

While thymic negative selection of self-reactive T cells has been shown to occur, not every self antigen is expressed in the thymus. Therefore, the primary encounter between some self-reactive T cells and autoantigens may occur in the periphery, resulting in either activation, clonal deletion or anergy. Pregnancy provides an excellent model to address the fate of these self-reactive T cells. Fetal antigens are expressed at physiologically relevant levels, and will never have been encountered in the thymus during T cell development. The fate of T cells specific for an antigen on male fetuses was evaluated in transgenic mice that express a T cell receptor (TCR) specific for H-Y + D^b. On day 7 and day 14 of pregnancy, and 5 days postpartum, lymphoid tissues were analyzed for changes in cell number, expression of the clonotypic TCR and other T cell markers. Numbers of both total splenic T cells, and clonotype-expressing T cells from H-2^b, H-Y specific TCR transgenic mice decreased steadily during pregnancy. This increase was not observed in H-2^d TCR transgenic mice that cannot recognize H-Y indicating an antigen specific response to H-Y. Interestingly, both the spleen and lymph nodes of H-2^b TCR transgenic mice continued to have decreased numbers of H-Y specific T cells when evaluated postpartum. This long-term decrease in fetal specific T cells argues against a transient downregulation of the TCR, and supports clonal deletion as a mechanism for the elimination of fetal reactive T cells.

1275 **Jacalin-Induced Apoptosis in Cultured PBLs from HIV+ Subjects is Reduced by Nitric Oxide Synthase Inhibitors.** *ML Quesada, S Saavedra, AM del Llano, JA Lavergne.* School of Medicine, UPR and Veterans Adm. Hospital, San Juan, P.R.

Jacalin (JAC), a lectin mitogenic for T lymphocytes has been reported to block *in vitro* HIV infection of human CD4+ lymphocytes (Favero et al., 1993). The proliferative responses and the apoptosis (Ao) induction of lymphocytes cultured with this lectin were studied in fifty samples from HIV+ patients and compared to those of ten uninfected subjects. These effects were assessed by flow cytometry and compared to those of PWM- and PHA-stimulated PBLs. Among uninfected subjects, PHA stimulation resulted in the highest proliferative response followed by PWM and JAC, while no mitogen induced Ao. In contrast, HIV+ patients showed decreased proliferation responses with the three mitogens when compared to normal values, and their unstimulated PBLs exhibited low percent values of spontaneous Ao (21.6 ± 10.4) which increased by stimulation with PWM (25.4 ± 13), JAC (29.1 ± 12.8) and PHA (34.2 ± 15.5). PBLs were also cultured in the presence of N-Monomethyl-L-arginine (L-NMMA) and 7-Nitroindazole (7-NI), two nitric oxide synthase inhibitors, resulting in a significant inhibition of Ao. The percent of inhibition with L-NMMA in JAC-stimulated PBLs was highest (67.3 ± 15.6 ; 36.7 ± 19.3 with 7-NI), followed by PHA-stimulated PBLs (61.9 ± 13.4) and PWM-stimulated (42.4 ± 20.4). These results indicate that JAC is a potent inducer of apoptosis in cultured PBLs from HIV+ patients. This phenomenon is reduced by L-NMMA and 7-NI, supporting the role of NO in apoptosis induction in this model. Supported by RCMI-RR03051.

1276 **Very low oxygen conditions distinguish between oxygen dependent and independent apoptosis.** *J F Torres-Roca, D R Greenwald, J Brown, I L Weissman, L A Herzenberg, L A Herzenberg and P D Katsikis.* Departments of Genetics, Radiation Oncology and Pathology, Stanford University School of Medicine, Stanford, CA. Supported by grants AG-07290 and NIH CA 42509-11.

Many of the stimuli that induce apoptosis are also known to elicit oxidative stress. This has led to the proposal of oxidative stress as a mediator of apoptosis. However, recently several investigators showed that anaerobic culture does not inhibit several forms of apoptosis. Using low

oxygen conditions. In contrast, anaerobic culture completely inhibits dexamethasone-induced apoptosis of immature mouse thymocytes. Although spontaneous apoptosis is increased in anaerobia (18% in aerobia vs 30% in anaerobia) dexamethasone did not have any effect above background death under low oxygen conditions (50% in aerobia vs 30% in anaerobia). Consistent with these results, inhibitors of two important intracellular oxyradical generating centers, mitochondrial respiratory complex I (rotenone) and cytochrome p 450 (metyrapone), were able to efficiently inhibit glucocorticoid-induced thymocyte death, while not affecting Fas-induced death. Rotenone and metyrapone, inhibited 75% and 78% respectively of dexamethasone-induced apoptosis. In conclusion low oxygen conditions and inhibitors of oxyradical generating centers distinguish between oxygen dependent and independent apoptosis. The level at which oxygen is involved in the oxygen dependent apoptosis is currently being investigated.

1277 **Suppressed lectin-induced proliferation of T lymphocytes in simulated microgravity is restored by direct activation of PKC.** *D Cooper^{1,2} and NR Pellis^{1,3}.* ¹The University of Texas, Graduate School of Biomedical Sciences; ²University Space Research Association; and ³The Biotechnology Program, NASA Johnson Space Center, Houston, Texas.

Several factors associated with spaceflight may be responsible for the immunosuppression observed in astronauts during and following spaceflight. We utilized Rotating Wall Vessel (RWV) bioreactors that simulate aspects of microgravity for cell cultures to assess the role of microgravity alone on polyclonal activation of lymphocytes. Previously we showed that PHA responsiveness was dramatically diminished in RWV culture as measured by [³H]-thymidine incorporation ($\geq 90\%$) and that T cells but not accessory monocytes are responsible for this impairment. In the current study, addition of exogenous IL-2 (10-100 U/ml) to these cultures did not restore PHA responsiveness in the RWV. However, activation of PBMC or column purified T cells with PMA and ionomycin is unaffected in RWV culture, indicating that signaling mechanisms downstream of PKC activation and calcium flux are unaffected by simulated microgravity. Furthermore, submitogenic doses of PMA alone, but not ionomycin alone (at any concentration), can restore PHA responsiveness in the RWV. Thus, a defect upstream of PKC activation and not calcium flux is most likely responsible for the suppression of polyclonal activation observed in simulated microgravity. This research was supported by NRA-94-OLMSA-02.