

SOLUBLE METAL DEBRIS INDUCE PRO-INFLAMMATORY CYTOKINE SECRETION IN HUMAN MONOCYTES SIMILAR TO PARTICULATE DEBRIS

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INTRODUCTION: It is well established that metal degradation products of prosthetic biomaterials include both particulate and soluble metal debris.(1) Previous studies have demonstrated the potential of metal particulate debris from orthopedic implants to induce monocyte/macrophage phagocytosis and activation with the concomitant release of pro-inflammatory cytokines in peri-implant tissue. This largely innate inflammatory response results in pathogenic bone homeostasis, ultimately leading to implant loosening. It is not known to what extent, if any, soluble implant metals are able to provoke macrophage/monocyte activation and thus contribute to implant pathogenesis. We hypothesized that particulate Co-Cr alloy debris will generate a pro-inflammatory cytokine response in primary human monocytes while soluble Co-Cr challenge will not. To test our hypothesis, we isolated and treated primary human monocytes with either Co-Cr alloy particulate debris or $\text{CoCl}_2\text{-CrCl}_3$ ions and measured the concentrations of secreted pro-inflammatory cytokines (IL-6, IL-1 β , GM-CSF, TNF- α) in a time point study. Culture supernatant cytokine concentrations were measured by enzyme-linked immunoassays (ELISA) at 0h, 2h, 4h, 8h, 16h, 24h, 48h and 72h after challenge.

MATERIALS AND METHODS: Primary human monocytes were isolated through a macrophage adhesion assay to assess their metal particle- and metal ion-induced inflammatory cytokine profile. Peripheral blood mononuclear cells (PBMCs) were collected intravenously from healthy volunteers (n=10) and PBMCs were isolated by ficol gradient separation. Isolated PBMCs (1×10^6) were cultured in 48 well plates in Dulbecco's modified Eagle medium (GIBCO, Grand Island, N.Y) with 10% autologous serum at 37° and 0.5% Co_2 for 24 hours. After 24 hours, non-adherent lymphocytes were removed and discarded, leaving adherent monocytes (1.0×10^6) at the bottom of the wells (80–90 % purity). Adherent monocytes/macrophages were washed twice with PBS and challenged with or without particles, Co-alloy (ASTM F-75), range mean particle diameter = 2 μm (volume and number based), range 1-10 μm (Bioengineering Solutions Inc, Chicago, IL) at a 10:1 (particles:monocytes) ratio and combined Co-Cr ions (from $\text{CoCl}_2\text{-CrCl}_3$) at a 0.1 mM concentration (Sigma, St Louis, MO) for 72 hours in fresh new medium. LPS (0.3 $\mu\text{g/ml}$)-treated monocytes were used as a positive control for cytokine production. **ELISA:** Culture supernatants were collected at 0h, 2h, 4h, 8h, 16h, 24h, 48h, 72h and assayed for IL-1 β , IL-6, GM-CSF and TNF- α production. Cytokine concentrations were normalized to their respective untreated controls at each specific time point. The increase for each cytokine at each time point was averaged for the 10 subjects tested. Statistical analysis was determined by students t-test.

RESULTS: Monocytes/macrophages treated with particulate and soluble metal debris displayed similar cytokine profiles over 72 hours of challenge. IL-6 and IL-1 β were not secreted in higher concentrations compared to untreated controls. TNF- α and GM-CSF were significantly elevated in both particulate- and soluble metal/ion-challenged monocytes/macrophages. Up-regulation of TNF- α by particle and ion challenged-monocytes/macrophages occurred between 0-2 hours. Co-Cr particles showed a 2.8 fold increase immediately after challenge (0 h) followed by a 2 fold increase after 2 hours and undetectable differences in the remaining time points (Fig.1). While no immediate (0h) cytokine production was detected with Co-Cr ions, they also induced an early up-regulation of TNF- α with a 2 fold increase at 2 hours followed by a decrease to baseline levels for the rest of the 72 hours (Fig.1). GM-CSF exhibited the greatest differences between treated and untreated cells. Co-Cr particles induced a seesaw type of response showing a gradual increase up to 17 fold by 8 hours, decreasing to homeostatic levels by 48 hours and rising back to 10 fold by 72 hours (Fig.1). A similar pattern was observed in Co-Cr ions treated cells. An 11.7 fold increase was observed by 4 hours with decreasing concentrations by 16 hours (2.6 fold) before gradually rising to a 10 fold increase by 72 hours (Fig.1). LPS-treated adherent monocytes exhibited higher concentrations of all cytokines tested at most time points. Soluble Co-Cr salt solutions produced an approximately 2 fold release of GM-CSF release at 24 and 48 hours when compared to Co alloy particle challenge.

DISCUSSION: The similar concentrations of TNF- α and GM-CSF released in response to both particulate and soluble debris refutes our original hypothesis and demonstrates the ability of soluble implant

metals to induce monocyte/macrophage activation. Other typical monocyte/macrophage cytokines, IL-1 β and IL-6, were not significantly elevated over the 72 hours of challenge. While the production of pro-inflammatory cytokines by macrophages upon metal particle-debris phagocytosis is well established, the same effect has not been previously shown in response to soluble implant metals. TNF- α was released early relative to GM-CSF thus synergistic co-stimulation in the peri-implant environment may not occur, and may represent a distinct pattern of cytokine release. The production of high concentrations of monocyte/macrophage derived GM-CSF from soluble and particulate metal debris would most likely act in-vivo to recruit and differentiate bone marrow monocytes to the site of insult. In addition, recent dendritic cell (DC) biology studies have shown TNF- α and GM-CSF are important factors responsible for dendritic cell maturation, thus compounding the chances of antigen presentation and adaptive immune system activation.(2) While the phenomenon of metal particle phagocytosis and subsequent cytokine/chemokine activation is expected, it is not understood how soluble metals at sub-toxic concentrations provoke similarly acute cytokine responses in monocyte/macrophage populations. The similar pattern of cytokine release (Fig 1) from particulate and soluble metal (where a temporally greater amount of GM-CSF to ions was found, suggests a similar mechanism of activation. This also suggests that particle induced activation/cytokine release may be due to soluble metal released from particles prior to or after phagocytosis. Further study is warranted to further elucidate how soluble metals induce monocyte/monocyte/macrophage cytokine production and how this may affect implant performance.

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REFERENCES:

- Hallab N.J., Jacobs J.J., Skipor A.; et al., J Biomed Mater Res, 49:353-361, 2000
- Manome H, Aiba S, Tagami H: Simple chemicals can induce maturation and apoptosis of dendritic cells. Immunology 1999;98:481–490.

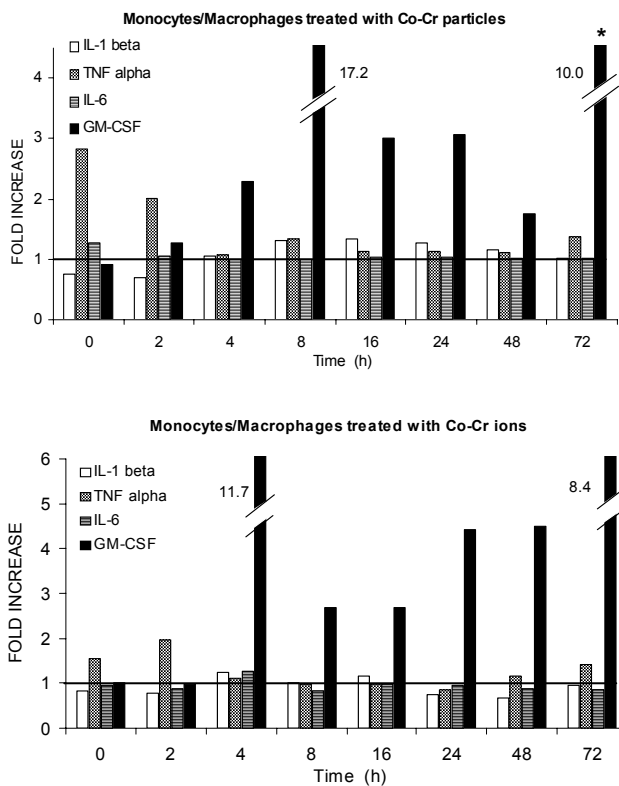


Figure 1. Pro-inflammatory cytokine profile of monocyte/macrophages treated with metal particulate (10:1) or soluble debris (1 mM) for 72 hours. Note * indicates significance at $p < 0.05$.