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The CD14 − 159C-to-T promoter polymorphism in periodontal disease

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Abstract

Background: A single-nucleotide promoter polymorphism in the CD14 gene was associated with various inflammatory conditions. The present study sought to determine the frequency of the CD14 − 159C-to-T polymorphism among subjects with periodontitis and healthy control individuals.

Methods: A total of 70 patients with periodontal disease and 75 healthy controls were genotyped for the CD14 − 159C-to-T polymorphism. Genotyping was performed by polymerase chain reaction and restriction fragment length polymorphism analysis. The allele frequencies and distribution of genotypes within both study groups were compared using Fisher's exact test at a level of significance of 5% ($p < 0.05$).

Results: Overall, the frequency for the CD14 − 159T allele in patients with periodontitis was 39.3% (55/140) and 48.0% (72/150) for the controls ($p = 0.135$). The CD14 − 159C allele was significantly more prevalent ($p = 0.013$) among females with periodontitis (33.3%; 24/72) as compared with healthy control subjects (55.6%; 30/54). In contrast, the distribution of the CD14 − 159C-to-T polymorphism showed no significant difference among males with and without periodontitis ($p = 0.816$).

Conclusion: Herein, the C − 159T promoter polymorphism of the CD14 gene was associated in female but not in male patients with periodontal disease.

Key words: innate immunity; lipopolysaccharide bacteria; PAMPs

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The CD14 pattern recognition receptor has a unique ability to bind lipoglycans, i.e. lipopolysaccharides (LPSs) that are expressed by Gram-negative bacteria (Wright et al. 1990). According to a recent biochemical model, CD14 interacts exclusively with bacterial LPS that is bound with high affinity to the systemically circulating LPS-binding protein. The signal transduction of the LPS/LBP/CD14 ternary complex on effectors cells is then transferred via the toll-like receptor (TLR)4/MD-2 (Da Silva Correia et al. 2001). Upon stimulation, the TLR4/MD-2 complex leads to the activation of innate host defense mechanisms via the nuclear factor κ B pathway and the release of proinflammatory cytokines, i.e. tumor necrosis factor α, interleukin-1β (IL-β), IL-6, and interferon γ (Aderem & Ulevitch 2000, Antal-Szalmas 2000).

Although CD14 exists as a single-copy gene, the CD14 protein is expressed in two phenotypic forms. The membrane-bound CD14 receptor protein (mCD14) is glycosylphosphatidylinositol anchored in the cell surface of monocytes/macrophages and neutrophils (Goyert et al. 1988). A soluble form of the CD14 receptor (sCD14) lacks the glycosylphosphatidylinositol anchor and circulates in high amounts in the serum (Bazil et al. 1986).

Recently, a single-nucleotide polymorphism in the proximal promoter region of the CD14 gene has been identified (Baldini et al. 1999). This polymorphism comprises a C-to-T substitution at position − 159 of the 5′-flanking region of the CD14 gene. An identical substitution has also been described as − 260C-to-T polymorphism by other authors because of differences in the translation start (Hubacek et al. 1999). Homozygotes for the CD14 − 159T allele (thymidine in position − 159) display significantly higher levels of circulating sCD14 proteins and a higher density of the membrane-bound mCD14 receptors on monocytes (Hubacek et al. 1999, Unkelbach et al. 1999).

Although periodontitis has been primarily defined as a chronic bacterial infection of the marginal periodontal tissue, a genetic predisposition to this entity has been suggested recently (Hart & Kornman 1997). Genetic factors were estimated to be of paramount importance in 50% of cases (Michalowicz et al. 1991). To elucidate the genetic background of the periodontal pathogenesis, members of the monocyte/macrophage

system along with the proinflammatory cytokines that are expressed upon stimulation by LPSs are excellent candidates (Graves et al. 2000). Since the − 159C-to-T promoter polymorphism has a considerable impact on the phenotypic expression of the CD14 receptor and thus on the functional activity of monocytes, it appears reasonable to assume that the CD14 promoter genotype is associated with the individual susceptibility for periodontal disease.

In order to assess whether this polymorphism contributes to the predisposition to periodontal disease, this study compared (i) the allele frequencies and (ii) the distribution of genotypes among patients with periodontal disease and healthy controls.

Material and Methods

Patient population

All participants provided written informed consent prior to their enrolment into the study. The study conformed to the ethical guidelines of the Helsinki Declaration and was approved by the local ethics committee (no. 290/01). Severe medical disorders including diabetes mellitus, immunological disorders, and increased risk for bacterial endocarditis, and also pregnant females were regarded as exclusion criteria.

Periodontitis group

Seventy patients from the Department of Periodontology, Ludwig-Maximilians University (Munich, Germany) were enrolled. The median age in the periodontitis group was 56 years (SD ± 8.98) and the age ranged from 35 to 74 years. The male-to-female ratio was 48.6% to 51.4%. All patients were adult Caucasians and presented with the diagnosis of periodontitis. The diagnosis was established using a standardized periodontal evaluation protocol including the evaluation of (1) the probing pocket depth measured at six locations on each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual) using a Michigan-type ''O'' probe, (2) the furcation involvement using a Naber-type probe, (3) the bleeding on probing registered as present or absent, and (4) bone loss as assessed by orthopantomographs. The probing pocket depth was determined from the free gingival margin to the base of the periodontal pocket keeping the probe in line with the long axis of the tooth. The furcation defects were examined by horizontal probing from the furcation entrance to the base of the defect. The furcation involvement was classified according to the protocol of Nyman & Lindhe (1997).

The patients fulfilled the following clinical criteria: (1) a total of at least 15 teeth in situ, (2) ⩾8 teeth with a probing pocket depth of ⩾5 mm at least at one location and/or a furcation involvement ⩾class II, and (3) evidence of bone loss manifested as the distance between the alveolar crest and the cemento-enamel junction of ⩾3 mm around the affected teeth.

According to the disease severity, patients with periodontitis were assigned to one of three groups using the following criteria: (1) mild group (maximum probing depth: 6 mm; attachment loss >30%: ⩽5 teeth; no attachment loss >50%); (2) moderate group (maximum probing depth: 8 mm; attachment loss >30%: ⩽8 teeth; attachment loss >50: ⩽5 teeth); and (3) severe group (maximum probing depth: >8 mm; attachment loss >30%: >8 teeth; attachment loss >50%: >5 teeth) (Table 1).

Control group

The healthy control group comprised a total of 75 unrelated, ethnically matched, Caucasian individuals without periodontitis. The absence of periodontal disease was documented according to the following criteria: (1) a minimum of 22 teeth in situ, (2) ⩽1 site with probing pocket depth ⩾3 mm, and (3) lack of any kind of furcation involvement at any tooth. None of the control subjects had a history of periodontitis or tooth loss because of pathogenic tooth mobility. Within the control group, the median age was 45.0 years (SD ± 9.46) and the age ranged from 35 to 73 years.

Blood samples and DNA isolation

Peripheral venous blood samples of 9 ml were drawn from each individual by standard venepuncture. Each blood sample was collected in sterile tubes containing K_3EDTA solution. DNA was isolated using partly the QIAamp® DNA Blood Midi Kit (Qiagen, Hilden, Germany), and partly the salting out procedure (Miller et al. 1998).

Genotyping of the CD14 − 159C-to-T promoter polymorphism

For genotyping of the − 159C-to-T polymorphism, an assay on the basis of polymerase chain reaction (PCR) and subsequent restriction fragment length polymorphism (RFLP) analysis was used. The total volume of the PCR was 50 μl containing 100 ng of DNA, 1 × PCR buffer (Qiagen), 0.2 mM of each dNTP (Sigma, Steinheim, Germany), 0.75 U of HotStarTaq™ DNA polymerase (Qiagen), and 7.5 pmol of each primer (TIB MOLBIOL, Berlin, Germany). The final concentration of $MgCl_2$ was 3 mM. The PCR was performed in a thermocycler UNO-Thermoblock (Biometra, Göttingen, Germany) with an initial denaturation step (95°C for 15 min), 35 cycles (94°C for 30 s,

Table 1. Characteristics of the study population and criteria as used for the classification of disease severity among periodontitis patients

	Study population	
	periodontitis	control
total number of patients	70	75
females	36 (51.4%)	26 (34.7%)
males	34 (48.6%)	48 (65.3%)
median age (± SD)	56 (± 8.98)	45 (± 9.46)
age range	35–74	35–73

	Classification of periodontal disease		
	periodontitis (mild)	periodontitis (moderate)	periodontitis (severe)
maximum pocket depth (mm)	6	8	>8
attachment loss >30%	⩽5 teeth	⩽8 teeth	⩾5 teeth
attachment loss >50%	–	⩽5 teeth	⩾8 teeth
number of patients	24	29	17

55°C for 30 s, 72°C for 30 s), and a final extension step (72°C for 10 min). A new primer pair was selected, the sequences were 5′-GCCTGAGTCATCAGGACA CTG-3′ (sense) and 5′-CTCTTCGGCT GCCTCTGACAG-3′ (antisense). The polymorphism was located within a recognition site for the restriction enzyme *Ava*II. The restriction assay in a total volume of 25 μl contained 1 × NEBuffer 4, 40 U of the enzyme *Ava*II (New England Biolabs, Beverly, MD, USA) and 18 μl of the PCR product. It was incubated overnight at 37°C and analyzed by agarose gel electrophoresis on a 2.5% gel. The length of the PCR product was 223 bp. In the case of an individual homozygous for the more common C allele, which is not digested by *Ava*II, only the full-length PCR product of 223 bp was present. In the case of an individual homozygous for the less common T allele, the PCR product was digested completely resulting in two fragments of 155 and 73 bp length. A heterozygous individual displayed all three fragments of 223, 155, and 73 bp length. Additionally, the results of restriction analysis had been confirmed by sequencing three individuals genotyped as CC, CT, and TT. These three samples served as controls for each restriction assay. For sequencing, the same primer pair as for genotyping was used. These three samples served as controls for each restriction assay.

Statistical analysis

To compare the distribution of the different genotypes of the CD14 −159C-to-T promoter polymorphism among periodontitis patients and healthy controls, the Pearson χ^2 test was used. The differences in the allele frequencies among both study groups were analyzed using Fisher's exact test. All statistical procedures were performed at a level of significance of 5% ($p < 0.05$).

Results

Allele frequencies

Overall, the CD14 −159T allele was prevalent in 39.3% (55/140) of periodontitis patients and in 48.0% (72/150) of the healthy control subjects. The overall allele frequencies were not significantly different between both study populations ($p = 0.135$) (Table 2). The prevalence of the CD14 −159T allele among females was 33.3% (24/72) for periodontitis patients and 55.6% (30/54) for control individuals ($p = 0.013$). Among male subjects, the prevalence of the CD14 −159T allele was 45.6% (31/68) as compared with 43.7% (42/96) for healthy controls ($p = 0.816$). Stratification analysis revealed no association between the allele frequencies and the severity of the disease (Table 3).

Distribution of genotypes

The distribution of genotypes in the periodontitis and the control group is depicted in Table 4. Homozygosity for the CD14 −159T allele (T/T) was observed in 12.8% of the individuals with periodontitis and 20.0% of the control subjects. Heterozygosity (C/T) was found in 52.9% of periodontitis patients and in 56.0% of healthy controls. Homozygosity for the CD14 −159C allele (C/C) was shown in 34.3% of periodontitis patients and in 24.0% of the control individuals. The distribution of genotypes of the CD14 −159C-to-T promoter polymorphism was not significantly different among both study groups ($p = 0.286$).

Discussion

Cells of the monocyte/macrophage lineage play a pivotal role in the manifestation of periodontitis-associated tissue

Table 2. Prevalence of the CD14 −159T allele and CD14 −159T allele among individuals with periodontitis and the healthy controls stratified according to the gender of subjects

Total	Periodontitis (%)	Control (%)	*p*-value (periodontitis versus control)	Odds ratio	Confidence interval
Frequency of alleles					
CD14 −159C	85 (60.7)	78 (52.0)			
CD15 −159T	55 (39.3)	72 (48.0)			
total	140 (100)	150 (100)	0.135	1.49	0.75–1.88
Female					
CD14 −159C	48 (66.7)	24 (44.4)			
CD15 −159T	24 (33.3)	30 (55.6)			
total	72 (100)	54 (100)	0.013	2.50	0.72–3.08
Male					
CD14 −159C	37 (54.4)	54 (56.3)			
CD15 −159T	31 (45.6)	42 (43.7)			
total	68 (100)	96 (100)	0.816	0.56	0.42–1.45

Table 3. Prevalence of the CD14 −159T allele and CD14 −159T allele among individuals with periodontitis stratified according to the disease severity and the healthy controls

	CD14 −159C	CD14 −159T	*p*-value (versus control)	Odds ratio	Confidence interval
periodontitis (total)	85 (60.7%)	55 (39.3%)	0.135	1.49	0.75–1.88
periodontitis (mild)	30 (62.5%)	18 (37.5%)	0.204	1.54	0.62–2.36
periodontitis (moderate)	35 (60.3%)	23 (39.7%)	0.279	1.40	0.63–2.13
periodontitis (severe)	20 (58.8%)	14 (41.2%)	0.472	1.32	0.53–2.39
control	78 (52.0%)	72 (48.0%)	–	–	–

Table 4. Frequencies of the different genotypes of the CD14 −159C-to-T promoter polymorphism as observed in the periodontitis and the healthy control group. T, CD14 −159T allele; C, CD14 −159C allele.

	CC	CT	TT	*p*-value (versus control)
control	18 (24.0%)	42 (56.0%)	15 (20.0%)	–
periodontitis (total)	24 (34.3%)	37 (52.9%)	9 (12.8%)	0.286
Gender				
female	15 (41.7%)	18 (50.0%)	3 (8.3%)	0.093
male	9 (26.5%)	19 (55.9%)	6 (17.6%)	0.939
Disease severity				
periodontitis (mild)	7 (29.2%)	16 (66.7%)	1 (4.1%)	0.186
periodontitis (moderate)	10 (34.5%)	15 (51.7%)	4 (13.8%)	0.506
periodontitis (severe)	7 (41.2%)	6 (35.3%)	4 (23.5%)	0.256

destruction (Page 1999). According to current pathogenetic models, the activity of monocytes/macrophages is predominantly controlled by the TLR4-MD2-CD14 receptor complex (Aderem & Ulevitch 2000).

Previous data have shown that the systemic level of the sCD14 receptor is significantly increased in patients with periodontal disease (Hayashi et al. 1999). In rodents, it was demonstrated that LPSs from the highly virulent bacteria *Porphyromonas gingivalis* stimulate bone resorption specifically through CD14 receptor signaling (Chiang et al. 2003). On the contrary, it was previously shown that the CD14 expression within the periodontal tissue is negatively correlated with the amount of attachment loss (Jin & Darveau 2001). Significantly lower levels of the sCD14 protein were observed at sites with advanced attachment loss, indicating a protective effect for CD14. Moreover, a reduced expression of CD14 on monocytes was suggested to be linked with an increasing susceptibility for early-onset periodontitis (Buduneli et al. 2001).

Altogether, these observations emphasize the strong relevance of the CD14 receptor activity in the clinical manifestation and development of periodontitis.

Recently, a single-nucleotide promoter polymorphism in the CD14 gene has been identified (Hubacek et al. 1999, Klein et al. 2002). The substitution of cytidine to thymidine at position − 159 leads to significant changes in the transcriptional activity of the CD14 gene for both the soluble and the membrane-bound receptor type. The CD14 − 159T allele was suggested to enhance the expression of the receptor protein (LeVan et al. 2001). It appears reasonable to assume that the respective genotype of the CD14 promoter polymorphism might influence the host response against inflammatory stimuli, i.e. LPSs.

Associations between the CD14 − 159C-to-T promoter polymorphism and several systemic diseases have already been previously reported. For example the TT genotype was positively correlated to the risk for Crohn's disease (Klein et al. 2002). Based on data from different populations, the CD14 − 159C-to-T promoter polymorphism seems to influence the risk for myocardial infarction and for microangiopathic stroke (Hubacek et al. 1999, Unkelbach et al. 1999, Shimada et al. 2000, Lichy et al. 2002). On the other hand, the CC genotype appears to be associated with atopy (Koppelman et al. 2001).

The frequency of the alleles and genotypes for the CD14 gene promoter polymorphism observed herein is consistent with previous data from other study groups (Haider et al. 2002). The most important result of the present study comprises the observation of a significant association between the CD14 gene promoter polymorphism and the susceptibility for periodontal disease among female subjects.

The positive correlation between periodontal disease and the CD14 − 159C allele frequency in females might be explained by the influence of estrogens. A previous study on rats found a 1.5-fold stronger expression of the CD14 receptor on Kupffer cells in females, which was suggested to be induced by the higher systemic estriol levels (Kono et al. 2000). Because of a higher overall expression of the CD14 receptor, the clinical relevance of the functional effective CD14 − 159C-to-T polymorphism might, therefore, be higher in females as compared with males.

Commonly, females have been considered to have a lower risk for periodontal disease than males (Albandar 2002). Apart from differences in oral hygiene measures between the two gender groups, it was assumed that other physiological factors contribute to the lower risk for periodontal disease in females than in males. The enhanced expression of the CD14 receptor probably comprises one of these factors leading to a reduced susceptibility of women for periodontitis. An estrogen-induced increasing density of CD14 receptors might provide a kind of protection against the manifestation of periodontal disease. At present, the pathophysiological role of CD14 is not fully understood. Apart from its role as a cell-activating receptor, CD14 might also play an important role in controlling bacterial infection by various mechanisms (Buduneli et al. 2001). For example CD14 can serve as an opsonin and thereby promote bacterial phagocytosis (Schiff et al. 1997). It was shown that the blockade of the CD14 receptor significantly increases intestinal tissue destruction as induced by Shigella infection (Wenneras et al. 2000). The CD14 − 159C allele leads to a reduced expression of the receptor in monocytes (LeVan et al. 2001). Hence, on the basis of the present findings, it can be assumed that females carrying this particular allele are exposed to a higher risk for periodontal disease since they are less effectively protected by the estrogen-associated increased CD14 receptor expression.

However, besides the relatively small study population, the selection criteria of the study groups might comprise a certain limitation of the present study. Specifically, in the control group, there have been included individuals showing no clinical or radiographic signs of periodontitis with a minimum number of 22 teeth. Although none of the subjects had any known history or symptoms, i.e. pathogenic tooth mobility, of periodontal disease, some of these individuals might nevertheless have lost some of their missing teeth because of periodontitis. Moreover, some of the individuals who have been actually classified as healthy might develop signs of periodontal disease in the future. Hence, the current findings should be confirmed using larger study populations in order to gain more reliable information on the association between periodontal disease in females and the CD14 − 159C-to-T polymorphism.

Within the limitations of this study, the present findings indicate that the susceptibility to periodontitis among females is significantly influenced by the CD14 − 159C-to-T promoter polymorphism but not in males. Stratification revealed no association between the polymorphism and the severity of periodontal disease.

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