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Exploring the role of Th1 cytokines: interleukin-17 and interleukin-18 in periodontal health and disease

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Abstract: There are conflicting data regarding the role of interleukin (IL)-17 in periodontal health and disease. However, IL-18 levels are known to increase with the severity of periodontal disease. The present study was performed to explore the role of these proinflammatory cytokines in periodontal disease progression, and also to clarify the effect of periodontal treatment on their concentration. Sixty age- and gender-matched subjects were divided into three groups each consisting of 20 subjects on the basis of gingival index (GI), probing pocket depth (PPD), clinical attachment loss (CAL) and radiological parameters (bone loss): healthy (group 1), gingivitis (group 2) and chronic periodontitis (group 3), while group 3 patients after treatment constituted group 4. GCF samples collected from all the groups were assayed by enzyme-linked immunosorbent assay to estimate the levels of IL-17 and IL-18. IL-18 levels in GCF increased proportionally with the severity of periodontal disease, and decreased after treatment. However, IL-17 levels in GCF were nearly zero. Since our data indicate an absence of IL-17 in GCF, it cannot be considered as a biomarker of periodontal disease progression, at least in Indian populations. However, IL-18 appears to be a good inflammatory biomarker. (J Oral Sci 51, 261-266, 2009)

Keywords: periodontal disease; cytokines; interleukin-17; interleukin-18; gingival crevicular fluid.

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Introduction

The incidence and progression rate of periodontal disease depends on complex interaction between periodonotopathic bacteria and cells of the host immune system (1-3). These interactions are mediated by cytokines and chemokines, which are produced by both resident and emigrant cells at the site of inflammation. Cells that produce cytokines include macrophages/monocytes, dendritic cells, lymphocytes, neutrophils, endothelial cells, and fibroblasts (4). Cytokines are central to the pathogenesis of an ever-increasing number of diseases, including periodontal disease. It has been postulated that "appropriate" cytokine production results in protective immunity, whereas "inappropriate" cytokine production leads to tissue destruction and disease progression (5). The balance between cellular and humoral responses, for example, is strongly regulated by the balance between Th1 and Th2 subsets (6). It is generally agreed that control of the Th1/Th2 balance is central to the immunoregulation of periodontal disease (7). It has also been suggested that stable periodontal lesions are mediated by Th1 cells, whereas lesion progression reflects a shift toward Th2 cells (5,8).

Interleukin-17 (IL-17) is a proinflammatory cytokine derived from T cells and produced mainly by cells of the Th1/Th0 phenotype but not cells of the Th2 phenotype (9). Recent findings have defined IL-17-producing cells as a new Th cell lineage, renamed Th17 (10). Th17 cells are highly proinflammatory and can mediate autoimmune diseases. It is proposed that induction of Th17 responses requires three distinct steps: induction, amplification and stabilization, whereby transforming growth factor (TGF)-β plus IL-6 induce differentiation of Th17 cells, IL-21 amplifies the frequency of Th17 cells, and IL-23 stabilizes the phenotype of previously differentiated Th17 cells. Loss of any of one of the members in this pathway (IL-6,

IL-21 or IL-23) severely limits the Th17 response (11,12). IL-17 has been shown to stimulate epithelial, endothelial and fibroblastic cells to produce IL-6, IL-8, and PGE2 (13). In addition, IL-17 induces receptor activator of nuclear factor kappa B ligand (RANKL) production by osteoblasts (14). It has been hypothesized that IL-17 is produced in periodontal lesions, which may be involved in Th1 modulation and enhance inflammatory reactions via gingival fibroblast-derived mediators in periodontal disease. IL-17 was found to be undetectable in gingival crevicular fluid (GCF) in periodontitis lesions (15). However, Vernal et al. showed that IL-17 was present at higher levels in the GCF and in supernatants from cellular cultures of gingival tissue from periodontitis patients than in those from healthy subjects, suggesting a role for IL-17 in the pathogenesis of chronic periodontitis (16). It was demonstrated that *Porphyromonas gingivalis* outer membrane protein (OMP) induced a significant increase in the production of IL-17 in periodontitis patients, and that after stimulation IL-17 was detected more frequently in patients with periodontitis than in those with gingivitis (17). Recently, Cardoso et al. demonstrated elevated levels of IL-17, TGF-β, IL-1β, IL-6, and IL-23 messenger RNA and protein in diseased tissues as well as the presence of Th17 cells in gingiva from patients with periodontitis. Moreover, IL-17 and the bone resorption factor RANKL were expressed abundantly in alveolar bone of diseased patients, in contrast to low expression in healthy controls (18).

Interleukin-18 (IL-18) is a proinflammatory cytokine of the IL-1 superfamily and was originally identified as interferon-γ (IFN-γ) inducing factor. It is unique in having a capacity to induce either Th1 or Th2 differentiation, depending on the immunological context (19). It is expressed by macrophages/monocytes and oral epithelial cells, and upregulated in various chronic diseases including periodontal disease. IL-18 could play an important role in gingival inflammation, as relatively high expression of IL-18 has been found in gingival samples with increasing sulcular depth (20). It has been shown that the levels of IL-18 in gingival crevicular fluid (GCF) are increased at sites of both gingivitis and periodontitis, suggesting an association between the severity of periodontal disease and the level of IL-18 (21). Recently, Figueredo et al. reported that levels of IL-18 were higher in patients with chronic periodontitis than in those with gingivitis, even at sites with similar pocket depths (22).

To date, no study has investigated IL-17 and IL-18 levels in GCF in diseased or healthy periodontal tissue before and after periodontal therapy, i.e. scaling and root planning (SRP). In this context, the present study was designed to assess the role of these cytokines in periodontal disease progression and also to clarify the effect of periodontal treatment on their concentrations.

Materials and Methods

The study population comprised 60 subjects (30 males, 30 females) aged between 23 and 49 years attending the outpatient section of the Department of Periodontics, Government Dental College and Research Institute, Bangalore. Approval for the study was obtained from the institutional ethics committee. The patients were given an explanation of the study procedure, and written informed consent was obtained from those who agreed to participate voluntarily in this study. Exclusion criteria included: a history of smoking, systemic diseases such as diabetes mellitus, rheumatoid arthritis, inflammatory bowel disease, pulmonary disease, multiple sclerosis, obesity, bacterial, viral and fungal systemic infections, aggressive periodontitis, and subjects who had received periodontal therapy, antibiotics or non-steroidal anti-inflammatory drugs within preceding 6 months.

Each subject underwent full-mouth periodontal probing and charting, along with periapical radiographs using the long-cone technique. Radiographic bone loss was recorded to differentiate chronic periodontitis (CP) patients from other groups. Furthermore, no delineation was attempted within the chronic periodontitis group based on the extent of alveolar bone loss. Based on the gingival index (GI) (23), probing pocket depth (PPD), clinical attachment loss (CAL) and radiograph evidence of bone loss, the subjects were categorized into three groups. Group 1 (Healthy) comprised 20 subjects with clinically healthy periodontium, with GI ≤ 1, CAL = 0 mm, and PPD ≤ 3 mm. Group 2 (Gingivitis) comprised 20 subjects who showed clinical signs of gingival inflammation, GI > 1, without any attachment loss, and PPD ≤ 3 mm. Group 3 (Chronic periodontitis) comprised 20 subjects who had signs of clinical inflammation, GI > 1, CAL ≥ 3 mm with radiographic evidence of bone loss, and PPD ≥ 4 mm. Patients with chronic periodontitis (group 3) were treated with a non-surgical approach, i.e., SRP and GCF samples collected from the same sites 6 to 8 weeks after treatment to constitute group 4 (post-treatment group).

Site selection and GCF collection

All the clinical and radiological examinations, group allocation and sampling site selection were performed by one examiner (ARP) and the samples were collected on the subsequent day by second examiner (PH) or (SC) to prevent contamination of GCF with blood associated with probing of inflamed sites on day 1. The subjects were seated comfortably in an upright position on the dental chair, and

the area to be examined was well illuminated. A sterile mouth mirror and a University of North Carolina periodontal probe (UNC 15, Hu-Friedy Chicago, IL, USA) were used to clinically examine the periodontal status. The test site for GCF sample collection was selected based on the highest scored sites in the oral cavity i.e., the site showing most severe inflammatory signs (in gingivitis cases) or greatest amount of attachment loss (in chronic periodontitis cases) along with radiographic confirmation of alveolar bone loss, and the same test site was selected for the after-treatment group. In the healthy group, to ensure adequate volume, GCF was pooled from multiple sites with no inflammatory signs. On the subsequent day, after gently drying the area with a blast of air, supragingival plaque was removed without touching the marginal gingiva. The area was isolated using cotton rolls to prevent saliva contamination, and GCF was collected by placing the microcapillary pipettes at the entrance of the gingival sulcus, and gently touching the marginal gingiva. From each test site, a standardized volume of 1 µl was collected using the calibration on white color-coded 1-5-µl calibrated volumetric microcapillary pipettes [Sigma-Aldrich Chemical Company, USA (Catalog no. p 0549)] using an extracrevicular approach ("unstimulated"). Each sample collection was allotted a maximum of 10 minutes, and some test sites in the healthy group that did not express any volume of GCF within the allotted time were excluded from the study. Furthermore, any micropipettes that were suspected to be contaminated with blood and saliva were also excluded. The GCF collected was immediately transferred to an airtight plastic vial and stored at -70°C until assay.

IL-17 and IL-18 assay

The samples were assayed for IL-17 levels using a Human IL-17 ELISA Kit [Quantikine R&D Systems, MN, USA (Catalog No. D1700)] and for IL-18 levels using a Human IL-18 ELISA Kit [Bender MedSystemsTM, Vienna, Austria (Catalog No. BMS 267/2)] in accordance with the manufacturer's instructions. Absorbance of each well was read on the ELISA reader using 450 nm and 405 nm as the primary wavelength for IL-17 and IL-18, respectively. The concentrations of IL-17 and IL-18 in the tested samples were estimated using the standard curve.

Statistical analysis

Statistical analysis was performed using the SPSSPC/ Windows version 10.5 software package (SPSS Inc., Chicago, IL, USA). Parametric tests were carried out for comparing the means of IL-18 concentration in different groups. Paired *t*-test was used to compare cytokine concentrations in GCF in groups 3 and 4. Pairwise comparison using Duncan's test for GCF cytokines was carried out to explore pairs that differed significantly at $P < 0.05$. Pearson's correlation test was used to observe any correlation between the GCF cytokine concentrations and clinical parameters.

Results

The descriptive data for concentrations of IL-17 and IL-18 in groups 1, 2 and 3 are shown in Table 1. The results indicated that the interleukin-17 concentration in GCF was close to 0 pg/µl in all groups. The mean IL-18 concentration in GCF was highest in group 3, i.e. 450.54 pg/µl, and lowest in group 1, i.e. 26.68 pg/µl. The mean concentration in GCF in group 2 (93.34 pg/µl) and that in group 4 (89.09 pg/µl) fell between the highest and the lowest values. The results of ANOVA showed that the difference in levels of IL-18 amongst these groups was statistically significant at $P < 0.05$. Patients with chronic periodontitis showed a significant decrease in IL-18 levels after the treatment (Table 2). The results suggested that IL-18 levels increased in GCF from a healthy state to periodontitis, and further as the severity of the disease increased. Pairwise comparison showed that these differences were statistically significant between Groups 1 and 2, Groups 1 and 3, and Groups 2 and 3 ($P < 0.05$) (Table 1). IL-18 levels in GCF were positively correlated with PPD and CAL in Groups 3 and 4 (Table 3).

Table 1 Descriptive statistics of the study population showing mean, standard deviation, GI, CAL, PPD, IL-17 and IL-18 levels in GCF

Study Group	GI ± SD	PPD ± SD (mm)	CAL ± SD (mm)	Mean IL-17 ± SD (pg/µl)	Mean IL-18 ± SD (pg/µl)	Pair-wise comparison using Duncan's test for IL-18
Group 1	0.31 ± 0.03	1.6 ± 0.52	0	0.00 ± 0.15	26.69 ± 12.76	Group 1 Vs 2*
Group 2	1.63 ± 0.18	2.4 ± 0.52	0	0	93.34 ± 46.03	Group 2 Vs 3*
Group 3	2.03 ± 0.24	5.3 ± 0.94	3.4 ± 1.07	0	450.54 ± 276.83	Group 1 Vs 3*

*Statistically significant ($P < 0.001$)

Table 2 Paired *t*-test to compare IL-18 concentrations in GCF in group 3 and group 4

Study Group	GI ± SD	PPD ± SD (mm)	CAL ± SD (mm)	Mean IL-17 ± SD (pg/µl)	Mean IL-18 ± SD (pg/µl)	paired *t*-test for IL-18 *P*-value
Group 3	2.03 ± 0.24	5.3 ± 0.94	3.4 ± 1.07	0	450.54 ± 276.83	0.001*
Group 4	0.82 ± 0.23	3 ± 0.66	1.8 ± 0.78	0.03 ± 0.12	89.09 ± 66.69	

* Statistically significant

Table 3 Pearson's correlation coefficient test comparing GCF IL-18 and other variables

Groups	IL-18 and GI	IL-18 and PPD	IL-18and CAL
Group 1	-0.025	0.376	---
Group 2	0.153	-0.242	---
Group 3	-0.018	0.771*	0.87*
Group 4	-0.286	0.725*	0.775*

* Statistically significant

Discussion

Investigations into the etiology of periodontal disease have suggested that infection by one or more virulent bacteria, interplay of host factors, or an interaction of both may be responsible. These interactions are mediated by cytokines produced by heavy lymphocytic infiltration into periodontal tissues, and these cytokines represent an important component of the immune response to bacterial lipopolysaccharides.

IL-17, produced mainly by cells with a Th0/Th1 profile, is a proinflammatory cytokine. IL-17 may affect osteoclastic bone resorption by stimulating osteoblasts to produce factors that affect the activity and/or formation of osteoclasts; osteoblasts are IL-17-responsive cells and express mRNA encoding the IL-17 receptor (24). Hence it is hypothesized that T cells in periodontal tissues produce and exacerbate inflammatory periodontal disease, activating gingival fibroblasts to produce inflammatory mediators and can playing a role in bone cell metabolism via T-cell-derived cytokines (15).

IL-18 may be one of the cytokines responsible for initiation and progression of periodontal destruction. Previous data indicate that IL-18 induces release of matrix metalloproteinase (MMP)-9 and IL-1β, which both have proinflammatory and tissue degradation effects (25). Since these events also occur in chronic periodontal inflammation, it seems worthwhile to evaluate IL-18 levels in normal and diseased periodontium.

The present study demonstrated that the total concentration of cytokine IL-17 in GCF of all subjects in the different groups was close to 0 pg/µl. This is contrary to the findings of Vernal et al. in a Chilean population, who showed a higher total amount of IL-17 in GCF in subjects with periodontitis than in healthy individuals (16). However, our results were in accord with a previous study by Takahashi et al., who also failed to detect IL-17 in GCF samples from Japanese patients with periodontitis (15). Recently it was found that endothelin-1 was also not detectable in GCF samples from the Indian population (26).

There are several possible explanations for the failure to detect IL-17 in GCF.

- IL-17 produced by activated T cells in periodontal lesions could have been bound to IL-17 receptor-positive cells.
- IL-17 may be degraded by both bacteria and host cell-derived proteases in the gingival crevice, as is the case for IgG A1 (27).
- The amount of IL-17 in GCF may be relatively low. Hence, more sensitive ELISA kits should be developed for estimation of IL-17 than those used in the present and previous studies, which have a sensitivity of 15 pg/ml.
- IL-17 was detected in a Chilean population but was undetectable in a Japanese population. The results for our (Indian) population were in accordance with the latter, and could be explained on the basis of a heterogeneous response due to racial variation in the study populations, as has been reported for other cytokines (26,28). Thus, it can be postulated that expression of IL-17 shows racial variation.

Also, the improvement in clinical parameters (GI, PPD and, CAL) in the present study could not have been correlated with IL-17 levels, in view of the absence of IL-17 in GCF. However, further studies should be conducted using a larger sample size or by testing subjects with different types of periodontitis, or by examining the mRNA expression of different forms of IL-17 (A-F).

The present study showed that the levels of IL-18 in GCF increased with the severity of periodontal disease. In addition, the levels of IL-18 were found to decrease significantly after periodontal therapy (SRP) in the chronic periodontitis group. The present study is the first to have investigated the concentrations of IL-18 in GCF in both periodontal health and disease, and to have assessed the effect of non-surgical periodontal therapy on IL-18 concentration. The influence of subject age and gender on the concentrations of IL-17 and IL-18 was minimized/ nullified by selecting subjects within a fairly narrow age

range of 23 – 49 years and including an equal number of males and females in each group. Our study comprised four groups (healthy, gingivitis, chronic periodontitis and chronic periodontitis after treatment), and in this respect differed from previous studies (21,22) that examined only subjects with gingivitis and periodontitis. Those studies also did not evaluate the effect of non-surgical periodontal therapy (SRP) on IL-18 levels in GCF, which might have further confirmed the role of IL-18 in periodontal disease. GCF was collected using microcapillary pipettes to avoid non-specific attachment of the analyte to filter paper fibers, which would have falsely reduced the levels of detectable cytokines, leading to underestimation of the correlation between IL-18 levels and disease severity/progression (29). The only disadvantage of this method is the possibility of trauma to the marginal gingiva, and therefore utmost care was taken to avoid this during GCF collection. The microcapillary pipette was placed at the entrance of the gingival crevice until it gently touched the gingival margin, and the collection time was restricted to no more than 10 min.

Our study demonstrated that the IL-18 concentration in GCF increased proportionally with the progression of periodontal disease. When GCF IL-18 values were considered, our results were in accord with those of previous studies (21,22). Additionally, we found a significant reduction in the levels of IL-18 in GCF, and there was a positive correlation among GCF IL-18 levels in PPD and CAL in Groups 3 and 4. The variability of IL-18 concentration among the patients in each group can be attributed to differences in disease stage at the time of GCF sampling. A few samples in group 2 (gingivitis) showed values close to those in group 3 (chronic periodontitis), which could have been attributable to near-conversion of gingivitis lesions to chronic periodontitis that was not clinically detectable. Furthermore, there was a significant overlap between the gingivitis group and the post-treatment (chronic periodontitis) group, due possibly to individual variation in the resolution of periodontitis after treatment.

Elevated plasma IL-18 concentrations have been associated with poor clinical outcome in severe inflammatory and septic conditions. For this reason, IL-18 has been proposed as a marker for monitoring severe inflammatory conditions and, in particular, suspected Gram-positive sepsis (30). Similarly, periodontal inflammation may not be resolved successfully if IL-18 accumulates. Furthermore, treatment aimed at arresting periodontal disease progression resulted in a statistically significant reduction of IL-18 concentration in GCF.

Since our data revealed an absence of IL-17 in GCF, it cannot be considered a biomarker of periodontal disease progression, at least in the populations we studied. However, controlled longitudinal prospective studies involving a larger population and using more solid-phase assays should be done to confirm this conclusion. On the other hand, the relatively high levels of IL-18 in GCF in patients with periodontal disease confirm that it is an important factor involved in periodontal destruction. Thus, IL-18 can be considered an inflammatory biomarker of periodontal disease and deserves further consideration in the development of methods for prevention and therapy.

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References

1. Payne WA, Page RC, Ogilvie AL, Hall WB (1975) Histopathological features of the initial and early stages of experimental gingivitis in man. J Periodontal Res 10, 51-64
2. Page RC (1986) Gingivitis. J Clin Periodontol 13, 345-355.
3. Schroeder HE, Münzel-Pedrazzoli S, Page RC (1973) Correlated morphometric and biochemical analysis of early chronic gingivitis in man. Arch Oral Biol 18, 899-923.
4. Abbas AK, Lichtman AH (2003) Cellular and molecular immunology. 5th ed, Saunders, Philadelphia.
5. Gemmell E, Seymour GJ (2004) Immunoregulatory control of Th1/Th2 cytokine profiles in periodontal disease. Periodontol 2000 35, 21-41.
6. Orozco A, Gemmell E, Bickel M, Seymour GJ (2007) Interleukin 18 and Periodontal Disease. J Dent Res 86, 586-593.
7. Belardelli F, Ferrantini M (2002) Cytokines as a link between innate and adaptive antitumor immunity. Trends Immunol 23, 201-208.
8. Seymour GJ, Taylor JJ (2004) Shouts and whispers: an introduction to immunoregulation in periodontal disease. Periodontol 2000 35, 9-13.
9. Aarvak T, Chabaud M, Moossec P, Natvig JB (1999) IL-17 is produced by some pro inflammatory Th1 cell /Th0 cells but not by Th2 cells. J Immunol 162, 1246-1251.
10. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT (2005) Interleukin 17-producing CD4+ effector T cells

develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 6, 1123-1132.
11. Bettelli E, Korn T, Kuchroo VK (2007) Th17: the third member of the effector T cell trilogy. Curr Opin Immunol 19, 652-657.
12. Weaver CT, Hatton RD, Mangan PR, Harrington LE (2007) IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu Rev Immunol 25, 821-852.
13. Fossiez F, Djoussou O, Chomarat P, Flores-Remo L, Ait-Yahia S, Maat C, Pin JJ, Garrone P, Garcia E, Saeland S, Blanchard D, Gaillard C, Das Mahapatra B, Rouvier E, Golstein P, Banchereau J, Lebecque S (1996) T cell interleukin-17 induces stromal cells to produce proinflammatory and heamtopoeitic cytokines. J Exp Med 183, 2593-2603.
14. Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, Saito S, Inoue K, Kamatani N, Gillespie MT, Martin TJ, Suda T (1999) IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest 103, 1345-1352.
15. Takahashi K, Azuma T, Motohira H, Kinane DF, Kitetsu S (2005) The potential role of interleukin-17 in the immunopathology of periodontal disease. J Clin Periodontol 32, 369-374.
16. Vernal R, Dutzan N, Chaparro A, Puente J, Antonieta Valenzuela M, Gamonal J (2005) Levels of interleukin-17 in gingival crevicular fluid and in supernatants of cellular cultures of gingival tissue from patients with chronic periodontitis. J Clin Periodontol 32, 383-389.
17. Oda T, Yoshie H, Yamazaki K (2003) *Porphyromonas gingivalis* antigen preferentially stimulates T cells to express IL-17 but not receptor activator of NF-κB ligand in vitro. Oral Microbiol Immunol 18, 30-36.
18. Cardoso CR, Garlet GP, Crippa GE, Rosa AL, Júnior WM, Rossi MA, Silva JS (2009) Evidence of the presence of T helper type 17 cells in chronic lesions of human periodontal disease. Oral Microbiol Immunol 24, 1-6.
19. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H (2001) Interleukin-18 is a unique cytokine that stimulates both Th1 and Th2 responses depending on its cytokine milieu. Cytokine Growth Factor Rev 12, 53-72.
20. Johnson RB, Serio FG (2005) Interleukin-18 concentrations and the pathogenesis of periodontal disease. J Periodontol 76, 785-790.
21. Orozco A, Gemmell E, Bickel M, Seymour GJ (2006) Interleukin-1β, interleukin-12 and interleukin-18 levels in gingival crevicular fluid and serum of patients with gingivitis and periodontitis. Oral Microbiol Immunol 21, 256-260.
22. Figueredo CM, Rescala B, Teles RP, Teles FP, Fischer RG, Haffajee AD, Socransky SS, Gustafsson A (2008) Increased interleukin-18 in gingival crevicular fluid from periodontitis patients. Oral Microbiol Immunol 23, 173-176.
23. Loe H, Silness J (1963) Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 21, 533-551
24. Van bezooijen RL, Farih-Sips HC, Papapoulos SE, Löwik, CW (1999) Interleukin-17: a new bone acting cytokine in vitro. J Bone Min Res 14, 1513-1521.
25. Jabłońska E, Izycka A, Wawrusiewicz N (2002) Effect of IL-18 on IL-1beta and sIL-1RII production by human neutrophils. Arch Immunol Ther Exp (warsz) 50, 139-141.
26. Pradeep AR, Guruprasad CN, Swati P, Shikha C (2008) Crevicular fluid endothelin-1 levels in periodontal health and disease. J Periodontal Res 43, 275-278.
27. Takahashi K, Mooney J, Frandsen EV, Kinane DF (1997) IgG and IgA subclass mRNA-bearing plasma cells in periodontitis gingival tissue and immunoglobulin levels in the gingival crevicular fluid. Clin Exp Immunol 107, 158-165.
28. Kinane DF, Winstanley FP, Adonogianaki E, Moughal NA (1992) Bioassay of interleukin-1 (IL-1) in human gingival crevicular fluid during experimental gingivitis. Arch Oral Biol 37, 153-156.
29. Griffiths GS (2003) Formation, collection and significance of gingival crevicular fluid. Periodontol 2000 31, 32-42.
30. Tschoeke SK, Oberholzer A, Moldawer LL (2006) Interleukin-18: a novel prognostic cytokine in bacteria-induced sepsis. Crit Care Med 34, 1225-1233.