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Serum levels of interleukin-10 and tumour necrosis factor-α in chronic periodontitis

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Abstract

Aims: To investigate, using a cross-sectional study design, whether the extent of periodontal inflammation associates with the serum levels of cytokine interleukin (IL)-10 and tumour necrosis factor (TNF)-α and their ratio.

Material and Methods: The study group consisted of 61 subjects with chronic periodontitis and 30 control subjects with minimally inflamed periodontal tissues. Probing pocket depth (PD), bleeding on probing (BOP) and periodontal attachment level (AL) were measured. The serum IL-10 (pg/ml) and TNF-α (U/l) levels were analysed using enzyme-linked immunosorbent assays. After categorization of the subjects, associations between serum IL-10 and TNF-α levels and the extent of periodontal inflammation were studied using linear regression models adjusted for age, gender, body mass index and smoking.

Results: A negative, partly dose-dependent association existed between the extent of BOP, PD ⩾ 4 mm and AL ⩾ 4 mm and serum IL-10 level. The subjects in the periodontitis group presented significantly higher serum TNF-α levels and their TNF-α/IL-10 ratio was approximately threefold when compared with the ratio in the control group.

Conclusions: The significantly higher serum TNF-α/IL-10 ratio in the subjects with chronic periodontitis when compared with the ratio in the controls is indicative of a stronger systemic pro-inflammatory state in chronic periodontitis.

Key words: IL-10; periodontal inflammation; serum; TNF-α

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The major factors regulating the destruction of tooth-supporting periodontal structures are inflammatory mediators released by host cells. Gaffen & Hajishengallis (2008) describe periodontal health as ''a dynamic state where the activity of pro-inflammatory/antimicrobial cytokines to control infection is optimally balanced by anti-inflammatory mechanisms to prevent unwarranted inflammation''. In a susceptible host, overreaction of the host response occurs, resulting in excessive production of pro-inflammatory cytokines such as interleukin (IL)-1, tumour necrosis factor (TNF)-α and IL-6 and the subsequent loss of periodontal attachment (Gemmel et al. 1997).

Both IL-10 and TNF-α are known for their central and contradictory roles in antibacterial immune responses. Besides the ability of IL-10 to directly inhibit the production of TNF (Fiorentino et al. 1989, De Waal Malefyt et al. 1991), it also affects several steps in antimicrobial immunity (reviews by Moore et al. 2001, Asadullah et al. 2003). IL-10 was long considered an inhibitory factor of cytokine synthesis, but recent data are more supportive of its immune-regulatory role. Both over-expression of IL-10 and its deficiency have been associated with autoimmune and inflammatory diseases. The major source of IL-10 are the macrophages, but certain T-cell subsets (Th2, Tc2, Tr1), monocytes and B cells are also known to produce IL-10. By inhibiting interferon (IFN)-γ production, IL-10 drives the Th1/Th2 balance towards the type 2 cytokine pattern. IL-10 is also known for its capacity to suppress monocyte differentiation to antigen-presenting cells, inhibit the expression of most inducible chemokines involved in inflammation, inhibit the production of prostaglandin E_2 (PGE_2) and enhance the production of anti-inflammatory mediators such as IL-1RA and soluble TNF-α receptors.

Conflict of interest and source of funding statement

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Other stimulatory effects of IL-10 include enhancement of the proliferation and differentiation of B cells and their maturation into plasma cells.

While IL-10 has a key role in maintaining the health and stability of periodontal tissues (Gemmel et al. 1997, Bozkurt et al. 2006, Dutzan et al. 2009), TNF-α fuels tissue pathology towards periodontal connective tissue destruction and bone loss (reviewed by Graves & Cochran 2003). Accordingly, TNF-α is a multi-potential pro-inflammatory cytokine produced as a result of bacterial stimulation by monocytes/macrophages, polymorphonuclear leucocytes, fibroblasts, epithelial cells, endothelial cells and osteoblasts. TNF-α may be regarded as an ''early'' cytokine that amplifies innate host responses. It acts on endothelial cells to increase the attachment of polymorphonuclear leucocytes and monocytes, and thus helps to recruit these cells into sites of inflammation. TNF-α enhances connective tissue and bone destruction via enhanced osteoclast formation and activity, induction of matrix-metalloproteinase expression and stimulation of PGE_2 production. In addition, apoptosis of fibroblasts is stimulated by TNF-α, resulting in limited repair of the periodontal tissues. The use of TNF-α antagonists results in a reduction of periodontal inflammation, indicating a cause–effect relationship between TNF-α and periodontal infection (Assuma et al. 1998, Graves et al. 1998, Delima et al. 2001).

Evaluation of the serum levels of pro- and anti-inflammatory cytokines not only helps in studying their role in host protection but is also crucial in determining the systemic inflammatory status of periodontitis patients. A growing body of evidence suggests that chronic periodontitis is associated with low-grade systemic inflammation (Nibali et al. 2007, Paraskevas et al. 2008, Saxlin et al. 2009), and some data indicating a positive association between the level of circulating TNF-α and the severity of periodontal inflammation already exist (Bretz et al. 2005, Engebretson et al. 2007). Contradictory to the reports of the local protective role of IL-10 in the periodontal area (Bozkurt et al. 2006, Dutzan et al. 2009), Górska et al. (2003) report that serum IL-10 levels in subjects with chronic periodontitis are higher than levels in periodontally healthy individuals. To clarify the role of serum IL-10 and TNF-α in periodontal inflammation, we examined whether any association exists between the extent of bleeding on probing (BOP), probing pocket depth (PD) $\geqslant$4 mm and attachment level (AL) $\geqslant$4 mm and the serum levels of IL-10 and TNF-α. Secondly, a possible deviation in the systemic inflammatory status, presented as the ratio of serum TNF-α to IL-10, from protective to destructive, was studied.

Material and Methods

Subjects

This study is part of a comprehensive investigation of the linkage between periodontal and general health in type 1 diabetic and non-diabetic subjects. All the subjects were recruited on a volunteer basis during 2001–2007. Originally, a total of 62 consecutive non-diabetic patients, referred to specialist periodontal therapy due to varying degrees of chronic periodontitis participated. Thirty non-diabetic age and gender-matched control subjects with either clinically healthy periodontal tissues or minimal signs of periodontal inflammation were also recruited. Because of lacking data on the serum IL-10 level of one subject, the final number of subjects in the present analysis was 91. All the subjects were examined by the same periodontal specialist (T. R.) at the Specialist Dental Health Care Unit, City of Oulu, Oulu, Finland. Informed consents were obtained from all subjects and the study protocol was accepted by the Ethical Committee of Oulu University Hospital, Oulu, Finland. Subjects needing prophylactic antibiotic medication in association with periodontal probing as well as those with rheumatoid arthritis and asthma, and those with immunosuppressive medication or antibiotics during the past 4 months were excluded from the study. In addition, none of the subjects had a diagnosis of cardiovascular disease. Smoking was recorded as dichotomized; smokers *versus* non-smokers. Originally, 20 control subjects and 25 periodontitis subjects were reported to be non-smokers. Smoking occasionally or $<$10 cigarettes per day was recorded for 10 control and 9 periodontitis subjects and $\geqslant$10 cigarettes per day for 27 periodontitis subjects. In addition, age (years) and body mass index (BMI) (weight of the subject divided by the square of the subject's height, i.e. kg/m^2) were recorded.

Clinical periodontal examination

Clinical measurements were made on four surfaces (mesiobuccal, midbuccal, distobuccal and midlingual) of each tooth, excluding third molars. A ball-point periodontal probe with 2 mm graduation was used to measure PD from the gingival margin to the base of the crevice/pocket. A positive score for BOP was registered if a site presented bleeding 20–30 s after probing. Periodontal AL from the cementoenamel junction to the base of the crevice/pocket was also registered. The extent of periodontal disease was expressed as the number of sites with a positive score for BOP, PD$\geqslant$4 mm and AL$\geqslant$4 mm (Table 1).

Subject categories

For statistical analyses of possible dose dependence between the extent of periodontal infection and serum cytokine levels, the subjects were categorized (Table 1). One category (reference category) was comprised of the control subjects, and the subjects with chronic periodontitis were categorized into tertiles based on the extent of BOP, PD$\geqslant$4 mm and AL$\geqslant$4 mm (I–III). The category limits are presented in Table 1.

Serum levels of IL-10 and TNF-α

A venous blood sample was drawn from each subject on the day of the examination. The serum IL-10 and TNF-α levels (Table 2) were analysed using commercially available enzyme-linked immunosorbent assays (Quantikine® Human IL-10, Quantikine® Human TNF-α, R&D Systems Europe Ltd., Abingdon, UK). The actual levels of IL-10 are expressed as picograms per millilitre (range 6.6–74.3 pg/ml) and the levels of TNF-α as units per litre (U/l) corresponding to optical density 450–540 nm × 10E+3 (range 0.0–62.5 U/l). Three subjects in the periodontitis group and six in the control group presented TNF-α levels that were under the detection limit (DL) (0.5 U/l). The individual TNF-α/IL-10 ratios were calculated by dividing the actual serum TNF-α levels by the IL-10 levels (Table 2).

IL-10^{-1082} and TNF-α^{-308} polymorphisms

The IL-10^{-1082} genotype *(G → A)* was determined using a simple bidirectional

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Table 1. Subject characteristics presented as mean values (± SD) or numbers of subjects

Subjects	*n*	No. of affected sites, mean (± SD)	No. of teeth, mean (± SD)	Age, mean (± SD)	Females, *n* (%)	Smokers, *n* (%)
Control subjects	30		27.4 (± 1.0)	41.9 (± 12.7)	19 (63.3)	10 (33.3)
BOP		15.5 (± 11.1)				
PD ⩾ 4 mm		1.0 (± 2.4)				
AL ⩾ 4 mm		0.6 (± 1.4)				
Periodontitis subjects						
BOP						
Tertile I (1–67)	20	55.0 (± 13.6)	23.2 (± 4.3)	56.0 (± 14.3)	13 (65.0)	10 (50.0)
Tertile II (68–95)	20	85.0 (± 8.5)	25.6 (± 2.5)	45.3 (± 10.2)	15 (75.0)	11 (55.0)
Tertile III (>95)	21	103.1 (± 4.6)	27.4 (± 0.9)	38.4 (± 9.3)	11 (52.4)	15 (71.4)
PD ⩾ 4 mm						
Tertile I (1–36)	20	24.3 (± 9.4)	24.4 (± 3.3)	54.9 (± 12.8)	13 (65.0)	10 (50.0)
Tertile II (37–58)	21	48.5 (± 6.7)	25.1 (± 4.2)	45.4 (± 13.9)	15 (71.4)	12 (57.1)
Tertile III (>58)	20	79.2 (± 14.7)	26.8 (± 1.5)	39.0 (± 8.1)	11 (55.0)	14 (70.0)
AL ⩾ 4 mm						
Tertile I (1–23)	20	12.5 (± 7.1)	26.2 (± 3.0)	45.8 (± 12.7)	12 (60.0)	9 (45.0)
Tertile II (24–43)	21	35.9 (± 6.0)	24.6 (± 3.8)	46.3 (± 15.3)	15 (71.4)	12 (57.1)
Tertile III (>43)	20	60.6 (± 15.8)	25.5 (± 3.0)	47.2 (± 12.6)	12 (60.0)	15 (75.0)

The characteristics are given separately for all control subjects and for the periodontitis subjects after dividing them into tertiles based on the numbers of sites presenting bleeding on probing (BOP), PD ⩾ 4 mm and AL ⩾ 4 mm. The tertile limits are given in the parentheses.
AL, attachment level; PD, pocket depth.

Table 2. The mean serum IL-10 (pg/ml) and TNF-α (U/l) levels and TNF-α/IL-10 ratios (the actual serum TNF-α levels divided by the IL-10 levels) in the control and the periodontitis subjects

Subjects	*n*	IL-10 (95% CI)	TNF-α (95% CI)	TNF-α/IL-10 (95% CI)
Control subjects	30	17.4 (12.9–21.9)	9.2 (5.9–12.4)	0.6 (0.4–0.8)
Periodontitis subjects				
BOP				
Tertile I	20	11.6 (10.1–13.1)	23.3 (15.4–31.2)	2.0 (1.3–2.8)
Tertile II	20	9.9 (8.9–11.0)	15.8 (9.1–22.6)	1.5 (1.0–2.0)
Tertile III	21	11.5 (8.8–14.3)	20.3 (12.0–28.6)	1.9 (1.1–2.8)
PD ⩾ 4 mm				
Tertile I	20	11.3 (9.8–12.9)	21.5 (14.0–29.1)	1.9 (1.2–2.5)
Tertile II	21	10.2 (9.2–11.2)	18.8 (11.5–26.2)	1.8 (1.2–2.4)
Tertile II	20	11.6 (8.7–14.5)	19.1 (10.6–27.7)	1.8 (0.9–2.6)
AL ⩾ 4 mm				
Tertile I	20	11.1 (9.9–12.4)	20.7 (12.9–28.5)	1.8 (1.2–2.4)
Tertile II	21	9.7 (8.6–10.7)	16.8 (10.2–23.5)	1.7 (1.0–2.3)
Tertile III	20	12.4 (9.5–15.3)	22.1 (13.3–30.9)	2.0 (1.1–2.8)

For TNF-α, the DL/2 method was used to substitute values under the detection limit (0.5 U/l).
The subjects in the periodontitis group were divided into tertiles according to the numbers of sites with bleeding on probing (BOP), probing depth ⩾ 4 mm (PD ⩾ 4 mm) and attachment level ⩾ 4 mm (AL ⩾ 4 mm). The tertile limits are presented in Table 1.
TNF, tumour necrosis factor; DL, detection limit; IL, interleukin.

allele-specific polymerase chain reaction method developed by our own group (Karhukorpi & Karttunen 2001). A total of 54 subjects (59%) carried the *AG/GG* genotype of IL^{-1082} previously associated with higher secretion of the regulatory cytokine IL-10 than the *AA* genotype (Turner et al. 1997). Genotyping for TNF-α^{-308} *(G → A)* was performed using the method described by Ozen et al. (2002). The genotypes of TNF-α^{-308} that contain A allele(s) are known to be associated with higher TNF-α transcription (Kroeger et al. 1997) and secretion (McGuire et al. 1994). In the present study population, only one subject carried the *AA* genotype of TNF-α^{-308}, whereas the genotype *AG* was presented by 28 subjects (31%). Comparisons of mean TNF-α levels were made between the *AG* and *GG* genotypes.

Statistical methods

The significance of the differences in the mean age between the periodontitis and control subjects was tested using the Student's *t*-test. The non-parametric Mann–Whitney test was used to study the significance of the differences in the actual mean levels of serum IL-10 and TNF-α between the IL-10^{-1082} and TNF-α^{-308} genotypes. Because of the skewness of the IL-10 and TNF-α data, a logarithmic transformation was then performed to yield symmetrical distributions. To process the TNF-α data below the DL, we used the DL/2 method, where the non-detections are reported as half of the DL (Uh et al. 2008). Linear regression models, adjusted for age, gender, BMI and smoking, were used to study the associations between the extent of periodontal disease (BOP, PD ⩾ 4 mm and AL ⩾ 4 mm) and the serum levels of IL-10 (Table 3), TNF-α (Table 4) and the TNF-α/IL-10 ratio (data not shown). In these models, smoking was used as a dichotomized variable and both age and BMI were used as continuous variables. The extent of periodontal disease was used as a categorized variable (Table 1) and the comparisons are presented both using the control group as a reference and between adjacent disease categories (Tables 3 and 4).

In the statistical analyses, the level of significance was set at $p < 0.05$. All calculations were carried out with the

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Table 3. Associations between IL-10 serum levels (the outcome variable, transformed to logarithmic scale) and the extent of BOP, PD ⩾ 4 mm and AL ⩾ 4 mm (numbers of affected sites) analysed using a multivariate linear regression model adjusted for age, gender, smoking and BMI

Extent of periodontal disease	*n*	β (95% CI for β)	*p*-value	β (95% CI for β) *p*-value	β (95% CI for β) *p*-value
BOP					
Control subjects	30	Reference			
Periodontitis group					
Tertile I	20	−0.35 (−0.58 to −0.13)	0.003	Tertile II *versus* I	
Tertile II	20	−0.47 (−0.67 to −0.26)	<0.001	−0.29 (−0.48 to −0.10)	Tertile III *versus* II
Tertile III	21	−0.38 (−0.59 to −0.17)	0.001	$p = 0.003$	−0.11 (−0.30 to −0.08)
					$p = 0.265$
PD ⩾ 4 mm					
Control subjects	30	Reference			
Periodontitis group					
Tertile I	20	−0.38 (−0.60 to −0.15)	0.001	Tertile II *versus* I	
Tertile II	21	−0.44 (−0.65 to −0.24)	<0.001	−0.26 (−0.44 −0.07)	Tertile III *versus* II
Tertile III	20	−0.38 (−0.59 to −0.17)	0.001	$p = 0.007$	−0.10 (−0.29–0.09)
					$p = 0.285$
AL ⩾ 4 mm					
Control subjects	30	Reference			
Periodontitis group					
Tertile I	20	−0.37 (−0.57 to −0.17)	0.001	Tertile II *versus* I	
Tertile II	21	−0.50 (−0.70 to −0.29)	<0.001	−0.31 (0.50 to −0.13)	Tertile III *versus* II
Tertile III	20	−0.32 (−0.54 to −0.11)	0.004	$p = 0.001$	−0.03 (−0.22–0.15)
					$p = 0.727$

On the left estimates of the analyses using the control group as reference, on the right estimates using adjacent categories.
IL, interleukin; AL, attachment level; PD, pocket depth; BOP, bleeding on probing; BMI, body mass index.

Table 4. Associations between TNF-α serum levels (the outcome variable, transformed to logarithmic scale) and the extent of BOP, PD ⩾ 4 mm and AL ⩾ 4 mm (numbers of affected sites) analysed using a multivariate linear regression model adjusted for age, gender, smoking and BMI

Extent of periodontal disease	*n*	β (95% CI for β)	*p*-value	β (95% CI for β) *p*-value	β (95% CI for β) *p*-value
BOP					
Control subjects	30	Reference			
Periodontitis group					
Tertile I	20	1.51 (0.68–2.35)	0.001	Tertile II *versus* I	
Tertile II	20	0.80 (0.03–1.57)	0.042	0.04 (−0.66–0.74)	Tertile III *versus* II
Tertile III	21	1.11 (0.33–1.90)	0.006	$p = 0.908$	0.34 (−0.36–1.05)
					$p = 0.336$
PD ⩾ 4 mm					
Control subjects	30	Reference			
Periodontitis group					
Tertile I	20	1.21 (0.37–2.10)	0.005	Tertile II *versus* I	
Tertile II	21	1.19 (0.42–2.00)	0.003	0.58 (−0.11–1.28)	Tertile III *versus* II
Tertile III	20	0.95 (0.16–1.75)	0.019	$p = 0.098$	0.15 (−0.55–0.86)
					$p = 0.664$
AL ⩾ 4 mm					
Control subjects	30	Reference			
Periodontitis group					
Tertile I	20	1.11 (0.35–1.88)	0.005	Tertile II *versus* I	
Tertile II	21	0.86 (0.09–1.63)	0.029	0.31 (−0.39–1.00)	Tertile III *versus* II
Tertile III	20	1.42 (0.61–2.23)	0.001	$p = 0.385$	0.76 (0.07–1.45)
					$p = 0.013$

On the left are estimates of the analyses using the lowest disease category as a reference, on the right are estimates using adjacent categories.
TNF, tumour necrosis factor; AL, attachment level; PD, pocket depth; BOP, bleeding on probing; BMI, body mass index.

aid of statistical software (SPSS, version 16.0; SPSS Inc., Chicago, IL, USA).

Results

The periodontitis and the control group were similar with regard to the mean age (± SD) of the subjects (46.4 ± 13.3 *versus* 41.9 ± 12.7 years, respectively, $p > 0.05$). The proportion of females was also similar in the two groups, 64% *versus* 63%, respectively, whereas the proportion of smokers was higher in the periodontitis group. The subjects' characteristics by disease category are presented in Table 1.

Based on the mean serum IL-10 and TNF-α levels, the control subjects presented higher IL-10 and lower TNF-α levels than the subjects in the periodontitis group (Table 2). Moreover, the TNF-α/IL-10 ratio of the subjects in the various categories of the periodontitis group was approximately three times the

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value presented by the control subjects. No statistically significant differences in the levels of IL-10 could be found between the IL-10^{-1082} genotypes (*AG/GG*, 12.8 ± 9.6 pg/ml *versus AA*, 13.6 ± 5.8 pg/ml, $p > 0.05$). The TNF-α levels were also similar between the *AG* and *GG* genotypes of TNF-α^{-308} (*AG*, 17.1 ± 17.5 U/l *versus* GG, 16.2 ± 14.3 U/l, $p > 0.05$).

The adjusted associations between serum IL-10 level and the extent of periodontal disease, using the control group as a reference, indicated that the subjects in the control group had significantly higher levels of IL-10 than the subjects in any of the BOP, PD ⩾ 4 mm and AL ⩾ 4 mm tertiles. According to the contrast results using adjacent BOP, PD ⩾ 4 mm and AL ⩾ 4 mm tertiles (I–III) in the model, the level of IL-10 was significantly higher in tertile II than in tertile I in all comparisons, whereas comparisons between tertiles II and III did not yield statistically significant results (Table 3).

The adjusted associations between serum TNF-α level and the extent of periodontal disease, on the other hand, indicated that the control subjects had significantly lower serum TNF-α levels than the subjects in each of the BOP, PD ⩾ 4 mm and AL ⩾ 4 mm tertiles (I–III). Except for the significantly higher TNF-α level in the AL ⩾ 4 mm tertile III compared with the level in the tertile II, the differences in the TNF-α levels between adjacent tertiles were not statistically significant (Table 4).

The age, gender, smoking and BMI-adjusted associations between the TNF-α/IL-10 ratio (the outcome) and the extent of periodontal disease indicated significantly higher ratios in all BOP, PD ⩾ 4 mm and AL ⩾ 4 mm tertiles when compared with the ratio in the control group (data not shown). Again, no statistically significant differences in the TNF-α/IL-10 ratio between adjacent BOP, PD ⩾ 4 mm and AL ⩾ 4 mm tertiles could be observed.

Discussion

According to the present results, a negative association exists between the serum level of IL-10 and the extent of BOP, PD ⩾ 4 mm and AL ⩾ 4 mm. The findings that the serum IL-10 level was significantly lower in the periodontitis subjects in tertile I when compared with the controls and also between the subjects in tertile II than in tertile I may be considered indicative of a dose-dependent association in these subjects. Furthermore, we also found that the serum TNF-α level was significantly higher in subjects with chronic periodontitis than in the control subjects; no dose-dependence was, however, found between the extent of BOP, PD ⩾ 4 mm and AL ⩾ 4 mm and the level of TNF-α. Single nucleotide polymorphism in the genes that regulate the expression of serum IL-10 and TNF-α has been reported (McGuire et al. 1994, Turner et al. 1997). In the present subjects, neither the IL-10^{-1082} *(G → A)* nor the TNF-α^{-308} *(G → A)* genotype had any influence on the serum levels of IL-10 or TNF-α.

Plausible arguments to support the down-regulation of IL-10 along with the heightened expression of serum TNF-α in the present subjects with chronic periodontitis can be presented. Consequently, in chronic periodontal infection, bacteria and/or their components disseminate from the inflamed areas into the circulation (Kinane et al. 2005, Forner et al. 2006, Oscarsson et al. 2008) to challenge the immune system, i.e. the circulating and resident immune cells of the body. A number of studies indicate that peripheral blood monocytes challenged by bacterial lipopolysaccharide produce inflammatory mediators like TNF-α (Pociot et al. 1993, Salvi et al. 1997, Pérez et al. 2004). Of other distant sources of TNF-α, adipose tissue is important (Ritchie 2007) and multiple immune cells in the liver (Kupffer cells, hepatocytes and T cells) are known for their capacity to produce cytokines, including IL-10 and TNF-α, as a response to lipopolysaccharides (LPS) from the intestinal tract (McClain et al. 2004, Tiegs & Lohse 2010). Interestingly, an imbalance between the pro- and anti-inflammatory cytokines is also characteristic for progression of liver diseases (Latvala et al. 2005). That LPS from the periodontal area can trigger an immune response in distant organs like the liver in humans is evident. This is supported by animal experiments in which mice have been inoculated by periodontal pathogens. In a recent study by Hyvärinen et al. (2009), intra-nasal inoculation of mice with *Actinobacillus actinomycetemcomitans* was followed by dissemination of the bacteria, their detection in the liver of the animals and a subsequent increase in the serum markers of inflammation. Using a subcutaneous chamber model with *Porphyromonas gingivalis* in a mouse, Lin et al. (2003) also demonstrated that bacterial dissemination (*P. gingivalis* DNA detection in maternal liver and uterus) and a systemic inflammatory response, measured as elevated serum TNF-α and decreased serum IL-10, occurred. In addition to being produced by distant immune cells, inflammatory mediators also leak from inflamed periodontal pocket areas into the circulation. In the context of the present results, one can only hypothesize that both of these pathways contributed to the down-regulation of serum IL-10 and the up-regulation of serum TNF-α.

The local pattern of cytokines in the periodontal area has been shown to contribute to the progression and severity of experimental periodontal disease (Garlet et al. 2004, 2006). Consequently, the expression of matrix metalloproteinases and receptor activator of nuclear factor-κβ ligand has been associated with the expression of pro-inflammatory cytokines (IFN-γ, TNF-α and IL-1β) and an increase in inflammatory reaction, whereas IL-10 and IL-4 were associated with tissue inhibitors of metalloproteinases and osteoprotegerin as well as reduced inflammation. The systemic pro-inflammatory status in subjects with periodontal disease may, therefore, enhance tissue pathology locally and thus constitute an additional susceptibility factor for periodontal disease progression. The above aspect was also discussed by Nishimura et al. (2003) and by Andriankaja et al. (2009) who suggest that the elevation of the levels of pro-inflammatory cytokines in serum, may contribute to increased tissue pathology locally in the periodontal area. Because of the interactions between cytokines, we studied the ratio of the level of TNF-α to the level of IL-10 and found out that the ratio was significantly higher in each of the BOP, PD ⩾ 4 mm and AL ⩾ 4 mm tertiles in the periodontitis group when compared with the ratio in the control subjects, indicating a stronger systemic pro-inflammatory state in chronic periodontitis. In this respect, periodontal infection appears to be similar to several other inflammatory/infectious conditions in which predominance of pro-inflammatory processes is observed (McClain et al. 2004, Goswami et al. 2009, Karpiński et al. 2009, Szkaradkiewicz et al. 2009).

Based on their biological characteristics, we chose to investigate the pro-inflammatory cytokine, TNF-α, and the

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regulative anti-inflammatory cytokine, IL-10, in the present study. However, host–pathogen interactions are complex and their influences are dependent on the strength and nature of the immune responses and the pathogenic properties of the invading microbes. This should be kept in mind when drawing conclusions on the associations between the serum levels of single cytokines like IL-10 and TNF-α and the severity of periodontal infection. According to our earlier finding in the present subjects, periodontal infection also contributed significantly to the serum level of IL-6, another pro-inflammatory cytokine (Raunio et al. 2007), a finding which together with the present results is indicative of chronic periodontitis-associated pro-inflammatory immune status.

We preferred to use the number of sites instead of their relative proportions to indicate the inflamed periodontal area. Roughly, the results were similar using any of the three periodontal parameters (BOP, PD $\geqslant$ 4 mm and AL $\geqslant$ 4 mm), which adds to the reliability of the results. Chronic periodontitis typically progresses with periods of exacerbation and quiescence (Quirynen et al. 2006) and the expression of both pro- and anti-inflammatory cytokines has been found to differ between active and inactive sites of inflammation (Dutzan et al. 2009). The activity of periodontal disease may also on its part explain the inter-individual variation in the levels of IL-10 and TNF-α. In the cross-sectional setting of the present study, we did not assess the activity of periodontal disease, which may be considered a shortcoming. Furthermore, although all the subjects were anamnestically healthy, we cannot fully exclude that some of them had subclinical inflammatory/infectious conditions affecting the serum levels of IL-10 and TNF-α.

Smoking was considered a confounding factor known for its capacity to influence the severity of periodontal disease (Bergström 2006) as well as serum cytokine levels (Klopocka et al. 2007, Reichert et al. 2009), and therefore adjustments were made for smoking in the linear regression models. Inferior to a more reliable measure of smoking such as pack-years, we used smoking as a dichotomized variable and therefore a need to control smoking in future studies remains. Because of previously observed associations between the levels of serum cytokines and obesity/BMI (Ziccardi et al. 2002, Karpiñski et al. 2009) on one hand, and BMI and periodontal destruction (Saxlin et al. 2009) on the other, adjustments were also made for BMI in all the regression models. By adjusting for BMI, we could also, at least partly, take into account the metabolic syndrome, in which obesity forms one important component.

The fact that the sample size was fairly small and no power analysis was performed for the sample size was a methodological limitation of this study. In the present material, nearly 10% of the population (5% of the periodontitis patients and 20% of the controls) showed TNF-α values, which were below the DL. Because such non-detects cannot be considered missing at random, a common practice in immunological assays has been to impute each non-detect with a single value as a half of the DL (DL/2) (Uh et al. 2008). This approach has been shown to perform well when immunological methods are constrained by DLs and the amount of non-detects is relatively small, as in the present material. Also, this is a cross-sectional study, which prohibits us from suggesting that a cause–effect relationship exists between the serum levels of IL-10 and TNF-α and the extent of periodontal inflammation. To ascertain a causal relationship, a prospective study of a possible change in the level of serum IL-10 and TNF-α in relation to the change in periodontal inflammation would be required. The higher serum TNF-α/IL-10 ratio observed in the subjects with chronic periodontitis when compared with the controls may, however, be considered indicative of a stronger systemic pro-inflammatory state in periodontitis.

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Clinical Relevance

Scientific rationale for the study: A high local level of IL-10 in the periodontal area is associated with the health of the tissues, whereas a high local expression of TNF-α, a pro-inflammatory cytokine, is known to fuel tissue pathology towards destructive processes. Serum TNF-α level is evidently associated with the severity of periodontitis, whereas the association between serum IL-10 and the extent of periodontal disease has not been studied before.

Principal findings: A negative, partly dose-dependent association existed between the extent of BOP, PD $\geqslant$ 4 mm and AL $\geqslant$ 4 mm and serum IL-10 level. Subjects in the periodontitis group presented significantly higher TNF-α levels and their TNF-α/IL-10 ratio was approximately threefold when compared with the ratio in the control group.

Practical implications: Regardless of the local nature of periodontal infection, it may exert systemic-level influences. Changes in the levels of serum cytokines may in turn be considered a susceptibility factor for further progression of chronic periodontitis.

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