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Effect of periodontal treatment on IL-1β, IL-1ra, and IL-10 levels in gingival crevicular fluid in patients with aggressive periodontitis

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Abstract

Aim: The aim of this study was to examine the effect of phase I periodontal treatment on the levels of interleukin (IL)-1β, IL-1ra, and IL-10 in gingival crevicular fluid (GCF) in patients with generalized aggressive periodontitis (G-AgP).

Material and Methods: Data were obtained from 15 patients with aggressive periodontitis and 15 healthy controls. GCF was collected from at least four pre-selected sites (one shallow, at least two moderate, or at least one deep pockets) in patients with G-AgP. In the healthy group, GCF samples were collected from one site. The cytokine levels were determined by an enzyme-linked immunosorbent assay. Probing depth, clinical attachment level (CAL), gingival and plaque indices, and bleeding on probing were measured. The GCF sampling and clinical measurements were recorded at baseline and 6 weeks later after periodontal treatment.

Results: IL-1β levels were significantly higher at the moderate and deep pocket sites compared with the shallow sites ($p<0.05$). After periodontal therapy, IL-1β levels were significantly reduced in the moderate and deep pocket sites ($p<0.05$). IL-1ra levels at baseline of the moderate and deep pocket sites were significantly lower than the control sites ($p<0.05$). IL-10 levels were similar in all pockets and did not change after periodontal therapy.

Conclusions: The periodontal treatment improves the clinical parameters in G-AgP, and this improvement is evident in deep pocket sites for pocket depth and CAL values. These results confirm that IL-1β is effective for evaluating the periodontal inflammation and can thus be used as a laboratory tool for assessing the activity of periodontal disease.

Key words: aggressive periodontitis; crevicular fluid; cytokine; IL-1; IL-10

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Generalized aggressive periodontitis (G-AgP) is an inflammatory disease that has its onset primarily during early-adult years and is characterized by rapid attachment loss and bone destruction (Armitage 1999). G-AgP patients display an inadequate host response to periodontopathic bacteria, which is due to increased expression of a wide variety of immunological and genetic risk factors (Gonzales et al. 2002, Kinane & Hart 2003). G-AgP can either be *localized*, with first molars and incisors, or *generalized*, affecting at least three permanent teeth other than first molars or incisors (Carranza et al. 2007). Although G-AgP is comparatively rare in the general population (0.1–5%) (Albandar & Tinoco 2002), there is considerable interest in studies aimed at understanding the aetiology and pathogenesis of this disorder. It is clear that chronic and aggressive periodontitis are initiated and sustained by bacterial plaque and that host defence mechanisms play an integral role in their pathogenesis (Gonzales et al. 2002).

The subgingival bacteria and their products stimulate host cells to release numerous inflammatory mediators such as prostaglandins and cytokines and cause

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periodontal inflammation (Birkedal -Hansen 1993, Gemmell et al. 1997). As a marker of active inflammation, interleukin (IL)-1 may contribute to the pathologic process of the inflammatory response (Yavuzyılmaz et al. 1995). Interleukin (IL)-1 occurs in two forms as IL-1α and IL-1β, of which IL-1β appears to be the most potent agent having a catabolic effect on bone approximately 10-fold compared with IL-1α. IL-1β has been found to be significantly increased in the periodontal tissues and gingival fluid from diseased sites, compared with healthy sites (Tsai et al. 1995, Ishihara et al. 1997, Giannopoulou et al. 2003). Various studies have shown a positive correlation between IL-1β gene polymorphisms and an increased risk of developing chronic and aggressive periodontitis (Kornman & di Giovine 1998, Diehl et al. 1999). In addition to the two agonist molecules, a third member of the IL-1 group was described and designated as IL-1 receptor antagonist (IL-1ra) (Dinarello & Wolff 1993 Tatakis 1993). Its only known function is to bind to IL-1 receptors, blocking IL-1 and preventing signal transduction (Dinarello & Wolff 1993). IL-1ra may play an important role in regulating the local effect of IL-1 in inflammatory periodontal disease (Ishihara et al. 1997, Rawlinson et al. 2000, 2003, Yoshinari et al. 2004). The administration of exogenous IL-1ra was found to be effective in reducing the inflammatory reactions mediated by IL-1 (Dinarello 2004).

As the traditional ''stop-signal'' for active inflammation, IL-10 plays a major role in suppressing immune and inflammatory responses (Al-Rasheed et al. 2003). It is mainly produced by T cells including human T-helper type 0 (Th0), Th1, and Th2 cells as well as B cells, monocytes, and macrophages (Gemmell et al. 1997). The synthesis of monocyte-derived pro-inflammatory cytokines, including IL-1, IL-6, IL-8, and TNF, is inhibited by IL-10, which also enhances the production of the IL-1ra in polymorphonuclear leucocytes stimulated with lipopolysacchride (De Waal Malefyt et al. 1993). IL-10 is expressed in both healthy and inflamed human periodontal tissues and is reported to be decreased in patients infected with *Actinobacillus actinomycetemcomitans* (Hirose et al. 2001), and the lack of IL-10 was linked to accelerated alveolar bone loss (Al-Rasheed et al. 2003). Thus, IL-10 can be a protective cytokine in periodontal diseases and regulates pro-inflammatory cytokines, including those implicated in alveolar bone loss (Hirose et al. 2001).

Periodontal inflammation may involve both an increase in inflammatory stimulators, e.g. IL-1, and a decrease in inflammatory inhibitors, e.g. IL-10 and such a double impact may be the underlying factor in the severe progressive changes inherent to periodontitis (Deschner et al. 2000). It is, however, not clear how this balance works in different stages of periodontitis and whether there is indeed such a multi-dimensional inflammatory response that may point to the involvement of various cell types in the progression of inflammation. Few studies have investigated the effect of these cytokines, especially IL-10, in patients with aggressive periodontitis (Yavuzyılmaz et al. 1995, Lappin et al. 2001, Kamma et al. 2004). Limited data also exist related to simultaneous changes in ''pro-inflammatory'' *versus* ''anti-inflammatory'' pathways in gingival crevicular fluid (GCF) in chronic periodontitis subjects only (Goutoudi et al. 2004). Therefore, in an attempt to quantify the kinetics of multiple cytokines as examples of pro- and anti-inflammatory pathways in G-AgP, we have examined the levels of IL-1β, IL-1ra, and IL-10 in GCF, compared with that of healthy controls, and studied the effect of phase I periodontal treatment on these targets.

Material and Methods

Study population and selection of the sampling sites

A total of 15 non-smoker subjects diagnosed with G-AgP and 15 periodontally and systemically healthy controls were selected consecutively for the study. Complete medical and dental histories were taken from all subjects. None of the subjects had a history of systemic disease and had received antibiotics or other medications or periodontal treatment within the past 4 months. Informed consent was obtained from all the subjects, and the study protocol was approved by the Medical Ethics Committee of Cumhuriyet University. Based on the previously described criteria (Armitage 1999), G-AgP subjects were defined as patients with at least 5 mm of attachment loss at a minimum of one site in more than eight teeth, three of which were other than first molars and incisors, and radiographic evidence of advanced alveolar bone loss. Control subjects were designated as healthy if they had full-mouth probing pocket depth (PD) <3 mm, gingival index (GI) scores equal to 0, and no radiographic evidence of alveolar bone loss. These individuals were systemically and periodontally healthy volunteers.

All subjects received a clinical periodontal examination including the measurement of probing PD and clinical attachment level (CAL) at six sites around each tooth with a manual probe (Williams probe, Hu-Friedy, Chicago, IL, USA). Based on the initial probing PD measurements, the study sites were further classified and selected as one shallow (1–3 mm), at least two moderate (4–6 mm), or at least one deep (>6 mm) pockets of teeth per subject (Pihlstrom et al. 1983). The PD, CAL, plaque index (PI), GI, bleeding on probing (BOP), and GCF samples were collected on the basis of site, only one site assessed and evaluated per tooth on the vestibular aspects (mesial or distal site) of maxillary incisors and canine teeth. One site in healthy controls was used for clinical measurements and GCF sampling as in the G-AgP patients.

Clinical measurements and phase I periodontal therapy

Before crevicular fluid collection, supragingival plaque was scored using the PI (Löe 1967). Gingival inflammation was scored following crevicular fluid collection using GI (Löe 1967). BOP was also recorded. PD and CAL measures were obtained using a Williams' periodontal probe. In all subjects, individual acrylic stents were fabricated with grooves as reference points for the clinical measurements. One researcher (H. T.), who was calibrated against a pressure-sensitive probe, performed all the clinical measurements. After recording the baseline measurements, phase I therapy, which consists of oral hygiene instructions, scaling, and root planing (SRP), was performed on subjects with periodontitis (Lindhe et al. 2003). The SRP procedure was performed quadrant per quadrant under local anaesthesia in four visits using specific curettes (Hu-Friedy, Chicago, IL, USA). Treatment was completed within at most 14 days. No antibiotics were prescribed during the treatment. The clinical measurements and GCF sampling from the sampling

sites were recorded at baseline and 6 weeks after the periodontal treatment.

Crevicular fluid sampling

GCF sampling was carried out during the same session as the clinical measurements. Only the upper anterior teeth were included in the study to improve the access and to reduce the risk of salivary contamination during these processes. GCF was collected from at least four pre-selected sites (one shallow, at least two moderate sites, and at least one deep site). Each sample site was carefully isolated using cotton rolls to avoid saliva contamination. The first paper strip (Pro Flow, Amityville, NY, USA) was placed in the pocket until mild resistance was felt and then left in place for 30 s. After removal of this first strip and waiting for 3 min, a second strip was inserted into the same site, in the same manner. The two strips were then placed in an Eppendorf tube and immediately frozen at $-70^{\circ}C$ until the day of analysis. In the case of visible contamination with blood, the strips were discarded.

Analysis of cytokine production

On the day of the assay, 250 μl of Hank's buffered salt solution containing 1% bovine serum albumin (Sigma, St Louis, MO, USA) was added to the tubes containing the sample strips. The tubes were gently shaken for 1 min. and then centrifuged at $2000 \times g$ for 5 min. After removal of the strips, the supernatants were divided into three aliquots for the determination of each cytokine. The amounts of IL-1β, IL-1ra, and IL-10 in the GCF were determined using ELISA assays (Immunotech, Beckman & Coulter Co., Marseille, France) in accordance with the manufacturer's instructions. After the colour development was stopped, the optical density was measured using a microtitre plate-computerized reader set to a wavelength of 450 nm. The GCF cytokine levels were calculated from the standard curves and defined as picograms/site for total cytokine levels. The sensitivities for IL-1β, IL-1ra, and IL-10 ELISA's were 1.5, 30, and 5 pg/ml, respectively. Sites with cytokine levels below the limits of the assay's detectability were scored as 0.

Statistical analysis

Baseline and 6-week PD and CAL values of shallow, moderate, and deep pocket sites in the G-AgP patients were analysed with Wilcoxon's rank test. Differences between baseline and 6-week PD and CAL values of shallow, moderate, and deep pocket sites in the G-AgP patients were analyzed with the Kruskal–Wallis ANOVA test, followed by post hoc Tukey's test. The ratio of BOP and suppuration in the G-AgP patients were analysed with the χ^2 test. IL-1β, IL-1ra, and IL-10 levels in different PDs in the G-AgP patients and healthy controls were analysed by an ANOVA test, followed by post hoc Tukey's test. Pearson's and Spearman's correlation analyses in the G-AgP patients and healthy controls were used for IL-1 β, IL-1ra, and IL-10 as appropriate. A p value of <0.05 was considered to be statistically significant.

Results

Clinical findings

The demographic data including gender and age in the G-AgP patients and healthy controls are summarized in Table 1. Table 2 presents the mean PD, CAL, PI, and GI values at baseline and 6 weeks and the changes in PD, CAL, PI, and GI values of the shallow, moderate, and deep pocket sites in the G-AgP patients and healthy controls. PD values at 6 weeks of all the sites were significantly lower than the baseline ($p<0.05$). Changes in PD and CAL values of the deep pocket sites were significantly higher than those of the moderate and shallow sites ($p<0.05$) and significantly higher in moderate sites compared with the shallow sites ($p<0.05$). CAL values of the moderate and deep pocket sites at 6 weeks were significantly lower than that at baseline ($p<0.05$). PI and GI values at 6 weeks of all the sites were significantly lower than those at baseline ($p<0.05$). There was no significant difference in the change of PI values of all the pocket sites ($p>0.05$) while GI values of the moderate and deep pocket sites were significantly higher than that of the shallow pocket site ($p<0.05$). There was no significant difference in the change in GI between the moderate and deep pocket sites ($p>0.05$).

The ratios of BOP of the moderate and deep pocket sites were significantly higher than that of the shallow pocket site ($p<0.05$). There was no significant difference in BOP between the moderate

Table 1. Demographic data on generalized aggressive periodontitis and control subjects

	Age (X ± SD)	Subjects	
		male	female
G-AgP	26 ± 3.9	4	11
Control	24.9 ± 3.9	5	10

G-AgP, generalized aggressive periodontitis.

Table 2. Clinical parameters of GCF sampling sites at baseline and 6 weeks in patients with G-AgP and healthy controls

	Healthy controls	Shallow pocket site (1–3 mm)			Moderate pocket site (4–6 mm)			Deep pocket site (>6 mm)		
		baseline	6-week	difference	baseline	6-week	difference	baseline	6-week	difference
PD	1.6 ± 0.4	3.0 ±	2.2 ± 0.5[a]	0.7 ± 0.5	5.1 ± 0.5	3.2 ± 0.7[a]	1.9 ± 0.7	7.3 ± 0.5	4.0 ± 0.9[a]	3.2 ± 0.9[b]
CAL	0	5.6 ± 0.9	5.6 ± 0.9	0	8.1 ± 0.9	7.0 ± 1.1[a]	1.0 ± 0.7	9.7 ± 0.4	7.9 ± 1.3[a]	1.8 ± 1.3[b]
PI	0.2 ± 0.4	0.8 ± 0.5	0.1 ± 0.3[a]	0.7 ± 0.4	1.4 ± 0.5	0.3 ± 0.4[a]	1.0 ± 0.6	1.6 ± 0.4	0.4 ± 0.5[a]	1.1 ± 0.7
GI	0	1.1 ± 0.3	0.1 ± 0.3[a]	1 ± 0.3	2.1 ± 0.5	0.6 ± 0.4[a]	1.5 ± 0.5[c]	2.3 ± 0.4	0.5 ± 0.5[a]	1.7 ± 0.5[c]
Percentage of BOP (%)	0	20	0		95	10		100	0	

[a] $p<0.05$ *versus* baseline.
[b] $p<0.05$ *versus* moderate and deep pocket sites.
[c] $p<0.05$ *versus* shallow pocket site.
GCF, gingival crevicular fluid; G-AgP, generalized aggressive periodontitis; PD, pocket depth; CAL, clinical attachment level; PI, plaque index; GI, gingival index.

and deep pocket sites at baseline ($p > 0.05$). The ratios of suppuration of the moderate and deep pocket sites were significantly higher (42.5% and 87.5% *versus* 0%, respectively) than that of the shallow pocket site ($p < 0.05$). There was no suppuration in any of the pocket sites at 6 weeks.

Cytokine levels in GCF

IL-1β was detected in 92.5–95.8% of pockets with no significant difference in recovery from shallow, moderate, or deep sites. Figure 1 presents the IL-1β levels at baseline and 6 weeks. IL-1β levels at 6 weeks of moderate and deep pocket sites were significantly lower than those at baseline ($p < 0.05$) while there was no significant difference in shallow sites between baseline and 6 weeks ($p > 0.05$). There were also no significant differences in IL-1β levels between all the pocket sites and control sites at 6 weeks ($p > 0.05$).

IL-1ra was detected in all GCF samples. Figure 2 presents the IL-1ra levels in shallow, moderate, and deep pockets and control sites at baseline and 6 weeks. IL-1ra levels at baseline of the moderate and deep pocket sites were significantly lower than the control sites ($p < 0.05$). There was no significant difference in IL-1ra levels of all the pocket sites between that at baseline and at 6 weeks ($p > 0.05$).

The detection ratios of IL-10 at shallow pocket sites and control sites (26.7% and 33.3%) at baseline were lower than that at moderate and deep pocket sites (57.5 and 41.7) but did not reach statistical significance. The detection ratios of IL-10 at shallow, moderate, and deep pocket sites at 6 weeks were 26.7%, 42.5%, and 45.8%, respectively. Figure 3 presents the IL-10 level of the shallow, moderate, and deep pocket sites and the control site at baseline and 6 weeks. There were no significant differences among the shallow, moderate, and deep pocket sites and healthy site with regard to IL-10 levels at baseline and 6 weeks ($p > 0.05$).

There were statistically significant negative correlations between IL-10 and IL-1ra levels in moderate and deep pocket sites ($p < 0.05$). There were no other significant correlations between any of these parameters ($p > 0.05$). We did not detect any significant correlations between IL-1β and clinical parameters ($p > 0.05$). There were negative but significant correlations between

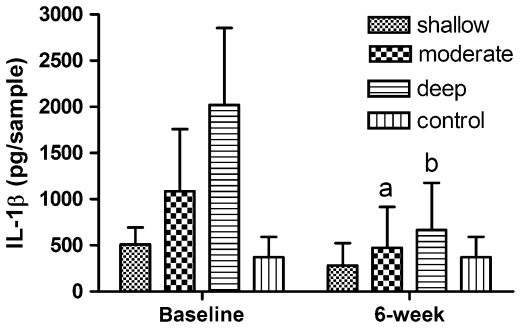

Fig. 1. Total amounts of IL-1β in shallow, moderate, deep pocket sites and the control site at baseline and 6 weeks. [a]$p < 0.05$ *versus* moderate pocket site at baseline. [b]$p < 0.05$ *versus* deep pocket site at baseline. IL, interleukin.

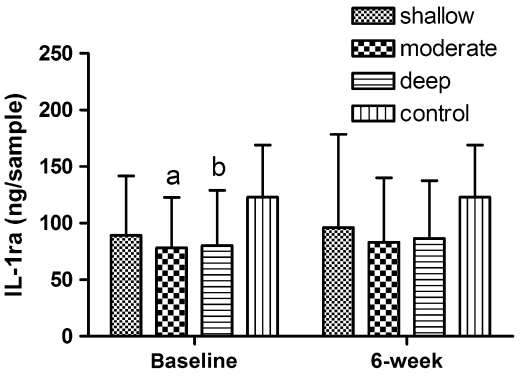

Fig. 2. Total amounts of IL-1ra in shallow, moderate, deep pocket sites and the control site at baseline and 6 weeks. [a]$p < 0.05$ *versus* control site at baseline. [b]$p < 0.05$ *versus* control site at baseline. IL, interleukin.

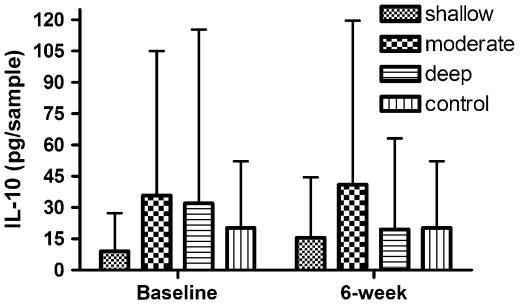

Fig. 3. Total amounts of IL-10 in shallow, moderate, deep pocket sites and the control site at baseline and 6 weeks. IL, interleukin.

IL-1ra and PI values in moderate and deep pocket sites ($p < 0.05$) and between IL-1ra and GI values in moderate pocket sites ($p < 0.05$). There was a positive significant correlation between IL-10 and PI values in moderate pocket sites ($p < 0.05$) and between IL-10 and GI values in moderate and deep pocket sites ($p < 0.05$).

Discussion

This study focuses on investigating the changes in IL-1β, IL-1ra, and IL-10 levels in GCF in patients with G-AgP undergoing phase I periodontal therapy. We found that there were significant improvements after periodontal treatment and this improvement was evident in deep pocket sites for PD and CAL values. The IL-1β levels were markedly reduced in moderate and deep pocket sites following periodontal therapy but there was no correlation between IL-1β and clinical parameters.

In a previous study, which has examined IL-1β and IL-8 levels in GCF from adult periodontitis patients and from clinically healthy subjects after

periodontal treatment, it was demonstrated that the total amounts of both cytokines were markedly reduced following periodontal treatment (Tsai et al. 1995). In other studies, decreased clinical inflammation as well parallel and reduced IL-1β levels were also shown (Alexander et al. 1996, Gamanol et al. 2000, Giannopoulou et al. 2003). Meanwhile, in a study that has investigated the relationship between the clinical changes after periodontal therapy and IL-1 in GCF and gingival tissues from patients with chronic periodontitis, the clinical parameters were improved after periodontal therapy and IL-1β levels in GCF were slightly increased (Yoshinari et al. 2004). In another recent report, the total amounts of IL-1β, IL-6, and IL-8 were higher in aggressive periodontitis patients than the chronic periodontitis and healthy groups. Our study had some limitations with respect to the small number of subjects in the G-AgP patients and healthy controls and one GCF sampling in the healthy controls. Still, when probing depths of the sampled sites were analysed, differences in GCF IL-1β levels between the different sites and controls at baseline were significant. This finding was in parallel with a previous study where deeper pockets (>4 mm) had significantly higher amounts of IL-1β than shallow pockets (<3 mm) (Hou et al. 1995) while another study found no significant differences in the concentrations of IL-1β in the GCF between deep and shallow pockets of the same patient (Figueredo et al. 1999). The reason for this difference may be due to the fact that the latter study compared sites with similar degrees of GI in shallow and deep pockets while our study and the parallel work (Hou et al. 1995) measured pockets with deeper probing levels as well as higher GI scores. This suggests that higher IL-1β levels in shallow sites are, in part, a ''normal'' trait of the host and not necessarily a function of clinical inflammation. More recent data from Engebretson et al. support this observation, demonstrating that PD and AL were each associated with increased GCF IL-1β levels (Engebretson et al. 2002).

The total amount of cytokines in GCF sample per sampling time has been suggested to be as a better indicator of relative GCF constituent activity rather than the GCF volume that might result in the decrease of the cytokine concentration (Figueredo et al. 1999, Gamanol et al. 2000). In the present study, we have collected the GCF samples for the same time period and reported the data as total amount per sample. In a study that has investigated the cytokine IL-1β and IL-1ra levels in GCF in patients with adult periodontitis, IL-1ra levels at non-bleeding and bleeding periodontitis sites were lower than the control sites. For healthy sites, a strong inverse relationship was found between IL-1β and IL-1ra levels in GCF (Birkedal-Hansen 1993). On the other hand, the highest IL-1ra levels were found at sites of medium bone destruction, whereas lower levels, both at sites of severe bone destruction and at healthy sites have been reported (Ishihara et al. 1997). Similar results were found in the study of (Boström et al. 2000). They analysed the GCF levels of IL-1β and IL-1ra levels in smoking patients with moderate to severe periodontal disease. They suggest that IL-1β and, in particular, IL-1ra are present in high levels in GCF and these cytokines can be detected in GCF in the vast majority of patients. However, the concentrations of these cytokines were not influenced by smoking. Our results demonstrated that IL-1ra can be detected in all the GCF samples and IL-1ra levels at baseline of the moderate and deep pocket sites were significantly lower than the healthy control site. After periodontal treatment, there was no difference in the IL-1ra level of all the pocket sites. Also, there was no correlation between IL-1β and IL-1ra levels but a negative significant correlation between IL-1ra and IL-10.

IL-10 is the predominant anti-inflammatory cytokine that decreases IL-1 production. Lappin et al. (2001) evaluated the role of proinflammatory and anti-inflammatory cytokines in periodontitis granulation tissue. They suggest that the numbers of inflammatory leucocytes that express the anti-inflammatory cytokine IL-10 were more increased in early-onset periodontitis than adult periodontitis. In a study that has investigated the relation between clinical parameters and concentrations of several cytokines (IL-1β, IL-4, IL-10, etc.) within the inflamed gingival tissue and serum samples from patients with severe chronic periodontitis, was demonstrated in patients sera with chronic periodontitis was higher IL-10 concentration than in the sera of the healthy controls (Gorska et al. 2003). Indeed, high ratios of IL-4 and IL-10 found in inflamed tissue biopsies were closely associated with periodontal disease severity and IL-10 was a predominant cytokine profile, observed in 60% of gingival tissue supernatants from periodontitis patients. However, in a previous study it was found that IL-10 was detected in only 43% of sites belonging to patients with periodontitis, while in healthy controls IL-10 was undetectable in GCF (Gamanol et al. 2000). The IL-10 concentration was found to be higher in sites with PD of 4–6 mm than in <3 mm and not detected in pockets >6 mm. In another study (Goutoudi et al. 2004) IL-10 was detected in 87.8% of all sites. The IL-10 level, however, remained unchanged after 6 weeks of therapy. In our results, the IL-10 was detected in 57.7% and 41.7% of the moderate and deep pocket sites and the detection ratio was higher in these pocket sites than shallow and control sites. Also, we did not see any difference in the IL-10 level between baseline and 6 weeks.

In conclusion, periodontal treatment significantly improved the clinical parameters and this improvement was accompanied by decreased IL-1β in GCF, and the results suggest an inverse correlation between IL-1ra and IL-10. We also suggested that the high ratio of IL-1β to IL-10 was the most characteristic finding for GCF and was found in almost all the GCF samples studied. This fact can be used as a laboratory tool for assessing periodontal disease.

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Clinical Relevance

Scientific rationale for the study: Aggressive periodontitis is an inflammatory disease that is characterized by rapid attachment loss and bone destruction. The subgingival bacteria and their products stimulate host cells to release numerous inflammatory mediators, such as prostaglandins and cytokines. The aim of this study was to investigate levels of IL-1β, IL-1ra, and IL-10 in GCF samples from subjects with G-AgP and to evaluate the effect of phase I periodontal treatment on these targets.

Principal findings: After periodontal therapy, IL-1β levels were significantly reduced in moderate and deep pocket sites. IL-1ra levels at baseline of the moderate and deep pocket sites were lower than at the control sites. IL-10 levels were similar in all pockets and did not change after periodontal therapy.

Practical implications: Periodontal treatment improves the clinical parameters with decreased IL-1β in aggressive periodontitis. These findings suggest that the high ratio of IL-1β to IL-10 is the most characteristic finding for GCF and is found in almost all GCF samples.