INTRODUCTION: Nuclear factor kappa-B (NF-kB) signaling mediates numerous physiological and pathological pathways including inflammatory cytokines, toll-like receptors, immunity, osteoclastogenesis, and osteolysis. Activation of the NF-kB signal transduction pathway is regulated by the IkB kinase (IKK) complex which contains catalytic subunits (IKKα and IKKβ) and a scaffold subunit (IKKγ/NEMO). It has been demonstrated that all members of this IKK complex are essential for osteoclastogenesis and selective inhibition of NEMO binding to IKKα and IKKβ impedes osteolysis. The carboxyl-terminal region of NEMO which contains a zinc finger motif is essential for NF-kB activation through ubiquitination events. Recently, it has been demonstrated that point mutation of Lys392 in mice (K392R) impaired TLR-induced signaling and NEMO-KR mice are more resistant to LPS-induced endotoxin shock. This study examines the osteoclastogenic response of cells derived from NEMO-KR mice and the effect of inflammatory agents and polymethylmethacrylate on such response.

METHODS: Commercially available PMMA microspheres (Polysciences, Inc.) 1-10 μm diameter (6.0 μm mean, 95% <10μm) were used for all experiments. Osteoclast Assay: Bone marrow macrophage from NEMO-KR mice or wild-type controls were maintained in the presence of RANKL (100ng/ml) and M-CSF (10ng/ml) for 3 days and then treated with control media or co-stimulated with LPS, TNF, IL-1, or PMMA particles for an additional 24 or 48 hours. Cultures were then fixed, Tartrate Resistant Acid Phosphatase (TRAP)-stained and average osteoclast (multinucleated TRAP positive cells) counts determined.

RESULTS: The results of three independent experiments show that basal osteoclastogenesis is reduced in NEMO-KR compared with wild-type cells. More importantly, the inflammatory response of NEMO-KR derived pre-osteoclasts to LPS, IL-1, TNF, and PMMA particles was significantly reduced compared with the exuberant response of wild type cells. Consistent with this result, we demonstrate that intracellular signaling of NEMO-KR cells is plummeted compared with wild type cells.

DISCUSSION: The results of this study show that the basal and inflammatory osteoclastogenic response of NEMO-KR is impaired compared with wild type cells. Given the fact that Lys392 undergoes K63-linked ubiquitination, it’s logical to assume such event supports propagation of cytokine-induced signals. Our finding that signaling by RANKL, PMMA, and inflammatory agents is impaired when Lys392 is mutated into Arginine suggests this residue is an essential mediator of inflammatory osteoclastogenesis. In vivo experiments are currently underway to determine if NEMO-KR mice are protected from endotoxins and PMMA-induced osteolysis. We conclude that Lys392 is potentially an important target for modulating inflammatory osteolysis.