Measuring the Biologic Reactivity of Implant Debris Using In Vitro Cellular Reactivity Correlated with In Vivo Serum Biomarkers

Marco Caicedo, Shelly Anderson, Anand Reddy, Joshua J Jacobs and Nadim James Hallab
Department of Orthopedic Surgery, Rush University Medical Center, Chicago, IL 60612

INTRODUCTION: Generally, lymphocyte reactivity to implant metals is present in 10% of the general population, 25% of individuals with well performing total joint arthroplasties (TJA) and 60% of individuals with failing TJAs(1). However, biologic linkage between in vitro metal-induced lymphocyte reactivity to in vivo inflammation and bone catabolism remains uncharacterized. Can in vitro metal sensitivity testing be correlated with biomarkers of inflammation and bone catabolism in vivo prior to the onset of clinical symtoms? We hypothesized that TJA subjects that demonstrate a high metal sensitivity index (in vitro) will also show elevated concentrations of pro-inflammatory cytokines and collagen catabolism markers in systemic serum. We tested this hypothesis by correlating metal reactivity using lymphocyte transformation testing (LTT) to serum markers of inflammation and bone catabolism in metal reactive and non-reactive people with TJA.

MATERIALS AND METHODS: *Patient groups*: Four groups of TJA subjects were created according to their metal stimulation index to one or more metals at 0.1 mM challenge. Reactive group: subjects with SI > 10 fold compared to untreated controls. Moderate group: subjects with SI between 5 - 10 fold compared to untreated controls. Mild group: subjects with SI between 2 - 5 fold compared to untreated controls. Non-Reactive (control) group: subjects with no SI compared to untreated controls.

Table 1. Study Group's profile.

		Reactive	Moderate	Mild	Non-Reactive
Number of subjects		8	8	9	9
Age	Mean (range)	59.3(48-80)	50.6(41-75)	56 (33-79)	54 (31-64)
Gender	M : F	3:5	6:2	5:4	3:6
Years w/ implant	Mean (range)	3.8 (2-6)	4.7 (3-7)	5.63(3-15)	5.25(3-10)

Lymphocyte proliferation: All subjects were lymphocyte transformation tested (LTT) with soluble metals. Human peripheral mononuclear cells (PBMC) were isolated from 30 ml. of blood (15-30 x 10^6 cells per subject) and incubated with DMEM and 10% autologous serum with either no metal (plain media) as a negative control, 0.01 mg/ml phytohemaglutinin (PHA) as a positive control, and Al, Co, Cr, Fe, Mo, Ni, V and Zr chloride solutions (Sigma, St Louis, MO) at 0.01, 0.1 and 1 mM concentrations. The amount of proliferating PBMC's was pulsed using [3H]-thymidine (1mCi / well) added over the last 12 hours of a 6-day culture period and counted with a Beckman Beta plate counter. Results were normalized to each subject's respective negative control (no treatment) providing a stimulation index (SI) of reactivity to each metal. *ELISAs*: Pro inflammatory cytokines IL-1 beta, IL-6, TNF alpha GMCSF (Granulocyte macrophage colony stimulating factor) and collagen catabolism marker Deoxypyridinoline (Dpd) crosslinks were measured with standard Enzyme Linked Immunoassay (ELISA) protocols. Statistical significance was determined using t-testing, $p<0.05$ and linear regression analysis.

RESULTS: MSP and Dpd concentrations in serum showed an increasing trend with increasing lymphocyte metal SI. TNF-alpha, GMCSF, IL-1 beta or IL-6 were not detectable in any of the groups in peripheral serum. IFN-gamma showed detectable levels in all groups, but no significant differences between them (69±4 pg/mL). The average SI for the reactive group subjects (n=8) was 32.5 fold proliferation above their non-treated base level of lymphocyte proliferation. Moderate (n=8) and mild (n=9) groups mean metal SI were 5.38 and 2.29 fold proliferation above their non-treated base level reactivity, respectively. Average metal SI for the subjects of the non-reactive control group (n=9) was 0.75 fold proliferation above their non-treated controls. The reactive group showed a significant ($p<0.05$) increased level of MSP (3.43 pg/mL) and Dpd (5.41 nmol/L) compared to the non-reactive (control) group, which had a MSP concentration of 1.30 pg/ml and Dpd at 3.85 nmol/L (Fig 1). There was also a significant difference in MSP concentrations between the mild and non-reactive groups ($p<0.05$). Even though all four groups showed detectable levels of TNF alpha, (Reactive= 65.7pg/mL, Moderate=71.6pg/mL, Mild=71.9g/mL, Non reactive=65.22pg/mL) there were no significant differences or correlations between the groups for this specific pro-inflammatory cytokine.

DISCUSSION: These results show that of the detectable serum markers of inflammation, MSP (a macrophage regulating protein) and Dpd (a collagen catabalolism marker) concentrations showed a linear correlation with metal reactivity (SI). While these results support our hypothesis, most pro-inflammatory cytokines were not detectable in any of the groups with the exception of TNF alpha (presumably from activated macrophages), which was constant in all groups, but showed no significant differences between them. The increased concentration of systemic serum MSP and Dpd in the reactive group however, lends support to a possible correlation between increased macrophage stimulation and bone (collagen type I) catabolism in vivo in association with elevated metal sensitivity in vitro (SI>2). This agrees with previous studies that show MSP can stimulate osteoclastic activity (2). Although GMCSF was not detectable in any of the groups, the presence of TNF alpha and MSP is consistent with an inflammatory response expected in metal sensitive TJA subjects. Limitations of this study included no correlation with clinical radiographic data, the lack of other systemically elevated cytokines and the presence of non-metal related immune mechanisms remains. None of the groups showed a correlation between age, gender, or age of implant and the level of cytokines detected (Table 1), which likely rules out demographic induced differences in cytokine levels and metal reactivity. Although further studies are needed to more fully support the findings of this investigation, this study supports the contention that immune reactivity to metals may play a role in more subtle and chronic inflammation associated with debris induced pathophysiology in humans, prior to the onset of symptoms of loosening. This correlation between lymphocyte stimulation and biomarkers of inflammation does not indicate whether lymphocyte sensitivity is a consequence or a cause of the correlated biomarker and any inflammation in vivo. Further follow-up of these individuals may determine if these early signs of peri-implant pathology become clinically relevant.

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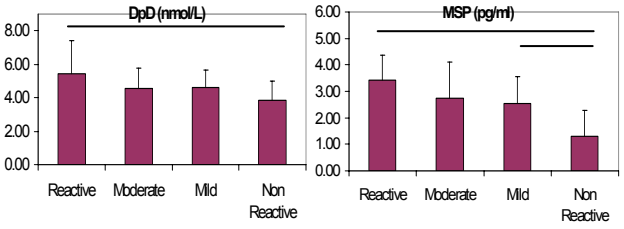

Figure 1. MSP and DPD serum concentrations in reactive groups. Note: Bars indicate significance at $p<0.05$, t-testing

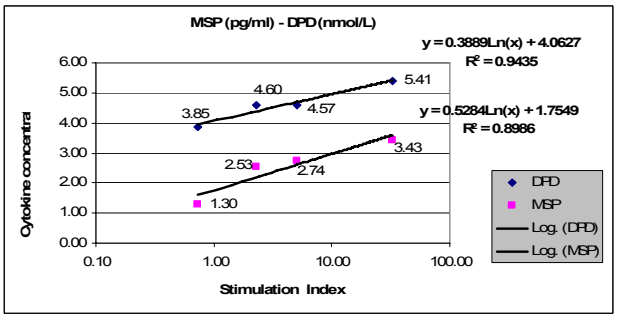

Figure 2. Logarithmic MSP and DpD metal- SI regression line prediction..