In Vitro Metal-Reactivity Is Associated With In Vivo Metal-Specific Antibodies (IgG) In Individuals with Metal Implants

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Introduction: Hypersensitivity to metallic implants occurs as a result of individually specific humoral and adaptive immune responses. Although adaptive cellular immunity has been correlated with metal sensitivity, other studies have suggested that metal-protein antibodies (humoral immunity) can be detected in subjects with total joint arthroplasty (TJA)(1). However, a possible correlation between metal hypersensitivity in TJA subjects with the presence of metal-protein complex specific antibodies in serum remains un-investigated, i.e. can increased metal sensitivity in TJA subjects be attributed to an enhanced specific antibody capability to recognize metal-protein conjugates? To address this question we hypothesized that implant soluble metals, in conjunction with peri-implant structural proteins (collagen I), will exhibit preferential antibody binding in metal-reactive TJA subjects when compared to non-reactive subjects in a concentration-dependent manner. We tested this hypothesis by measuring systemic serum IgG recognition (binding) of three implant metal-protein complexes at three concentrations in metal-reactive and non-reactive TJA groups.

MATERIALS AND METHODS: *Patient groups*: Two groups of TJA subjects were created according to their metal stimulation index to one or more implant metals at 0.1 mM concentration. Reactive group (n=4): consists of subjects with SI>10 fold to Co, Cr or Ni (0.1mM). Non-Reactive group (n=4): consist of subjects with SI < 2 (based on an 2n=8 at 80% power with average IgG of 180pg/mL and 20pg/mL standard deviation, with 50% group differences). *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 Co, Cr, and Ni, chloride solutions (Sigma, St Louis, MO) at 0.1and 1 mM concentrations. The amount of proliferating PBMC's was pulsed using [3H]-thymidine (1mCi/well) and analyzed with a Beckman Beta plate counter. *Protein metal complexes*: Ni, Co and Cr chloride solutions (Sigma) were conjugated to collagen in 96-well flat microtiter plates with 0.0 (background control) 0.01, 0.1 and 1 mM concentrations and incubated for 24 hours at 36C and 5% CO2. *IgG Analysis*: An indirect enzyme-linked immunoabsorvent assay was performed to detect IgG binding to metal-collagen complexes. Subjects' serum (100ul.well) was added to metal-collagen conjugates and incubated for 2 hours at 36°C. Goat anti-human IgG-HRP at 2ul/L (Biosource) was added to the metal-collagen human IgG complexes and incubated for 2 hours at 36°C and 5% CO2. Substrate solution (100ul) was added to each sample and incubated for 20 min. at room temperature. Samples were read at 450 nm with an ELISA plate reader. *Statistical analysis:* T-testing was used for statistical significance. P<0.05 was considered significant.

RESULTS: Reactive group had an average SI=20 and the NonReactive Group had an SI=0.4 (for the most reactive metal challenge agent). All three metal-collagen complexes tested (Ni, Co, Cr) exhibited higher serum IgG binding concentrations in the reactive group compared to the non-reactive group at 0, 0.01, 0.1 and 1 mM concentrations. IgG bound to Ni-collagen at 0.1mM (293.34 mg/ml) and 1mM (283.43 mg/ml) concentrations showed significant differences (p<0.05) compared to the non-reactive group, which showed 195.08 mg/ml IgG at 0.1 mM Ni-collagen and 191.28 mg/ml of IgG at 1 mM Ni-collagen. IgG binding to Co-collagen complexes showed similar results to Ni-collagen. Reactive subjects showed significant differences (p<0.05) in Co-collagen complexes at 0.1mM and 1mM concentrations compared to non-reactive subjects. Collagen-Co complex at 0.1mM and 1mM concentrations showed 306.42mg/ml and 295.15 mg/ml respectively. The non-reactive group showed 210.85 mg/ml IgG bound to Co-collagen at 0.1 mM and 192.37 mg/ml at 1mM concentrations. Chromium-collagen complex showed the least differences in IgG binding between the two groups. The only concentration that showed a significant difference for this complex was 1mM. The reactive group showed 353.29mg/ml of IgG bound to Cr-collagen at 1mM and 269.00 mg/ml for the same concentration in the non-reactive group. Overall, Ni-collagen and Co-complexes exhibited the highest differences between reactive and non-reactive groups. The highest binding concentrations were found at 0.01 mM for Ni, 0.1mM for Co and 0.01 mM for Cr.

DISCUSSION: The ability of IgG to bind metal indicates humoral immunity may be an important mechanism in triggering an adaptive response (via antigen presenting cells) in metal sensitivity. Typically, IgG binds antigenic determinants of metal-protein complexes (opsonization) and facilitates phagocyte-mediated engulfment of metal-protein antigen via IgG constant region receptors (2). More IgG binding to metal occurred in all metal-collagen complexes at all concentrations in the TJA reactive group compared to the nonreactive group, although only half of the metal-protein complexes exhibited significant differences between groups. These results lend support to our hypothesis that soluble implant metals, conjugated to peri-implant tissue proteins (collagen I), exhibit preferential IgG binding in metal-reactive TJA subjects compared to non-reactive subjects. However, contrary to our hypothesis, this IgG binding was not concentration dependent in either group. Even though the reactive group showed higher IgG specificity to all three metal-protein complexes, both groups showed a significant level of metal antigen recognition and the clinical implications of these findings are still unresolved. The results of this first stage of investigation need to be correlated with clinical performance to determine if this plays a significant role clinically. These results suggest that people demonstrating reactivity to metal implants produce greater amounts of metal-specific antibodies in vivo, implying certain subjects develop humoral and adaptive mechanisms creating a stronger metal-sensitive response. The specific antigenic determinants formed by self-proteins conjugated to metal degradation products remain unknown. Further studies are needed to determine the etiology of metal complexed with self-proteins that become antigenic.

ACKNOWLEDGMENTS: NIAMS / NIH

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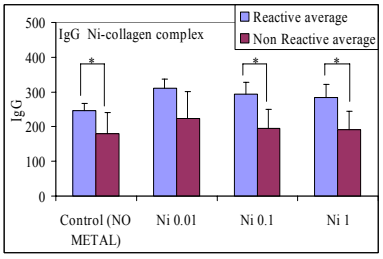

Fig1. IgG binding to Ni. Reactive group SI=20 , NonReactive SI=0.4. Note: * indicates significance at p<0.05.

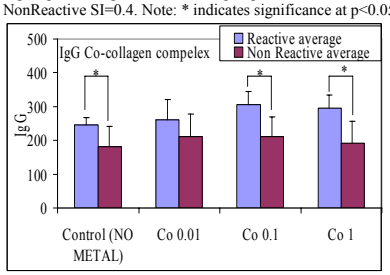

Fig2. IgG binding to Co. Reactive group SI=20 , NonReactive SI=0.4. Note: * indicates significance at p<0.05.

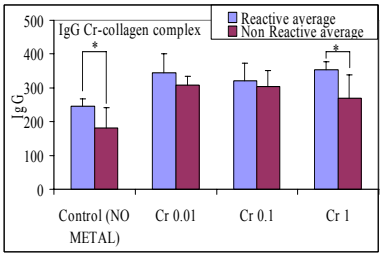

Fig3. IgG binding to Cr. Reactive group SI=20 , NonReactive SI=0.4 . Note: * indicates significance at p<0.05