INTRODUCTION: Osteolysis around orthopaedic implants is thought to be due to stimulation of osteoclast differentiation induced by bone resorptive cytokines produced in response to orthopaedic wear debris [1]. We have shown that stimulation of osteoclast differentiation by titanium particles in an in vitro cell culture system depends on IL-1α, IL-1β, IL-6, and TNFα, acting synergistically [2]. The in vivo role of TNFα in particle-induced osteolysis has been demonstrated previously [3-5]. The objective of this study was to compare the in vitro and in vivo roles of IL-1 in titanium particle-induced osteolysis.

MATERIALS AND METHODS: IL-1 receptor (−/-) mice were obtained from Jackson Laboratory. All mice were 4 to 6 weeks old and female. Age matched C57BL/6 female mice (Jackson Laboratory) were used as a wild-type control group with matched genetic background. All animal experiments were approved by the CWRU IACUC. Commercially pure titanium (cpTi) particles (Johnson Matthey, 75% <6.5μm [6]) were suspended in phosphate buffered saline (PBS) at a concentration of 12.6 x 10^5 particles/ml. 40 ul of PBS or cpTi were implanted on the surface of bilateral parietal bones of mice. After 5 days, mice were sacrificed and the parietal bones and overlying fibrous tissues were harvested. Quantitative analysis of osteolysis in the parietal bone was performed by histomorphometry of microradiographs [7]. Levels of TNFα and IL-6 in the fibrous tissue were measured by ELISA. All data are presented as means ± SEM. Statistical analyses were performed by ANOVA with Fisher’s Protected LSD post hoc test.

The effect of IL-1 receptor antagonist (IL-1ra, R&D System) on osteoclast differentiation was assessed in our previously described cell culture model [8]. Nucleated marrow cells from C57BL/6 mice were cultured with cpTi particles for 24 hours. The conditioned media was co-cultured with osteoclast precursors isolated from spleens of C57BL/6 mice and CMC-4 preosteoblastic cells with various concentrations of IL-1ra. Osteoclast differentiation was quantified by counting the number of tartrate-resistant acid phosphatase-positive multinucleated cells after 9 days of co-culture.

RESULTS: We have previously shown that neutralizing antibodies against IL-1α, IL-1β, IL-6, or TNFα each block cpTi particle-induced osteoclast differentiation in vitro by 60-80% [2]. Figure 1 shows that IL-1ra, which inhibits both IL-1α and IL-1β, almost completely blocks (p<0.0001) cpTi particle-induced osteoclast differentiation in vitro. In contrast to the in vitro results, IL-1 receptor (−/-) mice, which are unresponsive to both IL-1α and IL-1β, are not protected from osteolysis induced in vivo by cpTi particles (Fig. 2). In fact, the IL-1 receptor (−/-) mice showed about 60% more osteolysis (p<0.001) than the wild-type mice. We hypothesized that the lack of protection and the increased osteolysis in the IL-1 receptor (−/-) mice are due to compensatory increases in production of other cytokines. To test whether production of IL-6 or TNFα is increased in the (−/-) mice, we measured levels of these cytokines in the fibrous tissues overlying the calvaria. Figure 3 shows that TNFα production is indistinguishable between the IL-1 receptor (−/-) mice and the wild-type mice. In contrast, IL-6 production is reduced by ~50% in the IL-1 receptor (−/-) mice (Fig. 4).

DISCUSSION: Inhibition of IL-1 bioactivity with IL-1ra completely blocks cpTi particle-induced osteoclast differentiation in vitro. In contrast, inhibition of IL-1 bioactivity in IL-1 receptor (−/-) mice increases cpTi particle-induced osteolysis in vivo. The in vivo compensatory responses to IL-1 receptor ablation are not due to up regulation of IL-6 or TNFα production. Instead, it is likely that they are due to up regulation of other, as yet unidentified, pro-inflammatory mediators or down regulation of anti-inflammatory mediators.

IL-1 receptor ablation caused a reduction in cpTi particle-induced IL-6 production. These results could be due to a positive feedback effect of IL-1 that leads to an increase in cpTi particle-induced IL-6 production. Consistent with this hypothesis, IL-1 stimulates IL-6 production by many cell types, including macrophages [9].

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REFERENCES: