotic Cells (%) vs. Static and RWV conditions

- Control: 5% (P<0.0001)
- PHA: 40% (P<0.0001)
- PMA+IONO: 20% (P<0.01)
Apoptotic Cells (%) 18 hrs after PHA

<table>
<thead>
<tr>
<th></th>
<th>Static</th>
<th>RWV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-2</td>
<td>P&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>P&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.

Time of PHA Addition Related to μG Exposure (hr)
Figure 4: Graph showing the percent positive cells over culture time (hr) relative to PHA restimulation. The graph compares static conditions with RWV conditions.
Figure 5

Apoptotic Cells (%)

Static
RWV

FasL
Percent Positive Cells

Culture Time (hr) Relative to PHA Restimulation

No PHA

2

6

18

Static

RWV