Soluble Cobalt, Nickel and Co-Cr-Mo alloy particles induce monocyte T-cell co-stimulatory molecules CD-86, CD54 and pro-inflammatory cytokine secretion.

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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 the innate/adaptive immune system response by inducing monocyte up-regulation of lymphocyte co-stimulatory molecules and pro-inflammatory cytokines? We hypothesized that Co-Cr-Mo alloy particulate debris, but not soluble ions ($CoCl_2$, $CrCl_3$, $MoCl_5$, $NiCl_2$) will induce monocyte surface expression of pivotal lymphocyte co-stimulatory molecules as well as pro-inflammatory cytokines. To test our hypothesis, we treated THP1 monocytes and isolated human primary monocytes (n=10) with both $CoCl_2$, $CrCl_3$, $MoCl_5$, $NiCl_2$ ions and Co-Cr-Mo alloy particulate debris and measured the surface expression of co-stimulatory molecules CD80, CD86, ICAM-1, HLA-DR 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: THP-1 monocytes (ATCC) or freshly negatively isolated human primary monocytes from healthy volunteers (n=10) were cultured in Dulbecco's modified Eagle medium (GIBCO) supplemented with 10% fetal bovine serum (FBS) (Hyclone Laboratories, Inc) at 37°C and 0.5% Co2, 3.5x 10^5 cells/ well in 24 well plates with Co^{+2}, Cr^{+3}, Mo^{+5} ,Ni^{+2} chloride solutions (Sigma) at 0.0 (control) 0.1 mM or Co-Cr-Mo alloy particles (ASTM F-75), range mean particle diameter = 2um (volume and number based), range 1-10um (Bioengineering Solutions Inc, Chicago, IL) at a 10:1 (particles:monocytes) ratio for 48 hours before analysis. Immuno-staining: CD80 PE-CY5, CD86-APC, ICAM-1-PE and HLA-DR-FITC were analyzed after 48h of culture with or without metal challenge with standard flow cytometry protocols. (LPS 0.1 at 0.3 ug/ml was used as a positive control). Human pro-inflammatory 5-plex luminex cytokine analysis: Culture supernatants were collected at 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 for THP-1 experiments and paired t-test for primary monocyte experiments.

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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 $CoCl_2$, $NiCl_2$ and Co-Cr-Mo alloy particles were efficient in inducing CD86, ICAM-1 and IL-8 in both THP-1 and primary monocytes. $CrCl_3$ and $MoCl_5$ did not induce any significant differences in surface molecules or pro-inflammatory cytokines at any time point tested. $CoCl_2$-treated monocytes showed a significant up-regulation of ($p < 0.05$) CD86 detected at 109% MFI increase for THP-1 and 12.6% increase for primary monocytes compared to their respective untreated controls (Fig 1.A). CD54 (ICAM-1) was highly up-regulated in response to $CoCl_2$, $NiCl_2$ and Co-Cr-Mo alloy particles in THP-1 monocytes (252.6 %, 160.5% and 53.92% respectively), but were only significant in response to $CoCl_2$ and $NiCl_2$ in primary monocytes (12% and 15% respectively). Particulate debris decreased CD54 in primary monocytes to -13% MFI compared to untreated controls (Fig.1-A). None of the soluble or particulate metal challenges tested induced any significant production of TNF-α, IL-1β, GM-CSF or IL-6 in THP-1 monocytes. However, IL-8 production was highly up-regulated by $CoCl_2$, $NiCl_2$ and Co-Cr-Mo particles compared to their untreated controls (Fig 1.B). Untreated controls secreted 20.73 pg/ml of IL-8 at 48h. $CoCl_2$-treated monocytes secreted 8695.47 pg/ml of IL-8 and $NiCl_2$-treated THP1 secreted 64.23 pg/ml at 48h. Co-Cr-Mo particles also induced significant differences in IL-8 production compared to untreated controls (240.83 pg/ml compared to 20.73 pg/ml of 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 monocyte pivotal co-stimulatory molecules for inducing functional T-cell responses would be induced by particulate exposure was supported by data obtained from THP-1 monocytes, but not from primary human monocyte data. Also, metal ion challenge, which was predicted not to trigger a response in monocytes, highly induced co-stimulatory molecule up-regulation as well as cytokine secretion. However, elevated monocyte surface expression of CD86, CD54 and elevated IL-8 secretion was limited to specific metals, i.e. cobalt and Nickel ions and Co-Cr-Mo alloy particles. It is important to note that Cobalt and Nickel ions were consistent in up-regulating CD86 and CD54 in both THP-1 and primary monocytes (n=10). Interleukin-8 is a known neutrophil and lymphocyte chemoatractant 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 high level of CD80, CD86, CD54 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 (CD54), presentation and possible activation (CD86) of T-lymphocytes. Interestingly, while LPS-treated cells highly up-regulated CD80, CD86, CD54, as well as all pro-inflammatory cytokines (not shown), we observed that Cobalt ions up-regulated CD86, CD54, but not CD80 and it also induced high quantities of IL-8, but not of any other cytokines tested. 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.

ACKNOWLEDGEMENTS: NIAMS/NIH, Crown Family Chair of Orthopedics

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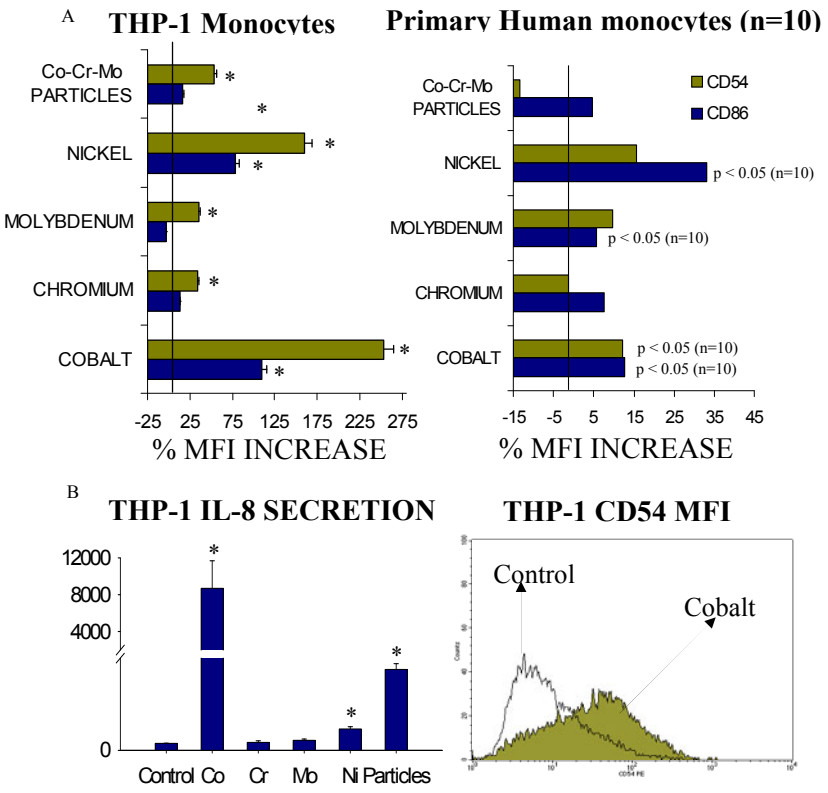

Figure.1. (A) CD86 and CD54 surface expression in THP1 and human primary monocytes (n=10) expressed as % increase in Mean Fluorescent Intensity (MFI). (B). IL-8 production in THP1 monocytes and Control vs. Cobalt histogram for THP-1 CD54 up-regulation. Note: * = $p<0.05$