Co-alloy implant debris (soluble and particulate) activate the macrophage inflammasome "danger" signaling pathway: A novel intracellular wear debris sensing mechanism

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INTRODUCTION

It is known that excessive immune reactivity to metal debris derived from the degradation of metal orthopedic implants is a cause for aseptic inflammation and osteolysis in certain individuals. Soluble and particulate metal debris has been shown to induce macrophage secretion of pro-inflammatory IL-1β, TNF-alpha and IL-6. However, the intracellular mechanisms that trigger macrophage activation and cytokine secretion in response to soluble or particulate metal challenge remain to be elucidated. It has been shown that macrophage intracellular danger/stress signals triggered by non-biologcial agents (e.g asbestos, DNTB) induce activation of the inflammasome complex of proteins (NALPs ASC) (1). Inflammasome activation induces caspase-1 activation allowing for cleavage of intracellular pro-IL-1β into its mature form, secretion and induction of a broader pro-inflammatory response. We hypothesized that Co-alloy particle- and/or ion-induced macrophage reactivity is mediated by the activation of the inflammasome, consequently leading to the secretion of mature IL-1β in a metal concentration dependent manner.

MATERIALS AND METHODS

Cell culture: THP-1 macrophages (ATCC) or freshly negatively isolated human primary monocytes (n=3) were cultured in RPMI-1640 10% fetal bovine serum (FBS) (Hyclone Laboratories, Inc) at 37°C and 0.5% Co2 with 3.4uM Nigericin (inflammasome positive control), Co^{+2}, Cr^{+3}, Mo^{+5} ,Ni^{+2} chloride solutions (Sigma) at 0.0 (control) 0.01, 0.1, 0.2 and 0.5 mM or Co-Cr-Mo alloy particles (ASTM F-75), mean diameter = 2um, range 1-10um (Bioengineering Solutions Inc, Chicago, IL) at a 5:1, 10:1, 20:1 (particles:monocytes) ratio for 24 hours. Z-Vad-FMK was used to block Caspase-1 activity. *siRNA*. THP-1 cells (2.0 x 10^5) were transfected with 50 pmol of ASC (putative inflammasome protein) siRNA and mock siRNA (control) using Lipofectamine RNAimax transfection reagent strictly following manufacturer's suggested protocols (Invitrogen). *Luminex cytokine analysis*: Supernatants were collected at 24h and assayed for IL1-β, IL-6 and TNF-α production. Statistical analysis was determined by paired t-tests

RESULTS

Cobalt, chromium, molybdenum, nickel and Co-Cr-Mo alloy particles induced a metal concentration-dependent secretion of IL-1β in THP-1 cells. Although IL-1β beta secretion was increased with increasing concentrations of metal challenge, Cobalt- and Nickel-induced IL-1β secretion peaked at 0.1 mM concentrations, whereas chromium- and molybdenum-induced IL-1β secretion peaked at 0.2 mM (Fig.1A). A similar concentration dependent effect was observed with Co-Cr-Mo alloy particles. While a 1:5 or ratio of particles per cell did not induce higher levels of IL-1β, a 10:1 ratio induced significantly higher IL-1β secretion compared to untreated controls by 24 hours (Fig.1A). Addition of Caspase-1 inhibitor zVAD to cell cultures completely abolished secretion of IL-1β in response to all metal challenges used confirming that metal ion- and metal particle-induced IL-1β production in macrophages is in fact dependent on the active form of caspase-1 (Fig. 1B). To further confirm inflammasome involvement, THP-1 cells knocked down for ASC expression with siRNA lost their ability to secrete IL-1β in response to cobalt, molybdenum, nickel, Co-Cr-Mo alloy particles and Nigericin compared to mock siRNA transfection (Fig.1C).

DISCUSSION:

We herein provide evidence of a novel pathway by which human macrophages may sense and respond upon contact with soluble and particulate implant metals that ultimately lead to pro-inflammatory and potentially bone resorbing responses. Our results show that soluble cobalt, chromium, molybdenum, nickel and Co-Cr-Mo alloy particles induce caspase-1- and ASC-dependent IL-1β secretion in human macrophages. This supports our hypothesis that Co-Cr-Mo alloy implant debris in its particulate and/or ionic components may induce human macrophage pro-inflammatory responses through the inflammasome multi-protein complex. In addition, our hypothesis that increasing concentrations of metal ions and particles will induce higher levels of IL-1β secretion was partially supported. Although higher metal concentrations induced higher THP-1 cell secretion of IL-1β in general, certain metal-specific concentrations rendered THP-1 cells unable to secrete IL-1β most likely due to a toxic effect at that particular concentration. Previous studies have shown that metal particles and several metal ions induce NADPH-mediated macrophage production of reactive oxygen species (danger signal). Taken this into consideration we blocked phagosome NADPH function in THP-1 cells and were able to render THP-1 cells deficient in their ability to secrete IL-1β (not shown). While we cannot confirm that metal-induced ROS is the cause of inflammasome activation and IL-1β secretion in our system, it lends some evidence for this theory. These findings give rise to several more questions for future research. There have been several inflammasome complexes identified that play a role in inflammation. While metal ion and metal particle induced activation of the inflammasome provides evidence of a novel pathway of metal debris-induced inflammation, if one specific or several inflammasomes complexes play a role in metal-induced IL-1 β remain to be elucidated

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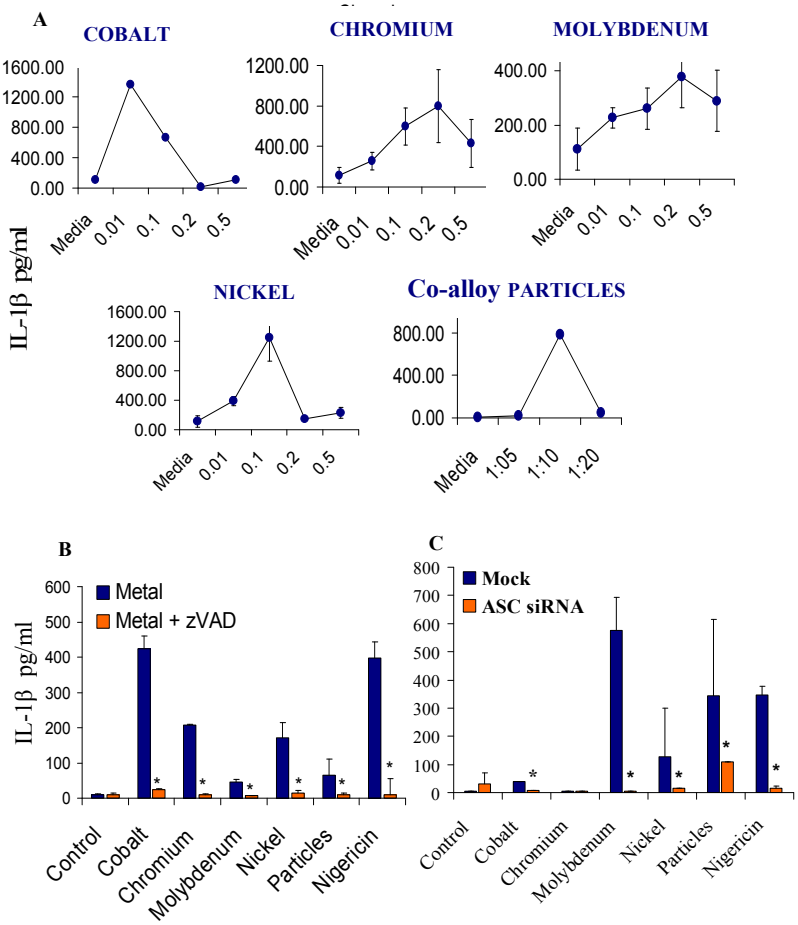

Figure.1. (A) IL-1β secretion in THP-1 cells with increasing metal concentrations. (B) Caspase-1 inhibition with zVAD of metal-induced IL-1β secretion in human monocytes (metals = 0.1 mM, particles 10:1, Nigericin 3.4uM). (C) Inflammasome blocking with siRNA for ASC (putative inflammasome protein) inhibits THP-1 cell productin of IL-1β. Note. * = p< 0.05 by student t-test.