Impact of *Staphylococcus aureus* infection on osteoclast differentiation

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**INTRODUCTION**

Osteomyelitis, mainly caused by *S. aureus*, is an incapacitating infectious disease which is associated with significant morbidity and mortality, characterized by progressive bone destruction. Interactions between *S. aureus* and osteoblasts, the cells responsible for bone formation, have been previously studied: *S. aureus*, via staphylococcal fibronectin binding protein (FnBP), is able to adhere to and invade these cells. Nevertheless, interaction between live *S. aureus* and osteoclasts, the cells responsible for bone resorption, have never been explored. We thus investigated the impact of *S. aureus* infection on osteoclastogenesis during the early and late stages of differentiation.

**MATERIALS AND METHODS**

Osteoclasts precursors were obtained from bone marrow of C57/B1/J6 mice and differentiated with osteoclastic inducers (RANKL and M-CSF).

In early phase experiments, osteoclast precursors were infected with live *S. aureus* strain 8325-4 and its isogenic mutant DUS883 (FnBP−) at a MOI of 50:1 during 2 hours. Extracellular bacteria were killed by addition of gentamicin (200µg/ml) for 1 h.

In late phase experiments, mature osteoclasts were infected as described above after 3 days of differentiation.

Osteoclasts were enumerated as multinucleated TRAP-positive cells. MTT assay (specific staining assay measured as OD595) was performed to assess cell viability. Morphologic analyses (including size) and level of multi-nucleation were performed using a dedicated image analysis algorithm (ImageJ) after staining of nuclei with DAPI and actin with phalloidin-alexa.

Viable adherent and internalized bacteria were enumerated by plate counting two-hours post infection.

Data were expressed as means ± SEM of three independent experiments performed in duplicate. Differences were tested for statistical significance using the non-parametric Mann-Whitney U-test with a 0.05 threshold.

**RESULTS**

Adherent and internalized viable bacteria counts were 60-fold lower in osteoclast precursors compared to mature osteoclast, independently of the presence of FnBP (data not shown).

In early phase experiments, infection of osteoclast precursors with *S. aureus* strain 8325-4 or DUS883 (FnBP−) significantly inhibited osteoclastogenesis (27±28 and 22±25 differentiated cells/well, respectively) as compared to uninfected cells (713±184 differentiated cells/well, *p<0.05*).

In late stage experiments, no significant difference in osteoclastogenesis was observed between uninfected and infected cells. However, 50% of infected osteoclasts displayed an atypical “big-round shape” phenotype, with a larger area and more nuclei, while this phenotype was observed in 8% of uninfected cells (*p<0.05*).

**CONCLUSIONS**

- Staphylococcal infection inhibited osteoclastic differentiation in early-stage progenitors, while it increased cell fusion events in late stage of osteoclastogenesis through an FnBP-independent mechanism.
- The functional impact of *S. aureus*-induced modifications of osteoclastogenesis on bone resorption is currently under investigation.