Modulation of Macrophage Polarization in vitro Using IL-4 Delivery by Osmotic Pumps


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Introduction: Aseptic loosening of total joint replacements 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 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. 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. 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 miniature osmotic pumps (Alzet, model 2006) were loaded with mouse IL-4. Pumps were connected to a collection vessel containing mouse bone marrow macrophage culture media, and IL-4 was then infused into this media for 4 weeks. To confirm the sustained outflow of IL-4 from the pump, the conditioned media was collected at seven day intervals (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 mouse M0 and M1 bone marrow derived macrophages either to fresh IL-4 (positive controls) or to week 1 or week 4 pump conditioned media for 3 days. Relative expression of TNF-α, IL-1RA, Mnr1, Arg1 and IRF4 was evaluated by qRT PCR and corresponding production of TNF-α and IL-1RA protein was assayed from cell culture supernatants by ELISA. A set of M0 and M1 cells, grown on chamber culture slides and then exposed to conditioned media or fresh IL-4, was stained for Mnr1 and Arg1 using immunofluorescence. Mean fluorescence intensity density per cell was quantified from 3 fields of view using ImageJ (NIH).

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 both M0 and M1 macrophages exposed to the pump conditioned media assumed 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).

Discussion: Our 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 possible strategies to mitigate wear particle-induced macrophage activation and subsequent peri-implant osteolysis by local modulation of macrophage polarization.

Significance: The modulation of macrophage polarization from M1 to M2 by local IL-4 delivery might be means to mitigate wear particle-induced macrophage activation and subsequent aseptic implant loosening.

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References: ORS 2014 Annual Meeting Poster No: 0944