Cytokine secretion in cultured human gingival fibroblasts in response to LPS or gingival overgrowth-inducing drugs

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Summary

Human normal, periodontitis and overgrown gingival fibroblasts were assayed concerning proinflammatory cytokines secreting ability. Stimulating effect of gingival overgrowth-inducing drugs such as phenytoin, nifedipine or cyclosporine A and LPS on the secretion ability of each fibroblast specimen was also estimated. The supernatant of cultured medium was assayed cytokines by ELISA techniques. IL-1B was not detected in any fibroblast specimen. Secretion of IL-6 and IL-8 were similar among the gingival specimens. Secretion of TGF-β was the most dominant among the cytokines in any gingival fibroblast. The largest amount of TGF-B was observed in overgrown gingival fibroblast. LPS stimulated the secretion of IL-6, IL-8 except of TGF-β. Phenytoin, nifedipine, cyclosporin A stimulated TGF-β secretion about 2 times of baseline in only normal gingival fibroblast. These results suggest that these drugs participate in the gingival overgrowth by secreting TGF-β.

Key Words: gingival fibroblast; cytokines; gingival overgrowth-inducing drugs.

Introduction

Proinflammatory cytokines, such as IL-6, IL-8 or IL-1|3 are thought to play the significant role on gingival inflammation process. Cytokines secretion capability and property from gingival fibroblasts depends on the pathological conditions of gingival [1] LPS from periodontitis relating bacteria or IL-1B stimulates the secretion of pro-inflammatory cytokines [2]. Gingival overgrowth induced by some drugs is one of the unwanted side effects. Long-term ingestion of some drugs, such as phenytoin [3, 4], nifedipine [5, 6], or cyclosporin [7-9] induces gingival overgrowth. The mechanisms involved in gingival overgrowth have not been clearly established. Williamson et al. [10] reported that messenger RNA of IL-6 expression was upregulated in overgrown gingival fibroblast induced by cyclosporin. Morton et al. [11] also observed that phenytoin, nifedipine and cyclosporin upregulated the secretion of IL-6 from gingival fibroblast.

TGF-β was significantly expressed in connective tissues of phenytoin- and nifedipine-induced gingival overgrowth [12] and cyclosporin-induced gingival overgrowth [13]. Thus, cytokines closely relates to pathogenesis of periodontitis or gingival overgrowth. The aim of the present study is to assay cytokines secreted from human gingival fibroblasts of normal, periodontitis and gingival overgrown. Furthermore, we estimated whether gingival overgrowth-inducing drugs would stimulate the cytokine secretion from gingival fibroblasts.

Materials and Methods

Gingival fibroblast (GF) specimens

Gingival tissues were excised from three clinically healthy donors, three adult periodontitis subjects having pocket depth > 4 mm and 1 epileptic patient with phenytoin-induced and two hypertensive patients having nifedipine-induced overgrown gingivae. The gingival specimens were placed into the plastic flask and primarily cultured at 37° in a humidified atmosphere of 5% CO2 and 95% air in Dulbecco's minimal essential medium (DMEM, Nissui, Tokyo) supplemented with 10% fetal calf serum until reached confluence. The cells were detached by 0.25% trypsin added 0.3% EDTA (Gibco BRL, N.Y.) and washed by PBS, resuspended in DMEM, and cultured in a flask. Fibroblasts cultured between

3rd to 8th passages were subjected to cytokine assay experiments.

Stimulation with gingival overgrowth-inducing drugs

Cell suspensions of 10^5/mL prepared by 1% MEM were seeded in 24-well plate, and incubated until the wells reached to confluence. The culture medium was replaced with 1 mL of fresh medium containing each gingival overgrowth-inducing drug, such as phenytoin (Tokyokasei, Tokyo), nifedipine (Wako-Pure-chemicals, Osaka), cyclosporine A (Sigma, St. Louis, MO) and *E. coli* LPS (Difco, Detroit, MI), and incubated for 24 h. The super-natant from each well was assayed cytokines.

Cytokine assay

ILl-B, IL-6, IL-8 (Endogen, Woburn, MA) and TGF-β (Amersham, Buckinghamshire) were assayed by ELISA techniques using ELISA kits.

Statistical analysis

Statistical analysis was performed with One-way analysis of valiance or Student's t-test for paired or unpaired samples in comparing the means. When each group had not equal variance, two samples were followed with Welch's correction.

Results

IL- l* was undetectable from any kind of GF. IL-6 and IL-8 were secreted less than 200 pg/mL from all kinds of GF. TGF-β secretion level was greater than the other cytokines. Especially, overgrown GF secreted the greatest level of TGF-β (about 1000 pg/mL). But statistical differences were not observed among kinds of GF or among the cytokines *(Figure 1).* LPS I greatly stimulated L-6 and IL-8 secretion about 10-times or more of basal levels, especially, it stimulated IL-6 in overgrown GF about 30-times of basal. On the other hand, TGF-β secretion was not stimulated by LPS in any GF *(Figure 2).* Phenytoin stimulated IL-6 secretion in periodontitis GF (about 3-times of basal) and TGF-β in normal GF (2-times of basal). None of cytokines secretion in overgrown GF was stimu-lated by Phenytoin *(Figure 3).* Nifedipine did not stimulate IL-6 and IL-8 secretion in normal GF. IL-6 from periodontitis GF and overgrown GF were slightly stimulated by nifedipine. Stimulation of TGF-β was slightly observed by nifedipine in only normal GF, but statistical significance was not observed *(Figure 4).* Cyclosporin A stimulated IL-6 secretion of 2.7 times of basal levels only from periodontal GF. TGF-β from normal GF was stimulated 2 times of basal level without statistical significance. None of cytokines secretion in overgrown GF was stimulated by cyclosporin A *(Figure 5).*

Discussion

IL-1B was not detected nor stimulated by all drugs tested in any gingival fibroblast specimen at all. And LPS stimulated the secretion of IL-6 and IL-8 except to IL-1B from all kinds of GF. This was agreed with the result of Reddi et al. [14] and Dongari et al. [1].

In contrast to our results, Agarwal et al. [2] reported that LPS originated from *A. a.* and *E. coli* greatly stimulated the secretion of IL-1* IL-6 and IL-8. According to Honig et al. [15], IL-1B might be secreted from neutrocytes • or lymph cells in inflammed tissues.

Anyway, LPS greatly participates in gingival inflammation process by stimulating IL-6 and IL-8 secretion. IL-6 and IL-8 were secreted from normal, periodontitis and overgrowth gingival fibroblast specimens, but there were no significant differences among the gingival specimens. This was different from the result of Dongari et al. [1]. They wrote that higher secretory capacity of fibroblast from periodontitis gingiva than that from healthy gingiva was due to the elevated proportion of subpopulations than healthy gingiva. We could not clarity the reason in this study.

Secretion of TGF-β was the most dominant among the cytokines in any gingival fibroblast. Especially, the largest amount of TGF-β was observed in overgrown gingival fibroblast. All drugs used in this experiment stimulated TGF-β secretion about twice of baseline from normal gingival, not from periodontitis nor overgrown gingival fibroblasts. TGF-β is known to upregulate collagen synthesis and to result in generating tissue fibrosis [16, 17]. This result suggests that these drugs participate in enlarging gingival tissues by secreting TGF-β following by producing the extracellular matrices. The great secretion of inflammatory cytokine such as IL-6 and IL-8 by LPS was observed from all types of gingiva, suggesting LPS participated in the pathogenesis of gingival inflammatory process.

Figure 1. Cytokine secretion levels in cultured medium from human gingival fibroblasts (GF) of normal (n = 3), periodontitis (n = 6) and overgrowth (n = 4)

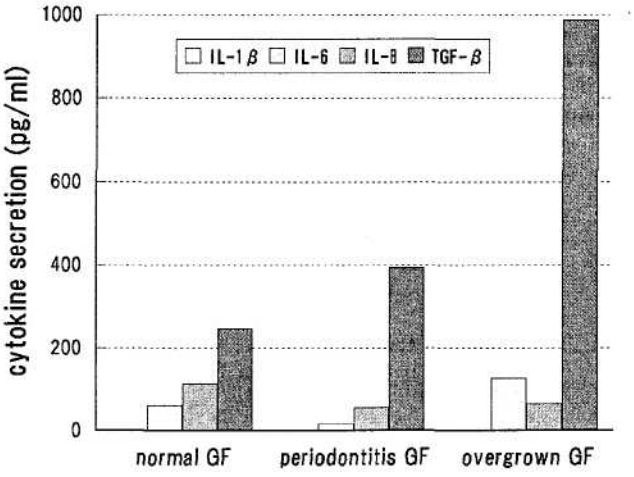


Figure 2. Effect of LPS on cytokine secretion from normal, periodontitis and overgrown GF. Results are expressed as the times of basal levels (non-stimulated level). Each cytokine was compared the mean to basal level. * $p<0.05$.

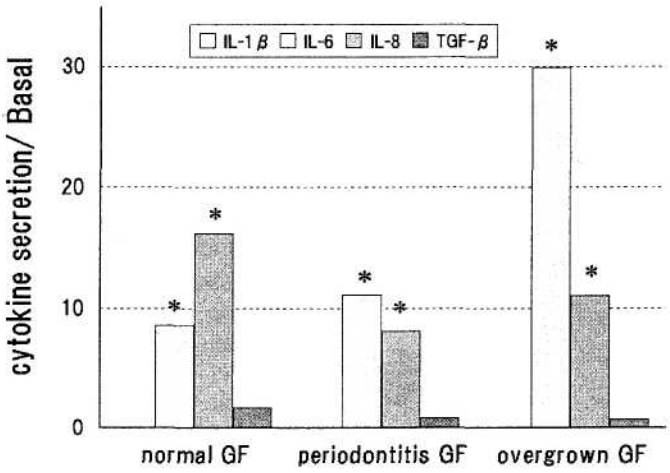


Figure 3. Effect of phenytoin on cytokine secretion from normal, periodontitis and overgrown GF. Results are expressed as the times of basal (non-stimulated level)

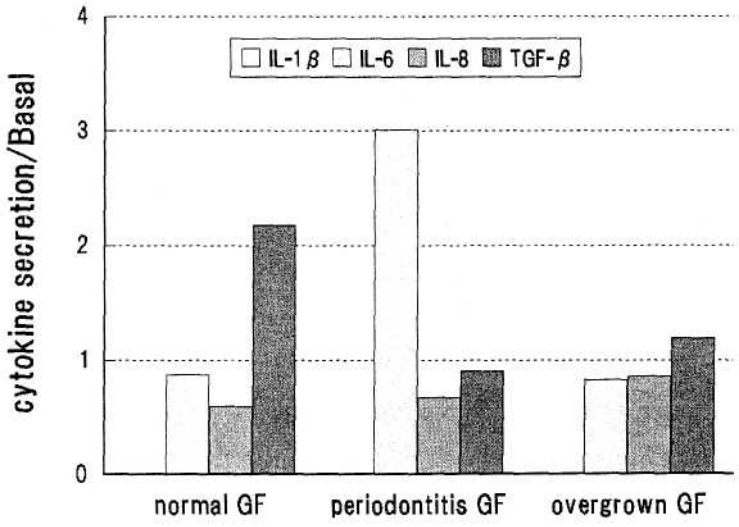


Figure 4. Effect of nifedipine on cytokine secretion from normal, periodontitis and overgrown GF. Results are expressed as the times of basal (non-stimulated level)

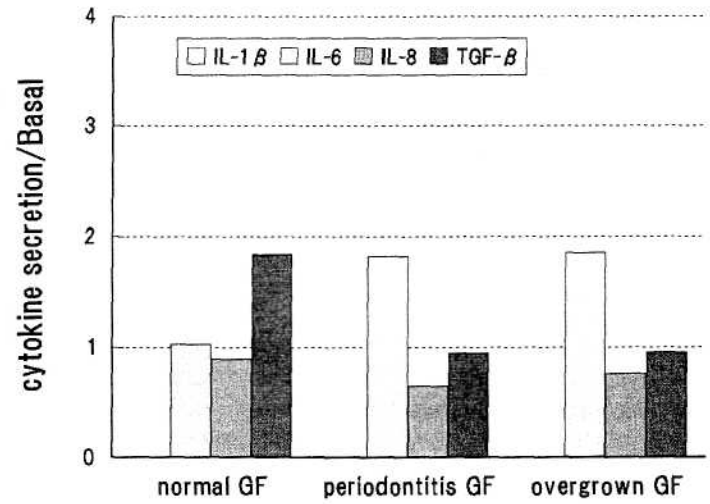
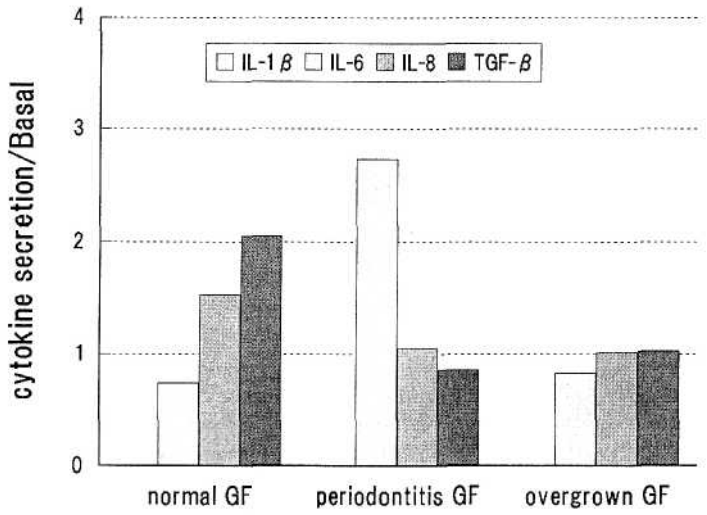


Figure 5. Effect of cyclosporin A on cytokine secretion from normal, periodontitis and overgrown GF. Results are expressed as the times of basal (non-stimulated level)



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