

Cytokine release and adhesion of immune cells to cardiac endothelial cells *

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Introduction

The cardiovascular system is nowadays well established as an organ of risk for accidental and therapeutic irradiation. Exposure of the cardiovascular system even with low doses (up to 2 Gy) can lead to an increased risk for the development of cardiovascular diseases (CVD) [1,2]. The underlying mechanism of developing CVD is putatively based on changed cytokine release of vascular cells (endothelial cells and smooth muscle cells), although their interactive pattern is still not understood [3]. In order to investigate the effect of low dosed X-irradiation on the first steps of CVD, we measured cytokine release and adhesion of immune cells after irradiation of cardiac endothelial cells, mimicking the inner layer of an *in vivo* blood vessel. Following we discuss the release of transforming growth factor β 1 (TGF- β 1), a cytokine which can have both profibrotic and anti-inflammatory effects, and its relevance on the adhesion of peripheral lymphocytes (PBL) to an endothelial monolayer.

Material and Methods

Human coronary artery endothelial cells (HCAEC) were immortalized by *est2* expression. For analysis of cytokine release, cells were cultured for 3 days prior irradiation. After irradiation with 0.05, 0.1, 0.5 and 2 Gy X-rays, medium supernatants were collected after 4 h, 24 h, 1 week and 2 weeks and frozen at -80°C . Medium was replaced after irradiation/24 h before each time point. TGF- β 1 concentrations were quantified using ELISA (eBioscience). For functional adhesion assays, FITC-tagged PBL were purified from donor blood and incubated with an EC monolayer 4 h/24 h after irradiation. The adhered PBL were microscopically quantified. Data were analyzed using a custom pipeline written in the R language.

Results and Discussion

In Figure 1A the release of TGF- β 1 after irradiation of an EC monolayer is shown 4 h after irradiation. In comparison to the unirradiated control, there is a trend towards an increased release after low doses (0.05 to 0.5 Gy), but no change after high doses (2 Gy). In comparison to those results, Figure 1B illustrates the relative adhesion of PBL to an EC monolayer. After irradiation with low doses (0.05 –

0.1 Gy), there is no change in adhesion of PBL detectable, whereas for high doses (0.5 – 2 Gy) increased adhesion takes place.

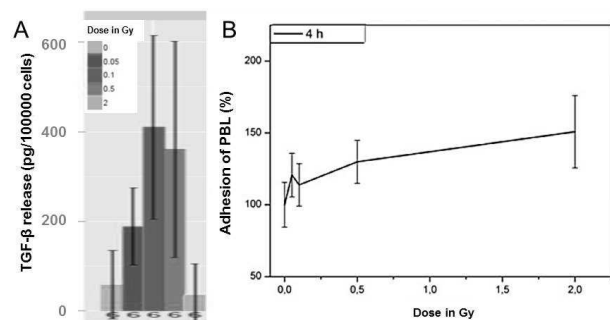


Figure 1: TGF- β 1 release and adhesion of PBL 4 h after irradiation of an EC monolayer. N=2, n=6.

Cytokine release is well described to mediate attraction of immune cells to damaged tissue, i.e. after irradiation [4]. TGF- β 1 has pleiotropic properties, since it is a known anti-inflammatory and profibrotic cytokine. The presented results 4 h after irradiation show correlation between no changes in relative adhesion of immune cells and increased TGF- β 1 release for low doses (0.05 – 0.1 Gy). After irradiation with the highest dose X-ray (2 Gy), no changes in TGF- β 1 release can be detected in parallel to an increased relative adhesion. This implies dependence between adhesion of immune cells and TGF- β 1 release, but cannot explain the increased TGF- β 1 release and in parallel increased adhesion after 0.5 Gy. This points toward an influence of TGF- β 1 on the adhesion of immune cells, but shows no exclusive interference between both mechanisms. This is in good agreement of the current dogma, that stimulation of the immune system is a complex process, orchestrated by multiple cytokines and other factors.

References

- [1] Boerma and Hauer-Jensen. *Cardiol Res Pract.* 2010 Oct 4;2011. pii: 858262.
- [2] Adams et al. *Semin Radiat Oncol.* 2003 Jul; 13(3):34656.
- [3] Schultz-Hector et al. *Int J Radiat Oncol Biol Phys* 2007 Jan 1; 67(1):108.
- [4] Libby et al. *Nature.* 2011 May 19;473(7347):317-25. doi: 10.1038/nature10146.

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