

RANK, with no effect on c-Fms. However, CysC inhibited osteoclast formation also in BMM cultures overexpressing *Rank* due to transfection with a lentivirus containing the *Rank* gene. Using fluorescent labelled CysC, the authors observed that CysC was taken up intracellularly in BMM by a process facilitated by RANKL. Similarly, all three CPIs decreased also LPS induced osteoclast formation.

**Conclusions** These data show that CPIs decrease osteoclast formation induced by either RANKL or LPS by interfering with early steps in signal transduction pathways downstream RANK or Toll-like receptor 4.

## 22 CYSTEINE PROTEINASE INHIBITORS DECREASE RANKL AND LPS INDUCED DIFFERENTIATION OF HUMAN AND MOUSE OSTEOCLAST PROGENITOR CELLS

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**Background and objectives** The authors have previously reported that the cysteine proteinase inhibitor (CPI) cystatin C (CysC) reduces osteoclast formation induced by signal pathways activated by either PTH receptor, vitamin D receptor or gp130 in crude mouse bone marrow cultures (ref). In the present study, the authors have investigated if CysC, E-64 (fungal CPI) and the tetrapeptidyl derivative Z-RLVG-CHN<sub>2</sub> (representing Arg<sup>8</sup>-Leu<sup>9</sup>-Val<sup>10</sup>-Gly<sup>11</sup> of the aminoterminal end of CysC) can inhibit osteoclast formation using purified mouse and human osteoclast progenitors stimulated by either RANKL or LPS *Escherichia coli*.

**Results** All three inhibitors concentration-dependently (IC<sub>50</sub> CysC=0.3 µM, E-64=3µM, Z-RLVG-CHN<sub>2</sub>=0.3 µM) inhibited RANKL induced osteoclast formation in mouse bone marrow macrophage (BMM) cultures; similar observations were made using human peripheral blood CD14<sup>+</sup> progenitors. These data were based upon (1) counting the number of TRAP<sup>+</sup> osteoclasts in cultures on plastic or (2) on bone, (3) assessing actin-ring expressing cells and (4) by analysing pit formation and release of CTX when progenitor cells were cultured on bone. The effect was induced early during differentiation as demonstrated by withdrawal and addition of CysC at different time points, by showing that a variety of RANKL induced osteoclastic genes (*Calcr*, *Acp5*, *Ctsk*, *Integrin b3*, *Mmp-9*) were downregulated and that BMM was maintained at a macrophage stage (capacity to phagocytose and increased *Irf-8*). The inhibitory effect was associated with decreased mRNA and protein expression of c-Fos and Nfatc1, and with decreased activation of NF-κB. Inhibition was also associated with decreased mRNA and protein expression of