Role of Pseudomonas aeruginosa Lipopolysaccharide on Osteoclastogenesis and Toll-like Receptor Activation

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ABSTRACT

In aural cholesteatoma, the accumulation of keratin debris and persistent infection cause an inflammatory response in the middle ear, which can then progressively erode surrounding bone, leading to hearing loss and vestibular dysfunction. It is now clear that bone loss accompanying chronic infections is the result of either osteoclast development and activity. While many bacterial products may be involved, lipopolysaccharide (LPS) is believed to be a major mediator of inflammation and osteolysis. LPS exerts its effects from recognition by toll-like receptors (TLRs), specifically TLR4 and TLR2. In light of the prevalence of Pseudomonas aeruginosa (PA) LPS in chronic ear infections, we have concentrated our research on the effects of PA LPS on osteoclastogenesis. Previously, we found that PA LPS interacts indirectly through osteoblasts to induce bone resorption. In addition, optimal osteoclastogenesis in vitro required functional TLR4 expression in both bone marrow monocytes (BMMs) and osteoclasts.

In this study, we isolated BMMs from wild type (C57BL/6J), TLR4-/- and MyD88-/- mice. PA LPS failed to induce osteoclastogenesis in wild type BMMs. Instead, we observed an up-regulation of macrophage markers, suggesting these cells develop towards an immune phenotype capable of fighting infections. On the contrary, TLR4-/- BMMs demonstrated a dose-dependent increase in osteoclast formation when exposed to PA LPS. This osteoclastogenic effect was even more pronounced in MyD88-/- BMMs exposed to PA LPS. These results demonstrated that PA LPS is able to activate both MyD88-dependent as well as MyD88-independent pathways. However, the effect of PA LPS stimulation on osteoclast precursors (BMMs) is based on the integrity of TLR signaling pathway. Future studies will compare cytokines and gene expression patterns from LPS activated MyD88-dependent as well as MyD88-independent pathway. By elucidating these signaling pathways, we can gain understanding into LPS-mediated inflammatory responses as well as osteoclast formation.

METHODS

Animals. Mice used in these experiments were the C57BL/6J wild type control strain and those strains with targeted deletions of TLR4, and MyD88 (obtained from Jackson Laboratories, Bar Harbor, ME, and from Dr. Shozo Akira, Osaka, Japan) on a C57BL/6J background. Use of these vertebrate animals was approved by the Washington University Animal Studies Committee.

LPS Serotypes. Pseudomonas aeruginosa LPS (serotype O1) were purchased from Sigma, St. Louis.

In Vitro Osteoclast Cultures Bone marrow monocytes (BMMs) were harvested from long bones of 4-week-old male B6C3F1 mice. Incubated in culture dishes in the presence of high doses of mCSF, along with osteoprotegerin (RANKL antagonist), to ensure an RANKL-free culture system. Osteoclasts were isolated from calvaria of 3 day old fetal mice. Purified BMM cultures were established at 5-10x10^6 cells/ml in o-minimal essential medium, supplemented with 10% heat-inactivated fetal bovine serum, antibiotics, and 10 ng/ml mCSF. Co-cultures were established with the addition of osteoclasts into BMM culture along with vit D and dexamethasone. Cultures were treated with varying concentrations of Pseudomonas LPS (Sigma, St. Louis, MO) for 7 days.

In Vitro Osteoclast Development Assay Osteoclast development was assessed by a tartrate resistant acid phosphatase (TRAP) solution assay. Cells were fixed and washed in PBS. TRAP solution was prepared according to manufacturers protocol (JAS Diagnostics, Miami, FL), added to cells and allowed to incubate for 30min at 37°C then analyzed at 405nm.

Osteoclast Visualization and Quantitation. For osteoclast visualization, fixed samples were stained for tartrate-resistant acid phosphatase (TRAP) using an osteoclast-specific marker, with a commercial kit (Sigma, St. Louis, MO). Positively stained cells containing the TRAP nuclei were considered osteoclasts. Osteoclasts were quantitated according to a method devised in our laboratory. Culture plates were digitally imaged using a digital camera coupled to an inverted microscope. These photographs were then converted into black and white images for analysis with ImageJ software to obtain both total osteoclast number in each well as well as area fractions of each well that were occupied by osteoclasts.

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REFERENCES

Available upon request.