Co-alloy Particles (DAMP's) Induce Stronger Inflammasome Activation (IL-1β) in Early Macrophages Compared to Fully Differentiated Macrophages

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INTRODUCTION:

It is well established that particulate debris derived from the degradation of orthopedic implants stimulates an innate inflammatory response that leads to osteolysis and loosening of the implant overtime. Previous *in-vivo* and *in-vitro* studies have shown that pro-inflammatory cytokines IL-1β, TNFα and IL-6 are secreted in response to particulate implant debris and contribute to the osteolytic process [1]. We have also reported that the Nalp3 inflammasome "danger" signaling pathway mediates the production of IL-1β in human monocytes and THP-1 macrophages in response to Co-alloy implant debris danger associated molecular patterns (DAMPSs) [2]. However, it is still unknown whether differential implant debris reactivity (i.e. quality of the pro-inflammatory response) exists between human early macrophages (monocytes that have just extravasated into tissue) vs. fully differentiated resident tissue macrophages (histiocytes) that come in contact with particulate implant debris. Do resident tissue macrophages (histiocytes) *in-vivo* require two signals (priming + metal debris stimulus) to produce inflammasome-mediated IL-1β secretion or do they reside in an endogenously primed state where only one signal (i.e. implant debris) is required to elicit inflammasome-dependent IL-1β secretion? We hypothesized that there are differences in particle-induced inflammasome activation (IL-1β secretion) between early macrophages compared to fully differentiated macrophages that come in contact with particulate implant debris, where early macrophages will require one signal only (particles) to induce IL-1β secretion, but differentiated macrophages need two signals (priming and stimulus, e.g. TLR agonist + particles). We tested this hypothesis by challenging matured and non-matured macrophages with Co-alloy particles and measuring inflammasome mediated IL-1β secretion.

MATERIALS AND METHODS:

Cell culture: *Early macrophages:* Human primary monocytes were negatively isolated with AutomacsPro micro beads (Miltenyi) from PBMCs (n=4) and were left to attach to the bottom of a 48 well plate in RPMI-1640 10% autologous serum at 37°C and 5% CO_2. After attachment, Early macrophages were challenged with Co-Cr-Mo alloy particles (ASTM F-75), mean diameter = 2um, range 1-10um (Bioengineering Solutions Inc, Chicago, IL), at a 20:1 (particles:macrophage) ratio for 24 hours. z-VAD-FMK was used to block Caspase-1 activity. *Differentiated Macrophages:* Human primary monocytes were negatively isolated with Automacs Pro micro beads (Miltenyi) from PBMCs (n=4) and were cultured in RPMI-1640 10% autologous serum supplemented with 50ng/ml M-CSF (R&D systems) for 6 days at 37°C and 5% CO_2. Fully differentiated macrophages were challenged with Co-Cr-Mo alloy particles (ASTM F-75), mean diameter = 2um, range 1-10um (Bioengineering Solutions Inc, Chicago, IL), at a 20:1 (particles:macrophage) ratio with or without priming by the addition of 10 ng/ml LPS (Sigma) for 24 hours.

Luminex cytokine analysis: Supernatants were collected at 24h and assayed for IL1-β and TNF-α production. Statistical analysis was determined by paired t-tests.

RESULTS:

Co-Cr-Mo alloy particles induced statistically significant elevated concentrations of IL-1β and TNFα in early macrophages (211 pg/ml and 1137 pg/ml respectively) compared to their untreated controls. Addition of Caspase-1 inhibitor zVAD-FMK to early macrophages completely abolished secretion of IL-1β in response to Co-alloy particles confirming that metal particle-induced IL-1β production in early macrophages is dependent on caspase-1 and is likely inflammasome dependent as we have previously reported [2]. While Co-alloy particles induced statistically significant levels of TNFα, there was no significant IL-1β secretion in fully differentiated macrophages. While LPS priming alone did not induce statistically significant higher levels of IL-1β in fully differentiated macrophages, treatment with Co-alloy particles in primed differentiated macrophages induced statistically significant elevated IL-1β (81 pg/ml) compared to untreated controls. LPS priming alone induced statistically significant TNFα production (3530 pg/ml) in differentiated macrophages, but did not induce higher TNFα when treated with Co-alloy particles.

DISCUSSION:

Our results indicate that early macrophages require only one signal (Co-alloy stimulus) to secrete caspase-1-dependent IL-1β, but fully differentiated macrophages require two signals (priming + Co-alloy stimulus) before IL-1β can be secreted. Interestingly, secretion of TNFα was significantly higher in both early macrophages and fully differentiated macrophages regardless of priming (only one signal required). This data indicates IL-1β production may be more regulated in fully differentiated macrophages (i.e. resident tissue macrophages), compared to early macrophages (recently extravasated monocytes), where priming by pattern associated molecular patterns (PAMPs) or other endogenous factors (i.e. necrotic HMGB1, high TNFα) may be necessary in addition to a second stimulus (i.e. Implant debris) to secrete IL-1β. This two-signal requirement is consistent with *in-vivo* conditions given that tissue macrophages are constantly exposed to endogenous and exogenous stimuli (e.g. cytokines, apoptosing cells, hematogenous bacteria etc.), that likely act to prime macrophages. Our results suggest that early macrophages that traffic to the site of particle inflammation may be the primary source of *in-vivo* sterile implant debris-induced inflammation due to their ability to immediately release IL-1β upon contact with debris. This is currently under further investigation by analyzing relative amounts and timing of NFκβ vs. Nalp3 activation in early and fully differentiated macrophages under 1 and 2 signal paradigms. Results from these studies will be discussed. Thus far, our results suggest a more targeted pharmacological approach to mitigate the amplifying affects of early macrophages in particle-induced osteolysis could employ anti-monocyte chemotactic therapy, e.g. anti-MCP-1.

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REFERENCES: 1-Hallab et al. Biologic effects of implant debris. Bull NYU Hosp 2009 2009
2- Caicedo et al. Soluble and particulate Co-Cr-Mo alloy .J Orthop Res. 2009

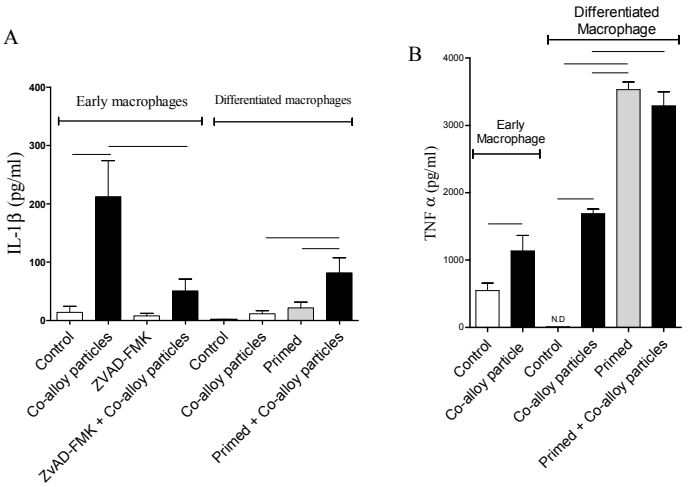

Figure 1. IL-1β (A) and TNFα (B) secretion in early macrophages and in primed and non-primed fully differentiated macrophages in response to Co-alloy particles (20:1) particles:cell ratio. Note. -- = p< 0.05 by student t-test.

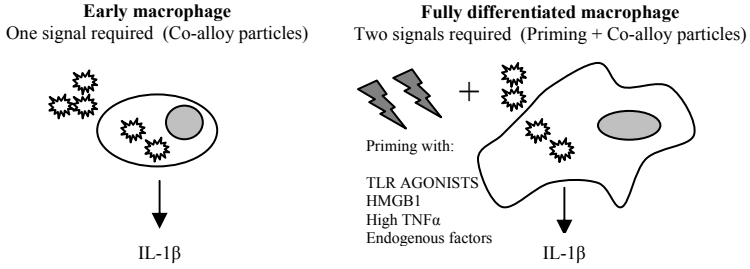

Figure 2. Schematic representation of early vs. differentiated macrophage IL-1β secretion