PP225

Inhibition of Lipopolysacharide Induced Osteoclast Formation And Bone Resorption *In Vitro* And *In Vivo* In Mice By Cysteine Proteinase Inhibitors

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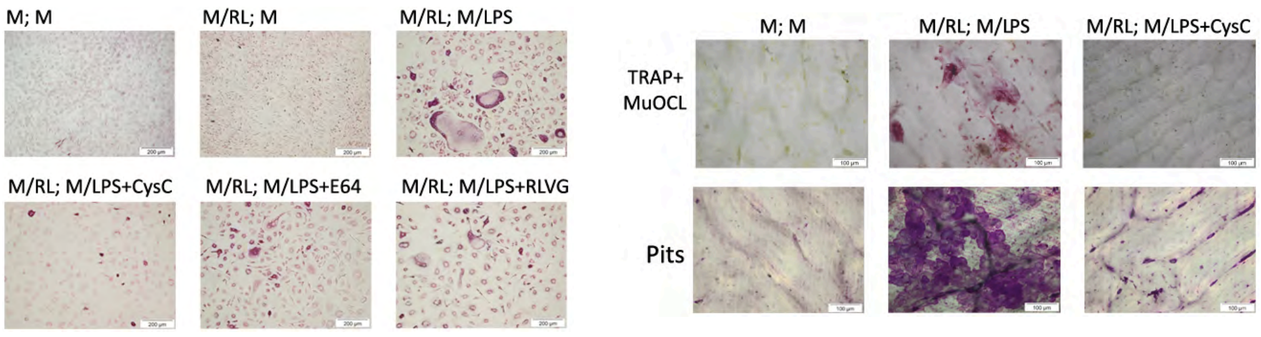
BACKGROUND

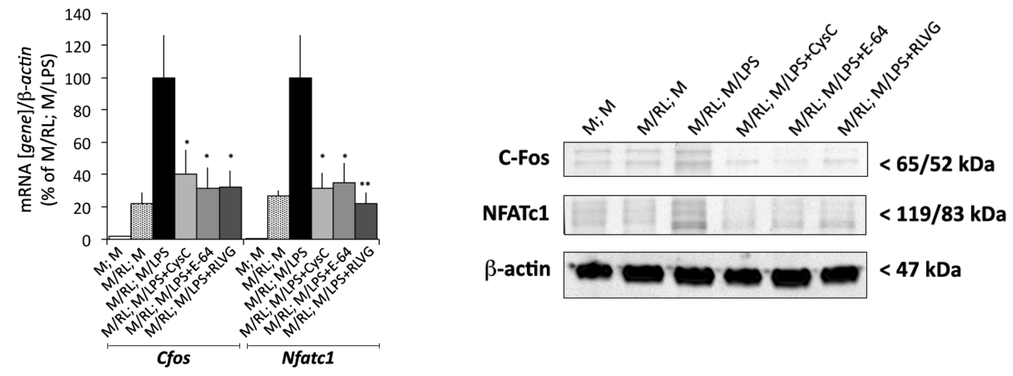
We recently reported that the signaling pathway downstream RANK in RANKL-induced osteoclastogenesis is inhibited by cysteine proteinase inhibitors (CPIs) (Strålberg *et al*., FASEB J, 2013). Here, we demonstrate that osteoclastogenesis stimulated by LPS *E.coli* in RANKL-primed mouse bone marrow macropages (BMM) is inhibited by CPIs cystatin C, E-64, and Z-RLVG-CHN_2. 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

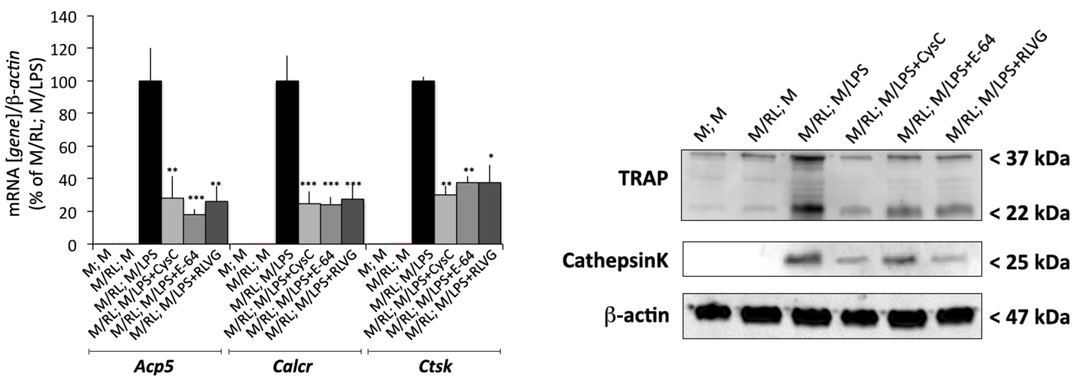
A) RL-primed LPS-induced osteoclast differentiation is inhibited by CPIs Cystatin C, E-64, and Z-RLVG-CHN_2



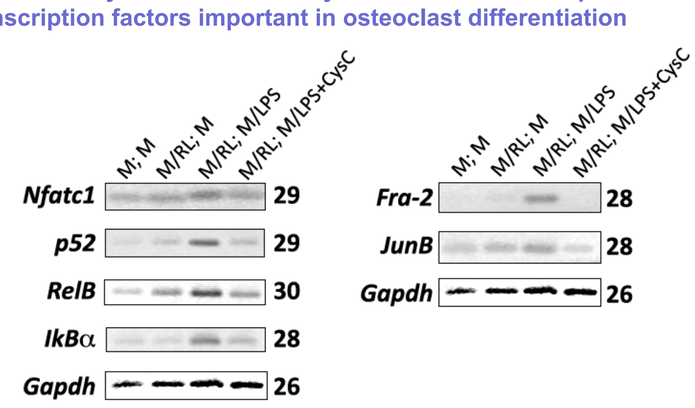
B) CPIs inhibit LPS-induced mRNA and protein expression of *Cfos* and *Nfatc1*



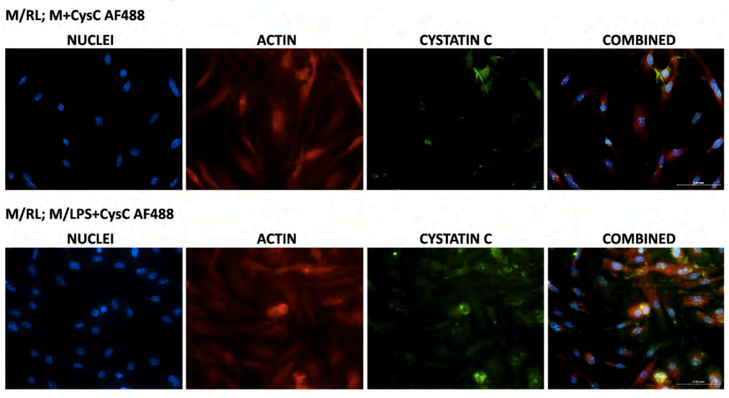
C) CPIs inhibit LPS-induced mRNA and protein expression of *Acp5, Calcr*, and *Ctsk*



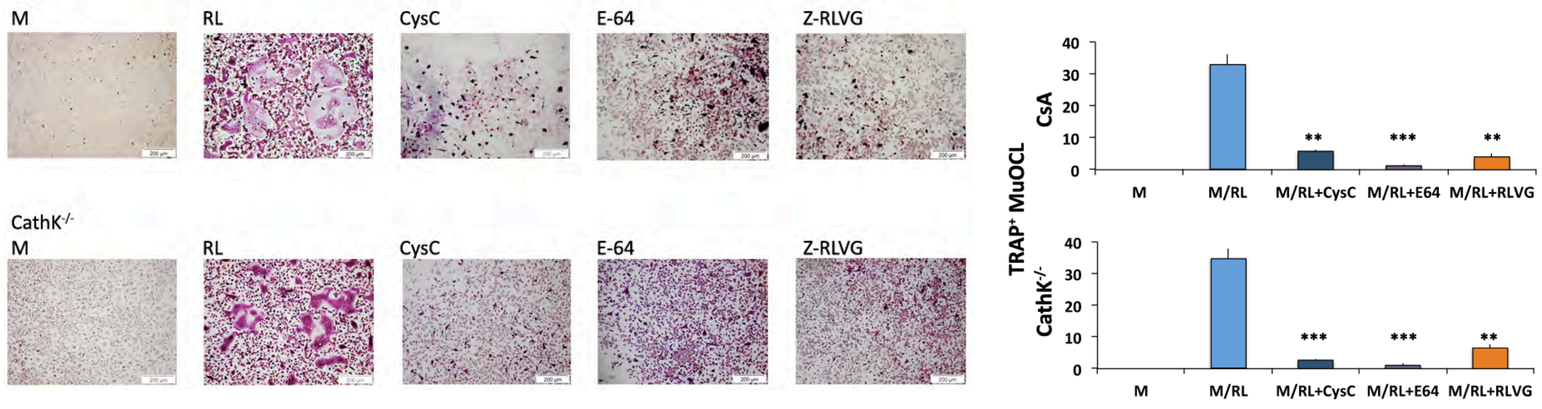
D) PCR analysis shows that Cystatin C inhibits RL-primed LPS-induced up-regulation of transcription factors important in osteoclast differentiation



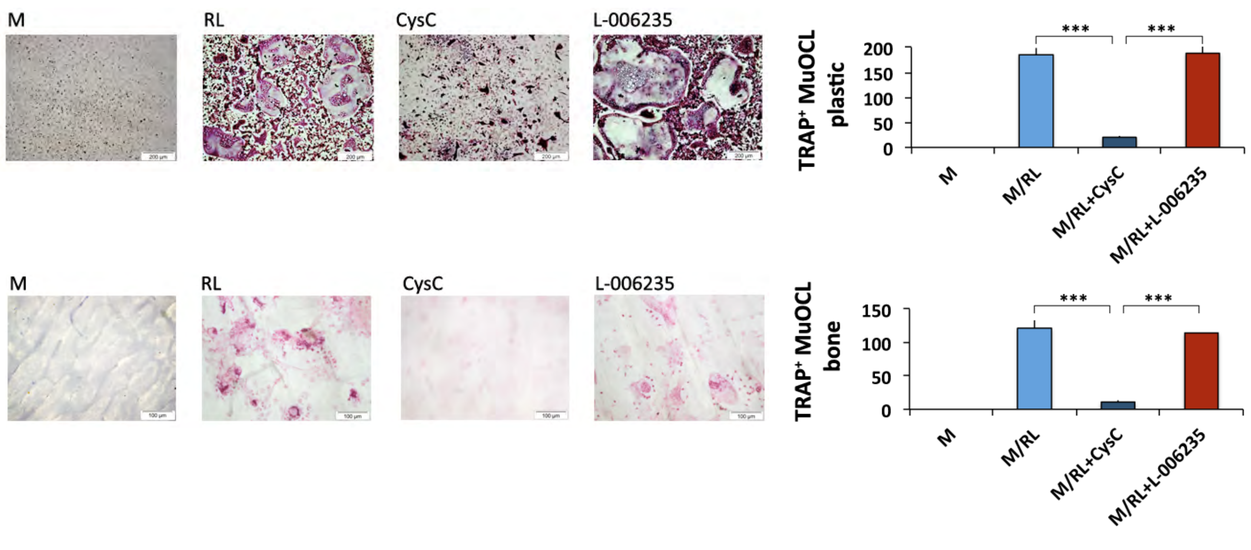
E) LPS potentiates the intracellular uptake of CysC in RL-primed osteoclast progenitors



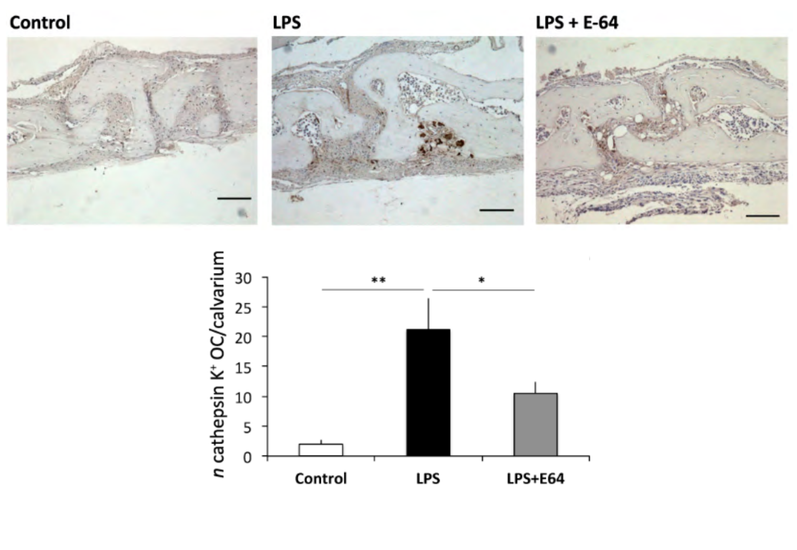
E) CPIs inhibit RL-primed LPS-induced osteoclastogenesis also in cathepsin K deficient mice



F) The cathepsin K inhibitor L-006235 does not inhibit osteoclast formation



G) LPS-induced osteoclast formation in skull bones is inhibited by E-64 *in vivo*



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 CsA and Cathepsin $K^{-/-}$ mice and cultured in the presence of macrophage colony-stimulating factor (M) and receptor activator for nucear 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