Suppression of Orthopaedic Wear Particle Induced Pro-inflammatory Cytokine Production in Macrophages via NFκB Signaling

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Disclosures:

Introduction: The production of wear particles from all total joint replacements (TJRs) is inevitable. Cells of the monocyte/macrophage lineage (macrophages, foreign body giant cells and osteoclasts) are among the key cells that perpetuate the inflammatory reaction to orthopaedic wear particles generated from TJRs. This adverse biological reaction is mediated primarily by the transcription factor Nuclear Factor kappa B (NFκB), a critical signaling molecule in the activation of pro-inflammatory genes. Thus, one possible strategy to curtail adverse events such as periprosthetic osteolysis associated with wear particles is to inhibit these processes far upstream within the inflammation pathway. The purpose of this study is to mitigate the adverse effects of particulate biomaterials and inflammatory stimuli by local delivery of an NFκB inhibitor, NFκB decoy Oligodeoxynucleotide (ODN). Fig. 1 summarizes these concepts.

Methods: 1. NFκB decoy ODN: Oligodeoxynucleotides (ODNs) can be used as “decoy” cis-elements to block the binding of nuclear factors to promoter regions of targeted genes, resulting in the inhibition of gene activation. Synthetic NFκB decoy cis-element ODNs bind to NFκB, resulting in the prevention of NFκB interaction and activation of NFκB-promoting target gene expression (Fig.1).

2. Anti-inflammatory effects of NFκB decoy ODN: We challenged murine bone marrow derived macrophages (BMDMs) or human THP-1 macrophage cells with ultra high molecular weight polyethylene (UHMWPE) particles 1.0±0.1 um (mean±SEM) in diameter. BMDMs were seeded onto 24-well plates (0.7×106 cells/well) and incubated with either NFκB decoy ODN (0.5 μM) or scrambled decoy ODN (S-ODN) for 12 hours before addition of the phagoytosable UHMWPE particles (6×108) and/or LPS (1μg/ml). Cell-seeded culture plates without particles/LPS served as negative controls. The supernatants were collected at 6, 12, 24 and 48 hours. The efficacy of NFκB decoy ODN was analyzed by Luminex Assay for specific cytokines. We also studied whether the effect of NFκB decoy ODN complemented with different transfection agents could improve the inhibition of TNF-α production. The transfection was performed in RAW 264.7 (mouse monocyte/macrophage cell line) cells using NFκB decoy ODN alone, or complemented with one of three commonly used transfection agents: a cationic polymer (C32-122), Lipidoid (NA114), or Lipofectamine 2000 (Invitrogen).

Results: Addition of UHMWPE particles to the cultures significantly increased the production of pro-inflammatory cytokines including TNF-α, IL-1β, IL-6 and chemokines such as MCP-1, whereas lipopolysaccharide (LPS) plus UHMWPE particles further enhanced the expression levels. Importantly, the functional decoy ODN significantly suppressed pro-inflammatory cytokine and chemokine expression in both mouse and human macrophages. (p<0.01, Fig. 2, 3 and 4). Addition of scrambled decoy ODN (nonfunctional) was ineffective. The NFκB decoy ODN alone, without an additional transfection agent down-regulated TNF-α production by macrophages in response to LPS stimulation without significant cytotoxicity. The use of other transfection agents did not lead to a further decrease in TNFα production.

Discussion: Local application of NFκB decoy ODN with particle challenged macrophages consistently decreased pro-inflammatory cytokine (TNF-α) production in particle/LPS induced inflammation. The small molecule NFκB decoy ODN alone was sufficient to produce this effect without the necessity of additional transfection agents.

Significance: The inflammatory and foreign body reaction associated with wear debris can interfere with initial prosthetic osseointegration and lead to periprosthetic osteolysis, jeopardizing long-term implant stability. Our study may provide a novel, translational strategy to mitigate wear particle-associated periprosthetic osteolysis by local delivery of NFκB decoy ODN.
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Fig. 1. Summary of biological processes involved in implant loosening and osteolysis.

Fig. 2: TNF-α mRNA (Real-Time PCR) expression at various time points by PE particles ± LPS and ± functional versus nonfunctional ODN. A, Murine bone marrow derived macrophages (BMDMs); B, Human THP-1 macrophage cells.