# 学 位 論 文 の 要 旨

氏名　Hossain Kazi Helal

学　位　論　文　名　Differential Expression of Pro-inflammatory and Pro-coagulant Genes in Endothelial Cells Induced by *Porhyromonas gingivalis* Lipopolysaccharide, *Escherichia coli* Lipopolysaccharide, and Zymosan.

発　表　雑　誌　名　Shimane Journal of Medical Science
（巻，初頁～終頁，年）　(In Press)

著　者　名　Kazi Helal HOSSAIN, Takayuki OKAMOTO, Haruki USUDA, Israt JAHAN, Tomomi NIIBAYASHI, Koichiro WADA

## INTRODUCTION

The recent studies have reported that infection of *Porhyromonas gingivalis* (*P. gingivalis*), one of the major periodontal pathogens, is closely correlated with progress of several vascular inflammatory diseases such as atherosclerosis, sepsis, hypertension, vascular hemorrhage, ischemic stroke, and Alzheimer's disease. The animal model studies and clinical studies that *P. gingivalis* is detected in intra-vascular vessels and injured tissues, suggest that *P. gingivalis* infiltrates from the periodontium to intravascular space and then disseminates systemic vascular vessels. Several studies indicated the possibility that intra-vascular infection of *P. gingivalis* potentially impairs endothelial function, endothelial cell barrier, and endothelium structure; however, the molecular mechanism of *P. gingivalis*-mediated endothelial cell dysfunction is poorly understood.

The lipopolysaccharide (LPS) of *P. gingivalis* is known as one of the major substances of this pathogen. It has been reported that *P. gingivalis*-LPS induces pro-inflammatory responses in monocytes and periodontal fibroblasts by acting as an agonist for toll like receptor (TLR) -2 or as an antagonist and/or agonist for TLR-4. In addition, several studies indicated that *P. gingivalis*-LPS might induce differential inflammatory signaling dependent on cell type specific pattern. Thus, studies about effect of *P. gingivalis*-LPS on endothelial cell hold promise for understanding how *P. gingivalis* triggers vascular inflammatory diseases.

In this study, we investigated the impact of *P. gingivalis*-LPS on the expression of pro-inflammatory and pro-coagulant genes in endothelial cells with comparison to TLR-4 agonist *Escherichia coli* (*E. coli*)-LPS and TLR-2 agonist zymosan. We identified the

differential expression pattern of pro-inflammatory and pro-coagulant genes in endothelial cells induced by *P. gingivalis*-LPS.

## MATERIALS AND METHODS

We employed a human hybrid endothelial cell line, EA.hy926 cell, was generated by the fusion of human umbilical vein endothelial cell with the human lung carcinoma cell line A549 cell. TLRs mRNA expression in EA.hy926 cells were detected by reverse transcription-polymerase chain reaction. In order to investigate the effect of *P. gingivalis*-LPS on the gene expression in endothelial cells, we stimulated EA.hy926 cells with 1 μg/ml and 10 μg/ml of *P. gingivalis*-LPS, *E. coli-LPS*, or zymosan in the presence or absence of fetal bovine serum (FBS) and evaluated inflammation- and blood coagulation-related mRNA expression by the quantitative real time PCR (qRT-PCR). The permeability of endothelial cells was evaluated by modified Boyden chamber assay. Briefly, monolayer EA.hy926 cells were grown to confluence in Boyden chambers. The lower chamber was filled with media containing 200 μg/mL FITC dextran and 10 μg/mL *P. gingivalis*-LPS or *E. coli*-LPS. Samples were collected at indicated time points from upper chamber and then the fluorescence intensity was measured by fluorescence microplate reader. For statistical analysis, Dunnett's t-test was performed for comparison to vehicle-treated cells. Statistical significance was defined as $P<0.05$.

## RESULTS AND DISCUSSION

Several reports indicated that *P. gingivalis*-LPS induces numerous pro-inflammatory responses in macrophages and periodontal fibroblasts via activation of TLR-4 and TLR-2. TLR-1 and TLR-6 are known as co-receptors of TLR-2 to promote unique signaling induced by specific pathogen binding. We, therefore, confirmed that EA.hy926 cells constitutively expressed TLR-4, TLR-2, TLR-1, and TLR-6 mRNA and used EA.hy926 cells in subsequent experiments.

In order to investigate the impact of *P. gingivalis*-LPS on endothelial cells, we analyzed mRNA expression pattern in EA.hy926 cells induced by *P. gingivalis*-LPS stimulation in comparison to TLR-4 agonist *E. coli*-LPS or TLR-2 agonist zymosan stimulation. We examined pro-inflammatory interleukin (IL)-6 and tumor necrosis factor-α (TNF-α) mRNA expressions that are typical genes induced by *E. coli*-LPS stimulation via TLR-4 activation in the presence of FBS. Although *E. coli*-LPS induced remarkably increases in IL-6 and TNF-α mRNA expressions, *P. gingivalis*-LPS induced weak expressions and zymosan did not. Notably, both *P. gingivalis*-LPS-induced IL-6 and TNF-α mRNA expression in the absence of FBS were weaker than those in the presence of FBS, suggesting that serum components might contribute to the effect of *P. gingivalis*-LPS as like as *E. coli*-LPS as a co-factor. Next, we evaluated IL-8

and hypoxia inducible factor (HIF)-2α mRNA expression that induced by TLR-2 activation. *P. gingivalis*-LPS slightly induced IL-8 expression in the presence of FBS compared with zymosan and *E. coli*-LPS. Our data that *P. gingivalis*-LPS attenuates IL-8, and HIF-2α mRNA expression in the absence of FBS, suggest that serum cofactors are required for *P. gingivalis*-LPS-mediated endothelial cell activation; however, further studies will be needed to identify any cofactors involved in *P. gingivalis*-LPS-mediated endothelial cell activation. Based on these findings, we speculated that *P. gingivalis*-LPS partially activates both TLR-2 and TLR-4 on EA.hy926 cells.

As endothelial cells induce increase of pro-coagulant molecules and decrease of anti-coagulant molecules on cell surface upon proinflammatory stimuli, endothelial cell is an important player for regulation of blood coagulation in intravascular vessels. It is thought that expression level of tissue factor (TF) and plasminogen activating inhibitor (PAI)-1 in endothelial cells directly links to intravascular blood coagulation state. We explored the impact of *P. gingivalis*-LPS on mRNA expressions of pro-coagulant factors, TF and PAI-1, in EA.hy926 cells. Notably, *P. gingivalis*-LPS significantly reduced both TF and PAI-1 mRNA expressions in the presence and absence of FBS. Thus, our data suggest the possibility that *P. gingivalis* might modulate the balance of blood coagulation and fibrinolysis. In addition, the endothelial cell permeability stimulated by *P. gingivalis*-LPS or *E. coli*-LPS was not altered in the presence and absence of FBS, suggesting that bacterial LPS may not affect the endothelial cell permeability in our experimental conditions. In this study, we have shown that *P. gingivalis*-LPS induces pro-inflammatory IL-6, TNF-α, IL-8, and HIF-2α mRNA and reduces pro-coagulant TF and PAI-1 mRNA without any alteration in endothelial cell permeability. Although further studies are required for understanding how *P. gingivalis* promotes vascular dysfunctions and vascular inflammatory diseases, this study provides insights into the activation of endothelial cell underlying vascular dysfunctions associated with infection of *P. gingivalis*.

## CONCLUSION

In this study, we investigated the differential gene expression pattern of *P. gingivalis*-LPS stimulation compared with stimulation of *E. coli*-LPS or zymosan. The differential gene expression pattern induced by *P. gingivalis*-LPS stimulation which is likely mediated by the activation of TLR-2 and TLR-4 may contribute to the development of the vascular inflammatory diseases.