Early Phase of Wear Particle Induced Inflammation was inhibited by NF-κB Decoy Oligodeoxynucleotide

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Introduction: Osteolysis, wear particle induced peri-prosthetic bone resorption, is a major cause of aseptic loosening and late implant failure in total joint replacement. Modulation of the transcription factor NFκB in immune cells has been proposed to abrogate wear particle induced inflammation and osteolysis. The aim of this study is to demonstrate that NF-κB decoy Oligodeoxynucleotide (ODN) inhibits the early phase of particle induced inflammation using the murine calvarial model.

Methods: This animal protocol was approved by the Stanford University Animal Care Committee. A total of 60 C57BL/6 male mice aged eight to ten weeks were divided into 4 different groups. Negative control mice received saline injection (Group1= Saline). The other three groups received ultra high molecular weight polyethylene (UHMWPE) particles alone suspended in PBS (Group 2=PE), with 5μM/100μl of NFκ ODN (Group 3= PE+ODN) or 5μM/100μl of Scrambled ODN (Group 4= PE+SC) in 100μl of saline given at the time of surgery and then every other day for 14 days into the subcutaneous bursa overlying the calvarium, respectively. To simulate the early phase of wear particle induced inflammation, we injected a comparatively small dose of particles (4 × 10⁸ particles in 3 mg) in this study. Animals were sacrificed 14 days after the surgical procedure. Frozen sections of 6μm were cut coronally to include the distal half of the frontal bones and proximal half of the parietal bones, the site of particle injection. Hematoxylin and eosin (H&E) stained sections was performed at 50μm increments from 100μm to the left and right of the midline suture using a digital ruler. The total tissue thickness (ThT) and the bone thickness (BT) were then manually measured at these 5 points. The measurements were expressed as a mean ratio of BT/ThT. Osteoclast-like cells were identified using a leukocyte acid phosphatase kit, TRAP (Sigma) as large multinucleated cells in order to evaluate the bone resorption activity.

Calvaria were removed as a whole under sterile conditions from five animals per group and randomly assigned for organ culture. Each calvarium was cultured with DMEM with glutamine, and 1% anti-mycotic/anti-biotic solution and incubated for 24 h at 37°C with 5% CO2. The culture supernatants were then collected for ELISA analysis of TNF-a, IL-1ra, RANKL, and OPG secretion. Micro CT scans were performed immediately before the first injection and at day 14 for all groups to detect changes in the bone volume (BV) and total volume (TV), and the ratio was defined as the bone volume fraction (BVF). Statistical analysis was performed using Graph-Pad Prism.

Results: Histomorphometric analysis showed no significance in BT/ThTs of 0.62±0.07, 0.58±0.13, 0.65±0.15 and 0.69±0.07 in Saline, PE, PE+ODN and PE+SC groups, respectively. However, the number of
TRAP positive cells in PE+ODN was significantly reduced compared to PE and PE+SC groups (13.0±2.6 versus 20.2±3.8 and 20.6±3.6, respectively, Figure. 1).

ELISA analyses delineated the effects of NF-κB ODN (Figure. 2). Tumor necrosis factor α (TNFα) secretion and Receptor activator of nuclear factor κ-B ligand (RANKL) were decreased in the PE+ODN group compared to PE group (7.77±2.29pg/ml versus 31.01±4.57pg/ml and 2.67±2.57pg/ml versus 15.13±5.77pg/ml, respectively). Increased osteoprotegerin (OPG) was also seen in the PE+ODN group.

Interleukin 1 receptor antagonist (IL-1ra) secretion was increased in the presence of NF-κB decoy ODN, however, this effect did not reach statistical significance. Micro CT analysis showed no significant difference in BVF among the groups (0.121±0.007mg/ml, 12.8±0.004mg/ml, 0.129±0.009mg/ml and 0.129±0.004mg/ml in Saline, PE, OPE+ODN, PE+SC groups, respectively).

**Discussion:** In order to understand the pathogenesis of wear particle inflammation, appropriate animals models must be developed to simulate the early phases of inflammation in which osteolysis is not present. This was the case in the present murine model. NF-κB decoy ODN injection mitigated the expression of pro-inflammatory cytokines in the presence of clinically relevant UHMWPE wear particles. This scenario is comparable to the early phases of particle induced inflammation in which bone resorption is not manifest and is the situation in which early intervention would optimize preservation of bone stock.

**Significance:** Mitigation of the innate immune response using local delivery of NF-κB decoy ODN appears to favorably modulate the early phase of inflammation due to clinically relevant wear particles.
Figure 2. Pro-inflammatory cytokines were suppressed by NF-κB decoy ODN (A,B). Conversely, OPG and IL-1ra were increased in the presence of NF-κB decoy ODN (C,D). *: p<0.05, **: p<0.005, ***: p<0.0005, ****: p<0.0001