

## LOW DOSE PHOTON RADIATION-INDUCED CHANGES IN T HELPER LYMPHOCYTES

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Health risks due to protracted low dose irradiation remain unclear. This project investigates T helper (Th) lymphocyte function and the cellular milieu in which they reside under conditions of low dose, low-linear energy transfer (LET) radiation exposure. The Th cells are important because they secrete cytokines essential for generating optimal immune defenses against tumor, virus-infected, and other aberrant cells. The overall *hypothesis* is that whole-body exposure to low dose photons will inhibit Th1 cell function as manifested by insufficient interferon- $\gamma$  (IFN- $\gamma$ ) secretion.

Female C57BL/6 mice (n = 125; 9-10 weeks old) were  $\gamma$ -irradiated to total doses of 0, 0.01, 0.05 and 0.1 Gy at a dose rate of 0.024 cGy/hr using <sup>57</sup>Co flood sources that provide high spatial uniformity (AEA Technology, Burlington, MA). Subsets per group were euthanized in CO<sub>2</sub> immediately after the end of irradiation (day 0, i.e. within 1-2 hours) and on days 4 and 21 thereafter. Analyses include: white blood cell (WBC) counts and 3-part differentials using a hematology analyzer, degree of DNA synthesis, flow cytometry analysis of major lymphocyte populations, quantification of cytokine expression within Th cells, Th1//Th2/Th3 gene expression using cDNA microarrays, and quantification of secreted cytokines using Luminex technology. To date, a portion of these assays have been performed on spleen and blood from photon-irradiation mice.

In the spleen, the WBC count was significantly elevated on day 0 in all irradiated groups, with means ranging from 17% to 25% above the non-irradiated controls (P<0.05); the enhancement was accounted for by both lymphocytes and monocyte-macrophages. Granulocytes were elevated only in the 0.1 Gy group (P<0.01). There were no significant differences in these parameters on days 4 and 21. Splenocyte DNA synthesis in all irradiated groups, based on <sup>3</sup>H-thymidine incorporation, was consistently above the control mice on days 0 and 4, although significance was not always obtained. Flow cytometry analysis of spleen cells showed that all major lymphocyte populations (CD3<sup>+</sup> T, CD3<sup>+</sup>/CD4<sup>+</sup>Th, CD3<sup>+</sup>/CD8<sup>+</sup> Tc, CD19<sup>+</sup> B, and NK1.1<sup>+</sup> NK cells) were affected by radiation at one or more of the time points. Splenocytes were activated with phorbol myristate acetate (PMA) and calcium ionophore (FastImmune Cytokine System, BD Biosciences); number of cells with intracellular IFN- $\gamma$  and IL-2 was based on the CD4<sup>+</sup> Th cell count. On day 0, cells expressing IFN- $\gamma$  were 68% and 60% higher after 0.05 Gy and 0.01 Gy compared to controls (P<0.05 and P=0.055, respectively). By day 4, cells positive for IFN- $\gamma$  were still elevated in the 0.01 Gy group (60% higher than control, P<0.05); by day 21, positive cells were 36% above control, but significance was lost. In contrast, the number of cells with intracellular IL-2 in the irradiated groups were consistently (but not significantly) below the control on days 0 and 4. However, by day 21, the IL-2-expressing cells in the 0.01 Gy group were significantly increased compared to all other groups (P<0.05); mean values ranged from 35% to 109% above the other groups. Differences in some measurements were also noted in blood, but these were far fewer and less pronounced.

Thus far, the data show significant perturbations in Th lymphocytes after protracted, whole-body exposure to photons. The lowest total dose of 0.01 Gy resulted in a high number of cells with intracellular IFN- $\gamma$  at the early time points, followed by increased cells with IL-2 on day 21, suggesting that this low level exposure may be radioprotective. However, additional studies, such as the ongoing quantification of cytokine secretion capacity, are obviously needed. Similar experiments will be performed with low dose protons in the near future and compared with results from the photon-irradiated animals.

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