Original

Interleukin-8 Production by Human Gingival Fibroblasts in Response to Periodontopathic Bacterial Lipopolysaccharides and Prostaglandin E₂ *In vitro*.

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Abstract: This study examined the modulating effect of PGE₂ on LPS-induced IL-8 production by human gingival fibroblasts *in vitro*. The expression of IL-8 mRNA, after stimulation with PGE₂, was determined by RT-PCR. IL-8 production by HGFs was shown to be related to the time of incubation and the dose of LPS. A slight increase in IL-8 levels was observed when cells were exposed to PGE₂ alone compared to controls. When HGFs were pre-stimulated with PGE₂ and then exposed to LPS, IL-8 production was increased 2-3 fold compared to non-treated cells. Herbimycin A completely abolished the secretion of IL-8. These results indicate that PGE₂ can modulate cytokine secretion from HGFs and increase IL-8 production from cells exposed to LPS. IL-8 production induced by LPS was dependent on the tyrosine phosphorylation pathway.

Key words: human gingival fibroblasts, interleukin-8, lipopolysaccharides, prostaglandin E₂.

Introduction

Human gingival fibroblasts (HGFs), which represent the major cellular component of the gingival connective tissues, have an important function in maintaining the integrity of the gingiva. HGFs may also play a significant role in the pathogenesis of periodontal disease. Indeed, over the past few years several studies have reported cytokine production by HGFs in response to bacterial components and inflammatory mediators. Thus, it is conceivable that these cytokines may participate in tissue remodeling which occurs during the active phase of periodontal destruction¹⁾.

The colonization of the tooth surface by micro-organisms triggers an inflammatory reaction within the gingival tissues accompanied by a destruction of the collagen fibers. In the early phase of disease, the inflammatory infiltrate is composed mainly of polymorphonuclear leukocytes. The migration of inflammatory

cells within the connective tissues could be due to chemotactic factors of bacterial origin. However, it is also possible that the reaction is host mediated and that inflammatory cells are attracted by mediators produced locally by resident cells. Several studies have demonstrated that human dermal fibroblasts have the ability to produce proinflammatory interleukins^{2,3)} including interleukin-8 (IL-8), a known neutrophil activating/chemotactic factor⁴⁾. Synthesis and secretion of IL-8 has also been demonstrated for human gingival fibroblasts⁵⁻⁹⁾ IL-8 production by HGFs may be induced by bacterial components as well as mediators such as IL-1 and TNF- α from immunocompetent cells⁶⁻⁸⁾.

It is generally agreed that oral bacteria such as *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* are important in the etiology of periodontal diseases. Lipopolysaccharides (LPS) from these pathogens have been implicated in pathogenesis of disease because of their ability to induce an inflammatory and immune response as well as periodontal tissue breakdown¹⁰⁻¹²⁾. However, little is known about the effect of LPS from periodontopathic bacteria on the production of proinflammatory cytokines by resident cells such as HGFs.

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Furthermore, the exact role of prostaglandin E₂ (PGE₂) in potentiating cytokine production is not well understood. PGE₂ is a potent mediator of inflammation which has been detected in inflamed gingival tissues and in gingival crevicular fluid¹³⁻¹⁶. Samples from inflamed sites showed elevated levels of PGE₂ as compared to non-inflamed sites¹⁷⁻¹⁹. One could speculate that increased levels of PGE₂ may be of importance in the disease process by regulating fibroblast functions.

The purpose of the present investigation was to explore the ability of periodontopathic bacterial LPS to modulate IL-8 production and to examine the effects of PGE₂ priming on LPS-induced production of IL-8 by HGFs *in vitro*.

Materials and Methods

Preparation of human gingival fibroblasts

Human gingival fibroblasts were obtained from nine healthy donors. Cells were derived from biopsies of the attached gingiva that were clinically free of periodontal disease. The gingival tissues were minced in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA). Tissue fragments were placed in 35mm plastic dishes (Falcon, Pharmacia Fine Chemicals, Uppsala, Sweden) and cultured in medium containing 100 µg/ml of streptomycin, 100 units/ml of penicillin G and 10% heat-inactivated fetal calf serum (FCS; Gibco) in a humidified atmosphere of 5% CO₂ at 37°C. Cells were used between the 2nd and the 8th passage. HGFs were seeded at a cell density of 1x10⁴ cells/well in 96-well microtiter plates 3 days prior to the experiments. The culture medium was then removed and replaced with DMEM supplemented with 1% FCS for 24 h. Cultures were washed with DMEM and exposed to LPS preparations or PGE₂. All experiments were performed in duplicate.

Lipopolysaccharides

Lipopolysaccharides from *Porphyromonas gingivalis* 381 (Pg-LPS) and *Actinobacillus actinomycetem-comitans* ATCC 29523 (Aa-LPS) were kindly provided by Dr. T. Nishihara, Department of Oral Science, The National Institute of Health, Tokyo, Japan. LPS was extracted from lyophilized cells of *P. gingivalis* and *A. actinomycetemcomitans* by the hot phenol-water procedure²⁰. LPS from *Escherichia coli* 0127:B8 (Ec-LPS) was purchased from Difco Laboratories (Detroit, Mich, USA) and used as standard.

Stimulation with LPS

Cultures were stimulated by exposing HGFs to LPS under different experimental conditions.

- HGFs were exposed for 4 h to Ec-, Pg-, or Aa-LPS (0.001-10 µg/ml) in the presence of 1% serum. After replacing the culture medium by medium containing no LPS, cultures were further incubated for 4 h.
- HGFs were exposed for 4 h to Ec-LPS or Aa-LPS (0.001-10 µg/ml) in the presence of 0-5% FCS.

- HGFs were exposed to herbimycin A (10 µg/ml) for 1 h before LPS stimulation.
- HGFs were exposed to LPS preparations (10 µg/ml) for various periods of time up to 24 h in the presence of 1% serum.
- HGFs were pre-stimulated with 10⁻⁵ M of PGE₂ for 2 h, washed twice with DMEM and then exposed to LPS for up to 24 h.

Activation of cells by PGE₂

To evaluate the effect of PGE₂ on LPS-induced IL-8 production, cultures were pre-stimulated with various concentrations (10⁻⁷ M to 10⁻⁵ M) of PGE₂ (Sigma) for up to 2 h before adding the LPS preparations. Cultures were then exposed to 10 µg/ml of Ec-, Pg- or Aa-LPS for various times up to 24 h.

Assay of IL-8

Following incubation, culture supernatants were collected and centrifuged at 3000g for 2 minutes and stored at 4°C until assayed for IL-8 content. The activity of IL-8 was measured using a commercial kit (PeliKine Compact™ human IL-8 ELISA Kit, Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). IL-8 concentration was determined by comparing the optical density of the samples to a standard curve. The cell culture supernatants incubated with medium alone without activating agents were used as controls.

IL-8 mRNA expression

Total RNA was extracted from control HGFs and cells exposed to PGE₂ using Trizol R and quantified by spectrophotometry. Reverse transcription reaction was carried out in a 20-µl volume containing 1.0 µg total RNA, 2.5 U RNasin, 5 mM MgCl₂, 50 mM KCl, 10 mM Tris (pH 8.3), 2.0 µM oligo (dT) 11-18 primer, and 10 U of reverse transcriptase. The samples were incubated at 37°C for 1 h. Reverse transcriptase was inactivated by incubation at 95°C for 7 minutes. For each amplification reaction (PCR), 2 µl cDNA (equivalent to 100 ng total RNA) was used. Each 100 µl reaction consisted of 10 mM Tris-HCl, 50 mM KCl, 0.2% Triton X-100, 1.5 mM MgCl₂, and 0.2 mM each of dATP, dCTP, dGTP, and dTTP. Sense and anti-sense IL-8 primer was purchased from CLONTECH Laboratories (Palo Alto, Ca, USA).

IL-8 sequence information:

Upstream(5')

primer; 5'-ATGACTTCCAAGCTGGCCGTGGCT-3'

Downstream (3')

primer; 5'-TC TCAGCCCTCTTCAAAAAGTTCTC-3'

Amplification was carried out for 30 cycles. The amplification reaction profile consisted of denaturation at 94°C for 45 sec, primer annealing at 60°C for 45 seconds, and primer extension at 72°C for 2 minutes. Additionally, sense and anti-sense primers for GAPDH were included in the reaction as internal controls. The

amplification for GAPDH was performed for 30 cycles. The amplification products were electrophoresed in 2% agarose gels containing 0.5 µg/ml ethidium bromide.

Statistical analysis

The paired *t*-test was used to assess the significance of the observed differences. The differences were considered significant when the probability (*p*) value was <0.05.

Results

IL-8 production by HGFs exposed to LPS

HGFs cultures exposed to *Ec*-, *Pg*-, and *Aa*-LPS showed a significant increase in IL-8 levels when compared to controls (Fig. 1A). In *Ec*- and *Pg*-LPS stimulated cultures IL-8 levels were dependent on the dose of LPS used with a maximum release at 10 µg/ml. At this concentration, *Pg*-LPS was more potent in stimulating IL-8 release compared to *Ec*-LPS with a 7.2 and 3.4 fold increase over control cells respectively (*p*<0.01). *Aa*-LPS also induced IL-8 secretion in a dose-dependent fashion at low concentrations but reached a plateau at 0.1 µg/ml.

When the incubation medium was removed and replaced by fresh medium containing no LPS, HGFs continued to release IL-8 (Fig. 1B). After 4 h, IL-8 levels were found to be significantly higher in LPS pre-stimulated culture supernatants than in control cultures (*p*<0.01). IL-8 release was dependent on the dose of LPS used during the stimulation period and was the highest with *Pg*-LPS at a concentration of 10 µg/ml. Cultures pre-exposed to *Aa*-LPS also showed a significant increase in IL-8 levels at low doses which leveled off at 0.1 µg/ml. There was no difference in the morphology of control cultures and cells exposed to LPS. Cell vitality, as assessed by violet blue staining, was >90% (data not shown).

Effect of serum on IL-8 production

The effect of serum was examined by exposing HGFs cultures to *Ec*- or *Aa*-LPS in the presence of various serum concentrations ranging from 0-5% FCS. In serum-free conditions, *Ec*-LPS and *Aa*-LPS induced low levels of IL-8, ranging from 118-1788 pg/ml and 332-1194 pg/ml respectively. With both LPS preparations, addition of 1% or 5% FCS to the culture medium greatly enhanced IL-8 secretion with levels 2-10 fold higher than in serum-free conditions (data not shown).

Effect of herbimycin A on IL-8 secretion

When herbimycin A treated cells were exposed to the various LPS preparations (concentrations 0.001-10 µg/ml *Ec*-, *Pg*-, *Aa*-LPS) IL-8 secretion by HGFs was completely inhibited. This was observed both during the exposure of cells to LPS as well as after removing LPS preparations from the medium (*p*<0.01) (data not shown).

IL-8 production by HGFs exposed to LPS for various periods of time

Fig. 1. IL-8 production by HGFs exposed to LPS (A) and after removing LPS (B). (A) HGFs were exposed for 4 h to various concentrations (0.001-10mmg/ml) of *Ec*-LPS, *Pg*-LPS or *Aa*-LPS. (B) Following LPS stimulation, medium was replaced by medium without LPS and cultures were further incubated for 4h. Data is reported as the mean plus standard deviation of 4 different experiments. *: Significant increase compared to control cultures (*p*<0.01).

Fig. 2. IL-8 production by HGFs exposed to LPS for various periods of time. Cells were exposed to 10mmg/ml of *Ec*-LPS or *Pg*-LPS or *Aa*-LPS for up to 24 h. Data is reported as the mean plus standard deviation of 5 different experiments.

Fig. 3. Effect of PGE₂ on IL-8 production by HGFs. Cells were stimulated with various concentrations of PGE₂ (10⁻⁷ M to 10⁻⁵ M) for up to 2 h. Each point represents the mean plus standard error of duplicate values.

Fig. 4. Effect of PGE₂ on IL-8 mRNA as assessed by RT-PCR. Each amplification was carried out for 30 cycles. (A) 0.5 h, (B) 1 h exposure to PGE₂ (10⁻⁷ M to 10⁻⁵ M).

As shown in Fig. 2, secretion of IL-8 increased in a time-dependent fashion with all LPS tested. *Pg*-LPS was the most potent in stimulating IL-8 production and was approximately 2 times higher than *Aa*-LPS at each observation time.

Effect of PGE₂ on IL-8 production

Cell cultures exposed to 10⁻⁵ M PGE₂ alone showed a modest but significant increase in IL-8 production compared to control cultures. The increase in IL-8 was dependent on the time of exposure as can be seen in Fig. 3. Cells were much less responsive to 10⁻⁶ M PGE₂, whereas 10⁻⁷ M PGE₂ had only a limited effect (data not shown). No difference in the expression of IL-8 mRNA between control and PGE₂ stimulated cells was detected after 0.5 h exposure (Fig. 4A). However, mRNA expression was clearly enhanced when cultures were stimulated with PGE₂ (10⁻⁵ M) for 1 h (Fig. 4B).

We also examined the response of PGE₂-activated cells to *Ec*-, *Pg*- or *Aa*-LPS by exposing the cultures to PGE₂ (10⁻⁵ M) for 2 h before adding the LPS preparations. As can be seen in Fig. 5, IL-8 production was time-dependent and increased up to 24 h with all LPS tested. Highest levels of IL-8 were found in cultures stimulated with *Pg*-LPS. When comparing the results obtained with PGE₂-treated cells (Fig. 5) and those with untreated cells (Fig. 2), production of IL-8 was significantly increased in activated cultures.

Table 1 summarizes IL-8 data of PGE₂-activated cells obtained from five experiments using four different cell lines. A 2-3 fold-increase in IL-8 levels was observed with PGE₂-activated cells compared to non-activated cells. Statistical significance was seen at 4 h stimulation with all LPS tested ($p < 0.05$) and at 8 h stimulation with *Ec*- and *Pg*-LPS ($p < 0.05$).

Discussion

LPS from periodontal pathogens have been shown to stimulate

Fig. 5. Effect of PGE₂ activation on LPS-induced IL-8 production. After incubation with PGE₂ for 2 h, cells were exposed to 10 mg/ml of *Ec*-LPS or *Pg*-LPS or *Aa*-LPS for up to 24 h. Data is reported as the mean plus standard deviation from five different experiments. *: Significant increase compared with non-activated cells ($p < 0.05$).

Table 1. Mean fold-increase of IL-8 production in PGE₂-pretreated HGFs

	4h	8h	24h
PGE ₂ + <i>Ec</i> -LPS	2.4 ± 0.6*	2.3 ± 0.6*	2.4 ± 1.1
PGE ₂ + <i>Pg</i> -LPS	1.8 ± 0.2*	2.3 ± 0.4*	1.9 ± 0.6
PGE ₂ + <i>Aa</i> -LPS	3.1 ± 0.7*	2.4 ± 0.3	3.0 ± 1.1

a Mean standard deviation (data from 5 different experiments)

* Significantly different from cultures stimulated with LPS alone ($p < 0.05$)

IL-8 mRNA expression and IL-8 synthesis in human gingival⁽⁶⁾ as well as periodontal ligament fibroblast cultures⁽²¹⁾ More recently, *A. actinomycetemcomitans* and *Campylobacter rectus* were reported to induce secretion of IL-8 as well as IL-6 by HGFs⁽¹⁰⁻¹²⁾. Under the present experimental conditions, *Pg*-LPS was more potent in inducing IL-8 production than the other LPS tested. These observations may be related to differences in the structure of LPS molecules between the bacteria tested or to variations in LPS receptors on mammalian cells⁽²²⁾. Several reports have indicated that the chemical composition and biological properties of LPS may vary among periodontopathic bacteria^(23,24) A recent study reported that *P. gingivalis* LPS contained larger amounts of carbohydrates than *A. actinomycetemcomitans*, *Fusobacterium nucleatum* and *Capnocytophaga ochracea* LPS whereas fatty acid content was lower in *P. gingivalis* compared to the other pathogens⁽²⁵⁾ Other characteristic features⁽²⁶⁾ have been described for the LPS structure of *P. gingivalis*^(27, 28) and *A. actinomycetemcomitans*^(29, 30). Thus, it is possible that differences in LPS structure may affect the recognition and interaction of the LPS molecule with the membrane receptors of the HGFs. Exposure of HGF cultures to herbimycin A before LPS stimulation resulted in a complete inhibition of IL-8 synthesis. Herbimycin A is known to be a potent inhibitor of the tyrosine kinase system as

it binds in an irreversible fashion to the thiol groups of tyrosine kinase³¹). It has been shown to be an effective inhibitor of LPS-induced protein tyrosine phosphorylation in human macrophages. Thus, the results suggest that the up-regulation of IL-8 production by HGFs in response to LPS is dependent on the tyrosine kinase system.

A previous report indicated that PGE₂ enhances IL-8 production by human synovial fibroblasts in cultures upon stimulation with IL-1³²). We also showed that pre-activation of HGFs with PGE₂ before exposure to LPS resulted in a 2-3 fold increase in the levels of IL-8 production compared to non-activated cells. IL-8 production patterns with PGE₂-treated cells were similar to those observed under standard experimental conditions: *Ec*-LPS and *Pg*-LPS were more potent than *Aa*-LPS in stimulating IL-8 release. These observations suggest that PGE₂ did not affect the binding affinity of sCD14-LPS complexes to HGFs but activated cell function¹). PGE receptor has been shown to be coupled to cell membrane G-proteins³³). PGE₂, through its binding to these specific receptors, can enhance cAMP levels through stimulation of adenylate cyclase metabolism³⁴). Lenardo *et al.*³⁵) suggested that increasing cAMP levels is essential for the expression and regulation of IL-1bb, IL-6, and TNF-aa genes. It is not known if production of IL-8 by PGE₂-treated HGFs is regulated by similar mechanisms. Our findings with PGE₂ are in contrast with previous observations with other human cell types^{36,37}). Others reported that PGE₂ had no effect on LPS-induced IL-8 production in human macrophages and that PGE₂ reduced macrophage IL-6 production³⁸). It is likely that macrophages and polymorphonuclear cells, whose origin differs basically from HGFs, are terminally differentiated phagocytes that may have lost the ability for PGE₂ to regulate their IL-8 expression.

There is strong evidence that LPS from *P. gingivalis* and *A. actinomycetemcomitans* have the potential to stimulate HGFs to release IL-8 as well as other cytokines. These observations indicate that HGFs can act as accessory immunocompetent cells in gingival tissues. The present results suggest that PGE₂ can modulate this role.

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References

- Wang P. and Ohura K.: *Porphyromonas gingivalis* lipopolysaccharide signaling in gingival fibroblasts CD14 and toll-like receptors. Crit Rev Oral Biol Med, 13: 132-142, 2002
- Larsen C.G., Anderson A.O., Oppenheim J.J. and Matsushima K.: Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumor necrosis factor. Immunology, 68: 31-36, 1989
- Boxman I.L., Ruwhof C., Boerman O.C., Lowik C.W. and Ponec M.: Role of fibroblasts in the regulation of proinflammatory interleukin IL-1, IL-6 and IL-8 levels induced by keratinocyte-derived IL-1. Arch Dermatol Res, 288: 391-398, 1996
- Baggiolini M., Walz A. and Kunkel S.L.: Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest, 84: 1045-1049, 1989
- Matsuki Y., Yamamoto T. and, Hara K.: Detection of inflammatory cytokine messenger RNA (mRNA)-expressing cells in human inflamed gingival by combined *in situ* hybridization and immunohistochemistry. Immunology, 76: 42-47, 1992
- Tamura M., Tokuda M., Nagaoka S. and Takada H.: Lipopolysaccharides of *Bacteroides intermedius* (*Prevotella intermedia*) and *Bacteroides* (*Porphyromonas*) *gingivalis* induce interleukin-8 gene expression in human gingival fibroblast cultures. Infect Immun, 60: 4932-4937, 1992
- Takashiba S., Takigawa M., Takahashi K., Myokai F., Nishimura F., Chihara T., Kurihara H., Nomura Y. and Murayama Y.: Interleukin-8 is a major neutrophil chemotactic factor derived from cultured human gingival fibroblasts stimulated with interleukin-1bb or tumor necrosis factor alpha. Infect Immun, 60: 5253-5258, 1992
- Takigawa M., Takashiba S., Myokai F., Takahashi K., Arai H., Kurihara H. and Murayama Y.: Cytokine-dependent synergistic regulation of interleukin-8 production from human gingival fibroblasts. J Periodontol, 65: 1002-1007, 1994
- Dongari-Bagtzoglou A.I. and Ebersole J.L.: Increased presence of interleukin-6 (IL-6) and IL-8 secreting fibroblast subpopulations in adult periodontitis. J Periodontol, 69: 899-910, 1998
- Fujiwara T., Ogawa T., Sobue S. and Hamada S.: Chemical, immunobiological and antigenic characterizations of lipopolysaccharides from *Bacteroides gingivalis* strains. J Gen Microbiol, 136: 319-326, 1990
- Takada H., Mihara J., Morisaki I. and Hamada S.: Production of cytokines by human gingival fibroblasts. In: Hamada S, Holt S.C. and McGhee J.R., eds. *Periodontal Disease; pathogens and host immune responses*. Quintessence Publishing Co., Tokyo; 265-276, 1991
- Wilson M., Reddi K. and Henderson B.: Cytokine-inducing components of periodontopathogenic bacteria. J Periodont Res., 31: 393-407, 1996
- Goodson J.M., Dewhirst F.E. and Brunetti A.: Prostaglandin E₂ levels and human periodontal disease. Prostaglandins, 6: 81-85, 1974
- El Attar T.M.A.: Prostaglandin E₂ in human gingival in health and disease and its stimulation by female sex steroids. Prostaglandins, 11: 331-341, 1976

15. El Attar T.M.A. and Lin H.S.: Prostaglandins in gingiva of patients with periodontal disease. *J Periodontol*, 52: 16-19, 1981
16. Offenbacher S., Farr D.H. and Goodson J.M.: Measurement of prostaglandin E in crevicular fluid. *J Clin Periodontol*, 8: 359-367, 1981
17. Dewhirst F.E., Moss D.E., Offenbacher S. and Goodson J.M.: Levels of prostaglandin E₂, thromboxane, and prostacyclin in periodontal tissues. *J Periodont Res*, 18: 156-163, 1983
18. Ohm K., Albers H-K. and Lisboa BP. Measurement of eight prostaglandins in human gingival and periodontal disease using high pressure liquid chromatography and radioimmunoassay. *J Periodont Res*, 19: 501-511, 1984
19. Offenbacher S., Odle B.M. and Van Dyke T.E.: The use of crevicular fluid prostaglandin E₂ levels as a predictor of periodontal attachment loss. *J Periodont Res*, 21: 101-112, 1986
20. Nishihara T., Ohsaki Y., Ueda N., Saito N. and Mundy G.R.: Mouse interleukin-1 receptor antagonist induced by *Actinobacillus actinomycetemcomitans* lipopolysaccharide blocks the effects of interleukin-1 on bone resorption and osteoclast-like cell formation. *Infect Immun* 62: 390-397, 1994
21. Yamaji Y., Kubota T., Sasaguri K., Sato S., Suzuki Y., Kumada H. and Umemoto T.: Inflammatory cytokine gene expression in human periodontal ligament fibroblasts stimulated with bacterial lipopolysaccharides. *Infect Immun*, 63: 3576-3581, 1995
22. Lei G., Flebbe L., Roeder D. and Morrison DC.: Identification and characterization of lipopolysaccharide receptor molecule on mammalian lymphoid cells. *Adv Exp Med Biol* 256: 445-466, 1990
23. Hamada S., Koga T., Nishihara T., Fujiwara T. and Okahashi N.: Characterization and immunobiologic activities of lipopolysaccharides from periodontal bacteria. *Adv Dent Res*, 2: 284-291, 1988
24. Hamada S., Takada H., Ogawa T., Fujiwara T. and Mihara J.: Lipopolysaccharides of oral anaerobes associated with chronic inflammation: chemical and immunomodulating properties. *Intern Rev Immunol*, 6: 247-261, 1990
25. Hara Y., Kaneko T., Yoshimura A. and Kato I.: Serum rheumatoid factor induced by intraperitoneal administration of periodontopathic bacterial lipopolysaccharide in mice. *J Periodont Res*, 31: 502-507, 1996
26. Novotny A. Heterogeneity of endotoxin. In Reitschel ET, ed. *Handbook of endotoxins*. Vol I. Amsterdam: Elsevier Science Publishers, pp308, 1984
27. Koga T., Nishihara T., Fujiwara T., Nishizawa T., Okahashi N., Noguchi T. and Hamada S.: Biochemical and immunological properties of lipopolysaccharide (LPS) from *Bacteroides gingivalis* and comparison with LPS from *Escherichia coli*. *Infect Immun*, 47: 638-647, 1985
28. Ogawa T.: Chemical structure of lipid A from *Porphyromonas (Bacteroides) gingivalis* lipopolysaccharide. *FEBS Lett*, 332: 197-201, 1993
29. Hoover C.I.: Electrophoretic heterogeneity of lipopolysaccharides of *Actinobacillus actinomycetemcomitans*. *J Dent Res*, 67: 574-576, 1988
30. Perry M.B., MacLean L.M., Brisson J.R. and Wilson ME.: Structures of the antigenic O-polysaccharides of lipopolysaccharides produced by *Actinobacillus actinomycetemcomitans* serotypes a, c, d and e. *Eur J Biochem*, 242: 682-688, 1996
31. Fukazawa H., Pei-Ming L., Chii Y., Murakami Y., Mizuno S. and Uehara Y.: Specific inhibition of cytoplasmic protein tyrosine kinases by herbimycin a *in vitro*. *Biochem Pharmacol*, 42: 1661-1671, 1991
32. Agro A., Langdon C., Smith F. and Richards C.D.: Prostaglandin E₂ enhances interleukin 8 (IL-8) and IL-6 but inhibits GM-CSF production by IL-1 stimulated human synovial fibroblasts *in vitro*. *J Rheumatol*, 23: 862-868, 1996
33. Negishi M., Ito S. and Hayashi O.: Prostaglandin E receptors in bovine adrenal medulla are coupled to adenylate cyclase via Gi and to phosphoinositide metabolism in a pertussis toxin-insensitive manner. *J Biol Chem*, 264: 3916-3923, 1989
34. Brunton L.L., Wiklund R.A., Van Arsdale P.M. and Gilmar AG.: Binding of [³H] prostaglandin E₁ to putative receptors linked to adenylate cyclase of cultured cell clones. *J Biol Chem*, 251: 3037-3044, 1976
35. Lenardo M.J. and Baltimore D.: NF-KB. A pleiotropic mediator of inducible and tissue-specific gene control. *Cell*, 58: 227-229, 1989
36. Standiford T.J., Kunkel S.L., Rolfe M.W., Evanoff H.L., Allen R.M. and Strieter R.M.: Regulation of human alveolar macrophage- and blood monocyte-derived interleukin-8 by prostaglandin E2 and dexamethasone. *Am J Respir Cell Mol Biol*, 6: 75-81, 1992
37. Wertheim W.A., Kunkel S.L., Standiford T.J., Burdick M.D., Becker F.S., Wilke C.A., Gilbert A.R., and Strieter R.M.: Regulation of Neutrophil-Derived IL-8: the role of Prostaglandin E2, Dexamethasone, and IL-4. *J Immunol*, 151: 2166-2175, 1993
38. Zhong W.W., Burke P.A., Drotar M.E., Chavali S.R. and Forse R.A.: Effects of Prostaglandin E2, cholera toxin and 8-bromo-cyclic AMP on lipopolysaccharide-induced gene expression of cytokines in human macrophages. *Immunology*, 84: 446-452, 1995