Titles: *Porphyromonas gingivalis* RagA protein induces RANKL-independent osteoclast generation

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Brief 1-Sentence Abstract Summary: RagA induces osteoclast generation in the absence of RANKL, possibly by the production of inflammatory cytokines.

Awards/Competitions:

Abstract (290/300 words)
Objective: Site-specific development of inflammatory bone resorption is characteristic of periodontal disease (PD), in association with increased levels of pathobiont, *Porphyromonas gingivalis* (*Pg*). Monocytes are critical for immune-pathophysiological responses in PD, protecting host from bacterial invasion, while differentiating into bone destructive osteoclasts. Rag gene locus in *Pg* is reported to be distinctively present in clinical isolates from deep periodontal pockets. However, possible pathogenic roles of RagA and RagB proteins encoded in rag locus are largely unknown. This study aims to investigate the possible pathogenic effects of RagA/B on inflammatory bone destructive responses by monocytes.

Methods: Recombinant RagA/B proteins generated in BL21 (DE3) competent *E. coli* were applied to RAW264.7 monocyte-like cells to detect proinflammatory cytokines and cytotoxicity/cell proliferation responses. RAW264.7 cells were stimulated with RagA/B proteins with or without RANKL, and OC-genesis was assessed with TRAP staining, pit-formation assay, and q-PCR, while phosphorylation of MAPK signaling proteins was detected by W-blotting.

Results: RagA significantly promoted cell proliferation and induced the production of TNF-α and IL-6, none of which was observed by stimulation with RagB. RANKL-induced OC-genesis was significantly promoted by RagA, but not RagB. Despite the absence of RANKL, RagA induced TRAP-positive multinucleated cells accompanied by elevated pit area, as well as mRNA expression of ocstamp, dcstamp, nfatc1, and mmp9. Nonetheless, both RagA and RagB promoted the phosphorylation of three key MAPKs, including ERK, JNK, and p38, indicating that RagA-mediated OC-genesis may involve a *de novo* cell signaling pathway.

Conclusion: It can be concluded that RagA, but not RagB, not only elicits proinflammatory responses, but also induces OC-genesis in an RANKL-independent manner. To the best of our knowledge, RagA is the very first bacterial factor able to induce OC-genesis in the absence of RANKL, while explaining the site-specificity of PD.

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