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ORIGINAL ARTICLE

Stimulation of prostanoids and IL-8 production in human gingival fibroblasts by *Porphyromonas gingivalis* LPS is associated with MEK/ERK signaling

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Abstract *Background/purpose:* Various toxic products are generated by periodontal pathogens. *Porphyromonas gingivalis* has been found to generate lipopolysaccharide (LPS) that may potentially affect periodontal health. However, the precise effects of *P. gingivalis* LPS on human gingival fibroblasts (GFs) await further investigation.
Materials and methods: Human GFs were cultured and exposed to different concentrations of *P. gingivalis* LPS (0.1–10 μg/mL) for 24 hours. Cytotoxicity was analyzed by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. Total ribonucleic acid (RNA) was isolated and subjected to reverse transcriptase-polymerase chain reaction (RT-PCR) using specific primer sets. Culture medium was collected for determination of prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, and interleukin-8 (IL-8) production by enzyme-linked immunosorbent assay. ERK1/2 phosphorylation in GFs was evaluated by Western blotting. In some experiments, U0126 (a MEK/ERK inhibitor) was added to the GFs culture 30 minutes before LPS and culture medium was also collected for analysis.

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Results: *P. gingivalis* LPS (0.1—10 μg/mL) showed little cytotoxicity and morphologic changes of GFs. *P. gingivalis* LPS obviously stimulated the cyclooxygenase-2 (COX-2) and IL-8 messenger RNA expression of GFs after 24 hours of exposure. Moreover, *P. gingivalis* LPS induced PGE_2, $PGF_{2\alpha}$, and IL-8 production in GFs. *P. gingivalis* LPS also induced ERK1/2 phosphorylation in GFs. Stimulation of PGE_2 production by *P. gingivalis* LPS was completely attenuated by U0126, whereas U0126 only partially inhibited the LPS-induced IL-8 production in the same condition.

Conclusion: Our data indicate that *P. gingivalis* LPS stimulates gene expression of differential inflammatory mediators (COX-2 and IL-8) as well as prostanoids and IL-8 production in GFs. These events are associated with MEK/ERK signaling and crucial in the pathogenesis of inflammatory periodontal diseases.

Introduction

Periodontitis is one of the most common chronic infectious diseases and is especially prevalent among elderly populations. It is a multifactorial disease caused primarily by microorganisms but significantly modified by the host response to bacterial invasion. Recent reports on the associations and potential biologic mechanisms between periodontitis and other diseases showed that periodontal diseases may be a risk factor of coronary heart disease, atherosclerosis, and cardiovascular diseases.[1—3] It means that the patient with periodontitis could be challenging to treat and treatment plans should include local and systemic considerations.

According to bacteriologic studies, *Porphyromonas gingivalis* has been demonstrated to be closely associated with the onset and progression of adult periodontitis.[4,5] *P. gingivalis* expresses a variety of pathogenic factors, including microstructures such as fimbriae, lipopolysaccharide (LPS), and flagella, as well as gingipain and endotoxin.[6] Previous *in vitro* experiments have shown that fimbriae and LPS of *P. gingivalis* may stimulate gingival epithelial cells to express a large amount of inflammatory cytokines, such as interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor-α (TNF-α), as well as to produce matrix metalloproteinases (MMP-1, -8, -9, -13).[7,8] The inflammatory factors may destroy tissue integrity, so that the bacteria can access deeper tissue. They are also related to the severity of periodontitis.[9] The fimbriae of *P. gingivalis* plays an important role in bacterial invasion into the cells and its pathogenic ability. In human monocytes, the fimbriae of *P. gingivalis* induces IL-6 production via TLR2, CD14, and CD11a/CD18 molecules, and it is also related to nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation.[10] Moreover, the protease expressed by *P. gingivalis*, for example, gingipain, breaks down cytokines produced by the host, such as IL-6, IL-8, IL-12, and TNF-α and suppresses the activation of the immune response. The experiments in rats also showed that *P. gingivalis* gingipain increases epithelial MMP-3 and MMP-8 expression, and affects the gingival metabolism and repair.[11]

In periodontal tissues, human gingival fibroblasts (GFs) are the most abundant cells and have the ability to secrete inflammatory cytokines such as IL-8 and prostaglandins such as PGE_2 when they are activated with stimuli.[12,13] IL-8 acts as a chemoattractant for neutrophils and can activate these cells.[12] In addition, PGE_2 plays important roles in the pathogenesis of periodontal diseases. It is associated with attachment loss and is a powerful stimulator of bone resorption.[13] As the inflammation begins, cyclooxygenase-2 (COX-2) is induced by a variety of cytokines, growth factors, and LPS, and consequently converts arachidonic acid to prostaglandins.[14] Therefore, the levels of prostaglandins including PGE_2 in inflamed gingival tissues are enhanced.[15] $PGF_{2\alpha}$ as well as PGE_2 could be detected in higher levels in periodontal lesions. It might involve in connective tissue degradation and bone resorption.[16] However, more information is necessary for our understanding the role of $PGF_{2\alpha}$ in the periodontal pathophysiology.

It has been reported that GFs produce a number of proinflammatory cytokines such as IL-1, IL-6, and IL-8 upon stimulation by *P. gingivalis* LPS.[12] However, the precise effects of *P. gingivalis* LPS on GFs await further investigation. In this study, we examined COX-2 and IL-8 gene expression, prostanoids, and IL-8 production in GFs after *P. gingivalis* LPS stimulation. The results of our study may facilitate our understanding of the pathogenesis of periodontitis.

Materials and methods

Materials

All cell culture materials were purchased from Gibco Laboratories (Life Technologies, Grand Island, NY, USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), was obtained from Sigma (Sigma Chemical Company, St. Louis, MO, USA). *P. gingivalis* LPS was obtained from InvivoGen (San Diego, CA, USA). PCR primers were synthesized from Genemed Biotechnologies, Inc (San Francisco, CA, USA). PGE_2 and $PGF_{2\alpha}$ enzyme-linked immunosorbent assay (ELISA) kits were from Cayman Chemical Company (Ann Arbor, MI, USA). IL-8 ELISA kits were from PeproTech Inc. (Rocky Hill, NJ, USA).

Culture of human gingival fibroblasts

Gingival tissues were obtained during crown-lengthening procedures by the approval of the Ethics Committee, National Taiwan University Hospital and the informed consent of the patients. GFs were cultured by a tissue explant technique as described previously and cultured in