Bioreactivity Responses to Bone Cement Are Dose Dependent

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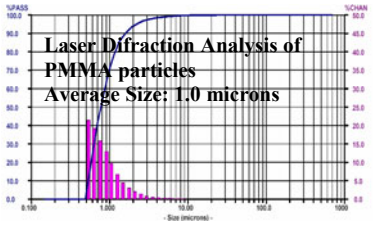
INTRODUCTION: Excessive immunologic reactivity (bioreactivity) to implant materials can lead to early failure. The benefit of bone cements' early fixation is tempered by the risk of increased biologic reaction to debris. The degree to which bone cement debris (monomer and/or particles) can activate lymphocytes in humans remains unknown. We hypothesized that a normal response of lymphocytes to bone cement debris will be both dose dependent and proportional, such that excessive reactivity will only occur at high concentrations of soluble and particulate debris. We tested this by exposing peripheral blood mononuclear cells (PBMCs) from young healthy individuals to increasing concentrations of bone cement monomer and polymeric particulate debris and measured the responses of lymphocytes using lymphocyte transformation testing (LTT).

MATERIALS AND METHODS:

Subjects: PBMCs from peripheral blood of n=6 young healthy adults was used (IRB approved), (average age=27, 4 males, 2 females). Group size (2N=11) was determined using power analysis at 80% and an expected 50% difference between reactive and nonreactive treatments with a <30% standard deviation. **Materials:** Soluble methyl-methacrylate and particulate PMMA bone cement particles (Surgical Simplex, Stryker) were size separated to an average size of 1 micron with 95% below 2 microns, see laser diffraction. All particles were verified to be endotoxin free (Limulus Assay). **Testing:** PBMC's were isolated from 30 mLs of blood (15-30 x 10^6 cells per subject) and incubated with DMEM and 10% autologous serum (plain media as a negative control, 0.01 mg/ml phytohemagglutinin PHA as a positive control). Proliferation assays were conducted as previously described.(1) The amount of PBMC lymphocyte proliferation was normalized to that of the negative control (no treatment), providing a stimulation index, SI where SI>2 was indicative of elevated reactivity. Proliferation assays were performed over a six-day period (delayed type response) in quadruplicate.



RESULTS: PMMA bone cement particles and monomer methacrylate solutions both stimulated lymphocytes in vitro (LTT) in 5 of 6 subjects challenged with soluble bone cement monomer (Fig 1) and 4 of 6 subjects challenged with PMMA particulate debris (Fig 2). Monomer PMMA reactivity was present at both low and high concentrations (0.0001% to 10% v/v in culture medium). Similarly particulate PMMA demonstrated dose dependent lymphocyte stimulation in each individual.

DISCUSSION: These results do not support our original study hypothesis. Although particulate and/or soluble bone cement demonstrated a dose dependent response in all 6 individuals tested, it was directly proportional to dose in only 2 of the 4 subjects reactive to particulate PMMA and in only 1 of the 5 subjects reactive to soluble methacrylate. That is, all reactive subjects (SI>2) demonstrated a dose dependence, but for some it was inversely proportional, for others it was directly proportional, and others demonstrated a mid-range (*Fig 1 Subject 5*) or a bi-modal (*Fig 1 Subject 6*) reactivity to dose. Limitations of this study include small group size, appropriate challenge agents and lack of differentiating innate from adaptive immune reactivity. However, this in vitro testing of primary human lymphocyte-monocyte populations from healthy young subjects demonstrates reactivity to soluble and particulate bone cement is a normal but highly variable response (6 orders of magnitude between optimal dose responses). This person dependent reactivity supports a hypothesis corollary that some individuals are more likely hyper-reactive to particulate and soluble bone cement debris at physiologically relevant concentrations (i.e. low doses).

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REFERENCES:

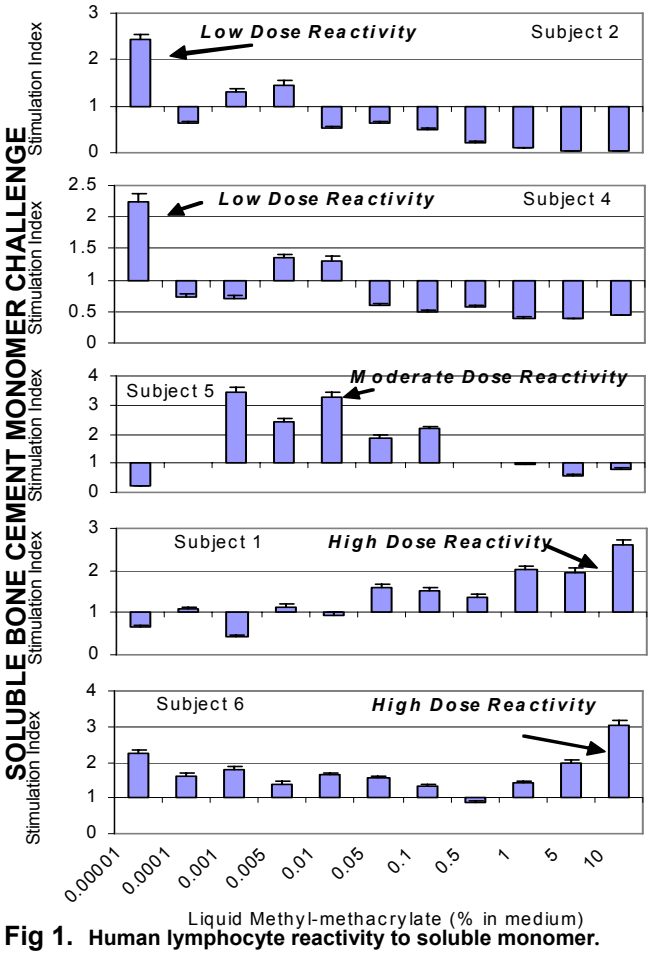

Fig 1. Human lymphocyte reactivity to soluble monomer.

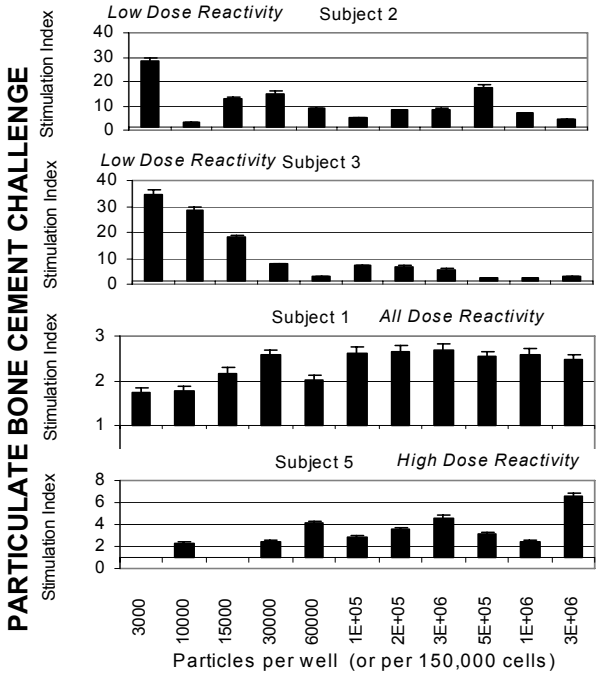

Fig 2. Human lymphocyte reactivity to PMMA particles.