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The extent of periodontal disease and the IL-6 $^{-174}$ genotype as determinants of serum IL-6 level

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Abstract

Aim: To study the extent of periodontal disease and the IL-6 $^{-174}$ genotype as determinants of serum and mouthwash IL-6 concentration in subjects with moderate to severe periodontal disease.

Material and Methods: Fifty-two generally healthy subjects volunteered to participate. Probing pocket depth (PD) and periodontal attachment level (AL) were clinically examined and alveolar bone level (BL) was measured on orthopantomographs. IL-6 concentrations in mouthwash, collected by rinsing with 3 ml saline for 30 s and in serum, obtained by venipuncture, were measured using ELISA. IL-6 $^{-174}$ polymorphism was studied using a polymerase chain reaction.

Results: Eleven subjects carried the GG genotype, and 41 subjects, carried the CG/CC genotype. The mean (± SD) concentration of IL-6 in serum was 1.6 (± 1.5) pg/ml and, 2.8 (± 5.04) pg/ml in mouthwash. The serum concentration of IL-6 was higher in subjects with the GG genotype than with the CG/CC genotype. In regression analyses the percentages of sites with PD ⩾ 6 mm, AL ⩾ 6 mm and BL ⩾ 8 mm, the IL-6 $^{-174}$ genotype, body mass index and gender associated significantly with serum IL-6 concentration.

Conclusions: The extent of moderate to severe periodontal disease and the IL-6 $^{-174}$ genotype contribute significantly to serum IL-6 concentration.

Key words: gene polymorphism; IL-6; periodontal disease; serum

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IL-6 is a proinflammatory cytokine produced by a number of cells, including monocytes/macrophages, activated T cells, endothelial cells, adipocytes and fibroblasts. It was originally identified as a B-cell stimulatory and differentiation factor, but is currently known as a regulator of the immune response, haematopoiesis, acute phase response and inflammation (Gabay 2006, Kishimoto 2006). IL-6 plays a role in the transition between acute and chronic inflammation through the recruitment, activation and clearance of individual leucocyte subpopulations (i.e. neutrophils, monocytes and macrophages). Other biological activities of IL-6 include the enhancement of T-cell proliferation and acceleration of bone resorption by increasing osteoclast formation (Ishimi et al. 1990, Tamura et al. 1993).

IL-6 is highly expressed in many autoimmune diseases, like rheumatoid arthritis and psoriasis (Kishimoto 1992, Fishman et al. 1998, Ishihara & Hirano 2002), and elevated serum levels of IL-6 have also been found in acute and chronic inflammatory diseases, such as sepsis (Schluter et al. 2002) and atherosclerosis (Beckman et al. 2005).

IL-6 is locally produced in inflamed tissues following cellular activation by bacterial lipopolysaccharide (LPS) or other cytokines such as IL-1β or tumour necrosis factor (TNF)-α (Kishimoto 1989, Ishihara & Hirano 2002). Local production of IL-6 also occurs in inflamed periodontal tissues (Takahashi et al. 1994, Guillot et al. 1995, Dongari-Bagtzoglou & Ebersole 1998), and significant correlations have been found between periodontal pocket depth (PD) and IL-6 content in gingival crevicular fluid (Geivelis et al. 1993, McGee et al. 1998). IL-6 has also been associated with attachment loss in refractory periodontitis (Reinhardt et al. 1993) and Guillot et al. (1995) found elevated levels of IL-6 in gingival connective tissue adjacent to intra-bony pockets representing poor response to phase I therapy.

Conflict of interest and source of funding statement

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The evidence that a causal relationship exists between periodontitis and systemic inflammatory response, measured as serum IL-6 levels, is based on the fact that subjects with no known medical disorders other than periodontitis have been reported to have elevated serum levels of IL-6 when compared with periodontally healthy controls (Loos et al. 2000, Buhlin et al. 2003). Secondly, immediately after subgingival instrumentation with hand instruments or either sonic or ultrasonic scalers, serum IL-6 is known to increase (D'Aiuto et al. 2004a, Ide et al. 2004, Forner et al. 2006). Thirdly, resolution of periodontal inflammation has been reported to result in significant decreases in serum IL-6 levels after periodontal therapy (D'Aiuto et al. 2004b).

Specific polymorphisms in the cytokine genes are known to modulate inflammatory response. D'Aiuto et al. (2004c) related serum IL-6 concentrations in subjects with severe periodontitis to the IL-6^{-174} genotype and reported higher serum IL-6 concentrations in subjects with the CG/CC genotype when compared with those with the GG genotype. On the other hand, Nibali et al. (2007) reported just recently that -174 G homozygous patients with generalized aggressive periodontitis exhibited higher detection rates of periodontopathogenic bacteria when compared with those with the CG/CC genotype. This finding was interpreted as higher susceptibility to periodontal tissue destruction among patients carrying this specific genotype. We have recently reported that the GG genotype of IL-6^{-174} associates with the extent of periodontal disease in subjects with moderate to severe periodontitis (Tervonen et al. 2007). We measured the serum and mouthwash IL-6 concentrations of the same subjects, and the main purpose of the present study was to evaluate the significance of the extent of periodontal disease and the IL-6^{-174} gene polymorphism as determinants of serum IL-6 levels.

Material and Methods

Subjects

A total of 52 patients of Caucasian origin (35 females and 17 males) with moderate to severe chronic periodontitis, originally referred to periodontal specialist therapy, volunteered to participate (Table 1). The mean age ($\pm$ SD) of the subjects was 43.2 years ($\pm$ 9.3) (range 22–64 years). All the subjects were examined by the same periodontal specialist (T. R.) at the dental specialist clinic of Oulu Municipal Health Center. The informed consent of all the subjects was obtained, and the study protocol was accepted by the Ethical Committee of the Faculty of Medicine, University of Oulu, Finland. Subjects needing prophylactic antibiotic medication in association with periodontal probing as well as those with rheumatoid arthritis, diabetes mellitus 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. Data concerning smoking habits were obtained by interviewing the subjects in conjunction with the clinical examination, and the subjects were categorized as non-smokers or smokers. There were altogether 18 non-smokers who had never smoked. The group of smokers comprised nine smokers who smoked either occasionally or <10 cigarettes/day and 25 who smoked $\geqslant 10$ cigarettes/day.

Table 1. Characteristics of the subjects in the study group

Parameter	
Age	
Mean ($\pm$ SD)	43.2 $\pm$ 9.3
Range	22–64
IL-6^{-174} genotype	
GG	11
CC+CG	41
Gender	
Females	35
Males	17
Smoking habits	
Non-smoker	18
Smoker	34
Periodontal parameters (mean% of sites/subject $\pm$ SD)	
PD $\geqslant$ 4 mm	51.5 $\pm$ 23.4
PD $\geqslant$ 6 mm	17.9 $\pm$ 21.8
AL $\geqslant$ 4 mm	46.3 $\pm$ 28.4
AL $\geqslant$ 6 mm	19.1 $\pm$ 24.0
BL $\geqslant$ 6 mm	31.0 $\pm$ 26.4
BL $\geqslant$ 8 mm	15.1 $\pm$ 18.5

PD, pocket depth; AL, attachment level; BL, bone level.

Clinical and radiographic parameters

For this report, probing PD from the gingival margin to the base of the crevice/pocket was measured at four sites (mesiobuccal, midbuccal, distobuccal and midlingual) on all teeth excluding third molars using a ball-pointed periodontal probe with 2 mm graduations. Periodontal attachment level (AL) was measured from the cementoenamel junction (CEJ) to the base of the crevice/pocket at the respective sites. Alveolar bone level (BL) was measured by one examiner (T. T.) from orthopantomograms of the mesial and distal sites of the teeth using a Dimaxis Planmega application. The actual distances measured from the CEJ to the level of the alveolar ridge where the periodontal ligament space started to be of uniform width were measured from the scanned OPTGs and then automatically calibrated to millimetres by the application. A more precise description of the method has been published previously (Tervonen et al. 2007).

Serum and mouthwash sampling and laboratory analysis

Mouthwash samples (available from 48 subjects) were collected before the clinical oral examination by rinsing with 0.9% saline (3 ml) for 30 s. After 1 h in ice, they were centrifuged at $9220 \times g$ for 15 min. and stored at -70°C until analysed. The blood samples were obtained by venipuncture (2×10 ml). Serum IL-6 levels were measured with sensitive ELISA Quantikine HS Immunoassay kits (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany) according to the manufacturer's instructions.

IL-6^{-174} polymorphism

Patient DNA was extracted from an EDTA blood sample and the IL-6^{-174} genotype was tested using a polymerase chain reaction as reported earlier (Tervonen et al. 2007). Genotypes GG and CG/CC were evaluated separately.

Data analysis

The severity of periodontal disease was expressed as the extent (percentage) of affected sites per subject using two different threshold values for each variable ($\geqslant$4 and $\geqslant$6 mm for PD and AL and $\geqslant$6 and $\geqslant$8 mm for BL). Correlations between the extent of periodontal disease and serum and mouthwash IL-6 levels were studied using Pearson's correlation coefficients (Table 2). Associations between serum IL-6 levels and the IL-6^{-174} genotype as well as the extent of periodontal disease

Table 2. Correlations between the extent of periodontal disease (percentages of affected sites) and serum and mouthwash IL-6 levels (pg/ml)

	PD ⩾ 4 mm	PD ⩾ 6 mm	AL ⩾ 4 mm	AL ⩾ 6 mm	BL ⩾ 6 mm	BL ⩾ 8 mm
Serum IL-6	0.354	0.524	0.376	0.487	0.390	0.551
	$p = 0.010$	$p < 0.001$	$p = 0.006$	$p < 0.001$	$p = 0.004$	$p < 0.001$
Mouthwash IL-6	0.147	0.104	− 0.170	− 0.053	− 0.206	− 0.132
	$p = 0.320$	0.482	$p = 0.248$	$p = 0.721$	$p = 0.160$	$p = 0.371$

PD, pocket depth; AL, attachment level; BL, bone level.

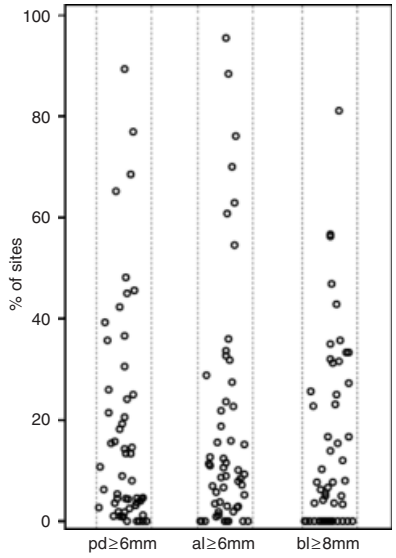

Fig. 1. Distribution of the subjects by the percentages of sites with PD ⩾ 6 mm, AL ⩾ 6 mm and BL ⩾ 8 mm.

(PD ⩾ 6 mm, AL ⩾ 6 mm and BL ⩾ 8 mm) were analysed using multiple regression analysis adjusted for age, gender, body mass index (BMI) and smoking (Table 4).

Results

Eleven subjects (20.0%) carried the GG genotype, and 41 subjects (80.0%) carried the CG/CC genotype of IL-6^{-174}. A high frequency of deepened periodontal pockets was found in the present subjects, as indicated by half of the sites (51.5 ± 23.4%) harbouring a PD ⩾ 4 mm. Advanced periodontal disease, measured as PD ⩾ 6 mm, AL ⩾ 6 mm and BL ⩾ 8 mm, was found on 17.9%, 19.1% and 15.1% of the sites, respectively (Table 1). Distribution of the subjects by the extent of advanced periodontal disease is shown in Fig. 1.

The mean concentration of IL-6 (± SD) in the serum was 1.6 pg/ml (± 1.5, median 1.4 pg/ml). The concentration of mouthwash IL-6 (± SD) was 2.8 pg/ml (± 5.04, median 1.03 pg/ml). Statistically significant correlations were observed between the extent of periodontal disease and serum IL-6 concentrations (Table 2). The correlations became more significant when the sites with early disease were excluded and only the sites with advanced periodontal disease (PD ⩾ 6 mm, AL ⩾ 6 mm and BL ⩾ 8 mm) were analysed. The concentration of IL-6 in mouthwash did not correlate with the extent of periodontal disease (Table 2) or with serum IL-6 concentrations (data not shown).

Table 3. Serum IL-6 levels (pg/ml) by genotype, BMI, gender, age and smoking status

	sIL-6 (pg/ml)
IL-6^{-174} genotype	
GG	2.8 ± 2.6
CG/CC	1.4 ± 0.9
BMI	
Low	1.1 ± 0.8
Middle	1.7 ± 2.3
High	2.1 ± 0.9
Gender	
Male	1.5 ± 1.0
Female	1.9 ± 1.7
Age (years)	
< 40	1.2 ± 0.7
40–49	1.6 ± 1.0
⩾ 50	2.2 ± 2.6
Smoking	
Non-smokers	1.6 ± 1.0
Smokers	1.8 ± 1.8

BMI, body mass index.

In bivariate comparisons, higher serum IL-6 levels were observed in subjects carrying the GG genotype than in those carrying the CG/CC genotype, in females than in males and in smokers than in non-smokers (Table 3). Serum IL-6 levels were also higher in subjects with a higher age and a higher BMI. The IL-6^{-174} genotype had no significant effect on the concentration of mouthwash IL-6 level (data not shown).

The estimates of the regression analyses used to study the associations between serum IL-6 level and its determinants are shown in Table 4. BMI, gender, age and smoking-adjusted associations between the percentages of sites with PD ⩾ 6 mm, AL ⩾ 6 mm and BL ⩾ 8 mm and serum IL-6 level were statistically significant. Significant associations were also found between serum IL-6 level and the IL-6^{-174} genotype. In each of the three models, BMI turned out to be a significant determinant of the serum IL-6 level, whereas age and smoking were not. The R^2 varied in the range of 44–51% in the three regression models.

We continued the analyses further, taking possible interaction between the IL-6^{-174} genotype and the extent of periodontal disease (PD ⩾ 6 mm, AL ⩾ 6 mm and BL ⩾ 8 mm in separate models) into consideration. In these analyses, serum IL-6 concentration associated significantly with the interaction term PD ⩾ 6 mm/IL-6^{-174} genotype ($p = 0.033$, $\beta = 0.037$, 95% confidence interval for β 0.003–0.071), AL ⩾ 6 mm/IL-6^{-174} genotype ($p = 0.035$, $\beta = 0.033$. 95% confidence interval for β 0.002–0.064) and BL ⩾ 8 mm/IL-6^{-174} genotype ($p < 0.001$, $\beta = 0.07$, 95% confidence interval for β 0.038–0.103). In these regression models, the extent of advanced periodontal disease (PD ⩾ 6 mm, AL ⩾ 6 mm and BL ⩾ 8 mm) remained a significant independent variable associated with serum IL-6 level, whereas the IL-6 genotype did not.

Discussion

In this study, our aim was to assess the significance of the extent of periodontal disease and IL-6^{-174} gene polymorphism to systemic inflammatory response as defined by serum IL-6 concentration in systemically healthy subjects with moderate to severe periodontal disease. One of the main findings was that in separate multiple regression analyses, the percentages of sites with PD ⩾ 6 mm, AL ⩾ 6 mm and BL ⩾ 8 mm emerged as the most significant independent determinants for serum IL-6 levels (Table 4). In line with earlier studies (Loos et al. 2000, D'Aiuto et al. 2004c, Ide et al. 2004, Ioannidou et al. 2006), we therefore conclude that periodontal disease contributes to the systemic pool of circulating IL-6 in a way that is dependent on the severity of periodontal inflammation.

Table 4. BMI, gender, age and smoking adjusted associations between serum IL-6 level and IL-6^{-174} genotype and the extent of periodontal disease (PD ⩾6 mm, AL ⩾6 mm and BL ⩾8 mm in the separate models)

	β	95% CI for β	p-value	R^2
PD⩾6 mm	0.033	0.017–0.049	<0.001	
IL-6^{-174} genotype*	0.761	−0.099–1.621	0.081	
BMI	0.136	0.038–0.233	0.007	
Gender†	0.799	0.056–1.541	0.036	
Age	0.014	−0.024–0.053	0.449	
Smoking‡	0.238	0.056–1.541	0.491	0.496
AL ⩾6 mm	0.025	0.010–0.041	0.002	
IL-6^{-174} genotype*	0.985	0.102–1.868	0.030	
BMI	0.144	0.041–0.247	0.007	
Gender†	0.622	−0.161–1.404	0.117	
Age	0.002	−0.039–0.043	0.916	
Smoking‡	0.106	−0.630–0.843	0.773	0.439
BL ⩾8 mm	0.042	0.023–0.061	<0.001	
IL-6^{-174} genotype*	0.900	0.078–1.721	0.033	
BMI	0.146	0.050–0.242	0.004	
Gender†	0.856	0.125–1.587	0.023	
Age	0.018	−0.021–0.058	0.353	
Smoking‡	0.047	−0.640–0.735	0.891	0.513

Continuing variables: percentages of sites with PD⩾6 mm, AL⩾6 mm and BL⩾8 mm, BMI (kg/m^2) and age (years).

*IL-6 genotype: GG compared with GC/CC.

†Gender: females compared with males.

‡Smoking: smokers compared with non-smokers.

BMI, body mass index; CI, confidence interval; PD, pocket depth; AL, attachment level; BL, bone level.

Another important finding of this study was that the IL-6^{-174} genotype was significantly associated with serum IL-6 level. Evidently, the IL-6^{-174} genotype is not only an independent determinant of serum IL-6 concentration (Table 4) but also modifies the association between serum IL-6 concentration and the extent of periodontal disease.

One advantage of our study was that subjects with varying degrees of periodontal disease (Fig. 1) were included, making it possible to relate serum IL-6 levels to the extent of periodontal disease varying up to approximately 90% of affected sites per person. In addition, we used three measures of periodontal disease, namely PD and attachment and bone level, and consistently found statistically significant associations between serum IL-6 levels and the extent of periodontal disease. This has not been the case in earlier studies in which serum IL-6 levels have been found to be related more or less occasionally to various periodontal parameters, like sites bleeding on probing and sites probing 4–6 mm (Ide et al. 2004), sites with attachment loss (Mengel et al. 2002) or with individual mean PD (D'Aiuto et al. 2004c, Ioannidou et al. 2006). In accordance with our results, Ioannidou et al. (2006) observed that the percentage of sites with a clinical AL⩾4 mm was a significant independent predictor of elevated serum IL-6 levels within a transplant patient group. They further showed that serum IL-6 levels were positively associated with the levels synthesized locally in the periodontal tissues.

The cumulative size of the dentogingival surface area may amount to up to 20 cm^2 in an untreated periodontitis patient (Hujoel et al. 2001, Loos 2005). In addition to the partly ulcerated epithelial surface, the underlying lamina propria adds to the volume of the inflamed tissue. Using methods of immunohistochemistry, IL-6-containing cells have been detected in lamina propria both adjacent to the pocket epithelium and in the area apart from it (Takahashi et al. 1994, Guillot et al. 1995, Dongari-Bagtzoglou & Ebersole 1998). Leakage of bacteria, bacterial products and locally produced inflammatory mediators and cytokines occurs into the circulation (Page 1998, Loos 2005). The increase in serum IL-6 may be explained by both the leakage of the locally produced IL-6 from the inflamed periodontal area and systemic production in response to the systemically dispersed antigen load.

As regards the IL-6^{-174} gene polymorphism, there is disagreement in the data concerning which genotype (GG, CC or GG/CG) is associated with increased IL-6 plasma concentrations (Endler et al. 2004). In addition to our previous finding of an increased extent of periodontal disease in subjects carrying the GG genotype of IL-6^{-174} (Tervonen et al. 2007), the present results indicate that also the serum concentration of IL-6 is higher in the same subjects. To our knowledge, the study by D'Aiuto et al. (2004c) is the only one in which serum IL-6 concentration has been related to the IL-6^{-174} genotype in periodontitis patients. In contrast to our result, they, however, report higher serum IL-6 concentrations in subjects with the CG/CC genotype when compared with those with the GG genotype. IL-6 is known to be among the cytokines that act on hepatocytes to induce acute-phase reactants including CRP (Baumann & Gauldie 1994). In fact, an important finding in the study by D'Aiuto et al. (2004c) was that also the levels of serum CRP were higher in subjects carrying the CG/CC genotype. Although no CRP data were included here, we conclude in line with the above that the IL-6^{-174} genotype is associated with systemic inflammation in periodontitis. According to Nibali et al. (2007), carriage of the IL-6^{-174} GG genotype favours the colonization of periodontopathogenic bacteria in aggressive periodontitis. In their study, significantly higher detection rates of *A. actinomycetemcomitans* were reported in IL-6^{-174} GG individuals (65.4%) when compared with homozygous CC (42.9%) and heterozygous CG (16.7%) individuals. The detection rates of *A. actinomycetemcomitans* together with *P. gingivalis* ($p = 0.019$, NS) as well as the detection of the two with *T. forsythensis* ($p = 0.042$, NS) were also higher in the homozygous GG individuals when compared with the CC/CG individuals. These findings are also in agreement with the present results.

Gender, age, smoking and body weight are known to contribute to serum IL-6 levels (Haack et al. 1999, Fischer et al. 2007), and therefore corresponding adjustments were made (Table 4). Serum IL-6 levels were higher in subjects with a higher BMI (Table 3), and BMI turned out to be significantly associated with serum IL-6 concentration in the present subjects, while age and smoking were not (Table 4). The mechanisms

behind the elevated IL-6 levels in obese people are not clear, but activation of circulating mononuclear cells to produce IL-6 has been suggested (Ghanim et al. 2004). On the other hand, adipocytes also produce IL-6 (Kershaw & Flier 2004).

The results obtained in the present study are preliminary, the severest limitation of this study being the low number of subjects included. In addition, although all the subjects were generally healthy, we cannot fully exclude the chance that some of them had subclinical medical conditions, which may have increased the concentrations of IL-6 in the serum. However, within the limitations of this study, we conclude that the extent of moderate to severe periodontal disease and the IL-6^{-174} genotype both contribute significantly to serum IL-6 concentration.

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Clinical Relevance

Scientific rationale for the study: Elevated serum concentrations of IL-6, a proinflammatory cytokine, have been reported in subjects with advanced periodontal disease. Our aim was to study the significance of the extent of periodontal disease and the IL-6^{-174} genotype as determinants of serum IL-6 concentration.

Principal findings: The extent of periodontal disease and the IL-6^{-174} genotype contributed significantly to serum IL-6 concentration in subjects with moderate to severe periodontal disease. The IL-6^{-174} genotype modified the association between serum IL-6 level and periodontal disease.

Practical implications: The IL-6^{-174} genotype should be taken into consideration when the systemic inflammatory response to periodontal disease is studied.