Inhibition of Lipopolysacharide Induced Osteoclast Formation And Bone Resorption

In Vitro And In Vivo In Mice By Cysteine Proteinase Inhibitors

Strålberg F1, Lindholm C2, Lindström E3, Kasprzykowski F4, Saftig P5, Abrahamson M6, Grubb A6, Lerner UH1,2


BACKGROUND

We recently reported that the signaling pathway downstream RANK in RANKL-induced osteoclastogenesis is inhibited by cysteine proteinase inhibitors (CPIs) (Strålberg et al., FASEB J, 2013). Here, we demonstrate that osteoclastogenesis stimulated by LPS E.coli in RANKL-primed mouse bone marrow macrophages (BMM) is inhibited by CPIs cystatin C, E-64, and Z-RLVG-CHN2. The inhibitory effect is associated with decreased gene and protein expression, and an active uptake of fluorescent tagged cystatin C dependent on LPS-stimulation. Osteoclast formation was inhibited by all three CPIs in cathepsin K deficient mice. Also, the cathepsin K inhibitor L-006235 did not inhibit osteoclast formation. LPS-stimulated osteoclast formation in skull bones of adult mouse was inhibited by E-64.

RESULTS

CONCLUSIONS

All together, these data show that:

✓ Cysteine proteinase inhibitors are important in LPS-induced osteoclastogenesis both in vitro and in vivo

✓ Osteoclast progenitors take up Cystatin C by a process facilitated by RL-primed LPS-stimulation

✓ inhibition of osteoclast formation by cysteine proteinase inhibitors is not explained by inhibition of cathepsin K

METHODS

✓ CELL CULTURE: Bone marrow macrophages (BMMs) were purified from bone marrow of mouse femur and tibia in 6-9w old Cia and Cathepsin K-/- mice and cultured in the presence of macrophage colony-stimulating factor (M) and receptor activator for nuclear factor κB ligand (RL) with or without LPS, cystatin C or related cysteine proteinase inhibitors. Osteoclasts were identified as multinuclear cells positively stained for the enzyme TRAP

✓ GENE TRANSCRIPTION ANALYSIS: RNA extracted from BMM cultures and mRNA expression analyzed using quantitative RT-PCR and semi-quantitative RT-PCR with β-actin and Gapdh as house keeping genes, respectively

✓ WESTERN BLOT: Protein expression analyzed using antibodies against c-Fos, NFATc1, Cathepsin K, and TRAP

✓ BONE SLICE CULTURES: BMM cells were cultured on bone slices, stained for TRAP and osteoclasts counted. Cells were then removed by sonication and toluidine-blue stained to assess pit formation

✓ CONFOCAL MICROSCOPY: BMMs incubated with 488nm fluorescent-labeled cystatin C and subsequently stained with DAPI (nuclei stain) and phalloidin-TRITC (actin-filament)

✓ IN VIVO: LPS injected above calvarial bone at day 0 with subsequent injections with saline or E-64 Day 0-4 and sacrificed on day 5. Calvarial bones were decalcified, sectioned and stained for cathepsin K+ osteoclasts

Disclosure of Interest: None Declared