

## Local Interleukin-4 Treatment Supports Osteoblast Function in a Murine Model of Particle Induced Osteolysis

Jukka Pajarinen<sup>1</sup>, Yasunobu Tamaki<sup>1</sup>, Allison Rao<sup>1</sup>, Heather Waters<sup>1</sup>, Katie Barcay<sup>1</sup>,  
Zhenyu Yao<sup>1</sup>, Michiaki Takagi<sup>2</sup>, Yrjö T. Konttinen<sup>3</sup>, Stuart B. Goodman<sup>1</sup>

1) Orthopaedic Research Laboratories, Department of Orthopaedic Surgery,  
Stanford University School of Medicine, Stanford, CA, USA

2) Department of Orthopaedic Surgery, Yamagata University School of Medicine, Yamagata, Japan

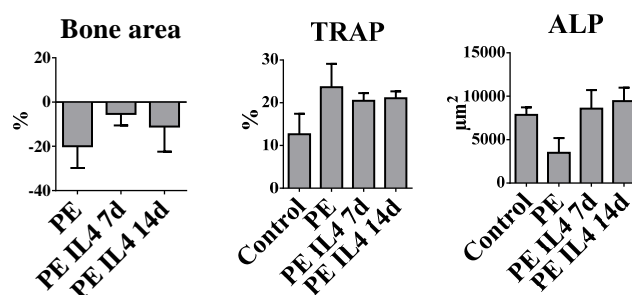
3) Department of Medicine, Institute of Clinical Medicine, University of Helsinki, Helsinki, Finland

**Statement of Purpose:** Aseptic loosening is one of the main long-term complications of total joint replacement. Peri-implant osteolysis is driven by chronic inflammation caused by implant derived wear particles. Biomaterial wear particles are phagocytosed by macrophages which are activated and secrete chemokines and pro-inflammatory cytokines. Macrophage-derived chemokines lead to further macrophage and osteoclast precursor cell recruitment while inflammatory cytokines increase local osteoclastogenesis and suppress osteoblast formation and function. Thus this wear particle-induced macrophage activation causes the balance of local bone remodeling to shift from bone formation to bone resorption, ultimately leading to peri-implant osteolysis. *In vitro* and *in vivo* studies have demonstrated that modulation of macrophage phenotype towards M2 alternative macrophage activation by local IL-4 treatment prevents this particle-induced macrophage activation with subsequent reduction in osteoclast formation and bone resorption (1, 2). The possible impact of IL-4 treatment on cells of the osteoblast-lineage and bone formation is, however, poorly understood. In this study, the effects of local IL-4 administration on the cells of the osteoblast-lineage were evaluated using the mouse calvarial model of particle-induced osteolysis.

**Methods:** Polyethylene particles suspended in saline were injected once over the sagittal suture of 15 C57BL/6, 8-10 week old, male mice. A control group of 5 similar mice received a saline injection only. Of the 15 particle treated mice, three groups were defined: 5 mice were left untreated and 10 mice were treated with daily IL-4 injections (1 µg) to the subcutaneous bursa overlying the calvarium. Of these IL-4 treated mice, 5 were treated with IL-4 for the whole duration and 5 for half of the duration of the experiment, with injections starting 7 days after particle injection. After 14 days, the mice were euthanized and the calvaria harvested for histomorphometric and immunohistochemical analyses. The extent of calvarial osteolysis was evaluated by measuring the total area of bone 700 µm left and right of sagittal suture. Osteoblasts and osteoclasts were identified by immunohistochemical alkaline phosphatase (ALP) and histochemical tartrate-resistant acid phosphatase (TRAP) staining respectively. The sections were imaged and the total area of ALP<sup>+</sup> cells and the percentage of bone surface covered by TRAP<sup>+</sup> cells quantified using imageJ (NIH).

**Results:** Particle treatment induced local osteolysis reflected by decreased bone area compared to controls (Figure 1). Corresponding increase in the percentage of

bone surface covered by TRAP<sup>+</sup> osteoclasts and decrease in the area of ALP<sup>+</sup> osteoblasts was observed in the particle treated group compared to controls (Figure 1). IL-4 treatment for either 7 or 14 days partially reversed these particle-induced effects so that bone loss and the percentage of bone surface covered by osteoclasts were reduced. IL-4 treatment for either 7 or 14 days also mitigated the particle-induced reduction in osteoblast expression (Figure 1).



**Figure 1.** Polyethylene particles were injected over the murine sagittal suture. Five mice each were treated with local IL-4 injections over the calvarium, either for the whole duration (14d), or for half of the duration (7d) of the experiment with injections starting 7 days after particle injection. After 14 days the extent of osteolysis was evaluated by quantifying bone area in histological sections. Osteoblasts and osteoclasts were identified by ALP and TRAP staining followed by image analysis. Bar diagrams show the percentage of bone area compared to controls; percentage of bone surface covered by TRAP<sup>+</sup> osteoclasts; and the total area of ALP<sup>+</sup> osteoblasts in different treatment groups

**Conclusions:** Wear particles caused local osteolysis with a reduction in calvarial bone area, increased numbers of osteoclasts and reduced osteoblast expression. Local IL-4 treatment either for 7 or 14 days partially reversed these particle-induced effects. These results suggest that IL-4 reduces particle-induced osteolysis not only by reducing osteoclast-mediated bone resorption but also by supporting osteoblast formation and function. Local IL-4 administration may provide a novel, minimally invasive treatment to mitigate particle-induced osteolysis.

**References:** 1) Rao AJ. J Biomed Mater Res A. 2013;101:1926-34. 2) Pajarinen J. Acta Biomater. 2013;9:9229-40.

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