Time Course of Macrophage Polarization In Response to Wear Particles *in vitro*

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Statement of Purpose: Usage of a total joint replacement leads to wear and the generation of particulate debris from the articulating surfaces. Phagocytosis of this wear debris by monocyte/macrophages leads to their differentiation into activated pro-inflammatory M1 macrophages in the periprosthetic tissues. This chronic inflammatory reaction is thought to contribute to downregulation of bone formation and increased bone resorption, a process known as periprosthetic osteolysis. Repolarization of these macrophages from an activated M1 phenotype to the anti-inflammatory M2 phenotype may provide a means of slowing osteolysis and promoting bone healing. M1 macrophages can be converted into M2 by the addition of IL-4 after exposure to polymethylmethacrylate (PMMA) particles in vitro. The objective of this work is to study the time course of murine macrophage polarization in response to PMMA particles, LPS, and IL-4 in vitro, and the ability to repolarize uncommitted and M1 macrophages into an M2 phenotype. For these experiments, the cytokine release profiles of macrophages will be delineated including markers for M1 (TNFα, IL-1β, IL-6), and M2 macrophages (IL-1ra, IL-10, IL-13).

Methods: Macrophages were harvested and isolated from the femora of 10 Jackson C57BL/6J mice aged 8-10 weeks old. The cells were expanded to confluency in augmented leukocyte conditioned media (RPMI 1640 medium + 30% LCM + 10% fetal bovine serum + 1% antibiotic-antimycotic) for 1 week, and plated in 24-well plates at an initial concentration of $1x10^5$ cells/well. 7 treatment groups were defined, receiving combinations of PMMA particles (0.15% v/v), LPS (1μg/mL), and IL-4 (20ng/mL). The culture media were collected at 6, 16, 24, 48, 72, and 120 hrs, and analyzed for TNFα and IL-1ra release using commercially available ELISAs. RNA was extracted by the TRIzol method at the same time points, and quantified by real-time PCR using primers for TNFα, IL-1ra, IL-1β, IL-10, RANK, OPG. RNA levels were normalized to the 18S housekeeping gene.

Results: Cell stimulation with PMMA particles and LPS resulted in significant TNFα release, suggestive of a strong pro-inflammatory response. This response was largely suppressed by the addition of IL-4, whether administered before or concurrent with particle challenge. The suppression was effective early, and well maintained over 5 days (Fig. 1). IL-1ra release was greatest in groups receiving IL-4, particularly when IL-4 was administered after macrophages were induced to an M1 phenotype (Fig. 2). Monocyte/macrophages pretreated with a pro-inflammatory stimulus and converted to an M1 phenotype before IL-4 administration and particle challenge showed the greatest increase in the ratio of IL-1ra to TNFα secretion. The levels approached or surpassed the normal balance evident in controls, suggesting a suppression of the inflammatory response (Fig. 3). IL-4 administration decreased the expression of pro-inflammatory cytokines in macrophages challenged with PMMA particles (Fig. 4).

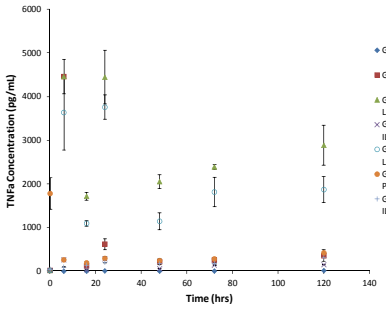

Figure 1. TNFα release in response to various treatments over time.

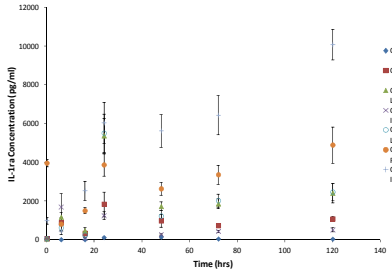

Figure 2. IL-1ra release in response to various treatments over time.

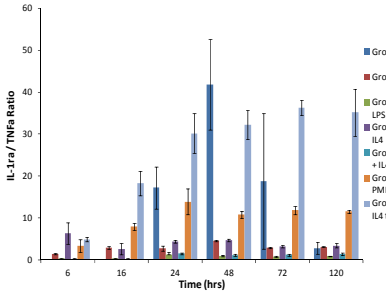

Figure 3. The ratio of IL-1ra /TNFα secretion in response to various treatments over time.

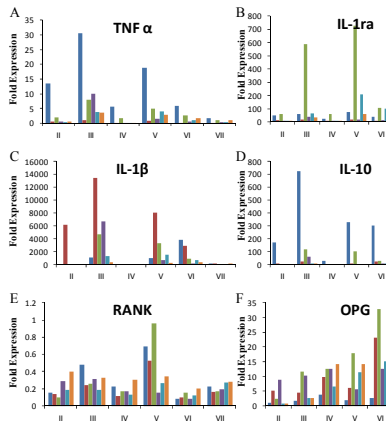

Figure 4. Fold gene expression of TNFα, IL-1ra, IL-1β, IL-10, RANK, and OPG compared to control over time.

Conclusions: Treatment of particle-challenged monocyte/macrophages with IL-4 led to repolarization into an M2 anti-inflammatory phenotype. This result was optimized when IL-4 was delivered to an M1 phenotype rather than uncommitted macrophages. The effects of this repolarization are sustained over a 5-day time course. Repolarization of M1 macrophages into an M2 phenotype may be a potential strategy to mitigate wear particle associated periprosthetic osteolysis.

Acknowledgements: Supported by NIH grants 2R01 AR055650-05 and 1R01AR063717-01.