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Effects of scaling and root planing on the amounts of interleukin-1 and interleukin-1 receptor antagonist and the mRNA expression of interleukin-1β in gingival crevicular fluid and gingival tissues

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Objective: The purpose of this study was to evaluate the relationship between the clinical changes after non-surgical periodontal therapy and interleukin 1 (IL-1) in gingival crevicular fluid (GCF) and gingival tissues from patients with chronic periodontitis.

Background: The inflammatory responses mediated by IL-1 play an important role in periodontal tissue destruction. Although numerous studies have attempted to elucidate the dynamic movement involved in chronic periodontitis, the results have often conflicted. Such discrepancies may have been due to the inability to determine clinical disease activity.

Methods: Seven patients with chronic periodontitis were examined. The severity of periodontal inflammation was expressed using clinical parameters before and after a scaling and root planing (SRP) procedure. The amounts and concentrations of IL-1α, IL-1β and IL-1 receptor antagonist in GCF were measured by enzyme-linked immunosorbent assay (ELISA) and IL-1 activity index was calculated. A needle biopsy in matching gingival tissues was also performed before and after the SRP procedure. The localization and mRNA expression of IL-1β were determined using histological methods.

Results: Clinical parameters improved slightly after the SRP procedure. Only the probing pocket depth (PPD) was reduced significantly ($p < 0.05$). However, the amount of IL-1β in GCF was slightly increased. The localization and mRNA

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expression of IL-1β could still be observed after the SRP procedure. Therefore, none of the clinical parameters showed a high sensitivity or specificity for evaluating subgingival inflammation.

Conclusion: These observations suggest that IL-1 is effective for evaluating in detail the state of subgingival inflammation.

Interleukin-1 (IL-1) is a polypeptide with a wide variety of activities and roles in tissue homeostasis, inflammation, immunity and tissue breakdown (1–5). Following activation, it is synthesized by various cell types, including monocyte, macrophage, T lymphocyte, fibroblast, vascular cell, brain cell, and skin cell. IL-1 is known to stimulate the proliferation of fibroblasts, keratinocytes, and endothelial cells, and to enhance fibroblast synthesis of type I procollagen, hyaluronate, and fibronectin. Therefore, IL-1 is a critical component in the homeostasis of periodontal tissues (5). However, unrestricted production of IL-1 may lead to tissue damage. IL-1 also stimulates gingival fibroblasts to produce collagenase and prostaglandin E_2 *in vitro* (6), which are thought to participate in the destruction of periodontal tissue. Furthermore, IL-1, as a potent inducer of bone demineralization, causes bone resorption *in vitro* and may also synergize with other cytokines such as tumor necrosis factor to stimulate bone resorption (7–9). Thus, IL-1 has been suggested to play a key role in the pathogenesis of periodontitis.

There are two subtypes of IL-1, designated IL-1α and IL-1β, produced mainly by monocytes and macrophages but also by other types of cells (10). Although IL-1α and IL-1β share only 27% homology at the amino acid level (11), they have similar biological functions. These two forms of interleukin bind to the same receptor, which is found on many cell types in various densities (12). In addition to the agonist molecules IL-1α and IL-1β, a third member of the IL-1 group was purified and designated IL-1 receptor antagonist (IL-1ra) (13). The isolation, cloning and characterization of a cDNA for a human IL-1ra that blocks the binding of IL-1 to its receptors have been reported (14). Many studies have demonstrated that IL-1ra contributes to the tight control over the production and activity of IL-1 (15, 16). Thus, IL-1 and IL-1ra may play important roles in regulating the pathogenesis of periodontitis.

We previously reported that both IL-1 activity in gingival crevicular fluid (GCF) and IL-1 activity index which we set up uniquely were closely associated with the severity of periodontal disease as classified by alveolar bone resorption (17). Many reports have indicated that IL-1 activity was higher in GCF obtained from inflamed gingival sites than in that from inflammation-free sites (17–26), and several reports have shown a relationship among the effect of periodontal therapy, IL-1 activity in GCF and the localization of IL-1 in inflamed gingival tissue (21, 23, 27–30). However, conflicting results have been reported regarding the effect of periodontal treatment on IL-1 levels.

One possible explanation for these conflicting results may be the dilution effects of increased GCF volumes in inflammation that may confound measures of IL-1α, β or IL-1ra. In this study, we add new insights into resolving this issue by presenting not only the amounts and concentrations of IL-1α, β and IL-1ra in GCF, but also a novel gingival needle biopsy and the quantitations of IL-1β by using immunohistochemical and *in situ* hybridization methods.

Therefore, the aim of the present study was to explore the precise relationship between the clinical changes after non-surgical periodontal therapy and IL-1 in GCF and gingival tissues from the same chronic periodontitis patients.

Material and methods

Patient selection

To evaluate the changes in clinical parameters between before and after a scaling and root planing (SRP) procedure, as well as the changes in the amount of IL-1 and the localization and mRNA expression of IL-1β in GCF and inflamed human gingival tissues, we examined seven patients (five female, two male) without any systemic disease, 36–58 years of age (mean age 48.4 ± 3.2), who were referred to Aichi-Gakuin University Dental Hospital, Japan for treatment of moderate to advanced chronic periodontitis. After achieving good plaque control, seven sites between two adjacent teeth were selected. At all of these sites, the mean value of the residual probing pocket depth (PPD) was 4 mm or more. None of the patients had used a mouth rinse or had taken systemic or local antibiotics for 6 months before the initiation of the study.

Informed consent was obtained from all patients in accordance with the Helsinki Declaration. The protocol was approved by the ethics committee of Aichi-Gakuin University, School of Dentistry, Nagoya, Japan.

Clinical examinations

The following clinical parameters were evaluated before and 1 month after the SRP procedure. The gingival index (GI) (31), PPD, clinical attachment level (CAL) and bleeding on probing (BOP) were assessed at the site of GCF sampling. Tooth mobility was also measured. PPD and CAL were measured to the nearest millimeter using a straight periodontal probe (PCP UNC15, Hu-Friedy, Chicago, IL, USA). As a reference for CAL

measurements, the cemento–enamel junction was used. If the cemento–enamel junction was destroyed by a restoration (filling, crown), the margin of this restoration served as a reference. GI, PPD, CAL and BOP were measured at four sites around the interdental area (mesial tooth: distobuccal, distolingual; distal tooth: mesiobuccal, mesiolingual) and the four measurements recorded on the mesial and distal aspects were then averaged. GI, PPD and CAL are given as average values and BOP is given as a rate of appearance. A reevaluation was done 1 month after the SRP procedure. All clinical parameters were assessed by one examiner.

GCF sampling

The sites to be sampled were isolated with cotton rolls and supragingival plaque was removed. After the sites were gently dried by blowing with air, GCF samples were collected with paper strips (PerioPaper Strips, Oraflow Inc., Plainview, NY, USA) that were inserted into the gingival crevice until mild resistance was felt and then allowed to remain there for 10 s. GCF was collected from the same four sites as in the clinical examination. Strips contaminated by bleeding were discarded. The amount of collected GCF was quantitated with a Periotron 8000 (Oraflow Inc., Plainview, NY, USA), which had been calibrated with pooled human serum. The strips were placed in 100 μl of phosphate-buffered saline containing 0.5% bovine serum albumin (Sigma, St Louis, MO, USA) and stored at −80°C until use. These samples were assayed for IL-1α, IL-1β and IL-1ra as described below.

Enzyme-linked immunosorbent assay (ELISA)

The Quantikine human IL-1α, IL-1β and IL-1ra ELISA kits (R & D Systems, Minneapolis, MN, USA) were used to detect the amounts of IL-1α, IL-1β and IL-1ra in the same sample according to the manufacturer's instructions. All IL-1 determinations were carried out in triplicate for each sample. IL-1 activity index was calculated as follows: (total amount of IL-1α + total amount of IL-1β)/total amount of IL-1ra × 10^3). All GCF sampling and ELISA assays were performed by one examiner blinded to the clinical results.

Needle biopsy

After the GCF sampling and clinical examination, we performed a needle biopsy to better understand the histological conditions elicited in inflammatory processes in periodontal tissue in matching gingival tissues from the same patient. Local anesthesia was applied at the circumference of the interdental area. Disposable soft tissue biopsy needles of 14G or 18G (Tru-Cut® Soft Tissue Biopsy Needles, Allengiance Health Care Corporation, McGaw Park, IL, USA) were used (32). The area of the biopsy was the center of the interdental gingiva almost above the alveolar bone before the SRP procedure (Fig. 1). The biopsy was performed before and 1 month after the SRP procedure at almost the same area. In the area of the biopsy, postoperative inflammation had recovered clinically 1 month after the SRP procedure.

The excised biopsy tissue plaques were fixed in 4% neutral buffered formalin for 24 h, embedded in paraffin, sectioned in a plane parallel to the long axis of the tooth at a thickness of 6 μm and placed on slides coated with poly L-lysine (0.01%, Sigma Chemical Co., St Louis, MO, USA). The sections were stained with hematoxylin and eosin using standard procedures or prepared for immunohistochemistry for IL-1β and *in situ* hybridization for IL-1β mRNA. The needle biopsy was performed by the doctor in charge. On the other hand, the section preparation and histological assay were performed by two examiners blinded to the clinical results.

SRP procedure

Immediately after the first needle biopsy, the SRP procedure was performed at each interdental area between two adjacent teeth under local anesthesia until the roots were free of detectable roughness. There were no restrictions on the time spent on each tooth using a suitable curette (Gracy curette, Hu-Friedy®, Chicago, IL, USA) for each site. One month after the SRP treatment, the clinical conditions of the patients were reevaluated. The patients also underwent a second GCF assay and a needle biopsy.

Immunohistochemistry

Immunohistochemical staining was performed using the streptavidin–biotin (SAB) method (Histofine SAB-PO Kit, Nichirei Co., Tokyo, Japan). A monoclonal antibody against human IL-1β (AM 11) was used as an IL-1β antigen (R & D Systems, Minneapolis, MN, USA). Trypsinization of sections increased the number of cells stained by the antibody and also enhanced the intensity of the labeling reaction.

This procedure was performed in all cases by incubating deparaffinized with xylene rehydrating in a graded series of alcohol, and then hydrating sections for 20–30 min in 0.1% trypsin and 0.1% calcium chloride solution (pH 7.6). Endogenous peroxidase activity was blocked by incubating sections with 1% hydrogen peroxidase/methanol solution for 20 min. After blocking serial sections with 5% normal rabbit serum for 30 min, the antibody was used at a dilution of 1 : 300 in phosphate-buffered saline, pH 7.4, containing 1% normal pig serum. Adjacent sections were used as the negative control, where the primary antibody was replaced by phosphate-buffered saline. Reaction products of immunostaining were developed by immersing the sections in 3,3′-diaminobenzidine tetrahydrochloride (DAB) solution containing 0.05% hydrogen peroxidase. Cell nuclei were stained with hematoxylin prior to dehydrating and mounting with 60% harleco synthetic resin solution (HSR solution, International Reagents Corporation, Kobe, Japan). All stainings were performed in parallel using serial sections.

Oligo-DNA probes

IL-1β antisense oligo-DNA (5′-ATCTTTGAAGAAGAACCTATCTTCTTTGAC-3′) complementary to the

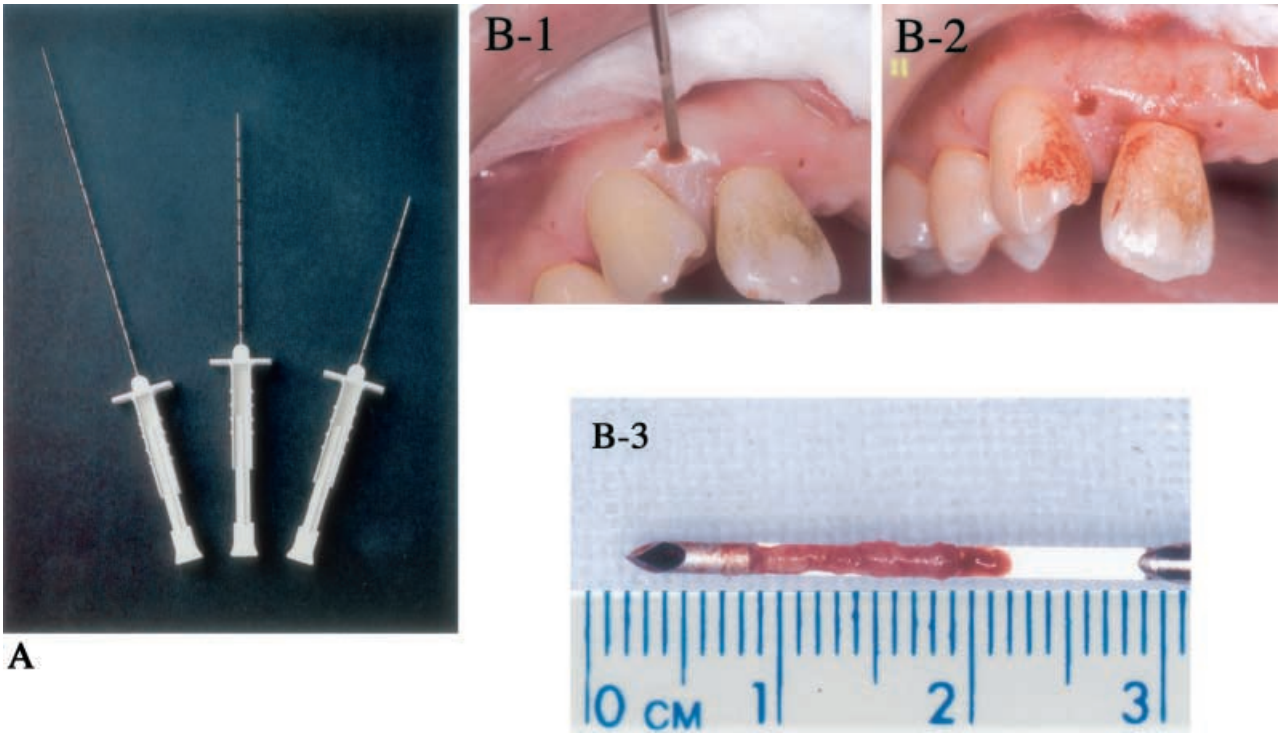

Fig. 1. Needle biopsy. (A) Tru-Cut® Soft Tissue Biopsy Needles. (B-1) Clinical features at the insertion of a Tru-Cut® Soft Tissue Biopsy Needle. (B-2) Clinical features immediately after taking the sample. (B-3) Gingival sample obtained by needle biopsy.

human IL-1β mRNA coding for amino acids 97–106 was synthesized. The sense oligo-DNAs corresponding to these mRNA sequences were selected as negative control probes. A computer-assisted search (GenBank release 113.0) of the oligo-DNA sequences found no significant homology with any known sequences. The oligo-DNAs were labeled by digoxigenin (Dig) with terminal transferase (Boehringer-Mannheim Co., Indianapolis, IN, USA).

In situ hybridization

For *in situ* hybridization with oligo-DNA probes, the deparaffinized and rehydrated sections (4 μm thick) were treated with 2 μg/ml proteinase K (37°C, 10min), and then immersed in 0.3% hydrogen peroxidase in methanol to block endogenous peroxidase activity. After probe DNA was denatured by heating the slides at 95°C for 5 min in an oven, hybridization was carried out in a humid chamber at 37°C overnight with 1 μg/ml of labeled probe dissolved in hybridization medium. After washing twice in 0.1 × saline sodium citrate (15 mM NaCl and 1.5 mM citric acid, pH 7.0) at 43°C for 20 min, a GenPoint Kit (#K 0620; DAKO, Kyoto, Japan) was used according to the manufacturer's instructions. First, samples were incubated for 30 min in primary anti-Dig antibody–horseradish peroxidase (HRP) at a dilution of 1 : 200. After washing in Tris-buffered saline with Tween 20 (50 mM Tris-HCl, 300 mM NaCl, 0.1% Tween 20), samples were incubated for 15 min in biotin–tyramide solution, and then in secondary streptavidin–HRP for 15 min. The HRP sites were visualized by DAB and hydrogen peroxidase in the presence of nickel and cobalt ions to enhance DAB staining (33). Methyl green was used for nuclear staining.

Histometrical analysis

The number of blots with positive signals for IL-1β mRNA was counted using image-analysis software (MacSCOPE®, Mitanishouji, Fukui, Japan). Briefly, the sections were captured into a computer system (Power Macintosh 7600/200, Apple, USA) by a CCD camera (CS 530 MD, Olympus, Tokyo, Japan) interfaced with a digital imaging microscope (BX50, Olympus, Tokyo, Japan). Three areas (40×50 μm^2) in the connective tissue were selected at random in each section. In each histologic sample, the measurement was performed at five different sections, at least 30 μm apart, and the average number was determined for each sample. The average values (mean ± standard error) before and after the SRP procedure were compared. The staining number for IL-1β mRNA was estimated semi-quantitatively by two separate observers blinded to the clinical results.

Statistical analysis

Data are shown as the mean ± standard error. Mean values for each clinical parameter, GCF volume, the amount and concentration of IL-1α, IL-1β and IL-1ra, IL-1 activity index, and the number of mRNA expression blots of IL-1β, were determined. Differences between baseline and reexamination were analyzed using the Student's *t*-test for paired comparisons

Table 1. Clinical parameters of sites

Sample no.	Sex	Age	Site	PPD (mm)	CAL (mm)	BOP (%)	GI	Tooth mobility
1	F	36	6, 7 interdental	4.5 ± 0.2	6.0 ± 0.6	25	1.5 ± 0.3	0
2	F	54	28, 29 interdental	5.0 ± 1.3	6.3 ± 1.3	0	0.8 ± 0.3	0
3	F	58	24, 25 interdental	6.3 ± 0.3	6.3 ± 0.6	100	2.0 ± 0.0	1.5 ± 1.1
4	F	47	27, 28 interdental	5.0 ± 0.6	6.0 ± 0.6	50	1.0 ± 0.0	0
5	M	50	24, 25 interdental	5.7 ± 0.2	12.0 ± 0.2	100	1.5 ± 0.3	1.0 ± 0.7
6	M	55	24, 26 interdental	5.8 ± 0.6	7.0 ± 0.5	100	1.0 ± 0.6	0.5 ± 0.4
7	F	38	10, 11 interdental	5.3 ± 0.2	5.8 ± 1.3	50	1.3 ± 0.8	0
Mean ± SE		48.4 ± 3.2		5.3 ± 0.2	7.0 ± 0.8	60.7 ± 15.3	1.3 ± 0.2	0.4 ± 0.3

PPD, probing pocket depth; CAL, clinical attachment level; BOP, bleeding on probing; GI, gingival index.

and p-values < 0.05 were considered significant.

Results

Patient characteristics and the data recorded at the baseline examination are shown in Table 1. There were five female patients. The mean age of the patients was 48.4 ± 3.2. All of the sample sites were the interdental areas between incisors and premolars. Mean PPD was 5.3 ± 0.2 mm and mean CAL was 7.0 ± 0.8 mm. The mean prevalence of BOP and GI scores were 60.7 ± 15.3% and 1.3 ± 0.2, respectively. The mean tooth mobility of the two adjacent teeth was 0.4 ± 0.3.

After SRP treatment, healing was uneventful in all cases. A significant reduction in PPD was seen between baseline (5.3 ± 0.2 mm) and at 1 month (3.6 ± 0.4 mm) ($p < 0.05$) (Fig. 2A). The mean CAL decreased from 7.0 ± 0.8 to 6.3 ± 1.3 mm (Fig. 2B). The mean incidence of BOP decreased from 60.7 ± 15.3% to 42.9 ± 16.1% (Fig. 2C). The mean GI value decreased from 1.3 ± 0.2 to 1.1 ± 0.2 (Fig. 2D).

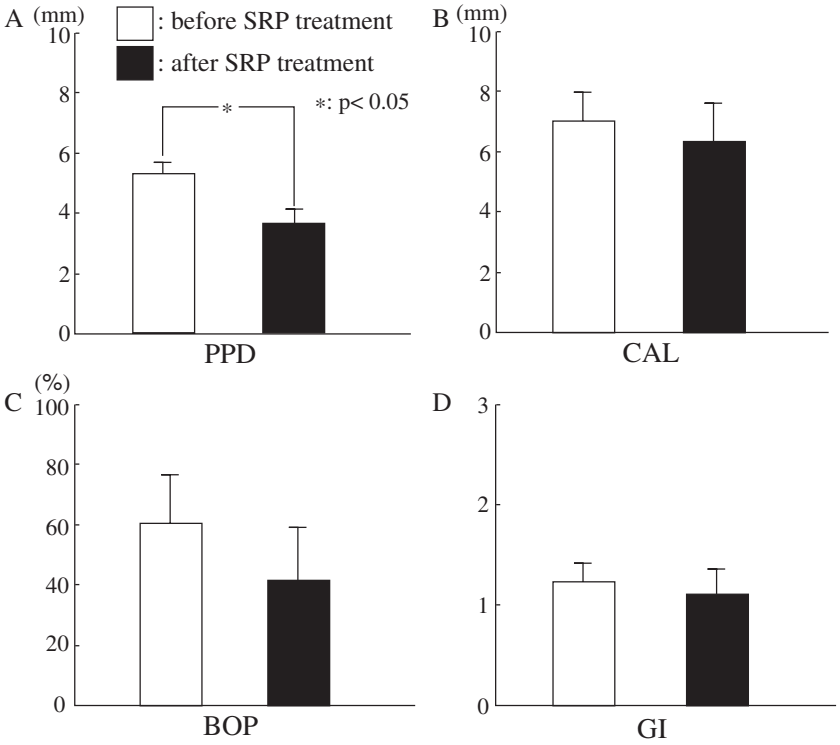

Fig. 2. Clinical parameters. All parameters were measured just before and 1 month after the scaling and root planing (SRP) procedure. Data are shown as the mean ± SE. (A) Probing pocket depth (PPD). (B) Clinical attachment level (CAL). (C) Bleeding on probing (BOP). (D) Gingival index (GI). A significant reduction in PPD was seen between baseline and 1 month ($p < 0.05$, $p = 0.0015$).

The mean GCF volume changed from 1.5 ± 0.2 μl to 1.4 ± 0.3 μl (Fig. 3). The mean amount of IL-1β increased slightly after the SRP treatment from 37.2 ± 26.8 to 41.8 ± 23.0 (Fig. 4). On the other hand, the mean concentrations of IL-1α, -1β and -1ra increased after the SRP procedure (Fig. 5). The mean value of IL-1 activity index decreased slightly (Fig. 6). Thus, the GCF assay showed that the mean amount and concentration of IL-1β slightly increased during the month after the procedure and this trend was different from results for the clinical parameters (Figs 4 and 5).

Histologically, inflammatory cell infiltration was slightly reduced in the connective tissue 1 month after the SRP treatment; however, the localization of IL-1β around inflammatory cells was still observed in immunohistochemically stained sections and the mRNA expression of IL-1β was recognized even after SRP treatment (Fig. 7).

In the histometrical analysis by *in situ* hybridization, the number of cells with positive signals for IL-1β mRNA expression slightly decreased from $56.5 \pm 14.4 \times 10^3$ blots/50×40 μm^2 to $41.0 \pm 6.4 \times 10^3$ blots/50×40 μm^2 (Fig. 8).

Discussion

Epidemiologic, retrospective and longitudinal studies have revealed that the progression of chronic periodontitis varies among patients and among sites within a patient. The methods that are currently used for periodontal diagno-

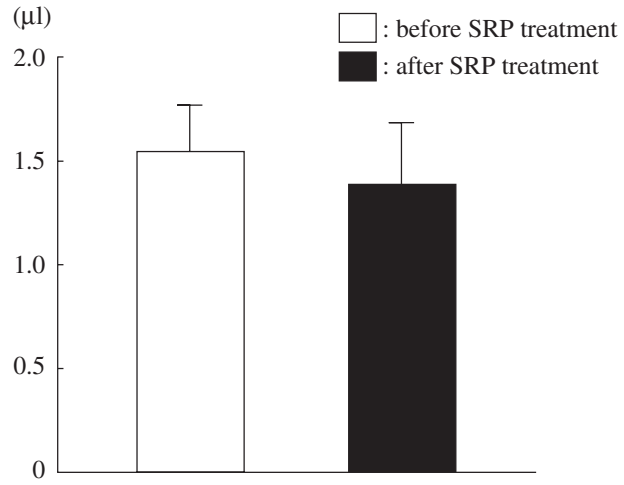

Fig. 3. Change in gingival crevicular fluid (GCF) volume following scaling and root planing (SRP) treatment. GCF was taken just before and 1 month after the SRP procedure. Data are shown as the mean ± SE.

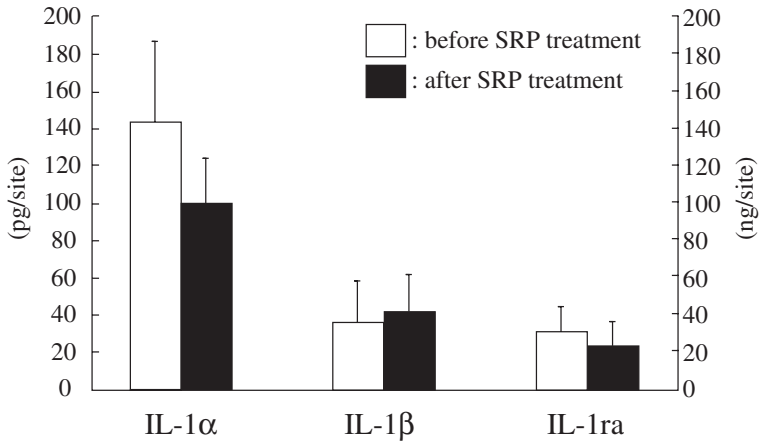

Fig. 4. Changes in the amounts of interleukin-1α (IL-1α), IL-1β and IL-1ra in gingival crevicular fluid following scaling and root planing (SRP) treatment. IL-1α and IL-1β are shown in units of pg/site, and IL-1ra is shown in units of ng/site. Data are shown as the mean ± SE.

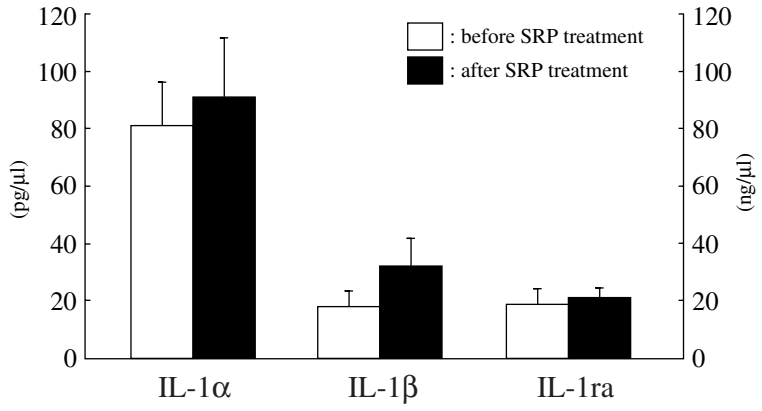

Fig. 5. Changes in the concentrations of interleukin-1α (IL-1α), IL-1β and IL-1ra in gingival crevicular fluid following scaling and root planing (SRP) treatment. IL-1α and IL-1β are shown in units of pg/μl, and IL-1ra is shown in units of ng/μl. Data are shown as the mean ± SE.

sis have been limited to clinical examination and radiographic evaluation. These methods essentially determine previous destruction, or a history of disease. The evaluation of disease activity has been limited to longitudinal evaluations of these parameters, which have limited accuracy, and predictors of future disease activity are not yet available (34–37). Future diagnostic methods should be able to provide an early diagnosis of disease, before significant destruction has occurred, as well as measures of successful treatment or disease arrest.

Lipopolysaccharide is a key microbial stimulus that initiates the host response at periodontal disease sites and triggers monocytes to release inflammatory mediators (prostaglandin E_2, thromboxane B, interleukin-1, -6 and -8, tumor necrosis factor, and collagenase) that increase the local destruction of connective tissue structural elements (38, 39). Therefore, the levels of monocytic inflammatory mediators in GCF may be ideal markers of disease activity at a particular site. In the present study, we examined IL-1, which is considered to be a cytokine that is also a powerful mediator of inflammation.

In a preliminary experiment, we compared clinical parameters and the volume of IL-1α, -1β and -1ra in GCF and IL-1 activity index. We found that both PPD and CAL increased, i.e. the severity of inflammation became clinically serious, and the amount of IL-1β in GCF also increased. However, the amounts of IL-1α, -1ra and IL-1 activity index did not change (data not shown).

Many studies have reported that GCF IL-1 levels are significantly elevated in all forms of periodontitis, compared to health or gingivitis (17–26). Studies have also confirmed an association between elevated GCF IL-1 levels and gingival inflammation, as well as a relationship between the severity of periodontitis and elevated GCF IL-1 levels (40–43). Matsuki *et al.* (21) reported that IL-1 activity in GCF from inflamed gingiva was higher than that extracted from healthy gingiva. Ishihara *et al.* (17) reported that the degree of periodontitis classified according to alveolar bone resorption

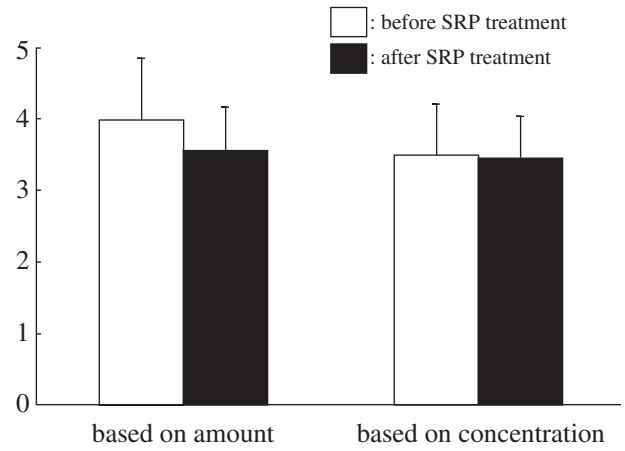

Fig. 6. Changes in interleukin-1 (IL-1) activity index following scaling and root planing (SRP) treatment. The values were calculated from the data for the volume and concentration of IL-1α, IL-1β and IL-1ra. Data are shown as the mean ± SE.

was correlated with the volume of IL-1α and IL-1β in GCF and the level of IL-1 activity index. Curtis *et al*. (22) also reported that the measurement of IL-1β in GCF was effective in the diagnosis of peri-implantitis. It has been reported that mouse macrophage stimulated by lipopolysaccharide produces either IL-1α in preference to IL-1β or the same quantity of IL-1α and IL-1β (44). On the other hand, it has been reported that human macrophage and monocyte produce IL-1β in preference to IL-1α (45). The host immune and inflammatory response in the connective tissue to a microbial challenge is a critical determinant of susceptibility to the development of a destructive periodontal disease, under the influence of multiple behavioral, environmental, and genetic factors. Based on our results and the findings of others, the measurement of GCF IL-1β may be useful for evaluating the host response in an inflammatory condition.

GCF is suitable for understanding the local cellular metabolism through the detection of biochemical indicators in subgingival periodontal tissues. Therefore, determination of the presence of inflammatory products in GCF may be useful for evaluating both periodontal disease status and the outcome of therapy. Some studies have reported positive correlations between level of GCF inflammatory mediators and clinical periodontal conditions (17, 30, 46–48), whereas other studies have reported poor correlations between such levels and the site clinical status (25, 28, 49–51). Published reports that have addressed this issue have been inconsistent. Since IL-1 occurs in only small amounts in GCF, and is transiently produced and consumed locally at the interface between the producing cells and target cells, the IL-1 level based on a measurement of GCF may change readily. Therefore, GCF may not be suitable for understanding the inflammatory events in periodontal tissue. To compensate for this weakness of GCF assay, a histological examination was carried out in this study using a needle biopsy. The examination of gingival biopsies is usually limited with regard to area and time, in that it is performed concomitant with tooth extraction or periodontal surgery. However, needle-biopsy sampling can be performed repeatedly in an area with a small amount of tissue. Okada *et al*. (52) reported that IL-1β, IL-6, IL-8, TNF-α and IFN-γ mRNA were detected in needle-punched biopsy samples of inflamed gingiva by reverse transcription polymerase chain reaction, and demonstrated the efficacy of needle biopsy in this context.

We examined the relationship between clinical parameters and IL-1 in GCF and the localization and mRNA expression of IL-1β in inflamed gingival tissue of chronic periodontitis patients to precisely evaluate the gingival inflammatory condition. One month after the SRP procedure, PPD was significantly improved, while there were slight improvements in CAL, GI score or incidence of BOP. In this study, the time between the two clinical examinations before and after SRP treatment was only 1 month. In an established periodontal lesion, it would require more time to observe any clinical changes. This means that gingival recession had occurred at the sites of the SRP procedure and it was in recovery process at the second examination. However, non-surgical mechanical therapy increased the amount and concentration of IL-1β in GCF even in recovery process after the SRP treatment. This suggests that the inflammatory condition may still be present during the 1 month after the SRP procedure.

The area of the needle biopsy was the center of the interdental gingiva almost above the alveolar bone. Since the periodontal pocket epithelium attaches to bacterial plaque and other antigenic stimulants, it is logical to expect that many IL-1β producing and mRNA-expressing macrophages and activated lymphocytes that produce IL-1 are close to the pocket epithelium. However, the area of lamina propria around the junctional epithelium could disappear by gingival recession after the SRP treatment. Therefore, we selected the area near the alveolar bone crest, since we wanted to compare almost the same site. Furthermore, we decided to observe the area because it has been reported that IL-1β is a potent stimulator of bone resorption and has been implicated in the pathogenesis of periodontal tissue destruction.

Histochemically, IL-1β was detected in the connective tissue at 1 month after the SRP procedure. We also examined the number of blots of IL-1β mRNA expression in the gingiva after the SRP procedure by *in situ* hybridization techniques, and found cells with intense IL-1β mRNA staining. There have been several studies on IL-1β mRNA expression that used periodontitis patients as a source for healthy or gingivitis tissue specimens, and these have shown higher levels of

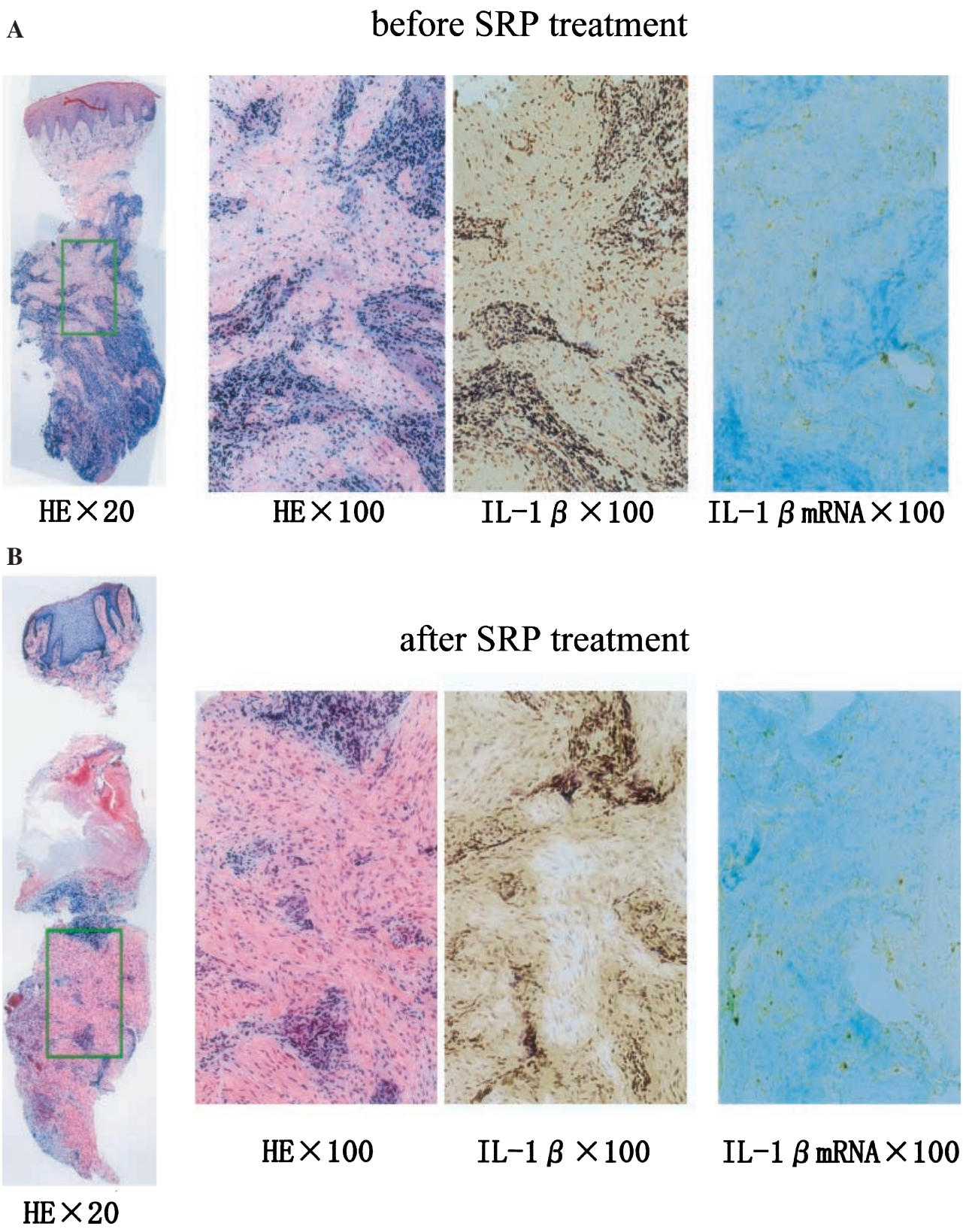

Fig. 7. Hematoxylin and eosin (HE) staining, immunohistochemistry and *in situ* hybridization for interleukin-1β (IL-1β) mRNA (A) before scaling and root planing (SRP) treatment and (B) 1 month after SRP treatment in the same patient. Serial sections were fixed and stained with hematoxylin and eosin or incubated with an antibody AM 11 for IL-1β or IL-1β-specific oligoprobe, and developed as described in the Material and Methods.

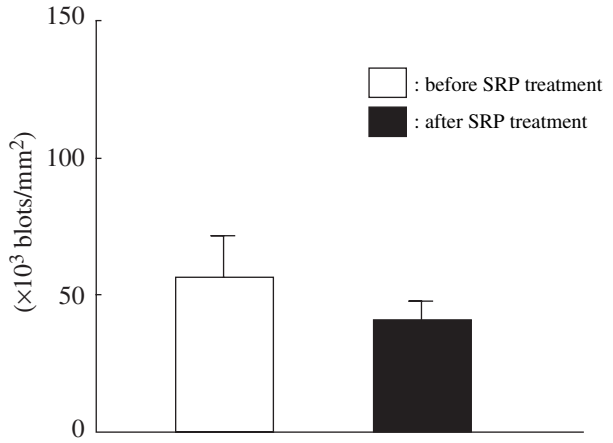

Fig. 8. Change in positive signals for interleukin-1β mRNA following scaling and root planing (SRP) treatment. Signals were measured within an area of 40 × 50 μm^2.

IL-1β mRNA expression in gingivitis tissue than have been seen in studies of healthy tissue from non-periodontitis donors. A possible explanation for these results is that the gingival cells from periodontitis patients may be hyperresponsive to lipopolysaccharide present in the subgingival plaque. Recent studies have shown that mononuclear cells, fibroblasts and epithelial cells isolated from periodontitis patients tend to be primed to respond to bacterial lipopolysaccharide, and show the *in vitro* production of higher levels of IL-1β in response to lipopolysaccharide challenge than such cells from unaffected patients. There was considerable inflammatory cell infiltration in the connective tissue even in the gingiva after the SRP procedure. IL-1β mRNA expression and the proteins predominate the area of inflammatory cell infiltration. Therefore, needle biopsy can help in determining the time-course changes in several stages of treatment and inflammation which cannot be examined clinically; for example, on the perimeter of an implantation.

In this study, in which we used ELISA, immunohistochemistry and *in situ* hybridization methods to compare gingival connective tissue before and after SRP treatment in the same patients, we found that the volume of IL-1β in GCF was not reduced, and the localization and mRNA expression of IL-1β occurred predominantly in the gingiva from patients, whereas PPD was improved at one month after the SRP treatment. However, the actual small magnitude of clinical improvement, small number of sites examined (one site each in seven patients), and most importantly, the time between the two GCF samples (one month) may be all probably insufficient to detect significant trends in IL-1 levels before and after SRP treatment. Moreover, the decision in this study to perform the biopsies at the alveolar crest rather than at the area of lamina propria around the junctional epithelium where significant IL-1 activity may occur, may also reduce the chances of detecting significant changes. Therefore, further study to address these concerns is needed.

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References

1. Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK. There is more than one interleukin-1. *Immunol Today* 1986;**7:**45–56.
2. Dinarello CA. Interleukin 1 and its biologically related cytokines. *Adv Immunol* 1989;**44:**153–205.
3. di Giovine FS, Duff GW. Interleukin 1: The first interleukin. *Immunol Today* 1990;**11:**13–20.
4. Havemose-Poulsen A, Holmstrup P. Factors affecting IL-1-mediated collagen metabolism by fibroblasts and the pathogenesis of periodontal diseases: a review of the literature. *Crit Rev Oral Biol Med* 1997;**8:**217–236.
5. Okada H, Murakami S. Cytokine expression in periodontal health and disease. *Crit Rev Oral Biol Med* 1998;**9:**248–266.
6. Richards D, Rutherford RB. The effects of interleukin-1 on collagenolytic activity and prostaglandin-E secretion by human periodontal-ligament and gingival fibroblast. *Arch Oral Biol* 1988;**33:**237–243.
7. Gowen M, Wood DD, Ihrie EJ, McGuire MKB, Russell GG. An interleukin 1-like factor stimulates bone resorption *in vitro*. *Nature* 1983;**306:**378–380.
8. Stashenko P, Dewhirst FE, Peros WJ, Kent RL, Ago JM. Synergistic interactions between interleukin 1, tumor necrosis factor, and lymphotoxin in bone resorption. *J Immunol* 1987;**138:**1464–1468.
9. Tatakis DN. Interleukin-1 bone metabolism: a review. *J Periodontol* 1993;**64:**416–431.
10. Dinarello CA. The interleukin-1 family: 10 years of discovery. *FASEB J* 1994;**8:**1314–1325.
11. Dinarello CA. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Int Rev Immunol* 1998;**16:**457–499.
12. Sims JE, Dower SK. Interleukin-1 receptors. *Eur Cytokine Netw* 1994;**5:**539–546.
13. Hannum CH, Wilcox CJ, Arend WP *et al.* Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature* 1990;**343:**336–340.
14. Eisenberg SP, Evans RJ, Arend WP *et al.* Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. *Nature* 1990;**343:**341–346.
15. Henderson B, Thompson RC, Hardingham T, Lewthwaite J. Inhibition of interleukin-1-induced synovitis and articular cartilage proteoglycan loss in the rabbit knee by recombinant human interleukin-1 receptor antagonist. *Cytokine* 1991;**3:**246–249.
16. Dinarello CA, Thompson RC. Blocking IL-1: interleukin 1 receptor antagonist in vivo and in vitro. *Immunol Today* 1991;**12:**404–410.
17. Ishihara Y, Nishihara T, Kuroyanagi T *et al.* Gingival crevicular onterleukin-1 and interleukin-1 receptor antagonist levels in periodontally healthy and diseased sites. *J Periodont Res* 1997;**32:**524–529.
18. Charon JA, Luger TA, Mergenhagen SE, Oppenheim JJ. Increased thymocyte-activating factor in human gingival fluid during gingival inflammation. *Infect Immun* 1982;**38:**1190–1195.

19. Mergenhagen SE. Thymocyte activating factor(s) in human gingival fluids. *J Dent Res* 1984;**63:**461–464.
20. Kobashima H, Maeda K, Iwamoto Y *et al*. Partial characterization of an interleukin-1-like factor in human gingival crevicular fluid from patients with chronic inflammatory periodontal disease. *Infect Immun* 1990;**58:**2621–2627.
21. Matsuki Y, Yamamoto T, Hara K. Localization of interleukin-1 (IL-1) mRNA-expressing macrophages in human inflamed gingival and IL-1 activity in gingival crevicular fluid. *J Periodont Res* 1993;**28:**35–42.
22. Curtis DA, Kao R, Plesh O, Finzen F, Franz L. Crevicular fluid analysis around two failing dental implants: a clinical report. *J Prosthodont* 1997;**6:**210–214.
23. Tsai C-C, Ho Y-P, Chen C-C. Levels of interleukin-1β and interleukin-8 in gingival crevicular fluids in adult periodontitis. *J Periodontol* 1995;**66:**852–859.
24. Salvi GE, Yalda B, Collins JG *et al*. Inflammatory mediator response as a potential risk marker for periodontal diseases in insulin-dependent diabetes mellitus patients. *J Periodontol* 1997;**68:**127–135.
25. Gonzales JR, Herrmann JM, Boedeker RH, Francz PI, Biesalski H, Meyle J. Concentration of interleukin-1beta and neutrophil elastase activity in gingival crevicular fluid during experimental gingivitis. *J Clin Periodontol* 2001;**28:**544–549.
26. Kinane DF, Winstanley FP, Adonogianaki E, Moughal NA. Bioassay of interleukin 1 (IL-1) in human gingival crevicular fluid during experimental gingivitis. *Arch Oral Biol* 1992;**37:**153–156.
27. Roberts FA, Hockett RD Jr, Bucy RP, Michalek SM. Quantitative assessment of inflammatory cytokine gene expression in chronic adult periodontitis. *Oral Microbiol Immunol* 1997;**12:**336–344.
28. Al-Shammari KF, Giannobile WV, Aldredge WA, *et al*. Effect of non-surgical periodontal therapy on C-telopeptide pyridinoline cross-links (ICTP) and interleukin-1 levels. *J Periodontol* 2001;**72:** 1045–1051.
29. Tuter G, Kurtis B, Serdar M. Interleukin-1beta and thiobarbituric acid reactive substance (TBARS) levels after phase I periodontal therapy in patients with chronic periodontitis. *J Periodontol* 2001; **72:**883–838.
30. Gamonal J, Acevedo A, Bascones A, Jorge O, Silva A. Levels of interleukin-1β, -8, and -10 and RANTES in gingival crevicular fluid and cell populations in adult periodontitis patients and the effect of periodontal treatment. *J Periodontol* 2000;**71:**1535–1545.
31. Löe H, Silness JL. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 1963;**21:**533–551.
32. Cusick JD, Dotan J, Jaecks RD, Boyle WT. The role Tru-cut® needle biopsy in the diagnosis of carcinoma of the breast. *Surg Gynecol Obstet* 1990;**170:**407–410.
33. Adams JC. Heavy metal intensification of DAB-based HRP reaction products. *J Histochem Cytochem* 1981;**29:**775–776.
34. Haffajee AD, Socransky SS, Goodson JM. Clinical parameters as predictors of destructive periodontal disease activity. *J Clin Periodontol* 1983;**10:**257–265.
35. Haffajee AD, Socransky SS, Goodson JM. Comparison of different data analyses for detecting changes in attachment level. *J Clin Periodontol* 1983;**10:**298–310.
36. Bandersten A, Nilveus R, Egelberg J. Effect of nonsurgical periodontal therapy (VII). Bleeding, suppuration and probing depth in sites with attachment loss. *J Clin Periodontol* 1985;**12:**432–440.
37. Lang NP, Joss A, Orsanic T, Gusberti FA, Siegrist BE. Bleeding on probing. A predictor for the progression of periodontal disease? *J Clin Periodontol* 1986;**13:** 590–596.
38. Taupin V, Gogusev J, Descamps-Latscha B, Zavala F. Modulation of tumor necrosis factor-alpha, interleukin-1 beta, interleukin-6, interleukin-8, and granulocyte/macrophage colony-stimulating factor expression in human monocytes by an endogenous anxiogenic benzodiazepine ligand, triakontatetraneuropeptide: evidence for a role of prostaglandins. *Mol Pharmacol* 1993;**43:**64–69.
39. He W, Pelletier JP, Martel-Pelletier J, Laufer S, Di Battista JA. Synthesis of interleukin 1beta, tumor necrosis factor-alpha, and interstitial collagenase (MMP-1) is eicosanoid dependent in human osteoarthritis synovial membrane explants: interactions with antiinflammatory cytokines. *J Rheumatol* 2002;**29:**546–553.
40. Ebersole JL, Cappelli D, Holt SC, Singer RE, Filloon T. Gingival crevicular fluid inflammatory mediators and bacteriology of gingivitis in nonhuman primates related to susceptibility to periodontitis. *Oral Microbiol Immunol* 2000;**15:**19–26.
41. Liu CM, Hou LT, Wong MY, Rossomando EF. Relationships between clinical parameters, Interleukin-1β and histopathologic findings of gingival tissue in periodontitis patients. *Cytokine* 1996;**8:** 161–167.
42. Masada MP, Persson R, Kenney JS, Lee SW, Page RC, Allison AC. Measurement of interleukin-1 alpha and – 1 beta in gingival crevicular fluid. implications for the pathogenesis of periodontal disease. *J Periodont Res* 1990;**25:**156–163.
43. Mogi M, Otogoto J, Ota N, Inagaki H, Minami M, Kojima K. Interleukin-1β, interleukin 6, β_2-microglobulin, and transforming growth factor-alpha in gingival crevicular fluid from human periodontal disease. *Arch Oral Biol* 1999;**44:**535–539.
44. Perregaux DG, Gabel CA. Post-translational processing of murine IL-1: evidence that ATP-induced release of IL-1 and IL-1 occurs via a similar mechanism. *J Immunol* 1998;**160:**2469–2477.
45. Demczuk S, Baumberger C, Mach B, Dayer JM. Expression of human IL-1 alpha and beta messenger RNAs and IL-1 activity in human peripheral blood mononuclear cells. *J Mol Cell Immunol* 1987;**3:**255–265.
46. Mathur A, Michalowicz B, Castillo M, Aeppli D. Interleukin-1α, interleukin-8 and interferon-α levels in gingival crevicular fluid. *J Periodont Res* 1996;**31:**489–495.
47. Nakashima K, Roehrich N, Cimasoni G. Osteocalcin, prostaglandin E_2 and alkaline phosphatase in gingival crevicular fluid: their relations to periodontal status. *J Clin Periodontol* 1994;**21:**327–333.
48. Rawlinson A, Dalati MH, Rahman S, Walsh TF, Fairclough AL. Interleukin-1 and IL-1 receptor antagonist in gingival crevicular fluid. *J Clin Periodontol* 2000; **27:**738–743.
49. Figueredo CM, Ribeiro MS, Fischer RG, Gustafsson A. Increased interleukin-1beta concentration in gingival crevicular fluid as a characteristic of periodontitis. *J Periodontol* 1999;**70:**1457–1463.
50. Nakashima K, Giannopoulou C, Andersen E *et al*. A longitudinal study of various crevicular fluid components as markers of periodontal disease activity. *J Clin Periodontol* 1996;**23:**832–838.
51. Wilton JM, Bampton JL, Griffiths GS *et al*. Interleukin-1β (IL-1β) levels in gingival crevicular fluid from adults with previous evidence of destructive periodontitis. A cross sectional study. *J Clin Periodontol* 1992;**19:**53–57.
52. Okada H, Kitamura M, Nozaki T, Shimauchi H, Murakami S. New diagnostic strategies to detect the disease susceptibility and activity in periodontitis. In: *Risk Factors in Asian Pacific Populations*. Brisbane, Australia: Merino Lithographics Pty Ltd., 1996: 104–115.