Skin Tissue Response to Low Doses of Ionizing Radiation

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Inflammatory responses following moderate- and high-dose exposure to ionizing radiation are well documented from *in vitro* and *in vivo* studies. Additional results indicate atomic-bomb survivors developed chronic increases in the levels of inflammatory markers, which may account for the increased risk of diseases in this population. Since there are few studies examining the effects of low doses of radiation on inflammatory responses, we have been investigating whether low doses of ionizing radiation influence the secretion of inflammatory proteins into the growth media using the MatTek EpiDerm FT, a human skin model. To accomplish this, we used both protein microarrays and ELISA assays to quantify numerous inflammatory proteins, including cytokines and chemokines. Initial microarray experiments indicated that a number of inflammatory proteins increased in the skin tissue following a 10cGy dose of ionizing radiation at 24, 48 and 72 hrs post irradiation. To validate the microarray data we used ELISA methods to quantify four proteins (G-CSF, GM-CSF, IL-8 and MIP-3 alpha) in media. Results from this study did not confirm the original response and instead showed considerable variability in the inflammatory response of the skin tissue after radiation exposure.

In an effort to identify the sources of variability we conducted studies where the MatTek tissue samples were allowed to acclimate for several days after arrival at our laboratory instead of the 30 hrs used for the first experiment. Based upon characterization studies it was decided that the ideal equilibration time for the skin tissue was five to six days prior to radiation exposure. Additionally, there were concerns about the skin tissue feeding method. MatTek recommends that the tissue be fed with 3.0 - 5.0 ml of media every 48 hours. In the previous experiments, 3.0 ml of media was completely changed every 24 hours. This complete change of media might result in a stress to the tissue and additionally it removed potentially important inflammatory signalling factors from the media. Therefore, we choose to adopt a feeding modification whereby one ml of media is replaced every 24 hours with one ml of fresh media, leaving 2ml of the original media in place at each feeding time.

Implementing the modifications outlined above, two additional experiments were undertaken. The tissue was equilibrated for six days before radiation exposure and starting the day of the exposure only 1 ml out of a total of 3 ml of media was exchanged for fresh media every 24 hours. Additionally, both a TPA (12-*O*-tetradecanoylphorbol-13-acetate) positive control and a

high dose (200cGy) of radiation was integrated into the experimental design. In the first experiment two proteins (IL-8 and MCP-2) were analyzed by ELISA, whereas in the second, three proteins (G-CSF, IL-8 and MCP-2) were analyzed by ELISA and 20 proteins were analyzed by ELISA protein microarray. In both experiments, radiation did not significantly alter the secretion of G-CSF, IL-8, or MCP-2 compared to the non-irradiated controls (Figure1). Additionally the higher dose of radiation (200 cGy) did not lead to changes in the secretion of these three proteins. However, TPA, a compound known to induce skin inflammatory responses in the mouse, resulted in a significant increase in all three proteins, demonstrating that the skin has the ability to mount an inflammatory response. Although analysis of the protein microarray and ELISA data is still preliminary, it appears that only one cytokine is induced following radiation exposure, IL-10, an anti-inflammatory cytokines. These results indicate that radiation has little or no effect on the inflammatory response of the MatTek skin tissue except to perhaps induce an anti-inflammatory response.

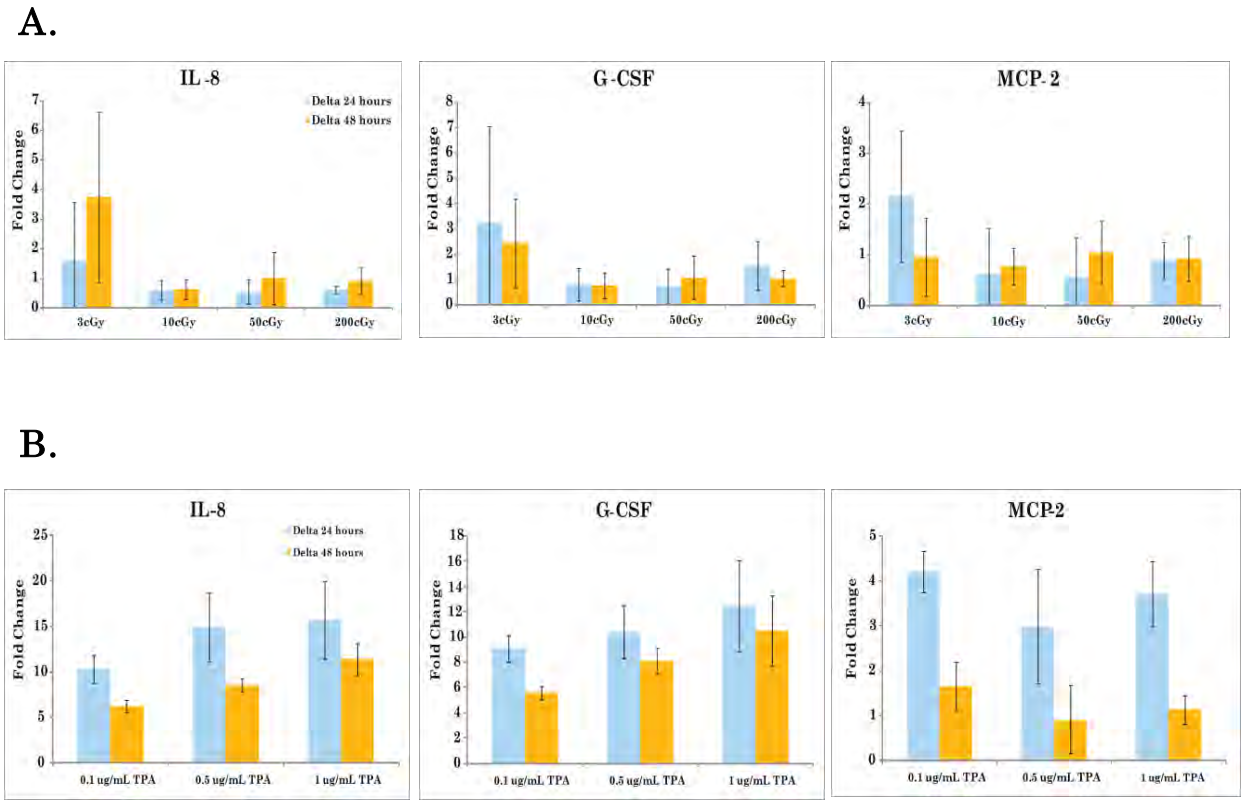

Figure 1. Effect of radiation (A) or TPA (B) on the secretion of IL-8, G-CSF, and MCP-2 by skin tissue. A) fold-changes of ratioed cytokine abundance data following radiation exposure versus non-irradiated controls. B) fold-changes of ratioed cytokine abundance data following TPA exposure versus non- exposed controls.