THE EFFECTS OF LIPOPOLYSACCHARIDE COATED CALVARIA BONE RESORPTION


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**Introduction**

Lipopolysaccharide (LPS) is an endotoxin that is a component of the outer membrane of gram-negative bacteria. Its role in the pathogenesis of aseptic implant loosening is controversial. Implants themselves may be coated with endotoxin and it has been suggested that the capacity of LPS to form a biofilm on implant surfaces impedes osseointegration. Adsorption of LPS to implant wear particles has been shown to increase osteoclast activity in vivo and in vitro by stimulating particle-induced release of pro-inflammatory mediators with osteoclast inducing activity. LPS administered systemically has also been shown to increase osteoclast differentiation. The direct effect of LPS on osteoclast-mediated bone resorption is complex. For example, soft tissue injection of LPS adjacent to bone surfaces induces osteoclast-mediated resorption. In addition, treatment of bone marrow cultures containing stromal and hematopoietic precursors with LPS at different concentrations has been shown to increase osteoclast activity in vitro. This suggests that the effects of LPS on osteoclast differentiation and activity. Two models were used; the first assessed the effect of soluble LPS on osteoclast differentiation using an in vitro model and the second assessed the effects of surface-associated LPS on bone resorption using an in vivo bone implantation model.

**Methods**

LPS model

Osteoclast formation was induced in a RAW264.7 cell clone (C6) by treatment with RANKL, (20 ng/ml) in the presence of increasing concentrations of soluble LPS (10-200 ng/ml, E. coli serotype Sigma, St Louis, MO) for four days. The effects on osteoclast-formation were assessed by analysis of TRAP enzymatic activity, as previously reported.

Surface-associated LPS model

4 mm diameter calvarial discs of bone were excised from euthanized CBAF/B6 mice. After devitalization by freeze-thawing, discs were treated with RANKL (20 ng/ml) and then incubated in the presence of increasing concentrations of soluble LPS (10-200 ng/ml, E. coli serotype Sigma, St Louis, MO) for four days. The effects on osteoclast-formation were assessed by analysis of TRAP enzymatic activity, as previously reported.

**Results and Discussion**

**Soluble LPS model**

Addition of LPS to RANKL treated cells produced a dose-dependent decrease in size and intensity of TRAP-positive multinuclear cells formation so that at the highest concentration of LPS only isolated mononuclear cells were present. These results support the observation of Takami et al. and provide additional evidence that LPS acts directly on mononuclear cell precursors to inhibit their differentiation into osteoclasts. The mechanism underlying this inhibitory effect has not been defined but data indicate that LPS directs precursors down a differentiation pathway leading to the formation of “activated” macrophage rather than osteoclasts.

**Surface-associated LPS model**

In the untreated samples, a granulomatous inflammatory reaction developed at the circumferential margins of the bone within several days after implantation. The initial cellular response consisted predominantly of macrophage-type cells, as indicated by positive staining for the lineage specific marker M4/80. In contrast, in the LPS and collagenase treated groups, F4/80 positive cells were detected on all of the discs surfaces. Over time, in the untreated samples TRAP, Cath-K and CTR positive osteoclast-like mono- and multinuclear cells formed on the bone surface at the disc margins. Similar osteoclast-like cells formed on the collagenase treated samples, but these cells were not restricted to the disc margins, as they were detected on all bone surfaces. In the LPS treated samples, although osteoclast-like cells were present on bone surfaces, their numbers were substantially reduced. Importantly, although collagenase treatment markedly increased bone disc resorption, as demonstrated by μCT, the LPS-treated discs showed almost complete absence of bone loss. These results are consistent with the direct inhibitory effects of LPS on RANKL-induced osteoclastogenesis observed in the soluble LPS model with purified osteoclast precursors. This suggests that surface bound LPS, like soluble LPS, shunts macrophage precursors to an activated macrophages rather than an osteoclast phenotype. These results also suggest that activated macrophage present on the LPS-treated discs have a limited capacity to directly resorb bone. These findings are in contrast to the results obtained when LPS is adsorbed to the surface of particles that can be internalized by monocyte macrophages or their precursors. Under these circumstances the particles enhance the release of pro-osteoclastogenic factors that can increase osteoclast formation. In addition, their contrast with the osteoclastogenic effects of LPS when stromal cells are present. Under these circumstances LPS acts indirectly to enhance osteoclast formation by stimulating the release of osteoclast-inducing factors from the stromal cells. Our findings provide further insights into the complex effects of LPS on osteoclast-mediated bone resorption, and stress that the “net” effect of LPS on osteoclast-mediated resorption is very much dependent on the specific cell targets and clinical circumstances in which it is introduced.

**References**