Orthopaedic Wear Particle Disease and NFκB Signaling

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Statement of Purpose: 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, NFkB decoy ODN. Fig. 1 summarizes these concepts.

Methods: *1. NFkB 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 NFkB decoy cis-element ODNs bind to NFkB, resulting in the prevention of NFkB interaction and activation of NFkB-promoting target gene expression (Fig.1). The NFkB decoy ODN was kindly provided by Dr. Egashira from Kyushu University, Japan.

2. Anti inflammation effects of NFkB decoy ODN: We challenged bone marrow derived macrophages (BMDMs) with ultra high molecular weight polyethylene (UHMWPE) particles 1.0±0.1 um (mean±SEM) in diameter. BMDMs were seeded onto 24-well plates (0.7×10^6 cells/well) and incubated with either NFkB decoy ODN (0.5 μM) or scrambled decoy ODN (S-ODN) for 12 hours before addition of phagoytosable UHMWPE particles (6×10^8) 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 NFkB decoy ODN was analyzed by Luminex Assay. Here we used the inhibition of TNF-α production by NFkB decoy ODN as representative data. We also studied whether the effect of NFκB decoy ODN complemented with different transfection agents could improve the inhibition of TNF-α production. Thus 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). TNF-α production in the supernatant was assayed by ELISA (R&D).

Results: Addition of UHMWPE particles to the cultures significantly increased TNF-α protein production. In contrast, addition of NFκB decoy ODN to the cultures after particle stimulation reduced TNF-α production by more than 50% at all time points ($p<0.01$, Fig. 2A). Addition of scrambled decoy ODN (nonfunctional) was ineffective. Addition of LPS with particles to the cultures significantly increased TNF-α protein production in the media of all groups as compared with respective particle alone groups. However, addition of NFκB decoy ODN to the cultures still significantly reduced TNF-α production (Fig. 2B). NFκB decoy ODN alone significantly down-regulated TNF-α production by macrophages in response to LPS stimulation. The use of other transfection agents did not lead to a further decrease in TNFα production.

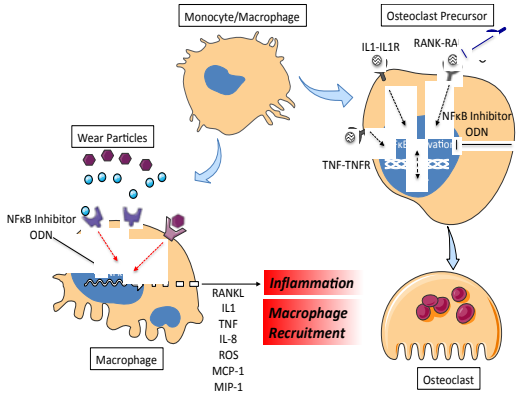

Fig. 1. Summary of biological processes involved in implant loosening and osteolysis.

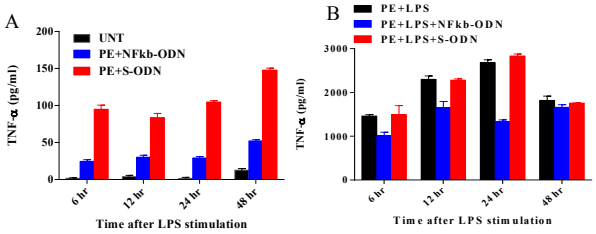

Fig. 2. Effect of NFκB decoy ODN on TNF-α production by BMDMs stimulated with UHMWPE particles alone (A) or UHMWPE particles with LPS (B) for 6, 12, 24 and 48 h.

Conclusions: 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. 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 NFkB decoy ODN.

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