The Effect of Continuous and Local IL-4 Delivery on Systemic Macrophage Trafficking and Polyethylene Particle Induced Bone Loss

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Introduction: Aseptic loosening remains one of the main long-term complications of total joint replacement surgery. Peri-implant osteolysis and subsequent total joint replacement loosening are driven by macrophage-mediated inflammation to implant-derived ultra-high molecular weight polyethylene (UHMWPE) and other biomaterial wear particles. Particle activated macrophages secrete chemokines and pro-inflammatory cytokines that lead to further macrophage recruitment, increased osteoclastogenesis, and suppression of osteoblast formation and function. Together these changes create a microenvironment which favors bone resorption over bone formation, thus ultimately leading to peri-implant osteolysis and implant loosening. Several studies have shown that induction of M2 macrophage polarization by IL-4 treatment mitigates this biomaterial particle-induced inflammation in vitro (1, 2). Similar results were obtained in vivo using a mouse calvarial model in which local IL-4 injections reduced polyethylene particle-induced inflammation and osteolysis (3). In this study, the effect of local, continuous, IL-4 delivery on systemic macrophage trafficking and UHMWPE particle induced osteolysis were investigated in a murine continuous femoral intramedullary particle infusion model (4) using bioluminescence imaging (BLI) and μCT.

Methods: The animal protocol was approved by the Stanford University Animal Care Committee. Alzet model 2006 miniature osmotic pumps were loaded either with carrier solution (control group); carrier solution with 0.48±0.10 μm diameter UHMWPE particles at a concentration of 15 mg/ml (PE group); or carrier solution with 15 ml/ml UHMWPE particles and 10 µg/ml mouse recombinant IL-4 (PE+IL-4 group). Pumps were then connected to hollow titanium rods via 6cm long vinyl tubing and implanted in the subcutaneous tissues in the backs of 8 to 12 weeks old, male BALB/cByJ mice under isoflurane anesthesia and buprenorphine analgesia. A subcutaneous tunnel reaching the right knee was made for the tubing. A lateral parapatellar arthrotomy was performed to access the intercondylar notch of the right distal femur after which a series of needles was used to drill through the notch to gain access to the medullary cavity. A titanium rod, connected from the one end to the pump via tubing, was then press fit into the drill hole resulting in a continuous delivery of UHMWPE particles with or without IL-4 to the medullary cavity (4, 5). Green fluorescent protein (GFP) and firefly luciferase (FLUC) expressing mouse primary macrophages were produced by infecting BALB/cByJ-derived bone marrow macrophages with pFU-Luc2-eGFP containing lentivirus vector. GFP and FLUC expressing reporter macrophages were then injected to the tail vein of the mice implanted with PE and PE+IL-4 containing pumps after one week of the surgery. Trafficking of the reporter cells to the distal femur was observed by obtaining lateral and prone whole body BLI images of the mice in 2 day intervals up to 20 days post injection. The BLI images were quantified by measuring the total flux (photons/second) from uniformly sized regions of
interest (ROI) drawn over the right distal femur. The amount of bone at the right distal femur was quantified by obtaining µCT images immediately prior to pump implantation and then, 28 days later, when mice were sacrificed and titanium rods removed prior to imaging. A 2x2x3mm ROI was defined to the distal femur and the bone volume fraction (BVF) determined.

**Results:** The continuous infusion of UHMWPE particles lead to the systemic recruitment of reporter macrophages as was evident from strong BLI signal developing to the right distal femur already two days post injection; although decreasing, the signal persisted and was clearly visible over the 20-day imaging period. The local IL-4 delivery with UHMWPE particles increased the reporter macrophage recruitment to the peri-implant tissue and this difference between the groups reached statistical significance on days 12, 14 and 17 post injection (Figure 1a). Using µCT, the continuous infusion of UHMWPE particles lead to decreased BVF compared to the controls, while continuous IL-4 delivery with UHMWPE particles lead to increased BVF with visible mineralization in µCT images, the difference between the PE and PE+IL-4 groups being statistically significant (Figure 1b and 1c).

**Discussion:** In agreement with previous reports, continuous infusion of UHMWPE particles led to local bone loss and systemic trafficking of reporter macrophages to the peri-implant tissue (4). IL-4 delivery prevented the particle induced osteolysis but somewhat surprisingly also led to increased macrophage trafficking and/or survival. It is tempting to speculate that continuous IL-4 delivery led to local M2 macrophage polarization with decreased inflammatory response to UHMWPE particles, increased macrophage survival and increased ability of macrophages to support local bone formation. We are currently undertaking efforts to investigate these hypotheses.

**Significance:** Continuous and local IL-4 delivery is an effective means to prevent UHMWPE particle induced bone loss thus providing a possible strategy to locally mitigate wear particle-induced macrophage activation and periprosthetic osteolysis.

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**Figure 1.** UHMWPE particles were infused in mouse distal femur with or without IL-4. Systemic trafficking of GFP and FLUC expressing reporter macrophages to the distal femur was followed and quantified by bioluminescence imaging and the amount of bone at the distal femur was quantified using µCT imaging. a) IL-4 led to increased macrophage trafficking to the peri-implant tissues at days 12, 14, 17 after the systemic injection of reporter cells compared to the particle only group. b) Coronal µCT images of right mouse femurs showing the red channel with decreased bone amount in PE group and increased bone formation in the presence of IL-4. c) Bone volume fraction (BVF) at distal femur expressed as logarithm-transformed fold change (fc) to control femurs. **p<0.01, *p<0.05** as determined by Student's t-test.

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