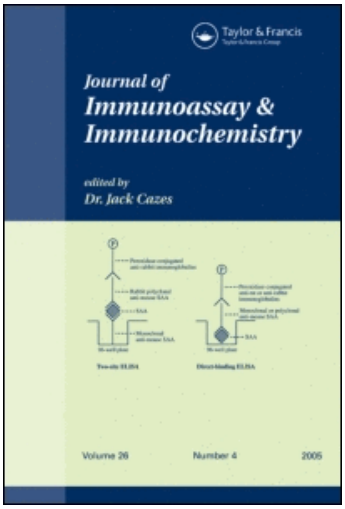

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Drop in Transforming Growth Factor-α and Osteoprotegerin Level in Gingival Crevicular Fluid from Patients with Gingivitis

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Abstract: Inflammatory mediators, especially cytokine, play a central role in the pathogenesis of gingivitis. The aim of this study was to identify and quantify the various growth factors, and cytokines in the gingival crevicular fluid (GCF) of patients with gingivitis, as compared with those of control subjects. The levels of cytokine in the samples were determined by their respective ELISAs. The transforming growth factor (TGF)-α and osteoprotegerin (OPG) level were significantly lower in patients with gingivitis than in the controls ($p < 0.05$). Also, there was a positive correlation between TGF-α and OPG levels ($r = 0.761$). These results suggest that the decrease in growth factor TGF-α is associated with the pathophysiology and/or the progress of gingivitis.

Keywords: EGF, GCF, Gingivitis, Osteoprotegerin, RANKL, TGF-α

INTRODUCTION

Whereas gingivitis is a relatively mild inflammation confined to the gingival tissue, periodontitis affects the ligaments and alveolar bone that support the root of the tooth and provide its anchorage to the maxilla or

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mandible. The role of proinflammatory cytokines in gingivitis has been evaluated in several studies,[1,2] and attention has been given to the significance of cytokines. Polypeptide growth factors are a class of natural biological mediators that regulate key cellular events in tissue repair, i.e., cell proliferation, chemotaxis (directed migration), differentiation, and matrix synthesis via binding to specific cell-surface receptors.[3] Examples of these factors found in bone, cementum, and healing wound tissues include epidermal growth factor (EGF) and transforming growth factor (TGF)-α, a member of the EGF superfamily.

The physiological effects of TGF-α are similar to those of EGF.[4] Both are known to effect epithelial and mesenchymal cell proliferation, migration, and differentiation. EGF appears to contribute to inflammatory responses, as well as to other physiological and pathological processes.[5]

Whereas a recent study also suggests the involvement of the receptor activator of NF-kB ligand (RANKL) and osteoprotegerin (OPG) in the pathogenesis of bone-destructive disease such as rheumatoid arthritis and periodontal disease,[6–11] no one has examined the level of RANKL and OPG in the body fluid of the patients with gingivitis except for one.[12]

Several investigators have examined gingival crevicular fluid (GCF) for cellular immune-response indicators, as the levels of such indicators are considered to serve as possible markers of active periodontal disease,[13–18] but, thus far, no one has looked at the level of growth factors, especially EGF and TGF-α in gingivitis. The present study was designed to investigate the levels of EGF, TGF-α, interleukin (IL)-1β, IL-6, interferon (IFN)-γ, β2-microglobulin (β2-MG), RANKL and OPG in GCF by use of highly sensitive ELISAs.

EXPERIMENTAL

Fifty patients with gingivitis around single-rooted teeth were selected from those referred to the Department of Periodontology of Matsumoto Dental University. Informed consent was obtained from all participants at the first visit. The present study was approved by the ethics committee of Matsumoto Dental University School of Dentistry, and was conducted in accordance with the declaration of Helsinki on Biomedical Studies Involving Human Subjects (WMA, 1997). All patients were in good general health with no history of anti-microbial or anti-inflammatory therapy or periodontal treatment during a 6-month period before the start of the study. One examiner performed all recordings. Gingival Index, Plaque Index, and Bleeding Index were recorded at preliminary examination. Since smoking is a risk factor for gingivitis and periodontitis, the current study did not include smokers. The gingivitis

Table 1. Demographic parameters of subjects and clinical parameters in the study groups

	Healthy control patients (45)	Gingivitis (50)
Age	31.2 ± 9.1	35.7 ± 14.6
Probing pocket depth (mm) (PPD)	1.76 ± 0.32	$2.79 \pm 0.51^*$
Clinical attachment loss (mm) (CAL)	0	0
Plaque index (PI)	0	$1.76 \pm 0.64^*$
Papilla bleeding index (PBI)	0	$2.18 \pm 0.73^*$

Mean ± S.E.M. is given. Significant difference from healthy group. Mann-Whitney *U*-test, $^*p < 0.05$.

group included 20 females and 30 males with varying degrees of gingival inflammation, but with no radiographic evidence of alveolar bone loss. These patients ranged in age from 13 to 60 years (mean age 35.7 ± 14.6 years). The healthy group consisted of 20 females and 25 males ranging in age from 18 to 54 years with a mean age of 31.2 ± 9.1 years. They had no clinical signs of gingival inflammation (no bleeding on probing), exhibited PPD <3 mm, and no radiographic evidence of alveolar bone loss. Table 1 shows clinical measurements and demographics for the study groups.

The GCF sampling was performed by the method of Offenbacher et al. (1986) with slight modification.[14,18–20] All clinically detectable supragingival plaque was removed carefully without touching the gingiva to minimize plaque contamination of the strips. The teeth were gently washed with water, and the sites under study isolated with cotton rolls and gently dried with an air syringe. One periopaper was used at each collection site. Paper strips (Periopaper, Harco, Tustin, CA, USA) were carefully inserted 1 mm into the gingival crevice and allowed to remain there for 30 sec. After a one-minute interval, a second strip was placed at the same site. Care was taken to avoid mechanical injury. The volume of GCF in the periopaper was measured with a Periotron (Harco, Tustin, CA, USA). The paper strips from the individual sites were stored at $-80°C$ for later processing. Periopapers for each subject were pooled, and the GCF extracted and assayed for the content of cytokines/growth factor. To free the GCF sample completely from the periopaper, we eluted the fluid by centrifugation with aliquots of buffer (50 mM phosphate buffer, pH 7.2, containing protease inhibitors, 0.1 mM phenylmethylsulphonylfluoride, 5 μg/mL each of leupeptin, pepstatin, amastatin, chemostatin, and antipain). In brief, 100 μL of the above

buffer was applied to each strip and the tube centrifuged at 15,000 *g* for 5 min. A further 100 μL was then applied and the centrifugation repeated. The GCF from the two strips was pooled to give a total volume of 200 μL and then stored at − 80°C for later assay. The protein concentration of the extract was estimated by the method of Bradford, with bovine serum albumin as a standard.[21] It has been previously reported that protein recovery rates from Periopaper points is not consistent, and depends on nature of the proteins and their concentrations.[22] But, we confirmed the reproducible evidence that protein recovery rates are relatively high and constant (80–87%) from Periopaper using IL-1*β*, *β*2-MG, and albumin in a preliminary way.

*β*2-MG was determined by a sandwich ELISA that consisted of solid-phase (polystyrene bead)-immobilized antibodies and antibodies labeled with *β*-D-galactosidase, as described previously.[18,19] The contents of EGF, IL-1*β*, IL-6, IFN-*γ* in the samples were measured by use of commercially available two-site sandwich ELISA kits (Cayman Chemical Co., USA; R&D System, MN, USA; MedSystems Diagnosis, Austria), as described previously.[18,23,24] The ELISA system also shows that the CV was less than 7%. The ELISA for TGF-α utilizing polyclonal antibodies that recognized limited epitopes of TGF-α in combination with a monoclonal anti-TGF-α IgG1 (detection limit: 1 pg/mL).[25] All of the coefficient variations (CV)s were less than 5.6%. Free soluble form of RANKL was measured by a two-site ELISA.[26] ELISA for human RANKL consists of a polyclonal antibody that recognizes human soluble RANKL and human osteoprotegerin as a capture. We could quantify the human RANKL level (detection limit: 0.05 ng/mL). OPG was also determined by use of a commercially available two-site sandwich ELISA kit (R&D System, MN, USA).[8]

All samples were assayed twice. Duplicate measurements were performed by ELISA on each sample and show the average data. Data were reported as the concentrations of growth factor or cytokine (pg/μL of GCF). The contents of *β*2-MG and total proteins were presented as ng/μL of GCF. Statistical analysis between gingivitis and control subjects was performed by using one- or two-way ANOVA.

RESULTS

We compared the levels of growth factor and cytokine in gingivitis and control subjects. A statistically significant lower concentration of TGF-α-level was observed in the gingivitis group, up to 47% of the control level ($p < 0.05$), whereas the EGF level showed no difference between control and gingivitis patients (Table 2). In contrast, the mean IL-1*β*, IL-6, IFN-*γ*, *β*2-MG, and RANKL values of the gingivitis patients were

Table 2. Immunochemical analysis of gingival cervical fluid in healthy controls and in gingivitis patients

Analyte	Units	Healthy control patients (45)	Gingivitis (50)
EGF	pg/μl	116.1 ± 24.8 (100)	108.2 ± 28.3 (93)
TGF-α	pg/μl	212.6 ± 29.4 (100)	99.7 ± 14.2 (47)*
IL-1β	pg/μl	38.3 ± 12.9 (100)	44.1 ± 10.0 (115)
IL-6	pg/μl	18.2 ± 11.2 (100)	17.6 ± 16.1 (97)
IFN-γ	pg/μl	11.3 ± 3.9 (100)	10.5 ± 3.2 (93)
β2-microglobulin	ng/μl	9.1 ± 6.8 (100)	9.5 ± 3.2 (104)
RANKL	pg/μl	24.1 ± 6.7 (100)	27.0 ± 5.2 (112)
OPG	pg/μl	358.2 ± 40.7 (100)	242.8 ± 45.5 (68)*
Total protein	ng/μl	203.3 ± 44.1 (100)	235.8 ± 47.0 (116)

Mean ± S.E.M. is given. Significantly different from control, $^{*}p < 0.05$. Percentage of the control is shown in parentheses.

slightly higher than those of the controls with no statistical significance. OPG values of the gingivitis patients were slightly lower than those for the controls with statistical significance ($p < 0.05$). There was a positive correlation between TGF-α and OPG levels ($r = 0.761$). The total protein content in the gingivitis subjects was moderately higher than that of controls, but with no statistical significance (Table 2).

DISCUSSION

This study clearly demonstrated a statistically significant decrease in the TGF-α level, but no change in the IL-1β, IL-6, β2-MG, EGF, IFN-γ or RANKL level, in the GCF from gingivitis patients. Although the levels of growth factors/interleukins in GCF in gingivitis have been studied,[27,28] our report is the first to show down regulation of TGF-α in the GCF of patients with gingivitis. As described previously,[18] we demonstrated that TGF-α levels were also significantly lower in patients with periodontitis than in the controls. In contrast, the concentrations of IL-1β, IL-6, β_2-MG and RANKL was significantly higher in the severe periodontitis group than in the controls.[8,11,18] While gingivitis is an inflammation confined to the gingival tissue, periodontitis affects the ligaments and alveolar bone that support the root of the tooth and provide its anchorage to the maxilla or mandible. Because TGF-α is able to exert a variety of effects on biological activities such as wound healing,[4] the lack of TGF-α might result in a delay in gingival regeneration during the progression of gingivitis, and finally lead to periodontitis. If this

hypothesis is correct, the current data suggest the administration of TGF-α to gingivitis patients as a possible therapy.

There still remain several questions to be asked about the role of TGF-α in gingivitis. First, the origin of TGF-α should be clarified. Although we earlier demonstrated the human submandibular gland as a rich source of TGF-α[26] we have no idea as to the origin of this growth factor in the GCF. We speculate that macrophage may release TGF-α at the inflammation sites. Secondly, it is questionable whether or not the decreased level of TGF-α may trigger the progression of gingivitis. Although current cross-sectional study indicates lower levels of TGF-α in gingivitis, a longitudinal study would be required to confirm that TGF-α levels dropped are associated with the development/progression of gingivitis.

In accordance with our previous report[8] and other,[12] OPG values of the gingivitis patients were significantly lower than those for the controls ($p < 0.05$). As to why the positive correlation between TGF-α and OPG levels in the GCF samples, we have no definite idea at this time. In earlier studies, we found an increase in the RANKL:OPG ratio in the synovial fluid from patients with other bone-destructive diseases, such as osteoarthritis and rheumatoid arthritis.[6,8,10] Since OPG decrease ratios are not so high in the GCF of gingivitis patients in comparison with those in other rheumatoid and periodontal diseases,[6–8] this might explain why gingivitis is a relatively mild inflammative disease and not bone-destructive disease. Previous data taken together with our current findings suggest that inflammation has a common mechanism to induce an increase in the RANKL:OPG ratio in the body fluid.

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REFERENCES

1. Dashash, M.; Blinkhorn, A.S.; Hutchinson, I.V.; Pravica, V.; Drucker, D.B. The relationship between interleukin-10 gene polymorphism at position-1082 and susceptibility to gingivitis in children. J. Periodontol. **2005**, *76*, 1455–1462.
2. Suárez, L.J.; Ocampo, A.M.; Dueñas, R.E.; Rodríguez, A. Relative proportions of T-cell subpopulations and cytokines that mediate and regulate the adaptive immune response in patients with aggressive periodontitis. J. Periodontol. **2004**, *75*, 1209–1215.

3. Baylink, D.J.; Finkelman, R.D.; Mohan, S. Growth factors to stimulate bone formation. J. Bone Miner. Res. **1993**, *8*, S565–S572.
4. Derynck, R. Transforming growth factor α. Cell **1988**, *54*, 593–595.
5. Matsuda, N.; Lin, W.L.; Kumar, N.M.; Cho, M.I.; Genco, R.J. Mitogenic, chemotactic, and synthetic responses of rat periodontal ligament cells to polypeptide growth factors in vitro. J. Periodontol. **1992**, *63*, 515–525.
6. Kotake, S.; Udagawa, N.; Hakoda, M.; Mogi, M.; Yano, K.; Tsuda, E. Activated human T cells directly induce osteoclastogenesis from human monocytes: Possible role of T cells in bone destruction in rheumatoid arthritis patients. Arthritis Rheum. **2001**, *44* (5), 1003–1012.
7. Romas, E.; Gillespie, M.T.; Martin, T.J. Involvement of receptor activator of NFkappaB ligand and tumor necrosis factor-alpha in bone destruction in rheumatoid arthritis. Bone **2002**, *30* (2), 340–346.
8. Mogi, M.; Otogoto, J.; Ota, N.; Togari, A. Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with Periodontitis. J. Dent. Res. **2004**, *83*, 166–169.
9. Ohazama, A.; Courtney, J.M.; Sharpe, P.T. Opg, Rank, and Rankl in Tooth Development: Co-ordination of Odontogenesis and Osteogenesis. J. Dent. Res. **2004**, *83*, 241–244.
10. Wakita, T.; Mogi, M.; Kurita, K.; Kuzushima, M.; Togari, A. Increase in RANKL:OPG ratio in synovial of patients with TMJ disorder. J. Dent. Res. **2006**, *85* (7), 627–632.
11. Mogi, M.; Otogoto, J. Expression of cathepsin-K in gingival crevicular fluid of patients with periodontitis. Arch. Oral Biol. **2007**, *52*, 894–898.
12. Bostanci, N.; Ilgenli, T.; Emingil, G.; Afacan, B.; Han, B.; Töz, H. Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: Implications of their relative ratio. J. Clin. Periodontol. **2007**, *34* (5), 370–376.
13. Cimasoni, G. Crevicular fluid updated. Monographs Oral Sci. **1983**, *12* 24–28; 104–123.
14. Offenbacher, S.; Odle, B.M.; Van Dyke, T.E. The use of crevicular fluid PGE_2 levels as a predictor of periodontal attachment loss. J. Periodont. Res. **1986**, *21*, 101–112.
15. Preiss, D.S.; Meyle, J. Interleukin-1β concentration of gingival crevicular fluid. J. Periodontol. **1994**, *65*, 423–428.
16. Reinhardt, R.A.; Masada, M.P.; Kaldahl, W.B.; DuBois, L.M.; Kornman, K.S.; Choi, J.-I. Gingival fluid IL-1 and IL-6 levels in refractory periodontitis. J. Clin. Periodontol. **1993**, *20*, 225–231.
17. Syrjanen, S.M.; Alakuijala, P.; Markkanen, S.O.; Markkanen, H. Gingival fluid, beta 2-microglobulin and protein levels as indicators of periodontal disease. Scand. J. Dent. Res. **1989**, *97*, 500–504.
18. Mogi, M.; Otogoto, J.; Ota, N.; Inagaki, H.; Minami, M.; Kojima, K. Interleukin 1β, interleukin 6, β2-microglobulin, and transforming growth factor-α in gingival crevicular fluid from human periodontal disease. Arch. Oral Biol. **1999**, *44*, 535–539.
19. Uematsu, S.; Mogi, M.; Deguchi, T. Interleukin (IL)-1β, IL-6, tumor necrosis factor-α, epidermal growth factor, and β_2-microglobulin levels are elevated in

gingival crevicular fluid during human orthodontic tooth movement. J. Dent. Res. **1996**, *75*, 562–567.
20. Uematsu, S.; Mogi, M.; Deguchi, T. Transforming growth factor (TGF)-β1 are elevated in gingival crevicular fluid during orthodontic tooth movement. Arch. Oral Biol. **1996**, *41*, 1091–1095.
21. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **1976**, *72*, 248–254.
22. Johnson, R.B.; Streckfus, C.F.; Dai, X.; Tucci, M.A. Protein recovery from several paper types used to collect gingival crevicular fluid. J. Periodont. Res. **1999**, *34*, 283–289.
23. Mogi, M.; Harada, M.; Kondo, T.; Riederer, P.; Inagaki, H.; Minami, M. Interleukin-1β, interleukin-6, epidermal growth factor and transforming growth factor-α are elevated in the brain from parkinsonian patients. Neurosci. Lett. **1994**, *180*, 147–150.
24. Mogi, M.; Matsuura, S.; Suzuki, K.; Inagaki, H.; Minami, H.; Kojima, K. Differential expression of transforming growth factor-α and epidermal growth factor during postnatal development of rat submandibular gland. Biochem. Biophys. Res. Commun. **1995**, *217*, 271–277.
25. Mogi, M.; Inagaki, H.; Kojima, K.; Minami, M.; Harada, M. Transforming growth factor-α in human submandibular gland and saliva. J. Immunoassay **1995**, *16* (4), 379–394.
26. Kinpara, K.; Mogi, M.; Kuzushima, M.; Togari, A. Osteoclast differentiation factor in human osteosarcoma cell line. J. Immunoassay **2000**, *21* (4), 327–340.
27. Wright, H.J.; Chapple, I.L.C.; Matthews, J.B. Levels of TGFβ1 in gingival crevicular fluid during a 21-day experimental model of gingivitis. Oral Diseases **2003**, *9*, 88–94.
28. Orozco, A.; Gemmell, E.; Bickel, M.; Seymour, G.J. Interleukin-1β, interleukin-12 and interleukin-18 levels in gingival fluid and serum of patients with gingivitis and periodontitis. Oral Microbiol. Immunol. **2006**, *21*, 256–260.

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