Background: Aseptic loosening is a common limitation of total joint arthroplasty and results in prosthetic failure in up to 20 % of patients. Currently there are no pharmacologic therapies for the prevention or treatment of aseptic loosening. Loosening of the implant is associated with the generation of microscopic wear debris particles, which become phagocytosed by local macrophages, resulting in production of pro-inflammatory cytokines such as TNF-α and Interleukin-6 (IL-6). These cytokines in turn activate osteoclasts, leading to periprosthetic bone resorption and implant failure. IL-6 has been implicated in several inflammatory bone resorptive diseases including rheumatoid arthritis, and is found in extremely elevated levels in periarticular tissues and synovial fluid in patients who have developed aseptic loosening. This study examines the role of IL-6 on wear debris induced inflammatory cytokine production as well as its direct effects on osteoclastogenesis in vitro. We also examined osteoclast formation and activity in vivo using IL-6 knockout mice in a well established mouse calvarial model of wear debris-induced osteolysis.

Materials and Methods: ANA-1 mouse macrophage cells were obtained from ATCC and cultured in 24-well plates in DMEM supplemented with 10% FBS and 1% Penicillin/Streptomycin. Cells were treated with various concentrations of PMMA particles (Wright Medical Technology, Inc., Arlington, TN), and culture media was collected after 24 hours, and analyzed for pro-inflammatory cytokine production by ELISA (BD Pharmigen, San Diego, CA). Mouse splenocytes were prepared from wild type and IL-6 knock out mice and treated with 1 X 10⁷ PMMA particles/ml. After 18 hours, cell supernatants were collected, and analyzed by ELISA for TNF-α, IL-1, and IL-6 production. The effect of IL-6 on osteoclastogenesis was examined using mouse splenocytes from wild type and IL-6 knock out mice. Splenocytes were plated in 96-well plates at an initial density of 1.75 X 10⁵ in complete media supplemented with mCSF (30 ng/ml) and RANKL (100 ng/ml). The cultures were incubated at 37 °C with 5% CO₂, and media was changed every 48 hours. Following 6 days of incubation, media was removed and cells were stained for TRAP (Sigma, St. Louis, MO). Multinucleated TRAP positive cells were counted manually for each well. Splenocytes were also taken from wild type and IL-6 knock out mice who had received titanium implantation and prepared as described above. TRAP stained histological specimens were manually inspected for osteoclasts.

Results: ANA-1 cells treated with various concentrations of PMMA particles showed a dose response in cytokine production, with a maximal effect at 1 X 10⁵ particles/ml. Cell cultures treated with fewer than 5 X 10⁵ particles/ml did not stimulate cytokine production above basal levels. Splenocytes from wild type and IL-6 knock out mice were stimulated with a maximally stimulatory dose of PMMA particles. Particle stimulation exhibited a marked increase in induced inflammatory cytokine production as well as its direct effects on osteoclastogenesis in vitro. We also examined osteoclast formation and activity in vivo using IL-6 knockout mice treated with PMMA particles. There was a significantly reduced number (2-fold) of TRAP+ multinucleated osteoclasts in splenocyte cultures taken from IL-6 knockout mice as compared to wild type cultures (Figure 1). In vivo titanium implantation resulted in a 2-fold increase in sagittal suture area (p < 0.05) over sham animals (Figure 2). IL-6 knock out mice exhibited a marked reduction in both sagittal suture area (p < 0.05), and osteoclast numbers (p < 0.05) as compared to controls. Splenocytes taken from IL-6 knockout animals also exhibited a significant reduction in TRAP+ multinucleated osteoclasts as compared to wild type animals.

Discussion: This study demonstrates that IL-6 plays an important role in osteoclastogenesis and inflammatory bone loss. Loss of function of IL-6 illustrated a marked reduction in both osteoclastogenesis and osteolysis both in vitro and in vivo. This study identifies IL-6 as a potential therapeutic target for the treatment and prevention of aseptic loosening, and implant failure. Further studies will be required to determine delivery methods of anti-IL-6 agents, as well as their safety and efficacy, and may potentially lead to clinical trials involving patients who exhibit evidence of aseptic loosening.

**Figure 1:** Loss of IL-6 inhibits osteoclast formation in splenocytes treated with PMMA particles. Splenocytes were incubated for 6 days and treated with M-CSF and RANKL. Data represents the mean of 4 independent experiments ± SEM.

**Figure 2:** Loss of IL-6 markedly reduces bone resorption in vivo. Mice were sacrificed on day 10, and bone resorption quantified. The graph depicts the mean of 5 separate animals ± SEM. Sham animals did not receive Ti during surgery.