NFATc1 MEDIATES ORTHOPEDIC PARTICLE-INDUCED OSTEOCLASTOGENESIS

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Introduction and Significance: Particle-induced peri-prosthetic osteolysis is the major cause for orthopedic implant failure. This failure is mediated mainly by the action of osteoclasts, the principal cells responsible for bone resorption and osteolysis. Therapeutic interventions to alleviate osteolysis have been focused on understanding and targeting mechanisms of osteoclastogenesis. The nuclear transcription factor NFAT is an essential terminal differentiation factor of osteoclastogenesis. This transcription factor is known to cooperate with c-jun/AP-1 in mediating RANKL-induced osteoclastogenesis. We have previously determined that RANKL is an essential cytokine mediator of particle-induced osteoclastogenesis, and that polymethylmethacrylate (PMMA) particles activate JNK and c-jun/AP-1 in bone marrow macrophages (osteoclast precursors). These results indicate that NFAT is also a key regulator of particle-stimulated osteoclastogenesis. Thus, the purpose of this study was to investigate the effect of PMMA particles on the NFAT signaling pathway in osteoclast precursor cells, and to test the effect of NFAT signaling blockade on particle-driven osteoclastogenesis in vitro.

Methods: Commercially available PMMA microspheres (Polysciences, Inc.) 1-10 μm diameter (6.0 μm mean, 95% <10μm) were used for all experiments. Immunoblot analysis: Equal amounts of cellular lysate protein were electrophoresed, transferred to nitrocellulose membrane and analyzed by immunoblot with antibodies to c-jun, phosphorylated c-jun and JNK. Osteoclastogenesis Assay: Murine (C57Bl/6) osteoclast precursor cells were isolated and maintained for three days in media supplemented with M-CSF (10ng/ml) and RANKL (10ng/ml). Cultures were then treated with control media, TNF (10ng/ml), PMMA (0.1mg/ml), TNF and CsA, PMMA and FK506 for 24 hours. FK506 treatment with the concentrations indicated (1nM, 10nM, 100nM, and 500μM) and then treated with control media or experimental conditions for additional 48 hours. Cultures were then fixed, Tartrate Resistant Acid Phosphatase (TRAP) stained and average osteoclast (multinucleated TRAP positive cells) counts were determined. Each condition was run in triplicates and all experiments performed three times. Results were compared with an unpaired t-test.

Results and Discussion: We demonstrate that PMMA particles stimulate nuclear translocation of the NFAT2 (also known as NFATc2). To this end, Cytosolic and nuclear protein extracts were isolated from bone marrow macrophages after treatment with the control media or PMMA (0.1mg/ml) for the times indicated. Equal amounts of extract lysates were subjected to electrophoresis, transferred to nitrocellulose membrane and analyzed by immunoblotting with antibodies to c-jun antibody. The data indicate that NFAT2 levels were marginally increased in the nuclei after 1 hour treatment with PMMA and significantly after 24 hours treatment. Nuclear translocation was further confirmed by electrophoretic mobility shift assay. We then tested specificity of the PMMA response on induction of NFAT2 and osteoclastogenesis using appropriate inhibitors. First, we show that cyclosporine-A (CsA) blocks PMMA particle-stimulated osteoclastogenesis. Specifically, osteoclast precursor cells were isolated and maintained for three days in media supplemented with M-CSF (10ng/ml) and RANKL (10ng/ml). Cultures were then treated with control media, TNF (10ng/ml) or PMMA (0.1mg/ml), TNF and CsA, PMMA and CsA for 24 hours. CsA treatment with the concentrations indicated (0.01μM, 0.1μM, 0.5μM, 1μM and 2μM) started 30 minutes prior to TNF or PMMA exposure. All conditions were run in quadruplicate. Significance compared to control * p<0.001 and **p<0.05. CsA blocked TNF-induced osteoclast formation significantly at the concentration of 0.1, 1, 2μM blocked PMMA-induced osteoclastogenesis at 0.1, 0.5, 1, 2μM concentrations. Next, we demonstrate that the compound FK506 blocks PMMA particle-stimulated osteoclastogenesis. Briefly, Osteoclast precursor cells were isolated and maintained for three days in media supplemented with M-CSF (10ng/ml) and RANKL (10ng/ml). Cultures were then treated with control media, TNF (10ng/ml), PMMA (0.1mg/ml), TNF and CsA, PMMA and FK506 for 24 hours. FK506 treatment with the concentrations indicated (1nM, 10nM, 100nM, and 500μM) started 30 minutes prior to TNF or PMMA exposure. All conditions were run in quadruplicate. Significance compared to control * p<0.001 and **p<0.05. FK506 blocked osteoclast formation significantly at the concentration of 100, 500nM both TNF and PMMA. Similarly, we show that VIVIT blocks PMMA particle-stimulated osteoclastogenesis. Under similar experimental conditions described above VIVIT (500nM) was added on day0, 2 or day2 or day3, All conditions were run in quadruplicate. Significant compared to control * p<0.01. VIVIT blocked osteoclast formation by a time-dependent manner (VIVIT added on day 0, 2 significantly blocked osteoclastogenesis).

These observations demonstrate that: 1) PMMA particles activate NFAT2 translocation to nuclei in osteoclast precursor cells. 2) Blockade of the calcineurin/NFAT signaling pathway by CsA abolishes PMMA particle-stimulated osteoclastogenesis in vitro. 3) Direct blockade of NFATc1 by NFAT inhibitor VIVIT abolishes PMMA particle-stimulated osteoclastogenesis in vitro. Thus, the NFAT activation pathway should be considered as an experimental target for particle-induced osteolysis.

FIGURE: Osteoclast precursor cells were isolated and maintained for three days in media supplemented with M-CSF (10ng/ml) and RANKL (10ng/ml). Cultures were then treated with control media, TNF (10ng/ml) or PMMA (0.1mg/ml), in the absence (upper panels) or presence of CsA (1μM) for 24 hours. PMMA-stimulated osteoclasts are clearly shown in the upper right panel.


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