Long-term Effects of Low-dose Photons on Treg and Other $CD4^+$ Lymphocyte Responses to High-dose Photons and Protons

Daila S. Gridley, Xian Luo-Owen, Asma Rizvi, Adeola Y. Makinde,
Jian Tian, and Michael J. Pecaut

Purpose: The increasing prevalence of low-dose irradiation under work-related and other conditions, e.g. space missions, diagnostic/therapeutic medical procedures, and the risk for high-dose irradiation due to accidents or terrorist attacks has raised concern regarding health consequences. This study investigated the impact of low-dose/low-dose-rate (LDR) photon exposure, alone and together with high-dose irradiation events, on a variety of cellular responses. This presentation emphasizes the $CD4^+$ T lymphocytes.

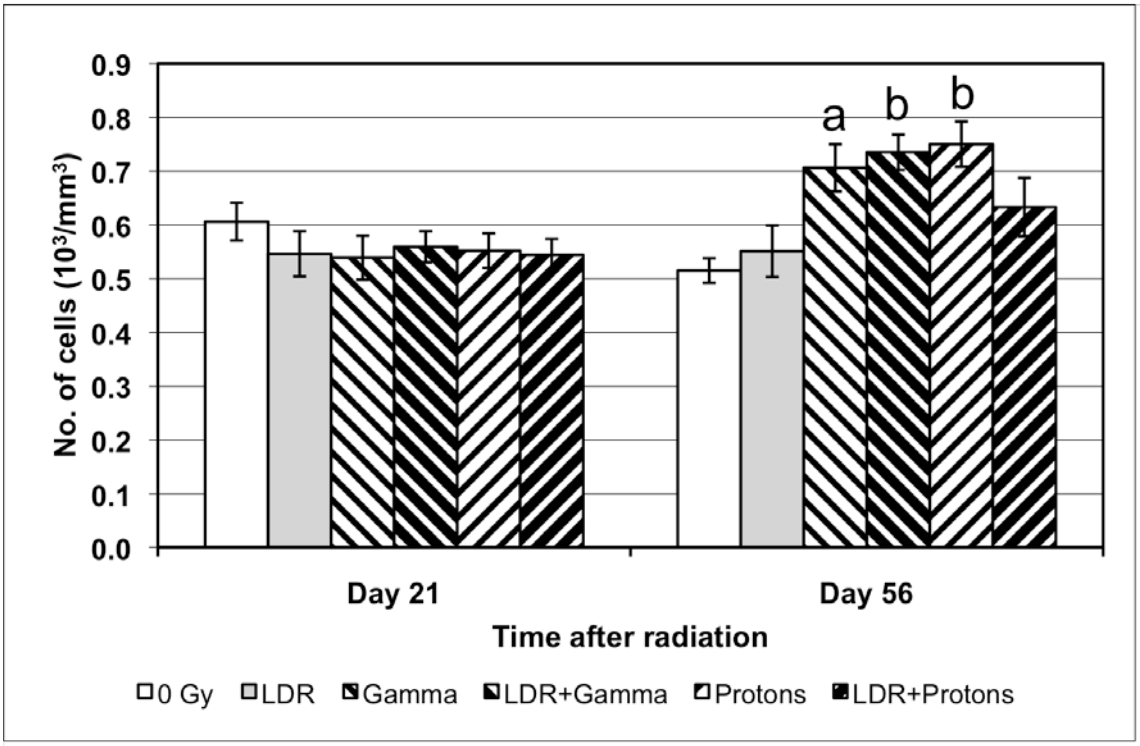
Materials and Methods: C57BL/6 mice (n = 120) were total-body irradiated with ^{57}Co γ-rays to a total dose of 0.01 Gy at 0.03 cGy/min. Different groups were exposed to 2 Gy photons (0.8 Gy/min), protons (0.9 Gy/min) alone or right after priming with LDR photons; 0 Gy controls were included. On days 21 and 56, the animals were euthanized and spleens were harvested. Analyses included tests for total white blood cell and major leukocyte population counts and proportions using an automated hematology analyzer and specific lymphocyte populations by flow cytometry. The following lymphocyte subsets were quantified: $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+CD25^+$ T cells, $CD4^+CD25^+Foxp3^+$ T regulatory (Treg) cells, $CD19^+$ B cells, and $NK1.1^+$ NK cells. The total and active forms of signaling proteins (JNK, Lck, p38MAPK) in negatively isolated $CD4^+$ T cells were quantified after activation with immobilized anti-CD3 antibody. Tissues in addition to spleen were harvested and archived; analyses are in progress.

Results: Only a portion of the data obtained for spleen are presented here. Total leukocyte counts were significantly reduced on day 21 in the LDR+Gamma, Proton, and LDR+Proton groups vs 0 Gy ($p<0.05$); lymphocytes were low in the LDR+Proton vs LDR group ($p<0.05$). Monocyte-macrophage and granulocyte numbers, as well as percentages of all three major leukocyte populations, were similar among groups at both time points. In contrast, although the $CD4^+CD25^+$Foxp3+ Treg cell counts were similar among groups on day 21, by day 56 the numbers were higher in the Gamma, LDR+Gamma and Proton groups compared to 0 Gy and the LDR+Gamma and Proton groups were higher than LDR alone ($p<0.05$). Overall patterns for the $CD4^+$ T cells, either with or without CD25, were similar to those of the $CD4^+CD25^+$Foxp3+ Treg cells. Significant differences ($p<0.05$) were also noted in the signaling proteins and either one or both time points:

Protein, form	**Day**	**Significant differences ($p<0.05$)**
JNK, active	21	LDR+Protons higher vs LDR+Gamma
JNK, active	56	LDR+Protons lower vs 0 Gy
JNK, active	56	Protons and LDR+Protons lower vs LDR
Lck, total	21	LDR+Gamma and Protons lower vs 0 Gy
Lck, total	56	LDR and Protons higher vs 0 Gy
Lck, active	56	Protons higher vs 0 Gy
p38MAPK, total	21	Protons lower vs 0 Gy
p38MAPK, total	56	LDR+Gamma and Protons higher vs 0 Gy
p38MAPK, active	21	LDR+Gamma lower vs LDR

Conclusions: The data demonstrate that protracted irradiation with low dose photons, as well as acute high-dose photons and protons, results in $CD4^+$ T cell alterations that can be detected nearly 2 months after exposure. The alterations include high levels of immunosuppressive $CD4^+CD25^+Foxp3^+$ Treg cells and differences in CD4+ T cell signaling proteins important in immune defenses.

$CD4^+CD25^+Foxp3^+$ Treg Cells in Spleen



a: $p<0.05$ vs 0 Gy
b: $p<0.05$ vs 0 Gy and LDR

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