Production of Cytokines in hPDL Cells Induced by *Porphyromonas gingivalis* and Mechanical Stress

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Abstract:
Objective: We previously reported that physiological mechanical stress and periodontal bacteria induced cytokine production in human periodontal ligament (hPDL) cells and that these cytokines influenced both local inflammation of the periodontal ligament and occlusal trauma. In this study, we examined the production of cytokines in hPDL cells induced by *Porphyromonas gingivalis* and mechanical stress or non-physiological mechanical stress.

Methods: The hPDL cells were obtained from a healthy erupted maxillary third molar. After the third to fourth passage, cells were exposed to various levels of mechanical stress (1MPa, 6MPa, 10MPa and 50MPa); mechanical stress group. A subset of the mechanically stressed cells was also stimulated with *P. gingivalis* (1 × 10^7 CFU/ml) for 24 hours; mechanical stress and *P. gingivalis* group. Total RNA was extracted and the cytokine mRNA expressions were determined by RT-PCR. We then analyzed the mRNA expressions of interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor (TNF)-α. Cytokines in the culture supernatants were assessed by ELISA, and morphologic changes of hPDL cells were observed using an inverted optical microscope. This experimental procedure was approved by the Ethics Committee, Kyoto Prefectural University of Medicine.

Results: IL-6, IL-8 and TNF-α mRNA were expressed in the mechanical stress group. Expressions of all inflammatory cytokines were detected in the mechanical stress and *P. gingivalis* group and the amounts of IL-6 and IL-8 in the mechanical stress group increased with mechanical stress. Moreover, the amounts of IL-6 and IL-8 in the mechanical stress and *P. gingivalis* group were higher than those in the mechanical stress group. The morphology of hPDL cells did not change after exposure to 6MPa or 10MPa, but hPDL cells were partly detached from the petri dish after exposure to 50MPa.

Key words: *Porphyromonas gingivalis*, Mechanical stress, Cytokines