



Rat periodontal fibroblast responses to bacterial lipopolysaccharide *in vitro*

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Rat periodontal ligament fibroblasts and gingival fibroblasts were stimulated with lipopolysaccharide from *Escherichia coli*. Cell proliferation and both interleukin-1 β and nitric oxide production were determined by a colorimetric, enzyme-linked immunosorbent assay and the Griess reaction, respectively. The results showed that at lipopolysaccharide concentration up to 100 ng per well, the cell proliferation of periodontal ligament fibroblasts was higher than that of gingival fibroblasts. No significant difference between the levels of interleukin-1 β produced by periodontal ligament and gingival fibroblasts can be observed. At high concentration of lipopolysaccharide (1000 ng/well), the levels of nitric oxide in the periodontal ligament fibroblasts cultures were higher than those in the gingival fibroblasts cultures. These results suggest those rat periodontal ligament and gingival fibroblasts may respond differently to lipopolysaccharide and, thus, may be different periodontal fibroblast subpopulations.

Key words: Fibroblast, gingiva, lipopolysaccharide, periodontal ligament, rat

Fibroblasts are the predominant cell type of the soft connective tissues of the periodontium. Based on the tissue localization, there are 2 types of periodontal fibroblasts—the gingival fibroblasts (GF) and periodontal ligament fibroblasts (PLF). Although GF and PLF are morphologically similar, previous comparative studies in humans and animals revealed that these types of fibroblasts are distinct cell subpopulations. For example, alkaline phosphatase activity and collagen production by the human PLF are higher than those by the human GF [1,2]. Gao and colleagues [3] showed that PLF had higher levels of alkaline phosphatase activity than GF in rats. Following topical applications of bacterial lipopolysaccharide (LPS) on the gingival sulcus in rats, the phagocytic activity of collagen fibrils by PLF was much higher than that by GF [4], indicating that in response to LPS, PLF may participate in collagen degradation during periodontal inflammation as compared with GF. Furthermore, interleukin (IL)-1 messenger RNA expression can be detected in both unstimulated human PLF and human GF [5]. Following stimulation with LPS from periodontopathogens, rat GF produced in a dose-dependent manner [6]. This cytokine

was also released by rat PLF when the cells was mechanically stressed [7]; yet, whether or not LPS stimulates the production of this cytokine by rat PLF remains unclear. Moreover, nitric oxide (NO) is a reactive gas produced by cells such as macrophages and though to play a crucial role in the course of periodontal disease [8]. Increased expression of nitric oxide synthase (iNOS) can be seen in human GF stimulated with LPS from *Porphyromonas gingivalis* [9]. Under mechanical forces, human PLF releases NO [10]. However, comparison between NO produced by LPS-stimulated GF and PLF in rats has not yet been reported. Lipopolysaccharide of gram-negative bacteria is known to play an important role in the development of periodontal disease [11]. The effects of this component of bacterial cell wall on the pathology of GF have been well reported [11], but its role in PLF remains to be elucidated. Therefore, the aim of this study was to compare between the levels of cell proliferation, IL-1 β , and NO production of rat PLF and those of rat GF following LPS stimulation.

Materials and Methods

Both PLF and GF were isolated from female Sprague Dawley rats of 4 to 6 weeks old. Following topical application of 5.25% NaOCl for 2 min, both maxillary and mandibular molars were extracted and immersed

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in RPMI 1640 medium containing 2 mg/100 mL of penicillin-streptomycin (Sigma, St. Louis, MO, US). Periodontal ligaments were obtained by digestion the teeth with 0.2% collagenase and 0.15% trypsin (Sigma) and the cell suspension was then washed 3 times as described elsewhere [3]. Pellets were resuspended in RPMI 1640, each 100 mL containing 10% fecal calf serum, 2% penicillin-streptomycin, and 2% amphotericin (all from Sigma) and cultured in 96 well plates (Nunc, Roskilde, Denmark). Pooled maxillary and mandibular gingival tissues were dissected after application of 5.25% NaCl and gingival connective tissues were separated from the epithelial layers under a microscope. The epithelial layer were subsequently digested enzymatically as above. After extensive washing, the cells were cultured in 96 well plates (Nunc). Fibroblasts were microscopically characterized by morphologic appearance. All experiments used both PLF and GF from passage 1.

Both PLF and GF (2×10^4 cells/well) were stimulated with various concentration of LPS from *Escherichia coli* 055:85 (Sigma) and incubated for 72 h. All cultures were in triplicate. Cell proliferation was determined by a colorimetric assay as previously described [12]. The levels of IL-1 β in the culture supernatants were determined by an enzyme-linked immunosorbent assay kit (R&D System, Minnesota, MN, US). Nitric oxide levels of the culture supernatants were determined by the Griess reaction as described elsewhere [13]. Briefly, 100 μ L of the Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid) were mixed with an equal volume of the 72 h culture supernatants and then read in an automated plate reader at 540 nm. Nitrite levels were calculated from a standard curve prepared with sodium nitrite. All reagents for NO assay were commercially obtained from Sigma. Nitric oxide levels of the samples were deduced from those of unstimulated cell cultures.

Each parameter was carried out in 3 experiments. Data was analyzed by a one-way analysis of variance followed by Fischer's least squared differences with a statistical software package (Minitab release 11 Inc., State College, PA, US).

Results and Discussion

Following stimulation with LPS for 72 h, the cell proliferation and the production of IL-1 β and NO by rat PLF and GF were determined. The results showed that when stimulated with concentration up to 100 ng/well of LPS, the proliferation of rat GF was significantly lower than that of rat PLF ($p < 0.05$)

(Fig. 1). However, no significant differences between rat GF and PLF proliferation could be seen when the cells were stimulated with 1000 ng per well of LPS ($p > 0.05$). In contrast, the levels of IL-1 β detected from the supernatants of the rat PLF and GF cultures stimulated with LPS up to 1000 ng per well were not significantly different ($p > 0.05$) (Fig. 2). Furthermore, as seen in Figure 3, the production of nitrite in the rat PLF cultures was significantly higher than that in the rat GF cultures only when the cultures were stimulated with 1000 ng per well of LPS ($p < 0.05$).

Results of this study showed that following LPS stimulation, both cell proliferation and NO but not IL-1 β production in rat GF were lower than those in rat PLF. These results are consistent with previous findings showing that the proliferation of human PLF proliferation is much higher than that of human GF [1]. Of interest, both rat PLF and GF produced IL-1 β at similar levels after stimulation with various concentration of LPS. These results are not surprising since the ability of both PLF and GF to produce this cytokine have been demonstrated in human study [5], suggesting that an attempt to functionally differentiate rat PLF and GF based upon the levels of IL-1 β may be irrelevant. Furthermore, the levels of NO production in rat PLF

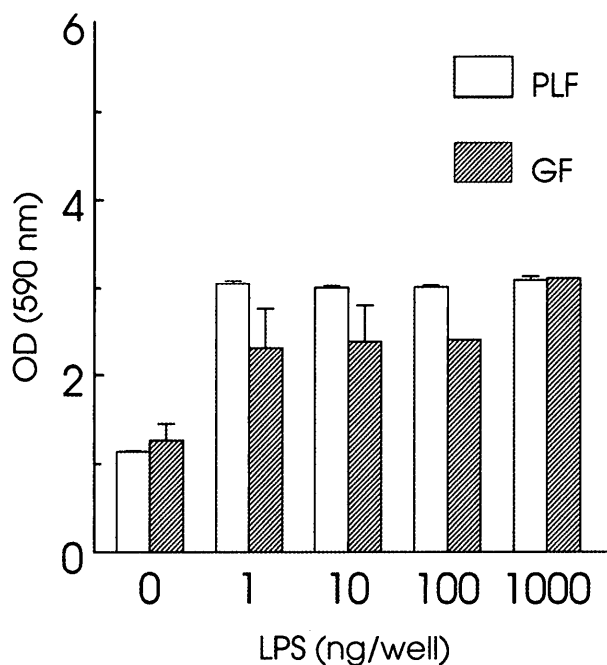


Fig. 1. The cell proliferation of lipopolysaccharide (LPS)-stimulated rat periodontal ligament fibroblasts (PLF) and gingival fibroblasts (GF). Both rat PLF and GF were stimulated with LPS from *Escherichia coli* for 72 h and the cell proliferation was determined by a colorimetric assay. Bar represents standard deviation.

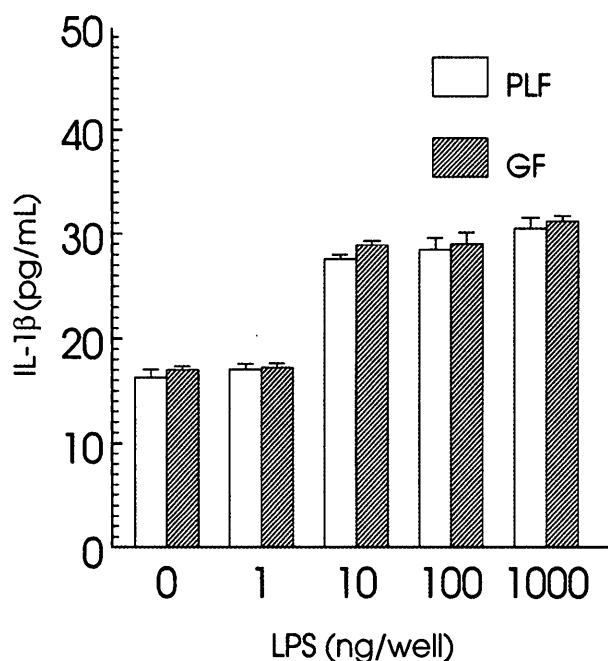


Fig. 2. The levels of interleukin (IL)-1 β production by lipopolysaccharide (LPS)-stimulated rat periodontal ligament fibroblasts (PLF) and gingival fibroblasts (GF). Both rat PLF and GF were stimulated with LPS from *Escherichia coli* for 72 h and the levels of IL-1 β detected from the culture supernatants were determined by enzyme-linked immunosorbent assay.

cultures were higher than those in rat GF cultures, particularly when stimulated with 1000 ng/mL of LPS. Similar results have also been documented that following mechanical forces, the production of NO by human PLF via the activities of neural NOS (nNOS) was higher than that by human GF [10]. No reports elucidating whether PLF express iNOS have yet been seen. Elevated expression of iNOS was observed in LPS-stimulated human GF [9]. No iNOS messenger RNA expression can be detected in human PLF [10]. Increased NO production by LPS-stimulated rat PLF and GF as seen in this study may indicate that both rat periodontal fibroblast subpopulations may also express iNOS, but the activity of this enzyme in rat PLF as compared with that in rat GF may be greater when stimulated with high concentration of LPS. To our knowledge, this is a first study to indicate that rat PLF are able to produce NO. A previous study indicated that the production of prostaglandin E₂ by LPS-stimulated human PLF was higher than that by LPS-stimulated human GF [14]. Indeed, this cytokine is capable to elevating NO production by murine macrophages stimulated with LPS from periodontopathogens [15]. We speculated that increased production of NO by rat

PLF as compared with that by rat GF seen in this study may be due to increased levels of prostaglandin E₂ in LPS-stimulated human PLF.

The pathophysiology of periodontal tissues *in vivo* explored in this study remains to be further investigated. Studies in human and animal models indicate that PLF and GF may be 2 distinct fibroblast phenotypes [1-4]. This study supports the view that rat GF and PLF may be distinct phenotypes as judged by different responses to LPS stimulation. Of interest, the levels of NO produced by rat PLF was lower than those produced by rat GF, suggesting that NO-produced PLF, in contrast with that produced by GF, may not participate primarily in the local immune defense to infectious agents. Several lines of evidence support the above notion that NO produced by PLF under catalization of endothelial and nNOS, but not iNOS, may play a crucial role on tissue repair and maintenance [10,16].

In conclusion, this study demonstrated that cell proliferation and NO but not IL-1 β production by LPS-stimulated rat PLF were higher than those by LPS-stimulated rat GF. Thus, these results support the view that rat PLF and GF may represent different periodontal fibroblast subpopulations.

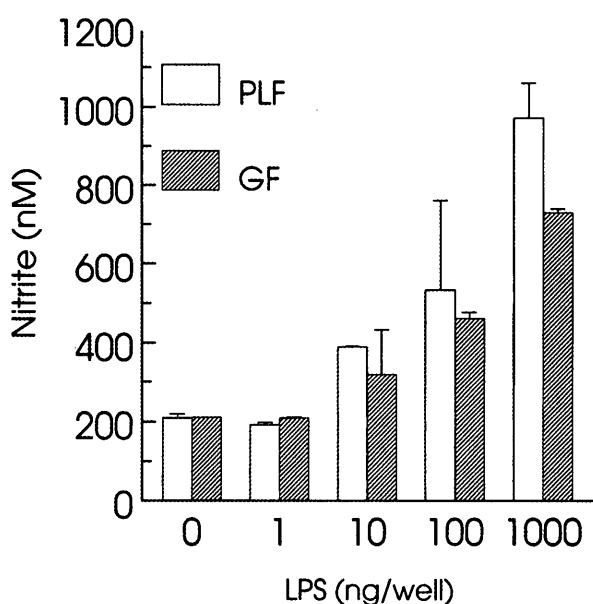


Fig. 3. The levels of nitric oxide production by lipopolysaccharide (LPS)-stimulated rat periodontal ligament fibroblasts (PLF) and gingival fibroblasts (GF). Both rat PLF and GF were stimulated with LPS from *Escherichia coli* for 72 h and the levels of nitrite detected from the culture supernatants were determined by the Griess reaction. Bar represents standard deviation.

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Errata

Correction: *J Microbiol Immunol Infect* 2002;35:68-70

Detection of Giardia cysts and Cryptosporidium oocysts in central Taiwan rivers by immunofluorescence assay

A production error occurred in this paper by Tai-Lee Hu. The header was inadvertently mistyped as "2002;34:68-70". It should read: 2002;35:67-70

Correction: *J Microbiol Immunol Infect* 2002;35:136-40

Expression of Epstein-Barr virus latent membrane protein 1 and B-cell leukemia-lymphoma 2 gene in nasopharyngeal carcinoma tissues

A production error occurred in this paper by John Jenn-Yenn Lu, Chi-Long Chen, Tsuey-Ying Hsu, Jen-Yang Chen, Ih-Jen Su, Winston CY Yu, and Czau-Siung Yang. The header was inadvertently mistyped as "Chu *et al.* Juvenile idiopathic arthritis". The header should read:

Lu *et al*

Expression of EBV LMP-1 and B-cell leukemia-lymphoma 2 gene in NPC tissue

Our apologies to the authors and readers.