Roles of TNFα Signaling and NFκB in Thiol- and Low Dose Radiation-Induced Adaptive Responses

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To better investigate the roles of tumor necrosis factor alpha (TNFα) signaling processes and nuclear transcription factor κB (NFκB) activation on the induction of manganese superoxide dismutase (SOD2) mediated adaptive responses, we employed a two by two experimental matrix that includes the use of both wild type C57BL/6 and C57BL/6 tumor necrosis factor receptor 1 and 2 ($TNFR1^-R2^-$) knockout mice, and *ras/c-myc* transfected wild type and $TNFR1^-R2^-$ knockout mouse embryo fibroblasts (MEF). MEF were immortalized in order to facilitate their use in our mouse models to test the role of normal or $TNFR1^-R2^-$ stromal cells and tissues on their responses to thiol and low dose radiation exposures. MEF $TNFR1^-R2^-$ cells grown in the hind legs of wild type C57BL/6 mice and irradiated *in vivo* first with a 2 Gy dose of ionizing radiation followed 30 min or 3 h later with 10 cGy and then challenged later with an additional 2 Gy dose exhibited an adaptive response. In a parallel study, splenocytes were harvested from $TNFR1^-R2^-$ knockout mice following *in vivo* irradiation with 2 Gy only, 10 cGy followed 24 h later with 2 Gy, and 400 mg/kg amifostine administered 24 h prior to exposure with 2 Gy. Micronuclei formation was used as a genomic instability related endpoint. Consistent with the lack of a low dose radiation-induced adaptive response observed for MEF $TNFR1^-R2^-$ using survival as an endpoint, splenocytes derived from $TNFR1^-R2^-$ knockout mice exhibited no significant adaptive response. In contrast, splenocytes from animals administered amifostine 24 h earlier exhibited a significant reduction in micronuclei formation, consistent with previous *in vitro* derived data demonstrating a thiol-induced adaptive response characterized by direct NFκB activation and subsequent elevation of *SOD2* gene expression. While our studies have identified SOD2 as an important mediator in both the low dose radiation- and thiol-induced adaptive responses, we identified a unique adaptive-like response that appears to be independent of SOD2. The demonstration of this phenomenon, under *in vitro* conditions, differed from the conventional treatment protocol whereby a low dose of radiation is first administered and then at a later time a larger challenge dose is administered. Rather, a low 10 cGy dose of radiation was administered at varying times either prior to or following two larger doses of radiation administered 24 h apart. MEF $TNFR1^-R2^-$ cells that exhibited no adaptive response under the conditions of a 10 cGy exposure followed 24 h later by a single 4 Gy challenge dose, demonstrated a robust adaptive response if the 10 cGy dose was administered at varying times either prior to or following the initial 2 Gy dose followed later by a second 2 Gy dose. Survival under each of these exposure conditions was significantly elevated over values obtained for cells irradiated with 2 Gy followed 24 h later with a second 2 Gy dose. Since we observed no elevation in SOD2, and the studies were performed with MEF $TNFR1^-R2^-$ knockout cells defective in TNFα signaling, we have identified a low dose radiation-induced adaptive response that appears not to be dependent on changes in SOD2 enzymatic activity.

This work was supported by DOE Low Dose Program/Project Grant DE-SC0001271 (D.J.G.).