Response of bone marrow progenitor cells to ionizing irradiation*

A.Groo[1], *D.Kraft*[1], *M.Hirschmann*[1], *L. Deloch*[3], *B. Frey*[3], *U.S. Gaipl*[3], *M.Durante*[1,2], *and C.Fournier*[1]

[1]Helmholtzzentrum für Schwerionenforschung GSI; [2]Technische Universität Darmstadt; [3]Universitätsklinikum Erlangen

Introduction

In the bone marrow, hematopoietic stem cells (HSC) and mesenchymal stem cells (MSCs) are responsible for the renewal of blood and skeletal structure of the body [1]. The bone metabolism is related to the tight balance between progenitor cells of MSCs and HSC- osteoblasts and osteoclasts (OCs), respectively. During chronic inflammatory diseases like rheumatoid arthritis (RA), the activity of bone resorbing OCs is enhanced and correlates with a high presence of inflammatory cells in the synovial fluid of RA patients [2]. An efficient treatment of RA is the exposure to low doses of ionizing irradiation, either photons or α-particles in radon galleries. Here we studied the impact of low dose X-irradiation on the differentiation and proliferation OCs and immune cells (Th17, Treg).

Material and Methods

OC and immune cells were generated from buffycoats of healthy donors (blood donor service Frankfurt). After X-irradiation, OC were generated from monocytes by adding RANKL and M-CSF. Treg/Th17 cells were differentiated from $CD4^+$ T cells in the presence of cytokines (IL-1β, IL-23, TGF-β) and stimulatory antibodies (CD3, CD8). Differentiated OC were identified by TRAP activity and F-actin ring formation, Th17 and Treg cells by immunophenotyping with anti-IL17 and anti-FOXP3 respectively.

Results and Discussion
Differentiation of OCs and immune cells (Th17, Treg) after Xray exposure

The measurement of the percentage of TRAP positive OCs 12 days following exposure to X rays (Fig. 1) showed that ionizing radiation has no influence on the differentiation of osteoclasts (osteoclastogenesis). The irradiated cells grew and fused to multinuclear OCs independent of the applied doses. The same applies for mouse osteoclastogenesis of C57BL/6 wild type and hTNF-alpha transgenic mice.

As IL-17 producing Th cells (Th17) are known to enhance osteoclastogenesis *in vitro*, we assessed the differentiation of Th17 and complementary Treg cells after irradiation. As shown in Fig. 2, after irradiation, the proportional distribution was shifted to a higher occurrence of Treg compared to inflammatory Th17 cells, which suggests an anti-inflammatory effect of low doses of X-irradiation.

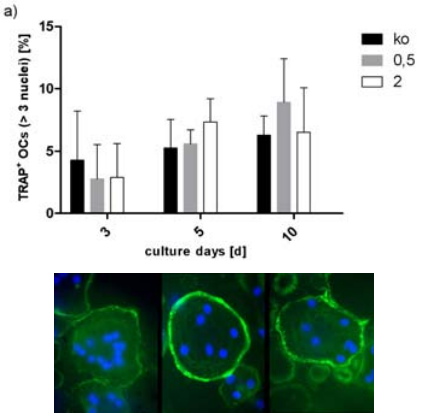

Figure 1: Frequencies of TRAP positive cells (OCs) within MNCs which were exposed to Xrays (0.5; 2 Gy) and stimulated for OCs differentiation. TRAP activity (a) and F-actin ring formation (b) were investigated. Only TRAP+ cells with >3 nuclei (DAPI) and intact F-actin ring were counted as OCs. (N=3,n=6).

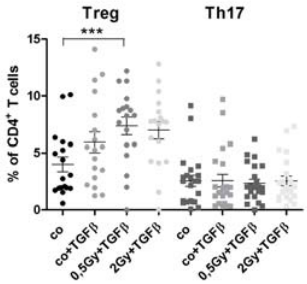

Figure 2: Human CD4+ T cells were irradiated with X-rays (0.5; 2Gy) and stimulated with CD3 and CD28 antibodies, in the presence of a cytokine cocktail (IL-1β, IL-23, and TGFβ) for 7 days. Distribution of Th17 and Treg cells were analysed by flow cytometry (N=4, n=8). Error bars represent SEM (***$p<0.001$).

References

[1] P. Bianco, 2011, Blood 117 (20) : 5281-5288

[2] Udagawa et al, 2002, Arthritis Res. 4: 281-289.

* This work was founded by the BMBF (GREWIS, 02NUK017A and 02NUK017G).

 doi:10.15120/GR-2014-1-BIOPHYSICS-08