IMPLANT METALS ACT SYNERGISTICALLY WITH BACTERIAL IMMUNOGENS (ENDOTOXINS, MITOGENS, AND SUPERANTIGENS) TO ACTIVATE LYMPHOCYTES INDICATING A NOVEL MECHANISM OF IMPLANT BIOREACTIVITY

Nadim James Hallab, Carlo Messina, Joshua J. Jacobs
Department of Orthopedic Surgery, Rush-Presbyterian-St. Lukes Medical Center, Chicago, IL 60612

INTRODUCTION: While approximately 0.5-2% of total joint arthroplasties (TJA’s) become infected within the first 6-10 years requiring revision,1 overall rates of revision arthroplasty are much higher approaching 7% at 10 years.2 It is well established that the degradation products of TJA have been associated with aseptic adverse tissue responses.3 The interaction(s) of TJA degradation products with bacteria and subsequent bioreactivity within the periprosthetic milieu remains unknown. Infection aside, some patients with TJA can tolerate large debris burdens for long periods of time (>8 years) with relatively little peri-implant reaction (i.e. inflammation and osteolysis), whereas other patients with seemingly the same particulate and ionic burdens demonstrate pronounced reactivity within 2-7 years, resulting in peri-implant bone resorption.2,5 Thus it is likely that immunologic responses associated with the release of metal from TJA components are partly responsible for differences in reactivity and that these metal induced alterations in local immune responses will also alter host defense and inflammatory responses to prosthetic infection. The factors which predispose to infection are primarily related to local alterations in host defense and the opportunity of, quantity of and exposure of microorganisms to the prosthesis.1 The biologic and immunologic consequences of the interaction of implant metal with bacterial immunogens previously implanted to be immunogens (e.g. Co, Cr, Ni and Ti4+) with prosthetic associated bacterial immunogens (e.g. endotoxins, mitogens and superantigens) remains undetermined. We hypothesized that specific implant alloy metals can act synergistically with bacterial immunogens to produce immunologic reactions (inflammation). We tested this hypothesis by evaluating primary human lymphocyte reactivity (isolated from 5 healthy individuals) to implant alloy metals and representative bacterial immunogens (i.e. endotoxin, mitogen and superantigen) both alone and in combination in varying doses.

METHODS AND MATERIALS: Subjects (n=5, 2 male, 3 female, average age 31 yrs) were used for lymphocyte reactivity testing to metals using lymphocyte transformation testing (proliferation assays). Human primary lymphocytes were isolated from 30 mls of blood using density gradient separation (Ficol-isopaque, Pharmacia, Piscataway, NJ) (15-30 x 10^6 cells per subject) at three different times, each testing one of the three bacterial immunogens. Lymphocytes in all assays were incubated with DMEM and 10% autologous serum with metals (NiCl2, CoCl2, and CrCl2 at 0, 0.01, 0.1, 1, and 10 mM) and representative bacterial immunogens (lipopolysaccharide [LPS] and phytohemagglutinin [PHA], each at 0, 0.0001, 0.001, 0.01mg/ml, and staphylococcal enterotoxin A [SeA] at 0, 0.00001, 0.01, and 1 ng/ml). Proliferation assays: Proliferation assays were conducted as previously described.4 The amount of lymphocyte proliferation was normalized to that of the negative control (no treatment), providing the stimulation index, SI. Proliferation assays were performed over a six-day period. All proliferation testing was conducted in quadruplicate.

RESULTS: All the metals tested were capable of eliciting a synergistic proliferation response with bacterial immunogens (LPS, PHA and SeA). An immunogenic response was defined as a greater than 2 fold (statistically significant, p<0.05) increase in proliferation. All subjects demonstrated immunogenic responses to Ni and Co, and 4 of the 5 subjects were reactive to Cr at concentrations between 0.01 and 10 mM. However, there was a large degree of variability in metal response of each individual at the three testing times. A synergistic effect of metal with bacterial immunogen was defined as a significantly greater (p<0.05) proliferative response when the metal and the bacterial immunogen were combined as compared to the additive effect of both treatments alone each at their respective doses. The synergistic effect of Cr is shown with LPS, PHA and SeA (Figs 1, 2, and 3 respectively), for a single individual. The columns representing the stimulation index demonstrate how Cr and bacterial immunogen can act synergistically to induce a proliferative response in lymphocytes when compared to each treatment separately at a specific concentration (indicated by the highlighted columns in Fig 1). The occurrence of this synergistic effect is summarized in Table 1 for all n=5 individuals. These results indicate that Cr together with LPS acted synergistically to activate lymphocytes of all 5 subjects, whereas Co and Ni did not. Co, Cr and Ni together with PHA and SeA activated lymphocytes in approximately half of the subjects tested.

DISCUSSION: All metals tested (Co, Cr, and Ni), alone and in combination with bacterial immunogens induced lymphocyte activation. Supporting our hypothesis, all metals tested (Co, Cr and Ni) were capable of acting synergistically with all bacterial immunogens tested (LPS, PHA and SeA) to activate certain individuals’ primary human lymphocytes. However, these metals acted synergistically with PHA and SeA, with only specific individuals’ lymphocytes, indicating an inherent variability. While Cr acted synergistically in all subjects, Co and Ni did not react similarly in any of the test subjects. Endotoxin (LPS) is characteristic of gram-negative bacteria, which contains LPS in the outer lipid layer. PHA typifies the action of bacterial lectins (carbohydrate binding molecules, found in plants and bacteria), which act as lymphocyte mitogens by cross-linking T cell receptors resulting in proliferation. SeA is a typical bacterial superantigen, which acts to directly bind class II molecules on immunogen presenting cells to the Vβ chain of T cell receptors resulting in activation of the T cells. That specific implant metals can act alone or synergistically with these three types of bacterial immunogens to activate lymphocytes indicates the need to further understand and predict this phenomena in patients with or receiving TJA where the use of various metal alloys is possible and underscores the various mechanisms by which metal debris may become bioreactive. Overall this data can be interpreted both positively and negatively in the context of TJA. Negatively: low grade infection not capable of eliciting an inflammatory reaction may do so in the presence of implant debris, leading to local persistent inflammatory reactions leading to osteolysis or and systemic reactions (e.g. metal sensitization). Positively: Metal implant debris may act synergically with bacterial immunogens to elicit immune response(s) acting to prophylactically enhance immune response to implant infection prior to a worsening of bacterial-prosthesis colonization. It may be that one or both of these scenarios can occur in vivo and which one dominates may vary with the individual. These findings represent a novel mechanism by which metal induced reactivity may be a potential participant in bioreactivity that previously has been largely attributed to macrophage/ particle mediated osteolysis of Type IV granulomatous hypersensitivity. Therefore we contend that the current findings may represent a new mechanism by which metals released from implant degradation may contribute to the etiology of poor implant performance.

ACKNOWLEDGMENTS: National Institutes of Health

REFERENCES: