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Cytokine levels in sites of chronic periodontitis of poorly controlled and well-controlled type 2 diabetic subjects

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Abstract

Aim: This study compared the levels of tumour necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-4, IL-17 and IL-23 in the gingival crevicular fluid (GCF) from well-controlled and poorly controlled type 2 diabetic subjects with chronic periodontitis, before and after periodontal therapy.

Material and Methods: Eighteen well-controlled (glycated haemoglobin levels $\leqslant 8\%$) and 20 poorly controlled (glycated haemoglobin levels $> 8\%$) diabetic subjects were enrolled in this study. All subjects were submitted to non-surgical periodontal therapy. GCF sampling and clinical periodontal parameters were assessed before, 3 and 6 months post-therapy. Total amounts and concentrations of TNF-α, IFN-γ, IL-4, IL-17 and IL-23 in the GCF were analysed by enzyme-linked immunosorbent assay (ELISA).

Results: The levels of IL-17 were higher in poorly than in well-controlled subjects ($p < 0.05$), whereas the levels of IFN-γ were increased in well- compared with poorly controlled subjects at all experimental groups ($p < 0.05$). In addition, IL-4 levels were lower in well- than poorly controlled diabetic subjects at baseline ($p < 0.05$). There were no differences between groups for TNF-α and IL-23 at any time points ($p > 0.05$).

Conclusion: These results indicate a predominance of pro-inflammatory T-helper type 1 (Th1)- or Th17-cytokines in sites of chronic periodontitis from type 2 diabetic subjects, according to their glycaemic control.

Key words: diabetes mellitus; chronic periodontitis; root planing; inflammation; cytokines; enzyme-linked immunosorbent assay

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Type 2 diabetes mellitus (DM), characterized by impaired insulin function due to changes in insulin molecules and/or their cell receptors (Kidambi & Patel 2008), is the most prevalent type of DM among middle-aged subjects (Israili 2009). Several studies have demonstrated that the prevalence, progression and severity of periodontal diseases are higher in diabetic subjects when compared with non-diabetic ones, supporting DM as a risk factor for periodontitis (Kinane & Bouchard 2008). In addition, clinical studies have demonstrated a positive association between poor glycaemic control and the severity of periodontal diseases (Seppälä & Ainamo 1994, Tsai et al. 2002, Lim et al. 2007, Bandyopadhyay et al. 2010, Chen et al. 2010).

Periodontitis is an infectious inflammatory disease that results from the interaction between biofilm and the host defense mechanisms and is sustained by a network of pro- and anti-inflammatory mediators that may play antagonist and/or synergic biological activities (Tatakis & Kumar 2005). After antigenic stimulation, naïve CD4+ T cells, one of the most important cell types in cell-mediated immune response, may differentiate into effector T-helper (Th) cells including Th1, Th2 and Th17 phenotypes, each with distinct profiles of cytokine production (Tesmer et al. 2008, Zhu et al. 2010). Interferon (IFN)-γ, the main cytokine secreted by Th1 subsets

Conflict of interest and source of funding statement

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and natural killer cells, induces the macrophage activation and the production of a series of pro-inflammatory mediators, such as tumour necrosis factor (TNF)-α. Increased numbers of IFN-γ-producing cells in periodontal tissues and elevated levels of IFN-γ in the gingival crevicular fluid (GCF) have been associated with the progression of chronic periodontitis (Ukai et al. 2001, Dutzan et al. 2009b). Conversely, interleukin (IL)-4, an anti-inflammatory cytokine secreted by Th2 cells, down-regulates the production of IFN-γ and other pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-1β (Mosmann & Coffman 1989, te Velde et al. 1990) and therefore, its presence has been related to healthy periodontal tissues (Shapira et al. 1992, Kabashima et al. 1996, Tsai et al. 2007). IL-17 is a pro-inflammatory cytokine, produced by Th17 subsets, which promotes recruitment of neutrophils (Tesmer et al. 2008) and stimulates the production of a series of pro-inflammatory mediators (Patel et al. 2007, Tesmer et al. 2008), matrix metalloproteinases (MMP) and osteoclastogenesis-related factors (Oda et al. 2003, Takahashi et al. 2005, Sato et al. 2006, Beklen et al. 2007). Elevated levels of IL-17 messenger RNA and protein, as well as the presence of Th17 cells, have been observed in diseased, when compared with healthy periodontal tissues (Takahashi et al. 2005, Cardoso et al. 2009). IL-23 is essential in maintaining and expanding the Th17 cell population and plays a critical role in driving an initial inflammatory immune response against pathogens or injuries by inducing IL-17 production and neutrophil recruitment (Tan et al. 2009). Increased levels of IL-23 in the gingival tissue have been observed in sites presenting clinical attachment loss (Lester et al. 2007).

Although the clinical relationship between periodontitis and DM is well established, few investigations have focused on the immunoinflammatory responses in sites with periodontitis in subjects with DM (Engebretson et al. 2004, 2006, Duarte et al. 2007, Navarro-Sanchez et al. 2007, Venza et al. 2010). In addition, the cellular and molecular mechanisms that could explain the more severe clinical periodontal destruction observed in poorly controlled diabetic subjects, when compared with well-controlled subjects, are still unclear (Seppälä & Ainamo 1994, Tsai et al. 2002, Lim et al. 2007, Bandyopadhyay et al. 2010, Chen et al. 2010). Therefore, the aim of this study was to compare the levels of TNF-α, IFN-γ, IL-4, IL-17 and IL-23 in the GCF from well-controlled and poorly controlled type 2 diabetic subjects with chronic periodontitis before and after non-surgical periodontal therapy. The hypothesis is that glycaemic control may alter the protective and destructive host immune and inflammatory responses to periodontal pathogens in type 2 diabetic subjects.

Material and Methods

Subject population

Thirty-eight subjects (age range: 40–67 years) diagnosed with type 2 DM and chronic periodontitis were selected from the population referred to the Periodontal Clinic of Guarulhos University, from July 2007 until March 2008. Detailed medical and dental records were obtained. Subjects who fulfilled the following inclusion/exclusion criteria were invited to participate in the study. All eligible subjects were thoroughly informed of the nature, potential risks and benefits of their participation in the study and signed their informed consent. This study protocol was approved previously by Guarulhos University's Ethics Committee in Clinical Research.

Inclusion and exclusion criteria

Data concerning the duration of DM and medications were retrieved from the medical records of the subjects at the beginning of the study. All subjects had presented diagnosis of type 2 DM during at least the past 5 years and were under insulin supplementation, diet regime and/or oral hypoglycaemia agents. Subjects were diagnosed with generalized chronic periodontitis, based on the clinical and radiographic criteria proposed by the 1999 World Workshop for Classification of Periodontal Diseases and Conditions (Armitage 1999). All subjects were >30 years old, had at least 15 teeth excluding third molars and teeth with advanced decay indicated to exodontias, and more than 30% of the sites had probing depth (PD) and clinical attachment level (CAL) $\geqslant 4$ mm at baseline.

Exclusion criteria were pregnancy, lactation, current smoking, smoking within the past 5 years, periodontal or/and antibiotic therapies during the previous 6 months, use of mouth rinses containing antimicrobials in the preceding 2 months, any systemic condition (except DM) that could affect the progression of periodontal disease (e.g. immunological disorders, osteoporosis) and long-term administration of anti-inflammatory and immunosuppressive medications. Subjects with periapical pathology, orthodontic appliances and multiple systemic complications of DM were also excluded from the study.

Blood analyses and experimental groups

A single laboratory (Guarulhos University Clinical Analysis Laboratory) performed all blood analyses. Blood samples were taken for each subject at baseline, 3 and 6 months post-therapy. The fasting plasma glucose (FPG), measured using the glucose oxidase method, was expressed in milligrams per decilitre. Glycosylated haemoglobin levels (HbA1c), measured by high-performance liquid chromatography, were expressed as a percentage. Subjects who had baseline HbA1c values $>8\%$ were assigned to the poorly controlled group ($n = 20$), whereas subjects who presented HbA1c levels $\leqslant 8\%$ were assigned to the well-controlled group ($n = 18$).

Clinical monitoring

All clinical examinations were performed by one examiner (V. R. S.), calibrated according to the method described by Araujo et al. (2003). The intra-examiner variability was 0.21 mm for PD and 0.25 mm for clinical attachment loss. This trained examiner was able to provide reproducible measurements of under 0.5 mm. The clinical parameters registered dichotomously, i.e. bleeding on probing (BoP) and suppuration (SUP), were calculated by the Kappa-Light test and the intra-examiner agreement was >0.85. The examiner was unaware of the glycaemic status of the subjects.

The following parameters were assessed at six sites of all teeth, excluding third molars (mesio-buccal, medio-buccal, disto-buccal, mesio-lingual, medio-lingual, disto-lingual), using a manual periodontal probe (UNC15, Hu-Friedy, Chicago, IL, USA): visible plaque accumulation (Ainamo & Bay 1975), BoP, SUP, PD (mm) and CAL (mm). Clinical examinations were assessed at baseline, 3 and 6 months after periodontal therapy.

GCF sampling

At baseline, GCF was sampled 1 week after clinical examination so as not to alter the nature of the GCF. Two non-contiguous sites per subject presenting PD and CAL $\geqslant$ 5 mm, BoP and no furcation involvement were chosen for sampling. After removal of the supragingival biofilm with sterile cotton pellets, the sites were isolated with cotton rolls and gently dried with an air syringe to eliminate the possibility of contamination with saliva. GCF was collected by inserting standard paper strips (Periopaper, Oraflow Inc., Smithtown, NY, USA) approximately 2 mm into the sulcus/pocket for 30 s. Strips visually contaminated with blood were discarded. The GCF sample volume was measured using a calibrated Periotron 8000 (Proflow Inc., Amityville, NY, USA) and the readings were then converted to an actual volume (μl) by reference to the standard curve. The strips from the two selected sites were immediately placed into separate microcentrifuge tubes containing 250 μl phosphate-buffered saline and protease inhibitor cocktail (Sigma-Aldrich, Saint Louis, MO, USA). The samples were stored at -20°C for subsequent assays. GCF samples were also taken from the same sites at 3 and 6 months after periodontal therapy. At these times, clinical parameter measurements and supportive therapy were performed after GCF sampling.

Cytokine enzyme-linked immunosorbent assay (ELISA)

GCF samples were analysed by ELISA for TNF-α, IFN-γ, IL-4, IL-17 and IL-23 using commercially available ELISA kits (Quantikine; R&D Systems Inc., Minneapolis, MN, USA). The tubes were vortexed for 30 s and centrifuged for 5 min. at 1500 *g* in order to elute. Assays were carried out according to the manufacturer's recommendations using human recombinant standards. The minimum detectable doses (sensitivity) for TNF-α, IL-4 and IL-23 assays range from 0.038 to 0.191, 0.03 to 0.22 and 2.7 to 16.3 pg/ml, respectively. The minimum detectable doses for IFN-γ and IL-17 are $<$ 8.0 and $<$ 15 pg/ml, respectively. The optical density was measured at 450 or 490 nm, according to each cytokine recommendation. Results were reported as total amount (pg) of each cytokine per site. Sites with cytokine levels below the detection limit of the assay were scored as 0 pg. Calculation of cytokine concentrations in each GCF sample (pg/μl) was established by dividing the total amount of each cytokine by the total volume of the fluid in the site.

Periodontal treatment

Subjects first received supragingival plaque and calculus removal, exodontia, provisional restoration and overhangs of fillings removal. They were instructed to perform a brushing technique using a soft toothbrush, dental floss and interdental toothbrushes, as necessary. Moreover, all volunteers received the same brand of toothpaste to use during the course of the study (Colgate Total®, Anakol Ind. Com. Ltda – Kolynos do Brasil – Colgate Palmolive Co., São Bernardo do Campo, SP, Brazil). Scaling and root planing (SRP) was performed during two to four appointments, lasting approximately 60 min. each, under local anaesthesia (3% prilocaine with felypressin) using periodontal curettes (Hu-Friedy) and an ultrasonic device (Jet Sonic, Gnatus, Ribeirão Preto, SP, Brazil). Treatment was concluded in a maximum of 21 days by the same operator without use of antibiotics or local antimicrobials. All subjects received supportive therapy, including professional plaque control with an abrasive sodium carbonate air-powder system (Jet Sonic) and re-instruction of oral hygiene, at 3 and 6 months post-therapy. The subjects were asked to report any changes in the DM treatment regimen in the follow-up appointments.

Table 1. Demographic characteristics of the study population

Characteristics	Poorly controlled ($n = 20$)	Well-controlled ($n = 18$)
Age (years)		
Mean $\pm$ SD	52.33 $\pm$ 7.0	52.2 $\pm$ 9.7
Range	41–66	40–67
Gender (n)		
Male	9	8
Female	11	10
Duration of DM		
Mean $\pm$ SD (years)	6.2 $\pm$ 0.8	6.1 $\pm$ 0.6

There were no differences between groups regarding age, duration of DM (Student's *t*-test; $p > 0.05$) and gender (χ^2-test; $p > 0.05$).
DM, diabetes mellitus.

Table 2. Full-mouth clinical parameters and glycaemic status of poorly controlled (HbA1c values $>$ 8%) and well-controlled (HbA1c levels $\leqslant$ 8%) diabetic subjects before and after scaling and root planing

Parameters	Poorly controlled ($n = 20$)			Well controlled ($n = 18$)		
	baseline	3 months	6 months	baseline	3 months	6 months
PI (%)	81.3 $\pm$ 21.4^{a*}	30.3 $\pm$ 20.5^{b}	29.0 $\pm$ 9.5^{b}	52.9 $\pm$ 22.3^{a}	21.3 $\pm$ 24.8^{b}	27.6 $\pm$ 22.5^{b}
BoP (%)	53.2 $\pm$ 30.5^{a}	7.8 $\pm$ 7.3^{b}	7.0 $\pm$ 7.3^{b}	49.9 $\pm$ 27.8^{a}	11.5 $\pm$ 10.2^{b}	13.2 $\pm$ 10.4^{b}
SUP (%)	3.0 $\pm$ 3.6	2.0 $\pm$ 3.6	0.7 $\pm$ 1.7	2.8 $\pm$ 5.7	1.0 $\pm$ 1.3	1.9 $\pm$ 1.3
PD (mm)	3.4 $\pm$ 0.4^{a}	2.5 $\pm$ 0.9^{b}	2.7 $\pm$ 0.4^{b}	3.4 $\pm$ 0.8^{a}	2.7 $\pm$ 0.4^{b}	2.5 $\pm$ 0.8^{b}
CAL (mm)	4.2 $\pm$ 0.6^{a}	3.5 $\pm$ 0.8^{b}	3.6 $\pm$ 0.8^{b*}	4.0 $\pm$ 0.9^{a}	3.2 $\pm$ 0.6^{b}	3.1 $\pm$ 0.5^{b}
HbA1c (%)	10.4 $\pm$ 1.1*	10.1 $\pm$ 2.2*	10.9 $\pm$ 2.0*	7.0 $\pm$ 0.9	8.3 $\pm$ 0.8	8.3 $\pm$ 0.2
FPG (mg/dl)	194.6 $\pm$ 56.7*	206.3 $\pm$ 72.5*	212.0 $\pm$ 79.0*	131.9 $\pm$ 38.8	145.2 $\pm$ 33.9	149.8 $\pm$ 51.0
GCF (µl)	0.40 $\pm$ 0.15^{a}	0.15 $\pm$ 0.05^{b}	0.15 $\pm$ 0.06^{b}	0.40 $\pm$ 0.16^{a}	0.17 $\pm$ 0.07^{b}	0.15 $\pm$ 0.05^{b}

Different letters indicate statistically significant differences over time within each glycaemic group (Friedman's and Wilcoxon's tests; $p < 0.05$).
*Differences between well-controlled and poorly controlled groups at each time point (Mann–Whitney *U*-test; $p < 0.05$).
PI, plaque index; BoP, bleeding on probing; SUP, suppuration; PD, probing depth; CAL, clinical attachment level; HbA1c, glycated haemoglobin; FPG, fasting plasma glucose; GCF, gingival crevicular fluid.

Sample size calculation

The number of sites for GCF sampling in this study was based on previous studies that found differences in the levels of cytokines in the GCF, when comparing different clinical periodontal status (Vernal et al. 2005, Tsai et al. 2007). The ideal sample size to assure adequate power for clinical parameter differences was calculated considering differences of at least 0.8 mm for CAL and a standard deviation of 0.94 mm between groups in initially deep periodontal pockets (>6 mm). Based on these calculations, it was decided that 17 subjects per group would be necessary to provide an 80% power at a significance level of 5%.

Statistical analysis

The statistical analysis was performed using a software program (BioEstat 5.0, Sociedade Civil Mamirauá, CNPq, Tefé, AM, Brazil). The biostatistician was unaware of the glycaemic status of the subjects. Data were first examined for normality by the Kolmogorov–Smirnov test and the data that did not achieve normality were analysed using non-parametric methods. The study unit for cytokine levels was the site rather than the subject because periodontitis is a site-specific disease. The primary variables were differences and changes in the levels of each evaluated cytokine. The secondary variables were clinical parameters, GCF volume and plasma levels of HbA1c and FPG. The percentage of sites with visible plaque accumulation, BoP and SUP, the mean PD, CAL, GCF volume and the levels of HbA1c and FPG were computed for each subject. Clinical parameters were averaged across subjects. Subsequently, all data were averaged in the glycaemic groups. The significance of clinical and glycaemic differences between groups was compared using the Mann–Whitney *U*-test. The Student *t*-test was used to compare age, duration of DM and cytokine levels between well-controlled and poorly controlled groups. The Friedman test was used to detect statistically significant differences within glycaemic groups among experimental periods in relation to clinical and glycaemic differences. When there were significant differences by the Friedman test, a pair-wise comparison was performed by the Wilcoxon test. Repeated measures ANOVA was used to detect statistically significant differences in cytokines within glycaemic groups among experimental periods. When there were significant differences by the repeated measures ANOVA, a pair-wise comparison was performed using Tukey's test. The χ^2-test was used to detect differences in the frequencies of gender between groups. Spearman's Rank correlation was used to test possible relationships between cytokine levels and PD and CAL in the same sampled sites. The cytokine levels were also correlated to HbA1c levels by Spearman's Rank correlation. The significance level established for all analyses was 5%.

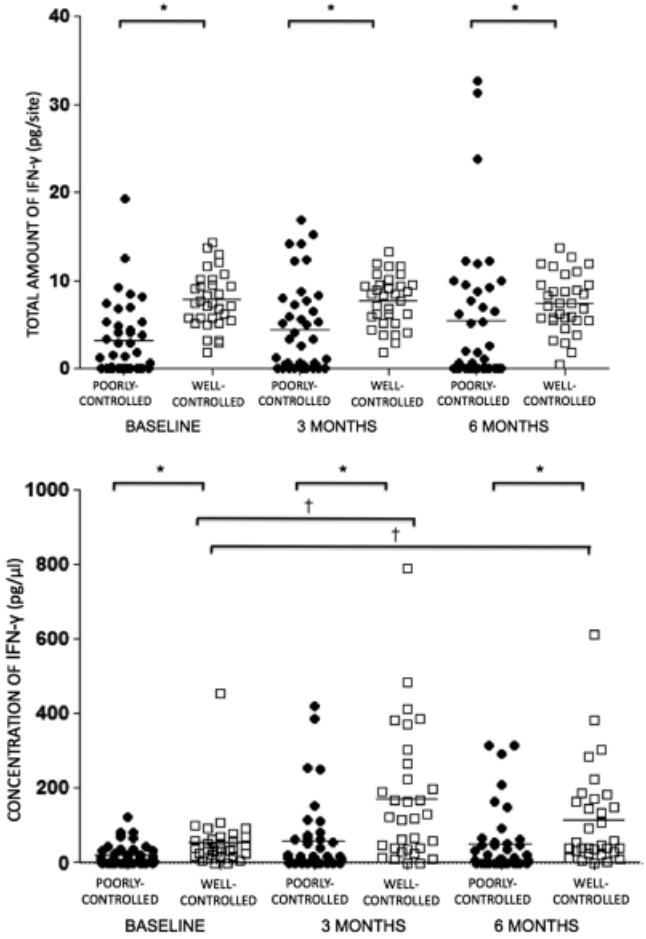

Fig. 1. Distribution of the total amount (pg/site) and concentration (pg/μl) of interferon (IFN)-γ in the gingival crevicular fluid of poorly and well-controlled subjects, before and after therapy. The horizontal bars show the median values. The individual dot represents the cytokine level at each site. *Differences between groups at each time point (Student's *t*-test; $p<0.05$). †Differences over time for each glycaemic group (repeated measures ANOVA and Tukey's test; $p<0.05$).

Results

Retention

There were no subject and sampling site dropouts during the course of the study. Thus, a total of 38 subjects completed the study, 20 poorly controlled and 18 well-controlled. Seventy-six samples of GCF were analysed per period, totaling 228 samples. The subjects from both groups reported no adverse effects such as fever and indisposition after treatment. No changes in the medication and diet were reported by the subjects during the study period.

Clinical results

No significant differences were observed between glycaemic groups for age, gender and duration of DM ($p<0.05$) (Table 1). The mean ($\pm$ SD) levels of PD and CAL of the sampled sites were 5.8 ± 1.0 and 5.8 ± 1.0 and, 5.7 ± 1.0 and 6.2 ± 1.6 for well-controlled and poorly controlled groups, respectively. At 3 months post-therapy, PD and CAL

of the sampled sites changed for 3.4 ± 0.8 and 5.2 ± 0.4, and 3.7 ± 0.9 and 5.5 ± 1.0 for well-controlled and poorly controlled groups, respectively ($p < 0.05$). At 6 months post-therapy, PD and CAL of the sampled sites changed for 3.3 ± 0.6 and 5.2 ± 0.4 and, 3.5 ± 0.8 and 5.6 ± 0.8 for well-controlled and poorly controlled groups, respectively ($p < 0.05$).

Statistically significant decreases in all full-mouth clinical parameters, except SUP, and in GCF volumes were observed for poorly controlled and well-controlled subjects at 3 and 6 months post-therapy (Table 2, $p < 0.05$). The mean levels of HbA1c and FPG did not change for either group over time ($p > 0.05$) and remained higher for poorly than for well-controlled subjects in all experimental periods ($p < 0.05$). Diabetic subjects with poor glycaemic control had significantly higher visible plaque accumulation than those with good glycaemic control at baseline ($p < 0.05$). Well-controlled subjects achieved a lower mean of CAL than poorly controlled subjects at 6 months post-therapy ($p < 0.05$).

Cytokine levels

In general, the cytokine levels for both diabetic groups did not change following periodontal therapy ($p > 0.05$), except for the concentration of IFN-γ, which increased in well-controlled subjects at 3 and 6 months post-therapy (Fig. 1; $p < 0.05$). In addition, total amounts and concentrations of IFN-γ were higher in well- than in poorly controlled subjects at baseline and 3 and 6 months post-therapy (Fig. 1; $p < 0.05$). The levels of IL-4 were lower in well- than poorly controlled diabetic subjects at baseline ($p < 0.05$; Fig. 2). Conversely, total amount and concentration of IL-17 were higher in poorly than well-controlled subjects in all experimental periods (Fig. 3; $p < 0.05$). There were no significant differences between groups, regarding the total amount and concentration of TNF-α (Fig. 4) and IL-23 (Fig. 5) at any time point ($p > 0.05$).

Correlations

Table 3 presents the correlation coefficients for total amounts and concentrations of cytokines and PD and CAL of the sampled sites and HbA1c level. Statistically significant positive correlations were found between total amounts and concentration of TNF-α ($p < 0.05$), IL-4 ($p < 0.001$) and IL-17 ($p < 0.001$) and HbA1c while the levels of IFN-γ were negatively correlated with HbA1c ($p < 0.001$). In addition, IL-17 was positively correlated with IL-4 ($p < 0.001$) and TNF-α ($p < 0.05$). There was a negative correlation between the concentration of IFN-γ and the total amount of IL-17 ($p < 0.05$).

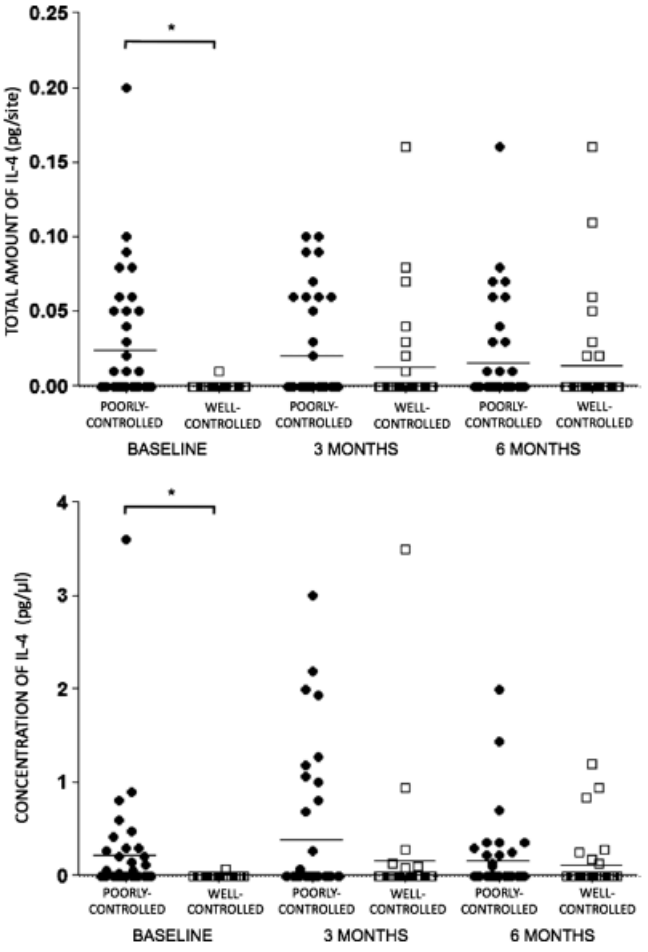

Fig. 2. Distribution of the total amount (pg/site) and concentration (pg/μl) of interleukin (IL)-4 in the gingival crevicular fluid of poorly and well-controlled subjects, before and after therapy. The horizontal bars show the median values. The individual dot represents the cytokine level at each site. *Differences between groups at each time point (Student's *t*-test; $p < 0.05$).

Discussion

It has been suggested that an imbalance among Th1-, Th2-, Th17- and T regulatory (Treg)-type cytokines in the immune-inflammatory response against periodontal pathogens is critical in the determination of the pattern of periodontal lesions (Seymour & Gemmell 2001, Ukai et al. 2001, Teng 2002, Garlet et al. 2003). This study examined, for the first time, the levels of pro- and anti-inflammatory cytokines related to the Th cells (TNF-α, IFN-γ, IL-4, IL-17 and IL-23) in chronic periodontitis sites of type 2 diabetic subjects presenting good or poor glycaemic control. In general, the results demonstrated that the pattern of cytokines in periodontitis sites, matched to disease severity, might be related to the glycaemic status, which, in turn, seems to play a crucial role in the host response against periodontal pathogens in diabetic subjects. Sites with chronic periodontitis of well-controlled type 2 diabetic subjects exhibited higher GCF levels of IFN-γ and decreased levels of IL-4, suggesting predominance of Th1-type cytokine in these subjects, while sites of poorly controlled individuals presented

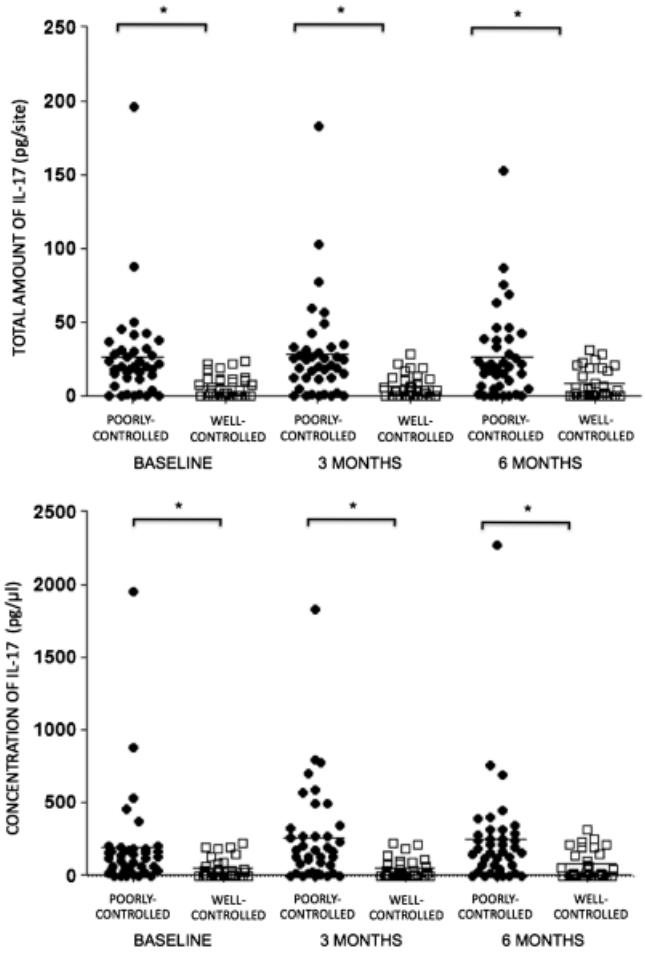

Fig. 3. Distribution of the total amount (pg/site) and concentration (pg/μl) of interleukin (IL)-17 in the gingival crevicular fluid of poorly and well-controlled subjects, before and after therapy. The horizontal bars show the median values. The individual dot represents the cytokine level at each site. *Differences between groups at each time point (Student's *t*-test; $p < 0.05$).

increased IL-17 levels, suggesting dominance of Th17-type cytokines in sites under the challenge of hyperglycaemia.

For many years, periodontitis was described as an imbalance between Th1 and Th2 cytokine profiles (Gemmell & Seymour 2004). Recently, this concept has been defied by the discovery of Th17 and Treg cells and their related cytokines in periodontal lesions (Takahashi et al. 2005, Lester et al. 2007, Cardoso et al. 2008, 2009, Dutzan et al. 2009a). Overall, clinical and in vitro evidence shows the presence of the Th17-type immune response in periodontal diseases and indicates that periodontal pathogens can stimulate IL-17 production from T cells (Oda et al. 2003, Lester et al. 2007, Cardoso et al. 2009). The role of Th17 cells and their related factors in autoimmune type 1 DM has been often investigated (Bradshaw et al. 2009). However, to date, there is little information regarding the function of these cells in type 2 DM (Arababadi et al. 2010). A recent study showed that the serum levels of IL-17 were higher in type 2 diabetic subjects, when compared with non-diabetic controls, suggesting a relationship between IL-17 and type 2 DM (Arababadi et al. 2010). The precise biological mechanisms that could explain the elevated levels of IL-17 in the GCF of poorly controlled subjects and the positive correlation between this cytokine and HbA1c levels remain to be truly evaluated. One hypothesis may be the non-enzymatic glycation of proteins under a hyperglycaemic condition and the subsequent accumulation of advanced glycation end products (AGEs) in periodontal tissues (Katz et al. 2005). In addition to the stimulation of pro-inflammatory mediators by pathogens in periodontal sites, AGEs, when attached to its receptors (RAGE), also stimulate the overproduction of pro-inflammatory cytokines, including IL-17 (King 2008). Accordingly, the destructive biological functions of IL-17 may amplify the severity of periodontal inflammation (Takahashi et al. 2005, Beklen et al. 2007) and link T-cell activation to bone resorption (Sato et al. 2006). In fact, it has been suggested that the Th17-type response may induce osteoclastogenesis, rather than Th1, possibly through IL-17-mediated induction of the receptor activator of NF-κB ligand (RANKL) (Kotake et al. 1999, Sato et al. 2006, Dutzan et al. 2009a). Interestingly, recent findings from our research group have shown that poor glycaemic control is related to an imbalance in the RANKL/OPG ratio in the GCF from type 2 diabetic subjects, favouring osteoclastogenesis (Santos et al. 2010). Together, these findings reinforce the hypothesis that chronic hyperglycaemia may interfere in the levels of specific immune-inflammatory mediators in periodontal tissues that could explain, at least in part, the more severe periodontal breakdown reported in poorly controlled, when compared with well-controlled diabetic subjects (Seppälä & Ainamo 1994, Tsai et al. 2002, Lim et al. 2007, Bandyopadhyay et al. 2010, Chen et al. 2010).

Elevated concentrations of IL-23 in diseased periodontal sites, compared with healthy tissues, suggest the involvement of this cytokine in the pathogenesis of periodontitis (Lester et al. 2007, Cardoso et al. 2009, Ohyama et al. 2009). However, to our knowledge, GCF levels of IL-23 have not been reported previously in periodontitis subjects with type 2 DM. Although IL-23 is able to differentiate and expand memory T cells and increase the production of IL-17 (Tan et al. 2009), in this study, no significant differences in the levels of IL-23 were found between groups at any time. It is important to note that, besides IL-23, other cytokines have been suggested to have a role in stabilizing the IL-17-producing T cells, supporting other pathways, differently from IL-23, in the induction of these types of response (Sutton et al. 2006).

IFN-γ and IL-4 correspond to Th1 and Th2 cytokines, respectively. In non-diabetic subjects, it has been demonstrated that low amounts of IL-4 and high levels of IFN-γ are involved in

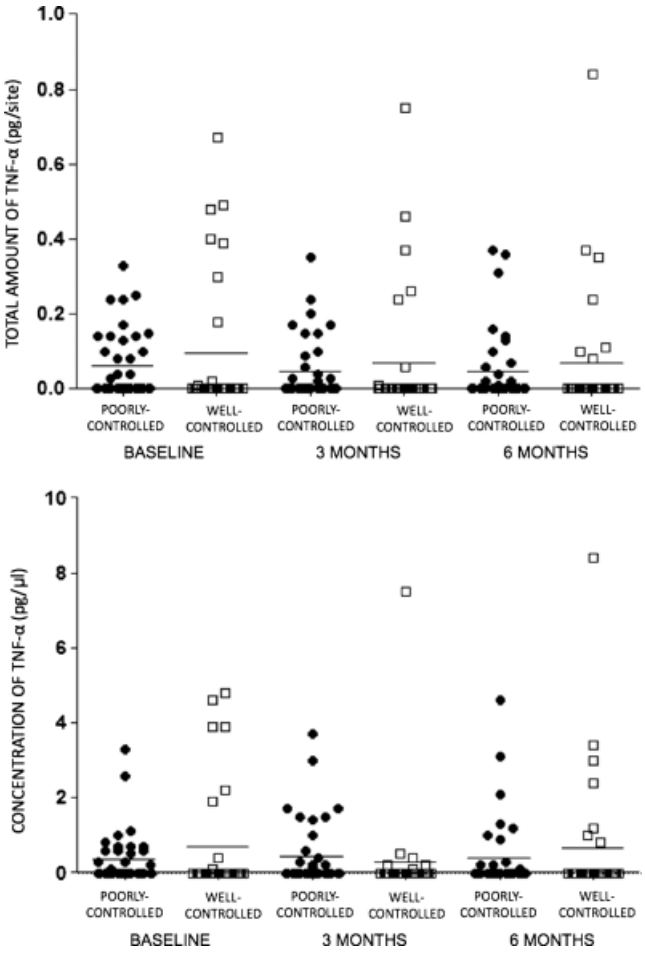

Fig. 4. Distribution of the total amount (pg/site) and concentration (pg/μl) of tumour necrosis factor (TNF)-α in the gingival crevicular fluid of poorly and well-controlled subjects, before and after therapy. The horizontal bars show the median values. The individual dot represents the cytokine level at each site.

the destruction of periodontal tissues, whereas an increased ratio of IL-4/IFN-γ is associated with healthy periodontal conditions (Shapira et al. 1992, Ukai et al. 2001, Tsai et al. 2007). In this study, periodontitis sites from well-controlled subjects exhibited higher levels of IFN-γ in all experimental periods and decreased levels of IL-4 at baseline, when compared with poorly controlled individuals, suggesting dominance of a Th1 and weakness of Th2 immune responses. Although the present study did not include a non-diabetic group, it seems that the periodontitis development in well-controlled subjects follows the same pattern of Th1/Th2 imbalance already described for non-diabetic subjects in the literature (Shapira et al. 1992, Ukai et al. 2001, Tsai et al. 2007). In addition, we found a slight but interesting negative correlation between levels of IFN-γ and IL-17. In fact, previous investigations have demonstrated that the Th1 cytokine, IFN-γ, may potently suppress the development of IL-17-producing Th cells from naïve CD4+ precursor cells, providing a mechanism by which Th1 development could antagonize Th17 expansion (Harrington et al. 2005, Park et al. 2005).

TNF-α is a well-recognized pro-inflammatory cytokine in periodontitis, able to stimulate the degradation of the connective tissue matrix and bone resorption directly and indirectly (Tervahartiala et al. 2001, Kurtiş et al. 2005). Although a slight significant positive correlation was found between TNF-α and HbA1c, in this study, TNF-α levels in the GCF were similar between poorly and well-controlled subjects in all experimental periods. This finding was somewhat expected because both IFN-γ and IL-17 are able to stimulate the production of TNF-α. Findings from a recent study (Venza et al. 2010) demonstrated that TNF-α gene expression was higher in poorly controlled (HbA1c levels $\geqslant$8%) than well-controlled (HbA1c levels $<$8%) type 2 diabetic subjects. Such divergences in both studies may be attributed to the differences in the accuracy of the methods used to detect TNF-α.

Besides the observed clinical improvements (Table 2), in general, cytokine levels for both diabetic groups did not change significantly following the non-surgical periodontal therapy. Only a significant increase in the concentration of IFN-γ was observed at 3 and 6 months for well-controlled subjects, probably due to the reduction in GCF volume after treatment, as a result of the remission of the inflammatory process (Buduneli et al. 2009). Few studies have evaluated the effect of periodontal therapy on the local levels of inflammatory markers in diabetic subjects and conflicting results have been shown (Talbert et al. 2006, Navarro-Sanchez et al. 2007, Correa et al. 2008). Talbert et al. (2006) demonstrated no changes in the TNF-α and IL-6 levels in GCF after non-surgical treatment of periodontitis in type 2 diabetic subjects. In contrast, some studies have demonstrated that the GCF levels of TNF-α, IL-1β, MMP-8 and MMP-9 were reduced significantly following periodontal treatment in type 2 diabetic subjects (Navarro-Sanchez et al. 2007, Correa et al. 2008). However, differences in experimental designs among studies, including the type of cytokines studied, periodontitis severity and ELISA sensitivity hampered a more meaningful comparison with the present results.

Interventional studies have evaluated the potential effects of periodontal therapies on glycaemic control of diabetic subjects (Stewart et al. 2001, Janket et al. 2005, Kiran et al. 2005, Navarro-Sanchez et al. 2007, Correa et al. 2010). Therefore, one important additional finding of this study is that the levels of HbA1c did not change significantly at 3 and 6 months following non-surgical periodontal therapy for any glycaemic group (Table 2). These results are in agreement with those from previous studies in which SRP resulted in periodontal clinical benefits without a significant reduction in the glycaemic control of diabetic subjects (Janket et al. 2005, Correa et al. 2010). On the other hand, these findings are in contrast to

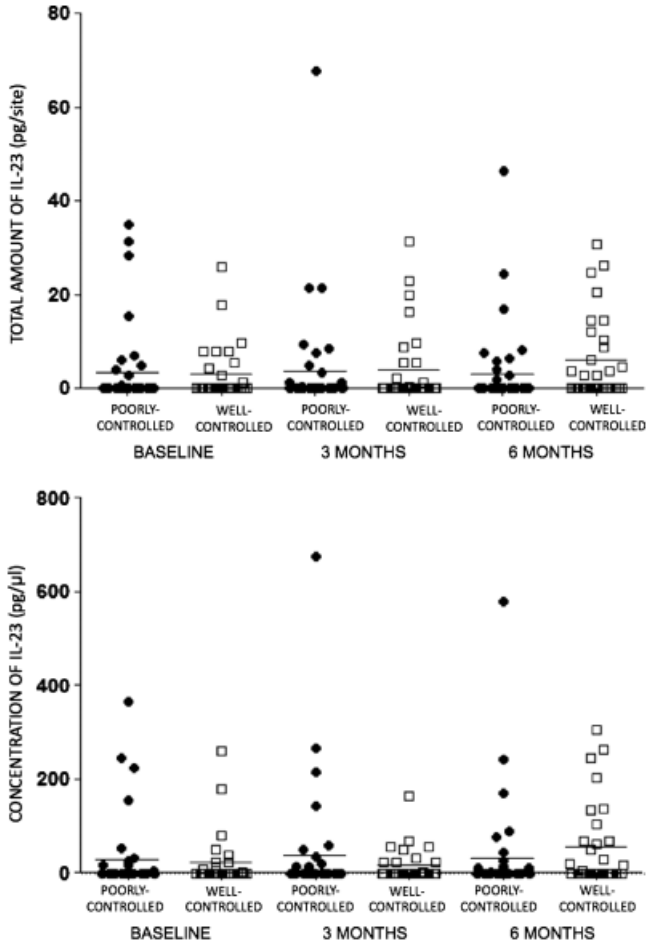

Fig. 5. Distribution of the total amount (pg/site) and concentration (pg/μl) of interleukin (IL)-23 in the gingival crevicular fluid of poorly and well-controlled subjects, before and after therapy. The horizontal bars show the median values. The individual dot represents the cytokine level at each site.

Table 3. Correlation coefficients for cytokine levels and PD and CAL of the sampled sites and, HbA1c levels ($n = 76$ periodontal sites)

	TNF-α		IFN-γ		IL-4		IL-17		IL-23	
	TA	CC	TA	CC	TA	CC	TA	CC	TA	CC
TNF-α										
CC	0.98*									
IFN-γ										
TA	−0.12									
CC	−0.25	−0.26	0.99*							
IL-4										
TA	0.03	0.67	−0.20	−0.10						
CC	0.04	0.08	−0.20	−0.11	0.99*					
IL-17										
TA	0.26†	0.30†	−0.20	−0.27†	0.51*	0.51*				
CC	0.20	0.25†	−0.08	−0.16	0.46*	0.46*	0.94*			
IL-23										
TA	0.15	0.16	0.20	0.04	0.23	0.22	0.20	0.20		
CC	0.15	0.16	0.18	0.03	0.22	0.22	0.19	0.19	0.99*	
HbA1c	0.25†	0.27†	−0.60*	−0.57*	0.41*	0.41*	0.50*	0.41*	−0.01	−0.01
PD	−0.13	−0.11	0.12	0.18	0.003	0.002	−0.10	−0.10	−0.03	−0.02
CAL	−0.04	−0.09	−0.10	0.20	−0.08	−0.08	−0.07	−0.05	−0.02	−0.01

*Correlations significant at the $p < 0.001$ level by Spearman's Rank correlation test.

†Correlations significant at $p < 0.05$ level by Spearman's Rank correlation test.

TA, total amount (pg/site); CC, concentration (pg/μl); HbA1c, glycated haemoglobin (%); PD, probing depth (mm); CAL, clinical attachment level (mm); TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; IL-4, interleukin-4; IL-17, interleukin-17; IL-23, interleukin-23.

those from intervention studies that showed improvements in metabolic control of diabetic subjects following periodontal therapy (Stewart et al. 2001, Kiran et al. 2005, Navarro-Sanchez et al. 2007). Overall, recent meta-analyses have demonstrated positive effects of periodontal therapies in the glycaemic control of diabetic subjects (Darré et al. 2008, Simpson et al. 2010, Teeuw et al. 2010). Darré et al. (2008) suggested that periodontal treatment could improve glycaemic control after analysing randomized and controlled interventional studies performed in type 1 and 2 diabetic subjects. Similarly, Teeuw et al. (2010) and Simpson et al. (2010) proposed that there may be a modest but significant improvement in glycaemic control after periodontal treatment in type 2 diabetic subjects. Conflicting results among studies may be explained by differences in study designs and interventions, types of DM, initial levels of HbA1c, methods for determining HbA1c values, severity of periodontitis and the role of other variables on the glycaemic condition such as diet, physical activity and compliance to medications and changes in hypoglycaemia medications during study period. In addition, it has been recognized that a large sample size is required for the observation of any significance reduction in the HbA1c level (Janket et al. 2005, Darré et al. 2008).

In conclusion, the results of the present study indicated a trend towards a domination of pro-inflammatory Th1- or Th17-cytokines in sites of chronic periodontitis from type 2 diabetic subjects, according to their glycaemic control. Because only cytokine profiles were evaluated and no characterization of T-cell phenotypes in the periodontal lesions was performed, these initial findings are still not enough to define the predominant pattern of the Th immune response in each glycaemic condition. Therefore, further studies are required to better characterize the Th subsets that control the periodontitis development in well- and poorly controlled diabetic subjects. Furthermore, because Treg cells regulate the effector functions of activated Th cells, further studies are also needed to assess the role of these cells and related cytokines in the periodontal lesions of type 2 diabetic subjects and determine the impact of glycaemic control in the modulation these responses.

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References

Ainamo, J. & Bay, I. (1975) Problems and proposals for recording gingivitis and plaque. *International Dental Journal* **25**, 229–235.

Arababadi, M. K., Nosratabadi, R., Hassanshahi, G., Yaghini, N., Pooladvand, V., Shamsizadeh, A., Hakimi, H. & Derakhshan, R. (2010) Nephropathic complication of type-2 diabetes is following pattern of autoimmune diseases? *Diabetes Research and Clinical Practice* **87**, 33–37.

Araujo, M. W., Hovey, K. M., Benedek, J. R., Grossi, S. G., Dorn, J., Wactawski-Wende, J., Genco, R. J. & Trevisan, M. (2003) Reproducibility of probing depth measurement using a constant-force electronic probe: analysis of inter- and intraexaminer variability. *Journal of Periodontology* **74**, 1736–1740.

Armitage, G. C. (1999) Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology* **4**, 1–6.

Bandyopadhyay, D., Marlow, N. M., Fernandes, J. K. & Leite, R. S. (2010) Periodontal disease progression and glycaemic control among Gullah African Americans with type-2 diabetes. *Journal of Clinical Periodontology* **37**, 501–509.

Beklen, A., Ainola, M., Hukkanen, M., Gurgan, C., Sorsa, T. & Konttinen, Y. T. (2007) MMPs, IL-1, and TNF are regulated by IL-17 in periodontitis. *Journal Dental Research* **86**, 347–351.

Bradshaw, E. M., Raddassi, K., Elyaman, W., Orban, T., Gottlieb, P. A., Kent, S. C. & Hafler, D. A. (2009) Monocytes from patients with type 1 diabetes spontaneously secrete proinflammatory cytokines inducing Th17 cells. *Journal of Immunology* **183**, 4432–4439.

Buduneli, N., Buduneli, E. & Kütükçüler, N. (2009) Interleukin-17, RANKL, and osteoprotegerin levels in gingival crevicular fluid from smoking and nonsmoking patients with chronic periodontitis during initial periodontal treatment. *Journal of Periodontology* **80**, 1274–1280.

Cardoso, C. R., Garlet, G. P., Crippa, G. E., Rosa, A. L., Júnior, W. M., Rossi, M. A. & Silva, J. S. (2009) Evidence of the presence of T helper type 17 cells in chronic lesions of human periodontal disease. *Oral Microbiology and Immunology* **24**, 1–6.

Cardoso, C. R., Garlet, G. P., Moreira, A. P., Júnior, W. M., Rossi, M. A. & Silva, J. S. (2008) Characterization of CD4+CD25+ natural regulatory T cells in the inflammatory infiltrate of human chronic periodontitis. *Journal of Leukocyte Biology* **84**, 311–318.

Chen, L., Wei, B., Li, J., Liu, F., Xuan, D., Xie, B. & Zhang, J. (2010) Association of periodontal parameters with metabolic level and systemic inflammatory markers in patients with type 2 diabetes. *Journal of Periodontology* **81**, 364–371.

Correa, F. O., Gonçalves, D., Figueredo, C. M., Bastos, A. S., Gustafsson, A. & Orrico, S. R. (2010) Effect of periodontal treatment on metabolic control, systemic inflammation and cytokines in patients with type 2 diabetes. *Journal of Clinical Periodontology* **37**, 53–58.

Correa, F. O., Gonçalves, D., Figueredo, C. M., Gustafsson, A. & Orrico, S. R. (2008) The short-term effectiveness of non-surgical treatment in reducing levels of interleukin-1beta and proteases in gingival crevicular fluid from patients with type 2 diabetes mellitus and chronic periodontitis. *Journal of Periodontology* **79**, 2143–2150.

Darré, L., Vergnes, J. N., Gourdy, P. & Sixou, M. (2008) Efficacy of periodontal treatment on glycaemic control in diabetic patients: a meta-analysis of interventional studies. *Diabetes and Metabolism* **34**, 497–506.

Duarte, P. M., Neto, J. B., Casati, M. Z., Sallum, E. A. & Nociti, F. H. Jr. (2007) Diabetes modulates gene expression in the gingival tissues of patients with chronic periodontitis. *Oral Diseases* **13**, 594–599.

Dutzan, N., Gamonal, J., Silva, A., Sanz, M. & Vernal, R. (2009a) Over-expression of forkhead box P3 and its association with receptor activator of nuclear factor-kappa B ligand, interleukin (IL) -17, IL-10 and transforming growth factor-beta during the progression of chronic periodontitis. *Journal of Clinical Periodontology* **36**, 396–403.

Dutzan, N., Vernal, R., Hernandez, M., Dezerega, A., Rivera, O., Silva, N., Aguillon, J. C., Puente, J., Pozo, P. & Gamonal, J. (2009b) Levels of interferon-gamma and transcription factor T-bet in progressive periodontal lesions in patients with chronic periodontitis. *Journal of Periodontology* **80**, 290–296.

Engebretson, S. P., Hey-Hadavi, J., Ehrhardt, F. J., Hsu, D., Celenti, R. S., Grbic, J. T. & Lamster, I. B. (2004) Gingival crevicular fluid levels of interleukin-1beta and glycemic control in patients with chronic periodontitis and type 2 diabetes. *Journal of Periodontology* **75**, 1203–1208.

Engebretson, S. P., Vossughi, F., Hey-Hadavi, J., Emingil, G. & Grbic, J. T. (2006) The influence of diabetes on gingival crevicular fluid beta-glucuronidase and interleukin-8. *Journal of Clinical Periodontology* **33**, 784–790.

Garlet, G. P., Martins, W. Jr., Ferreira, B. R., Milanezi, C. M. & Silva, J. S. (2003) Patterns of chemokines and chemokine receptors expression in different forms of human periodontal disease. *Journal of Periodontal Research* **38**, 210–217.

Gemmell, E. & Seymour, G. J. (2004) Immunoregulatory control of Th1/Th2 cytokine profiles in periodontal disease. *Periodontology 2000* **35**, 21–41.

Harrington, L. E., Hatton, R. D., Mangan, P. R., Turner, H., Murphy, T. L., Murphy, K. M. & Weaver, C. T. (2005) Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nature Immunology* **6**, 1123–1132.

Israili, Z. H. (2009) Advances in the Treatment of Type 2 Diabetes Mellitus. *American Journal of Therapeutics*, doi: 10.1097/MJT.0b013e3181afbf51.

Janket, S. J., Wightman, A., Baird, A. E., Van Dyke, T. E. & Jones, J. A. (2005) Does periodontal treatment improve glycemic control in diabetic patients? A meta-analysis of intervention studies. *Journal of Dental Research* **84**, 1154–1159.

Kabashima, H., Nagata, K., Hashiguchi, I., Toriya, Y., Iijima, T., Maki, K. & Maeda, K. (1996) Interleukin-1 receptor antagonist and interleukin-4 in gingival crevicular fluid of patients with inflammatory periodontal disease. *Journal of Oral Pathology and Medicine* **25**, 449–455.

Katz, J., Bhattacharyya, I., Farkhondeh-Kish, F., Perez, F. M., Caudle, R. M. & Heft, M. W. (2005) Expression of the receptor of advanced glycation end products in gingival tissues of type 2 diabetes patients with chronic periodontal disease: a study utilizing immunohistochemistry and RT-PCR. *Journal of Clinical Periodontology* **32**, 40–44.

Kidambi, S. & Patel, S. B. (2008) Diabetes mellitus: considerations for dentistry. *Journal of the American Dental Association* **139**, 8S–18S.

Kinane, D. & Bouchard, P.Group E of European Workshop on Periodontology (2008) Periodontal diseases and health: consensus Report of the Sixth European Workshop on Periodontology. *Journal of Clinical Periodontology* **35**, 333–337.

King, G. L. (2008) The role of inflammatory cytokines in diabetes and its complications. *Journal of Periodontology* **79**, 1527–1534.

Kiran, M., Arpak, N., Unsal, E. & Erdoğan, M. F. (2005) The effect of improved periodontal health on metabolic control in type 2 diabetes mellitus. *Journal of Clinical Periodontology* **32**, 266–272.

Kotake, S., Udagawa, N., Takahashi, N., Matsuzaki, K., Itoh, K., Ishiyama, S., Saito, S., Inoue, K., Kamatani, N., Gillespie, M. T., Martin, T. J. & Suda, T. (1999) IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *The Journal of Clinical Investigation* **103**, 1345–1352.

Kurtiş, B., Tüter, G., Serdar, M., Akdemir, P., Uygur, C., Firatli, E. & Bal, B. (2005) Gingival crevicular fluid levels of monocyte chemoattractant protein-1 and tumor necrosis factor-alpha in patients with chronic and aggressive periodontitis. *Journal of Periodontology* **76**, 1849–1855.

Lester, S. R., Bain, J. L., Johnson, R. B. & Serio, F. G. (2007) Gingival concentrations of interleukin-23 and -17 at healthy sites and at sites of clinical attachment loss. *Journal of Periodontology* **78**, 1545–1550.

Lim, L. P., Tay, F. B., Sum, C. F. & Thai, A. C. (2007) Relationship between markers of metabolic control and inflammation on severity of periodontal disease in patients with diabetes mellitus. *Journal of Clinical Periodontology* **34**, 118–123.

Mosmann, T. R. & Coffman, R. L. (1989) Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Advances in Immunology* **46**, 111–147.

Navarro-Sanchez, A. B., Faria-Almeida, R. & Bascones-Martinez, A. (2007) Effect of non-surgical periodontal therapy on clinical and immunological response and glycaemic control in type 2 diabetic patients with moderate periodontitis. *Journal of Clinical Periodontology* **34**, 835–843.

Oda, T., Yoshie, H. & Yamazaki, K. (2003) *Porphyromonas gingivalis* antigen preferentially stimulates T cells to express IL-17 but not receptor activator of NF-kappaB ligand in vitro. *Oral Microbiology and Immunology* **18**, 30–36.

Ohyama, H., Kato-Kogoe, N., Kuhara, A., Nishimura, F., Nakasho, K., Yamanegi, K., Yamada, N., Hata, M., Yamane, J. & Terada, N. (2009) The involvement of IL-23 and the Th17 pathway in periodontitis. *Journal Dental Research* **88**, 633–638.

Park, H., Li, Z., Yang, X. O., Chang, S. H., Nurieva, R., Wang, Y. H., Wang, Y., Hood, L., Zhu, Z., Tian, Q. & Dong, C. (2005) A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nature Immunology* **6**, 1133–1141.

Patel, D. N., King, C. A., Bailey, S. R., Holt, J. W., Venkatachalam, K., Agrawal, A., Valente, A. J. & Chandrasekar, B. (2007) Interleukin-17 stimulates C-reactive protein expression in hepatocytes and smooth muscle cells via p38 MAPK and ERK1/2-dependent NF-kappaB and C/EBPbeta activation. *The Journal of Biological Chemistry* **14**, 27229–27238.

Santos, V. R., Lima, J. A., Gonçalves, T. E., Bastos, M. F., Figueiredo, L. C., Shibli, J. A. & Duarte, P. M. (2010) Receptor activator of NF-ÊB ligand/osteoprotegerin ratio in sites of chronic periodontitis of poorly-controlled and well-controlled type 2 diabetic subjects. *Journal of Periodontology*, doi: 10.1902/jop.2010.100125.

Sato, K., Suematsu, A., Okamoto, K., Yamaguchi, A., Morishita, Y., Kadono, Y., Tanaka, S., Kodama, T., Akira, S., Iwakura, Y., Cua, D. J. & Takayanagi, H.

(2006) Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. *The Journal of Experimental Medicine* **27**, 2673–2682.

Seppälä, B. & Ainamo, J. (1994) A site-by-site follow-up study on the effect of controlled versus poorly controlled insulin-dependent diabetes mellitus. *Journal of Clinical Periodontology* **21**, 161–165.

Seymour, G. J. & Gemmell, E. (2001) Cytokines in periodontal disease: where to from here? *Acta Odontologica Scandinavica* **59**, 167–173.

Shapira, L., van Dyke, T. E. & Hart, T. C. (1992) A localized absence of interleukin-4 triggers periodontal disease activity: a novel hypothesis. *Medical Hypotheses* **39**, 319–322.

Simpson, T. C., Needleman, I., Wild, S. H., Moles, D. R. & Mills, E. J. (2010) Treatment of periodontal disease for glycaemic control in people with diabetes. *Cochrane Database of Systematic Reviews* **12**, CD004714.

Stewart, J. E., Wager, K. A., Friedlander, A. H. & Zadeh, H. H. (2001) The effect of periodontal treatment on glycemic control in patients with type 2 diabetes mellitus. *Journal of Clinical Periodontology* **28**, 306–310.

Sutton, C., Brereton, C., Keogh, B., Mills, K. H. & Lavelle, E. C. (2006) A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *The Journal of Experimental Medicine* **203**, 1685–1691.

Takahashi, K., Azuma, T., Motohira, H., Kinane, D. F. & Kitetsu, S. (2005) The potential role of interleukin-17 in the immunopathology of periodontal disease. *Journal of Clinical Periodontology* **32**, 369–374.

Talbert, J., Elter, J., Jared, H. L., Offenbacher, S., Southerland, J. & Wilder, R. S. (2006) The effect of periodontal therapy on TNF-alpha, IL-6 and metabolic control in type 2 diabetics. *Journal of Dental Hygiene* **80**, 7.

Tan, Z. Y., Bealgey, K. W., Fang, Y., Gong, Y. M. & Bao, S. (2009) Interleukin-23: immunological roles and clinical implications. *The International Journal of Biochemistry and Cell Biology* **41**, 733–735.

Tatakis, D. N. & Kumar, P. S. (2005) Etiology and pathogenesis of periodontal diseases. *Dental Clinics of North America* **49**, 491–516.

Teeuw, W. J., Gerdes, V. E. & Loos, B. G. (2010) Effect of periodontal treatment on glycemic control of diabetic patients: a systematic review and meta-analysis. *Diabetes Care* **33**, 421–427.

Teng, Y. T. (2002) Mixed periodontal Th1-Th2 cytokine profile in *Actinobacillus actinomycetemcomitans*-specific osteoprotegerin ligand (or RANK-L)-mediated alveolar bone destruction in vivo. *Infection and Immunity* **70**, 5269–5273.

Tervahartiala, T., Koski, H., Xu, J. W., Häyrinen-Immonen, R., Hietanen, J., Sorsa, T. & Konttinen, Y. T. (2001) Tumor necrosis factor-alpha and its receptors, p55 and p75, in gingiva of adult periodontitis. *Journal Dental Research* **80**, 1535–1539.

Tesmer, L. A., Lundy, S. K., Sarkar, S. & Fox, D. A. (2008) Th17 cells in human disease. *Immunological Reviews* **223**, 87–113.

te Velde, A. A., Huijbens, R. J., Heije, K., de Vries, J. E. & Figdor, C. G. (1990) Interleukin-4 (IL-4) inhibits secretion of IL-1 beta, tumor necrosis factor alpha, and IL-6 by human monocytes. *Blood* **76**, 1392–1397.

Tsai, C., Hayes, C. & Taylor, G. W. (2002) Glycemic control of type 2 diabetes and severe periodontal disease in the US adult population. *Community Dentistry and Oral Epidemiology* **30**, 182–192.

Tsai, C. C., Ku, C. H., Ho, Y. P., Ho, K. Y., Wu, Y. M. & Hung, C. C. (2007) Changes in gingival crevicular fluid interleukin-4 and interferon-gamma in patients with chronic periodontitis before and after periodontal initial therapy. *The Kaohsiung Journal of Medical Sciences* **23**, 1–7.

Ukai, T., Mori, Y., Onoyama, M. & Hara, Y. (2001) Immunohistological study of interferon-g and interleukin-4-bearing cells in human periodontitis gingiva. *Archives of Oral Biology* **46**, 901–908.

Venza, I., Visalli, M., Cucinotta, M., De Grazia, G., Teti, D. & Venza, M. (2010) Proinflammatory gene expression at chronic periodontitis and peri-implantitis sites in patients with or without type 2 diabetes. *Journal of Periodontology* **81**, 99–108.

Vernal, R., Dutzan, N., Chaparro, A., Puente, J., Antonieta Valenzuela, M. & Gamonal, J. (2005) Levels of interleukin-17 in gingival crevicular fluid and in supernatants of cellular cultures of gingival tissue from patients with chronic periodontitis. *Journal of Clinical Periodontology* **32**, 383–389.

Zhu, J., Yamane, H. & Paul, W. E. (2010) Differentiation of effector CD4 T cell populations. *Annual Review of Immunology* **28**, 445–489.

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Clinical Relevance

Scientific rationale for the study: Evidence indicates that the host response to periodontal infection in diabetic subjects may be influenced by glycaemia. It is important to clarify these mechanisms and determine the Th-derived cytokine profile in periodontitis of diabetic subjects, according to their glycaemic condition.

Principal findings: Periodontitis in well-controlled type 2 diabetic subjects presented a predominance of a Th1-type cytokine (IFN-γ), while in poorly controlled individuals exhibited dominance of Th17-type cytokine (IL-17).

Practical implications: The understanding of host immunoinflammatory response may be useful to suggest modulation agents as adjunctive to anti-infectious therapies, especially in groups at risk, such as diabetic subjects.