SWEDISH NATIONAL
GRADUATE SCHOOL IN
ODONTOLOGICAL SCIENCE

# STIMULATION OF BONE RESORPTION IN CALVARIAL BONES BY TOLL-LIKE2 RECEPTORS THROUGH ENHANCED RANKL


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## BACKGROUND

Bone loss in inflammatory diseases like periodontitis, rheumatoid arthritis, septic arthritis and loosened joint prosthesis or tooth implants is being considered a consequence of cytokine induced RANKL and subsequent enhanced osteoclast formation. During the last decade it has been recognized that a variety of cells express receptors (pattern-recognition receptors=PRPs) for specific signatures (pathogen-associated molecular patterns=PAMPs) of different pathogens, including Toll-like receptors (TLR). It is also known that these receptors recognize a variety of endogenous stress signals (Danger-associated molecular pattern=DAMPs) that is released or expressed during the cellular stress/apoptosis.
LPS from the perio-pathogenic bacteria *Porphyromonas gingivalis* and heat-shock protein 60 (HSP-60) are examples for PAMPs and DAMPs involved in periodontitis and osteoporosis, respectively.

## AIM

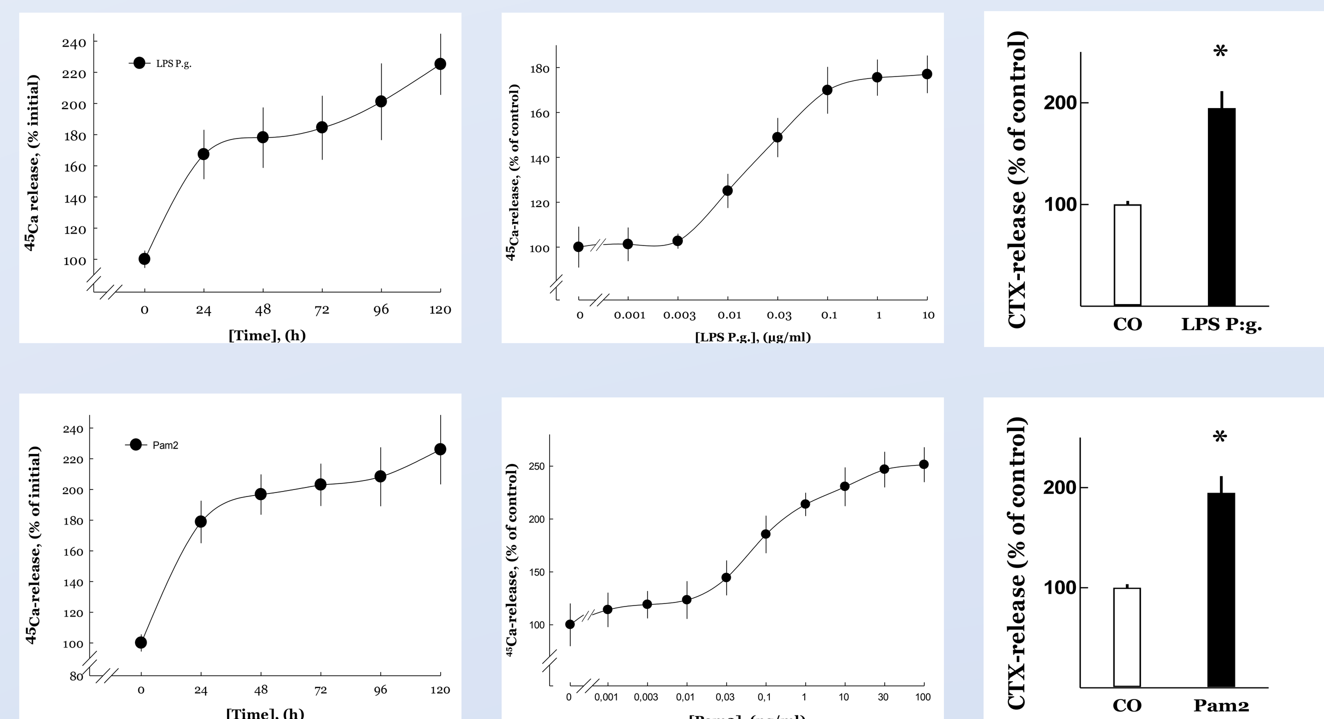
The aim of this study was to elucidate the effect of TLR2 agonists on periosteal bone resorption in mouse calvarial bones including the importance of TLR2 in periosteal osteoblasts.

## METHODS

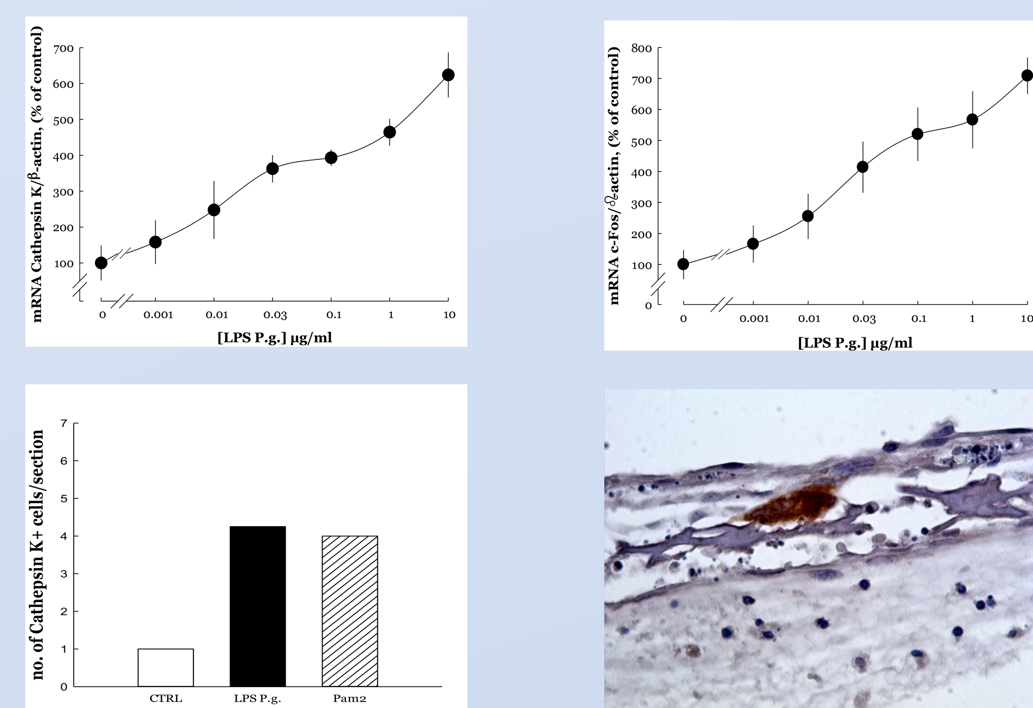
- Organ culture of neonatal mouse calvarial bone
- Bone resorption assessed by the release of:
  - $^{45}$Ca
  - CTX
- Gene expression analyzed by qPCR
- Protein expression determined by ELISA
- Immunohistochemical demonstration of osteoclasts by using *Cathepsin K* abs
- Calvarial osteoblasts isolated by collagenase digestion

## RESULTS

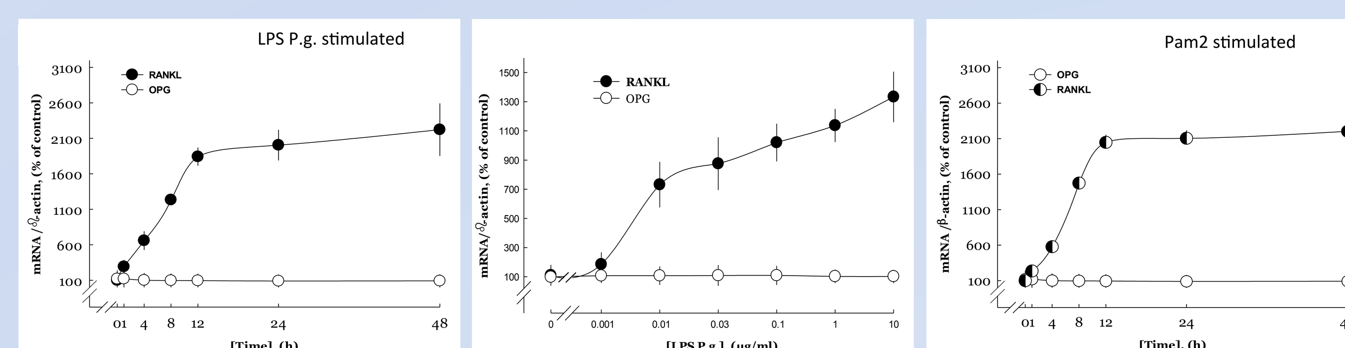
TLR2 activation by both LPS P. gingivalis & Pam2 (synthetic ligand for TLR2) results in increased bone resorption as assessed by enhanced released **$^{45}$Ca** and **CTX**



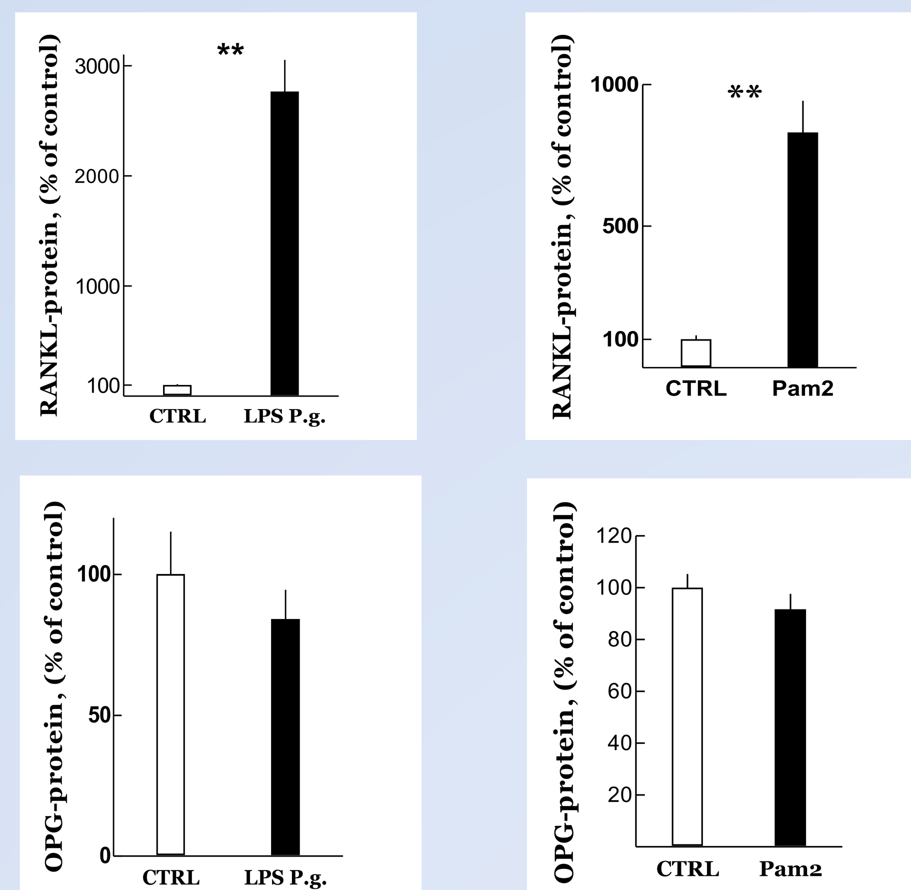

TLR2 activation by LPS P. gingivalis enhances osteoclast differentiation as assessed by increased mRNA expression of **Cathepsin K** and **c-Fos** and the number of **Osteoclasts**



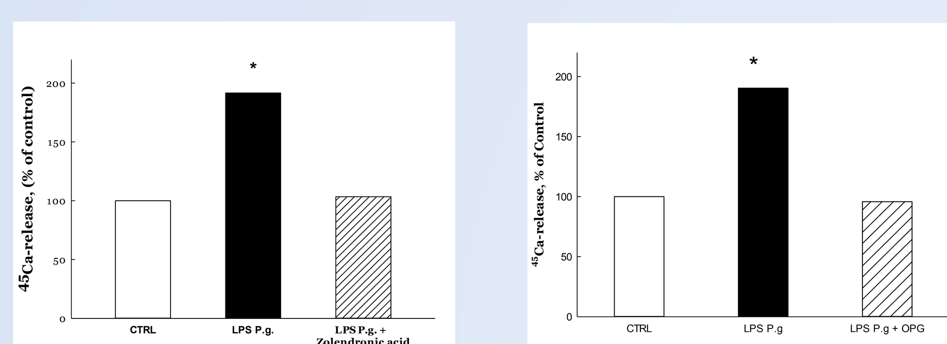
TLR2 activation by LPS P. gingivalis & Pam2 results in increased mRNA expression of **RANKL**, time- and concentration-dependently, without affecting **OPG** mRNA



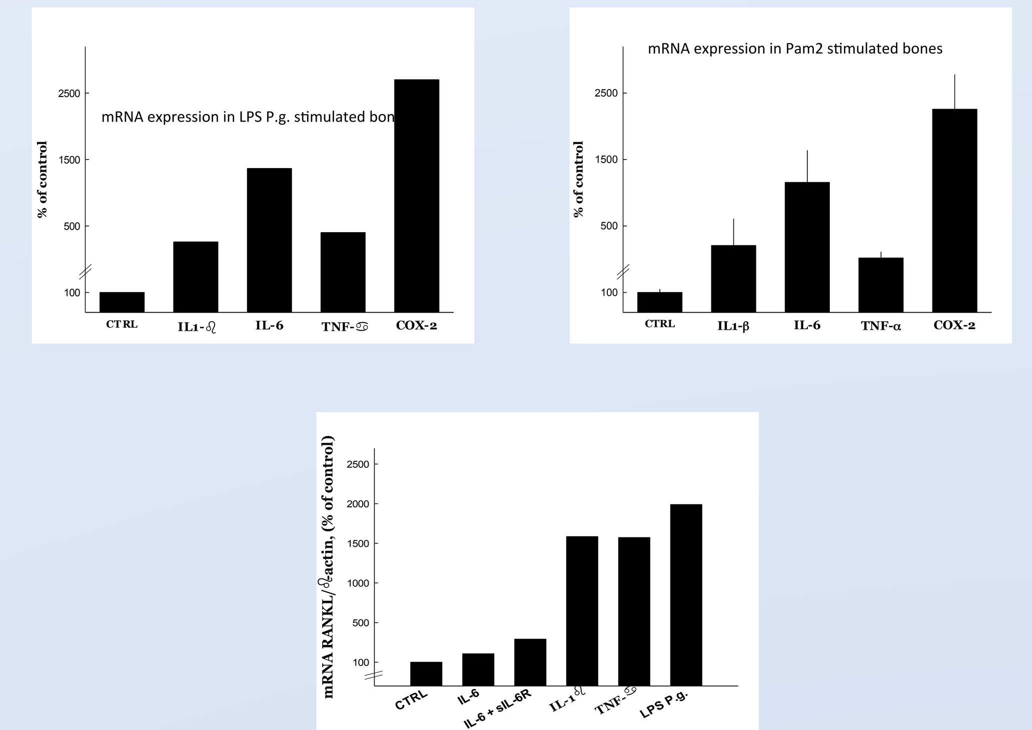

TLR2 activation by LPS P. gingivalis & Pam2 results also in increased **RANKL-protein** without affecting the **OPG-protein**



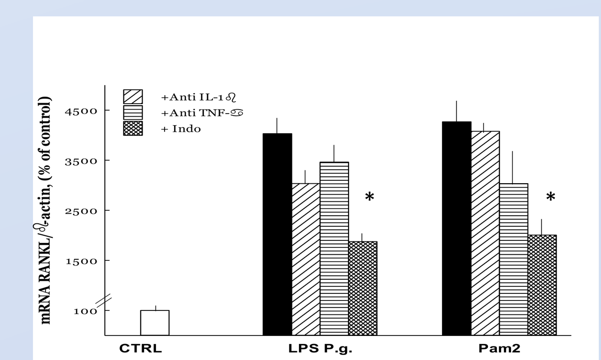

Inhibited bone resorption ($^{45}$Ca-release) in presence of :
***1. Zolendronic acid*** showing the importance of *Osteclasts*
***2. OPG*** showing the crucial role of **RANKL** in the process



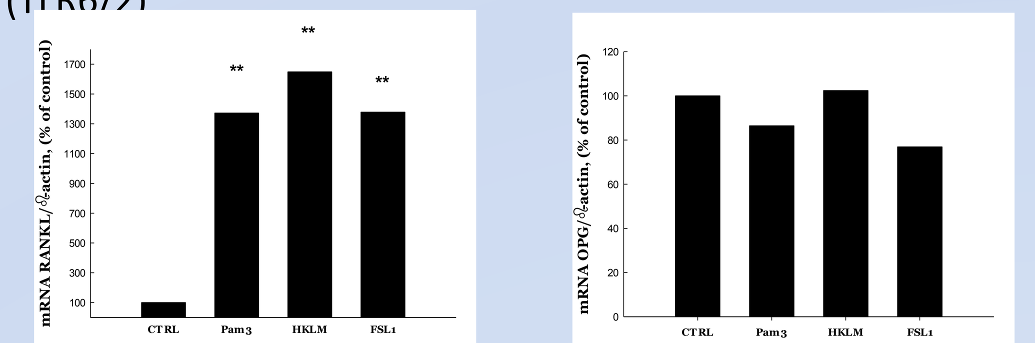
LPS P.gingivalis & Pam2 enhance the mRNA expression of several cytokines and of COX-2 which can stimulate RANKL mRNA in calvarial bones



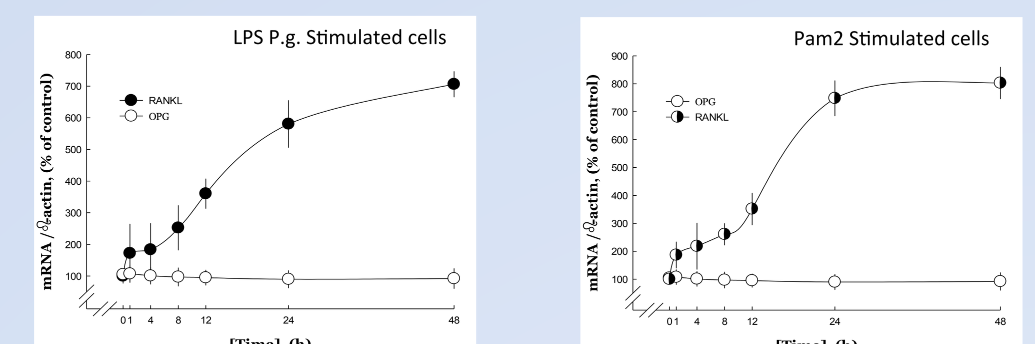

The stimulatory effect by LPS P.gingivalis & Pam2 is independent on IL-1β and TNF-α but partly dependent on $PGE_2$



Also other agonists for TLR2 stimulate RANKL mRNA expression in mouse calvarial bones
**Pam3**, synthetic ligand (TLR1/2)
**HKLM**, heat killed *Listeria Monocytogenes* (TLR2)
**FSL1**, synthetic lipoprotein representing the N-terminal part of the 44-kDa lipoprotein LP44 of *Mycoplasma salivarium* (TLR6/2)



Activation of TLR2 on calvarial *Osteoblasts* by LPS P.gingivalis and Pam2 enhances the mRNA expression of **RANKL**, without affecting that of **OPG**




## CONCLUSION

LPS P. gingivalis & Pam2 cause bone resorption in calvarial bones through TLR2 on *Osteoblasts* by enhancing **RANKL** without affecting **OPG**.