

Differential induction of MAPK signaling pathways by *Porphyromonas gingivalis* and *Escherichia coli* lipopolysaccharide in human monocytes

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Abstract

Periodontal disease is the leading reason for tooth loss in adult and is characterized by a chronic inflammation caused by oral bacteria including *Porphyromonas gingivalis* (*P. gingivalis*). The lipopolysaccharide (LPS) of *P. gingivalis* is a crucial virulence factor critically involved in the regulation of immune inflammatory responses.

The aim of the study is to investigate the production of interleukin (IL)-1 β and IL-8 and the activation of mitogen-activated protein kinase (MAPK) signaling by LPS of *P. gingivalis* and *Escherichia coli* (*E. coli*) in human monocytes. THP-1 cells and PBMC-derived monocytes were challenged with *P. gingivalis* and *E. coli* LPS. Expression of MAPK family members were examined using immunoblotting. IL-1 β and IL-8 secretion were measured by enzyme-linked immunosorbent assay (ELISA).

The results showed that *P. gingivalis* and *E. coli* LPS had the ability to promote the production of IL-1 β and IL-8 from monocytes. However, *P. gingivalis* LPS induced higher levels of c-Jun N-terminal kinases (JNK) and extracellular-signal-regulated kinases (ERK)1/2 phosphorylation, whereas p38 MAPK phosphorylation was greater increase upon *E. coli* LPS stimulation. The finding suggests that *P. gingivalis* and *E. coli* LPS may use different MAPK signaling pathways to induce IL-1 β and IL-8 production in human monocytes.

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Introduction

Porphyromonas gingivalis, an anaerobic Gram-negative rod, is recognized as a key pathogen associated with periodontitis¹. *P. gingivalis* LPS has been proposed to play an important role in mediating inflammation and stimulating production of pro-inflammatory cytokines by immune and inflammatory cells.^{2,3} These cytokines particularly IL-1 β and IL-8 are important mediators in the inflammatory response to periodontopathic bacteria resulting in the initiation and progression of periodontal disease.⁴

Monocytes produce several cytokines in response to periodontal pathogens which are involved in the local inflammation and connective tissue destruction.⁵ Toll-like receptors (TLRs) are expressed by innate immune cells known to be stimulated by bacterial pathogens. LPS from *P. gingivalis* binds mainly to TLR-2 of the innate host defense system.⁶ LPS derived from enteric bacteria such as *E. coli* is structurally and functionally distinct from *P. gingivalis* LPS and has been shown to interact with TLR-4.⁷

After binding to the TLR, LPS can trigger pro-inflammatory cytokine gene expression by activation of intracellular signaling components, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), p38 MAPK, and JNK.^{8,9}

Previous studies have demonstrated that the major signaling pathway of cytokine production by *P. gingivalis* LPS-stimulated THP-1 cells was mainly via JNK, while *E. coli* LPS was

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mainly dependent upon NF-KB and p38 MAPK activation.¹⁰

However, data regarding the signaling pathways of *P. gingivalis* LPS-induced cytokine production in human monocytes are still limited. Therefore, the objective of this study is to investigate the activation of three MAPK family members as well as IL-1 β and IL-8 production by *P. gingivalis* LPS in comparison to LPS from *E. coli* in THP-1 cells and PBMC-derived monocytes.

Materials and methods

Cell culture

This study was approved by the Naresuan University Institutional Review Board (COE No. 55 01 04 0025). PBMC were acquired from buffy coats obtained from the Blood Bank Center of Naresuan University Hospital. The isolation of monocytic cells was conducted by adherence method, as described previously.¹¹

THP1 cells (ECACC) and PBMC-derived monocytes were cultured in RPMI 1640 (Sigma Chemical Co., Dorset, UK) containing 10% fetal calf serum (Sigma Chemical Co.), 2 mM L-glutamine, 50 U/ml of penicillin G, and 50 μ g/ml of streptomycin sulfate.

LPS-Stimulation

THP1 cells and PBMC-derived monocytes were seeded at a density of 4.0×10^6 cells/well into 6-well plates with a final volume of 2 ml medium per well. Cells were stimulated by *P. gingivalis* ATCC 33277 strain LPS-TLR2 ligand and *E. coli* K12 strain LPS (InvivoGen, San Diego, CA, USA) with 1 μ g/ml of final concentration for 6 h.

Estimation of IL-1 β and IL-8 levels using ELISA

Cytokine concentrations in the culture supernatant were assayed using a sandwich ELISA kit (Biolegend, San Diego, CA, USA). Briefly, the samples were added into the 96-well plates coated with a human IL-1 β or IL-8 specific monoclonal antibody and incubated for 2 h at RT. Next, the detection antibody was added for 1 h. The wells were then incubated with avidin-horse radish peroxidase, followed by substrate solution in the dark. After the addition of stop solution, microwell absorbance was detected using a microplate reader (PerkinElmer Life Sciences, Waltham, MA, USA) set at 450 nm. All measurements were performed in duplicate and

were repeated at three different occasions. Mean values of the three measurements were used for statistical analysis.

Analysis of JNK, ERK1/2, and p38 MAPK phosphorylation using Western Blotting

Western blot analysis was performed as previously described.¹² After treatment, THP1 cells and PBMC-derived monocytes were lysed and sonicated in Brij96 lysis buffer containing protease and phosphatase inhibitors (20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 2 mM ethylenediamine tetraacetic acid (EDTA), 10% glycerol, 10 mg/ml leupeptin, 10 mg/ml aprotinin, 1 mM phenylmethylsulphonyl fluoride (PMSF), 500 mM sodium orthovanadate, 1 mM sodium fluoride (NaF) and 0.5% Brij96). 80 μ g of total protein were subjected to SDS-PAGE. β -actin was used as the loading control. After protein separation by electrophoresis, samples were transferred to Immobilon-P membrane (Millipore) followed by immunoblotting with the anti-phospho-JNK (Cell Signaling Technology, Danver, MA, USA), anti-phospho-ERK1/2 antibody (Upstate Biotechnology, Lake Placid, NY, USA), and anti-phospho-p38 MAPK antibodies (Cell Signaling Technology). Immunoreactive proteins observed under CCD camera (Image Quant LAS 4000; GE Healthcare Life Sciences, Pittsburgh, PA, USA). Relative band intensity was quantified by ImageJ software and shown as the mean \pm SE of three separate experiments.

Statistical analyses were performed using SPSS software version 22. All data were expressed as mean \pm standard deviation (SD). Differences between experimental groups were analyzed with paired samples t-tests. A *p* value <0.05 was considered statistically significant.

Results

IL-1 β and IL-8 production in response to *P. gingivalis* and *E. coli* LPS stimulation

To investigate the ability of *P. gingivalis* LPS and *E. coli* LPS to induce cytokine production, the culture supernatants were collected for IL-1 β and IL-8 determination by ELISA.

The results showed that both *P. gingivalis* LPS and *E. coli* LPS significantly upregulated IL-1 β and IL-8 production (*p*<0.05) compared with the unstimulated THP-1 cells (Figure 1a,b) and

PBMC-derived monocytes (Figure 2a,b). In THP-1 cells, *E. coli* LPS stimulation significantly more IL-1 β and IL-8 production as compared with *P. gingivalis* LPS stimulation.

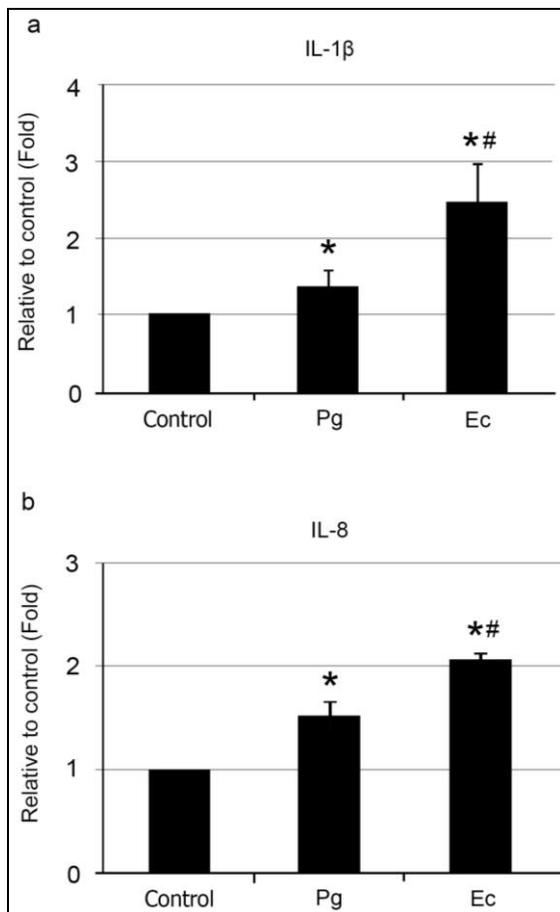


Figure 1. *P.gingivalis* LPS and *E. coli* LPS upregulated IL-1 β (a) and IL-8 (b) production in THP-1 cells. *significant differences ($p < 0.05$) compared with control (untreated cells); #significant differences ($p < 0.05$) compared with *P.gingivalis* LPS.

P. gingivalis and E. coli LPS-mediated MAPK activation

To examine the activation of JNK, ERK1/2, and p38 MAPK, The total protein of THP-1 cells and PBMC-derived monocytes exposed to *P. gingivalis* LPS and *E. coli* LPS were extracted and subjected to immunoblot analysis. It was found that *P. gingivalis* LPS stimulation resulted in the higher levels of JNK and ERK1/2 phosphorylation in THP-1 cells, whereas the higher p38 MAPK phosphorylation was found upon *E. coli* LPS stimulation (Figure 3a,b,c,d). Similar to these results, the higher p38 MAPK phosphorylation level was found in *E. coli*

LPS stimulated-PBMC derived monocytes (Figure 4a,b).

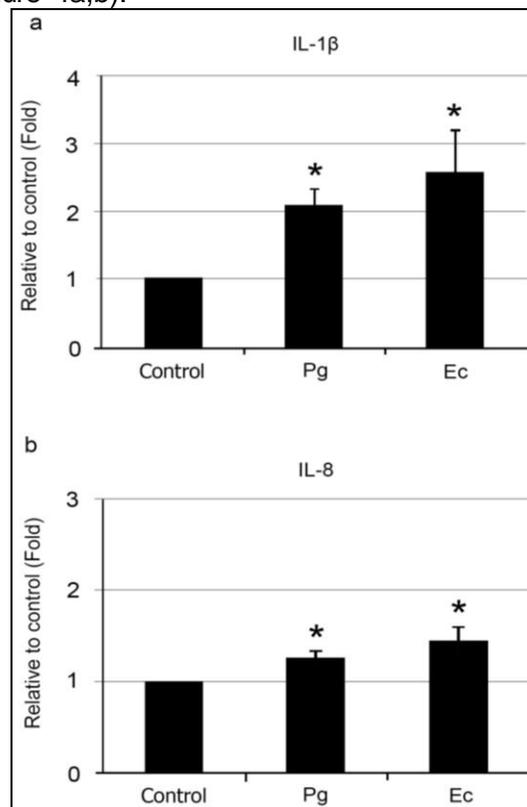


Figure 2. *P.gingivalis* LPS and *E. coli* LPS upregulated IL-1 β (a) and IL-8 (b) production in PBMC-derived monocytes. *significant differences ($p < 0.05$) compared with control (untreated cells).

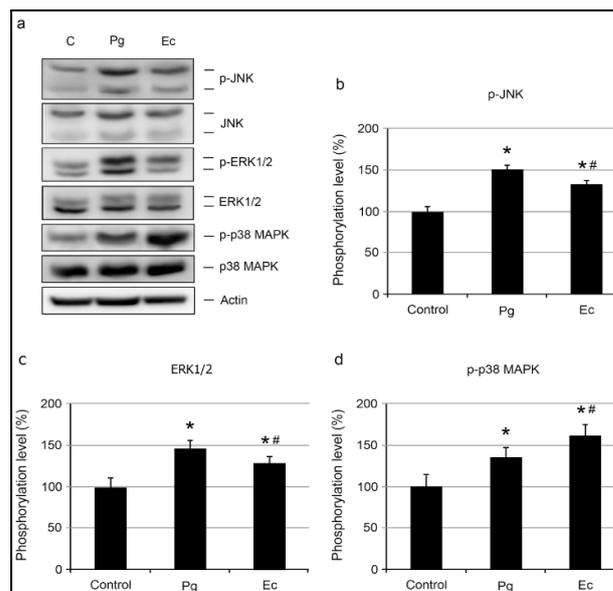


Figure 3. *P.gingivalis* LPS upregulated higher levels of JNK (a, b), ERK1/2 (a, c) phosphorylation, while *E. coli* LPS upregulated

higher levels of p38 MAPK (a, e) phosphorylation in THP-1 cells. *Significant differences ($p < 0.05$) compared with control (untreated cells); #significant differences ($p < 0.05$) compared with *P.gingivalis* LPS.

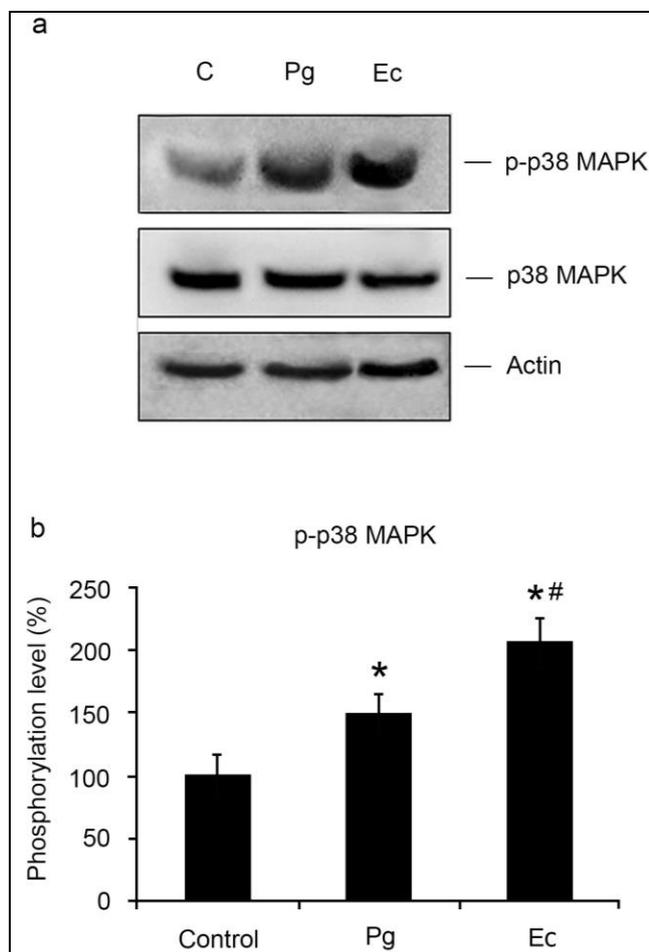


Figure 4. *E. coli* LPS upregulated higher levels of p38 MAPK (a, b) phosphorylation in PBMC-derived monocytes. *Significant differences ($p < 0.05$) compared with control (untreated cells); #significant differences ($p < 0.05$) compared with *P. gingivalis* LPS.

Discussion

P. gingivalis induce cytokine expression through the activation of signaling molecules ultimately result in the periodontal tissue destruction. Differential activation of TLRs by LPS from different bacteria elicits distinct pathways of intracellular signaling.^{13,14} In this study, we compared the effect of *P. gingivalis* LPS and *E. coli* LPS stimulation on the intracellular signaling pathways as well as IL-1 β and IL-8 production in human monocytes. It has

been reported that LPS from *P. gingivalis* and *E. coli* may elicit the activation of p38 MAPK in PBMC-derived monocytes.¹⁵ However, in THP-1 cells, *P. gingivalis* LPS-stimulated cytokine production was mainly through JNK pathways, while the major signaling pathways of *E. coli* LPS stimulation were NF- κ B and p38 MAPK.¹⁴ Consistent with these findings, we found that *P. gingivalis* LPS differs from *E. coli* LPS in that it induces different MAPK family members in both THP-1 cells and PBMC derived monocytes. In THP-1 cells, *P. gingivalis* LPS induced higher levels of JNK phosphorylation, whereas higher p38 MAPK phosphorylation was found upon *E. coli* LPS stimulation. Moreover, we have extended previous findings that *P. gingivalis* LPS also induced higher levels of activated ERK1/2 compared with *E. coli* LPS in THP-1 cells.

IL-1 β and IL-8 play important roles in inflammatory processes, including progression of periodontal disease. IL-1 β and IL-8 levels in gingival crevicular fluid have been shown to correlate with severity of periodontitis.¹⁶ Previous studies have shown that *P. gingivalis* LPS and *E. coli* LPS can upregulate IL-1 β production in THP-1 cells.¹⁴

The present study also demonstrated that both types of LPS had the ability to promote the production of IL-1 β and IL-8 from THP-1 cells and PBMC-derived monocytes. Understanding the biological mechanisms involved in expression of pro-inflammatory cytokines might lead to a novel approach to control chronic inflammatory diseases, including periodontitis. However, we only investigated the phosphorylation of MAPK signaling and IL-1 β and IL-8 production in human monocytes stimulated by *P. gingivalis* LPS. Therefore, inflammatory signaling pathways in various cells of the periodontium upon *P. gingivalis* stimulation should be further investigated.

Conclusions

The present investigation indicated that *P. gingivalis* LPS and *E. coli* LPS stimulation may lead to activation of different MAPK family members. *P. gingivalis* LPS induced IL-1 β and IL-8 production mainly through JNK and ERK1/2 signaling pathways, whereas cytokine induction by *E. coli* LPS was mainly via p38 MAPK pathway in human monocytes.

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Declaration of Interest

The authors declare that they have no conflict of interest.

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