17 STIMULATION OF BONE RESORPTION IN CALVARIAL BONES BY TOLL-LIKE2 RECEPTOR 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 recognised that a variety of cells express receptors (pathogen-recognition receptors=PRP) for specific signatures of different pathogens (pathogen-associated molecular patterns PAMP) and endogenous stress signals (danger-associated molecular patterns DAMP), including Toll-like receptors (TLR).

Material and methods/results The authors used organ cultured neonatal mouse calvarial bones and isolated periosteal osteoblasts which express TLRs to study the role of TLR2 in bone resorption. LPS from the perio-pathogenic bacterium *Porphyromonas gingivalis* (*Pg;* which is a weak agonist for TLR4 but a strong for TLR2 because of the contaminating lipoprotein), enhanced number of osteoclasts, ^{45}Ca release and bone matrix degradation (CTX) by a process inhibited by osteoprotegerin and zolendronic acid. LPS *Pg* enhanced the expression of osteoclastic genes (c-Fos, trap, oscar and cathepsin K) and reduced the expression of osteoblastic genes (osteocalcin, runx2, alp and procollagen α1). The effects were associated with increased mRNA and protein expression of RANKL, whereas OPG mRNA and protein were unaffected. Similar to LPS *Pg*, $Pam2CSK_4$ (synthetic ligand for TLR2/TLR6), $Pam3CSK_4$ (ligand for TLR1/TLR2), HKLM (a heat killed preparation of *Listeria monocytogenes,* a TLR2 agonist) and FSL1 (a synthetic lipoprotein representing the N-terminal part of the 44-kDa lipoprotein LP44 of *Mycoplasma salivarium*) stimulated ^{45}Ca release and increased the mRNA expession of cathepsin K and RANKL, without affecting OPG. Pam2 also increased CTX release and RANKL-protein expression without affecting that of OPG. All TLR agonists increased the mRNA expression of IL-1β, TNFα, IL-6 and COX-2. LPS *P.g.* and Pam2 enhanced the PGE_2 release from the bones. IL-1β and TNFα enhanced RANKL mRNA to a similar extent as LPS *Pg* and PGE_2 caused a 50% response, whereas IL-6 had no effect. Anti-IL-1β and anti-TNFα did not affect LPS *Pg* induced RANKL mRNA. In the presence of an inhibitor of prostaglandin biosynthesis, the response to LPS *Pg* was reduced by 50% but still causing a considerable enhancement of RANKL mRNA. Stimulation of isolated calvarial osteoblasts with all TLR agonists increased RANKL mRNA expression, without affecting OPG mRNA.

Conclusion These data show that stimulation of TLR2 results in bone resorption mediated by increased RANKL in osteoblasts and may be one mechanism for developing inflammatory bone loss.