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Characterization of cellular infiltrate, detection of chemokine receptor CCR5 and interleukin-8 and RANTES chemokines in adult periodontitis

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Leukocyte migration is essential for immune surveillance of tissues by focusing immune cells to sites of antigenic challenge. The control of leukocyte migration depends on the combined actions of adhesion molecules and a vast array of chemokines and their receptors. The purpose of the present study was to investigate the involvement of Interleukin-8 (IL-8), RANTES, the associated infiltrating cells and expression of CCR5 chemokine receptors in periodontitis; furthermore, the effect of periodontal therapy on these parameters was evaluated. Patients included in the study had moderate to advanced periodontal disease with at least 5–6 teeth with probing depth >6 mm, attachment loss ≥ 3 mm and extensive radiographic bone loss. The inflammatory infiltrate was analyzed by immunohistochemistry in gingival biopsies obtained from subjects at the beginning of the study and 2 months after periodontal treatment. Gingival crevicular fluid (GCF) was collected for 30 seconds using periopaper strips, and chemokines were quantified by ELISA. The cellular components of the inflammatory infiltrate included B (CD19) and T (CD3, CD4+ and CD8+) lymphocytes and monocytes/macrophages (CD11c). CCR5 chemokine receptor expressing cells were exclusively found in periodontitis gingiva. IL-8 and RANTES were detected in the periodontitis group, obtaining a total amount of 212.5 pg and 42.0 pg, respectively. However, IL-8 was also detectable in the GCF of the healthy group (total amount of 44.8 pg). Periodontal therapy reduced the cell number in the infiltrate and the levels of IL-8 and RANTES, suggesting a relationship between these chemokines and periodontal status. We propose that the presence of these chemokines and the expression of chemokine receptors may represent a marker of lymphocyte subsets with the ability to migrate to inflammatory sites.

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Chronic periodontitis is an inflammatory disease of the supporting tissues of the teeth, that comprise gingiva, periodontal attachment fibers and the alveolar bone. The clinical entity of the disease results from the interaction of periodontopathic plaque bacteria and host immune response mechanisms (1). The infiltrate in periodontal disease contains mononuclear cells, which are mainly transmigrated mononuclear phagocytes and lymphocytes. Whereas T lymphocytes predominate in the established chronic lesion, the proportion of B cells and plasma cells increases with the progression of the disease (2–5). Upon stimulation by bacterial products, it has been shown that macrophages,

lymphocytes and local resident cells, such as fibroblast and vascular endothelial cells, synthesize and secrete a variety of cytokines.

During the last decade, chemokines, a family of structurally related cytokines showing chemotactic activity for specific types of leukocyte populations, have been identified. The molecular hallmark of the chemokine family is the conservation of four cysteine residues which affect the tertiary structure of the proteins. Depending on whether the first two cysteines are separated by one amino acid or not, chemokines can be divided into CXC or CC subfamilies. CXC chemokines mainly attract and activate neutrophils, whereas CC chemokines attract and activate monocytes, lymphocytes, basophils, eosinophils, natural killer cells and dendritic cells (6). Lymphotactin is the only member of a third subclass, the C chemokine subfamily, and it lacks two of the four cysteines. Recently, a fourth chemokine subclass that contains the CX3C motif has been identified (7). Interleukin-8 (IL-8) is one of the CXC chemokines, and is considered an important mediator of granulocyte accumulation (8). IL-8 has been detected in the gingival crevicular fluid (GCF) (9–11) and gingival tissue sections of patients with periodontitis and IL-8 mRNA is expressed at the same sites (12, 13).

Recent data demonstrate the presence of RANTES in the GCF of patients with periodontitis (14). RANTES is a member of the CC chemokine family which displays significant chemotactic activity for eosinophils (15), monocytes (16) and CD45+ T cells (17). RANTES interacts with CCR3 and CCR5 chemokine receptors, which are present in monocytes, eosinophils, basophils, leukocytes and activated T cells (18–22). Recently it was shown that CCR5 is expressed almost exclusively by T helper type 1 cells (Th1) (23). Furthermore, RANTES is an efficient chemoattractant of Th1 cells, inducing their transmigration in a dose response manner, whereas Th2 cells are not attracted by this chemokine (24).

The aim of our study was to evaluate the total amount and local concentration of the CXC chemokine IL-8 and the CC chemokine RANTES in GCF. We have also identified the cell populations and expression of the CCR5 chemokine receptor in biopsies of gingival tissue. Finally, short-term variations of IL-8, RANTES and cell populations have been evaluated following periodontal therapy.

Materials and methods

Patients

Patients for this study were selected from Primary Attention Service, Facultad de Odontologia, Universidad Complutense de Madrid. Criteria for entry were a minimum of 14 natural teeth, excluding 3rd molars, and including at least 10 posterior teeth. Patients with chronic inflammatory periodontal disease (CIPD) had moderate to advanced periodontal disease (at least 5–6 teeth had sites with probing depth >6 mm and with attachment loss ≥ 3 mm and extensive radiographic bone loss) and had received no treatment at the time of examination. Subjects did not suffer from systemic illness and they had not received antibiotics or non-steroid anti-inflammatory therapy in the 6-month period prior to the study. The control group, also selected from Primary Attention Service, consisted of subjects with no evidence of periodontal disease. Subjects were free from periodontal disease as determined by the absence of clinical attachment loss or increased probing pocket depths. The protocol was clearly explained to all patients and Institutional Review Board-approved informed consents were signed. Within 2 weeks of the detection of disease, all patients were provided with periodontal treatment.

Clinical measurement

Prior to the beginning of the study, all subjects received supragingival prophylaxis to remove gross calculus and allow probing access. Attachment level and pocket depth measurements were taken at 6 sites per tooth by a single calibrated investigator. Measurements were made at the mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual positions. Dichotomous measurement of supragingival plaque (PI) and bleeding on probing (BOP) were also performed at 6 sites per tooth. Attachment level and pocket depth measurements were obtained with two models of the Florida Probe (Florida Probe Corporation, Gainesville, FL, USA). The Florida Disk Probe was used for relative attachment level recordings and the Pocket Depth probe was used to obtain probing depth recording. In order to monitor the effect of periodontal therapy on the total amount and concentration of cytokines and cell population, the clinical parameters mentioned previously were recorded at the same sites after 60 days of therapy. Therapy consisted in scaling, root planing and oral hygiene instruction.

Collection of gingival crevicular fluid (GCF)

If patients met entry criteria, GCF samples were obtained from six independent sites (72 samples). These sites derived from three different types of locations based on the probing depth: 2 sites with ≤ 3 mm, 2 sites with 4 to 6 mm and 2 sites with ≥ 6 mm, of randomly selected teeth. Briefly, 24 samples were obtained from each of the three different probing depth sites in each patient. GCF

samples were subsequently collected two months after periodontal therapy, from the initial sites to evaluate the effect of therapy on cytokine levels (72 samples). GCF samples in control groups were collected from the mesiobuccal gingival sulci at teeth 16 and 26 ($n=12$ per group).

After isolating the tooth with a cotton roll, supragingival plaque was removed with curettes (Hu Friedy, Gracey, USA), without touching the marginal gingiva. The crevicular site was then dried gently with an air syringe. GCF was collected with Periopaper® filter paper strips (ProFlow, Amityville, New York, 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. A calibrated Periotron-6000® (ProFlow) was used for volume determination of the strips. Then, the Periopaper® strips were immediately placed inside a sterile vial and stored at -70°C until further analysis.

Analysis of GCF

Following collection of GCF, the volume of the sample on the Periopaper® strips was measured using a calibrated Periotron-6000®. A standard curve correlating digital readout to volume was constructed for each calibration with standard human serum. Each volume was applied 3 times to a Periopaper® and the respective periotron units were recorded. No re-calibration of the Periotron-6000® was necessary throughout the study period. The readings from the Periotron-6000® were converted to an actual volume (μl) by reference to the standard curve. After GCF collection, strips were placed in Eppendorf vials containing 50 μl of phosphate buffered saline with 0.05% Tween-20 (PBS-T). GCF was extracted by centrifugation at 10,000 ***g*** for 5 min at 4°C (Biofuge 17RS, Heraeus, Sepatech, Osterode, Germany) and the procedure was repeated three times (25).

Quantification of cytokines

Aliquots of each GCF sample were assayed by an enzyme linked immunosorbent assay (ELISA) to determine the levels of IL-8 and RANTES using matched antibody pairs and according to the manufacturer's recommendations (ENDOGEN Inc., Cambridge, USA). Briefly, 96-well micro plates (F16 Maxisorp Loose, Nunc A/S, Roskilde, Denmark) were coated with anti-human monoclonal antibodies (ENDOGEN Inc.) overnight at 4°C. They were then washed 3 times with washing buffer (50 mM TRIS, 0.2% Tween-20, pH 7.9–8.1), and 15 μl of GCF samples in 100 μl phosphate buffered saline-Tween-20 (PBS-T) were added to the plate in duplicate and incubated for 1 hour at room temperature (RT). 100 μl of appropriate diluted biotin-labeled antibody (ENDOGEN Inc.) were added to each well, covered and incubated for 1 hour at RT. Plates were washed 3 times, and incubated with 100 μl HRP-conjugated Streptavidin (ENDOGEN Inc.) for 30 min at RT. After extensive washing, 100 μl TMB (ENDOGEN Inc.) substrate solution were added. The reaction was stopped after 30 min by addition of 50 μl 0.18 M sulfuric acid and color was measured at 450 nm using an automated micro-plate spectrophotometer (Labsystems Multiskan, BICHROMATIC, UK). Cytokine and chemokine concentrations in the samples were calculated with a standard curve (15.6–1000 pg) obtained with recombinant cytokine and chemokine (ENDOGEN Inc.). Values below 15.6 pg were not considered. Cytokine concentration was calculated according to the following formula: Cytokine concentration (pg/μl) = total cytokine (pg)/volume (μl).

Collection of gingival tissues

After informed consents were obtained, two soft tissue gingival biopsy samples were taken from each patient with periodontal disease ($n=12$). The incisions were made 1 to 2 mm subgingivally; therefore, the specimens consisted of the gingival margin, sulcular epithelium and gingival connective tissue. They were divided into two groups according to therapy: pre- and post-periodontal therapy samples. For controls, biopsies from 6 patients (aged 20 to 32 years) with normal gingiva were taken during the surgical removal of wisdom teeth. Soft tissue biopsy specimens were immediately embedded in issue freezing medium OCT Compound (Miles Inc., Diagnostics Division, Elkhart, IN, USA) and snap-frozen in liquid nitrogen slurry. Cryostat sections (4–6 μm thick) were obtained (Minotome Damon, IEC/Division, USA), briefly prefixed in acetone (Merck, Whitehouse Station, NJ, USA) and stored desiccated at -70°C until use.

Monoclonal antibodies

Monoclonal antibodies employed included SPT-T3b anti-CD3 (26), B.9.4.2. anti-CD8 (27), Hp2.6 anti-CD4 (28), HC1/1 anti-CD11c (29), BU12 anti-CD19 (30) and 45531.111 anti-CCR5 (R&D Systems Inc., Wiesbaden-Nordenstadt, Germany)

Immunohistochemistry

Tissue sections were fixed for 15 min in acetone at 4°C. A standard three-stage immunoperoxidase ABC technique was used to detect CD3, CD19,

CD4, CD8, CD11c and CCR5. Briefly, nonspecific tissue binding was blocked by incubation with 1.5% horse serum for 30 min. Specimens were incubated for 60 min with 40 μl of diluted primary antibody. All rinsing steps were performed with Tris-buffered saline (TBS pH 7.2–7.6). Biotinylated horse anti-mouse IgG secondary antibodies at a 1:200 dilution were incubated for 30 min (Dako, Glostrup, Denmark). Endogenous peroxidase activity was quenched by exposure for 30 min to 0.3% H_2O_2 in methanol. Sections were then incubated for 45 min with preformed avidin biotin-horseradish peroxidase macromolecular complexes (Dako). A black color was developed by exposure for 6 to 8 min to 0.5 mg/ml of the chromogen 3′,3′diaminobenzidine tetrahydrochloride (Dako). Sections were counterstained with hematoxylin Carazzi, dehydrated and permanently mounted. Positive and negative controls were processed with each series. Standard tissue sections of human tonsils were used as positive controls for immunohistochemistry. Supernatant from X63-myeloma cells was used as a negative control. Specificity and sensitivity of each immuno-histochemical staining were determined by comparison with the appropriate positive and negative controls included in each series.

Data analysis

The clinical parameters as well as the total amount and concentration of cytokines at healthy and diseased sites were expressed as subject means ± standard deviation. The unpaired Student *t*-test was used to analyze differences in clinical and biochemical parameters between patients with periodontitis and control groups. The significance of differences within each group was assessed using the analysis of variance (ANOVA) test. The correlation of cytokine levels with clinical parameters, probing depth and degree of activity in healthy and diseased subjects was calculated using Pearson's correlation.

The topographical distribution of positively immunostained cells in the infiltrating connective tissue (ICT) was established, using consecutive tissue sections as previously described (31, 32). A range between 0 and 4 was used to establish the proportion of total inflammatory cells present in the inflammatory infiltrate: 0 = no detectable cells; 1+ = less than 10%; 2+ = approx. 25%; 3+ = approx. 50%; and 4+ = more than 50%. The degree of histological inflammation in the connective tissue underlying the junctional epithelium (JE) was established on hematoxylin and eosin stained sections, according to the density and distribution of the inflammatory cells and assigned a Tagge inflammation score (TIS) (33). Briefly, each histological section was scored according to the following criteria: 0 = scattered areas of chronic inflammatory cells adjacent to the crevicular epithelium; 1 = an accumulation of chronic inflammatory cells forming a thin, continuous band, the infiltrate extended between and around the epithelial rete ridges; 2 = a dense infiltrate of chronic inflammatory cells replacing the gingival fibers and extending towards the oral side; 3 = a generalized, dense accumulation of chronic inflammatory cells in all zones. All slides were coded by one person (AS) and analyzed by another person (JG), making the measurements double-blind. The results were verified by a second examiner (AA).

Results

Degree of histological inflammation

The clinical characteristics of patients with periodontitis and the control group are indicated in Table 1. Inflammatory associated cells could be detected in the perivascular area underneath the JE and adjacent to the localization of the bacterial plaque. The biopsies of tissues from healthy patients showed a low degree of inflammation (type TIS 1), which was characterized by the presence of a poorly defined infiltrate. A significantly higher degree of inflammation could be observed in the gingiva of patients with periodontal disease. TIS 2/3 degree of inflammation could be observed in biopsies from sites with periodontitis, with a well organized and dense cell infiltration in the connective tissue (Fig. 1).

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

	Periodontitis group ($n=12$)	Control groups ($n=6$)
Age (years)	47.16 ± 11.15	40.16 ± 3.71
% males	33.33	33.33
Mean probing depth (mm)	3.17 ± 0.53	1.12 ± 0.8
Mean attachment level (mm)	3.6 ± 1.15	–
% sites with plaque	81.50 ± 11.1	45.40 ± 8.7
% sites with bleeding on probing	56.28 ± 15.7	4.56 ± 1.10
GCF volume (μl):		
mean volume	0.74 ± 0.3*	0.26 ± 0.10*
range	0.11–1.54	0.11–0.48

*Gingival crevicular fluid (GCF) volume, periodontitis *vs* control groups $p=0.0001$

Characterization of the infiltrate

The cellular composition of the infiltrate was defined by immunohistochemistry with specific antibodies against the different cellular subsets. Table 2 shows the degree of histological inflammation and characterization of inflammatory infiltrate according to the time of biopsy. The highest inflammation score was observed in periodontitis sites, which additionally presented the most abundant cellularity in the infiltrate. The specific analysis of the inflammatory infiltrate of periodontitis patients with monoclonal antibodies to distinguish different cell types showed significant levels of T cells (CD3+, CD4+, CD8+), B cells (CD19) and monocytes/macrophages (CD11c) (Fig. 2). These cell subpopulations were no longer present in biopsies from sites after two months of periodontal treatment (Fig. 3). Cells expressing the chemokine receptor CCR5 were exclusively found in periodontitis gingiva (Figs 2 and 3). The presence of CCR5+ cells was expected considering our previous finding reporting detection of RANTES in gingival crevicular fluid from patients with periodontitis (14).

Cytokines in the GCF from periodontal patients and controls

The majority of patients with periodontitis had detectable levels of IL-8 (100%, 72/72 samples) and RANTES (87%, 63/72 samples). However, IL-8 (75%, 9/12) was also the only detectable cytokine in GCF of healthy controls. Total amounts and concentrations of IL-8 and RANTES are indicated in Table 3. RANTES was undetectable in healthy subjects (<15.6 pg), whereas significant levels of this cytokine were present in periodontitis patients. In the case of IL-8, although present in GCF of the control group, it was significantly increased in the diseased group ($p<0.05$).

As shown in Table 4, the association of cytokine secretion with the severity of periodontitis was evaluated according to the probing depth. Total amount of IL-8 was higher in pockets with probing depth

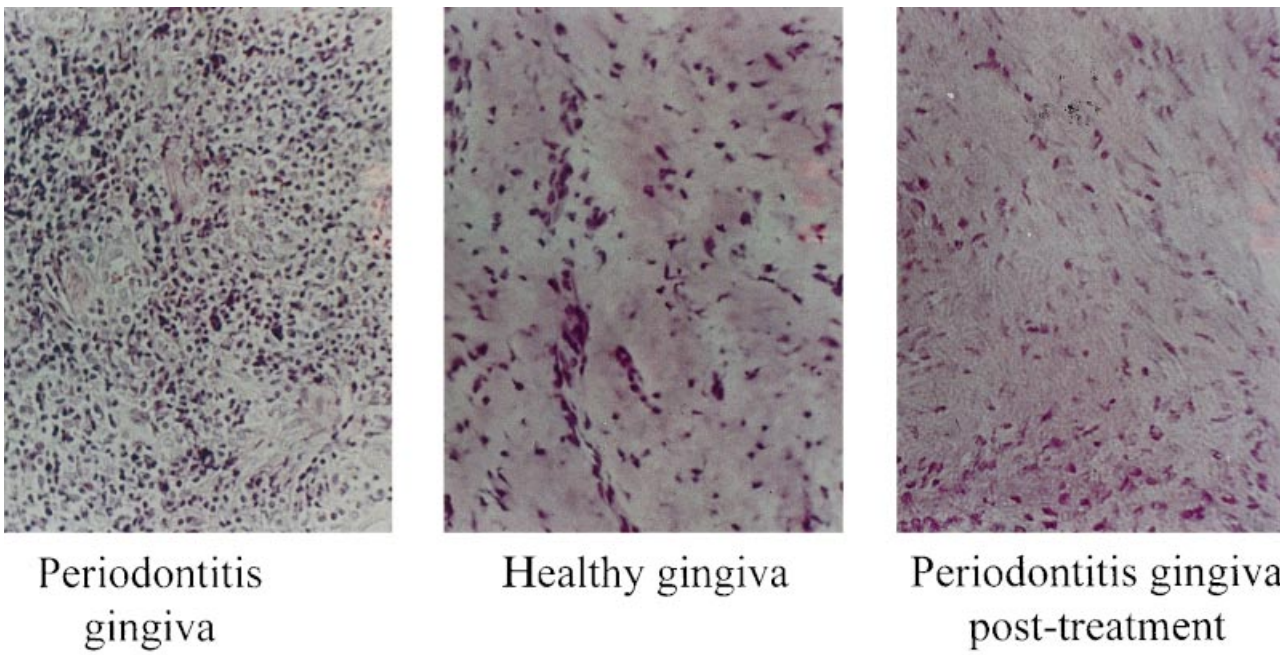

Fig. 1. Degree of histological inflammation in frozen sections of gingiva from periodontitis patients, post-treatment periodontal and control biopsies, counterstained with hematoxylin and eosin. Original magnification ×20.

Table 2. Characterization of inflammatory infiltrate from periodontitis patients (before and after periodontal therapy)

	Degree of inflammation	Inflammatory infiltrate (cells)					
Site	TIS score*	CD3	CD4	CD8	CD19	CD11c	CCR5
Before therapy	3	++/+++	++	+	+	+	+
After therapy	1	++/+++	++	−/+	−/+	−/+	−

*TIS: Tagge's inflammation score.
+: less than 10%; ++: approx. 25%; +++: approx. 50%, ++++: more than 50%.

>6 mm than in those with probing depth <3 mm, but this was not significant ($p>0.05$). In contrast, the total amount of RANTES detected according to the different probing depths showed no differences. However, both IL-8 and RANTES concentrations decreased with the probing depth ($p>0.05$).

Considering that cytokines could be related to the degree of the disease, we tested whether the elevated levels of IL-8 and RANTES would revert to control values following periodontal treatment. Therefore, we determined the levels of RANTES and IL-8 in sites before and after periodontal treatment. Table 5 shows that total amounts of IL-8 decreased significantly in the post-treatment sites ($p<0.05$) and RANTES became undetectable after periodontal treatment, which resulted as well in a significant decrease in GCF volume ($p=0.001$).

Correlation between cytokines and clinical parameters

The correlation between the total amount and concentration of cytokines and the clinical parameters is presented in Table 6. There was a weak correlation between the clinical parameters and the total amount of cytokines. However, a significant correlation between total amounts of IL-8 and

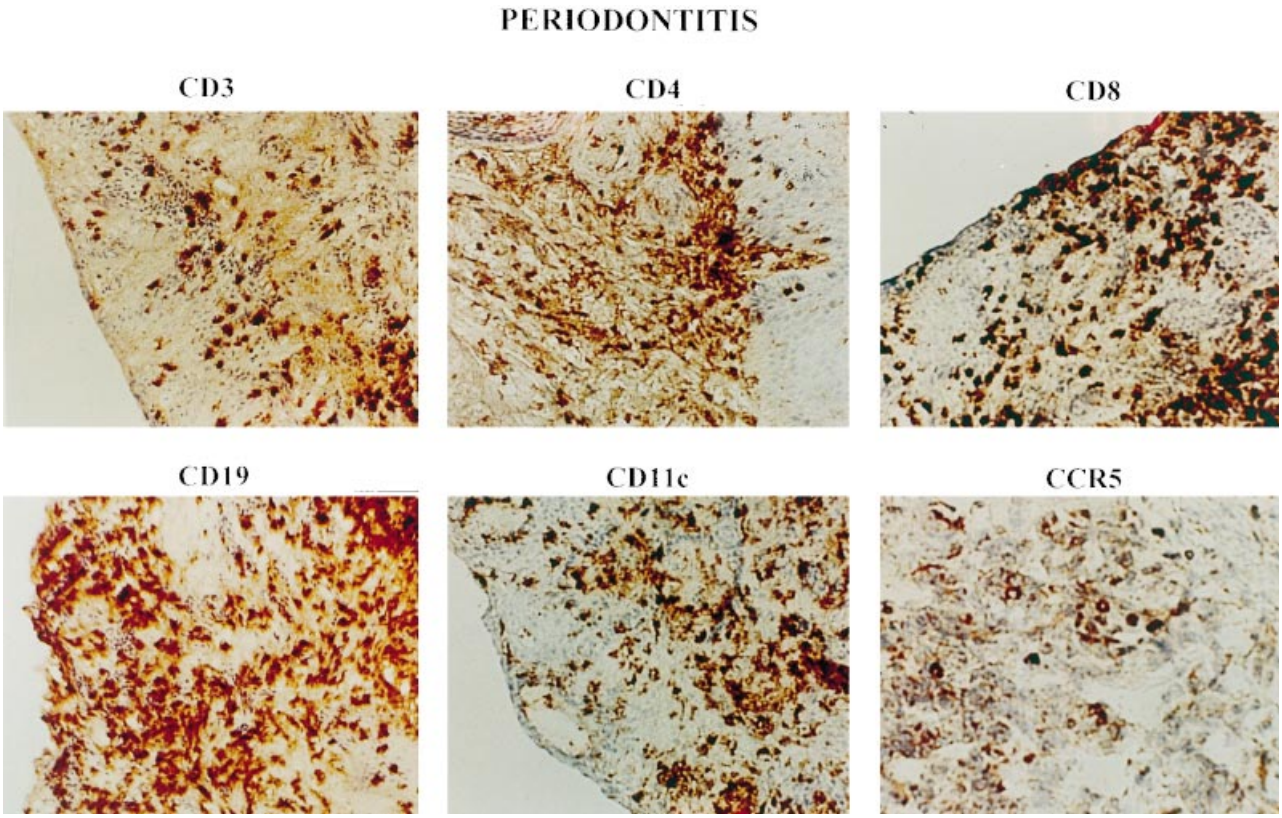

Fig. 2. Characterization of inflammatory infiltrate by immunohistochemistry on frozen sections of gingiva of periodontitis patients. Detection of T cells (anti-CD3, -CD4, -CD8), B cells (anti-CD19), monocyte/macrophages (anti-CD11c) and CCR5 chemokine receptor. Original magnification $\times 20$.

Table 3. Cytokine quantitation in GCF from periodontitis and control patients (mean $\pm$ SD)

	IL-8		RANTES	
Quantitation	Control ($n=12$)	Periodontitis ($n=72$)	Control ($n=12$)	Periodontitis ($n=72$)
Total (pg)	44.8 ± 17[a]	212.5 ± 133[a]	bkg	42.0 ± 20
Range	28.9–75.8	24.1–534.1	–	28.8–126
Concentration (pg/μl)	188.9 ± 98[b]	316.7 ± 209[b]	bkg	64.8 ± 39
Range	96.3–361.6	36.1–886	–	19.0–249.2

[a]IL-8 total amount (control *vs* periodontitis), p value $=0.025$.
[b]IL-8 concentration (control *vs* periodontitis), p value $=0.03$.
bkg, background level (<15.6 pg); GCF = gingival crevicular fluid.

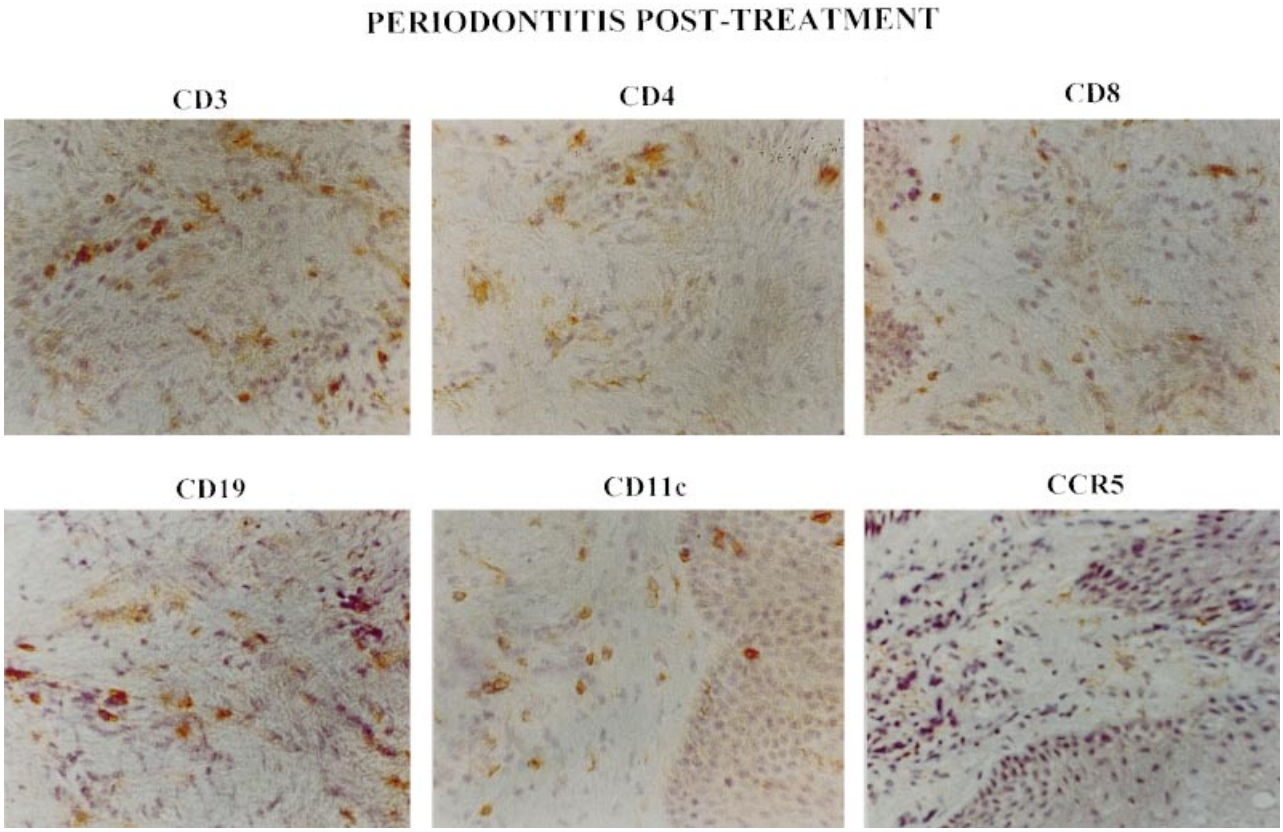

Fig. 3. Characterization of inflammatory infiltrate by immunohistochemistry on frozen sections of post-treatment periodontal disease. Detection of T cells (anti-CD3, -CD4, -CD8), B cells (anti-CD19), monocytes/macrophages (anti-CD11c) and chemokine receptor CCR5. Original magnification $\times 20$.

Table 4. Cytokines in GCF from periodontitis patients according to probing depth (mean $\pm$ SD)

		IL-8		RANTES		
Probing	No. of samples	Total amount (pg)	Concentration (pg/μl)	Total amount (pg)	Concentration (pg/μl)	GCF (μl)
$\leq$3 mm	24	193.3 $\pm$ 155	304.2 $\pm$ 244	42.1 $\pm$ 28	65.8 $\pm$ 45	0.6 $\pm$ 0.3
4–6 mm	24	194.4 $\pm$ 97	339.8 $\pm$ 198	38.6 $\pm$ 20	63.5 $\pm$ 44	0.6 $\pm$ 0.3
$\geq$6 mm	24	256.1 $\pm$ 148	302.8 $\pm$ 201	41.6 $\pm$ 16	54.3 $\pm$ 35	0.9 $\pm$ 0.3

GCF = gingival crevicular fluid.

Table 5. Cytokines in GCF before and after periodontal treatment (mean $\pm$ SD)

		IL-8		RANTES		
Treatment	N	Total amount (pg)	Concentration (pg/μl)	Total amount (pg)	Concentration (pg/μl)	GCF (μl)
Before	72	212.5 $\pm$ 133[a]	316.7 $\pm$ 209	42.0 $\pm$ 20	64.8 $\pm$ 39	0.72 $\pm$ 0.3[b]
After	72	85.4 $\pm$ 49[a]	241.4 $\pm$ 163	bkg	bkg	0.38 $\pm$ 0.1[b]

[a]IL-8 total before *vs* after treatment, p value = 0.011.
[b]Gingival crevicular fluid (GCF) before *vs* after, p value = 0.001 bkg, background level (< 15.6 pg).

Table 6. Correlation between total amount of cytokines and clinical parameters

Clinical parameters	IL-8	RANTES
PI	0.115	0.018
BOP	0.013	0.019
PD	0.049	0.052
GCF	0.706*	0.736*

*p value < 0.05.
PI: Plaque index; BOP: Bleeding on probing; PD: Probing depth; GCF: Gingival crevicular fluid.

RANTES and GCF volume was obtained ($r = 0.706$, $r = 0.736$, $p < 0.05$).

Discussion

The immune response to periodontopathic bacteria is mediated by specific adhesion molecules. Selective migration and accumulation of leukocytes is determined by the recently discovered chemokines, a family of low-molecular-weight cytokines with cell type-specific chemoattractant properties. This study demonstrates that the development of chronic adult periodontitis is related to the secretion of IL-8 and RANTES and to the expression of CCR5+ cells in inflamed gingival tissue, as determined by immunohistochemistry. Periodontal treatment, which consisted of the removal of the bacterial plaque, reduced the degree of inflammation and the total amount of cytokines present in the GCF.

The immune response is promoted by secretion of bacterial products and involves T and B lymphocytes and macrophages. In fact, clear differences in the degree of inflammation and secreted cytokines were observed when periodontitis patients were compared with normal healthy individuals. Periodontal disease histopathological classification includes four different stages (34–36). Our results demonstrate an increased number of T cells, B cells and macrophages in the inflammatory infiltrate from biopsies of periodontitis patients as compared to healthy individuals.

IL-8 (100%) and RANTES (87%) were detected in the GCF of periodontitis patients. However, IL-8 was the only cytokine detected in GCF from healthy individuals (75%). The presence of IL-8 in healthy individuals could be related with the steady state of the gingiva, considering this is a site of permanent antigenic insult, requiring the presence of neutrophils, macrophages and antigen presenting cells, which could be chemoattracted towards the gingival micro-environment by IL-8. The increase in IL-8 levels in periodontitis patients, shown by our findings, has been previously described (9, 11). However, other authors found that IL-8 concentrations were increased in healthy donors (25, 37). Our findings differ from the concentration data reported by other authors (25); as already suggested (37), one possible explanation could be attributed to as yet unidentified mediators of IL-8 secretion accounting for the difference in concentration observed. Additionally, it could be due to the increased GCF volume found in diseased sites, which in our study varies significantly between periodontitis patients and healthy controls. The difference in GCF volume could depend on the duration of sample collection; in certain studies (37), GCF was collected with filter strips for a 3 minute period, whereas in other reports (9), strips were maintained in the crevice for 20 seconds. In our study, GCF was collected with filter strips for 30 seconds, in order to avoid cytokine secretion induced by the mechanical irritation due to longer collection periods.

Our results show that RANTES cytokines are detected exclusively in periodontitis patients. RANTES is a member of the CC chemokine subfamily, implicated in selective attraction of different leukocyte subsets (8, 38). The presence of RANTES in GCF could be involved in the development of the gingival inflammatory response by mediating leukocyte recruitment and activation. It has been clearly established that the production of specific chemokines is associated with the selective migration of subsets of effector T cells. Activated T cells, expressing CCR3 and CCR5, are specifically attracted by RANTES, MCP-1 and MIP-1β chemokines, which have been reported to be ligands for these receptors (39). Recent data demonstrate that RANTES is an efficient chemoattractant for Th1 cells (but not for Th2 cells), inducing a dose response transmigration of Th1 cells (24). Therefore, RANTES may play a significant role in the regulation of local immune reactions controlling the balance between pro-inflammatory and anti-inflammatory T cell subsets. The presence of T cells and macrophages in biopsies of connective tissue in periodontitis patients (Table 2, Figs 2 and 3) implies their potential role in the mechanisms of tissue destruction associated with periodontitis, suggesting that RANTES could contribute to the increased infiltration of macrophage/monocytes in periodontal tissues observed in this pathological condition.

Presumably, expression of chemokine receptors is also important for the selective migration of T cells, especially certain subsets of effector T cells. In this study, we detected CCR5 expressing T cells, a marker of lymphocyte subsets with the ability to migrate to inflammatory sites.

The marked reduction of cytokines in GCF following treatment observed in the present study clearly suggests a relationship between disease and cytokine production. Periodontal treatment also led to significant variation in the cell populations present in the inflammatory infiltrate.

As shown by our results, higher cytokine levels were detected in GCF from inflamed sites than from healthy sites. Moreover, cytokine levels in GCF could not be correlated with any of the clinical parameters tested in the present study. This finding is in agreement with other studies (40, 41) and may be explained by the fact that clinical parameters such as pocket depth, attachment loss and bleeding on probing do not necessarily reflect current disease activity.

Our results suggest that chemokine production could be critical to control the type of T cell immune response in a given tissue. An attempt to correlate the absence or presence of Th1 or Th2 cells with the progression of the disease could deserve further study.

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