INTRODUCTION: Past studies have shown the potential of released metal particulate debris from implants to alter the normal function of cells in the periprosthetic space including osteoblasts, fibroblasts and lymphocytes. The metal degradation products of prosthetic materials may be present as particulate wear debris, colloidal organometallic complexes (specifically or non-specifically bound), or inorganic metal salts/oxides, free metallic ions, or in an organic storage form. It is not known to what extent the biologic effects of metal debris are mediated by particulate or soluble forms of metal. Our hypothesis is that specific metals released in ionic form affect the biologic response of certain types of periprosthetic cells differently. We tested this hypothesis by treating human osteoblast-like MG-63 cells, primary human fibroblasts and primary human lymphocytes with 8 different concentrations of Na, Cr, Mg, Mo, Al, Co, Ni, Fe, Cu, Mn, and V chloride solutions. The degree to which these metal ions affected cell function was assessed using viability assays, proliferation assays, and cytokine assays. In this fashion, we determined which cell types were most affected by metals, which metals were responsible for these effects, and what metal concentrations were required to interfere with the homeostasis of these three selected cell types.

MATERIALS AND METHODS: Cells: Human osteoblast-like cell lines MG-63 (ATCC, Rockville, MD), primary human fibroblasts (19 yr old female, ATCC) and primary human lymphocytes (n=6, 3 male, 3 female healthy volunteers, no implants, average age 29) were used to test the effects of metal ions. Lymphocytes were isolated using Ficol gradient separation. The cells were cultured in Dulbecco's modified Eagle medium (GIBCO, Grand Island, N.Y.) at 37°C and 0.5% CO2, containing 10% fetal bovine serum (FBS; HyClone Laboratories, Inc., Logan, UT). MG-63 cells were cultured at 8 concentrations of Co, Cr, Mo, Ni, Al, V, Fe, Mg, Mn and Cu chloride solutions (Sigma, St Louis, MO) at 0.0, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, and 10.0 mM. Despite its prominence as an implant alloy, titanium was not tested due to the insoluble nature of Ti at physiologic pH (Ti precipitates as TiO2 at a pH > 1), and its subsequent inability to form an ionic solution. Viability Assays: Cell viability was determined by flow cytometry (FACScan, Becton Dickinson Co., Franklin Lakes, NJ) using Propidium Iodide (Becton Dickinson Co.). The Propidium Iodide (PI) staining solution, a fluorescent vital dye that stains DNA, is used to assess plasma membrane (PM) integrity and was composed of 50 μg Pl/ml in PBS, pH 7.4 and 10 μl/test of 1 x 10⁶ cells in flow cytometric assays. Proliferation Assays: Proliferation assays were performed using [3H]-thymidine (1 μCi/1 ml-thymidine/well) (Amersham International, Arlington Heights, IL) for 48 hours (osteoblasts and fibroblasts) and 6 days (lymphocytes). Inflammatory Cytokine concentrations in supernatants of osteoblast, and lymphocyte cultures were measured using high sensitivity assay kits for IFN-γ, IL-2, IL-6, and TNF-α (R&D Systems).

RESULTS: There was a wide range of cell reactivity to different metal ions. The concentration effects are shown in the proliferation results of Fig. 1. Lymphocytes demonstrated significantly elevated proliferation responses to high concentrations of Mo, Co, Fe, Cr, and Al (data shown for a representative individual, Fig. 1). Fibroblasts demonstrated increases in proliferation to Cu, Ni, V and Fe, while osteoblast proliferation was completely inhibited by these same metals at concentrations (1.0mM) which provoked lymphocyte proliferative responses. Fe, Al and Cr had the least inhibitory effects on osteoblasts, lymphocytes and fibroblasts. Viability Assays (data not shown) demonstrated similar results to proliferation assays. Metal ions influenced MG-63 osteoblast viability in a similar manner to proliferation effects when ranked from least to most "toxic" using a 50% viability measure of concentrations, i.e. Na, <Cr, <Mo, <Al, <Co, <Ni, <Fe, <Cu, <Mn, <V. Below concentrations of 0.01 mM none of the metals significantly reduced osteoblast, lymphocyte or fibroblast viability. However, above 0.1-1 mM the more "toxic" metals (i.e. V, Mn, Cu, and Ni) significantly reduced cell viability to levels less than 30%. Cytokine release of metal ion-stimulated lymphocytes and osteoblasts: Lymphocytes and osteoblasts demonstrated distinct patterns of cytokine release in response to specific metals. These effects were in proportion to reactivity indicated in proliferation assays. For osteoblasts the more toxic metals (e.g. Ni) induced a 2-fold greater (at 0.1 mM concentration) and 4-fold (at 1 mM concentration) greater IL-6 release after 48 hours. The less toxic metals (e.g. Co and Cr) had no effect on IL-6 release of MG-63 cells. In contrast, there was no IL-1β, TGF-β1 or TNF-α detected in the culture medium even after 24 or 48-hours of culture. Lymphocytes demonstrated increased release of IFN-γ and IL-2 at metal concentrations that induced proliferative responses.

DISCUSSION: The effects of soluble metals on the cells in the periprosthetic space is a complex function of cell type, composition and concentration of metal. Most notable was the stimulatory effect some metals (e.g. Al and V) had on lymphocytes and fibroblasts, while suppressing the viability and proliferation of osteoblast-like cells. This differential impact of metal ions on fibroblasts relative to osteoblasts may potentially explain how fibrous membranes so readily form around implants initially placed in intimate contact with bone (i.e. osteoblasts). The levels of metal which produced decreased cellular responses are above those observed systemically (e.g. in serum) in patients with implants. However, they are within the concentrations reported to exist locally around implants, thereby increasing the likelihood that similar effects are produced on peri-implant cells in-vivo. This investigation quantitatively provides the critical concentrations at which implant alloy metal ions affect peri-implant human cell types such as lymphocytes, fibroblasts and osteoblasts. These effects support the contention that adverse local and remote tissue responses purportedly associated with particulate debris may be due in part to specific soluble metals resulting from implant degradation.

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REFERENCES: