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CORRELATION OF INTERLEUKIN-1 β, INTERLEUKIN-6, AND PERIODONTITIS

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In order to understand the role of IL-1β and IL-6 in the periodontal tissue destruction coincident to periodontitis, we assessed the levels of these two mediators in both the gingival tissue and the serum of patients with periodontal disease and of periodontally healthy subjects. In addition, production of IL-6 by six healthy human gingival fibroblast (HGF) strains in response to IL-1β was also investigated. The levels of IL-1 β and IL-6 in gingival tissues and in serum were examined by ELISA. Both mediators were observed to increase in diseased tissues of patients with adult periodontitis, and there was a positively significant relationship between both mediators and clinical assessments of periodontal destruction. Moreover, a significant correlation was also noted between levels of IL-1β and IL-6 in gingival tissues of periodontitis patients ($r = 0.4334$, $p < 0.01$). However, there was no significant difference in the serum levels of IL-1β and IL-6 between periodontitis patients and periodontally healthy controls. In fibroblast cultures, confluent monolayers of HGF were incubated with recombinant human IL-1β for 48 h at 37 °C in 5% CO_2 and air. At the end of the culture period, supernatants were collected and assayed for IL-6 activity by inducing proliferation in the IL-6-dependent hybridoma cell line 7TD1. A dose-dependent stimulatory effect of IL-1β on IL-6 production by HGF was noted, wherein 3 strains exhibited higher IL-6 activity than the other 3. These data indicate that the levels of IL-1β and IL-6 in gingival tissues are closely related to the severity of periodontal disease and that the IL-1β and IL-6 produced in gingival tissues may not reflect these two mediators levels in serum. Moreover, IL-1 β responsiveness of HGF in IL-6 production depends on both the concentration of IL-1 β and cells of individual subjects. Since HGF are present in periodontal lesion, it is possible that IL-6 secretion stimulated by exposure to inflammatory cell products such as IL-1β may participate in the destruction of periodontal tissue in periodontitis.

Key words: interleukin-1β, interleukin-6, periodontitis

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Interleukin-1 β (IL-1 β) is a pro-inflammatory cytokine which has extensive biological activities, such as the induction of lymphocyte proliferation, chemotaxis of macrophages, regulation of cell metabolism of connective tissue, stimulation of prostaglandin and collagenase release from fibroblasts, and stimulation of bone resorption[1-3]. Recently, a role for IL-1β in the pathogenesis of the periodontal disease has been explored. The level of IL-1 β was found to be elevated in gingival tissues from patients with periodontitis as compared to clinically healthy subjects[4,5]. A similar result was observed from active periodontitis sites versus inflamed stable sites[6]. Also, it has been observed to be increased in human gingival crevicular fluid (GCF) from the inflamed sites but decreased after periodontal treatment[7-9].

Interleukin-6 (IL-6) is also a pleiotropic cytokine that stimulates B cell differentiation

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and T cell activation as well as hepatocyte production of acute phase proteins[10]. In addition, it can induce bone resorption, both by itself and in conjunction with other bone-resorbing agents[3,11]. Spontaneous production of IL-6 has been reported in mononuclear cells isolated from inflamed gingival tissues of patients with periodontitis[12]. The presence of IL-6 bearing cells in inflamed gingival tissue also has been demonstrated by other researches[13,14]. In a longitudinal study, IL-6 content in GCF was found to be correlated with the severity of periodontal disease[15]. On the contrary, Guillot *et al.*[16] revealed that, following Phase I periodontal therapy, IL-6 levels in GCF were significantly greater at resolved than at unresolved gingival sites; however, IL-6 tissue levels were significantly greater at unresolved than in resolved gingival sites. Thus, IL-6 accumulation within gingival connective tissue may be a factor in failure of sites of periodontal disease to resolve following conservative therapy.

So far, few studies have attempted to correlate gingival tissue IL-6 with clinical periodontal parameters, and to examine IL-6 production locally and systemically in order to investigate the possible role of IL-6 in the pathogenesis of periodontal disease.

Recent studies have demonstrated that IL-1, tumor necrosis factor-α (TNF-α) and bacterial lipopolysaccharide (LPS) can activate in human gingival fibroblasts (HGF), which are the predominant cells in gingiva, to release IL-6[10,13,17,18]. IL-6 mRNA and protein have been observed to be increased in inflamed gingival tissues, which notably included fibroblasts[19]. Thus, HGF can be included in the cytokine network, and IL-6 derived from HGF may play an important role in the mediation of inflammatory and immune responses. However, there is little information regarding the regulation and the donor variability in IL-6 production by HGF treated with IL-1β.

The present study was designed to quantify the levels of IL-1 β and IL-6 in the gingival tissues and serum obtained from patients with adult periodontitis. In addition, we also examined IL-6 production by six primary cultures of HGF following stimulation with IL-1β.

MATERIALS AND METHODS

Patients and biopsies

This study consisted of 48 patients including 33 with adult periodontitis (20 males and 13 females, ages 33 to 50 years, mean age 41.5 years) and 15 periodontally healthy controls (9 males and 6 females, ages 22 to 49 years, mean age 38.7 years). All subjects were systemically healthy and had not received either periodontal therapy or antibiotics in the 3-month period prior to the study. Before treatment for periodontitis patients, replicate probing depth (PD) and attachment level (AL) measurements were obtained from 6 sites per tooth[20] .The sites were classified as diseased on the basis of the following criteria: PD and AL ≧ 4mm, bleeding on probing, and radiographic evidence of more than 30% bone loss (BL) using the technique of Schei *et al.*[21]. Diseased gingival tissues were obtained at the time of periodontal surgery approximately four weeks after initial therapy consisting of scaling, root planing and oral hygiene instruction. Most of the sampling sites still exhibited bleeding on gentle probing. Prior to surgery, PD and AL were re-recorded at the sampling sites, and the values were taken as a basis for the analysis. Fifteen controls without gingival inflammation required crown lengthening procedures (CLP) for crown restoration and served as a source of healthy tissue. All healthy sites had probing depth less than 3 mm and no bleeding was observed on probing. The informed consent of each patient to use tissue which would otherwise be discarded was obtained at the time of surgery. A written explanation of the purpose of the study was provided for each subject and signed consent was obtained.

Tissue sample collection and extract preparation

Fifteen tissue samples from the periodontally healthy subjects were obtained during CLP. Thirty-three tissue samples were also obtained from the diseased sites of periodontitis patients while carrying out the periodontal surgery. Additionally, 8 non-diseased gingival tissues from the periodontitis patients were also taken. In this study, the periodontal conditions of non-diseased sites were observed to fulfil the criteria of periodontally healthy sites. However, since the patients in this study had extensive disease, completely healthy sites were rare and were often found to be located outside the area of surgery. Moreover, many of the healthy sites

sampled during surgery yielded tissue which was insufficient for analysis. Thus, tissue samples from only 8 healthy sites were available for analysis. All the tissue samples were removed and processed individually. Tissues were rinsed in RPMI 1640 medium containing gentamycin (50 μ g/ml), freed of clots and debris, blotted, weighed, and immediately frozen and stored in liquid N2.

For extract preparation, the tissues were finely minced with a scalpel and the resulting fragments were homogenized in RPMI 1640 by using a homogenizer. The homogenate was then placed on ice and was subjected to 60 seconds sonication to further disaggregate the tissues. This procedure caused complete tissue dispersion and cell lysis, and liberated cytokines from both inter- and intracellular compartments. After centrifugation, the supernatant was collected. The protein concentration of the supernatants was determined (Bio-Rad Laboratories, Inc., California, USA) by measuring the absorbance at 595 nm. The supernatants were analysed for IL-1 β and IL-6 content by using ELISA.

Sera

We also took the serum samples from 10 periodontitis patients and 10 periodontally healthy subjects at the time of the periodontal surgery. Immediately prior to surgery, a 5 ml sample of intravenous blood was drawn with a vacutainer from the antecubital fossa of each patient. Blood was centriguged at 400 x g for 10 min. and serum was separated. All serum samples were stored at-70°C for later determination of IL-1β and IL-6 levels.

Immunoassay of IL-1β and IL-6 for tissue supernatants and sera

Assays for IL-1 β and IL-6 were carried out according to the manufacturer's instructions (Amersham International, Buckinghamshire, England) and optical densities were measured at 450 nm using a spectrophotometer (SLT Labinstruments, Grodig, Austria). The lower limit of detection in the IL-1 β assay was 0.3 pg/ml, and that in the IL-6 assay was 0.7 pg/ml. All samples were tested in duplicate, and the amount of cytokine present in each sample was determined by comparison with a standard curve constructed for each assay. The IL-1 β and IL-6 contents were expressed as pg/mg protein in tissue samples, and pg/ml in sera.

Fibroblast cultures

Fibroblasts derived from healthy gingiva were obtained during CLP of six periodontally healthy subjects. Each tissue explant was incubated in a 60 mm petri-dish in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 μg streptomycin/ml, 100 units penicillin/ml, and 50 μ g/ml gentamycin at 37 °C in humidified air containing 5% CO_2. The cultures were incubated until cells migrating from pieces had formed a confluent monolayer. The monolayers were passaged by treatment with trypsin, and cells in passage 4, 5 and 6 were used separately for these experiments.

Conditioned culture medium to be tested for IL-6 activity was obtained by plating the cells in triplicate into 24-well plates for each experiment at an initial density of 50,000 cells per well. The cells were allowed to attach and to reach confluence in an incubator for approximately 4 days at 37 °C in a 5% CO_2 atmosphere. After cultivation, the medium was removed and replaced with fresh DMEM containing 10% FCS and recombinant human IL-1 β (rIL-1 β) (Boehringer Mannheim Biochemica, Germany) at a final concentration of 0.05, 0.5, 5, 50 or 500 units/ml (rIL-1 β : 0 unit/ml as a control). The cells were further cultured for 48 h. The culture supernatants (conditioned medium) were harvested after being centrifuged at 800 x g for 10 min, and immediately frozen at-70 °C until assayed.

Bioassay of IL-6 for culture supernatants

This assay is based on the ability of IL-6 to induce proliferation in the IL-6-dependent hybridoma cell line 7TDl[22]. Briefly, 50μl supernatant was added to 2 x 10^3 7TDl cells in 50 μl RPMI 1640 supplemented with 10% FCS. Each control and sample in the microwell plates comprised 6 replicate wells. After 3 days, the proliferation of cells was evaluated by the MTT dye (Boehringer Mannheim Biochemica, Germany) technique using an ELISA plate reader[23]. Results were expressed as IL-6 units/ml by inclusion of a standard curve of recombinant human IL-6 (rIL-6) (Boehringer Mannheim Biochemica, Germany) in each assay. The sensitivity of 7TDl cells to IL-1β was determined by adding rIL-1 β in various concentra-

tions (0.05 to 500 units/ml) to serial dilutions of rIL-6 (1000 to 0.001 units/ml) and testing these in the 7TDl assay. We found that rIL-1β had no effect on the 7TDl assay irrespective of the extent of dilution of rIL-6.

Neutralization of IL-6 activity by anti-IL-6 antibody

To identify the IL-6 activity, serial dilutions of HGF culture supernatant (incubated with 50 units/ml rIL-1β) were incubated with and without anti-IL-6 antibody (0.1 μg/ml) (Boehringer Mannheim Biochemica, Germany) at 4 °C overnight. 7TDl cells were then added and cultured as described above.

Statistical analysis

Analysis of the data was performed by using the statistical package SPSS (SPSS Inc., Microsoft Corp., Chicago, USA). The Mann-Whitney U-test was used to find the differences in IL-1β and IL-6 levels. The correlation among the levels of cytokines and clinical periodontal measurements were assessed by using the Pearson product moment correlation coefficient. The relationship between IL-1β and IL-6 was examined by linear regression analysis.

RESULTS

Levels of IL-1β and IL-6 were measured in extracts of gingival tissues obtained from 33 diseased and 8 non-diseased sites of individuals with periodontitis as well as from 15 sites of individuals without periodontitis. Using ELISA, all the tissue samples were found to contain IL-1β and IL-6. From Table 1 we can observe that the IL-1β level in the diseased sites of the periodontitis group was significantly greater than both that in the non-diseased sites of the periodontitis group ($p < 0.05$) and that in the healthy gingiva group ($p < 0.05$).

It can be noted from Table 2 that the tissue level of IL-6 in the diseased sites of the periodontitis group was higher than both that in the non-diseased sites of the periodontitis group and that in the healthy gingiva group. However, a significant difference in IL-6 levels was only noted between the diseased sites of the periodontitis group and the healthy gingiva group ($p < 0.05$).

When the data on IL-1β and IL-6 levels of all tissue samples were pooled for analysis, a significant relationship between the levels of IL-1β and IL-6 was noted. The observed relationship can be expressed as IL-6 level = -0.89103 +0.16274 x IL-1β level ($r=0.4334$, $p < 0.01$, Fig. 1).

The correlation of IL-1β or IL-6 levels and the clinical periodontal assessments in the sampling sties of the periodontitis group is given is Table 3. The IL-1β level was found to be positively correlated with PD, AL and BL ($P<0.001$).

Table 1. Interleukin-1β (IL-1β) levels in tissues of adult periodontitis and healthy gingiva groups

Site designation	No. of samples	IL-1β concentration (pg/mg protein)
Periodontitis group		
Diseased sites	33	166.96 ± 62.33* †‡
Non-diseased sites	8	86.81 ± 38.92
Healthy gingiva group		
Healthy sites	15	57.70 ± 33.82

* Mean ± standard deviation.
† $p<0.05$, comparison with healthy gingiva group.
‡ $p<0.05$, comparison with non-diseased sites of periodontitis group.
Statistical analysis: Mann-Whitney U test.

Table 2. Interleukin-6 (IL-6) levels in tissues of adult periodontitis and healthy gingiva groups

Site designation	No. of samples	IL-6 concentration (pg/mg protein)
Periodontitis group		
Diseased sites	33	27.97 ±32.55* †
Non-diseased sites	8	10.78 ±7.60
Healthy gingiva group		
Healthy sites	15	6.10 ± 7.11

* Mean ± standard deviation.
† $p<0.05$, comparison with healthy gingiva group by Mann-Whitney U test.

The level of IL-6 also had a positive correlation with AL and BL ($P < 0.05$), but had only a borderline correlation with PD ($r=0.3041$, $p=0.053$).

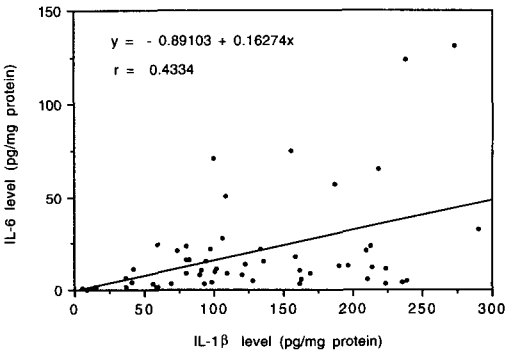

Fig. 1. Regression analysis of IL-1 β and IL-6 levels in tissue samples obtained from periodontitis patients and healthy gingiva individuals. Each point represents a single tissue sample. The correlation between IL-1 β and IL-6 was found to be statistically significant ($p < 0.01$).

Table 3. The correlation between the levels of cytokines(IL-1 β , IL-6) and clinical periodontal assessments (PD, AL, BL)* in all the tissue samples of periodontitis group

Cytokine	PD	AL	BL
IL-1β	0.7531 † ‡	0.6972 ‡	0.7978 ‡
IL-6	0.3041	0.3764 §	0.3498 §

*PD: probing depth. AL: attachment level. BL: bone loss.
† Pearson correlation coefficient (r)
‡ $p<0.001$
§ <0.05

In Table 4, the levels of serum IL-1 β and IL-6 of each subjects, as well as means with standard deviations are presented. IL-1 β was measurable in 5 of 10 periodontitis patients and in 6 of 10 periodontally healthy control subjects, with no statistically significant difference between these 2 groups. However, IL-6 was measurable in all periodontitis and control subjects. No statistically significant difference was noted between these 2 groups, either.

The effect of various concentrations of rIL-1 β on IL-6 production of six HGF strains are shown in Fig. 2. Upon the addition of rIL-1 β into DMEM containing 10% FCS, the IL-6 production was found to be stimulated greatly for all the strains studied. However, HGF-1, 3,5 seemed to exhibit higher IL-6 activity as compared to the other three strains. The IL-6 activity also increased linearly in all HGF strains with the addition of 50 units/ml rIL-1 β. Moreover, when the concentration of rIL-1 β was increased to 500 units/ml, and increase in the IL-6 activity of HGF-1,3 was noted, but the activity in the other four strains was, however, negligible. Control experiments using unconditioned media containing identical concentrations of rIL-1 β did not show any detectable IL-6 activity.

Table 4. IL-1 β and IL-6 levels in the serum of periodontitis patients and periodontally healthy subjects

case	Periodontally healthy subjects		Periodontitis patients	
	IL-1β*	IL-6*	IL-1β	IL-6
1	ND †	12.98	1.58	5.76
2	ND	9.75	0.30	3.16
3	0.33	4.80	0.65	8.70
4	0.47	16.88	ND	4.86
5	1.45	1.68	ND	10.06
6	2.11	6.45	ND	9.67
7	0.87	5.02	ND	18.56
8	ND	8.06	ND	2.11
9	3.48	5.53	2.67	6.71
10	ND	2.59	4.05	12.40
	0.87 ±1.16‡	7.37 ±4.71	0.93 ±1.41	8.20 ±4.86

*Concentration: pg/ml
† ND: not detectable
‡ Mean ± standard deviation

In order to ascertain that the activity under investigation was indeed from IL-6, the culture supernatants were incubated with anti-IL-6 antibody prior to performing the IL-6 bioassay. Anti-IL-6 antibody at a final concentration of 0.1μg/ml reduced the IL-6 activity of HGF supernatants stimulated with 50 units/ml rIL-1 β by more than 96% (Fig. 3).

DISCUSSION

The present study showed a significant difference in the levels of IL-1 β and IL-6 between diseased gingival tissues of patients

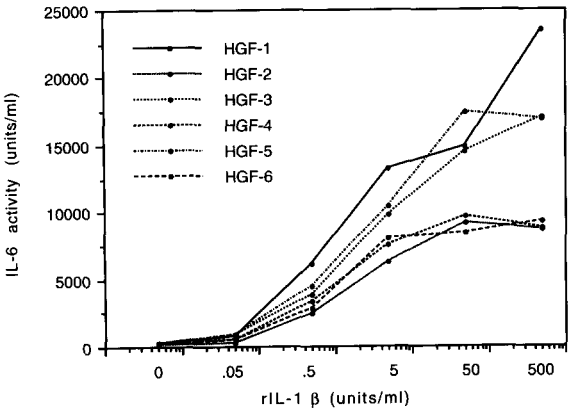

Fig. 2. The effect of rIL-1 β on IL-6 production as demonstrated by six cases of healthy gingival fibroblasts (HGF). The cells were incubated for 48 h with rIL-1 β at various concentrations (0.05, 0.5, 5, 50 and 500 units/ml). The supernatants were then harvested and IL-6 activity was determined by Bioassay as described in the Materials and Methods. Results are expressed as mean values of triplicate determinations with six replicate assays per well. SD < 10% of the mean. The results are representative of three separate experiments.

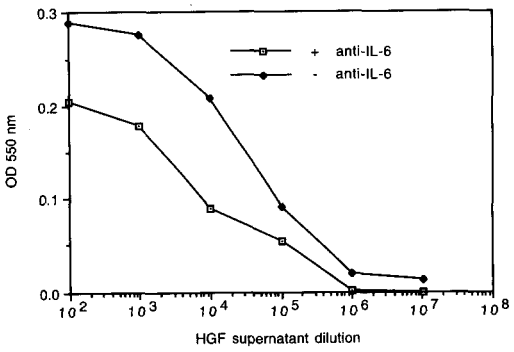

Fig. 3. Inhibition of IL-6 activity with anti-IL-6 antibody. Serial dilutions of healthy gingival fibroblast (HGF) culture supernatant (incubated with 50 units/ml rIL-1 β) reacted overnight with anti-IL-6 antibody (0.1 μ g/ml) at 4 °C. The supernatants were then tested for inducing proliferation in the IL-6-dependent hybridoma cell line 7TD1, which were evaluated by the MTT dye technique.

with periodontitis and healthy gingival tissues of controls. A significant difference was also observed in IL-1 β levels between diseased and non-diseased tissues of patients with periodontitis. However, there was no significant difference in the serum levels of IL-1 β and IL-6 between periodontitis patients and healthy controls. These data suggest that both IL-1 β and IL-6 levels are highly correlated with the locally inflammatory conditions of the periodontium and that the differences in serum and gingival IL-1 β or IL-6 levels were due to local cytokine synthesis within the gingiva. The large numbers of inflammatory cells in the connective tissues and connective tissue cells per se (i. e., fibroblasts and endothelial cells) can lead to the release of IL-1 β and IL-6, stimulated by bacterial products and by interaction with the host cells[1,3,10]. Several researchers have reported the increased IL-1 β level in gingival tissues in periodontitis patients[4-6]. Bartold *et al.*[13] observed more intense IL-6 staining in the section of inflamed human gingiva than in the healthy gingival tissue. Furthermore, Matsuki *et al.*[14] have indicated that there were prominent IL-1 β and IL-6 mRNA-expressing cells in the inflamed gingival tissue. These reports support the fact that the IL-1 β and IL-6 levels are higher in diseased tissue than in healthy tissue as observed in the present study.

It was not surprising that IL-1 β and IL-6 could also be detected in healthy gingival tissues. It is a well established fact that small numbers of macrophages and mononuclear cells are usually present in clinically healthy gingival tissues[24]. All these cells and resident fibroblasts, endothelial cells, etc. could synthesize and release IL-1 β and IL-6. Jandinski *et al.*[25] demonstrated by the immunofluorescent technique that IL-1 β positive staining cells were also present in normal gingival tissues, but are much less than in the inflamed tissues.

The results show that the tissue level of IL-1 β in the periodontitis group is positively correlated with the PD, AL and BL (r=0.7531, 0.6972, 0.7978; $p < 0.001$). These data suggest that a significant relationship exists between the amount of a bone resorptive cytokine (IL-1 β) and the destruction of periodontal connective tissues. The present results are in general agreement with those of Stashenko *et al.*[6], who reported that IL-1 β levels were higher in sites with greater pocket depth or attachment level. The present study also observed that the IL-6 level was positively correlated with AL and BL (r=0.3764, 0.3498; $p < 0.05$). Borderline correlation between PD and IL-6 level was also noted. Thus the presence of IL-1 β and IL-6 in the gingival tissues along with the significant correlation

with clinical assessments of periodontal tissue destruction strongly suggests an important role for the two mediators in the pathogenesis of periodontal disease.

Our statistical analysis also revealed that the tissue levels of IL-1 β and IL-6 were positively correlated. This finding suggests that coordinate expressions of IL-1 β and IL-6 exist, and the tissue levels IL-6 may also be influenced by local IL-1 β activities. In Fig. 2, we demonstrated that IL-1 β was a stimulator for IL-6 production by HGF. However, other factors, e. g. LPS, TNF-α, colony stimulating factors, etc. may also be responsible for the synthesis of IL-6[10,13,17,18]. Considered together from the clinical and in vitro findings of the present study, we may infer that, it is likely that IL-1 β, produced primarily by macrophages in inflamed gingiva[14], may lead to the activation of HGF for the production of increased level of IL-6 and that both IL-1β and IL-6 are capable of inducing alveolar bone resorption and soft tissue destruction[1-3,10,11].

It is noted in the present study that the stimulatory effect of IL-1β on the IL-6 production by HGF varies directly in a dose-dependent manner. Although the results obtained from heterogeneous cultures may not reflect the true nature of the effect of IL-1β, it may be an average of the overall IL-6 production by HGF of three separate experiments after stimulated with IL-1β. This is in agreement with various other studies which have also indicated that such a mediator stimulates the synthesis of IL-6 in a wide variety of cells[10,13,18,26]. We also confirmed here that the release of IL-6 from each cell of HGF strain, under identical culturing conditions, had somewhat individual variability. This implies that individual cell responses to IL-1 β are different, suggesting a possible relationship between sensitivity to IL-1 β and relative risk of periodontal disease[27]. However, it is more likely that populations of HGF consist of heterogeneous phenotypes[28]. In addition, if our finding reflects a natural difference among these HGF individuals, it leads us to speculate that either HGF-1, 3, 5 may have a larger number of IL-1β receptors or receptors of higher affinity than the other 3 strains, or that the expression and stability of IL-6 mRNA are different among these cells. Thus, fibroblasts from different subjects do not respond similarly to IL-1β, suggesting that individual variability is important in recognition of IL-1β.

In conclusion, the present study demonstrates that the levels of IL-1β and IL-6 in gingival tissues are closely related to the severity of periodontal disease. Also, there are coordinated expressions of these two mediators in the disease process. However, the IL-1β and IL-6 produced in gingival tissues may not reflect these two mediators levels in serum. Moreover, IL-1 β-treated HGF are capable of secreting considerable amounts of IL-6 in vitro, and may therefore contribute to the elevated levels of IL-6 identified in diseased periodontal tissues in vivo. Collectively, IL-1β and IL-6 play a critical role in the pathogenesis of periodontal disease.

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介白質1β、介白質6與牙周炎之關係

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本研究之目的，第一部分：在比較牙周炎患者與牙周健康者之牙齦組織與血清中介白質1β(IL-1β)和介白質6(IL-6)之濃度，並探討牙齦組織中此兩種介質濃度與牙周臨床參數之關係，以便實際瞭解IL-1β與IL-6在發炎組織中所占的角色。第二部分：以體外實驗，探討IL-1β刺激健康牙齦造纖維細胞產生IL-6之程度，藉以瞭解二者之互動關係。第一部分實驗取材包括：(1)成年型牙周炎病患組，33個病變組織與8個健康組織標本，以及10個病患之血清標本；(2)牙周健康對照組，15個正常組織和10個健康者之血清標本。所獲得之組織與血液經處理與離心後，以酵素免疫分析法測定細胞激素濃度。結果顯示：(1)IL-1β與IL-6在牙周炎病患組之病變組織中，有顯著增加，(2)IL-1β與IL-6和牙周臨床參數（囊袋探測深度、附連高度、齒槽骨吸收高度）呈有意義之正相關，(3)在牙周炎病患組之組織中，IL-1β與IL-6濃度呈有意義之正相關（$r = 0.4334, P < 0.01$），(4)牙周炎病患組與牙周健康對照組之間，血清中IL-1β與IL-6濃度沒有統計上之差異。第二部分實驗用造纖維細胞，分別培養自6位牙周健康者之牙齦組織。實驗時，細胞培養於24孔培養皿中，達融合時加入濃度分別爲0.05、0.5、5、50、500單位／毫升之重組人類介白質1β(rhIL-1β)，作用48小時後，取上層培養液經離心、過濾，然後利用促進IL-6-dependent hybridoma cell line 7TD1增殖能力之生物分析法來測量IL-6活性。結果顯示：(1)6株造纖維細胞分泌IL-6濃度，在添加rhIL-1β後均比未添加rhIL-1β時（對照組）呈有意義增高，且均隨rhIL-1β濃度增加而增加，(2)各細胞株之間，對rhIL-1β感受性互有差異，其中3株細胞顯示具有較高分泌IL-6之能力。因此，由本研究可得以下結論：牙齦組織中IL-1β與IL-6濃度多寡和牙周炎之嚴重程度有密切關連，但牙周炎患者血清中IL-1β與IL-6濃度並沒有增加的現象；另外，IL-1β刺激牙齦造纖維細胞產生IL-6之程度，除了與IL-1β之濃度有關係外，也會因細胞來源個體之不同而有所差異。因此，可以推測，在牙周疾病的過程中，發炎細胞產生之IL-1β可能會刺激牙齦造纖維細胞產生大量IL-6，進而有助於牙周組織之破壞。

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