Continuous Interleukin-4 Delivery by Osmotic Pumps Modulates Macrophage Polarization *in vitro*

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Statement of Purpose: Aseptic loosening is one of the main long-term complications of total joint replacement. Peri-implant osteolysis is driven by macrophage-mediated inflammation to implant-derived wear particles. Particle activated macrophages secrete chemokines and pro-inflammatory cytokines that lead to further macrophage recruitment, increased osteoclastogenesis, and suppression of osteoblast function. *In vitro* and *in vivo* studies have shown that induction of M2 macrophage polarization by IL-4 treatment mitigates this biomaterial particle-induced and macrophage-mediated chronic inflammatory reaction (1, 2). As a model for continuous local drug delivery, we used miniature osmotic pumps to deliver IL-4 in order to modulate macrophage polarization *in vitro* from non-activated M0 and inflammatory M1 phenotypes towards an anti-inflammatory and tissue regenerative M2 phenotype.

Methods: 12 Alzet miniature osmotic pumps were loaded with murine IL-4. IL-4 was then infused into collection vessels containing murine bone marrow macrophage (mBMM) media. This pump-conditioned media was collected in seven-day intervals up to four weeks (week 1, 2, 3 and 4 samples). IL-4 concentration in the conditioned media was measured using ELISA and its biological activity confirmed by exposing M0 and M1 mBMMs, to week 1 to week 4 pump conditioned media or fresh IL-4 for 3 days. Relative expression of TNF-α, IL-1Ra, CD206, Arg1 and IRF4 was evaluated by qRT PCR. Corresponding production of TNF-α and IL-1Ra protein was assayed from cell culture supernatants by ELISA. CD206 and Arg1 proteins were stained using immunocytochemistry followed by quantification of fluorescence intensity using image analysis (Image J).

Results: During the first week of IL-4 infusion, osmotic pumps delivered IL-4 at a rate that closely approximated the theoretical delivery rate. In subsequent weeks, IL-4 dosage was reduced to about half of the theoretical maximum (Figure 1a). Despite this reduction in the weekly dosage delivered, the biological activity of the infused IL-4 was well retained, as M0 macrophages exposed to the pump conditioned media assumed an M2-like phenotype as indicated by downregulation of TNF-α and upregulation of several M2 marker genes (Figure 1b). The magnitude of these phenotypic changes was similar in positive controls and both in macrophages exposed to week 1 and week 4 conditioned media. These changes were generally observed both at the level of mRNA and protein production (Figure 1c and 1d). Corresponding phenotype change was observed in M1 macrophages exposed to fresh IL-4 or to pump conditioned media (data not shown).

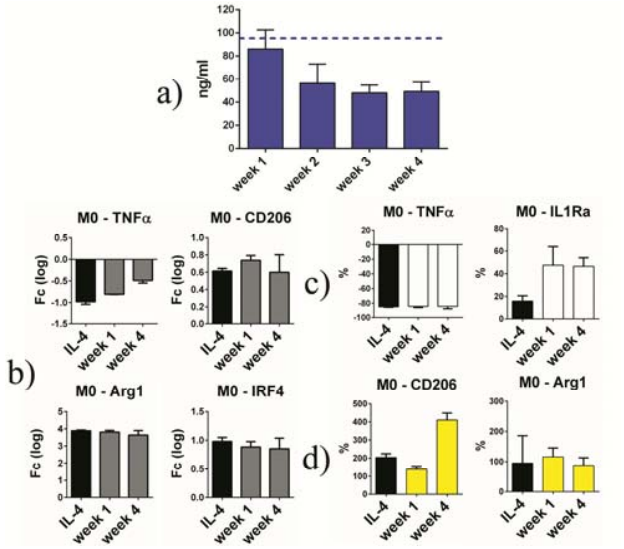

Figure 1 a) IL-4 was infused into collection vessels containing macrophage culture media for 4 weeks using osmotic pumps. Samples were collected at 7 day intervals and the IL-4 concentration in conditioned media was determined using ELISA. Blue line indicates the expected IL-4 concentration at each time point. b-d) M0 mouse macrophages were exposed to fresh IL-4 (positive controls, black bars) or either one of week 1 or week 4 pump conditioned media for 3 days after which b) relative expression of indicated M1 and M2 markers was evaluated using qRT PCR; c) production of TNF-α and IL-1Ra protein was assayed from cell culture supernatants using ELISA; and d) CD206 and Arg1 were stained using immunocytochemistry followed by quantification of fluorescence intensity using image analysis. Results expressed either as logarithm transformed fold chance to (b), or percent of (c, d), corresponding untreated cells.

Conclusions: results show that IL-4 can be locally delivered using osmotic pumps, and that IL-4 so delivered can modulate macrophage phenotype both from M0 and M1 phenotypes towards an M2 phenotype. Results build a foundation for further *in vivo* studies and provide strategies to mitigate particle-induced macrophage activation and subsequent peri-implant osteolysis by local modulation of macrophage polarization.

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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