Porphyromonas gulae LPS activates inflammatory responses in gingival epithelial cells

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Background: *Porphyromonas gulae*, a Gram-negative black-pigmented anaerobe, is an oral pathogen known to be associated with periodontal disease in humans and dogs. Recent reports have revealed that this bacterium possesses a number of potential virulence factors, such as lipopolysaccharide (LPS), fimbriae, and proteases, which may contribute to the pathogenesis of periodontitis. However, molecular responses by human gingival epithelial cells to *P. gulae* LPS have yet to be investigated. The purpose of the present study was to identify inflammatory responses and signaling pathways mediated by *P. gulae* LPS.

Material & Methods: LPS from the *P. gulae* D049 strain was extracted using an LPS extraction kit. Cells from a human gingival epithelial cell line (Ca9-22) were incubated with *P. gulae* LPS for 0-24 hours, then expressions showing inflammatory responses, including *IL-6*, *IL-8*, COX_2, and *TNF-α*, were analyzed using a real-time polymerase chain reaction assay. mRNA Expression values were quantified by the ΔΔCt method with GAPDH as the control. In addition, activation of signal transduction components was determined by western blotting analysis.

Result:

P. gulae LPS stimulation was associated with rapid upregulation of the Toll-like receptors *TLR2* and *TLR4* in Ca9-22 cells. At 6 hours after stimulation, expressions of *IL-6*, *IL-8*, *TNF-α*, and COX_2 were observed in response to *P. gulae* LPS, while their mRNA levels were diminished by knockdown of TLR2 and/or TLR4. In addition, ERK1/2, p38, and IkB in Ca9-22 cells were shown to be phosphorylated by *P. gulae* LPS.

Discussion: The present results suggest that *P. gulae* LPS activates TLR2 and TLR4 to modulate the activities of multiple signaling pathways, resulting in activation of inflammatory responses by Ca9-22 cells. We speculate that *P. gulae* LPS plays an important role in establishment of chronic inflammatory periodontal disease.