Introduction: Metal hypersensitivity is found in approximately 10% of the general population, 25% of individuals with well performing total joint arthroplasties (TJA) and 60% of individuals with failing TJAs (1). Antigen presenting cells (e.g. Monocytes, dendritic cells and B cells) play an important role in the uptake of soluble and particulate antigens that can initiate a pro-inflammatory response (2).

However, the mechanisms by which soluble and particulate metals trigger dendritic cell-mediated inflammatory responses initially remains poorly understood. Can soluble and/or particulate metal implant debris induce dendritic cell responses used to recruit and activate peripheral T cells? We hypothesized that soluble metal-implant debris will induce a dendritic cell pro-inflammatory response, but particulate debris will not, because of lower phagocytic capabilities compared to macrophages. To test our hypothesis we generated monocyte-derived dendritic cells (MDDC) from human PBMC’s and challenged them with CoCl, CrCl, MoCl, NiCl ions and Co-Cr-Mo alloy particulate debris. Their T cell co-stimulatory surface marker expression and pro-inflammatory cytokine production was analyzed after 48 hours.

Materials and Methods: Monocyte Derived Dendritic cells (MDDC): Peripheral blood mononuclear cells (PBMCs) were isolated by ficoll gradient separation from healthy volunteers (n=6). Highly pure (95%) unlabeled monocytes were collected by depletion of labeled cells in a magnetic column. Isolated monocytes were cultured in RPMI media supplemented with 10% autologous serum, 50 ng/ml of GM-CSF and 100 ng/ml of IL-4 for 6-7 days. 50% of media was replaced every 2 days. At day 7 MDDC were collected and challenged with or without Co-Cr-Mo alloy (ASTM F-75) particles, range mean particle diameter = 2 μm (volume and number based), range 1-10 μm (Bioengineering Solutions Inc, Chicago, IL) at a 10:1 (particles:MDDC) ratio or with CoCl2, CrCl3, MoCl, NiCl ions at a 0.1 mM concentration (Sigma, St Louis, MO) for 48 hours. LPS (0.3μg/ml)-treated MDDC were used as a positive control. Human Pro-inflammatory 5-plex Luminex assay: MDDC culture supernatants were collected at 48 hours and assayed for IL1 beta, IL-6, IL-8, GM-CSF and TNF alpha concentrations using a bead-based multi-plex system for cytokine analysis. Flow Cytometry: MDDC-T-cell co-stimulatory surface molecules were analyzed using standard flow cytometry Statistical significance was determined using paired t-testing, p<0.05.

Results: Surface Marker Expression: Both soluble and particulate metal debris induced MDDC up-regulation of T cell co-stimulatory molecules and pro-inflammatory cytokines. Surface marker up-regulation and cytokine production was found to be metal-dependent. Cobalt ions induced the greatest response in MDDC up-regulation of CD80 and CD54 (MFI 58.47, 447.99 respectively) compared to untreated controls, which showed an MFI of 43.22 for CD80 and an MFI of 210.39 for CD54. No other soluble metal or particles induced significant up-regulation of CD80. However, in addition to Cobalt, Nickel and Co-Cr-Mo alloy particulate debris highly up-regulated CD54 in MDDC after 48 hours. Control MDDCs showed a CD54 MFI of 210.39, Nickel and Co-Cr-Mo alloy particle debris showed an increased MFI of 340 and 391 respectively (fig.1B). CD86 and HLA-DR were not significantly up-regulated in response to any of the metals tested. LPS (positive control) significantly up-regulated all surface markers tested. Pro-inflammatory cytokine production: MDDCs exhibited high production of TNF alpha and IL-6, but no significant production of IL-8, GM-CSF or IL-1 beta in response to any metal challenge. Cobalt induced the highest MDDC production of TNF alpha (3690 pg/ml), compared to untreated controls, which only showed a basal level of 1278 pg/ml (fig.1A). Nickel and Co-Cr-Mo alloy particles also showed a significant increase in TNF alpha compared to controls, producing 2525 pg/ml and 2088 pg/ml respectively. IL-6 production was only increased significantly in response to Cobalt and Molybdenum (292 pg/ml and 214 pg/ml respectively) compared to their untreated controls (26 pg/ml) (Fig 1.A). LPS-treated MDDCs highly up-regulated all cytokines tested.

Discussion: Our results suggest that monocyte derived dendritic cells up-regulate important T cell surface co-stimulatory molecules and pro-inflammatory cytokines in a metal-dependent manner. While our results indicated that soluble metal can induce a pro-inflammatory response in MDDCs, contrary to our original hypothesis, Co-Cr-Mo alloy particles also induced the up-regulation of CD54 and high production of TNF-alpha. Each metal tested had a slightly different effect on surface marker expression or cytokine secretion. While Cobalt was the highest inducer of both CD80, CD54, IL-6 and TNF alpha, only Nickel showed significant differences for CD54 and TNF alpha production. Neither CD80 nor the production of IL-6 were significant in Nickel-treated MDDCs. Molybdenum uniquely induced significant IL-6 production, but no TNF alpha compared to untreated controls. The different responses induced by Cobalt, Nickel, Molybdenum and Co-Cr-Mo alloy particles suggest different mechanisms of dendritic cell (MDDC) reactivity to soluble and particulate debris. CD83 (a marker of dendritic cell maturation) was not up-regulated by any of these metal challenges, (unlike LPS). This suggests that soluble and particulate metal debris may induce a pro-inflammatory response via dendritic cells, but differently than endotoxins (i.e. LPS). Thus peri-implant dendritic cells may be targets for pharmacologically mitigating exuberant reactivity to metal implant debris.


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Figure 1. A. TNF-alpha and IL-6 secretion by MDDCs in response to metal debris. B. CD54 (ICAM-1) surface expression in MDDCs expressed as Mean Fluorescent Intensity (MFI).

Note: bars and * = p<0.05