COMPARISON OF THE ROLES OF IL-1, IL-6, AND TNFα IN CELL CULTURE AND MURINE MODELS OF ASEPTIC LOOSENING

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INTRODUCTION: Aseptic loosening is thought to be initiated by pro-inflammatory cytokine production in response to wear particles. Thus, inhibition of TNFα or IL-1 activity inhibits particle-induced responses. Moreover, polymorphisms in TNFα and IL-1RA are associated with an increased frequency of aseptic loosening. Less is known about IL-6, the third major pro-inflammatory cytokine. The current study compared the roles of IL-6, IL-1, and TNFα during induction, by titanium particles, of osteoclast differentiation in vitro and osteolysis in vivo.

METHODS: In vitro: Conditioned media were harvested from murine marrow cells cultured for 24 hours ±commercially pure titanium particles (cpTi, Lot G11G04, Catalog #00681, Johnson Matthey, 90% <3.6 μm, 20-40 Endotoxin Units/10^9 particles). Osteoclast differentiation was induced by adding the conditioned media to co-cultures of osteoclast precursors and CIMP-4 calvaria cells [1]. The role of specific cytokines was assessed using neutralizing antibodies or IL-1RA (R&D Systems). N = 5-6 for all groups.

In vivo: Knock out (KO) mice were compared with wild-type mice with matched age, gender, and genetic background. IL-1 receptor single KO, TNF receptor-1/TNF receptor-2 double KO mice were from Jackson Labs. IL-1 receptor/IL-6 double KO mice were derived by crossing the two single KO mice strains. Osteolysis induced by the cpTi particles was assessed using the murine calvarial model [2-3]. Osteolysis was analyzed by 4 methods: in the entire parietal bones + subtraction of the sham controls and in the central suture region + subtraction of the sham controls. Statistics: ANOVA w/ Fisher’s LSD post hoc tests (SigmaStat, SPSS).

RESULTS: In vitro: All of the cytokine antibodies dose-dependently inhibited particle-induced osteoclast differentiation. Maximal inhibition was 77% by the anti-IL-1α antibody (Fig. 1A, p<0.0001), 71% by the anti-IL-1β antibody (Fig. 1A, p<0.0001), 66% by the anti-IL-6 antibody (Fig. 1B, p<0.0001), and 84% by the anti-TNFα antibody (not shown, p<0.0001).

DISCUSSION: Osteoclast differentiation induced in vitro by titanium particles depended on IL-1α, IL-1β, IL-6, and TNFα. In contrast, KO of IL-6 or receptors for IL-1 or TNFα did not convincingly reduce particle-induced osteolysis in vivo. The difference between the in vivo and in vitro results is likely due to two factors. First, the cytokines are more likely to compensate for each other in KO mice than in antibody experiments. Second, the low signal to noise ratio in vivo makes it difficult to detect the effects of gene KO. Thus, the cell culture method is more useful for demonstrating the role of individual cytokines.

The low signal to noise ratio in vivo makes it necessary to study relatively large numbers of mice. For example, two groups initially reported that IL-6 KO significantly protects mice from particle-induced osteolysis [4-5]. However, both groups found that examination of larger numbers of mice did not replicate the initial findings (this study and O’Keefe, personal communication). Thus, studies of osteolysis with small numbers of mice should be considered preliminary. In fact, a power analysis showed that the required N is 11 mice per group for the entire bone method and 10 mice per group for the suture region method (α = 0.05, power = 0.8, Δ=0.5).

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