How Estrogen Receptor Signaling Modulates the Response of Human Macrophages to Wear Particles

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Statement of Purpose: Periprosthetic osteolysis induced by particulate wear debris is the leading cause of long-term complications and failure in joint replacement surgery. Once phagocytized by macrophages, wear particles generated by the articulating surfaces will initiate the release of a series of pro-inflammatory mediators, which leads to the recruitment and maturation of osteoclast precursors and the development of periprosthetic osteolysis. In our previous study, suppression of estrogen receptor (ER) signaling in ER-deficient mice or mice receiving an estrogen receptor antagonist (ERA), showed both osteo-protective and anti-inflammatory effects in wear particle induced osteolysis. Therefore, administration of ERA may be a potential treatment for patients receiving joint replacement surgery[1]. In our current study, we further explored the effect of ER blockade on human macrophages in response to either polyethylene (PE) or polymethylmethacrylate (PMMA) particles.

Methods: Human monocyte/macrophages were isolated from buffy coats (Stanford Blood Center) of healthy female donors (F, premenopausal, age 20-40) using Ficoll-Paque PLUS (GE Healthcare) and cultured for 5 days in culture media supplemented with 5% human serum (Sigma-Aldrich) and 10 ng/mL Granulocyte-macrophage colony-stimulating factor (GM-CSF, Biolegend). Cells were then cultured for 3 days in phenol-red-free media to eliminate the effect of phenol red, a potent regulator of estrogen receptors. After maturation, macrophages were pre-treated with either 17β-estradiol (E2, 1 nM), ERA (1 μM), or phosphate-buffered saline (PBS) control for 24 hours. Then, cells were plated into 24-well plates ($1X10^5$/well) and exposed to stimulation with either PE (0.3 mg/mL) or PMMA (0.05%, v/v) particles for another 24 hours. Culture medium was collected and TNF-α release was analyzed by ELISA (Biolegend). Human macrophages were lysed and RNA was extracted using TRIzol Reagent (Invitrogen). mRNA expression was evaluated by Real-time PCR (Applied Biosystem) using primers for IL-1, IL-4, IL-6, IL-10, IL-13 and TNF-α. The 18S housekeeping gene was employed for internal normalization.

A specimen from a healthy male donor (M, age 20-40) was processed as control.

Results: ELISA analysis of TNF-α release from human macrophages showed no clear response pattern to E2 or ERA administration (Figure 1). In macrophages from female donors (F) receiving either E2 or ERA treatment, both pro- and anti-inflammatory effects were observed relative to PBS control. Response to treatment varied greatly between donors. Macrophages isolated from a male donor showed only a minor response to either pre-treatment. RT-RCR analysis was performed on the samples with the most significant TNF-α release (F 24). No obvious suppression of pro-inflammatory response was noted.

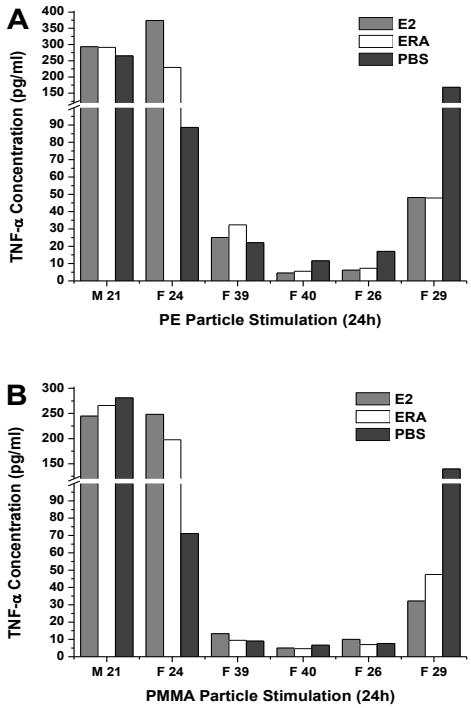

Figure 1. TNF-α release from macrophages of different donors stimulated with (A) PE or (B) PMMA particles.

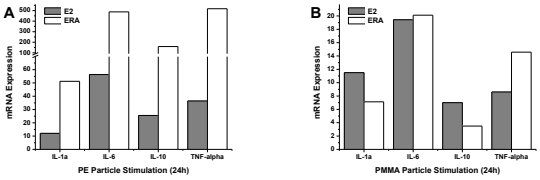

Figure 2. Relative gene expression of macrophages (F 24) stimulated with (A) PE or (B) PMMA particles.

Conclusions: Our hypothesis of the anti-inflammatory effect of ERA administration on human macrophages under wear particle stimulation was not supported by the current results. The variability observed between donors may be a consequence of individual medical history, genetic background, or menstrual cycle stage, as neither E2 nor ERA treatment had significant effects on male-derived macrophages. It is also possible that certain factors modulating ER signaling in humans were not considered or examined in this study. Further exploration upon this issue is needed.

References: [1] *Biomaterials*. 2013 Jan; 34(3):641-50.

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