

Suppression of UHMWPE Wear Particle- induced Pro-Inflammatory Cytokine and Chemokine Production by Macrophages using an NF- κ B Decoy Oligodeoxynucleotide

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Statement of Purpose: Total joint replacement (TJR) is a cost-effective surgical procedure for end-stage arthritis. As TJR has been extended to younger more active patients, one important goal is to decrease the surgical revision rate due to wear of the bearing surfaces. Continuous production of UHMWPE wear particles induces macrophage infiltration and chronic inflammation, which can lead to peri-prosthetic osteolysis. Targeting individual pro-inflammatory cytokines directly has not reversed the osteolytic process in clinical trials, due to compensatory upregulation of other pro-inflammatory factors. We hypothesized that targeting the important transcription factor NF- κ B could mitigate the inflammatory response to wear particles, potentially diminishing osteolysis.

Methods: RAW264.7 murine reporter cells were generated by stably transfecting the cells with pGL4.32[luc2P/NF- κ B-RE/Hygro] reporter plasmid (Promega). Human peripheral blood macrophages were isolated from healthy male, caucasians, age 20-40. In addition, cells from the human macrophage cell line THP1 were activated by treating the cells with 50 nM Phorbol-12-myristate-13-acetate for 3 days. UHMWPE particles were obtained from joint simulation test samples provided by Dr. Tim Wright at the Hospital of Special Surgery. Decoy NF- κ B oligo-deoxynucleotide (ODN), which interferes with the transcription of NF- κ B, was used to modulate NF- κ B activity. Murine and human macrophages were cultured with various combinations of UHMWPE particles, lipopolysaccharide, decoy or scrambled NF- κ B ODN with appropriate controls, and then analyzed for luciferase activity and cytokine expression profiles using the multiplex-panel cytokine array (Invitrogen). Chemotaxis assays were performed using a ChemoTx® Disposable Chemotaxis System (NeuroProbe) containing 5 μ m pore. Conditioned media from THP1 macrophage cultures with different treatments was used as a chemo-attractant to recruit THP1 cells. Statistical analysis was performed using Graph-Pad Prism. The animal protocol was approved by the Stanford University Animal Care Committee.

Results: The luciferase expression level increased 6-fold (indicating NF- κ B activation) with 1 μ g/ml LPS treatment. The NF- κ B activity was increased 50% in response to UHMWPE particles; addition of the decoy ODN significantly decreased the particle-induced cell activation compared with scrambled ODN (Fig.1). Exposure of macrophages to UHMWPE particles induced the expression of multiple pro-inflammatory cytokines

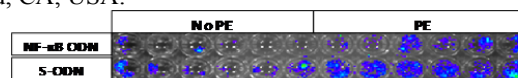


Fig.1 NF- κ B decoy ODN suppressed NF- κ B activation in RAW264.7 NF- κ B luciferase reporter cells response to UHMWPE particles. Luciferase activity in the reporter cells response to UHMWPE particles (PE) or control (No PE), which combined with decoy ODN (NF- κ B ODN) or scramble ODN (S-ODN).

Classification	Name	Mouse	THP1
Chemokine -Macrophage	<i>MCP1</i>	*↓ 27%	*↓ 20%
	<i>MIP1α</i>	*↓ 83%	*↓ 65%
	<i>MIP1β</i>	N.A.	*↓ 66%
	<i>RANTES</i>	N.A.	*↓ 63%
	<i>IL-8</i>	N.A.	*↓ 54%
-Neutrophil	<i>CXCL1</i>	*↓ 36%	N.A.
	<i>TNF-α</i>	*↓ 41%	*↓ 49%
Pro-inflammatory cytokines	<i>IL-1α</i>	N.S.	N.A.
	<i>IL-1β</i>	*↓ 38%	N.S.
	<i>IL-1Ra</i>	N.S.	*↓ 27%
	<i>IL-6</i>	*↓ 84%	*↓ 68%
	<i>IL-12</i>	N.S.	*↓ 37%

Table 1 NF- κ B decoy ODN suppressed the expression of multiple cytokines by macrophages exposed to UHMWPE particles or endotoxin. Primary mouse macrophage and human macrophage THP1 cells were exposed to particles or endotoxin (1 μ g/ml LPS) for 48 hrs. *Significant suppressions compared with scrambled ODN are indicated. N.A.: Not available, N.S. Not Significant

and chemokines including TNF- α , MCP1, MIP1 α and others. The neutrophil attracting chemokines IL-8 and CXCL1, were both induced by exposure to UHMWPE particles. Importantly, the decoy ODN significantly suppressed the induced pro-inflammatory cytokine and chemokine expression in both murine and human macrophages (Table 1). Conditioned media from cells treated with decoy ODN induced less THP1 cell migration after 24h and 48h exposure to particles with/without LPS.

Conclusions: Targeting the NF- κ B pathway simultaneously suppressed the expression of multiple pro-inflammatory cytokines induced by UHMWPE particles and endotoxin in macrophages. This strategy could potentially block wear particle-mediated osteolysis by several mechanisms. First, decoy NF- κ B ODN inhibited the production of pro-inflammatory cytokines such as TNF- α and IL-1 β , which could mitigate particle-induced tissue damage. Second, inhibition of macrophage attracting chemokines could further reduce the infiltration of circulating macrophages to the inflammatory site. Third, RANK/RANKL mediated osteoclastogenesis is also regulated by the NF- κ B pathway and is critical for the osteolytic process. Thus, the strategic use of NF- κ B ODN, delivered locally, could potentially diminish particle-induced peri-prosthetic osteolysis.

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