CHANGES IN GINGIVAL CREVICULAR FLUID INTERLEUKIN-4 AND INTERFERON-GAMMA IN PATIENTS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER PERIODONTAL INITIAL THERAPY

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Cytokines are pivotal to the immune response of chronic periodontitis. The present study investigated the changes of gingival crevicular fluid (GCF) interleukin-4 (IL-4) and interferon-gamma (IFN-γ) in patients with chronic periodontitis before and after initial nonsurgical periodontal therapy (NSPT). GCF was collected from 17 patients by means of Periopaper® at baseline and 1 month after NSPT. IL-4 and IFN-γ were measured by enzyme-linked immunoabsorbent assay. NSPT resulted in decreased total amount of IFN-γ, increased concentration of IL-4, and increased ratio of IL-4 to IFN-γ levels. We suggest that a low ratio of IL-4 to IFN-γ levels might be involved in the destruction (diseased sites) of periodontal tissue, whereas an increased ratio of IL-4 to IFN-γ levels could be related to the improvement of clinical periodontal health.

Inflammatory cell infiltrate containing lymphocytes and macrophages is often observed in periodontal lesions. It is hypothesized that T-lymphocytes are predominant in stable lesions, whereas B-lymphocytes and plasma cells are increased in progressive lesions [1–6]. This suggests that Th_1 cytokine expression T-cells are the main regulators of inflammation in the early/stable lesions. Production of interferon-gamma (IFN-γ) can increase the phagocytosis of polymorphonuclear neutrophils and macrophages, therefore inhibiting the progression of infection. The predominance of B-lymphocytes and plasma cells in advanced progressive lesions indicate the role of Th_2 cells in the destruction of periodontal tissue. If the immune regulation is inadequate, reduced level of interleukin-12 (IL-12) often leads to low expression of Th_1 and uncontrolled infection. Stimulation of plasma cells can lead to the production of IL-4 and promote the Th_2 response, activation of B-cells, and antibody production. Antibody can be protective by helping the elimination of infectious agents; however, antibody can also be destructive and lead to the progression of disease. Also, the continuous activation of B-cells promotes the production of IL-1 that results in the destruction of supporting osseous and connective tissues of the teeth.

Many studies have tried to elucidate the profile of Th_1/Th_2 cell expression in periodontal disease by various materials and methods. Some investigators

suggest the downregulation of Th$_1$ cytokine in gingival crevicular fluid (GCF) [7], gingival mononuclear cells [8], and peripheral blood monocytes [9,10] from periodontitis patients. Others reported increased Th$_2$ response in GCF [11], gingival mononuclear cells [12], peripheral blood monocytes [13,14], and gingival tissue [15–17] of periodontitis patients. These results support the relationship of Th$_1$ cells with stable periodontal lesions, whereas the Th$_2$ response is associated with disease progression. However, many reports suggest the predominance of Th$_1$ response and, therefore, a decreased Th$_2$ response in periodontal diseased tissues [18–20]. Studies [21,22] reporting that localized low IL-4 in periodontal diseased tissue is related to disease activity and progression led to the hypothesis by Shapira et al [21] that the localized absence of IL-4 can trigger periodontal disease activity. Giannopoulou et al reported an inverse relationship between GCF IL-4 level and periodontal status [23]. IL-4 was higher in the periodontal healthy group, but very minute in the periodontal diseased group [23]. This is in agreement with Kabashima et al, i.e. lack of IL-4 in GCF from severe inflammatory sites [22]. Similar results were also reported in the mononuclear cells isolated from inflamed gingival tissues [23,24]. All these findings suggest that localized absence of IL-4 in gingival tissue might lead to the development of periodontitis from gingivitis [21].

IL-4 and IFN-γ together may regulate the immunoinflammatory response [25]. Ukai et al investigated the IFN-γ- and IL-4-bearing cells in human periodontitis gingival tissue and reported that the ratios of IL-4- to IFN-γ-bearing cells in the severe inflammation or deep pocket depth (PD) groups were significantly lower than those in the moderate inflammation or shallow PD groups [26]. The authors suggested that a low ratio of IL-4- to IFN-γ-bearing cells might be involved in the destruction of periodontal tissue. Salvi et al reported that advanced adult periodontitis patients showed higher GCF IFN-γ than IL-4, suggesting that longitudinal follow-up in the levels and ratio of IFN-γ to IL-4 in GCF might be a useful indicator of periodontal tissue destruction [19].

IFN-γ and IL-4 represent Th$_1$ and Th$_2$ cytokines produced by lymphocytes. Both cytokines play a role in the regulation of the immunoinflammatory response [27]. The aim of this study was to determine the influence of nonsurgical periodontal treatment (NSPT) on the levels of IFN-γ and IL-4 in GCF.

MATERIALS AND METHODS

Subject selection

Seventeen Chinese adult non-smoking patients (11 males, 6 females; age range, 32–64 years) were selected from those being treated at the Department of Periodontics, Dental Clinic, Kaohsiung Medical University, Taiwan. The enrolment criteria were at least 20 teeth, no antibiotic or non-steroidal anti-inflammatory drug usage within 3 months, and no periodontal treatment within 6 months. Additional exclusion criteria were pregnancy or lactation, diabetes, HIV infection, bleeding disorders, and any condition necessitating antibiotic premedication for dental appointment. The institutional review board of Kaohsiung Medical University approved the study. Informed consent was obtained from all subjects.

GCF collection and clinical parameters

Each clinical evaluation was preceded by collection of GCF from the mesiobuccal surfaces of maxillary teeth (right second premolar to left second premolar, excluding teeth with dental restoration) to avoid saliva contamination. Briefly, teeth were air dried and isolated with cotton rolls, supragingival plaque was gently removed, and GCF samples were collected with Periopaper® (GCF collection strips, Proflow, Amityville, NY, USA) for 30 seconds. Paper strips were measured for fluid volume with a calibrated Periotron 8000 (Oraflow Inc., Plainview, NY, USA), and then removed to separate microcentrifuge tubes containing 100 μL physiologic saline 0.1% Tween 20. The tubes were stored at –70°C until eluted. Following elution, each GCF sample was analyzed separately.

Clinical measurement was performed immediately after the GCF collection. Probing PD is the distance in millimeters from the most coronal margin of free gingival to the most apical penetration of the probe. Probing attachment level (PAL) is the distance in millimeters from the cementoenamel junction to the most apical penetration of the probe. PD and PAL were measured using a Williams periodontal probe (Hu-Friedy™, Chicago, IL, USA) at six sites per tooth. The gingival index (GI) and the plaque index (PLI) were recorded dichotomously during GCF collection [28,29]. The sample sites were categorized into groups of PD <6 mm and ≥6 mm, PAL <6 mm and ≥6 mm. Only sites of PD and PAL ≥4 mm (4–10 mm) at baseline were subjected to data analysis.

Assays of IL-4 and IFN-γ

After grinding paper strips with microcentrifuge pestles, each sample was eluted in 500 μL phosphate buffered saline containing 0.1% Tween 20 by centrifugation (1,500 g, 10 min, 4°C). Following elution, GCF samples were analyzed for IL-4 and IFN-γ using commercially available enzyme-linked immunoabsorbent assays (ELISA, R&D System Inc., MN, USA). Analyses were performed according to the manufacturer's recommended protocol. Concentration of each GCF substance was calculated with a standard curve obtained with each standard recombinant GCF substance. Total amount of each GCF substance was obtained by multiplying concentration and GCF volume. Cytokine concentration of site lower than the detectable range was estimated statistically as one-half of the lowest value of the detectable site at the time of ELISA.

After the baseline clinical periodontal data were collected, patients were arranged to receive NSPT consisting of scaling and root planning with ultrasonic cavitron and curette ranging from five to seven sessions. Clinical periodontal parameters were reassessed 1 month after the completion of NSPT.

Statistical analysis

All data analyses were done using JMP and SAS (SAS Institute, Cary, NC, USA). Data are presented as mean ± standard deviation. Significance of mean differences between sites were tested by paired t test, assigning significance for two-tailed $p < 0.05$. Relationships among mean clinical parameters and GCF cytokines were tested by analysis of variance followed by a *post hoc* comparison using Tukey's pair-wise t test. The associations between the levels of various GCF substances and clinical parameters were calculated using Pearson's correlation and expressed by Pearson's correlation coefficient.

RESULTS

Baseline levels of IL-4 and IFN-γ in GCF

Non-significant differences for IL-4 and IFN-γ (Table 1) were observed for different PD or PAL site-groups.

Influence of NSPT on levels of IL-4 and IFN-γ in GCF

Significant improvements ($p < 0.0001$) in clinical periodontal parameters (GCF volume, PLI, GI, PD, PAL) were achieved by NSPT (Tables 2 and 3); however, only total amount of IFN-γ was significantly decreased ($p < 0.05$), and concentration of IL-4 was significantly increased ($p < 0.001$).

Influence of NSPT on ratios of IL-4 levels and IFN-γ levels in GCF

The ratios of the total amounts and concentrations of IL-4 to IFN-γ after NSPT were significantly higher ($p < 0.0001$) than at baseline (Table 4).

	IL-4 (pg) (n = 47)	IFN-γ (pg) (n = 47)	IL-4 (pg/μL) (n = 47)	IFN-γ (pg/μL) (n = 47)
Table 1. Baseline levels of interleukin-4 (IL-4) and interferon-gamma (IFN-γ) in gingival crevicular fluid*				
PD				
< 6	0.155 ± 0.188 (n = 22)	0.150 ± 0.240 (n = 22)	0.153 ± 0.171 (n = 22)	0.161 ± 0.242 (n = 22)
≥ 6	0.163 ± 0.167 (n = 25)	0.204 ± 0.277 (n = 25)	0.172 ± 0.227 (n = 25)	0.211 ± 0.260 (n = 25)
p	0.8754	0.4803	0.7500	0.5046
PAL				
< 6	0.160 ± 0.197 (n = 17)	0.164 ± 0.252 (n = 17)	0.153 ± 0.155 (n = 17)	0.168 ± 0.241 (n = 17)
≥ 6	0.159 ± 0.165 (n = 30)	0.188 ± 0.266 (n = 30)	0.168 ± 0.225 (n = 30)	0.198 ± 0.258 (n = 30)
p	0.9790	0.7707	0.8142	0.6980

*Data are presented as mean ± standard deviation. IL-4 (pg) = total amount of IL-4; IFN-γ (pg) = total amount of IFN-γ; IL-4 (pg/μL) = concentration of IL-4; IFN-γ (pg/μL) = concentration of IFN-γ; n = number of sample sites; PD = probing pocket depth (mm); PAL = probing attachment level (mm).

Table 2. Influence of nonsurgical periodontal therapy (NSPT) on periodontal parameters*

	PLI ($n = 47$)	GI ($n = 47$)	PD ($n = 47$)	PAL ($n = 47$)
Baseline	1.489 ± 0.804	1.915 ± 0.620	6.255 ± 1.621	6.660 ± 1.773
Post-NSPT	0.766 ± 0.865	1.511 ± 0.505	4.191 ± 1.597	5.574 ± 1.862
Difference	-0.723 ± 0.772	-0.404 ± 0.712	-2.064 ± 1.436	-1.085 ± 1.472
p	< 0.0001	0.0003	< 0.0001	< 0.0001

*Data are presented as mean ± standard deviation. PLI = plaque index; GI = gingival index; PD = probing pocket depth (mm); PAL = probing attachment level (mm); n = number of sample sites.

Table 3. Influence of nonsurgical periodontal therapy (NSPT) on the levels of interleukin-4 (IL-4) and interferon-gamma (IFN-γ) in gingival crevicular fluid (GCF)*

	GCF (μL) ($n = 47$)	IL-4 (pg) ($n = 47$)	IFN-γ (pg) ($n = 47$)	IL-4 (pg/μL) ($n = 47$)	IFN-γ (pg/μL) ($n = 47$)
Baseline	1.039 ± 0.349	0.159 ± 0.175	0.179 ± 0.259	0.163 ± 0.201	0.188 ± 0.250
Post-NSPT	0.342 ± 0.232	0.127 ± 0.121	0.120 ± 0.180	0.463 ± 0.667	0.377 ± 0.798
Difference	-0.697 ± 0.384	-0.032 ± 0.195	-0.069 ± 0.203	0.301 ± 0.619	0.189 ± 0.649
$p^{\dagger}$	< 0.0001	0.2606	0.0235	0.0017	0.0514

*Data are presented as mean ± standard deviation; †Wilcoxon signed rank test. IL-4 (pg) = total amount of IL-4; IFN-γ (pg) = total amount of IFN-γ; IL-4 (pg/μL) = concentration of IL-4; IFN-γ (pg/μL) = concentration of IFN-γ; n = number of sample sites.

Table 4. Influence of nonsurgical periodontal treatment (NSPT) on the ratios of interleukin-4 (IL-4) levels to interferon-gamma (IFN-γ) levels in gingival crevicular fluid*

	IL-4 (pg)/ IFN-γ (pg) ($n = 47$)	IL-4 (pg/μL)/ IFN-γ (pg/μL) ($n = 47$)
Baseline	4.8 ± 5.8	2.5 ± 2.7
Post-NSPT	12.4 ± 19.4	18.8 ± 29.9
Difference	7.7 ± 18.6	16.4 ± 29.6
p	0.0068	0.0004

*Data are presented as mean ± standard deviation. IL-4 (pg) = total amount of IL-4; IFN-γ (pg) = total amount of IFN-γ; IL-4 (pg/μL) = concentration of IL-4; IFN-γ (pg/μL) = concentration of IFN-γ; n = number of sample sites.

DISCUSSION

After NSPT, the total amount of IL-4 was not changed, but the concentration of IL-4 was significantly increased. This is different from the results of Kabashima et al who reported no detectable IL-4 in GCF from severe inflammation sites, but IL-4 could be detected after periodontal treatment [22]. However, Kabashima et al and we are in agreement that IL-4 might play a role in regulating the degree of local inflammation in periodontal disease [22].

Giannopoulou et al reported an inverse relationship between GCF IL-4 and periodontal status, i.e. the healthy group showed higher IL-4 than the diseased group, which had only minute IL-4 [23]. This is in agreement with our results. Ginnopoulou et al also indicated that IL-4 is negatively related with PD [23]. After NSPT, the total amount of IFN-γ was significantly decreased. NSPT resulted in the improvement of periodontal health; therefore, we suggest that the decrease in IFN-γ was related to the manifestation of periodontal lesions. This is in agreement with the suggestion of Ebersole and Taubman [18]. Salvi et al, in their study of inflammation mediators of the terminal dentition in adult and early-onset periodontitis, reported that the concentrations of GCF IFN-γ is higher than IL-4 in severe advanced adult periodontitis [19]. They concluded that Th$_1$ is significantly higher than Th$_2$ in severe advanced periodontitis.

In the present study, we compared the ratios of GCF IL-4 to IFN-γ in the baseline and post-NSPT sites and found that IL-4 to IFN-γ ratios were increased after NSPT. We suggest an association of the increased IL-4 to IFN-γ ratio with the improvement of periodontal status. Since IL-4 and IFN-γ represent Th$_1$ and Th$_2$ cytokines, the expression of IL-4 and IFN-γ has some relationship with the subpopulations of T-cells; in other words, Th$_1$ might be predominant

in periodontal lesions, and Th$_2$ might be more likely to be related with the remission of periodontal inflammation. Ukai et al discovered that ratios of IL-4- to IFN-γ-bearing cells in the severe inflammation or deep PD (≥5 mm) groups were significantly lower than those in the moderate or shallow PD groups [26]. These investigators suggest that a low ratio of IL-4- to IFN-γ-bearing cells might be involved in the destruction of periodontal tissue and support our current findings. In addition, our present study found that the change in IL-4 to IFN-γ ratio is mainly due to the significant decrease of IFN-γ by NSPT. Zong et al reported that the level of blood serum IFN-γ and IL-4 in patients with chronic periodontitis was significantly higher than the control group; however, only IFN-γ (not IL-4) concentration was reduced significantly in spite of the improvement in sulcular bleeding index and probing depth by initial periodontal therapy. This finding seems to be in agreement with or supporting our present results.

NSPT could significantly improve periodontal status. After NSPT, the total amount of IFN-γ decreased significantly, but IL-4 to IFN-γ ratios of both total amount and concentration were increased.

In conclusion, we suggest that Th$_1$ cytokine as represented by IFN-γ might be predominant in chronic periodontitis lesions; Th$_2$ cytokine as represented by IL-4 was associated with the remission or improvement of periodontal inflammation. A longitudinal observation may be helpful to more clearly define the role of GCF IL-4 and IFN-γ in the process of periodontal treatment.

ACKNOWLEDGMENTS

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非手術性牙周治療對牙齦溝液中 IL-4 及 IFN-γ 的影響

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近來的研究顯示細胞激素對慢性牙周炎的免疫反應的重要性，尤其是由 T 細胞所製造的細胞激素，將來或許可以作為診斷的指標。我們研究的主要目的是針對中度以及重度慢性牙周炎的患者，探討牙齦溝液中 IL-4 和 IFN-γ 的表現與臨床牙周指數之關係，以及其對牙周非手術性治療的反應。研究包括了 17 位慢性牙周炎患者口腔中共 47 個病灶，利用 Periopaper®，分別在非手術性牙周治療前及非手術性牙周治療後一個月，收集選定病灶中的牙齦溝液。利用 R&D 公司所生產的 ELISA (enzyme-linked immunosorbent assay) 套件測定治療前後共 94 個樣本中 IL-4 和 IFN-γ 的總量與濃度。研究發現，治療前患者 IL-4 與 IFN-γ 的總量與濃度在 PD、PAL < 6 的部位與 PD、PAL ≥ 6 的部位間沒有統計學上的差異。經過牙周非手術性治療，臨床牙周指數有顯著的改善，IFN-γ 的總量會顯著下降，IL-4 的濃度會顯著增加，且 IL-4/IFN-γ 比值顯著增加。因此我們推論，Th_1 的代表性細胞激素 IFN-γ 在牙周病灶區是佔優勢的，而 Th_2 的代表性細胞激素 IL-4 則是和牙周發炎的緩解有關，IL-4/IFN-γ 的高比值意味著牙周狀況的改善。

關鍵詞：牙齦溝液，IFN-γ，IL-4，非手術性牙周治療，牙周炎

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