Introduction: Periprosthetic osteolysis stimulated by implant particulate debris is mediated by various pro-inflammatory cytokines (TNF-alpha, IL-1, and IL-6) that enhance osteoclast differentiation and activity, yet TNF-alpha (TNF) has recently been identified as playing a critical role in this process (1,2). Specifically, in osteoclast precursor cells implant particles activate the nuclear transcription factor NF-κB via a TNF-dependent pathway (3). This is a fundamental signalling mechanism since NF-κB is mandatory for normal osteoclast development and function. RANKL (receptor activator of NF-κB ligand, also known as ODF, OPGL) is a member of the TNF superfamily that also stimulates osteoclastogenesis and has recently been implicated in having a role in particle-induced bone resorption. The activity of RANKL is inhibited by a soluble decoy receptor called osteoprotegrin (OPG). Presently, the specific roles and interaction of TNF and RANKL in particle-induced osteoclastogenesis are not understood. Thus, we have investigated the distinct actions of these potent osteocorticogenic factors in polydimethylacrylate (PMMA) particle-induced osteoclastogenesis in vitro.

Methods: PMMA particles/Reagents: Commercially available PMMA microspheres (Polysciences, Inc.) 1-10 um diameter (mean, 4.5um) were used for all experiments. Particles tested negative for endotoxin contamination with a Limulus Amebocyte Lysate assay (BioWhittaker, Inc.). OPG and TNF-alpha were obtained from R&D Systems Inc.

NF-κB Electrophoretic Mobility Shift Assay (EMSA): Osteoclast precursor cells in the form of bone marrow macrophages were isolated from C3H/HeN mice according to established protocols, treated at confluence and analyzed by EMSA (4). Nuclear extracts were prepared and incubated with an end-labeled double stranded oligonucleotide probe derived from the KB-3 site of the TNF promoter. Samples were fractionated by 4% TBE gel electrophoresis and analyzed by autoradiography. Findings were verified in three independent experiments with similar results.

Osteoclastogenesis Assay: Whole bone marrow (WBM) cultures for the osteoclastogenesis assay were obtained from C57Bl wild type or TNF knockout mice (TNF−/−) and maintained in the presence of 10nM 1,25-(OH)2D3 for 8 days as previously described (4). Cultures were treated on day 8 with control media (alpha-MEM, 10% FBS) or experimental media and maintained in culture for an additional 24 hours. Cultures were then fixed (day 10), TRAP stained and average osteoclast (multinucleated TRAP positive cells) number was determined. Each condition was run in triplicate and all experiments performed three times with similar results. Experimental conditions were compared to controls with an unpaired t-test.

Results: Since NF-κB is known to be an essential mediator of osteoclastogenesis, we first utilized EMSA to determine the effect of PMMA particles, TNF and RANKL on NF-κB activity in murine osteoclast precursor cells. All three of these stimuli are potent activators of NF-κB activity, suggesting that the effect of particles may be mediated by TNF and/or RANKL. To further distinguish the role of RANKL and TNF in particle-induced osteolysis we turned to an in vitro osteoclastogenesis assay. In WBM cultures, PMMA particles markedly induced osteoclast number (7 fold, p<0.002) and osteoclast size (Figure) compared to controls. This response was dose-dependent and maximal in 8 day old WBM cultures treated for an additional 2 days with particles. Under these same conditions, TNF and RANKL were also potent activators of osteoclastogenesis (11 and 6 fold increase respectively, p<0.001). The role of RANKL in the particle response was tested by addition of the soluble inhibitor OPG to the WBM cultures. OPG completely (99%) blocked basal and particle-induced osteoclastogenesis (Figure) in a dose-dependent manner with an optimal effect at 50ng/ml. To further understand the mechanism of OPG action, this inhibitor was added to WBM cultures at various time points (day 0, 2, 4, 6, 7 and 8) and maintained in the cultures through day 10. The inhibitory effect remained complete with addition of OPG to cultures on days 0, 2, 4, 6 and 7. OPG treatment on day 8 had a 90% inhibitory effect on osteoclastogenesis. Therefore, relative short-term inhibition of RANKL also had a major inhibitory effect on particle-directed osteoclastogenesis. Additionally, the OPG effect was reversible as demonstrated by cultures treated with OPG for the first 4 days and then treated for the remainder of the experiment with OPG-deficient media. In these cultures the osteocorticogenic effect of PMMA treatment on day 8 was maintained. We next investigated the role of TNF by utilizing WBM cultures derived from TNF−/− mice. In TNF−/− WBM cultures basal osteoclastogenesis was intact, but the stimulatory effect of particles was abolished (1.3 fold increase versus 11 fold in wild type) indicating a major role of TNF in particle-enhanced osteoclastogenesis.

Significance: These data demonstrate that: 1) PMMA particles, TNF and RANKL are all potent activators of NF-κB in osteoclast precursor cells, 2) PMMA particles induce a robust osteocorticogenic response in WBM cultures, 3) basal and particle-induced osteoclastogenesis are absolutely dependent upon RANKL activity and 4) basal osteoclastogenesis is independent of TNF action, yet particle-enhanced osteoclastogenesis is dependent upon TNF. These findings suggest TNF and RANKL participate in particle-induced osteoclastogenesis at different stages. TNF and other pro-inflammatory cytokines are likely to enhance particle-directed osteoclastogenesis under RANKL "primed" conditions. In contrast, RANKL has an essential and fundamental role in mediating basal and inflammatory osteoclastogenesis. In our system, blockade of RANKL activity creates a WBM microenvironment that does not respond to other particle-induced pro-osteocorticogenic factors. Thus, in addition to the TNF signalling pathway, RANKL and its associated molecules should be considered an excellent target for therapeutic interventions designed to block particle-induced osteolysis.

References:

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