Co-alloy Particle Induced Macrophage Activation May Not Occur Via Tlr4 Dependent Mechanism

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Introduction: It is well established that a macrophage inflammatory response to particulate implant-derived debris is the main cause of periprosthetic osteolysis and aseptic loosening of implants. Inflammamome danger signaling has been identified as a the primary means of innate immune system recognition to non pathogen derived stimuli (Danger associated molecular patterns, DAMPs); however, the role of pathogen-associated receptors such as Toll-like receptors (TLRs) that detect various pathogen-associated molecular patterns (PAMPs) remains controversial1. Can highly immunogenic implant debris such as Co-alloy particles activate receptors such as the TLR4 signaling pathway that specifically sense gram negative bacteria (e.g. E. coli) surface lipopolysaccharides (LPS)? We hypothesize that TLR4 receptors are not directly activated by non-pathogenic implant debris such as Co-alloy particles and that furthermore, abrogating TL4 reactivity does not prevent Co-alloy debris reactivity. We tested this hypothesis by challenging THP-1 macrophages and human primary monocytes/macrophages with Co-alloy particles alone and in combination with lipopolysaccharide (LPS) under both control and TLR4 inhibited conditions (i.e. neutralizing activation of TLR4 receptor and it’s co-receptor CD14), and subsequently measured DAMP and PAMP reactivity using IL-1β and TNF-α, respectively.

Methods: Cell Culture: THP-1 macrophages (ATCC) and negatively isolated human primary monocytes/macrophages (using IRB approved consent) using AutomacsPro micro-beads (Miltenyi) from human PBMCs (n=2) were cultured in RPMI-1640 10% fetal bovine serum (FBS) (Hyclone Laboratories, Inc) at 37°C 0.5% Co2 and were challenged with CoCrMo alloy particles (mean diameter 0.8µm; endotoxin cleaned at <0.01Eu Kinetic QCL, provided by BioEngineering Solutions Inc, Chicago, IL) at a 5:1 (particle:cell) ratio and/or positive control LPS at 100ng/ml. PAb control (InvivoGen) was used as control isotype antibody and polyclonal antibody to human TLR4 (PAb hTLR4; InvivoGen) was used to neutralize TLR4 in THP-1 cells and human primary monocytes/macrophages according to manufacturer’s instructions. Neutralizing antibody to human CD14 (Anti-hCD14-IgA; Invivogen) or recombinant human CD14 (R&D Systems) was used to neutralize/block CD14 in THP-1 cells or human primary monocytes/macrophages respectively according to manufacturer’s instructions Supernatants were collected 16 h and assayed for IL-1β and TNF-α production. Statistical analysis was determined by standard unpaired t-tests to determine statistical significance between treated cells to their respective controls at p<0.05.

Results: THP-1 Macrophage responses: Single treatment of Co-alloy particles induced statistically significant elevated concentrations of IL-1β compared to their untreated controls (Fig.1). However, addition of anti-TLR4 or anti-CD14 did not significantly decrease IL-1β secretion in response to Co-alloy particles (Fig.1). Dual challenge of Co-alloy particles plus LPS resulted in the significant increase of IL-1β over untreated controls that was reduced to Co-alloy challenge alone levels, when TLR4 blockers were used (Fig.1). That is, IL-1β production in response to Co-alloy particles plus LPS was significantly reduced to control levels in anti-TLR4 treated cells compared to control dual challenged cells (Fig.1). Moreover, IL-1β levels were similar to that of control and anti-TLR4 Co-alloy particle treated cells (Fig.1). LPS induced significant increases in IL-1β secretion in control cells, whereas, it was significantly reduced compared to LPS treated plus anti-TLR4 or anti-CD14 cells (Fig.1). Primary monocyte/macrophage responses: Co-alloy particle challenge alone or plus LPS produced significantly more IL-1β and TNF-α secretion for both subjects (n=2; one subject shown Fig. 2). IL-1β secretion controlling by the DAMP pathways was not significantly decreased due to Co-alloy particle and LPS challenge in anti-TLR4 or soluble CD14 treated primary human monocytes/macrophages (Fig.2A). However, TNF-α production in both anti-TLR4 and soluble CD14 treated (TLR4 inhibited) cells was severely attenuated for both subjects to dual challenge of Co-alloy particles plus LPS challenge (subject one shown. Fig.2B).

Discussion: Our results support our hypothesis that Co-alloy induced macrophage activation is not due to a TLR4-dependent mechanism, but can be exacerbated by TLR challenge. Thus, dual challenge of Co-alloy particles and LPS resulted in a greater pro-inflammatory response as measured by IL-1β and TNF-α secretion levels; however, when TLR4 is inhibited, IL-1β and TNF-α production is reduced to similar levels as non-TLR4 inhibited or anti-TLR4 Co-alloy treated cells as shown by both THP-1 and human primary monocytes/macrophage cell data. In addition, inhibition of TLR4 co-receptor CD14 resulted in attenuated secretion levels of IL-1β and TNF-α as compared to control Co-alloy particles plus LPS challenged cells. Overall, our results indicate that IL-1β danger associated macrophage recognition and response to metal wear debris is not dependent on LPS related TLR membrane receptors. Further investigation with more primary human samples and other intracellular blocking agents is required to examine the role of other non-membrane bound PAMP associated pathway activators (not membrane TLR) that play a role in LPS+Particle synergistic response profiles.
Significance: Our findings suggest that TLR4 is not required or used in the recognition or induction of a pro-inflammatory response to clean Co-alloy particles in macrophages thus identifying DAMP associated pathways as potentially therapeutic.

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References: Medzhitov R. Origin and physiological roles of inflammation. Nature 2008; 454 428-