MK2 Signaling Regulates Osteoclastogenesis and Pro-resorptive Cytokines During Aggregatibacter actinomycetemcomitans-induced Bone Loss

Bethany A Herbert¹, Heidi Steinkamp¹, Keith L Kirkwood¹,²

Department of Oral Health Sciences and the Center for Oral Health Research¹, Department of Microbiology and Immunology², MUSC

Objectives: The objective is to demonstrate that MK2 signaling is critical for osteoclastogenesis and bone loss during A.a. infection.

Background: Aggregatibacter actinomycetemcomitans (A.a.) is associated with aggressive periodontal disease (PD) characterized by inflammation coupled with bone loss. Monocyte precursors differentiate into macrophages or bone resorbing osteoclasts, both of which are critical in PD pathogenesis. Furthermore, macrophages secrete osteoclastogenesis driving cytokines. Interestingly, a mitogen activated protein kinase, MK2, regulates pro-inflammatory cytokines and osteoclast formation.

Methods: Mk2⁺/⁺ and Mk2⁻/⁻ mice were treated with live A.a. or PBS control at the mid-sagittal suture for 3-5 days. Calvariae were harvested for uCT, histological staining, RNA, and protein. Peripheral blood (PB) and bone marrow (BM) were harvested for flow cytometry and cytokine multiplex assays. In vitro, BM derived osteoclasts were differentiated by CD11blo magnetic bead sorting, priming with M-CSF and RANKL into pre-osteoclasts, and driven to form mature osteoclasts with A.a. lipopolysaccharide (LPS).

Results: Calvarial tissue stimulated with A.a. had increased MK2 levels (P≤0.05). Nanostring analysis of tissue RNA revealed that macrophage Emr1/F4/80 (P≤0.01) and Itgam/CD11b (P≤0.05) were reduced in Mk2⁻/⁻ mice during A.a. challenge. Osteoclastogenesis stimulating Tnfsf11/RANKL, Tnf, and Il1a were attenuated in the absence of MK2 (P≤0.05). Additionally, osteoclastogenesis-driving cytokines IL-1α and CXCL1 were regulated by MK2 in calvarial tissue. Calvariae resorption pits enumerated by uCT were reduced in Mk2⁻/⁻ compared to Mk2⁺/⁺ mice after A.a. treatment (P≤0.01). TRAP positive osteoclasts were also positively regulated by MK2 in vivo. In vitro, MK2 deficiency reduced A.a. LPS driven osteoclast size (P≤0.01) and number (P≤0.05). During A.a. challenge flow cytometry showed that PB CD11b⁺Ly6C⁴⁰CCR2⁺ osteoclast progenitors were upregulated independent of MK2 signaling.

Conclusions: Thus, MK2 signaling locally regulates osteoclast formation and pro-osteoclastogenic cytokines during A.a. infection. These results provide insight into modulation of MK2 as a potential PD therapeutic.

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