Levels of interleukin-17 in gingival crevicular fluid and in supernatants of cellular cultures of gingival tissue from patients with chronic periodontitis

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Abstract

Background and Aims: Interleukin-17 (IL-17) is a T-cell-derived cytokine that may play an important role in the initiation or maintenance of the pro-inflammatory response and has recently been found to stimulate osteoclastic resorption. The purpose of the present study was to determine the presence of IL-17 in gingival crevicular fluid (GCF) samples and in the culture supernatants of gingival cells from patients with chronic periodontitis.

Method: GCF samples were collected during 30 s from two sites in 16 patients from periodontally affected sites (probing depth $\geqslant$5 mm, attachment loss $\geqslant$3 mm). The comparison with healthy controls was carried out by collecting GCF samples from eight healthy volunteers. GCF was collected using a paper strip and ELISA was performed to determine the total amount of IL-17. Supernatant cellular cultures of gingival cells were obtained from periodontal biopsies taken from 12 periodontitis patients and from eight healthy control subjects during the surgical removal of wisdom teeth. Spontaneous and phytohaemagglutinin (PHA)-stimulated levels of IL-17 were determined by ELISA.

Results: The total amount of cytokine IL-17 was significantly higher in the periodontitis group than the control group (45.9 *versus* 35.6 pg, $p = 0.005$). Significantly higher GCF volume and amount of total proteins were obtained from periodontitis patients as compared with control subjects (0.98 *versus* 0.36 μl, $p = 0.0005$; 0.12 *versus* 0.05 μg, $p = 0.0005$, respectively). A higher concentration of IL-17 was detected in culture supernatants from periodontitis patients compared with healthy subjects, either without stimulation (36.28 $\pm$ 8.39 *versus* 28.81 $\pm$ 1.50 μg/ml, $p = 0.011$) or with PHA stimulation (52.12 $\pm$ 14.56 *versus* 39.00 $\pm$ 4.90 μg/ml, $p = 0.012$). Treatment with PHA induced a significant increase in the production of IL-17 in healthy subjects and periodontitis patients ($p = 0.001$ and 0.003).

Conclusions: The total amount of cytokine IL-17 in GCF samples and in the culture supernatants of gingival cells are significantly increased in periodontal disease.

Key words: culture supernatants; cytokine; gingival crevicular fluid; periodontitis

Chronic periodontitis is defined as an infectious disease leading to slowly or moderately progressive loss of attachment and bone (Armitage 1999). Chronic periodontitis is associated with a bacterial infection and there is evidence that only some bacterial species of the complex biofilm adhering to the tooth surface contribute to the disease (Clark & Löe 1993, Haffajee & Socransky 1994, Socransky et al. 1998). The clinical features of perio-

dontitis include clinical attachment loss (CAL), alveolar bone loss, periodontal pockets, and gingival inflammation (Position Paper 2003). There is abundant evidence that major tissue destruction in periodontitis lesion results from the recruitment of host cells via activation of monocytes/macrophages, lymphocytes, fibroblasts, and other cell types (Seymour 1987, 1991, Saglie et al. 1988, Zappa et al. 1991). Considerable effort has been made to study the cytokines released by different host cells when exposed to components of periodontopathogenic bacteria (Birkedal-Hansen 1993, Offenbacher 1996). These studies have demonstrated that a number of pro-inflammatory cytokines are synthesized in response to periodontopathogenic bacteria and their products, hence inducing and maintaining an inflammatory response in the periodontium (Meikle et al. 1986, Page 1991).

Interleukin-17 (IL-17) is a T-cell cytokine that exhibits pleiotropic biological activities on various types of cells, such as fibroblasts, endothelial cells, and epithelial cells to produce other inflammatory cytokines and chemokines (Rouvier et al. 1993, Yao et al. 1995). It is expressed by a restricted set of cells, and the major source of IL-17 in the activated memory CD4+ T-cell subset (CD4+CD45RO) (Yao et al. 1995, Fossiez et al. 1996). IL-17 may affect osteoclastic bone resorption by stimulating osteoblasts to produce factors that affect the activity and/or formation of osteoclasts; osteoblasts are IL-17-responsive cells and express mRNA encoding for the IL-17 receptor (Von Bezooijen 1999).

Results from several clinical studies indicate that IL-17 may be involved in many inflammatory diseases. IL-17 is secreted by synovial T cells from rheumatoid arthritis patients and stimulates the production of inflammatory mediators (Chabaud et al. 1998, 1999). Elevated IL-17 mRNA expression has been found in mononuclear cells from patients with multiple sclerosis, a myelin-directed autoimmune disease (Matusevicius et al. 1999). In addition, the expression level of IL-17 mRNA is increased in T cells isolated from lesional psoriatic skin (Teunissen et al. 1998). The above findings have led to the suggestion that IL-17 may play a pivotal role in the initiation or maintenance of an inflammatory response (Jovanovic et al. 1998). While the role of IL-17 in inflammation has been studied by a number of investigators, the direct effects of IL-17 on periodontal diseases have not been well characterized. The aim of our study was to determine the total amount and concentration of the cytokine IL-17 in samples of gingival crevicular fluid (GCF) from chronic periodontitis patients and determine spontaneous and PHA-stimulated secretion of IL-17 in supernatants of the gingival cells.

Materials and Methods

Patients

The study group consisted of 16 adult patients (five males and 11 females; with age range 35–51 years old; mean age 38.4 ± 8.2 years) with moderate-to-advanced periodontitis selected from patients of the Graduate Periodontal Clinic at the Faculty of Dentistry of the University of Chile and the Center of Diagnostic and Treatment of Northern Metropolitan Health Services. The criteria for entry were a minimum of 14 natural teeth, excluding third molars, and including at least 10 posterior teeth. Patients with chronic periodontitis had moderate-to-advanced periodontitis (at least five to six teeth had sites with probing depth (PD) $\geqslant 5$ mm and with attachment loss $\geqslant 3$ mm and extensive bone loss in radiography), and had no received any periodontal treatment before the time of examination. Subjects did not suffer from systemic illness and had not received antibiotics or non-steroid anti-inflammatory therapy in the 6-month period prior to the study. Eight periodontal healthy subjects were recruited for the control group (two males and six females; with age range 35–53 years; mean age 36.4 ± 7.9 years). They did not have periodontal disease as determined by the absence of CAL or increased probing pocket depths. Prior to the study, all subjects received supragingival prophylaxis to remove gross calculus and allow PD. The protocol was clearly explained to all patients and controls, and Institutional Reviews Board-approved informed consents were signed. The protocol stated that, within 2 weeks of the detection of disease, all patients would be provided with periodontal treatment. Periodontal therapy consisted of scaling, root planing, and oral hygiene instruction.

Clinical measurement

Clinical parameters were evaluated in all teeth, excluding third molars, and included the following: PD, CAL and dichotomous measurements of supragingival plaque accumulation, and bleeding on probing (BOP) to the base of the crevice. Six sites were examined for each tooth: mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual. An automated disk probe was used for attachment level and PDs (Florida Probe Corporation, Gainesville, FL, USA). One calibrated examiner monitored the patients and collected the clinical reports.

Collection of GCF

After isolating the tooth with a cotton roll, supragingival plaque was removed with curettes (Hu Friedy, Gracey, IL, USA), without touching the marginal gingiva. The crevicular site was then dried gently with an air syringe. GCF was collected with paper strips (ProFlow, Amityville, NY, USA). Strips were placed into the sulci/pocket until mild resistance was sensed and left in place for 30 s. Strips contaminated by saliva or blood were excluded from the sampled group. Following collection of GCF, the volume of the sample on the paper strips was measured using a calibrated Periotron 8000 (ProFlow). The readings from the Periotron 8000 were converted to an actual volume (μl) by reference to the standard curve. After GCF collection, strips were placed in Eppendorf vials containing 100 μl of phosphate-buffered saline (PBS) with 0.05% Tween-20 (PBS-T). GCF was extracted by centrifugation at 10,000 g for 5 min. at 4°C (Hermle Labortech Nik. Z-233 MK-2. Gostheimerstr 56, 78564 Wehingen. Germany), the elution procedure was repeated twice, and samples were stored at -20°C until further analysis.

GCF samples were obtained from 2 sites in the periodontally affected sites (PD $\geqslant 5$ mm, attachment loss $\geqslant 3$ mm) from 16 patients with periodontal disease, and from 2 sites in the 8 healthy controls at the mesiobuccal gingival sulci at teeth 16 and 26.

Quantification of cytokine IL-17

Aliquots of each GCF sample were assayed by ELISA to determine the level of IL-17, according to the manufac-

turer's recommendations (Quantikine®, R&D Systems, Minneapolis, MN, USA). Briefly, 100 μl of standards and GCF samples were added in the respective wells in duplicate. One hundred microliters detection antibody was added to all wells, except blank, mixed gently, covered with strips of plastic film, and incubated overnight (16–24 h) at 4°C. Plates were washed 5 × and incubated with 200 μl conjugate for 50 min. at room temperature (18–26°C). Plates were washed 5 × and 200 μl of substrate was added and incubated for 30 min. at room temperature (18–26°C) in the dark. The reaction was stopped by the addition of 50 μl stop solution, and colour was measured in an automated microplate spectrophotometer (Bio-Tek Instruments Inc., Elx 800, Highland Park, VT, USA).

The amount of IL-17 was determined in picograms (pg). Results were calculated using the standard curves created in each assay. Values lower than the detection limit in our assay (below 15.6 pg) were considered undetectable. Cytokine concentration (pg/μl) was calculated from the volume of GCF estimated from the calibration unit reading, according to the following formula: cytokine concentration (pg/μl) = total amount cytokine (pg)/volume GCF (μl). Concentrations were also normalized for the protein content of the samples and given as pg/μg protein, according to the following formula: Cytokine concentration (pg/μg) = total amount cytokine (pg)/protein concentration in GCF (μg).

Protein determination

Protein concentration: amount of total proteins was measured using the Bio-Rad microassay (Bio-Rad Laboratories, Hercules, CA, USA) following the instructions of the manufacturer, with bovine serum albumin as standard.

Gingival tissue biopsies and preparation of gingival cells

Gingival tissues were obtained from periodontal biopsies taken from 12 periodontitis patients and eighty healthy control subjects during the surgical removal of wisdom teeth. The base incisions were made 1–2 mm subgingivally; therefore, the specimens consisted of the gingival margin, sulcular epithelium, and gingival connective tissue. Soft tissue biopsy specimens were washed extensively in PBS and immediately placed in a vial containing 5 ml of cold sterilized transport media: RPMI 1640 supplemented with 50 IU/ml penicillin, 50 μg/ml streptomycin, and 200 mM L-glutamine (Sigma Chemical Co., St. Louis, MI, USA). Vials with samples were transported at 4°C to the Biostructure Laboratory of the Faculty of Dentistry, University of Chile and processed immediately.

Samples of gingival tissues were weighed, minced into approximately 1 mm^3 pieces, and incubated in tissue digestion medium in a water bath at 37°C for 90 min. The tissue digestion medium consisted of RPMI 1640 supplemented with 50 IU/ml penicillin, 50 μg/ml streptomycin and 200 mM L-glutamine, plus 200 U/ml of type IV collagenase (Gibco Invitrogen Corporation, Grand Island, NY, USA), in a relation of milliliter of tissue digestion medium by 50 mg of gingival tissue.

Cells obtained were washed twice with PBS, cell counting was performed in a Neubahuer chamber using a phase contrast microscopy (Axiovert 100, Zeiss Co., Göttingen, Germany), and cell viability equal to or greater than 90% was calculated by Trypan blue dye exclusion. Cells were resuspended in cellular culture medium RPMI 1640 supplemented with 50 IU/ml penicillin, 50 μg/ml streptomycin, 200 mM L-glutamine and 10% foetal bovine serum (Gibco Invitrogen Corporation) to be immunotypified and immediately cultured.

Total cells were immunotypified and quantified to determine the percentage of leucocytes, granulocytes, and monocytes, and the relation CD4 and CD8 T cells of each sample. Fifty microliters of cellular culture medium containing 2,00,000 total cells was incubated separately with 10 μl of LeucoGATE (CD45 FITC/CD14 PE) and TriTEST (CD4 FITC/CD8 PE/CD3 PerCP) during 30 min. at room temperature in the dark (Becton Dickinson Immunocytometry Systems, San José, CA, USA). A sample incubated without antibodies was used as a negative control. After being washed once in PBS, samples were resuspended in 300 μl of PBS and were analysed by flow cytometry (FACSort Becton Dickinson Immunocytometry Systems).

Cells were cultured at a concentration of 1×10^6 cells/well in a 96-well culture plate and stimulated with 5 μg/ml of phytohaemagglutinin (PHA) for the stimulation of T-lymphocyte cells (Roche Molecular Biochemicals, Mannheim, Germany), for 24 h in a humidified atmosphere, 5% CO_2, and at 37°C.

Aliquots of supernatants cellular cultures were assayed by ELISA to determine the level of IL-17 according to the manufacturer's recommendations (Quantikine®, R&D Systems).

Data analysis

The clinical parameters and the total amount and concentration of cytokine IL-17 in healthy and diseased sites were expressed as subject mean ± standard deviation. Sites of patients were used as the experimental unit of observations and statistical data were analysed considering subjects as the experimental unit. Clinical measurements and the total amount and concentration of IL-17 were compared between healthy and diseased sites using an unpaired Student's *t*-test. The significance of differences within each group was assessed using the ANOVA test. The significance ($\alpha = 0.05$) of differences was assessed using the Tukey test.

Results

The clinical characteristics of patients and volunteers included in this study are grouped in Table 1. No statistically significant differences in age or gender existed between both groups. A significantly higher percent sites with plaque, sites with BOP, and higher PD and attachment level could be observed in the periodontal conditions in subjects with chronic periodontitis ($p < 0.05$).

The mean variations of total amounts and concentrations of cytokine IL-17 in the periodontitis group and in the control group are shown in Table 2. IL-17 in GCF was analysed in 32 samples from 16 patients with periodontal disease and in 16 samples from eight healthy subjects. The total amount of cytokine IL-17 was significantly higher in the periodontitis group than the control group (45.9 *versus* 35.6 pg) (p-value = 0.005). When cytokine concentration (pg/μl) was calculated from the volume of GCF estimated from the calibration unit reading, levels were higher in healthy subjects than in periodontitis patients (107.17 ± 46.32 *versus* 46.08 ± 11.86, $p = 0.03$). However, when cytokine concentration was normalized for the protein content of the samples and given as pg/μg protein, no significant variations were

Table 1. Clinical characteristics of periodontitis and control groups (mean ± SD)

	Periodontitis group ($n = 16$)	Control group ($n = 8$)
Age (years)	38.4 ± 8.2	36.4 ± 7.9
Woman (%)	68.8	75.0
Mean probing depth (mm)	3.2 ± 0.6*	2.2 ± 0.6*
Mean attachment level (mm)	3.2 ± 0.4†	0.5 ± 0.5†
Percentage sites with plaque	52.0‡	23.1‡
Percentage sites with bleeding on probing	40.9§	7.4§

Unpaired Student's *t*-test, ANOVA, and Tukey's test.
*Mean probing depth in periodontitis *versus* control groups: $p = 0.002$.
†Mean attachment level in periodontitis *versus* control groups: $p = 0.001$.
‡Percentage sites with plaque in periodontitis *versus* control groups: $p = 0.0005$.
§Percentage sites with bleeding on probing in periodontitis *versus* control groups: $p = 0.007$.

Table 2. Cytokine interleukin 17 (IL-17) in gingival crevicular fluid (GCF) of periodontitis and control groups (mean ± SD)

	Periodontitis group ($n = 16$)		Control group ($n = 8$)	
	mean ± SD	range	mean ± SD	range
IL-17 (pg)*	45.9 ± 17.4†	26.3–96.4	35.6 ± 2.4†	28.8–35.8
IL-17 (pg/μl)‡	46.1 ± 11.9§	29.8–72.5	107.2 ± 46.3§	56.5–288.4
IL-17 (pg/μg)¶	4.4 ± 2.6	1.9–11.5	7.0 ± 1.6	4.6–9.0
GCF (μl)	1.0 ± 0.2‖	0.8–1.4	0.4 ± 0.2‖	0.1–0.6
Total proteins (μg)	0.1 ± 0.04**	0.04–0.2	0.05 ± 0.01**	0.04–0.1

Unpaired Student's *t*-test and Tukey's test.
*Total amount of IL-17.
†Total amount of IL-17 in periodontitis *versus* control groups: $p = 0.005$.
‡Concentration of IL-17 in GCF.
§Concentration of IL-17 in periodontitis *versus* control groups: $p = 0.03$.
¶Concentration of IL-17 in the amount of total proteins.
‖Gingival crevicular fluid volume in periodontitis *versus* control groups: $p = 0.0005$.
**Amount of total proteins in periodontitis *versus* control groups: $p = 0.0005$.

Table 3. Gingival cells obtained by tissue digestion of periodontitis and healthy gingival samples and immunotypified by flow cytometry

	Periodontitis group ($n = 12$)	Control group ($n = 8$)
Lymphocytes (%)	58.5 ± 19.6	41.5 ± 1.3
Granulocytes (%)	34.9 ± 20.3	49.5 ± 3.9
Monocytes (%)	6.6 ± 4.1	9.1 ± 2.6
CD4 T cells (%)	29.1 ± 19.9	4.6 ± 0.4
CD8 T cells (%)	13.7 ± 7.7	3.3 ± 0.2
CD4/CD8 (ratio)	1.9 ± 0.9	1.4 ± 0.02

found (6.96 ± 1.56 *versus* 4.36 ± 2.58, $p > 0.05$). Significantly higher GCF volume and amount of total proteins were obtained from periodontitis patients as compared with control subjects (0.98 *versus* 0.36 μl, $p = 0.0005$; 0.12 *versus* 0.05 μg, $p = 0.0005$, respectively).

Table 3 and Figure 1 show gingival cells obtained by tissue digestion of periodontitis and healthy gingival samples and immunotypified by flow cytometry (Fig. 1).

Levels of IL-17 in culture supernatants of gingival cells are shown in Table 4. A higher concentration of IL-17 was detected in culture supernatants from periodontitis patients compared with healthy subjects, either without stimulation (36.28 ± 8.39 *versus* 28.81 ± 1.50 μg/ml, $p = 0.011$) or with PHA stimulation (52.12 ± 14.56 *versus* 39.00 ± 4.90 μg/ml, $p = 0.012$). Treatment with PHA induced a significant increase in the production of IL-17 in healthy subjects and periodontitis patients ($p = 0.001$ and $p = 0.003$, respectively).

Discussion

This study examined the total amount and concentration of the cytokine IL-17 in GCF of adult patients with chronic inflammatory periodontal disease. Our data demonstrate that the total amount of cytokine IL-17 is present in GCF of patients with periodontitis in levels significantly higher than in healthy subjects. Because of the role that IL-17 appears to play in the regulation of the immune system and its possible contribution to clinical disorders, identification and characterization of related molecules have been of particular interest (Lee et al. 2001). IL-17 has been found to stimulate the production of many cytokines: tumour necrosis factor α (TNF-α) and IL-1β from macrophages (Jovanovic et al. 1998); IL-6, IL-8, and the intracellular adhesion molecule-1 (ICAM-1) from human fibroblasts (Yao et al. 1995, Fossiez et al. 1996) and granulocyte colony-stimulating factor (G-CSF) and prostaglandin 2 (PGE_2) from synoviocytes (Fossiez et al. 1996). Considering the levels of IL-17 found in periodontitis patients, it is possible to speculate that this cytokine is involved in the development of the gingival inflammatory response by mediating activation of immune response.

Our results also show that IL-17 was detected in supernatants from cellular cultures of gingival tissue of both periodontitis patients and healthy subjects without stimulation, with levels significantly higher in the culture supernatants from periodontitis patients. After stimulation with PHA, the level of IL-17 in culture supernatants from periodontitis patients was significantly higher than that of healthy subjects. PHA is a plant lectin, glycoprotein that binds specifically to certain sugar residues on TCR and CD3 proteins, and thereby stimulates T cells. PHA induces a polyclonal functional response and it is commonly used for studying T cell activation (Abbas et al. 2000); however, it has been proposed that other kinds of cells as fibroblast are able to respond to PHA stimulation (Mustafa et al. 2000). Our data show that PHA stimulation induces significantly higher levels of IL-17 in both periodontitis and control groups. By flow cytometry, we demonstrate that lymphocytes are the predominant cells obtained by tissue digestion of periodontitis samples, and CD4T cells are in higher number than CD8T cells, with a ratio of 1.86. Furthermore, it has been determined that the majority of the $CD4^+$ T cells infiltrating in periodontitis lesion are $CD45RO^+$ memory T cells, cells expressing IL-17 (Gemmel et al. 1992, Yama-

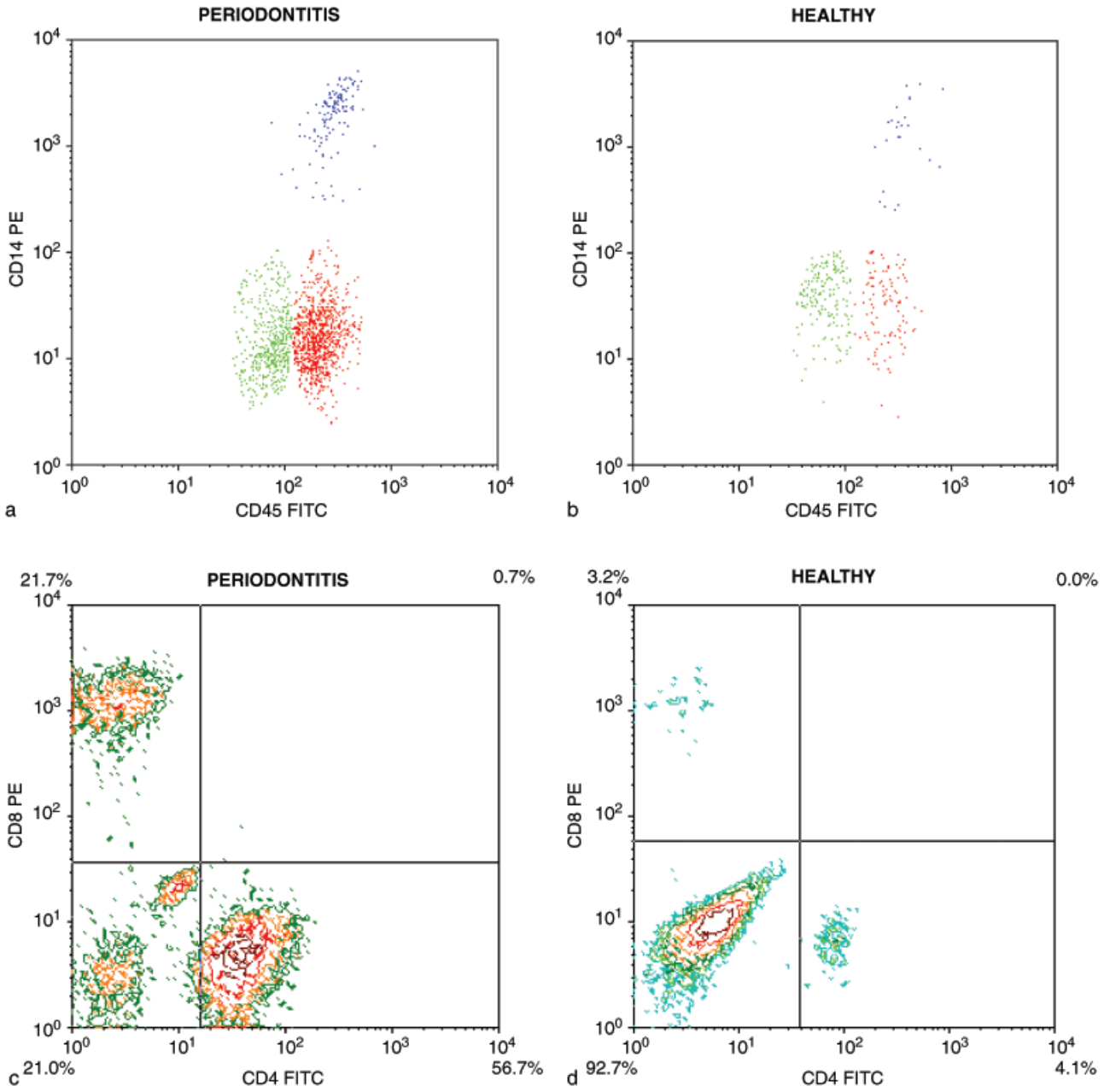

Fig. 1. Immunotypification by flow cytometry of total gingival cells obtained by tissue digestion of gingival samples. (a, b) LeucoGATE dotplot of a representative sample of periodontitis and control group. Red: lymphocytes (55.83% periodontitis *versus* 40.57% control), green: granulocytes (52.18% *versus* 34.94%), and blue: monocytes (9.22% *versus* 7.24%). (c, d) TriTEST CD4/CD8/CD3 contourplot of a representative sample of periodontitis and control group. Periodontitis: CD4 cells 56.7% and CD8 cells 21.7%, control group: CD4 cells 4.1% and CD8 cells 3.2%.

saki et al. 1993). Taken together, these results allow us to suggest that T cells are involved in the production of IL-17 in gingival tissue. These findings are in agreement with a recent study where *Porphyromonas gingivalis* OMP induced a significant increase in the production of IL-17 in periodontitis patients and after stimulation, IL-17 was more frequently detected in periodontitis patients than in gingivitis patients (Oda et al. 2003).

Although it has been established that IL-17 is a cytokine mainly produced by T $CD4^+$, from our results it cannot be demonstrated because PHA could stimulate other cellular components obtained by gingival digestion, such as fibroblast. On the other hand, it is still uncertain whether all $CD4^+$ cells or a subset of them produce IL-17.

Th cells can be divided into Th1 and Th2 subtypes according to their cytokine profiles and have different functional properties (Mosmann et al. 1986). Th1 cells produce IFN-γ, IL-2, and TNF-β, which induce cellular immunity and production of pro-inflammatory cytokines (Romagnani 1991). Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13 and favour B-cell-mediated humoral immunity and anti-inflammatory cytokine patterns (Romagnani 1991). Studies at a clonal level demon-

Table 4. Cytokine interleukin 17 (IL-17) levels in gingival cell culture supernatants (mean ± SD)

	Periodontitis group ($n = 12$)		Control group ($n = 8$)	
	spontaneous	PHA stimulated	spontaneous	PHA stimulated
IL-17 (pg/ml)	$36.3 \pm 8.4^{*,\dagger}$	$52.1 \pm 14.6^{\ddagger,\dagger}$	$28.8 \pm 1.5^{*,\S}$	$39.0 \pm 4.9^{\ddagger,\S}$
Range	29.1–58.2	33.5–83.3	26.8–31.2	33.2–46.6

Unpaired Student's *t*-test and Tukey's test.
*Concentration of spontaneous IL-17, periodontitis *versus* control $p = 0.011$.
†PHA stimulation, periodontitis group $p = 0.003$.
‡Concentration of PHA-stimulated IL-17, periodontitis *versus* control $p = 0.012$.
§PHA stimulation, control group $p = 0.001$.

strate that IL-17 is produced by some $CD4^+$ T cells exclusively of the Th1/Th0 type and not of the Th2 type (Aarvak et al. 1999). A number of studies have attempted to delineate the Th1/Th2 profile in periodontal disease; however, results are difficult to interpret due to differences in material examined and methodologies used. It is likely that different T-cell subsets predominate at different phases of disease and the inability to determine disease activity clinically is a major limitation in all these studies (Seymour and Gemmel 2001). Consequently, these IL-17-producing TH1/Th0 cells may define a new subset of pro-inflammatory Th1/Th0 cells; thus IL-17 appears to be a key marker in identifying these cells.

In our study, we have observed a significant decrease in IL-17 concentration in GCF from periodontitis patients compared with healthy subjects. However, considering that the volume of GCF produced in sites with probing >5 mm depth is higher than that in sites from healthy subjects, it explains the lower IL-17 concentration detected in sites with higher PD. However, when cytokine concentration was normalized for the protein content of the samples and given as pg/μg protein, no significant variations were found.

IL-17 acts on osteoblasts, resulting in COX-2-mediated PGE_2 synthesis and RANKL expression, the latter directly inducing differentiation of osteoclast progenitors into mature osteoclasts by binding to RANKL receptor present in osteoclast progenitor (Kotake et al. 1999). Destruction of the osseous support of the dentition is a hallmark of periodontal diseases. This localized bone resorptive process has been the target of therapeutic intervention and preventive strategies; hence, understanding the underlying mechanisms is critical for the effective treatment of periodontitis.

In summary, our data show higher cytokine IL-17 levels in the GCF and in supernatants from cellular cultures of gingival tissue from periodontitis patients than in healthy subjects, suggesting a role for IL-17 in the pathogenesis of chronic periodontitis.

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