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Polymorphisms in the CD14 and IL-6 genes associated with periodontal disease

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Abstract

Aim: To compare the frequencies of cytokine and receptor molecule genotypes in patients with chronic periodontitis with the corresponding frequencies in a reference population and to study the relationship between periodontal disease severity and polymorphisms in the studied genes.

Subjects and methods: CD14, IL-6, TNF-α, IL-10, IL-1α, IL-1β, and TLR-4 polymorphisms of 51 periodontitis patients were studied using polymerase chain reaction. The genotype frequencies in the periodontitis patients and a reference population ($n = 178$) were compared. Probing pocket depth (PD), periodontal attachment level (AL), and alveolar bone level (BL) were related to the genotypes.

Results: No statistically significant differences could be found between the frequencies of the cytokine genotypes in the periodontitis patients and in the reference group. The extent of periodontal disease was higher in subjects with the T-containing genotype of CD14^{-260} and the GG genotype of IL-6^{-174} when compared with the extent in the rest of the group. Subjects carrying the composite genotype of the above two were most severely affected by periodontal disease.

Conclusion: According to the present results, an evident association exists between the carriage of the T-containing genotype of CD14^{-260} and the GG genotype of IL-6^{-174} and the extent periodontal disease.

Key words: CD14; cytokines; IL-6; IL-10; IL-1α; IL-1β; periodontal disease; receptor molecules; TLR-4; TNF-α

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The progression of periodontal diseases, initially originated by bacteria in the dental biofilms, from gingivitis to advanced forms of periodontitis is modified by several factors including environmental, systemic, and local factors (Albandar 2002). Inter-individual variations in periodontal disease experience have also been explained by variations in the genetically determined immunoinflammatory response. One of the virulence factors common to all Gram-negative bacteria is lipopolysaccharide (LPS), and obviously the genetically determined responsiveness of an individual to an LPS challenge might have a role in determining susceptibility to periodontal infection. Accordingly, a hyperresponsive individual secretes increased amounts of inflammatory mediators including destructive cytokines. In progressive periodontal disease, excessive amounts of ''inappropriate'' cytokines are obviously secreted, inducing a loss of periodontal attachment and bone (Gemmel et al. 1997). Among others, Engebretson et al. (2002) demonstrated that a significant correlation exists between the IL-1β levels of gingival crevicular fluid (GCF) and periodontal parameters such as probing pocket depth (PD) and attachment level. Patients with severe periodontitis had higher levels of IL-1β in each probing depth category than patients with less severe disease, the differences being more pronounced in shallow pockets. The authors conclude that the IL-1β expression is in part a host trait and not only a function of clinical parameters. Other cytokines such as IL-10 and IL-11, on the other hand are known to down-regulate IL-1 production indicating that suppression of the immune-inflammatory response occurs in the inflamed tissue (Seymour & Gemmel 2001).

The biological basis for the association of cytokine gene polymorphism and periodontal disease is that carriage of certain alleles of a cytokine gene is related to increased production of a given cytokine. The risk of having

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periodontal disease has been related to carriage of the rare alleles of single cytokines such as IL-1, TNF-α, and IL-6 (Taylor et al. 2004, Loos et al. 2005, Shapira et al. 2005, Takashiba & Naruishi 2006, Yoshie et al. 2007). One of the most extensively studied composite cytokine gene polymorphisms originally identified by Kornman et al. 1997 is a composite genotype where allele 2 of the IL-1A −889 polymorphism was present with allele 2 of the +3953 polymorphism of the IL-1B gene. When compared with the risk of genotype negatives, this periodontitis-associated genotype significantly increased the risk of periodontitis in non-smokers. Laine et al. (2001) took both smoking and the presence of *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans* into account to find out that carriage of the rare alleles of the IL-1 cluster genes was associated with severe adult periodontitis in the absence of the above risk factors. On the other hand, lower susceptibility to periodontal disease has been associated with protective gene polymorphisms (Trevilatto et al. 2003, Holla et al. 2004, Komatsu et al. 2005).

As described above the relationship between gene polymorphism and periodontal disease expression has been studied by comparing the frequencies of genotypes or alleles between subjects with various degrees of periodontal disease or between periodontitis patients and reference subjects. In other studies severity of periodontitis, presence of periodontal pathogens, response to periodontal therapy, loss of teeth or failure of dental implants have been related to genotype (Taylor et al. 2004, Shapira et al. 2005).

We have studied polymorphisms in the genes encoding the following cytokines and inflammatory molecules: CD14, IL-6, TNF-α, IL-10, IL-1α, IL-1β, and TLR-4. The primary purpose of our study was to compare the frequencies of the cytokine genotypes in patients with moderate to severe chronic periodontitis with the corresponding frequencies in reference subjects. A second objective was to find out whether any relationship exists between periodontal disease severity and polymorphisms in the studied genes.

Material and Methods

Subjects

A total of 51 subjects with moderate to severe chronic periodontitis, originally referred to periodontal specialist therapy volunteered to participate. The mean age (± SD) of the subjects was 42.9 (± 9.3) years (range 22–61 years) (Table 1). The patients were in principle periodontally untreated. The group consisted of 34 females and 17 males of Caucasian origin (Table 1). All the subjects were examined by the same periodontal specialist (TR) 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. Data concerning smoking were obtained by interviewing the subjects in association with the clinical examination.

Table 1. Subject characteristics of the periodontitis group

Parameter	
Age	
Mean (± SD)	42.9 ± 9.3
Range	25–61
Gender	
Females	34
Males	17
Smoking habits	
Non-smoker	15
Smoker	36
Periodontal parameters (mean percentage of sites ± SD)	
Subgingival calculus	74.9 ± 24.6
PD ⩾ 4 mm	51.8 ± 23.9
PD ⩾ 6 mm	17.6 ± 21.7
AL ⩾ 4 mm	47.4 ± 28.4
AL ⩾ 6 mm	18.8 ± 24.2
BL ⩾ 6 mm	35.0 ± 29.0
BL ⩾ 8 mm	15.0 ± 20.0

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

The reference group ($n = 178$) representing normal population consisted of university staff and students of Caucasian origin (56 males and 122 females aged 39.4 ± 13.4 years) who were informed of the study and volunteered to give their blood samples to be used as reference samples (Karhukorpi et al. 2002). The genotype frequencies of the two groups were compared, and no data concerning their general health or periodontal status were collected.

Clinical and radiographic parameters

For this report, probing PD was measured using a ball-pointed periodontal probe with 2 mm graduations from the gingival margin to the base of the crevice/pocket at four sites (mesiobuccal, midbuccal, distobuccal, and midlingual) of all teeth excluding third molars. Periodontal attachment level (AL) was measured from the cementoenamel junction (CEJ) to the base of the crevice/pocket at the respective sites. The Björby & Löe (1967) retention index was applied for the registration of calculus, with scores of 2 and 3 corresponding to the presence of subgingival calculus. Alveolar bone level (BL) was measured by one examiner (T. T.) from orthopantomograms at 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. Any site where the CEJ or the alveolar crest could not be identified was excluded as non-measurable. Duplicate measurements of the distance were made at each site, and if the difference between these two measurements was ⩽ 1 mm, the first value was used in the analyses. If the difference was > 1.0 mm, a third measurement was made and the one of the first two measurements that was closer to the third measurement was used.

Cytokine gene polymorphism

Cytokine and receptor gene polymorphisms were tested using the primers and methods taken from the literature and/or developed by our own group: $CD14^{-260}$ earlier known as $CD14^{-159}$ (Hubacek et al. 1999, Karhukorpi et al. 2002), $IL\text{-}6^{-174}$ (Fishman et al. 1998), $TNF\text{-}\alpha^{-308}$ (Ozen et al. 2002), $IL\text{-}10^{-1082}$ (Karhukorpi & Karttunen 2001), $IL\text{-}1\alpha^{-889}$ (McDowell et al. 1995), $IL\text{-}1\beta^{+3954}$ (Luomala et al. 2001), and $TLR4^{+896}$ (Lorenz et al. 2001). Polymerase chain reaction conditions were optimized by comparing various annealing temperatures. The genotypes in each cytokine gene were grouped according to their known biological significance, so that in a proinflammatory cytokine, a genotype/genotypes known to produce higher

levels of the cytokine (positive genotype) were estimated separately from the one(s) known to be associated with lower secretion. Genotypes of CD14^{-260} containing T allele are known to be associated with a higher soluble amount of CD14 (Baldini et al. 1999, Karhukorpi et al. 2002). The GG genotype of IL-6^{-174} is known to be associated with higher IL-6 secretion (Fishman et al. 1998). Genotypes of TNF-α^{-308} containing A allele(s) are known to be associated with higher TNF-α secretion (McGuire et al. 1994). There is some indication that the genotypes of IL-1α^{-889} and IL-1β^{+3954} containing T allele are associated with higher IL-1 secretion (Taylor et al. 2004). A genotype of a regulatory cytokine IL-10 associated with lower secretion (IL-10^{-1082}, AA) was analysed separately (Turner et al. 1997). For the TLR4^{+896} polymorphism, a mutant genotype containing G is associated with a weaker receptor action (Arbour et al. 2000) with a lessened response to LPS.

Data analysis

Comparisons of the distribution of the periodontitis/reference subjects (Table 2), females/males and non-smokers/smokers (Table 4) by gene polymorphism were tested using the Fisher exact test. The severity of periodontal disease was expressed as the extent (percentages) of affected sites using two different threshold values for each variable ($\geqslant$4 and $\geqslant$6 mm for PD, $\geqslant$4 and $\geqslant$6 mm for AL, and $\geqslant$6 and $\geqslant$8 mm for BL). The differences in the mean percentages of sites with PD$\geqslant$4 mm, AL$\geqslant$4 mm, and BL$\geqslant$6 mm in the subjects with positive genotypes and in the rest of the periodontitis group were tested using Student's *t*-test (Table 3). The Student *t*-test was also used to test possible differences in the mean age, the mean number of teeth and the mean percentage of sites with subgingival calculus between genotypes (Table 4). Owing to the skewness of the distributions, the non-parametric Mann–Whitney test was used to compare the frequencies of sites with PD$\geqslant$6 mm, AL$\geqslant$6 mm, and BL$\geqslant$8 mm (Fig. 1). Because of multiple testing adjusted *p*-values were calculated as suggested by Benjamini & Hochberg (1995).

Analysis of variance, adjusted for smoking and gender, was used to assess the relationship between PD$\geqslant$6 mm, AL$\geqslant$6 mm, and BL$\geqslant$8 mm, and single nucleotide polymorphisms (SNPs) of CD 14^{-260} and IL-6^{-174} (Table 5). Interaction between the CD 14^{-260} and IL-6^{-174} polymorphisms, on one hand, and the CD14^{-260} and TLR4^{+896} polymorphisms, on the other hand, was studied using separate models with the two genotypes and their interaction term.

Table 2. Distribution of the genotypes in the adult periodontitis and reference subjects

Genotype	Adult periodontitis subjects *N* (%)	Reference subjects *N* (%)
CD 14^{-260}		
CT/TT	24 (47.1)	101 (56.7)
CC	27 (52.9)	77 (43.3)
IL-6^{-174}		
GG	11 (21.6)	37 (20.8)
CG/CC	40 (78.4)	141 (79.2)
TNF-α^{-308}		
GG	35 (68.6)	137 (77.0)
AG/AA	16 (31.4)	41 (23.0)
IL-10^{-1082}		
AA	19 (37.3)	54 (30.3)
AG/GG	32 (62.7)	124 (69.7)
IL-1α^{-889}		
CT/TT	28 (54.9)	76 (42.7)
CC	23 (45.1)	102 (57.3)
IL-1β^{+3954}		
CT/TT	25 (49.0)	78 (43.8)
CC	26 (51.0)	100 (56.2)
TLR4^{+896}		
AG/GG	13 (25.5)	36 (20.2)
AA	38 (74.5)	142 (79.8)
CD14^{-260} CT/TT +IL-6^{-174}GG	8 (15.7)	23 (12.9)
Any other combination	43 (84.3)	155 (87.1)

In the first column the positive genotype is bolded.

No statistically significant differences in the distribution of subjects by various genotypes of each gene.

Results

The periodontal health status of the present subjects was poor, as indicated by the high mean percentages of sites with PD$\geqslant$4 mm (51.8%) (Table 1). Subgingival calculus was detected on three out of every four surfaces (74.9%). Advanced periodontal disease (PD$\geqslant$6 mm, AL$\geqslant$6 mm, and BL$\geqslant$8 mm) was detected on nearly 20% of the sites. As regards smoking, 15 subjects never smoked and the rest of the subjects ($n = 36$) were current smokers.

SNPs

No statistically significant differences could be found in the frequencies of the cytokine genotypes between the periodontitis and reference subjects when any of the SNPs was considered (Table 2).

When compared with the rest of the periodontitis group, the subjects with the T-containing genotype of CD14^{-260} had significantly more sites with PD$\geqslant$4 mm and BL$\geqslant$6 mm, and those with the GG genotype of the IL-6^{-174} had a higher extent of AL$\geqslant$4 mm and BL$\geqslant$6 mm (Table 3).

When compared with the subjects carrying the CC genotype of CD 14^{-260}, the subjects with the T-containing genotype had a higher extent of advanced periodontal disease, e.g. sites with PD$\geqslant$6 mm and AL$\geqslant$6 mm (Fig. 1). Subjects with the positive genotype of IL-6^{-174} had more sites with AL$\geqslant$6 mm compared with subjects carrying the CC/CG genotype.

Subjects carrying the GG genotype of the TNF-α^{-308} had significantly more sites with PD$\geqslant$4 mm than subjects carrying the A-containing genotype ($p = 0.048$) (Table 3). No statistically significant differences were observed in the severity of periodontal disease between subjects with positive genotypes of IL-10^{-1082}, IL-1α^{-889}, IL-1β^{+3954}, or TLR-4^{+896} and the rest of the periodontitis group, either when the frequencies of sites with PD$\geqslant$4 mm, AL$\geqslant$4 mm, and BL$\geqslant$6 mm (Table 3) or sites with advanced disease (data not shown) were analysed. The above result was the same when smoking was considered.

Subjects carrying various genotypes of the CD14^{-260} and IL-6^{-174} were similar with regard to age, gender, number of teeth, and percentage of sites with subgingival calculus (Table 4). Although there were relatively more smokers among subjects carrying the GG genotype of IL-6^{-174} than among the rest of the periodontitis group (81.8% *versus* 67.5%), the difference was not statistically significant. In the analysis of variance with smoking and gender as co-variates (Table 5), CD14^{-260} genotype turned out to be a significant variable associated with the percentage of sites with PD$\geqslant$6 mm ($p = 0.032$), AL$\geqslant$6 mm ($p = 0.024$), and BL$\geqslant$8 mm ($p = 0.045$). After the same adjustments, IL-6 genotype was significantly associated with the frequency of PD$\geqslant$6 mm ($p = 0.01$).

Combinations of SNPs

The relative proportions of subjects carrying positive genotypes of both

Table 3. Percentages of sites with PD ⩾ 4 mm, AL ⩾ 4 mm and BL ⩾ 6 mm by genotype

Genotype	*N*	PD ⩾ 4 mm (%)	AL ⩾ 4 mm (%)	BL ⩾ 6 mm (%)
CD14^{-260}				
CT/TT	24	58.9 ± 26.6	54.3 ± 31.4	39.7 ± 30.1
CC	27	45.5 ± 19.5	41.2 ± 24.4	22.7 ± 20.6
p-value		0.044*	0.102	0.022*
IL-6^{-174}				
GG	11	62.1 ± 27.4	61.8 ± 30.6	44.2 ± 31.5
CC/CG	40	49.0 ± 22.4	43.4 ± 26.8	27.0 ± 24.3
p-value		0.107	0.056	0.058
TNF-α^{-308}				
AG/AA	16	42.1 ± 21.9	40.1 ± 24.2	23.1 ± 23.9
GG	35	56.2 ± 23.7	50.7 ± 29.8	34.2 ± 27.4
p-value		0.048*	0.218	0.169
IL-10^{-1082}				
AA	19	57.3 ± 23.4	53.0 ± 29.2	27.7 ± 23.6
AG/GG	32	48.5 ± 23.9	44.1 ± 27.9	35.8 ± 31.2
p-value		0.207	0.283	0.300
IL-1α^{-889}				
TT/CT	28	50.7 ± 26.4	44.2 ± 30.7	30.0 ± 30.3
CC	23	53.1 ± 20.9	51.3 ± 25.4	31.5 ± 22.1
p-value		0.730	0.381	0.841
IL-1β^{+3954}				
TT/CT	25	52.7 ± 24.8	44.4 ± 31.7	30.8 ± 30.0
CC	26	50.9 ± 23.4	50.2 ± 25.1	30.6 ± 23.7
p-value		0.787	0.472	0.976
TLR-4^{+896}				
AG/GG	13	49.1 ± 25.1	45.0 ± 30.3	24.3 ± 23.7
AA	38	52.7 ± 23.5	48.2 ± 28.1	32.9 ± 27.6
p-value		0.639	0.727	0.325
CD14^{-260} CT/TT+IL-6^{-174} GG	8	67.9 ± 29.2	70.1 ± 31.2	51.2 ± 33.5
Any other combination	43	48.8 ± 21.8	43.2 ± 26.1	26.9 ± 23.8
p-value		0.036*	0.012*	0.016*
CD14^{-260} CT/TT+TLR4^{+896} AG/GG	7	52.1 ± 32.9	52.2 ± 33.3	30.2 ± 27.1
Any other combination	44	51.7 ± 22.6	46.6 ± 27.9	33.7 ± 25.3
p-value		0.966	0.635	0.582

In the first column the positive genotype is bolded.
Statistical significances were tested using Student's *t*-test.
*Adjusted *p*-values: CD14^{-260}: CT/TT *versus* CC, PD ⩾ 4 mm, $p = 0.196$, BL ⩾ 6 mm, $p = 0.196$; TNF-α^{-308}: AG/AA *versus* GG, PD ⩾ 4 mm, $p = 0.196$; CD14^{-260} CT/TT+IL-6^{-174} GG *versus* any other combination: PD ⩾ 4 mm, $p = 0.196$, AL ⩾ 4 mm, $p = 0.196$, BL ⩾ 6 mm, $p = 0.196$.
AL, attachment level; BL, bone level; PD, pocket depth.

Table 4. Periodontal disease associated factors by significant cytokine and receptor gene polymorphisms

Genotype	*n*	Females/males, *n* (%)	Non-smokers/ smokers, *n* (%)	Mean ± SD		Subgingival calculus, mean percentage of sites ± SD
				age (years)	no. of teeth	
CD-14^{-260}						
CT/TT	24	16/8 (66.7/33.3)	7/17 (29.2/70.8)	42.1 ± 7.9	25.3 ± 3.7	79.8 ± 25.7
CC	27	18/9 (66.7/33.3)	8/19 (29.6/70.4)	43.6 ± 10.5	25.7 ± 3.0	70.6 ± 23.2
IL-6^{-174}						
GG	11	8/3 (72.7/27.3)	2/9 (18.2/81.8)	41.3 ± 6.4	25.4 ± 3.4	81.2 ± 24.5
CC/CG	40	26/14 (65.0/35.0)	13/27 (32.5/67.5)	43.3 ± 10.0	25.5 ± 3.3	73.2 ± 24.7
CD14^{-260} CT/TT+IL-6^{-174} GG	8	6/2 (75.0/25.0)	2/6 (25.0/75.0)	43.3 ± 3.9	24.4 ± 3.6	73.6 ± 23.9
Any other combination	43	28/15 (65.1/34.9)	13/30 (30.2/69.8)	42.8 ± 10.0	25.7 ± 3.3	81.9 ± 29.1
Total	51	34/17 (66.7/33.3)	15/36 (29.4/70.6)	42.9 ± 9.3	25.5 ± 3.3	74.9 ± 24.6

In the first column the positive genotype is bolded.
No statistically significant differences between genotypes in any of the variables.

CD14^{-260} and IL-6^{-174} were similar in the periodontitis and the reference group (Table 2). In the pairwise comparison the eight subjects with this composite genotype had significantly higher percentages of sites with PD ⩾ 4 mm, AL ⩾ 4 mm, and BL ⩾ 6 mm than the rest of the periodontitis group (Table 3). In the same subjects the extent of sites with PD ⩾ 6 mm was approximately three times and the extent of AL ⩾ 6 mm and BL ⩾ 8 mm was more than 2.5 times that observed in the rest of the periodontitis group (Fig 1). Females and males and smokers and non-smokers were evenly distributed between subjects carrying positive genotypes of both CD14^{-260} and IL-6^{-174}

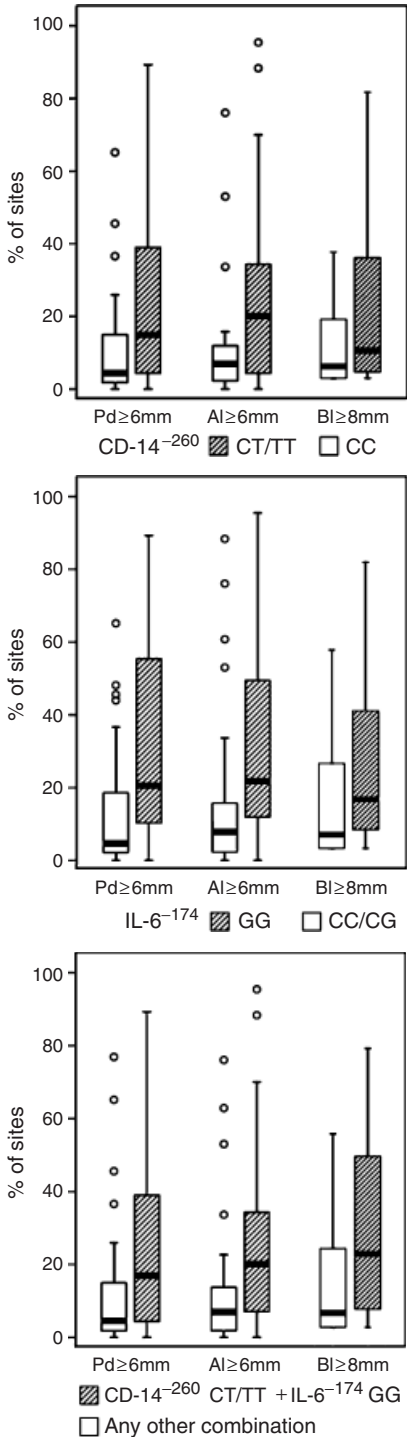

Fig. 1. Box-plots presenting the percentages of sites with PD≥6 mm, AL≥6 mm and BL≥8 mm by genotype. Significances of differences between genotypes using the non-parametric Mann–Whitney test (adjusted *p*-values in parentheses): $CD14^{-260}$: PD ≥6 mm, $p = 0.053$, AL≥6 mm, $p = 0.045$ (0.073), BL≥8 mm, $p = 0.065$. $IL\text{-}6^{-174}$: PD ≥6 mm, 0.06, AL≥6 mm, $p = 0.048$ (0.073), BL≥8 mm, $p = 0.089$. $CD14^{-260}$/$IL\text{-}6^{-174}$: PD≥6 mm, $p = 0.014$ (0.063), AL≥6 mm, $p = 0.009$ (0.063), BL≥8 mm, $p = 0.056$.

and the rest of the periodontitis group and the age, the mean number of teeth as well as the amount of subgingival calculus were similar in the two groups. In a multivariate analysis no interaction between the $CD14^{-260}$ and $IL\text{-}6^{-174}$ polymorphisms could be found (data not shown). The severity of periodontal disease in those seven subjects carrying a positive genotype of both $CD14^{-260}$ and $TLR\text{-}4^{+896}$ was similar to that found in the rest of the periodontitis group (Table 3) and in a multivariate model no interaction could be found between these two polymorphisms either (data not shown).

Discussion

We could not find statistically significant differences between the frequencies of the cytokine genotypes in the chronic periodontitis and reference subjects (Table 2). We chose to compare the genotype frequencies of the periodontitis group with the frequencies in a randomly selected sample of the normal population. By doing this we were able to see whether any accumulation of risk genotypes occurs in the periodontitis group. In addition to genotype other possible risk factors such as smoking and presence of periodontal pathogens have been found to mask the influence of cytokine gene polymorphism on periodontal disease expression (Kornman et al. 1997, Laine et al. 2001). We took smoking into consideration, but a lack of microbial data are a shortcoming of this study. In the absence of these two risk factors, namely smoking and colonization by *A. actinomycetemcomitans* and *P. gingivalis*, Laine et al. (2001, 2005) demonstrated that carriage of allele 2 in IL-1A, IL-1B, and IL-1RN on one hand, and the CD14 -260T/T genotype on the other hand, contributed to the susceptibility to severe periodontitis.

The main finding of this study was that inside the periodontitis group, carriage of the T-containing genotype of $CD14^{-260}$ and the GG genotype of $IL\text{-}6^{-174}$ were separately associated with advanced periodontal disease (Table 5, Fig. 1). In previous studies increased frequencies of the -159 TT homozygotes have been found among subjects with severe periodontitis when compared with a subgroup with moderate periodontitis (Holla et al. 2002) or periodontally healthy controls (Laine et al. 2005). In addition, when compared with the CC or CT genotypes, the homozygotes of the allele 159 T have been observed to express a higher density of the membrane-bound mCD14 receptors on monocytes (Hubacek et al. 1999, Unkelbach et al. 1999, Le Van et al. 2001, Yamazaki et al. 2003), to have higher levels of soluble CD14 in the serum (Baldini et al. 1999) and to produce higher amounts of TNF-α after induction by *P.* gingivalis LPS (Yamazaki et al. 2003). It can therefore be speculated that carriage of the T allele is associated with higher activation of the immune response to the LPS/LBP complex, and thus to increased progression of periodontal disease. Also in agreement with our results an association has been found between the GG genotype of the IL-6 gene and susceptibility to chronic periodontitis in a Caucasian population in Brazil (Trevilatto et al. 2003) and patients with juvenile rheumatoid arthritis carrying the GG genotype have been observed to have twice as high IL-6 serum levels as patients with the CC genotype (Fishman et al. 1998). Contradictory to the previous findings high serum levels of IL-6 in periodontitis patients have been associated with carriage of the C allele (D'Aiuto et al. 2004).

We further found out that the highest extent of periodontitis was observed in subjects carrying a positive genotype of both $CD14^{-260}$ and $IL\text{-}6^{-174}$ (Table 3, Fig. 1). As no significant interaction could be found between the $CD14^{-260}$ and $IL\text{-}6^{-174}$ polymorphisms in the multivariate model, we conclude that the severe periodontal disease in these subjects reflects the load of the two separate SNPs. As CD14 and TLR4 together recognize the LPS-LPB complex, it was reasonable to study the possible interaction of these two polymorphisms, as well. No such interaction could, however, be found. Owing to the low numbers of subjects carrying various composite genotypes, the above findings should, however, be verified in larger samples.

The role of the $TNF\text{-}\alpha^{-308}$ polymorphism in periodontal disease is somewhat obscure (Galbraith et al. 1998, 1999, Graandijk et al. 2002, Folwaczny et al. 2004, Donati et al. 2005), and recently other polymorphisms of TNF-α have been studied (Loos et al. 2005, Shapira et al. 2005, Yoshie et al. 2007). In line with the result of Galbraith et al. (1999), we found a

Table 5. Parameter estimates of the analyses of variance using percentages of sites with PD ⩾ 6 mm, AL ⩾ 6 mm and BL ⩾ 8 mm as dependent variable

Genotype	PD ⩾ 6 mm (percentage sites)			AL ⩾ 6 mm (percentage sites)			BL ⩾ 8 mm (percentage sites)		
	β	95% CI	p	β	95% CI	p	β	95% CI	p
CD-14 **CT+TT**	13.1	1.2–25.1	0.032	15.1	2.1–28.1	0.024	10.6	0.2–20.9	0.045
CD-14 CC	0			0			0		
IL-6 **GG**	19.3	4.9–33.6	0.010	15.3	0.9–31.6	0.064	11.1	− 1.7–23.9	0.088
IL-6 CC+CG	0			0			0		

In the first column the positive genotype is bolded.
Adjusted by gender and smoking.
AL, attachment level; BL, bone level; PD, pocket depth.

significantly higher extent of PD ⩾ 4 mm in subjects carrying the GG genotype than in those carrying the AA/AG genotype (Table 3). Associations between SNPs in IL-10^{-1082}, IL-$1\alpha^{-889}$, IL-$1\beta^{+3954}$ and TLR-4^{+896} and periodontal disease reported elsewhere (Taylor et al. 2004, Kinane et al. 2005, Agerbaek et al. 2006, Yoshie et al. 2007) could not be confirmed in this study (Tables 2 and 3).

In the pairwise comparisons (Table 3, Fig. 1) both periodontal disease associated confounding factors and, in this special case, multiple testing should be considered. Because of multiple testing unadjusted *p*-values should be interpreted with caution and also the adjusted *p*-values are presented in Table 3 and Fig. 1. The gender, the age of the subjects, the number of remaining teeth or the amount of subgingival calculus were not confounding factors in this study (Table 4). The fact that subjects with a positive genotype of CD14^{-260} or IL-6^{-174} and their combination generally had more sites with periodontal disease (Table 3), but a similar amount of local aetiology (Table 4) when compared with the rest of the subjects, supports the hypothesis that a positive genotype predisposes to periodontitis. Smoking was common among the present subjects, as indicated by 71% of the subjects smoking currently. There were, however, no statistically significant differences in the distribution of smokers/non-smokers between various genotypes (Table 4) and in the analysis of variance, adjusted for smoking and gender, an association was found between the CD14^{-260} and IL-6^{-174} polymorphisms and the severity of periodontal disease (Table 5). It is possible that smoking and a positive genotype act in synergy, as suggested by Meisel et al. (2002).

In the current study we related the severity of periodontal disease rather than the absence/presence of chronic periodontitis to various genotypes. Our principal finding was that the CD14^{-260} and IL-6^{-174} polymorphisms associated with the severity of periodontal disease after controlling for significant confounding factors. Secondly, subjects carrying both the T-containing genotype of the CD14^{-260} and the GG genotype of the IL-6^{-174} were most severely affected by periodontal disease. Verification of the above results is, however, needed.

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Clinical Relevance

Scientific rationale for the study: It has been suggested that polymorphisms in the genes encoding proinflammatory cytokines contribute to the variation in the susceptibility to periodontal diseases. We studied a number of cytokine and receptor molecule gene polymorphisms in periodontitis patients and in a reference population.

Principal findings: The genotype distributions were similar in the periodontitis and the reference subjects. Inside the periodontitis group carriage of the T-containing genotype of the CD14^{-260} and the GG genotype of the IL-6^{-174} were associated with an increased extent of periodontal disease.

Practical implications: Information concerning gene polymorphism as a susceptibility factor of periodontal disease is useful in assessing disease progression and in treatment planning.