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Polymorphisms of the interleukin-18 gene in periodontitis patients

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Abstract

Background: Interleukin (IL)-18 regulates the expression of the proinflammatory cytokine interferon (IFN)-γ. The present study sought to test the putative involvement of six different IL-18 gene polymorphisms in pre-disposition to destructive periodontal disease.

Methods: A total of 123 patients with periodontitis and 121 healthy controls were genotyped for six IL-18 polymorphisms at position -656, -607, -137, $+113$, $+127$ and codon 35/3. Genotyping has been performed by PCR and restriction fragment length polymorphism analysis. The frequencies of alleles and genotypes as well of haplotypes within both study groups were compared using the Pearson Chi-square test at a level of significance of 5% ($p<0.05$).

Results: Coseggregation was found to be 100% for the two polymorphisms at position -656 and -607 as well as for the polymorphisms at position -137, $+113$, and $+127$. The distribution of genotypes for the IL-18 gene polymorphism at position $-656/-607$ ($p=0.854$), at position $-137/+113/+127$ ($p=0.320$), and at codon 35/3 ($p=0.481$) was not significantly different among periodontitis patients if compared with healthy control subjects. The distribution of haplotype combinations for the -607 and -137 polymorphism also showed not significant difference between the both study groups ($p=0.545$).

Conclusion: Herein the six different IL-18 gene polymorphisms were not associated with destructive periodontal disease.

Key words: destruction; immunity; inflammatory; innate; interferon-γ (IFN-γ); tissue

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Interleukin (IL)-18 is a pleiotropic cytokine that is involved in the regulation of both the innate and the acquired immune response (Dinarello & Fantuzzi 2003). The most prominent biologic property of IL-18 is its ability to induce the production of interferon (IFN)-γ in the presence of IL-12 (Okamura et al. 1995). Consequently, IL-18 was originally designated as *IFN-γ inducing factor*. Moreover, it stimulates the expression of tumor necrosis factor (TNF)-α and IL-1, enhances the differentiation of T cells to the Th1 (proinflammatory) phenotype and impairs the synthesis of the anti-inflammatory cytokine IL-10 (Puren et al. 1998, Dinarello 1999, Nakahira et al. 2002). The IL-18 molecule shares structural homology with the IL-1 family and among others is produced by macrophages, monocytes, and osteoblasts (Di Marzio et al. 1994, Gerdes et al. 2002). Similar to its analogue IL-1β, IL-18 requires the processing of the 24-kDa precursor by the IL-1β-converting enzyme, caspase-1, to the 18-kDa mature form to reach biologic activity (Ghayur et al. 1997). IL-18 is recognized by binding to a heterodimeric receptor complex that is composed of the IL-1 receptor-related protein, termed IL-18Rα, and the IL-1 receptor accessory protein-like chain, termed IL-18Rβ (Dinarello & Fantuzzi 2003). The activity of IL-18 begins with the low-affinity binding to the IL-18Rα chain. Afterwards a high affinity tricomplex is formed with the IL-18Rβ chain that mediates the intra-cellular signal transduction (Gerdes et al. 2002).

Recently six different polymorphisms within the IL-18 gene have been identified. Three single nucleotide polymorphisms (SNPs) were found in the promoter region (-656 G/T; -607 C/A, -137 G/C) and two of the polymorphisms were observed in the 5′-untranslated region ($+113$ T/G, $+127$ C/T) (Giedraitis et al. 2001). Moreover, one polymorphism was found in the coding region of the IL-18 gene at position 105 (Higa et al. 2003). Although the functional significance of the aforementioned polymorphisms is yet not fully

established it was suggested that particularly the polymorphisms at position −607 and −137 are associated with considerable changes in IL-18 expression (Giedraitis et al. 2001, Sugiura et al. 2002).

Periodontitis is an infectious disease induced by the colonization of the dentogingival interface with pathogenic bacteria. Upon stimulation by pathogen associated molecular patterns (PAMPs), i.e. lipopolysaccharides or peptidoglycans, an inflammatory reaction is induced within the periodontal tissue (Page 1999). The local inflammatory response is inevitably bound to the destruction of alveolar bone and connective tissue (Baker et al. 1999, Graves et al. 2000). The inflammatory process is maintained and amplified by the in situ production of pro-inflammatory cytokines, including IFN-γ, TNF-α and IL-1β (Van Dyke et al. 1993). For the delineation of the genetic background of periodontitis, functional effective polymorphisms in genes coding for cytokines that are involved in the regulation of the inflammatory process are attractive candidates.

The present study aimed to assess whether polymorphisms of the IL-18 gene contribute to the pre-disposition to periodontitis and compared (i) the allele frequencies and (ii) the distribution of genotypes among patients with periodontitis and healthy controls.

Materials and Methods

Patient population

Individuals with severe medical disorders including diabetes mellitus, autoimmune diseases (e.g. rheumatoid arthritis), immunological disorders, increased risk for bacterial endocarditis and pregnant females were excluded from the study. The study conformed to the ethical guidelines of the Helsinki Declaration and was approved by the local ethics committee (No. 290/01). All participants provided written informed consent prior to their enrolment into the study.

Periodontitis group

A total of 123 patients from the Department of Periodontology, Ludwig-Maximilians University (Munich, Germany) were enrolled. The median age in the periodontitis group was 52.9 years (SD ± 12.3) and the age ranged from 25 to 74 years. The male to female ratio was 54–46%. All individuals in the periodontitis group were adult Caucasians. The diagnosis of periodontitis was made on basis of a standardized periodontal examination 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 defined as the distance from the free gingival margin to the base of the periodontal pocket keeping the probe in line with the long axis of the tooth. For the examination of furcation defects horizontal probing from the furcation entrance to the base of the defect was used. 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, (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. Subjects providing clinical, radiographic, and/or microbiologic symptoms of aggressive periodontitis according to the classification of Tonetti & Mombelli (1999) were excluded from the study.

Control group

A total of 121 unrelated, ethnically matched, Caucasian individuals without periodontitis comprised the healthy control group. The absence of periodontitis was determined according to the following criteria: (1) a minimum of 22 teeth in situ, (2)⩽1 site with probing pocket depth ⩾3 mm, (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 40.0 years (SD ± 13.3) and the age ranged from 18 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), partly the salting out procedure (Miller et al. 1998).

Genotyping of the polymorphisms within the IL-18 gene

The polymorphisms at positions −656, −607, −137, +113, +127 and the third position of codon 35 (+105) were genotyped using PCR and restriction fragment length polymorphism (RFLP) analysis. The total volume of the PCR was 20 µl containing 100 ng of genomic DNA, 1 × PCR-buffer (Qiagen, Hilden, Germany), 0.5 mM of a dNTP-Mix (Sigma, Steinheim, Germany), 0.5 units of HotStarTaq™ DNA polymerase (Qiagen) and 5 pmol of each primer (TIB MOLBIOL, Berlin, Germany). The final $MgCl_2$ concentrations are given in Table 2. The PCR comprised an initial denaturation step (95°C for 15 min.), 35 cycles (94°C for 30 s, primer annealing temperature as given in Table 2 for 30 s, 72°C for 30 s (90 s for sequencing of the promotor region) and a final extension step (72°C for 10 min.). The volume of the restriction assays was 25 µl containing the appropriate 1 × NEBuffer and restriction enzyme (New England Biolabs, Beverly, MD, USA) and 20 µl of the PCR product. It was incubated overnight at 37°C or 60°C (*Mwo* I) and analyzed by electrophoresis on a 2.5% agarose gel. Additionally, the results of restriction analysis were confirmed by sequencing individuals displaying all possible genotypes. All Primer sequences, primer annealing temperatures, final $MgCl_2$ concentrations and restriction enzymes used are given in Table 1.

Statistical analysis

The differences in the distribution of the genotypes of the different IL-18 gene polymorphisms among periodontitis patients and healthy controls were analysed with the Pearson Chi-squared test. All statistical procedures were performed at a level of significance of 5% ($p < 0.05$).

Results

Allele frequencies and genotype distribution

The allele frequencies and genotype distributions for the six different IL-18 gene polymorphisms among both study

Table 1. Primer sequences, PCR conditions, final $MgCl_2$ concentrations, restriction enzymes and fragment sizes in RFLP analyses of IL-18 promotor and codon 35 polymorphisms

Polymorphism	Primer sequences	Primer annealing (°C)	$MgCl_2$ concentration (mM)	Restriction enzyme	Fragment sizes (bp)
IL-18 – 656 G → T	AGGTCAGTCTTTGCTATCATTCCAGG CTGCAACAGAAAGTAAGCTTGCGGAGAGG	60	1.5	*Mwo* I	G: 96+24 T: 120
IL-18 – 607 C → A	GCCCTCTTACCTGAATTTTGGTAGCCCTC AGATTTACTTTTCAGTGGAACAGGAGTCC	60	1.5	*Mse* I	C: 171 A: 101+70
IL-18 – 137 G → C	CACAGAGCCCCAACTTTTACGGCAGAGAA GACTGCTGTCGGCACTCCTTGG	60	1.5	*Mbo* II	G: 116 +39 C: 155
IL-18+113 T → G	CCAGCTTGCTGAGCCCTTTGCTCC GCAGGTGGCAGCCGCTTTAGCAGCTAG	60	1.5	*Nhe* I	T: 120 G: 93+27
IL-18+127 C → T	CCAGCTTGCTGAGCCCTTTGCTCC CTGTGTAGACTGCAGCAGGTGGCGGCC	60	1.5	*Eag* I	C: 113 +21 T: 134
IL-18 codon 35 A → C	AGATTTAATGTTTATTGTAGAAAACCTGGACTC CAGTCATATCTTCAAATAGAGGCCG	55	2.5	*Dde* I	A: 109+32 C: 141
IL-18 (sequencing of promotor region)	AGGTCAGTCTTTGCTATCATTCCAGG CTGCGACAAATAGTTTGTTGCGAGAG TGCTTCTAATGGACTAAGGAGGTGC (internal) GCACCTCCTTAGTCCATTAGAAGCA (internal)	65	1.5	–	968
IL-18 (sequencing of codon 35 region)	GCTACTTCTGGAACAGAAGATTGTC TTCATCTGAGGATTGGGACTAGCAC	65	3	–	356

Underlined bases in the primer sequences are different from the original sequence and serve for introduction of a recognition site for the restriction enzymes or for disruption of an additional recognition site within the PCR amplificate.
RFLP, restriction fragment length polymorphism; IL, interleukin; PCR, polymerase chain reaction.

groups are summarized in Table 2. The genotype frequencies were in agreement with the Hardy–Weinberg equilibrium ($p > 0.1$ for all analyses). Regarding the polymorphism at position – 607 the AA genotype was found in 20 (16.3%), the AC genotype in 58 (47.2%), and the CC genotype in 45 (36.5%) of the periodontitis patients. In comparison 22 (18.2%) of the healthy controls showed the AA genotype, 53 (43.8%) the AC genotype, and 46 (38.0%) the CC genotype ($p = 0.854$). At position – 137 the CC genotype was observed in 8 (6.5%), the GC genotype in 51 (41.5%), and the GG genotype in 64 (52.0%) of the subjects with periodontitis. Among healthy individuals the CC genotype was found in 8.3% ($n = 10$), the GC genotype in 32.2% ($n = 39$), and the GG genotype in 59.5% ($n = 72$) ($p = 0.320$). Overall, the allele frequencies and genotype distribution were not significantly different between both study populations. Stratification analyses according to severity revealed also no significant difference of the genotype distribution among the patients (data not shown).

Table 2. Allele frequencies and distribution of genotypes for the polymorphisms of the interleukin (IL)-18 gene at position – 656, – 607, – 137, +113, +127, and codon 35/3 among periodontitis patients and healthy controls

Position		Control (%)	Periodontitis (%)	χ2	*p*-value
– 607					
Genotype distribution	AA	22 (18.2)	20 (16.3)	0.315	0.854
	AC	53 (43.8)	58 (47.2)		
	CC	46 (38.0)	45 (36.5)		
Allele frequencies	A	97 (40.1)	98 (39.8)	0.003	0.956
	C	145 (59.9)	148 (60.2)		
– 656					
Genotype distribution	TT	22 (18.2)	20 (16.3)	0.315	0.854
	GT	53 (43.8)	58 (47.2)		
	GG	46 (38.0)	45 (36.5)		
Allele frequencies	T	97 (40.1)	98 (39.8)	0.003	0.956
	G	145 (59.9)	148 (60.2)		
– 137					
Genotype distribution	CC	10 (8.3)	8 (6.5)	2.277	0.320
	GC	39 (32.2)	51 (41.5)		
	GG	72 (59.5)	64 (52.0)		
Allele frequencies	C	67 (27.2)	59 (24.4)	0.378	0.539
	G	179 (72.8)	183 (75.6)		
+113					
Genotype distribution	GG	10 (8.3)	8 (6.5)	2.277	0.320
	TG	39 (32.2)	51 (41.5)		
	TT	72 (59.5)	64 (52.0)		
Allele frequencies	G	67 (27.2)	59 (24.4)	0.378	0.539
	T	179 (72.8)	183 (75.6)		
+127					
Genotype distribution	TT	10 (8.3)	8 (6.5)	2.277	0.320
	CT	39 (32.2)	51 (41.5)		
	CC	72 (59.5)	64 (52.0)		
Allele frequencies	T	67 (27.2)	59 (24.4)	0.378	0.539
	C	179 (72.8)	183 (75.6)		
codon 35/3					
Genotype distribution	AA	65 (59.7)	58 (47.2)	1.464	0.481
	AC	43 (35.5)	53 (43.1)		
	CC	13 (10.8)	12 (9.7)		
Allele frequencies	A	173 (71.5)	169 (68.7)	0.452	0.501
	C	69 (28.5)	77 (31.3)		

p-values as analyzed with the Pearson chi-squared test ($p < 0.05$).

Haplotype frequencies of IL-18 gene polymorphisms

The distribution of haplotypes of the IL-18 gene polymorphisms in the periodontitis and the control group is depicted in Table 3. The haplotype 1 (– 607C/ – 137G) was found in 45

Table 3. Distribution of haplotypes for the polymorphism at position − 607 and − 137 among periodontitis patients and healthy controls

Distribution of haplotypes								
	11 (− 137GG/ − 607CC)	12 (− 137GC/ − 607CA)	13 (− 137GG/ − 607CA)	22 (− 137CC/ − 607AA)	23 (− 137GC/ − 607AA)	33 (− 137GG/ − 607AA)	$\chi 2$	*p*-value
Control	46 (38.0)	31 (25.6)	22 (18.2)	10 (8.3)	8 (6.6)	4 (3.3)	–	–
Periodontitis (total)	45 (36.6)	40 (32.5)	18 (14.6)	8 (6.5)	11 (8.9)	1 (0.9)	13.943	0.545

Haplotype 4 (− 607C/ − 137C) was not observed in any of the study subjects. *p*-value as analysed with the Pearson chi-squared test ($p < 0.05$).

(36.6%) of periodontitis patients as compared with 46 (38.0%) of the healthy controls ($p = 0.545$). Haplotype 4 (− 607C/ − 137C) was not observed in any of the study subjects.

Discussion

Unraveling the pathophysiology of destructive periodontal disease comprises one of the major challenges in periodontology (Page 1999). Currently, genetic factors causing imbalances in pro-and anti-inflammatory cytokine production along with an ineffective immune response against bacterial invasion were thought to increase the susceptibility for periodontitis (Baker et al. 1999).

Among the different cytokines that are typically expressed in periodontal lesions specifically the level of IFN-γ was suggested to be positively correlated with the progression of the disease (Ukai et al. 2001). A considerably higher level of IFN-γ was found in aggressive periodontitis patients and in active periodontal pockets suggesting a destructive role for IFN-γ (Garlet et al. 2003). Moreover, studies in rodents reported a significantly decreased bone loss for mice lacking the cytokine IFN-γ suggesting an important role for IFN-γ in periodontitis associated tissue destruction (Baker et al. 1999, Houri-Haddad et al. 2002).

IL-18 plays an important role in the regulation of IFN-γ production. IL-18 protein expression is regulated by the IL-18 promoter gene (Sugiura et al. 2002). Two single nucleotide polymorphisms within the promoter region have recently been described that were suggested to impair IL-18 expression. At position − 607 the change from cytosine to adenine disrupts a binding site for CREB (cyclic AMP-responsive element-binding protein) and at position − 137 the change from guanine to cytosine affects the H4TF-1 (human histone H4 gene-specific transcription factor-1) binding site (Sivalingam et al. 2003). Upon stimulation low promoter activity was observed for A and C alleles at positions − 607 and − 137, respectively (Giedraitis et al. 2001).

Consistently, it was shown that the polymorphisms that have been investigated herein reach significant impact on the pathophysiology of various inflammatory and/or autoaggressive conditions, e.g. rheumatoid arthritis, sarcoidosis, and type 1 diabetes (Kretowski et al. 2002, Sivalingam et al. 2003, Ide et al. 2004). However, the functional influence of the polymorphisms investigated herein still remains to be proved.

The frequency of the alleles and genotypes for the IL-18 gene polymorphisms observed herein is consistent with previous data from other study groups (Kretowski et al. 2002, Stassen et al. 2003). The present study revealed no association between any of the IL-18 gene polymorphisms and the susceptibility for periodontitis. Both the frequencies for the alleles and the distribution of genotypes were almost equal among the two study groups.

Regarding the polymorphisms at position − 607 and − 137 only three of the possible haplotypes were found in both of the study populations. This observation is consistent with previous studies in populations with different ethnic backgrounds (Giedraitis et al. 2001, Takada et al. 2002, Ide et al. 2004). Specifically, the haplotype 1 that bears C at position − 607 and G at position − 137 was shown to be associated with significant higher expression of the IL-18 protein (Giedraitis et al. 2001). The haplotype 1 was reported to be linked with type 1 diabetes (Ide et al. 2004). Herein the frequency of the different haplotypes among periodontitis patients and healthy controls was not significantly different.

In conclusion, the present findings indicate that the susceptibility for periodontitis is not influenced by the six IL-18 gene polymorphisms that have been investigated in this study. Herein the distribution of genotypes among periodontitis patients and healthy control individuals was not significantly different for any of the six polymorphisms.

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