University of Applied Sciences and Arts Northwestern Switzerland
School of Life Sciences

Effect of gamma irradiation in the structural and functional properties of splenic cells response

Deana Yanik

Bachelor thesis, Bachelor of Science in Life Sciences, Molecular Bioanalysis

Principal: Prof Dr Pieles Uwe, University of Applied Sciences Northwestern Switzerland ICB
Expert: Dr Mutz Michael, Novartis Basel
Supervisor: Dr Burgara Alexel, Universidad de Sonora

ABSTRACT

An increase in remaining dendritic cells, B- and T-lymphocytes were measured, assuming a positive effect to a better induced immune response. We have demonstrated that γ-irradiation have no effect on membrane structure in roughness (102 ± 4 nm) and a few variations in biochemical fingerprint of 3 Gy on adherent splenic cells. Decreased protein expression of anti-inflammatory marker IL-10 and increased transcriptional level of pro-inflammatory cytokines IL-1β, IFN-γ and CD36 in higher irradiation doses were shown. Cytokine profile showed the shift towards more pro-inflammatory phenotype. Furthermore, we reported tendentially increases of cytotoxic effect and phagocytosis activity of splenic cells.

"We assume that γ-irradiation will affect splenic cells phenotype, membrane roughness, biochemical fingerprint, cytokine profiles, increase of cytotoxic effect and phagocytosis activity. This effects will be dose-dependent."

INTRODUCTION

Ionizing radiation is nowadays effectively used in cancer treatments, however the effect of low-dose irradiation is poorly understood[1]. Splenic cells are mainly responsible for immune response. Irradiated macrophages have shown increased cytotoxic and phagocytosis activation and production of inflammatory cytokines[2][3]. The aim of this work was to determine the effect of γ-irradiation in different doses in structural and functional properties of splenic cells response.

RESULTS

The population mainly containing lymphocytes were clearly affected by irradiation independent of the doses, resulting in a massive reduction of its population. In contrast, the percentage of population including mainly monocytes and macrophages was increasing with rising irradiation doses. All marked cell populations for remaining dendritic cells (DCs), B- and T-lymphocytes showed an increasing tendency of cell percentage with increasing irradiation, assuming a positive effect to a better induced immune response. No structural changes of adherent splenic cell's membrane roughness measured by AFM could be found. All treatments of 0, 3, 7 and 27 Gy showed similar values of 102 ± 4 nm with high variations. The molecular composition of investigated adherent splenic cells by Raman spectroscopy were not dose-depended affected by γ-irradiation. Slightly differences in Raman peaks of 1311, 1338, 1365 1581, 1635 and 1662 cm^{-1} for irradiation dose 3 Gy were evaluated, however not significant (data not shown).

An increasing tendency of cytotoxic effect for splenic cells to HeLa in ratio 1:1 could be investigated. In contrast, HepG2 cells were not equally affected by co-culturing with splenic cells. In conclusion, the stimulation of cytotoxic and tumoricidal activity of splenic cells by irradiation as shown in previous studies[4], could be verified, however not significant. An increasing tendency of phagocytosis activity of irradiated splenic cells up to 7 Gy was evaluated, however not significant. These findings correlated with previous studies in macrophages[3]. Taken together, functional properties of splenic cells were not-significant but dose-depended affected by γ-irradiation showing increasing tendencies of cytotoxic and phagocytosis activity (Figure 1).

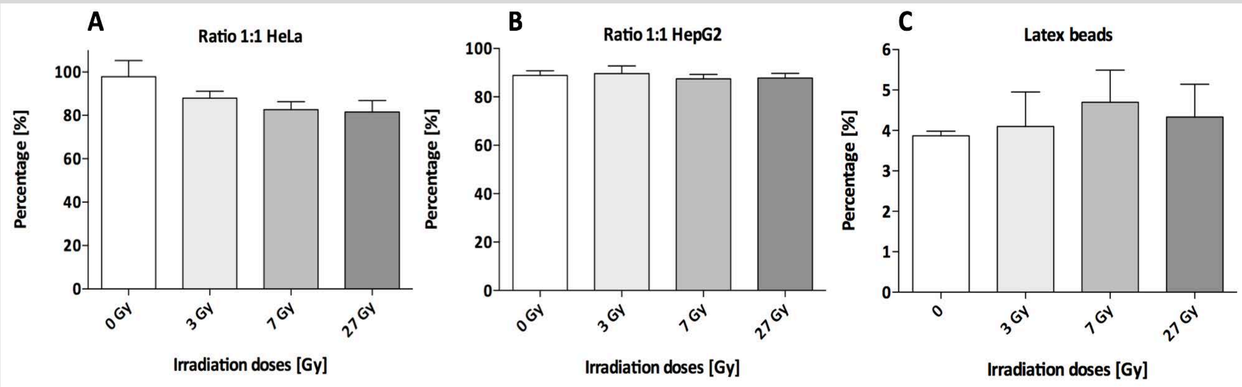

Fig. 1 | Graphs showing remaining percentage of living HeLa (A) or HepG2 (B) cells after co-culturing with splenic cells in ratio 1:1, respectively. With P-values ≥ 0.7 for HeLa and ≥ 0.38 for HepG2, no statistically relevant differences could be defined. Nevertheless, there is a decreasing tendency in percentage of HeLa cells and therefore an increasing tendency in cytotoxic effect of splenic cells. Graph C shows average percentage of splenic cells with absorbed latex beads for phagocytosis activity. An increasing tendency was observed up to 7 Gy, however not significant (P-values ≥ 0.21).

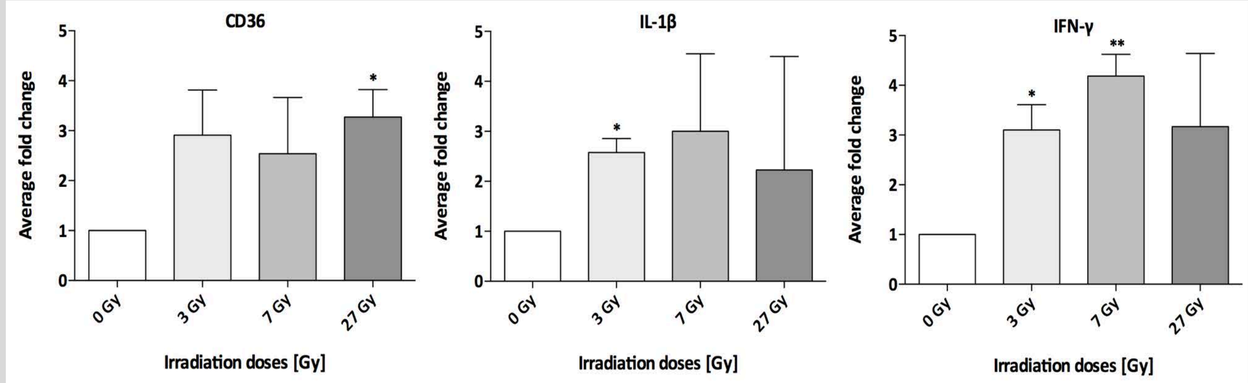

Fig. 2 | RT-qPCR-graphs of calculated average fold changes (Fc) out of mice (n = 3) for four treatments (0, 3, 7 and 27 Gy) for the cytokines CD36, IL-1β and IFN-γ, compared to non-irradiated control (0 Gy). CD36 is upregulated (Fc = 3.27 ± 0.45) in 27 Gy (P-value = 0.019), Il-1β is upregulated (Fc = 2.58 ± 0.23) in 3 Gy (P-value = 0.0103), IFN-γ is upregulated in 3 Gy (Fc = 3.11 ± 0.41; P-value = 0.0187) and 7 Gy (Fc = 4.18 ± 0.36; P-value = 0.0063). For all three cytokines, an upregulating tendency is shown with increasing irradiation doses.

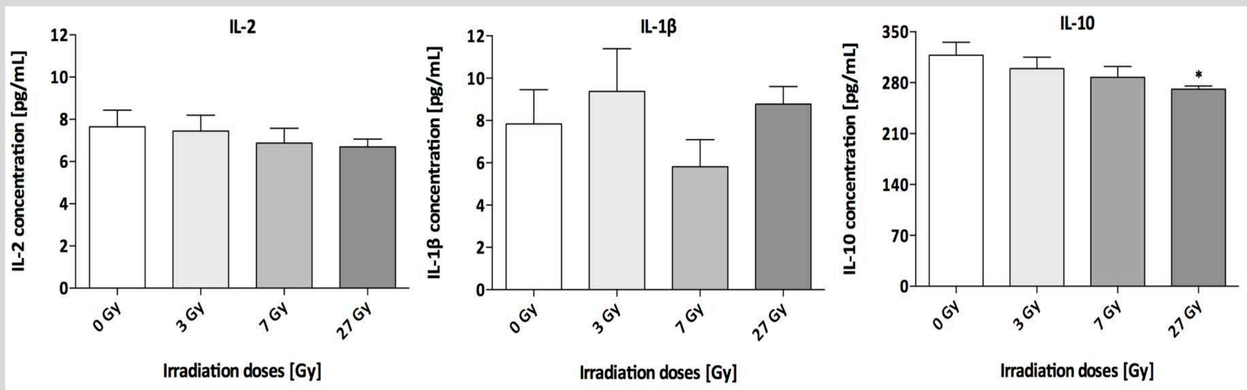

Fig. 3 | ELISA graphs show the average concentrations of IL-2, IL-1β and IL-10 of mice (n = 3) in duplicates for four treatments (0, 3, 7 and 27 Gy). IL-2 showed a non-significant decreasing tendency of its concentration with increasing irradiation doses. No clear tendency for up- or downregulation of IL-1β could be evaluated. For IL-10, a downregulating tendency was showed with increasing irradiation doses, only significant for 27 Gy (P = 0.0403).

Altogether, all three cytokines were upregulated on transcriptional level compared to non-irradiated control. In conclusion, the expression of pro-inflammatory cytokine IL-1β, auto-inflammatory cytokine IFN-γ and scavenger receptor CD36 were affected by γ-irradiation, leading to an upregulating tendency (Figure 2). Our findings verified the data of recent studies[5] showing no significant increase in expression of pro-inflammatory macrophage marker IL-1β and decreasing tendency of anti-inflammatory marker IL-10 and cytokine IL-2 (Figure 3). Higher expression of IL-1β protein in 3 Gy correlated with its upregulation in transcriptional level at same irradiation dose. The increased IFN-γ expression in combination with the decreased production of IL-10 suggests that increasing γ-irradiation is correlation with higher appearance of pro-inflammatory macrophage M1 polarization. Overall, this data suggests that irradiation directs macrophages towards an increased pro-inflammatory phenotype.

CONCLUSION

Taken together, our findings have demonstrated that doses of γ-irradiation lead to an increase in remaining cell populations such as DCs, B- and T-lymphocytes, γ-irradiation have no effect on membrane structure in roughness and only a few variations in structural biochemical fingerprint with high variations on adherent splenic cells such as monocytes and macrophages. Transcriptional cytokine profiles showed a skew towards a more pro-inflammatory phenotype with higher expression levels of pro-inflammatory cytokine IL-1β and auto-inflammatory cytokine IFN-γ, and CD36. Moreover a decreased expression of protein anti-inflammatory marker IL-10 was shown. Furthermore, we observed tendentially increases in cell functions measured as cytotoxic effect and phagocytosis activity of splenic cells displaying its function with increasing irradiation doses, assuming this changes have a positive effect to a better induced immune response.

REFERENCES

[1] Somosy et al., Scanning Microscopy **3(3)**, 895–906 (1989)
[2] Swirski et al., Science (New York, N.Y.) **325(5940)**, 612–6 (2009)
[3] Sablonnière et al., International Journal of Radiation **44(6)**, 575–584 (1983)
[4] Nowosielska et al., International Hormesis Society **10(4)**, 500–15 (2012)
[5] Pinto et al., Scientific Reports, **6(1)** 18765 (2016)

ACKNOWLEDGEMENTS: Dr Montalvo Maricela (CIAD) for support.

CIAD