Lipoteichoic acid inhibits bone destruction by inhibition through TLR2/MyD88-independent pathway

Yeongkag Kwon1, Jihyun Yang1,2, Ok-Jin Park1, Chaeyeon Park1, Dongwook Lee1, Cheol-Heui Yun3, and Seung Hyun Han3

1Department of Oral Microbiology and Immunology, and Dental Research Institute, School of Dentistry, Seoul National University, Seoul, Republic of Korea; 2Infectious Disease Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea; 3Department of Agricultural Biotechnology, Research Institute for Agriculture and Life Sciences, and Center for Food and Bioconvergence, Seoul National University, Seoul, Republic of Korea

Abstract
Bone resorption is mainly caused by excessive differentiation and/or activation of bone-resorbing osteoclasts. Although microbe-associated molecular patterns can influence the differentiation and activation of bone cells, little is known about the role of lipoteichoic acid (LTA), which is a major cell wall component of Gram-positive bacteria, in the regulation of bone metabolism. In this study, we investigated the effect of LTA on bone metabolism using wild-type Staphylococcus aureus and its LTA-deficient mutant strain. Wild-type S. aureus induced bone loss in vivo and osteoclast differentiation in vitro, while LTA-deficient S. aureus increased bone loss and osteoclast differentiation more potently than wild-type S. aureus. LTA isolated from S. aureus (SaLTA) inhibited osteoclast differentiation from committed osteoclast precursors in the presence of osteoclastogenesis-inducing factors, including anti-CSK-inducible S. aureus, a synthetic lipopeptide Pam3CSK4, receptor activator of nuclear factor-kB ligand (RANKL), tumor necrosis factor-α, or lipopolysaccharides. In addition, SaLTA alleviated Pam3CSK4- or RANKL-induced bone resorption in the calvarial bone model. Furthermore, SaLTA potently inhibited the expression of NFATc1 and gelsolin-actin dissociation, which are critical factors in osteoclastogenesis. Interestingly, the inhibitory effect of SaLTA on osteoclast differentiation was maintained in TLR2- or MyD88-deficient osteoclasts as well as wild-type committed osteoclasts. Notably, LTAs purified from other Gram-positive bacteria including Bacillus subtilis, Enterococcus faecalis, and Lactobacillus species, also suppressed Pam3CSK4- or RANKL-induced osteoclast differentiation. Taken together, these results suggest that LTAs from S. aureus and probiotics inhibit osteoclast differentiation through TLR2/MyD88-independent mechanism.

Results
Figure 1. LTA-deficient S. aureus induces massive bone resorption through activation of osteoclast differentiation compared with wild-type S. aureus

Figure 2. SaLTA inhibits osteoclast differentiation without affecting osteoclast differentiation in osteoblast-osteoclast co-culture system

Figure 3. SaLTA directly attenuates the differentiation of committed osteoclast precursors into giant mature osteoclasts

Figure 4. SaLTA containing γ-glutamyl moieties inhibits differentiation of committed osteoclast precursors into mature osteoclasts in response to various osteoclastogenic factors in vitro

Figure 5. SaLTA suppresses the expression of NFATc1 and gelsolin-actin dissociation in TLR2/MyD88-independent pathway

Figure 6. LTAs purified from other Gram-positive bacteria inhibit the differentiation of committed osteoclast precursors into mature osteoclasts

Summary & Conclusion
▶ SaLTA attenuated the bone loss via down-regulation of osteoclastogenesis in various in vivo models.
▶ SaLTA directly inhibited the differentiation of committed osteoclasts by various osteoclastogenic factors through TLR2/MyD88-independent pathway.
▶ SaLTA bound with newly committed osteoclasts and interrupted the gelsolin-actin dissociation, which is critical for the osteoclastogenesis.
▶ LTAs purified from other Gram-positive bacteria also inhibited the osteoclastogenesis.
▶ LTAs from probiotics and even from pathological S. aureus might be an important factor capable of inhibiting osteoclastogenesis through TLR2/MyD88-independent pathway in committed osteoclasts.