

Low-dose, low-LET γ -radiation alters carcinogen-induced splenic cytokine production and immune cell phenotype of A/J mice.

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Introduction: Low-dose, low-linear-energy-transfer (LET) radiation (LDR; < 100 mGy) activates the immune response, presumably via epigenetic pathways (Scott *et al.* 2009) and may lead to cancer suppression (Nowosielska *et al.* 2006). One of the mechanisms by which it might do so is by altering the cytokines produced after carcinogen and/or radiation exposure. Alternatively, LDR may activate splenocytes which would presumably increase their anti-cancer surveillance function (Liu 2007; Bogdándi *et al.* 2010). We investigated the effect of LDR exposure and carcinogen (benzo[a]pyrene (BaP)) injection on spleen cell number and phenotype as well as cytokine secretion in an A/J mouse model.

Methods: Mice were administered a single, whole-body gamma radiation dose (10, 100, 1000 mGy) via a Gammacell 1000 irradiator (Atomic Energy of Canada, LTD., Kanata, Ontario, Canada) one day prior to intraperitoneal injection with BaP (100 mg/kg i.p.) or vehicle (tricapryllin, 0.2 ml). The 1000 mGy dose was administered as a high-dose reference. One group of mice was left unirradiated for a control group. Splenocytes were harvested at days (d) 2, 7, 9 and 14 post-irradiation to determine viable cell counts and phenotype. Splenocyte phenotype was measured by flow cytometry using antibodies for 1) T (CD4 and CD8) and B cell (CD19) lymphocytes and their activation markers (CD69, FasL, IFN- γ), 2) regulatory T cells (CD4, CD25 and FoxP3), 3) NK cells (CD49b) and their activation marker (CD107a), 4) macrophages (F4/80) and dendritic cells (CD11c) and their activation marker (MHC Class II). Splenocytes (5×10^6 /ml) were also cultured for 48 hours +/- the T cell mitogen Concanavalin A (5 μ g/ml) and cytokine secretion was measured by Luminex technology. A total of 16 cytokines were measured.

Results: BaP treatment decreased total body weight and total splenocyte number by Day 2 post-injection. BaP also decreased activated (CD69+) CD4+ T lymphocytes in the spleen by d7. The reduction in activated T cell number may be conducive to reduced anti-cancer surveillance. However, the reduction in activated T cell number was attenuated in mice treated with 10 mGy radiation. Treatment of mice with 1000 mGy, but not 10 mGy, in combination with BaP decreased total cell, B cell, and T regulatory cell number in the spleen by Day 9.

Regarding changes in cytokine production potential *ex vivo*, BaP treatment decreased Th2 cytokines (IL-4, IL-13) on d7 and/or d9 and increased IL-1 β and IL-17 production by Con A-stimulated splenocytes harvested on d2 and d7. These data indicate that BaP induces a pro-inflammatory cytokine milieu as Th2 cytokines are generally considered to be anti-inflammatory while IL-1 β and IL-17 are pro-inflammatory. Effects of radiation on Con-A stimulated cytokine secretion varied with day post-irradiation and treatment group (Vehicle vs. BaP). On d2 post-radiation, 10 mGy treatment increased IL-2 (BaP group), IL-4 (Vehicle group), and IL-17 (BaP group) secretion while IL-6 (Vehicle group) and IL-10 (BaP group) production was decreased by higher radiation doses.

Conclusions: These data suggest that treatment with BaP and LDR significantly affect the immune system with changes being manifested in splenocyte phenotype as well as function (cytokine secretion). Interestingly, 10 mGy, but not 1000 mGy, treatment often mitigated the deleterious effect of BaP (i.e. by preventing the inhibition of T cell activation and inflammatory cytokine secretion). The long term consequences of these effects are currently under investigation in our laboratories. A/J mice injected with BaP have been subjected to repetitive dosing with LDR (10, 20 and 100 mGy; every 2 weeks for a total of six sessions of irradiation starting at 4 weeks post-BaP injection) in order to determine whether LDR inhibits lung cancer prevalence by 46 weeks post-BaP. Independent studies in our group (Belinsky and Lin) indicate that control mice injected with carcinogens such as BaP develop adenocarcinomas by this time point. It is our hypothesis that LDR will attenuate the induction and/or progression of lung cancer in these mice. In parallel, we will investigate the consequence of repetitive LDR on the immune system of similarly treated A/J mice. Our findings on the effects of BaP and/or LDR on the immune system in the short follow-up setting (days 2 – 14) will guide the future studies on the effects of repetitive LDR by serving to fine-tune and narrow our endpoints that will be under investigation. In these future studies, mice will be euthanized at specific time points after each LDR exposure to examine the effect on the immune system of multiple exposures to LDR.

Acknowledgments: This research was supported by the Office of Science (BER), U.S. Department of Energy Grant No. DE-FG02-09ER64783.

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