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Effect of inflammation, smoking and stress on gingival crevicular fluid cytokine level

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Abstract

Background: Recent studies have shown that cytokines are pivotal to the pathogenesis of periodontal diseases and may be used as markers in diagnosis.

Aim: The aim of the present study was to determine the levels of interleukin (IL)-1β, IL-4, IL-6 and IL-8 in gingival crevicular fluid of periodontally healthy and diseased individuals and to study their association to smoking, stress and clinical periodontal parameters.

Material and methods: A total of 80 patients were included in the study : 20 patients with early onset or aggressive periodontitis (EOP), 20 with chronic adult periodontitis (AP), 20 with gingivitis (G) and 20 patients with healthy periodontium (H). GCF was collected by means of Durapore strips, from four sites per patient, randomly selected in each quadrant. The contents of IL- 1β, IL-4, IL-6 and IL-8 were measured in 320 samples by use of commercially available sandwich enzyme-linked immunoadsorbent assays.

Results: In periodontally diseased subjects the total amounts of IL-1β, IL-6 and IL-8 were significantly elevated as compared to healthy subjects, whereas IL-4 showed an inverse relationship to periodontal status and higher amounts were found in the healthy group. The amounts of all four cytokines were positively correlated with probing depths. IL-4, IL-6 and IL-8 were significantly correlated to smoking while stress was associated with IL-1β, IL-6 and IL-8 levels.

Conclusions: The present data suggest that crevicular IL-1β, IL-6 and IL-8 reflect the activity of periodontal destruction, whereas IL-4 shows an inverse correlation to it. The enhanced production of inflammatory cytokines in the presence of smoking and stress may have clinical consequences.

Key words: chronic adult periodontitis; cytokines; early onset or aggressive periodontitis; gingival crevicular fluid; gingivitis; IL-1β, IL-4, IL-6, IL-8; smoking; stress

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In the last decades, the local host response to periodontitis has been studied by biochemical analysis of gingival crevicular fluid (GCF). Among many inflammatory and immune mediators identified in GCF, cytokines have attracted particular attention and are suspected to be involved in both inflammation-related alteration and repair of the periodontal tissues. Certain cytokines have been proposed as potentially useful diagnostic, or prognostic markers of periodontal destruction (Birkedal-Hansen 1993, Genco 1992). Interleukin (IL)-1β, IL-4, IL-6 and IL-8 have been shown to function in concert with other members of the cytokine network in order to regulate the cellular inflammatory response in the periodontium.

IL-1β is a multifunctional inflammatory mediator able to modulate bone resorption by the activation of osteoclasts (Dewhirst et al. 1985) and by stimulating prostaglandin E_2 synthesis (Tatakis et al. 1988). Although this cytokine was originally considered to be a product of mononuclear phagocytes, evidence suggested that both keratinocytes and gingival fibroblasts can also produce it, in response to stimulation by bacterial products (Dinarello 1988).The observation that IL-1β can act on a large number of cells, such as fibroblasts, chondrocytes, bone cells, neutrophils and lymphocytes, suggests that periodontal destruction and repair in periodontitis may in part be associated with this cytokine (Jandinski

1988). Site-specific increases were also observed in untreated periodontitis (Preiss & Meyle 1994) and in experimental gingivitis models (Kinane et al. 1992). Furthermore, treatment of periodontitis resulted in dramatic local decrease of IL-1β, suggesting that this molecule is crucial in periodontal tissue destruction (Masada et al. 1990, Reinhardt et al. 1993a).

IL-4, originally described as B-cell growth factor, is a potent down-regulator of macrophage function. It down-regulates the CD14 lipopolysaccharide membrane receptor of macrophages (Lauener et al. 1990), subsequently diminishing the production of cytokines by the macrophages and is known to induce apoptosis to monocytes (Mangan et al. 1992). It also inhibits the secretion of PGE_2 by human monocytes which leads to bone resorption (Shapira et al. 1992, Corcoran et al. 1992). Furthermore, localized absence of IL-4 in diseased periodontal tissues is associated with periodontal disease activity and progression (Kabashima et al. 1996, Shapira et al. 1992). This led to the hypothesis of Shapira et al. (1992) that the absence of IL-4 triggers periodontal disease.

IL-6 is a pleiotropic cytokine that stimulates immunoglobulin secretion by human B-lymphocytes, activates T cells, stimulates the synthesis and secretion of acute phase proteins by hepatocytes, and activates the complement cascade (Revel 1989). Its major role is the terminal differentiation of B-lymphocytes to plasma cells, the predominant infiltrate cells in established and advanced periodontal disease (Page & Schroeder 1976). Of particular significance is the ability of IL-6 to induce bone resorption, both by itself and in conjunction with other bone-resorbing agents (Ishimi et al. 1990, Mundy 1991).

Finally, IL-8, produced by a wide variety of cells (polymorphonuclear leukocytes, monocytes, macrophages and fibroblasts), plays a key role in the accumulation of leukocytes at the sites of inflammation (Bickel 1993, Baggiolini & Lewis 1992) and its level is known to increase in the GCF of inflamed as compared to healthy sites (Tsai et al. 1995).

The release of the above cytokines from several cell types, is the result of the immune response to the bacterial challenge under inflammatory conditions (Darveau et al. 1997). Bacterial invasion, however, can be modulated by several environmental risk factors. Cigarette smoking and stress, for instance, may be important contributors in the development of periodontal disease. Tobacco smoking is strongly associated with destructive periodontal disease, alveolar bone loss and poor response to periodontal therapy, although the mechanisms of its negative influence are not well understood (Haber & Kent 1992, Feldman et al. 1983, Grossi et al. 1994, Bergström & Preber 1986, Kamma et al. 1999). Upregulation of LPS-mediated monocyte secretion of PGE_2 by nicotine, has been suggested to play an important role in the pathogenesis of periodontal disease (Payne et al. 1996).

Stress has also been suggested to affect both immune functions (Herbert & Cohen 1993) and susceptibility to infectious diseases (Cohen & Williamson 1991), thus contributing to periodontal inflammation (Monteiro da Silva et al. 1996). The pathways mediating this mechanism are still unexplored. However, Deinzer et al. (1999) suggested that stress might affect periodontal health by increasing levels of IL-1β locally, especially when oral hygiene is neglected.

Although associations have been established between levels of cytokines and presence of periodontal disease in general, large inter- and intra-individual variations suggest that these parameters are influenced by a multitude of other factors which, so far, have been poorly quantified. In order to further establish their diagnostic value, cytokines should be determined in a large spectrum of periodontally healthy and diseased subjects exposed to environmental conditions such as smoking and stress.

The aim of the present investigation was to determine the levels of IL-1β, IL-4, IL-6 and IL-8 in the GCF of periodontally healthy and diseased individuals and to investigate the relationship between these cytokines and environmental factors such as smoking and stress.

Material and methods

Subject population

A total of 80 subjects were entered into the study, classified as healthy (H), gingivitis (G), adult periodontitis (AP) and early onset periodontitis (EOP) patients. They were selected from a private practice limited to periodontics in Athens, Greece. All patients were systemically healthy, had not received antibiotics during the 6 months prior to entering the study and had no periodontal therapy during the previous year. Individuals who were pregnant or required premedication with a systemic antibiotic were excluded. Patients were divided in four groups consisting each of 20 individuals, according to clinical and radiographic criteria:

- the healthy group (H) comprised 7 males, 13 females, mean age: 38 ± 11 years with clinically healthy gingiva;
- the gingivitis group (G) comprised 5 males, 15 females, mean age: 31 ± 8 years, with gingival inflammation but no evidence of bone loss;
- the adult periodontitis group (AP) comprised 6 males, 14 females, mean age: 52 ± 8 years, showing radiographic evidence of bone loss and attachment loss of more than 5 mm in at least eight sites;
- the early onset or aggressive periodontitis group (EOP) comprised 10 males, 10 females, mean age: 32 ± 2 years. These patients, diagnosed as having EOP, were under 35 years old and exhibited severe periodontal destruction, with loss of attachment exceeding 5 mm at two to three sites in more than 14 permanent teeth (at least three of them were not first molars and incisors) and radiographic evidence of advanced alveolar bone loss.

The patients' smoking habits (packs of cigarettes/day) and stress were recorded during the initial periodontal examination.

The stressful social events experienced by the patients, were assessed by the Modified and Perceived Stress Scale (MAPS) (Linn 1986), based on the total perceived stress.

Periodontal examination

The clinical and radiographic evaluations were performed by one periodontist (J.K.). The clinical examination included assessment of probing depth (PD), attachment loss (AL) (Glavind & Löe 1967), plaque (Pl) (O'Leary et al. 1972), bleeding upon probing (BOP) (Ainamo & Bay 1975) and suppuration (SUP) (Singh et al. 1977) at four sites around each tooth, excluding 3rd molars. Measurements of PD and AL were carried out to the nearest mm

using a Goldman/Fox Williams periodontal probe. The number of teeth present in each patient was also recorded.

Full mouth standardized periapical radiographs were taken in all patients. Destruction of alveolar bone was assessed by the Schei method on the mesial and distal aspects of all teeth (Schei et al. 1959).

Clinical measurements were recorded and GCF sampling sites preselected 1 week before sampling. Clinical parameters were registered again after GCF sampling and these values were used in the analysis.

Gingival crevicular fluid sampling

The gingival crevicular fluid was collected in four preselected sites in each patient by means of durapore filter membranes (pore size = 0.22 mm; Millipore Corp., Bedford, MA, USA). In H and G patients, one experimental site was randomly chosen in each quadrant. In AP and EOP patients one deep periodontal pocket (PD > 5 mm) was randomly chosen in each quadrant. In case of contamination of the strip with blood another site fulfilling the same criteria in the same quadrant was sampled. After isolation of the test sites from saliva, a first Durapore strip was inserted 1 mm into the sulcus or pocket and left in place for 15 s. Three minutes after removal of the first strip, a second Durapore strip was similarly inserted in the same site for 15 s. The two strips were then placed into a microcentrifuge tube and immediately frozen at −70 °C until the day of the analysis. In case of visible contamination with blood, the strips were discarded.

Analysis of cytokine production

The amount of IL-1β, IL-4, IL-6 and IL-8 in the GCF was determined after centrifugal elution, by using enzyme-linked immunoadsorbent assays (ELISAs), specific for each cytokine. All kits were purchased from Ruwag Diagnostics (Zürich) and the assays carried out in accordance with manufacturer's instructions.

Total cytokine amounts per 30 s samples were calculated, based on ELISA concentration values. Sites with cytokine levels below the limits of assay's detectability were scored as 0 pg.

Statistical analysis

The hypothesis of no difference in the levels of the four cytokines studied, between the different groups of patients was tested using the non-parametric ANOVA (Mann–Whitney U test) in which subjects were treated as blocks (Shirley 1987).

The values in Table 3 given for each cytokine represent the median and range values.

In order to compensate for the multiple comparisons, the significance level was set at $P < 0.05$ and a Bonferroni correction (Brown & Swanson-Beck 1988) was made according to the following formula (c: number of comparisons).

$$P_B = \frac{P}{\sqrt{c}} = \frac{0.05}{\sqrt{0.25}} = 0.010$$

Multiple linear regression analysis was performed to reveal correlations between cytokines level and clinical and environmental parameters, such as PD, smoking and stress. Finally, the interrelationship between levels of IL-β and IL-8 was determined using the Spearman rank correlation coefficient.

Table 1. Demographic and behavioral data of the subjects with healthy periodontium (H), gingivitis (G), adult periodontitis (AP) and early onset periodontitis (EOP)

	H	G	AP	EOP
n	20	20	20	20
Mean age	38 ± 11	31 ± 8	52 ± 8	32 ± 2
Male/Female	7/13	5/15	6/14	10/10
Smokers	1	6	10	14
Cigarettes/day (for smokers)	10	22 ± 11	29 ± 15	36 ± 14
Stress (tps)	2.5 ± 0.9	4 ± 8	16 ± 19	24.5 ± 22

Table 2. Periodontal status of the subjects with healthy periodontium (H), gingivitis (G), adult periodontitis (AP) and early onset periodontitis (EOP).

	H *n* = 20	G *n* = 20	AP *n* = 20	EOP *n* = 20
No. of teeth	30 ± 1.7	32 ± 0.22	29.6 ± 2.63	26.4 ± 2.07
PD (mean ± SD)	2.25 ± 0.9	2.29 ± 0.48	5.32 ± 1.07	5.8 ± 1.54
AL (mean ± SD)	0	1.3 ± 1.05	5.96 ± 1.60	6.28 ± 2.08
% of sites with:				
plaque accumulation	37.5	97.8	95	95
bleeding on probing	0	97.5	95	97.5
suppuration	0	0	0	2.5
PD?4mm	0	1.4	89	85
% of bone loss	0	0	43.57 ± 11.1	40.45 ± 5.34

n, number of subjects.

Table 3. GCF inflammatory mediator levels (median, range) in the 4 groups of patients : healthy (H), gingivitis (G), adult periodontitis (AP) and early onset periodontitis (EOP). Results are expressed as pg/30s sample

	IL-1β *n* = 80	IL-4 *n* = 80	IL-6 *n* = 80	IL-8 *n* = 80
H	6.90 (1.6−25)	11.50 (1.3–27)	0.90 (0.1–3.5)	22.60 (8.2–52.5)
G	19.5 (14–70)	1.61 (0–4.7)	0.77 (0.1–5.2)	42 (13.6–100)
AP	43 (16.3–139)	1.17 (0–3.3)	1.24 (1.2–10.3)	59.55 (12.9–76)
EOP	50 (15–126)	0.89 (0–5)	2.2 (0.2–12)	66.68 (23–171)
*H/G	*< 0.001*	*< 0.001*	NS	*< 0.001*
*H/AP	*< 0.001*	*< 0.001*	*0.004*	*< 0.001*
*H/EOP	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
*G/AP	*< 0.001*	*< 0.001*	*0.007*	*< 0.001*
*G/EOP	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
*AP/EOP	0.001	NS	0.001	*< 0.001*

n = number of sites.
*P, Comparison between groups.

Table 4. Multiple linear regression (GLM). Subset of predictor variables for each biochemical mediator (pg/30sample) in all patients (n = 80).

Marker	Predictor variable	Estimate	SE	*P*
IL-1*β*	*smoking*	3.75	2.67	0.16
	stress	0.34	0.07	**<0.001**
	PD	7.45	0.69	**<0.001**
IL-4	*smoking*	−1.89	0.65	**0.004**
	stress	−0.02	0.018	0.268
	PD	−0.97	0.168	**<0.001**
IL-6	smoking	1.28	0.56	**0.024**
	stress	0.05	0.016	**0.001**
	PD	0.50	0.146	**0.001**
IL-8	*smoking*	9.21	2.71	**0.001**
	stress	0.18	0.076	**0.016**
	PD	4.57	0.702	**<0.001**

*Bold face denotes statistically significant differences.

Results

Patient biographical and clinical data

The demographic and behavioral data, including age, gender, smoking habits and stress characteristics are summarized in Table 1. Interestingly, significantly more patients of the AP and EOP groups were smokers and under more stressful conditions as compared to those in groups H and G.

Table 2 shows the periodontal status of each group, including PD, AL, percentage of sites with plaque accumulation bleeding on probing (BOP), suppuration, PD74 mm, and percentage of bone loss. In this table, clinical parameters of the four selected sites from each patient are presented. Overall, PD and AL were slightly higher in the EOP group vs AP group, but failed to reach a level of statistical significance.

Gingival crevicular fluid mediator levels

Table 3 presents the median total amount of each mediator/30 s in each group of patients. IL-1*β*, IL-6 and IL-8 were significantly higher in the G, AP and EOP groups as compared to healthy subjects. Significant differences on the level of the above cytokines were observed between G and AP+EOP groups, as well as between AP and EOP groups. IL-4 showed an inverse association with the periodontal status: its highest amounts were observed in the healthy group (11.5 pg/30 s), followed by group G (1.61pg/30s), AP (1.17 pg/30 s) and EOP (0.89 pg/30 s). The difference between AP and EOP on the IL-4 total amount was not statistically significant.

Correlations between PD, smoking, stress and cytokine levels

Multiple linear regression analysis showed significant correlations between GCF cytokine levels and PD, smoking and stress. As shown in Table 4, PD was significantly correlated to the amounts of all four cytokines, smoking was associated with IL-4, IL-6 and IL-8 levels and stress was mostly associated with IL-1*β*, IL-6 and IL-8 levels.

Correlation analysis of IL-1β versus IL-8

The scatter plot showing the correlation of IL-1*β* versus IL-8 total amounts, is shown in Fig. 1. The highly significant relationship between the levels of these two mediators suggests that IL-1*β* and IL-8 may be coordinately expressed by macrophages and possibly other cell types in gingival tissues.

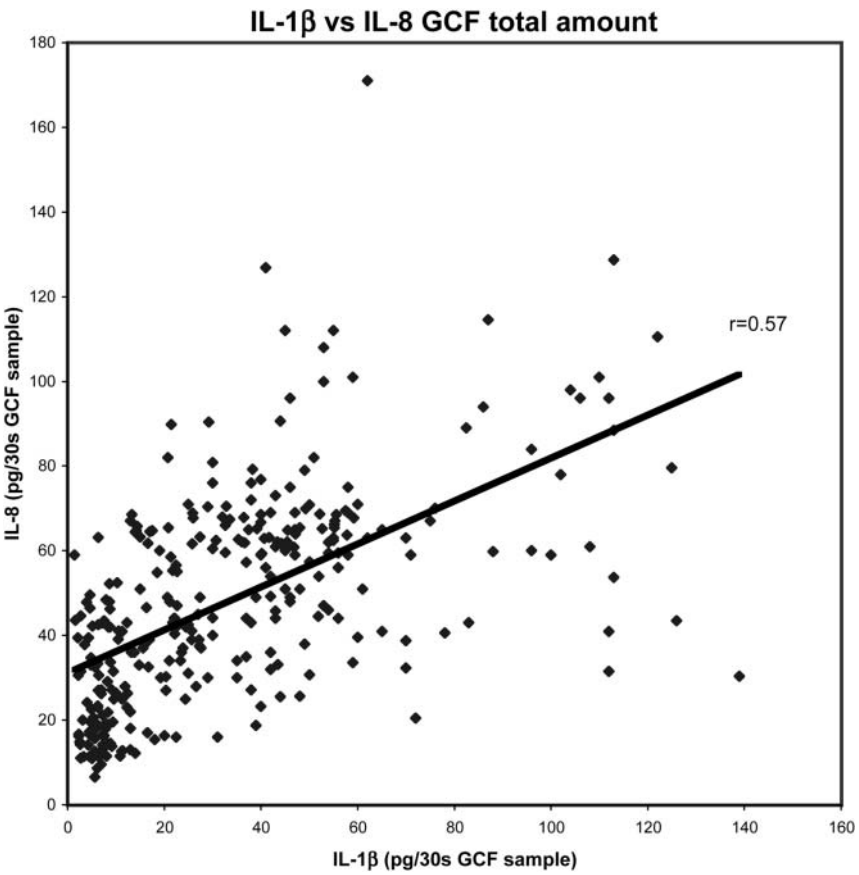

Fig. 1. Scatter plot of IL-1*β* and IL-8 in GCF. Statistical analysis using Spearman rank correlation coefficient.

Discussion

In the present study, the total amounts of IL-1*β*, IL-4, IL-6 and IL-8 were analyzed in the gingival crevicular fluid of periodontally healthy, gingivitis, adult periodontitis and early onset or aggressive periodontitis patients, and significant differences were observed.

Due to the inability of measuring the extremely small quantities of GCF available from healthy sites, the levels of the biochemical compounds have been

reported as total amounts per 30-s sample, as an alternative to concentrations. This is in accordance with the findings of several authors (Nakashima et al. 1996, Eley & Cox 1992, Smith et al. 1992), suggesting that total amounts rather than concentrations of GCF components should be used when estimating periodontal disease activity.

Our data indicate that elevated total amounts of IL-1β, IL-6 and IL-8 are associated with sites showing periodontal destruction: indeed, the levels of these three markers increased significantly in sites belonging to the AP and EOP patients, compared to those belonging to the H and G groups.

Marked differences of IL-1β were observed in the different disease categories groups, as compared to the healthy group; a 3-fold increase was noticed in the gingivitis patients, a 6-fold increase in the AP patients and an almost 9-fold increase in the EOP group. This finding is in agreement with those of Kinane et al. (1992), who, using the experimental gingivitis model, reported that the level of GCF IL-1β increased rapidly with plaque accumulation, prior to the clinically recognizable gingival changes.

Several other studies have reported higher levels of GCF IL-1β in periodontitis sites as compared to healthy sites (Wilton et al. 1992, Tsai et al. 1995, Liu et al. 1996, Ishihara et al. 1997, Kido et al. 1999, Figueredo et al. 1999, Rasmussen et al. 2000). Also, increased levels of IL-1β were found in GCF from active, as compared to inactive sites, suggesting that this proinflammatory cytokine may serve as possible indicator of disease activity in refractory periodontitis (Lee et al. 1995), the more so, that significant reductions in the level of IL-1β were observed in patients undergoing periodontal therapy (Reinhardt et al. 1993a; Alexander et al. 1996).

Another cytokine analyzed in GCF samples is IL-4, a 20-kDa product of Th2 cells, known to suppress synthesis of a number of pro-inflammatory cytokines, including IL-1, tumor necrosis factor (TNF)-α, IL-6 and IL-8. In our study, IL-4 showed an inverse relationship with the periodontal status, as significantly higher amounts were observed in the healthy group and only trace amounts in the other three groups of patients. This finding confirms previous observations regarding the lack of IL-4 in severe periodontal lesions (Kabashima et al. 1996), as well as in gingival mononuclear cells isolated from inflamed sites (Fujihashi et al. 1993a, 1993b). Moreover, Shapira et al. (1992) have suggested that the localized lack of the regulator cytokine IL-4 in the gingival tissues, predisposes susceptible individuals to progress from gingivitis to periodontitis.

IL-6 was successfully detected in almost all GCF samples of the present investigation. The level of this cytokine in the healthy and gingivitis groups was extremely low, but showed a 2-fold and 7-fold increase in the AP and EOP groups, respectively. IL-6 has been described as a 'lower tier' mediator, often undetectable in less severe periodontal conditions (Salvi et al. 1998). Our results concerning IL-6 are in agreement with those of other studies, showing higher amounts of IL-6 in patients with periodontitis (Mogi et al. 1999, Kurtis et al. 1999), in active sites of refractory periodontitis (Lee et al. 1995, Reinhardt et al. 1993b), as well as in sites undergoing orthodontic movement, emphasizing the role of IL-6 in the bone remodeling process (Uematsu et al. 1996).

The same trend was observed for IL-8, whose levels exhibited statistically significant differences between the groups. In general, the total amounts of this cytokine were much higher as compared to the three cytokines described above. IL-8 is a potentially important mediator through recruitment and functional activation of polymorphonuclear leukocytes (Ribeiro et al. 1991, Walz et al. 1991, Wozniak et al. 1993). IL-8, like IL-1β, is highly related to the inflammatory status of the periodontium, where large amounts of inflammatory cells are led to release it, upon stimulation by bacterial products. However, conflicting results concerning the association of IL-8 in GCF and the severity of periodontitis have been reported. Indeed, two studies suggested an inverse relationship between IL-8 activity and PMN recruitment. Jin et al. (2000) showed lower concentrations of IL-8 in patients with periodontitis as compared to healthy controls. Furthermore, Chung et al. (1997) observed a lower GCF IL-8 concentration in patients with deeper pockets and a higher percentage of sites which bled on probing. On the contrary, two other studies, along with the present one, suggested a positive relationship between GCF IL-8 activity and periodontal disease. According to Mathur et al. (1996), total amounts of IL-8 were significantly higher in GCF from diseased sites of patients with adult periodontitis as compared to GCF from healthy sites of control patients. Moreover, Tsai et al. (1995) observed that total amounts of GCF IL-8, significantly decreased after therapy in adult periodontitis patients. Such conflicting results may be related to varying factors. As reported earlier, one possibility is the mode of expressing cytokines in GCF. In the studies of Jin et al. (2000) and Chung et al. (1997), the results have been expressed as concentrations, whereas the results of Mathur et al. (1996), those of Tsai et al. (1995), and our own, were given as total amounts. One should keep in mind that in the presence of inflammation, lower concentrations of a given parameter may correspond to a significant increase of crevicular fluid volume whereas in health, higher concentrations may only reflect the minimal amounts of crevicular fluid available. Another important factor is the method used for the collection of the gingival fluid. In some studies the paper strip was placed at the orifice, while in others the strip was inserted into the crevice 'until resistance is felt'.

A parallel expression of IL-1β and IL-8 was observed in the present study, thus confirming earlier findings that these two cytokines are highly related to the inflammatory conditions of the periodontium (Kjeldsen et al. 1993, Tsai et al. 1995). Both IL-8 and IL-1β may be synthesized and secreted by the local periodontal connective tissue cells: fibroblasts, endothelial cells, or by infiltrating leukocytes, mononuclear cells, macrophages and neutrophils after bacterial stimulation.

Cytokine profiles are of considerable value when studying periodontal tissue destruction. The penetration of bacteria and/or bacterial products into the tissues results in recruitment and activation of the monocyte/T lymphocyte axis. This in turn leads to the enhanced monocytic release of TNF-α ,IL-1β, IL-6, associated with periodontal tissue destruction. IL-8, secreted by monocytic cells but also from keratinocytes, endothelial cells and fibroblasts, induces release of Matrix-Metalloproteinase (MMP) 8 pocket formation by neutrophils. This potent collagenase plays a critical role in degrading connective tissue at the inflammed site.

In summary, in diseased sites an imbalance in the cytokine network is locally induced and this may contribute to

the development of elevated B-cell responses in the inflamed gingival tissue. The fact that all cytokines were also detected in periodontally healthy sites is attributable to the presence of small numbers of macrophages and mononuclear cells in the gingival tissues and/or to neutrophils in the GCF. Finally, the wide range in total amounts obtained for all four cytokines, is mostly related to the diversity of cell types which can produce these mediators.

Smoking is a major risk factor which contributes to the pathogenesis of periodontitis. In the present study, associations were observed between smoking and the total amounts of GCF IL-4, IL-6 and IL-8 but not with the level of IL-1β. This is in agreement with the observations of Boström et al. (2000), who analyzed GCF levels of IL-1β and its receptor antagonist IL-1ra with respect to smoking in patients with moderate to severe periodontal disease. IL-1β was detected in almost all GCF samples but smoking showed no association with GCF levels of this cytokine nor with those of IL-1ra. *In vitro* application of nicotine on peripheral blood monocytes and lymphocytes, and on gingival mononuclear cells from patients with periodontitis, had no effect on IL-1β secretion, suggesting that nicotine cannot activate more cells, in the periodontitis lesion possibly due to maximal previous stimulation (Bernzweig et al. 1998; Payne et al. 1996). It seems that cigarette smoke contains potent inhibitors of cytokine production, at least for IL-1β, IL-2, interferon (IFN)-γ and TNF-α (Ouyang et al. 2000). In individuals who smoke, an imbalance in cytokine production seems to occur. However, when the influence of smoking was studied on the IL-6 content of GCF in patients with moderate to severe forms of periodontal disease, no statistically significant differences were observed between smokers and non-smokers (Boström et al. 1999). On the contrary, elevated concentrations of IL-6 were observed in the plasma of smokers (Tappia et al. 1995), as well as in the alveolar cells of healthy donors stimulated by TGP, a phenol-rich glycoprotein present in tobacco leaves and cigarette smoke condensate (Francus et al. 1992).

As for IL-8, its mRNA levels in the epithelial cells of smokers were positively correlated with the extent of smoking history (packs/day $\times$ no. of years of smoking) (Takizawa et al. 2000) and exposure to cigarette smoke induced bronchial epithelial cells to release IL-8 in a concentration and time-dependent manner (Moi et al. 1997). Other studies showed negative correlations between IL-8 levels and smoking (Sher et al. 1999).

Stress has been suggested as an important disruptive factor in the homeostatic regulation between oral bacteria and the host's immune system through alteration in the production of cytokines (Genco 1992, Genco et al. 1998, Ainamo & Ainamo 1996, Seymour et al. 1993). Indeed, in the present investigation, stress was found to be a good predictor of the GCF IL-1β, IL-6 and IL-8 levels. Paik et al. (2000) has shown that academic stress significantly increased serum IL-1β, IL-6 and IL-10 and decreased IFN-γ production. Also, in patients with posttraumatic stress disorders or after speaking and exercise tasks, IL-6 serum concentration were increased (Maes et al. 1998, 1999, Goebel et al. 2000). IL-6 is often called the 'stress-inducible cytokine'.

To our knowledge, the only cytokine measured in GCF in association with stress is IL-1β. Deinzer et al. (1999, 2000) observed significantly higher amounts of GCF IL-1β levels in a group of medical students participating in a major medical exam as compared to a group of students not participating in the exam, concluding that stress might affect periodontal health by increasing local IL-1β levels. Interestingly, most of the known periodontal disease risk factors, including bacterial LPS, heavy smoking and depressive mood states, are known to induce Hypothalamic Pituitary Adrenal axis hypersensitivity (Genco et al. 1998, Breivik et al. 2000). Recent research has provided evidence that products from the nervous and neuroendocrine system, released by emotional stress, may influence immune activities by immune cells, via alterations in the production of cytokines (Blalock 1994), thus affecting the Th1/Th2 balance (for review, see Breivik et al. 1996). The overall effect of glucocorticoids seems to be the suppression of the synthesis of pro-inflammatory and T-helper 1 (Th1) cytokines and the induction of a T-helper 2 (Th2) pattern of cytokine production by the CD4 T lymphocytes (Ramirez et al. 1996). In summary, our observation that higher amounts of GCF cytokines are associated with stressful events, strongly supports the hypothesis that aggravation of periodontal conditions is the consequence of increased cytokine production.

In conclusion, the present study showed a strong positive association between levels of IL-1β, IL-6, IL-8 in GCF and the periodontal disease status. Furthermore differences in total amounts of cytokines may be useful in distinguishing different forms of periodontal diseases.

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Zusammenfassung

Effekt der Entzündung, des Rauchens und von Stress auf das Cytokinniveau in der gingivalen creviculären Flüssigkeit

Hintergrund: Kürzliche Studien haben gezeigt, dass Cytokine zentral in der Pathogenese der parodontalen Erkrankungen sind und als Marker in der Diagnostik genutzt werden könnten.

Ziel: Das Ziel der vorliegenden Studie war die Bestimmung der Level von IL-1β, IL-4, IL-6 und IL-8 in der gingivalen creviculären Flüssigkeit bei parodontal Gesunden und erkrankten Individuen und das Studium ihrer Verbindung zu Rauchen, Stress und klinischen parodontalen Parametern.

Material und Methoden: Insgesamt wurden 80 Patienten in die Studie einbezogen: 20 Patienten mit früh beginnender oder aggressiver Parodontitis (EOP), 20 mit chronischer Erwachsenen-Parodontitis (AP), 20 mit Gingivitis (G) und 20 Patienten mit gesundem Parodontium (H). Die GCF wurde mit Durapore-Streifen von 4 Flächen pro Patient gesammelt, zufällig ausgewählt in jedem Quadranten. Der Gehalt von IL-1β, IL-4, IL-6 und IL-8 wurde in 320 Proben gemessen unter Nutzung kommerziell verfügbarer Sandwich ELISA.

Ergebnisse: Bei parodontal erkrankten Personen war der totale Gehalt von IL-1β, IL-6 und IL-8 signifikant erhöht verglichen mit gesunden Personen, während IL-4 eine inverse Beziehung zum parodontalen Status zeigte sowie höhere Mengen in der gesunden Gruppe gefunden wurde. Die Mengen aller 4 Cytokine waren positiv korreliert mit der Sondierungstiefe. IL-4, IL-6 und IL-8 waren signifikant korreliert zum Rauchen, während der Stress mit IL-1β, IL-6 und IL-8 verbunden war.

Zusammenfassung: Die vorliegenden Daten zeigen, dass das creviculäre IL-1β, IL-6 und IL-8 die Aktivität der parodontalen Destruktion reflektiert, während IL-4 eine inverse Korrelation dazu zeigt.

Résumé

Effet de l'inflammation, du tabagisme et du stress sur les niveaux de cytokines du fluide gingival

Contexte: De récentes études ont montré que les cytokines ont un rôle pivot dans la pathogénie des maladies parodontales et peuvent être utilisées comme marqueurs pour le diagnostic.

But: Le but de cette étude était de déterminer les niveaux d' IL-1β, IL-4, IL-6 et IL-8 dans le fluide gingival d'individus sains et au parodonte atteint et d'étudier leur association avec le tabagisme, le stress et les paramètres parodontaux cliniques.

Matériel et Méthodes: 80 patients furent inclus dans cette étude : 20 patients présentant une parodontite évoluant de façon précoce ou agressive (EOP), 20 présentant une parodontite chronique de l'adulte (AP), 20 avec une gingivite (G) et 20 patients au parodonte sain (H). GCF fut prélevé à l'aide de bandelettes Durapore sur 4 sites par patient, sélectionné au hasard dans chaque quadrant. Leur contenu en IL-1□, IL-4, IL-6 et IL-8 fut mesuré dans 320 échantillons grâce à un test ELISA commercialisé.

Résultats: Chez les sujets au parodonte malade la quantité totale d' IL-1□, IL-6 et IL-8 était significativement élevée par rapport à celle des sujets sains alors qu'IL-4 présentait une relation inverse par rapport à l'état parodontal et de plus grande quantités furent trouvées dans le groupe de patients sains. Les quantités des 4 cytokines étaient corrélées positivement avec la profondeur de poche. IL-4, IL-6 et IL-8 étaient significativement corrélées au tabagisme alors que le stress était associe avec IL- 1β, IL-6 et IL-8.

Conclusions: Ces données suggèrent que les niveaux créviculaires d' IL- 1β, IL-6 et IL-8 reflète l'activité de la destruction parodontale alors qu'IL-4 présente une corrélation inverse à cette activité. De plus, quelques conséquences cliniques du tabagisme et du stress pourrait être initiée et augmentée par la production de ces cytokines inflammatoires.

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