Introduction: Metal prosthetic biomaterials release both particulate and soluble metal degradation products (1). Largely innate inflammatory responses result in pathogenic bone homeostasis, ultimately leading to osteoclastogenesis and loosening of the implant. It is still unclear what role particulate and/or soluble debris play in inducing adaptive immune responses. Can soluble metal and metal particles contribute to an the innate/adaptive immune system response by inducing in monocytes up-regulation of lymphocyte co-stimulatory molecules? We hypothesized that soluble ions (CoCl2, CrCl3, MoCl5) and Co-Cr-Mo alloy particulate debris may induce monocyte surface expression of pivotal lymphocyte co-stimulatory molecules as well as pro-inflammatory cytokines in a human monocyte cell line. To test our hypothesis, we treated THP1 monocytes with both CoCl2, CrCl3, MoCl5 ions and Co-Cr-Mo alloy particulate debris and measured the surface expression of co-stimulatory molecules CD80, CD86, ICAM-1 as well as the concentrations of secreted pro-inflammatory cytokines IL-6, IL-1β, GM-CSF, TNF-α and IL-8.

Materials and Methods: Cell culture: Human monocytes (ATCC) Monocytes were cultured in Dulbecco’s modified Eagle medium (GIBCO) supplemented with 10% fetal bovine serum (FBS) (HyClone Laboratories, Inc) at 37°C and 5% CO2 (Sigma) at 0.0 (control) 0.1 mM or Co-Cr-Mo alloy particles (ASTM F-75), ranging mean particle diameter = 2um (volume and number based), range 1-10um (Bioengineering Solutions Inc, Chicago, IL) at a 10:1 (particles:monocytes) ratio for 24 and 48 hours before analysis. Immunofluorescence: CD11c-PE-Cy5, CD86-APC and ICAM-1-PE and analyzed at 24 and 48h with standard flow cytometry protocols. (LPS was used as a positive control). Human pro-inflammatory 5-plex luminex cytokine analysis: 24h and 48h and assayed for IL1-β, IL-6, GM-CSF, TNF-α and IL-8 production. Statistical analysis was determined by student’s t-test.

Results: Up-regulation of surface co-stimulatory molecules and pro-inflammatory cytokine production was metal-specific and not observed with all metal challenges tested. Only CoCl2 and Co-Cr-Mo alloy particles were efficient in inducing CD86, ICAM-1 and IL-8. CrCl3 and MoCl5 did not induce any significant differences in surface molecules or pro-inflammatory cytokines at any time point tested. CoCl2-treated monocytes showed a significant increase (p < 0.05) in CD86 detected at 82.68 and 137.1 MFI at 24 and 48 hours compared to untreated controls at 77.5 and 65.3 MFI respectively. While differences in CD86 in Cobalt-treated monocytes were significantly higher at 48h, ICAM-1 was highly up-regulated at both time points. Untreated controls showed 18.8 and 17.6 MFI at 24h and 48h respectively increasing to 46.3 MFI at 24h and 62.3 MFI at 48h after treatment with CoCl2. Surprisingly, Co-Cr-Mo particle debris induced a significant decrease in CD86 at both time points tested, however, surface expression of ICAM-1 was significantly higher at 24 and 48 hours, increasing from 18.8 to 29.7 MFI at 24h and 17.6 to 27.2 MFI at 48h compared to untreated controls. None of the soluble or particulate metal challenges tested induced any significant production of TNF-α, IL-1β, GM-CSF or IL-6. However, IL-8 production was highly up-regulated by CoCl2 and Co-Cr-Mo particles compared to their untreated controls. Untreated controls produced 8.69 and 20.73 pg/ml of IL-8 at 24 and 48h respectively. CoCl2-treated monocytes secreted 1482.43 and 8695.47 pg/ml of IL-8 at 24 and 48h respectively. Co-Cr-Mo particles also induced significant differences in IL-8 production compared to untreated controls. LPS was used as a positive control and up-regulated all cytokines and all surface markers tested at significant levels compared to their untreated controls (data not shown).

Discussion: Our hypothesis that pivotal co-stimulatory molecules for inducing functional T-cell responses were induced by metal ion and particulate exposure in monocytes (antigen presenting cells), was largely supported by this study. However this induction of elevated monocyte surface expression of CD86, ICAM-1 and elevated IL-8 secretion was limited to specific metals, i.e. cobalt ions and Co-Cr-Mo alloy particles. Interleukin-8 is a known neutrophil and lymphocyte chemoattractant that has been shown to stimulate osteoclastogenesis and bone resorption in an in-vitro model in previous studies (2). While soluble Chromium and Molybdenum did not induce any significant CD80, CD86, ICAM-1 or IL-8 up-regulation in monocytes, it is important to point out that from Co-Cr-Mo alloy implants, at least one of the three metals (in soluble form) and particle debris as a whole, up-regulated lymphocyte co-stimulatory molecules required for cell to cell adhesion (ICAM-1), presentation and possible activation (CD86) of T-lymphocytes. Interestingly, while LPS-treated cells highly up-regulated CD80, CD86, ICAM-1 as well as all pro-inflammatory cytokines (not shown), we observed that Cobalt ions up-regulated CD86, ICAM-1, but not CD80 and it also induced high quantities of IL-8, but not of any other cytokines. Similar was the case with Co-Cr-Mo alloy particles. Our data suggests a possible specific effect of different metal ions and/or particles on the up regulation of pivotal co-stimulatory molecules in antigen presenting cells. Further study is warranted to further elucidate different mechanisms of soluble and particulate metal effects at the transcription level of co-stimulatory molecule and cytokine production.


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