Involvement of the Innate Immune System in Wear Debris Particle Induced Inflammation

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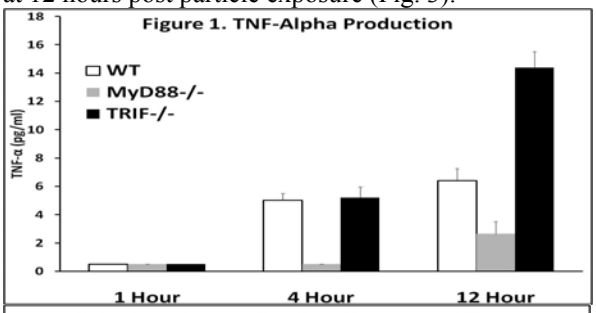
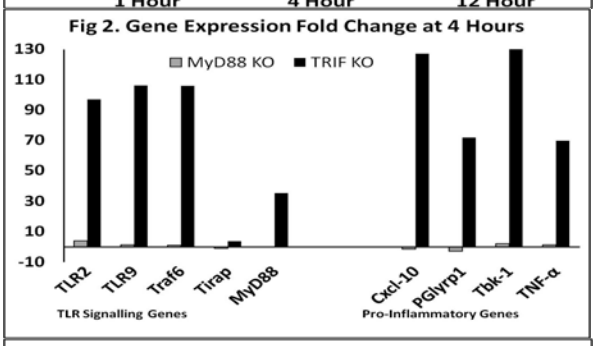
Statement of Purpose: Aseptic loosening of prosthetic joints is the most common cause of revision surgery. A major factor is the shedding of particles from the bearing surfaces, resulting in a chronic inflammatory response producing periprosthetic osteolysis with loss of native bone. Wear debris-induced osteolysis occurs in part by an increase in cytokine production and osteoclast differentiation, both of which involve the NFκB pathway. Macrophages are a key inflammatory cell involved in the process. One pathway for macrophage activation involves the innate immune system via activation of Toll-Like Receptors (TLR). When TLRs are stimulated, they interact either with TRIF or MyD88, both of which are adapter proteins coupling TLRs to downstream signaling kinases, producing activation of NFκB. To investigate the role of the TLR pathway in recognition of wear debris particles we exposed cells with either MyD88 or TRIF disrupted to wear-debris particles, comparing their *in vitro* production of the pro-inflammatory cytokine TNF-α and expression of 84 genes involved in the TLR signaling pathway and subsequent inflammatory response.

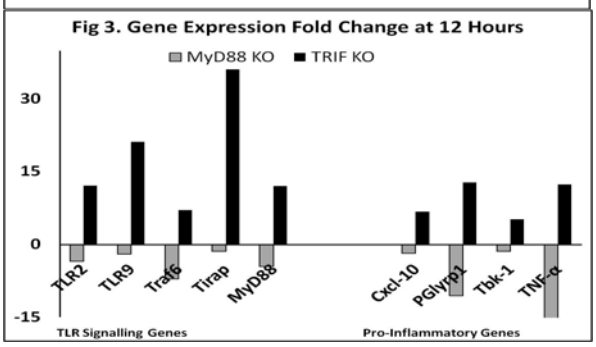
Methods: PMMA particles (Polysciences) were washed with ethanol and demonstrated free of endotoxin by a Limulus amoebocyte lysate assay (BioWhittaker). *KO cell experiments***:** Bone marrow derived macrophages isolated from C57BL/6 wild type (WT), MyD88-/- and TRIF-/- mice were cultured at $8x10^5$ cells/well for 24 hours to allow adherence. The media was then replaced with fresh media containing PMMA particles at a dose of 0.30% v/v. *Quantification of TNF-α release:* WT, MyD88 -/-, TRIF-/- cells were exposed to PMMA particles and samples from the culture media were collected at 1, 4, and 12 h post challenge. TNF-α levels were quantified using ELISA kits. *TLR Signaling Pathway Microarray:* Reverse-transcriptase polymerase chain reaction (RTPCR) was performed using probes from the TLR RT² *Profiler*™ PCR Array (SA Biosciences). Relative quantification was measured with the delta comparative threshold (CT) method after determining the CT values for reference (GAPDH) and target genes. Data are expressed as fold change of KO cells relative to WT cells.

Results: TNF-α release from WT and KO cells: WT macrophages exposed to PMMA particles exhibited a time-dependent increase in the release of TNF-α that was significant at 4 h post particle exposure and remained elevated at 12 h (Fig. 1; N=6 per group). There was a marked decrease in TNF-α release in the MyD88 -/- cells and increase at 12 hours in the TRIF-/- cells compared to WT cells (fig. 1). Each group contained an N=6.

Comparison of Expression Profiles: Relative to the WT cells, addition of PMMA particles to TRIF-/- cells markedly increased the expression of numerous pro-inflammatory genes as well as components of the TLR signaling pathway both at 4 and 12 hours (Fig. 2, 3; N=6 per group). In contrast, disruption of the MyD88 gene mitigated the PMMA particle induced increased expression of pro-inflammatory and TLR signaling genes at 12 hours post particle exposure (Fig. 3).







Conclusions: This study demonstrates the response to PMMA particles is partly dependent upon MyD88, presumably as part of TLR signaling. TNF-α production was markedly decreased with disruption of MyD88. In contrast disruption of TRIF increased TNF-α production, likely due to a compensatory increase in MyD88 expression resulting in a more robust response upon PMMA particle induced stimulation. This hypothesis is supported by the gene expression data demonstrating that TRIF-/- cells had increased expression of numerous TLR signaling pathway components, most notably MyD88. The expression of TLRs 2 and 9 which are MyD88 dependent and Tirap which is a component of the MyD88 adapter complex were markedly increased relative to WT cells. This is likely a compensatory mechanism due to lack of TRIF signaling and may explain the observed increase in both production of TNF-α and expression of numerous pro-inflammatory genes. This data indicates that TLR signaling through MyD88 may be a novel therapeutic target for prevention of particle induced periprosthetic osteolysis.