Journal of Dentistry (2004) **32**, 511-520

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Journal of Dentistry

www.intl.elsevierhealth.com/journals/jden

Effect of periodontal therapy on crevicular fluid interleukin-1β and interleukin-10 levels in chronic periodontitis

Paschalina Goutoudi[a,*], Evdoxia Diza[b], Malamatenia Arvanitidou[c]

[a]*Department of Preventive Dentistry, Periodontology and Implant Biology, Dental School, Aristotle University, Thessaloniki, Greece*
[b]*Laboratory of Microbiology, Medical School, Aristotle University, Thessaloniki, Greece*
[c]*Laboratory of Hygiene, Medical School, Aristotle University, Thessaloniki, Greece*

Received 10 February 2004; received in revised form 21 April 2004; accepted 21 April 2004

KEYWORDS
Interleukin-1β;
Interleukin-10;
Crevicular fluid;
Cytokines; Periodontal therapy

Summary *Objectives*. This study aimed to analyse the levels of the proinflammatory cytokine IL-1β and the anti-inflammatory cytokine IL-10 in gingival crevicular fluid (GCF) of patients with chronic periodontitis prior to, and following, periodontal therapy for a period of 32 weeks.

Material and methods. GCF samples were obtained from 24 non-diseased and 72 diseased sites of 12 periodontal patients prior to as well as at 6, 16 and 32 weeks post-periodontal therapy. All sites received conventional periodontal treatment and IL-1β and IL-10 levels (concentration and total amount) were determined by enzyme linked immunosorbent assay (ELISA). Additionally, probing pocket depth (PD), clinical attachment loss (CAL), gingival (GI) and plaque (PlI) indices were evaluated pre-and post-therapy.

Results. IL-1β was detected in 382 out of 384 samples, while IL-10 was detected in 337 out of 384 samples. The total amount of IL-1β was significantly higher at diseased compared to non-diseased sites ($p < 0.01$). Following therapy, IL-1β total amounts were reduced, while IL-1β concentration gradually increased. IL-10 total amounts (per 30 s sample) were similar in diseased and non-diseased sites, and following therapy they remained almost unchanged. By contrast, IL-10 concentration was significantly higher in non-diseased sites ($p < 0.01$) and displayed a significant increase post-therapy. Moreover, IL-1β concentration and total amount were significantly greater in smokers following therapy, while IL-10 total amount was significantly higher in non-smokers both prior to and following therapy. Total IL-1β amounts were positively correlated with GI and PlI. A weak negative corretation between IL-1β and IL-10 levels was noted ($p > 0.05$).

Conclusions. The data suggest that the total amount rather than the concentration of IL-1β in GCF seemed to be closely associated with periodontal disease severity. Moreover, smoking status influenced IL-1β and IL-10 levels. An inverse relationship between IL-1β and IL-10 was evident.
© 2004 Elsevier Ltd. All rights reserved.

*Corresponding author. Address: 70 Tsimiski Str., Thessaloniki 54622, Greece. Tel.: +30-2310-279-560.
E-mail address: sferopoulos@in.gr (P. Goutoudi).

0300-5712/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jdent.2004.04.003

Introduction

In chronic periodontitis, the interplay between periodontal pathogens and the host inflammatory–immune system is responsible for the destruction of connective tissues, loss of periodontal attachment and resorption of alveolar bone.

Several inflammatory and immune mediators implicated in periodontal destruction have been identified in gingival crevicular fluid (GCF). Among these are cytokines, which are cellular immune-response indicators induced by periodontopathic bacteria that are intimately involved in a variety of biological responses seen in periodontal disease. Many investigations have focused on the proinflammatory cytokine interleukin-1β (IL-1β) due to its pleiotropic role in the initiation and development of periodontitis. IL-1β is a glucoprotein of 17 KDa[1] which is structurally related to interleukin-1α (IL-1α), the other form of IL-1 that has been identified. Both forms possess proinflammatory properties, with IL-1β being the more potent.[2] Additionally, IL-1β is usually produced at 10–50 fold higher levels than IL-1α.[2] IL-1β is produced predominantly by monocytes/macrophages[3,4] but also by fibroblasts and bone cells.[5] Its production may be induced by microorganisms, microbial products, inflammatory agents and antigens.[6] Some of its biological effects include stimulation of T-lymphocytes and lymphokine production,[7] proliferation of B-lymphocytes and antibody production,[8] fibroblast proliferation, stimulation of prostaglandin (PGE_2) release by monocytes and fibroblasts, and release of metalloproteinases that degrade extracellular matrix proteins.[9] IL-1β also promotes osteoclast formation and bone resorption,[10] and it affects neutrophil chemotaxis and activation[11,12] and endothelial cell function.

In periodontitis patients, increased IL-1β levels have been reported in both GCF[13,14] and periodontal tissues.[15,16] The amounts of IL-1β have been closely associated with periodontal disease severity,[17] while according to Masada et al.[13] IL-1β may serve as a marker of periodontal tissue destruction. Kornman and co-workers[18] demonstrated an association between a specific composite genotype of the IL-1 gene cluster and periodontal disease severity, while in a more recent study[19] it was shown that among patients of similar disease severity those with the periodontitis associated genotype demonstrated elevated levels of IL-1β in GCF and gingival tissues.

Interleukin-10 (IL-10) is also a pleiotropic cytokine known for its immunosuppressive properties.[20] It is produced by T-cells, B-cells, monocytes/macrophages after activation.[21] IL-10 has been implicated in the suppression of tissue destruction. The synthesis of monocyte-derived proinflammatory cytokines, including IL-1, IL-6, IL-8 and TNF, is inhibited by IL-10, which also enhances the production of the IL-1 receptor antagonist (IL-1ra) in polymorphonuclear leukocytes stimulated with lipopolysaccharide (LPS).[22,23] Therefore, it was suggested that IL-10 might have an important regulatory role in limiting the duration and extent of the acute inflammatory response.[22] IL-10 suppresses the production of metalloproteinases, while increasing the synthesis of tissue inhibitors of metalloproteinases in macrophages.[24] In addition, it has been suggested that IL-10 suppresses the differentiation of cells with resorptive function in hemopoietic cell cultures, representing an important matrix protective cytokine during inflammation.[25] Stein and Hendrix[26] suggested that gingival mononuclear cells extracted from adult periodontitis patients constitutively produced more IL-10 than gingival mononuclear cells derived from non-inflamed tissues. However, Gemell and Seymour [27] found a lower percentage of gingival IL-10 + CD8 cells exctracted from adult periodontitis lesions compared to healthy tissues. Furthermore, they suggested that the presence of a high percentage of IL-10 + T-cells might indicate a stable lesion, while a low percentage of IL-10 + T-cells might indicate a progressive lesion.

The purpose of this study was to examine GCF concentrations and total amounts of these proinflammatory (IL-1β) and antiinflammatory (IL-10) cytokines in periodontal patients and to investigate the longitudinal effect of periodontal therapy on GCF IL-1β and IL-10 levels of periodontal sites with varying degrees of periodontal destruction and inflammation.

Materials and methods

Patients with chronic periodontitis were recruited into this randomised, longitudinal, split-mouth, interventional study, from patients referred to the Department of Periodontology, Aristotle University of Thessaloniki. All of them were Caucasians. The selection criteria were:

- Patients aged 35–65 years for males and 35–45 years for females.
- Pregnant or lactating females were excluded. Post-menopausal females or others on estrogen therapy were excluded.[28]
- Good general health with no history of systemic disease.

- No medication was taken.
- No periodontal therapy received in the preceding 1 year.
- More than 20 remaining teeth.
- Moderate to advanced periodontal disease as evidenced by multiple sites with a probing depth of 5 mm or more, extensive radiographic bone loss and bleeding on gentle probing.

Informed consent was obtained from each patient prior to enrolment in the study, and ethical approval was obtained from the Aristotle University of Thessaloniki ethics committee.

In each patient, two quadrants of either the mandible or maxilla were randomly assigned as experimental. In each experimental quadrant, 4 periodontal sites in single-rooted teeth were selected. Three sites displayed probing depths (PD) $\geq$5 mm and a gingival index (GI)[29] of 2 or 3 and were defined as diseased sites and 1 site with PD $\leq$ 3 mm and GI = 0 or 1 was defined as a non-diseased control site. A total of 96 test sites were included in the study, 72 of them as diseased and 24 as non-diseased sites. Sites in one experimental quadrant received conventional periodontal treatment consisting of oral hygiene insctructions, scaling and root surface debridement, while the contralateral sites received concentional followed by surgical periodontal treatment, using a modified Widman flap.[30] At 6, 16 and 32 weeks following treatment the dentition received supragingival polishing with a rubber cup and pumice.

Prior to as well as 6,16 and 32 weeks following periodontal therapy, a GCF sample was taken from each test site, and IL-1β and IL-10 were quantified. The following clinical measurements were also evaluated: (1) plaque index (PlI), according to Silness and Löe,[31] (2) gingival index (GI), according to Löe,[29] (3) probing pocket depth (PD) and (4) clinical attachment loss (CAL), to the nearest millimetre with a Williams probe. The PD score in each site was evaluated in duplicate and mean values were, then, recorded. The same examiner performed all measurements. In all patients, individual acrylic stents were fabricated with reference grooves as reference points for the above clinical measurements and for GCF sampling.

Patients were all asked about their smoking habits and were classified as smokers or never-smokers. Former smokers, i.e. patients who had stopped the habit, were not included in this study.

GCF sampling

Both experimental quadrants were isolated with cotton rolls, and clinically detectable supragingival plaque was removed using a curette without touching the marginal gingiva. Sites were gently dried with an air syringe, and a sterile paper strip (Periopaper, OraFlow, Plain View, NY, 11803, USA) was inserted into the gingival crevice, until mild resistance was felt, and was kept there for 30 s. Strips contaminated by bleeding were discarded. The amount of GCF collected was quantitated using Periotron 6000 (Siemens Medical Systems, Inc., Iselin, NJ, USA), which had been calibrated with 1:5 diluted serum.[32] Each paper strip was placed into a coded sealed plastic tube containing 250-μl phosphate buffered saline (PBS). The samples were left at 4 °C for 2 h and, then, they were frozen at −70 °C and stored until cytokine analysis.

Enzyme linked immunosorbent assay (ELISA)

GCF was eluted from each filter paper strip into PBS as follows: before the IL-1β and IL-10 assays were performed, samples were left at 4 °C for 2 h. Then, each strip was lifted to the surface of the eluent, and another 350 μl of PBS was added to the strip (600 μl final volume). Samples were then, refrigerated at 4 °C for another 20 min and centrifuged at 10,000 rpm for 10 min. Finally, the strips were discarded.

Commercial ELISA Kits (R and D systems, Abingdon, Oxon, UK) were used to analyse IL-1β and IL-10 levels. The kit employs a quantitative 'sandwich' enzyme immunoassay technique. A murine anti-human monoclonal antibody specific for IL-1β or IL-10 was pre-coated onto a 96-well microplate. All reagents were brought to room temperature, and assay diluent (50 μl) was added to each well. Then, 200 μl of the standard or the sample was added and the plate was incubated at room temperature for 2 h. Any IL-1β or IL-10 present was bound by the immobilised antibody. After washing of unbound proteins, an enzyme-linked (horseradish peroxidase) polyclonal antibody (200 μl) specific for IL-1β or IL-10 (goat anti-human) was added to each well. The plate was incubated at room temperature for 2 h, and the wells re-washed. Then, 200 μl of a substrate solution was added and any colour developed was proportional to the amount of IL-1β or IL-10, respectively, bound in the initial step. After a 20 or 30 min incubation, a stop solution (sulfuric acid) was added and the reaction arrested. The intensity of the colour (optical density) was measured using a microplate reader at 450 nm (wavelength correction set to 540 nm) within 30 min. A standard curve was prepared by plotting the concentration of the IL-1β (standards 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9 and 0 pg/ml) or the concentration of the IL-10 standards (500, 250,

125, 62.5, 31.2, 15.6, 7.8 and 0 pg/ml) against their optical density and the concentration of IL-1β or IL-10, respectively, was determined. Then, the pg of IL-1β or IL-10 in each sample (total amount) were calculated and the IL-1β or IL-10 concentration (pg/μl) was determined by dividing the amount of IL-1β or IL-10 by the GCF volume (μl). The ELISA assays were run in duplicate, and mean values were used to calculate total amounts and concentrations of each cytokine. The minimum detectable level of IL-1β (sensitivity of ELISA) was typically less than 1 pg/ml, while the minimum detectable level of IL-10 was less than 2 pg/ml.

Statistical analysis

Data analysis was performed using the statistical package SPSS ver. 7.5 (1997, SPSS Inc., Chicago Illinois, USA). For all intervals, the mean cytokine and clinical data for the two healthy sites and similarly the mean data for cytokines and clinical measurements from 6 diseased sites in each patient were used for the purposes of analysis. Negative samples were considered zero for the calculations. Differences in cytokine levels between diseased and non-diseased sites as well as between smokers and non-smokers were evaluated by the Mann–Whitney Wilcoxon Rank Sum *W* test. In each case the level of significance was set at $p < 0.05$. Comparison of the clinical measurements prior to and following therapy was performed by student *t*-test. Paired samples *T*-test (except PlI and GI) was used to investigate the differences in GCF volume and in GCF IL-1β or IL-10 levels before as well as 6,16 and 32 weeks following periodontal treatment. Finally, the Kendall's correlation coefficient was used to study the correlation between IL-1β, IL-10 levels and clinical parameters, while a Bonferroni correction was also made.

Results

Twelve volunteers (7 females and 5 males, mean age 45.4 years) took part in this study.

Frequency of detection. Interleukin-1β was detected in all GCF samples with the exception of two samples taken from non-diseased sites 6 weeks following therapy (22/24, or 91.66% of sites).

At baseline, interleukin-10 was detected in GCF samples from 61/72 diseased sites (84.72%) and from 19/24 non-diseased sites (79.16%). The respective values following therapy were: 63/72 (87.5%) and 20/24 (83.33%) at the 6th week, 59/72 (81.94%) and 22/24 (91.66%) at the 16th week, while the frequency of detection was higher at the 32nd week of maintenance 70/72 (97.22%) and 23/24 (95.83%), respectively.

GCF volumes. The GCF volumes, expressed in μl, of 72 diseased and 24 non-diseased sites both prior to and following therapy are presented in Table 1. Mean GCF values were significantly higher in diseased compared to non-diseased sites ($p < 0.01$ at baseline, $p < 0.05$ following therapy). Periodontal therapy resulted in a significant decrease in GCF volume in both diseased and non-diseased sites ($p < 0.01$ and $p < 0.05$, respectively).

Concentration and total amount of IL-1β and IL-10. The concentration (IL-1β, IL-10) and the total amount (TAIL-1β, TAIL-10) of interleukin-1β and -10 in GCF expressed in pg/μl and pg/30 s, respectively, are presented in Fig. 1. Mean TAIL-1β values were significantly higher in diseased compared to non-diseased sites ($p < 0.01$), while there was no significant difference between diseased and non-diseased sites concerning IL-1β concentration. Following therapy, TAIL-1β levels were reduced (significantly at 6 weeks, $p < 0.01$), while IL-1β concentration was gradually increased (significantly at 6 weeks, $p < 0.01$). IL-10 concentration was significantly higher in non-diseased compared to diseased sites ($p < 0.01$), while mean TAIL-10 was similar in diseased and non-diseased sites. Periodontal treatment resulted in a significant increase in IL-10 ($p < 0.05$ at 6 weeks), while TAIL-10 levels displayed a slight decrease during the first 6 weeks post-therapy followed by a non-significant increase at 32 weeks.

Both treatment modalities resulted in similar IL-1β as well as IL-10 levels.

Clinical parameters. In diseased sites, periodontal treatment lead to improvements in all clinical

Table 1 GCF volume (μl) of diseased and non-diseased sites in 12 periodontal patients prior to and following therapy.

Periodontal sites	Baseline (mean ± SD)	6 weeks (mean ± SD)	16 weeks (mean ± SD)	32 weeks (mean ± SD)
Diseased	0.186 ± 0.112**	0.05 ± 0.023*	0.032 ± 0.024*	0.036 ± 0.019*
Non-diseased	0.033 ± 0.017	0.014 ± 0.011	0.016 ± 0.012	0.013 ± 0.009

** $p < 0.01$ between diseased and non-diseased sites; * $p < 0.05$ between diseased and non-diseased sites.

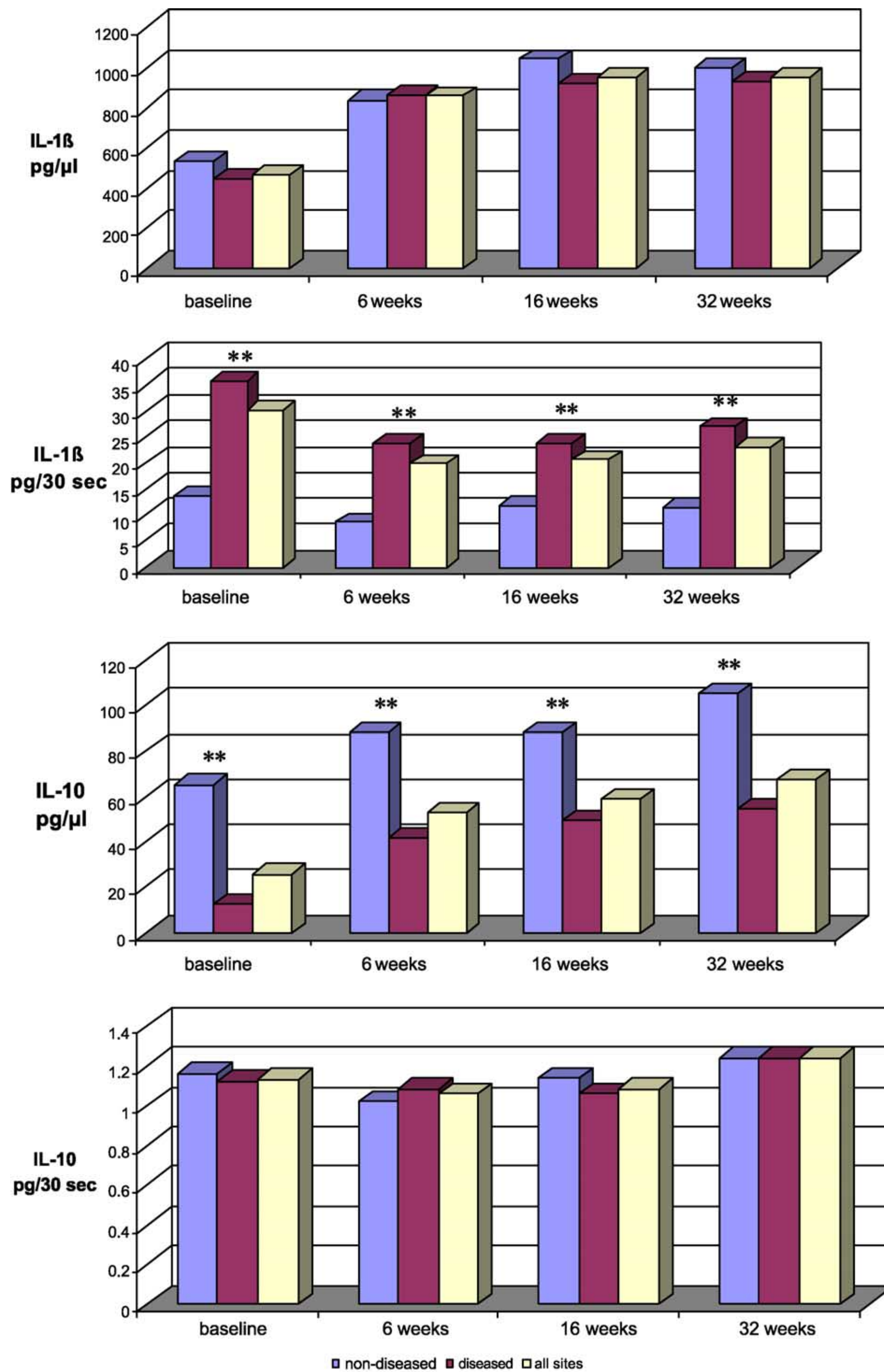

Figure 1 Concentration and total amount of IL-1β and IL-10 in GCF of non-diseased and diseased sites in 12 periodontal patients prior to and following therapy.

parameters. At 6 weeks, mean PD, CAL, PII and GI scores were significantly decreased ($p < 0.01$). The PII and GI scores were also decreased ($p < 0.05$) at 16 weeks. However, by 32 weeks post-therapy an increase in PD, CAL, GI and PlI scores was noted ($p < 0.05$ for GI and PlI) (Fig. 2). By contrast, in non-diseased sites periodontal therapy resulted in a significant decrease only in PlI and GI scores at 6 weeks ($p < 0.01$).

Smoking status. The concentration and the total amount of IL-1β and IL-10 as well as the mean values of PD and CAL in seven smokers and five

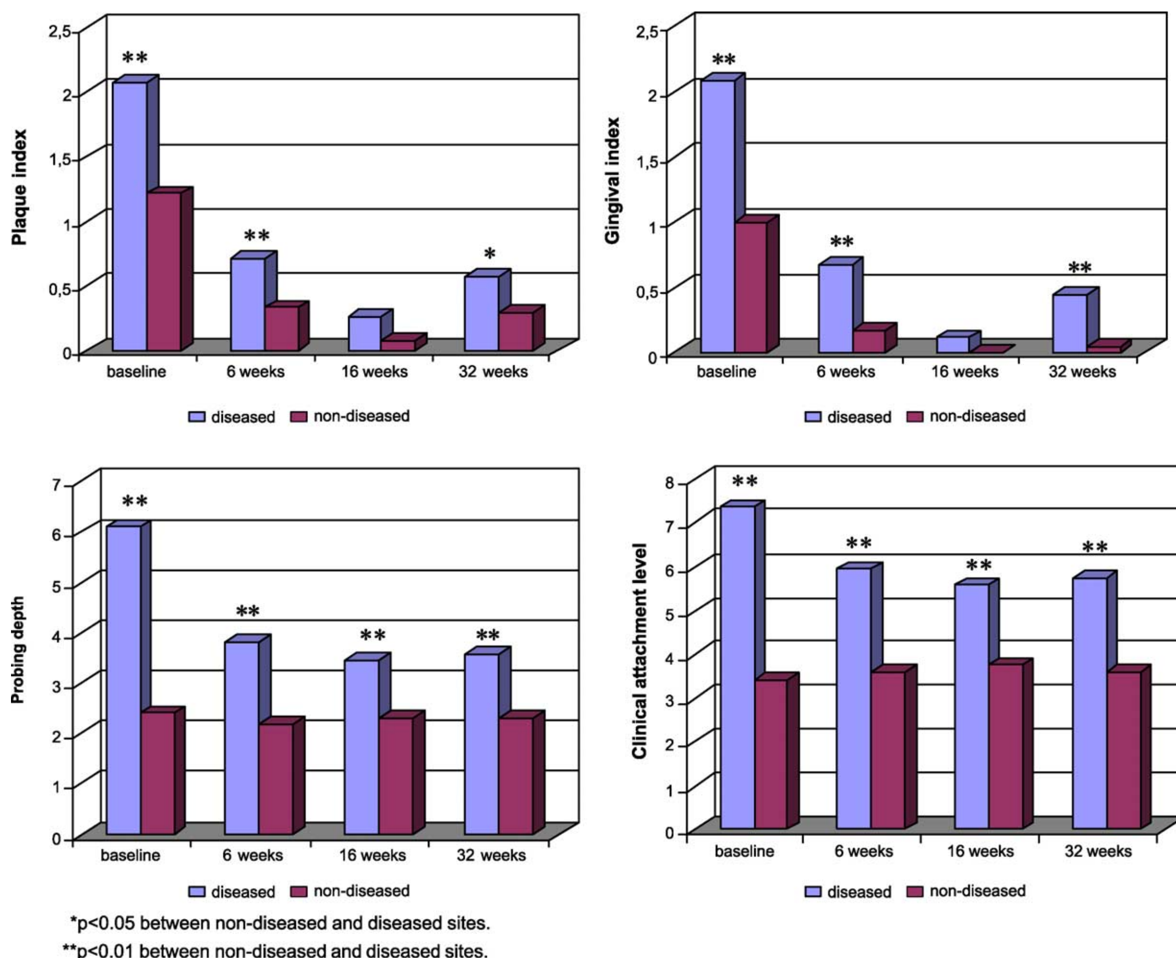

Figure 2 Clinical parameters of non-diseased and diseased sites in 12 periodontal patients prior to and following therapy.

non-smokers are presented in Table 2. In diseased sites, IL-1β concentration was significantly greater in smokers following therapy ($p < 0.01$). Similarly, TAIL-1β levels were significantly higher in smokers at 6 and 16 weeks post-therapy ($p < 0.05$). On the contrary, TAIL-10 levels were significantly higher in non-smokers at baseline ($p < 0.01$) as well as at 6 ($p < 0.05$) and at 16 weeks ($p < 0.01$). IL-10 concentration was also higher in non-smokers but without reaching statistical significance. Moreover, smokers displayed significantly greater PD and CAL scores at 32 weeks ($p < 0.05$). In non-diseased sites, both TAIL-1β and IL-1β were statistically higher in smokers following periodontal treatment ($p < 0.01$). At baseline, mean TAIL-10 and IL-10 levels were significantly higher in non-smokers ($p < 0.01$ and $p < 0.05$, respectively). Following therapy, TAIL-10 levels were numerically greater in non-smokers at 6 and 16 weeks, while IL-10 concentration was greater in smokers (significantly at 16 and 32 weeks ($p < 0.01$). In addition, the mean PD score was statistically higher in smokers at 16 and 32 weeks ($p < 0.05$), while GI and Pll scores were similar in both smokers and non-smokers therefore, they are not presented in Table 2.

Clinical parameters and IL-1β, IL-10. At baseline, there were significant positive correlations between TAIL-1β and GI, Pll, while strong inverse relationships between IL-10 concentration and PD, CAL were observed (Table 3). In addition, the correlation between total amount and concentration of IL-1β and IL-10 is presented in Table 4. A weak negative correlation between TAIL-1β and TAIL-10 was noted ($p > 0.05$). Moreover, a good correlation between IL-1β and IL-10 concentration was obtained ($p > 0.05$).

Discussion

In this study, IL-1β was detected in almost all GCF samples. Our findings are in agreement with previous studies,[33-37] where IL-1β was detected in

Table 2 Concentration and total amount of IL-1β and IL-10 in GCF as well as PD, CAL prior to and following therapy in diseased and non-diseased sites of 7 smokers and 5 non-smoker periodontal patients.

Parameters	Baseline (Mean ± SD)	6 weeks (Mean ± SD)	16 weeks (Mean ± SD)	32 weeks (Mean ± SD)
Diseased sites				
IL-1β NS	482.20 ± 301.97	464.55 ± 273.07**	345.40 ± 167.21**	598.10 ± 217.63**
IL-1βS	420.28 ± 283.44	1147.80 ± 801.33**	1332.52 ± 509.99**	1171.7 ± 664.92**
TAIL-1β NS	33.04 ± 17.12	19.89 ± 10.79*	14.04 ± 0.80*	27.19 ± 16.26
TAIL-1β S	37.36 ± 20.05	26.36 ± 14.50*	30.39 ± 22.73*	26.37 ± 13.81
IL-10 NS	14.98 ± 8.76	39.23 ± 23.61	42.04 ± 21.01	44.10 ± 20.37
IL-10 S	11.09 ± 5.49	42.44 ± 32.17	53.06 ± 38.62	61.83 ± 53.52
TAIL-10 NS	1.39 ± 0.75**	1.25 ± 0.67*	1.35 ± 0.94**	1.39 ± 0.72
TAIL-10 S	0.93 ± 0.46**	0.96 ± 0.54*	0.86 ± 0.39**	1.14 ± 0.67
PD-NS	6.03 ± 0.81	3.67 ± 1.03	3.30 ± 0.79	3.27 ± 1.05*
PD-S	7.52 ± 1.85	3.95 ± 1.25	3.57 ± 1.06	3.79 ± 1.02*
CAL-NS	7.10 ± 1.40	5.53 ± 1.61	5.03 ± 1.63	5.13 ± 1.61*
CAL-S	7.52 ± 1.85	6.21 ± 2.08	5.95 ± 2.05	6.19 ± 1.81*
Non-diseased sites				
IL-1β NS	592.7 ± 298.66	292.68 ± 117.34**	541.34 ± 487.21**	475.92 ± 301.87**
IL-1β S	487.40 ± 280.03	1225.74 ± 799.20**	1393.69 ± 469.90**	1362.75 ± 1112.06**
TAIL-1β NS	18.42 ± 13.17	4.42 ± 2.83**	7.31 ± 3.48**	7.43 ± 5.83**
TAIL-1β S	10.32 ± 7.55	11.42 ± 7.68**	14.84 ± 8.62**	14.19 ± 12.01**
IL-10 NS	83.34 ± 64.39*	76.38 ± 43.52	63.85 ± 35.70**	77.86 ± 53.62**
IL-10 S	52.57 ± 30.42*	96.09 ± 88.13	105.28 ± 69.22**	125.44 ± 90.02**
TAIL-10 NS	1.42 ± 0.88**	1.12 ± 0.52	1.28 ± 0.88	1.10 ± 0.61
TAIL-10 S	0.98 ± 0.53**	0.95 ± 0.39	1.03 ± 0.60	1.25 ± 0.77
PD-NS	2.30 ± 0.67	2.10 ± 0.32	2.00 ± 0.00*	2.00 ± 0.47*
PD-S	2.50 ± 0.52	2.21 ± 0.70	2.50 ± 0.52*	2.50 ± 0.52*
CAL-NS	3.20 ± 1.03	3.20 ± 1.03	3.40 ± 0.97	3.40 ± 0.84
CAL-S	3.57 ± 1.45	3.86 ± 1.23	4.00 ± 1.18	3.71 ± 1.33

NS: non-smokers, S: smokers, IL-1β, IL-10: concentration (pg/μl), TAIL-1β, TAIL-10: total amount (pg/30 s), $*p < 0.05$, $**p < 0.01$.

a high percentage (94, 95, 97, 98 and 100%) of GCF samples from adult periodontitis patients, while in a more recent study involving experimental gingivitis all GCF samples contained IL-1β.[38] In our study, IL-10 was detected in 87.8% of all sites. Gamonal et al.[34] found IL-10 in only 43% of sites from periodontal patients, while in healthy controls IL-10 was undetectable in GCF. A partial explanation for the disagreement in the frequency of IL-10 detection in GCF could be attributed to the sensitivity and the specificity of the immunoassay used as well as to the differences in the study population since high cytokine expression in GCF may be in part a host trait and not only a function of clinical parameters.[39] Sampling time may also affect the frequency of cytokine detection in GCF since, according to Reinhardt et al.[28] collection time of 30 s may be not enough to collect some cytokines in detectable amounts.

IL-1β has been implicated in the pathogenesis of periodontal disease, and it has been suggested that its levels in GCF are strongly influenced by the degree of periodontal tissue inflammation and destruction.[34,37] In periodontitis patients, it was found[33,36] that the average total amount of IL-1β

Table 3 Correlation between concentration, total amount of IL-1β and IL-10 and clinical parameters of 12 periodontal patients.

Parameters	IL-1β	IL-10	TAIL-1β	TAIL-10
PlI	−0.079	−0.183	0.469*	0.117
GI	−0.069	−0.097	0.387*	0.059
PD	−0.203	−0.312*	0.211	−0.048
CAL	−0.146	−0.446*	0.214	−0.142

$*p < 0.05$, IL-1β, IL-10: concentration (pg/μl), TAIL-1β, TAIL-10: total amount (pg/30 s).

Table 4 Correlation between total amount and concentration of IL-1β and IL-10.

	IL-1β	TAIL-1β	IL-10	TAIL-10
IL-1β	1.000			
TAIL-1β	0.467	1.000		
IL-10	0.331*	−0.274	1.000	
TAIL-10	0.267	−0.198	0.570	1.000

IL-1β, IL-10: concentration (pg/μl), TAIL-1β, TAIL-10: total amount (pg/30 s); $*p < 0.05$.

was significantly higher in diseased than in non-diseased sites, while following therapy it declined. In a more recent study,[34] IL-1β was much higher in periodontitis patients than in healthy subjects, while periodontal treatment resulted in numerically lower IL-1β total amount and significantly higher IL-1β concentration. In agreement with these studies, we found that both prior to and following treatment the total amount of GCF IL-1β was significantly higher in diseased compared to non-diseased sites. At 6 weeks following periodontal therapy, the amounts of IL-1β were found to be significantly reduced in all sites; however, there was an increase at 32 weeks. Engebretson et al.[39] also found that IL-1β levels returned almost to baseline levels in patients with severe disease at 24 weeks following therapy. In experimental gingivitis, Gonzàles et al.[38] demonstrated no significant correlation between the clinical estimates of inflammation or plaque load and the presence of IL-1β in GCF. Additionally, in chronic periodontitis patients IL-1β levels could not be correlated with the clinical parameters.[33] However, in other studies the amounts of IL-1β were positively correlated with Pll, GI, PD and CAL[33,36] or with alveolar bone loss.[17] In our study, there was a significant positive correlation between Pll, GI and IL-1β total amount.

IL-10 is also thought to play a role in periodontitis, especially by down regulation of the production of monocyte derived proinflammatory cytokines and stimulating protective antibody production.[40,41] Yamazaki et al.[42] suggested that IL-10 levels might be critical in immune regulation, controlling the balance between innate inflammatory and acquired humoral responses. In gingival tissues, the expression of IL-10 in periodontitis lesions was found to be lower than that from healthy gingiva; IL-10 was also lower at the sites that were positive for bleeding on probing.[43] In GCF, Gamonal et al.[34] found that IL-10 was only detected in samples from periodontal patients, while little variation was observed according to active sites or the sulcular depth. In agreement with the above findings, we found that IL-10 total amounts were similar in non-diseased and diseased sites of the periodontal patients, while IL-10 total amounts remained almost unchanged during the 32-week period of follow-up. There was weak correlation between IL-10 amounts and clinical parameters. Therefore, it might be speculated that IL-10 levels could be systematically regulated by a certain 'type' of immunologic response, while tissue inflammation and periodontal destruction seemed rather unlikely to significantly influence its levels.[34]

Following periodontal treatment, IL-1β and IL-10 concentration significantly increased. This increase could be due to the reduction of GCF volume following successful therapy (Table 1). It has been suggested that in GCF the total cytokine amount might be more representative of the disease status as compared to the concentration.[44] According to Chapple et al.[45] GCF volumes are very variable irrespective of inflammatory status, while the large variation in GCF volume obtained from each periodontal site may result in a wide range of marker concentrations. Thus, it was proposed[45] that the total marker activity per 30 s GCF sample rather than the concentration of the marker might provide a better correlation with health or disease status.

Smoking is an important risk factor for attachment loss and loss of alveolar bone in adult periodontitis.[46,47,48] In agreement with previous results,[35] our findings showed that smoking did not influence GCF IL-1β levels at baseline. However, at 6 and 16 weeks following therapy both concentration and total amount of IL-1β were significantly higher in smokers in diseased sites. By contrast, the IL-10 total amounts were found to be significantly higher in non-smokers prior to treatment as well as at 6 and 16 weeks following therapy in diseased sites. In addition, in diseased sites a better clinical result was achieved in non-smokers following therapy, as evident from the PD and CAL values. This is in agreement with previous observations that the outcome of periodontal therapy was significantly compromised in smokers.[46,47] Although one should consider the small number of patients studied when interpreting the results, our observations suggest that smoking influenced the response to therapy and this was reflected in baseline and post-therapy levels of total IL-1β and IL-10. Indeed, one may speculate that the reduced IL-10 levels pre-therapy may play a role in the diminished healing response seen in smokers relative to non-smokers.

Finally, IL-10 is predominantly an antiinflammatory cytokine that decreases IL-1 production.[49] Culter et al.[50] observed a clinical benefit in periodontal patients using oral irrigation with water plus routine oral hygiene accompanied by a decrease in GCF IL-1β levels and a trend for increased GCF IL-10 levels. Moreover, Deschner et al.[51] found that IL-1β suppressed IL-10 release from periodontal ligament cells in vitro. In our study, IL-1β total amounts were significantly decreased following periodontal therapy, whilst IL-10 total amounts were almost unchanged. Although not significant, a negative correlation between IL-1β and IL-10 levels was also found supporting the antagonistic effect of these two cytokines.

In conclusion, both treatment modalities improved significantly the clinical indices; this

improvement was accompanied by a down regulation of the proinflammatory cytokine IL-1β in GCF. The total amount rather than the concentration of IL-1β seemed to be closely associated with periodontal disease severity. IL-10 total amounts, however, remained almost unchanged during the 32 weeks following therapy. An antagonistic relationship between IL-1β and IL-10 was obvious in our study. Moreover, smoking status seemed to influence IL-10 concentration and total amount in GCF of periodontal patients prior to as well as following therapy and IL-1β concentration and total amount following periodontal treatment. Despite the bulk of evidence concerning the role of cytokines in GCF of periodontal patients, further studies are required to clarify the exact role of IL-10 in periodontal tissue destruction, the value of monitoring its levels in GCF as well as the relationship between IL-10 and the proinflammatory IL-1β.

Acknowledgements

The authors are grateful to Professor ILC Chapple for his scientific advice and guidance in the preparation of the manuscript and also for the final review.

References

1. Dinarello CA. Interleukin-1 and interleukin-1 antagonism. *Blood* 1991;**77**:1627—52.
2. Tatakis DN. Interleukin-1 and bone metabolism: a review. *Journal of Periodontology* 1993;**64**:416—31.
3. Matsuki Y, Yamamoto T, Hara K. Interleukin-1 mRNA-expressing macrophages in human chronically inflamed gingival tissues. *The American Journal of Pathology* 1991; **138**:1299—305.
4. Matsuki Y, Yamamoto T, Hara K. Detection of inflammatory cytokine messenger RNA (mRNA)-expressing cells in human inflamed gingiva by combined in situ hybridization and immunohistochemistry. *Immunology* 1992;**76**:42—7.
5. Horowitz MC. Cytokines and estrogen in bone: anti-osteoporotics effects. *Science* 1993;**260**:626—7.
6. Dagmar SP, Meley J. Interleukin-1β concentration of gingival crevicular fluid. *Journal of Periodontology* 1994;**65**:423—8.
7. Mizel SB. Interleukin-1 and T cell activation. *Immunological Reviews* 1982;**63**:51—72.
8. Chiplunkar S, Langhorne J, Kaufmann SHE. Stimulation of B cell growth and differentiation by murine recombinant interleukin-1. *Journal of Immunology* 1986;**137**:3748—52.
9. Dewhirst FE, Stashenko PP, Mole JE, Tsurumachi T. Purification and partial sequence of human osteoclast-activating factor: identity with interleukin-1β. *Journal of Immunology* 1985;**135**:2562—8.
10. Gowen M, Mundy GR. Actions of recombinant IL-1, IL-2 and interferon-a on bone resorption in vitro. *Journal of Immunology* 1986;**136**:2478—82.
11. Sauder DN, Mounessa NC, Katz SJ, Dinarello CA, Gallin JJ. Chemotactic cytokines: the role of leukocytic pyrogen and epidermal cell thymocyte-activating-factor in neutrophil chemotaxis. *Journal of Immunology* 1984;**132**:828—32.
12. Westmacott D, Wadsworth J, Bloxham DP. Chemotactic activity of recombinant human interleukin-1. *Agents Actions* 1987;**21**:323—4.
13. Masada MP, Persson R, Kenney JS, Lee SW, Page RC, Allison AC. Measurement of interleukin-1α and 1β in gingival crevicular fluid: implications for the pathogenesis of periodontal disease. *Journal of Periodontal Research* 1990;**25**: 156—63.
14. Reinhardt RA, Masada MP, Kaldhal WB, DuBois LM, Kornman KS, Choi J-I, Kalkwarf KL, Allison AC. Gingival fluid IL-1 and IL-6 levels in refractory periodontitis. *Journal of Clinical Periodontology* 1993;**20**:225—31.
15. Honing J, Rordorf-Adam C, Siegmund C, Wiedemann W, Erard F. Interleukin-1 beta (IL-1b) concentration in gingival tissue from periodontitis patients and healthy control subjects. *Journal of Periodontal Research* 1989;**24**:362—7.
16. Tokoro Y, Yamamoto T, Hara K. IL-1β mRNA as the predominant inflammatory cytokine transcript: correlation with inflammatory cell infiltrate into human gingiva. *Journal of Oral Pathology and Medicine* 1996; **25**:225—31.
17. Ishihara Y, Nishihara T, Kuroyanagi T, Shirozu N, Yamagishi E, Ohguchi M, Koide M, Veda N, Amano K, Noguchi T. Gingival crevicular interleukin-1 and interleukin-1 receptor antagonist levels in periodontally healthy and diseased sites. *Journal of Periodontal Research* 1997;**32**:524—9.
18. Kornman KS, Crane A, Wang HY, di Giovine FS, Newman MG, Pirk FW, Wilson Jr TG, Higginbottom FL, Duff GW. The interleukin-1 genotype as a severity factor in adult periodontal disease. *Journal of Clinical Periodontology* 1997;**24**: 72—7.
19. Engebretson SP, Lamster IB, Herrera-Abreu M, Celenti RS, Timms JM, Chaudhary AG, di Giovine FS, Kornman KS. The influence of interleukin gene polymorphism on expression of inteleukin-1beta and tumor necrosis factor-alpha in periodontal tissue and gingival crevicular fluid. *Journal of Periodontology* 1999;**70**:567—73.
20. Moore KW, O'Garra A, de Waal Malefyt R, Vieira P, Mosmann TR. Interleukin 10. *Annual Reviews of Immunology* 1993;**11**: 165—71.
21. De Waal Malefyt R, Yssel H, Roncarolo M-G, Spits H, de Vries JE. Interleukin-10. *Current Opinion in Immunology* 1992;**4**: 314—20.
22. Cassatella AM, Meda L, Bonora S, Ceska M, Constantin G. Interleukin 10(IL-10) inhibits the release of proinflammatory cytokines from human polymorphonuclear leukocytes. Evidence for an autocrine role of tumor necrosis factor and IL-1β in mediating the production of IL-8 triggered by lipopolysaccharide. *Journal of Experimental Medicine* 1993;**178**:2207—11.
23. Cassatella AM, Meda L, Gasperini S, Calzetti F, Bonora S. Interleukin 10 (IL-10) upregulates IL-1 receptor antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNa degradation. *Journal of Experimental Medicine* 1994;**179**: 1695—9.
24. Lacraz S, Nicod LP, Chicheportiche R, Welgus HG, Dayer J-M. IL-10 inhibits metalloproteinase and stimulates TIMP-1 production in human mononuclear phagocytes. *Journal of Clinical Investigation* 1995;**96**:2304—11.
25. Owens MJ, Gallagher CA, Chambers JT. IL-10 modulates formation of osteoclasts in murine hemopoietic cultures. *Journal of Immunology* 1996;**157**:936—40.

26. Stein SH, Hendrix CL. Interleukin 10 promotes anticollagen antibody production in gingival mononuclear cells. *Journal of Dental Research* 1996;**75**:158. abstr 1122.
27. Gemell E, Seymour GJ. Cytokine profiles of cells extracted from humans with periodontal diseases. *Journal of Dental Research* 1998;**77**:16–26.
28. Reinhardt RA, Masada MP, Payne JB, Allison AC, DuBois LM. Gingival fluid IL-1β and IL-6 levels in menopause. *Journal of Clinical Periodontology* 1994;**21**:22–5.
29. Löe H. The gingival index, the plaque index and the retention index systems. *Journal of Periodontology* 1967;**38**:610–6.
30. Ramfjord SP, Nissle RR. The modified Widman flap. *Journal of Periodontology* 1974;**45**:601–7.
31. Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. *Acta Odontologica Scandinavica* 1964;**22**:121–35.
32. Chapple ILC, Landini G, Griffiths GS, Patel NC, Ward RSN. Calibration of the Periotron 8000® and 6000® by polynomial regression. *Journal of Periodontal Research* 1999;**34**:79–86.
33. Hou L-T, Liu C-M, Rossomando EF. Crevicular interleukin-1β in moderate and severe periodontitis patients and the effect of phase I periodontal treatment. *Journal of Clinical Periodontology* 1995;**22**:162–7.
34. 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. *Journal of Periodontology* 2000;**71**:1535–45.
35. Boström L, Linder LE, Bergström J. Smoking and GCF levels of IL-1β and IL-1rα in periodontal disease. *Journal of Clinical Periodontology* 2000;**27**:250–5.
36. Tsai C-C, Ho Y-P, Chen C-C. Levels of interleukin-1β and interleukin-8 in gingival crevicular fluids in adult periodontitis. *Journal of Periodontology* 1995;**66**:852–9.
37. Rawlinson A, Dalati MHN, Rahman S, Walsh TF, Fairclough AL. Interleukin-1 and IL-1 receptor antagonist in gingival crevicular fluid. *Journal of Clinical Periodontology* 2000;**27**: 738–43.
38. Gonzáles JR, Herrmann JM, Boedeker RH, Francz PI, Biesalski H, Meyle J. Concentration of interleukin-1β and neutrophil elastase activity in gingival crevicular fluid during experimental gingivitis. *Journal of Clinical Periodontology* 2001;**28**:544–9.
39. Engebretson SP, Grbic JT, Singer R, Lamster IB. GCF IL-1β profiles in periodontal disease. *Journal of Clinical Periodontology* 2002;**29**:48–53.
40. Rousset F, Garcia E, Defrance T, Perone C, Vezzio N, Hsu DH, Kastein R, Moore KW, Banchereau J. Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proceedings of the National Academy of Science of the United States of America* 1992;**89**: 1890–3.
41. De Waal Malefyt R, Yssel H, de Vries JE. Direct effects of IL-10 on subsets of human CD4 + T cell clones and resting T cells. *Journal of Immunology* 1993;**150**:4754–65.
42. Yamazaki K, Tabeta K, Nakajima T, Ohsawa Y, Ueki K, Itoh H, Yoshie H. Interleukin-10 gene promoter polymorphism in Japanese patients with adult and early-onset periodontitis. *Journal of Clinical Periodontology* 2001;**28**:828–32.
43. Hirose M, Ishihara K, Saito A, Nakagawa T, Yamada S, Okuda K. Expression of cytokines and inducible nitric oxide synthase in inflamed gingival tissue. *Journal of Periodontology* 2001;**72**:590–7.
44. Lamster IB, Oshrain RL, Gordon JM. Enzyme activity in human gingival crevicular fluid: Considerations in data reporting based on analysis of individual crevicular sites. *Journal of Clinical Periodontology* 1986;**13**:799–804.
45. Chapple ILC, Matthews JB, Thorpe GHG, Glenwright HD, Smith JM, Saxby M. A new ultrasensitive chemiluminescent assay for the site-specific quantification of alkaline phosphatase in gingival crevicular fluid. *Journal of Periodontal Research* 1993;**28**:266–73.
46. Ah MK, Johnson GK, Kaldahl WB, Patil KD, Kalkwarj B. The effect of smoking on the response to periodontal therapy. *Journal of Clinical Periodontology* 1994;**21**:91–7.
47. Grossi SG, Zambon JJ, Ho AW, Koch G, Dunford RG, Machtei EE, Norderyd OM, Genco RJ. Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *Journal of Periodontology* 1994;**65**:260–7.
48. Boström L, Linder LE, Bergström J. Smoking and crevicular fluid levels of IL-6 and TNF-α in periodontal disease. *Journal of Clinical Periodontology* 1999;**26**:352–7.
49. Leon LR, Kozak W, Kluger MJ. Role of IL-10 in inflammation. Studies using cytokine knowckout mice. *Annals of the New York Academy of Sciences* 1998;**29**:69–75.
50. Cutler CW, Stanford TW, Abraham C, Cederberg RA, Boardman TJ, Ross C. Clinical benefits of oral irrigation for periodontitis are related to reduction of proinflammatory cytokine levels and plaque. *Journal of Clinical Periodontology* 2000;**27**:134–43.
51. Deschner J, Arnold B, Kage A, Zimmermann B, Kanitz V, Bernimoulin J-P. Suppression of intereleukin-10 release from human periodontal ligament cells by suppression of interleukin-10 release from human periodontal ligament cells by interleukin-1β in vitro. *Archives of Oral Biology* 2000;**45**: 179–83.