Interaction of endothelial cells and lymphocytes after X-ray exposure*

N. Erbeldinger[1], *S. Meyer*[2], *J. Zimmermann*[1], *T. Dettmering*[1], *M. Durante*[1], *M. C. Cardoso*[2], *F. Rödel*[3], *and C. Fournier*[1]

[1]GSI, Darmstadt, Germany; [2]Technische Universität Darmstadt, Germany; [3]Universitätsklinikum Frankfurt, Germany

Introduction

Low-dose irradiation is an effective therapy for chronic inflammatory diseases [1,2]. We currently investigate radiation induced lowered adhesion of immune cells on the endothelial layer of the blood vessel walls as one possible modification of the in inflammatory processes. Beside alterations in the inflammatory pathways in irradiated cells, an increased frequency of apoptosis of exposed immune cells has been considered as responsible for a lowered adhesion [3].

Materials and Methods

X-ray irradiation (250 kV, 16 mA) was performed with isolated peripheral blood lymphocytes (PBL) or endothelial cells (EC). After exposure of either PBL or EC, the adhesion of unirradiated or irradiated PBL to TNF-α-stimulated (1 ng/ml) EC (primary HMVEC or Ea.hy.926 cells) was tested at 24h under static conditions. Cell death of PBL was quantified by flow cytometric analysis of annexin/propidium-iodide stained PBL. In irradiated EC, TNF-α induced NFκB signalling was analysed in parallel by quantification of the nuclear translocation of p65.

Results and Discussion

Fig. 1 shows that the adhesion of unirradiated PBLs to HMVEC is enhanced by TNF-α treatment (Fig. 1A), mimicking an inflammatory environment. Previously, we have shown for TNF-α treated HMVEC, which have been exposed to low dose irradiation, that the adhesion of PBL is decreased [4]. However, as shown in Fig. 1A, the adhesion of irradiated PBL to stimulated HMVEC displays also a discontinuity at low doses (minimum at 0.5 Gy). This is not reflected by the induction of apoptosis (Fig. 1B), indicating that cell death can only be a factor for the lowered adhesion at high doses, not at low doses.

Furthermore, TNF-α induced NFκB signalling was assessed after radiation exposure of EC (Ea.hy.926) as a putative molecular basis of the lowered adhesion of PBL to EC. In the presence of TNF-α, in 40% of unirradiated EC show nuclear translocation of p65, but irradiation did not modify this (Fig. 2), although adhesion was lowered (not shown). These results show that low dose exposure to PBL or EC reduce the adhesion of PBL to EC, but not based on radiation induced cell-death and modification of NFκB signaling.

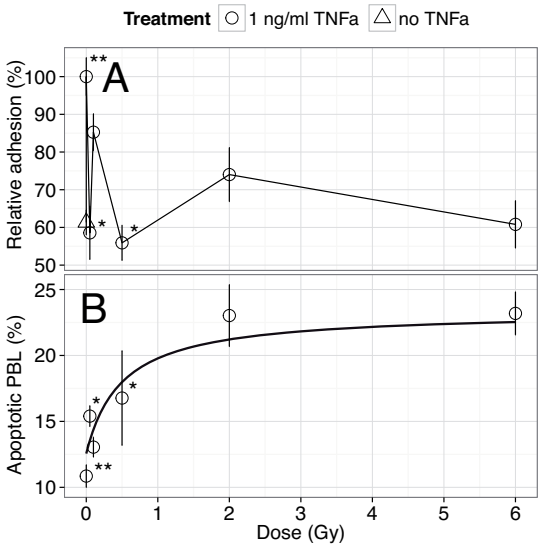

Figure 1: (A) Adhesion of irradiated PBL to stimulated and non-stimulated EC under static conditions. (B) Apoptotic frequencies of irradiated PBL under static conditions. N = 3 (triplicates); *N = 2; **N = 7

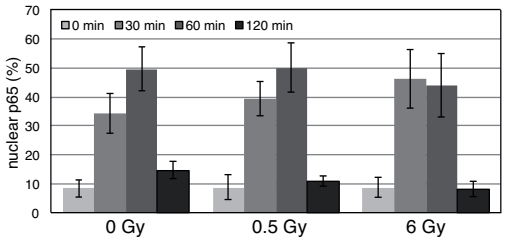

Figure 2: Ea.hy.926 cells expressing GFP-tagged p65(RelA). TNF-α treatment results in a 40% increased p65 transient activation. N = 2

References

[1] Rödel *et al.* (2007), Int J Radiat Biol 83(6): 357–366.

[2] Trott *et al.* (1999), Radiother Oncol 51(3): 197–203.

[3] Kern *et al.* (2000), Radiother Oncol 54(3): 273–282.

[4] Klinger *et al.* (2013), GSI Report 2013-1.

* Financially supported by GREWIS (02NUK017A and 02NUK017D) and FOI Bad Gastein

doi:10.15120/GR-2014-1-BIOPHYSICS-10