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Original

Lipopolysaccharide-Treated Human Gingival Fibroblasts Continuously Produce PGE_2

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Abstract: The aim of this study was to examine whether human gingival fibroblasts (HGFs) sustain to produce prostaglandin E_2 (PGE_2) in the presence of lipopolysaccharide (LPS). HGFs were treated with or without 10 ng/ml of LPS from *Porphyromonas gingivalis* (PgLPS) for 7 days collecting the culture supernatants for every 24 h, and PGE_2 concentration in the supernatants was measured by ELISA. HGFs produced PGE_2 at low level without PgLPS treatment. In contrast, HGFs continuously produce PGE_2 with PgLPS treatment. At each day, the amount of PGE_2 production by HGFs with PgLPS treatment was higher than that without treatment. These results demonstrate that the characteristic of HGFs, which continuously produce PGE_2 in the presence of LPS, sustains inflammatory response in periodontal disease.

Key words: Human gingival fibroblast, Lipopolysaccharide, Prostaglandin E_2

Introduction

Caries and periodontal disease are two major oral diseases and both are considered to be infections caused by biofilms[1,2]. In particular, periodontal disease is highly prevalent and can affect most of the world population. Periodontal disease is accompanied by inflammation of the gingiva and destruction of periodontal tissues, leading to alveolar bone loss in severe clinical cases. *Porphyromonas gingivalis* (*P. gingivalis*), a gram-negative bacterial species with black pigmentation, is one of the suspected periodontopathic bacteria and is frequently isolated from the periodontal pockets of patients with chronic periodontal disease [3,4]. Periodontopathic bacteria produce many virulence factors such as lipopolysaccharide (LPS) and peptidoglycan, and these bacterial factors induce host responses including the production of pro-inflammatory cytokines and prostaglandin E_2 (PGE_2). In particular, because PGE_2 has several functions in vasodilation and the enhancement of vascular permeability and pain, the induction of osteoclastogenesis, PGE_2 plays an important role in inflammatory response and alveolar bone resorption in periodontal disease[5].

IL-6, IL-8 and PGE_2 are produced by not only inflammatory cells (monocyte and macrophage) but also human gingival fibroblasts (HGFs) [6-9]. Among these cells, we assume that HGFs are the most important cells that produce inflammatory cytokines and PGE_2 because HGFs are the most prominent cells in periodontal tissue.

It has been well documented that the LPS-induced production of inflammatory cytokines was down-regulated by LPS-pretreatment in inflammatory cells such as peripheral blood mononuclear cells[10,11], monocytes[12,13] and macrophages[14,15]. Moreover, LPS-induced PGE_2 production was also down-regulated in macrophage-like cells[16]. Recently, we reported that HGFs sustain to produce IL-6 and IL-8 in the presence of LPS[17]. However, it remains elucidated that whether HGFs sustain to produce PGE_2 production. Therefore, to investigate whether HGFs sustain to produce PGE_2 in the presence of LPS is of interest in the viewpoint of the pathology of periodontal disease. To reveal to this point, we examined PGE_2 production by HGFs treated with LPS continuously.

Materials and methods

Reagents and cells

LPS from *P. gingivalis* 381 (PgLPS) was provided by Drs. Tatsuji Nishihara and Nobuhiro Hanada (National Institutes of Public Health, Wako, Japan). HGFs were prepared as described previously[18,19]. HGFs were maintained in Dulbecco's modified Eagle's medium (D-MEM; Sigma) containing 10% heat-inactivated fetal calf serum (FCS), 100 units/ml penicillin and 100 mg/ml streptomycin, at 37°C in a humidified atmosphere of 5% CO_2. This study was approved by the Ethical Committee of

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our institution. Informed consent was obtained from each subject for the collection of HGFs.

Cytokine measurement

HGFs were seeded in 96-well plates (Asahi Techno Glass Corp., Tokyo, Japan) at 10,000 cells/ml. The next day, HGFs were treated with or without 10 ng/ml of PgLPS (200 μl per each well) for 7 days collecting the culture supernatants for every 24 h (Fig. 1A), and the concentration of PGE_2 was measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacture's instructions (Cayman Chemical, Ann Anbor, MI).

Results

To investigate whether prolonged exposure to LPS affects the production of inflammatory cytokine, HGFs were treated with or without 10 ng/ml of PgLPS for 7 days. HGFs produced basal level of PGE_2 (< 100 pg/ml) at each day without LPS treatment (Fig. 1). When treated with PgLPS, HGFs produced a large amount of PGE_2 (Fig. 1B). At each day, the amount of PGE_2 with LPS treatment is apparently higher than without PgLPS treatment although PGE_2 levels at day 3 or 4 are lower than that at day 1. These results indicate that HGFs sustain to produce PGE_2 after exposure to PgLPS for at least 7 days.

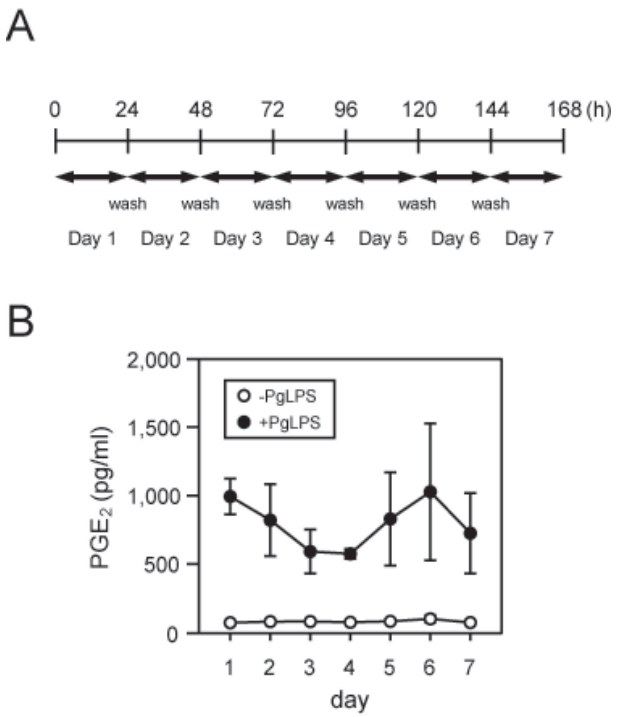

Figure 1.PGE_2 production after prolonged exposure to LPS. (A) HGFs were treated with or without 10 ng/ml of PgLPS for 7 days. For every 24 h, culture supernatants were collected, and cells were washed with PBS and culture medium with PgLPS was added. (B) The level of PGE_2 in culture supernatants at from day 1 to day 7 was measured by ELISA. Results are the mean ± SD of triplicate samples.

Discussion

Until now, many reports have demonstrated that the secondary LPS-induced production of inflammatory cytokines and PGE_2 was down-regulated by primary LPS in inflammatory cells such as PBMC, monocytes and macrophage (*i.e.* LPS tolerance)[10-16]. In this study, we demonstrated that in the presence of LPS, HGFs sustain to produce PGE_2 as well as inflammatory cytokines (IL-6 and IL-8)[17]. Because (i) HGFs are the prominent cells in periodontal tissue, (ii) HGFs continuously produce PGE_2 and (iii) macrophages show LPS tolerance[16], we consider that HGFs rather than monocytes/macrophages are the primary cells responsible for the production of PGE_2 in periodontal tissue and that continuous PGE_2 production leads to persisting inflammatory response. This assumption is supported by the previous report that cyclooxygenase (COX)-2 was detected in fibroblasts as well as other cells in inflamed gingiva[5].

Down-regulation of cell-surface toll-like receptor (TLR)4 expression is one of the LPS tolerance mechanisms [20]. Moreover, several negative-regulators such as suppressor of cytokine signaling-1 (SOCS-1)[21,22], interleukin-1 receptor-associated transducer for interleukin (IRAK)-M[23] and SH2 domain-containing inositol-5-phosphatase (SHIP)-1[24] also play important roles in LPS tolerance. SOCS-1 and IRAK-M, especially, inhibit LPS-TLR signaling pathway by suppression of adaptor molecules [25], resulting in down-regulation of p38, Jun N-terminal kinase (JNK) and nuclear factor (NF)-κB signaling pathways. These signal pathways, at least in part, are essential to IL-6, IL-8 and COX-2 expressions[26,27]. However, from the finding that (i) HGFs treated with LPS for 24 h expressed similar level of TLR4 expression compared to without treatment[28] and that (ii) LPS-treated HGFs failed to induce SOCS-1 and IRAK-M[17], it is suggested that COX-2 expression and following PGE_2 production are not suppressed in LPS-treated HGFs.

In conclusion, we demonstrated that HGFs sustain to produce PGE_2 in the presence of LPS. From this and our previous findings, we suggest that HGFs are critical in the pathology of periodontal disease. Our findings may provide the basic and clinical evidences that this characteristic of HGFs sustains inflammatory response in the presence of virulence factors and that the elimination of biofilm is essential to the treatment of periodontal disease.

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