INTRODUCTION:
Lipopolysaccharide (LPS), a major constituent of Gram-negative bacteria, has been suggested to stimulate bone loss in osteomyelitis. LPS was reported to increase the number of osteoclast precursors via TNF-alpha-TNFFR1 action in vivo. TNF-alpha stimulates osteoclastogenesis directly or indirectly via activation of RANK-RANKL signaling. Recently, it was reported that LPS can enhance the survival, fusion and activation of osteoclast directly. Furthermore, LPS stimulation was reported to increase osteoclast survival via NF-kB activation. On the other hand, it was reported that Toll-like receptors stimulated by LPS inhibits differentiation of osteoclast precursors. These data suggests that LPS signaling in osteoclastogenesis has at least two different pathways, such as RANK-RANKL dependent and independent. However, the hierarchy of LPS function in bone metabolism in vivo remains to be elucidated. The purpose of this study was to investigate the role of RANK-RANKL pathway and TNF-alpha -TNFR1 pathway in LPS induced bone loss in vivo.

MATERIALS AND METHODS:
C57BL6 background wild type mice or TNFR1-/- mice were used. All mice were injected LPS (20mg/kg body weight) SC on day 0. wild type mice were injected intraperi PBS or osteoprotegerin (OPG) (0.05, 0.5, 5mg/kg body weight) on day 0 and day 1. TNFR1-/- mice were injected intraperitoneally PBS or OPG (5mg/kg body weight) on day 0 and day 1. All mice were sacrificed 48 hours after the LPS injection. Tibiae or femora of each mouse were analyzed by histological examination. Trabecular bone 200um apart from growth plate was evaluated for such parameters as bone volume/tissue volume (BV/TV), bone surface (BS/TV), osteoblast surface (Ob.S/BS), osteoid surface (OS/BS), osteoclast number (N.Oc/BS) and osteoclast surface (Oc.S/BS).

RESULTS:
BV/TV was suppressed by LPS injection about 40% in wild type mice. OPG administration dose dependently reversed the LPS induced bone loss and OPG (0.5mg/kg) was sufficient for the maintenance of bone volume within basal levels. Histological examination indicated that Oc.S/BS and N.Oc/BS were increased in LPS treated mice. In addition, there were many TRAP positive cells apart from bone surface in bone marrow of LPS treated mice. The increase in N.Oc/BS, Oc.S/BS and the number of non-osteoclast TRAP positive cells induced by LPS injection were suppressed by OPG administration in dose dependent manner. As for bone formation, Ob.S/BS and OS/BS were increased by LPS injection, suggesting that LPS induced high turn over osteopenia. OPG treatment also reduced these parameters to basal levels. To investigate the roles of TNF-alpha and RANKL in LPS signaling, TNFR1-/- mice were injected LPS with OPG or PBS. BV/TV was reduced significantly in LPS injected TNFR1-/- mice. The reduction in bone volume was reversed by OPG treatment. Interestingly, Ob.S/BS and OS/BS were remarkably reduced in LPS injected TNFR1-/- mice, whereas both parameters were increased in LPS injected wild type mice. OPG had no or little effects on Ob.S/BS or OS/BS. As for osteoclastogenesis, administration of LPS and OPG also had less effect on N.Oc/BS nor Oc.S/BS in TNFR1-/- mice compared with wild type mice. Individual osteoclasts in LPS treated mice were thinner and smaller compared with control TNFR1-/- mice or LPS treated wild type mice. The shape of osteoclasts in LPS treated TNFR1-/- mice was not affected by OPG treatment. LPS increased non-osteoclast TRAP positive cells in bone marrow cavity of TNFR1-/- mice as observed in LPS treated wild type mice. However, OPG treatment had less effect on the increase in number of TRAP positive cells in TNFR1-/- mice compared with wild type mice.

DISCUSSION:
Our experiments indicated that OPG inhibited LPS induced high turn over osteopenia via suppression of N.Oc/BS and Oc.S/BS. This result suggested that OPG suppressed LPS induced osteoclastogenesis at least via RANK-RANKL signaling. Moreover, the increase in the number of TRAP positive cells in bone marrow of LPS injected mice suggested that LPS might promote proliferation of the osteoclast precursor cells. Surprisingly, LPS injection did induce low turn over osteopenia in TNFR1-/- mice. Remarkable reduction in Ob.S/BS and OS/BS in LPS administrated TNFR1-/- mice indicated that TNF-alpha has some roles in maintenance of osteoblast number and function in LPS stimulated inflammation. OPG administration reversed the bone volume reduction in TNFR1-/- mice, but had little effect on N.Oc/BS or Oc.S/BS, suggesting that OPG reduced the function rather than the number of osteoclasts in TNFR1 deficiency. In addition, the existence of osteoclasts in OPG treated TNFR1-/- mice suggested there might be osteoclastogenic pathway independent of RANK/RANKL/TNF-alpha. The thinner and smaller shape of osteoclasts in LPS treated TNFR1-/- mice suggested that LPS might inhibit osteoclast differentiation without TNF-alpha activation in vivo as previously reported in vitro.

In conclusion, OPG suppressed LPS induced bone loss via both TNF-alpha dependent and independent pathways. Our data suggested that OPG could rescue bone loss in the region of infection such as osteomyelitis and periodontitis.