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The interleukin-10 promoter haplotype ATA is a putative risk factor for aggressive periodontitis

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Background and Objective: Interleukin-10 has been described as an anti-inflammatory cytokine and a B-cell proliferation factor. Promoter polymorphisms of the interleukin-10 gene have been associated with altered interleukin-10 expression. Therefore, the aim of this study was to evaluate three polymorphisms at positions −1082G > A, −819C > T and −590C > A in patients with generalized chronic periodontitis ($n = 27$) and generalized aggressive periodontitis ($n = 32$) in comparison with periodontitis-free controls ($n = 34$).

Material and Methods: Interleukin-10 promoter polymorphisms were analyzed by polymerase chain reaction with sequence-specific primers (PCR-SSP). Distributions of single alleles, genotypes and haplotypes were calculated by the chi-square test. Risk factor analyses were carried out by logistic regression. Subgingival bacteria were subjected to molecular biological analyses using the micro-Ident® test.

Results: The combination ATA/ATA was found only in patients with aggressive periodontitis (15.6 vs. 0.0%, $p = 0.023$). Taking into account age, gender, smoking and plaque level, an increased odds ratio (3.7, $p = 0.04$) for aggressive periodontitis was shown for subjects with the haplotype ATA. *Prevotella intermedia* was found to be decreased in ACC- positive (41.3 vs. 66.7%, $p = 0.022$), ATA-positive (33.3 vs. 57.1%, $p = 0.032$) and ACC/ATA-positive (20.0 vs. 55.9%, $p = 0.002$) individuals. In GCC/GCC-positive subjects, *P. intermedia* occurred more frequently (86.7 vs. 42.3%, $p = 0.002$).

Conclusion: The haplotype ATA, which is known as a 'low interleukin-10 producer' is a putative risk indicator for generalized aggressive periodontitis.

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Generalized chronic and generalized aggressive periodontitis have primarily a bacterial aetiology (1–5). There is increasing evidence that besides certain environmental factors, the onset and progression of periodontitis can be influenced by different genetic factors (6–8).

As there is a complex network of pro- and anti-inflammatory cytokines acting in the diseased periodontal tissue (9), several cytokine polymorphisms have been identified as disease-modifying genetic variants. On the one hand, these cytokine polymorphisms influence the cytokine production and consequently the host reaction to bacterial lipopolysaccharides (10–12). On the other hand, differences in cytokine production might be able to effect the growth and/or virulence activity of bacterial species (13,14).

Interleukin-10, which is produced primarily by macrophages and to a lesser extent by lymphocytes, has pleiotropic effects on immunoregulation and inflammation (15). Interleukin-10 terminates secretion of cytokines such as interleukin-12, interleukin-18 and tumor necrosis factor-α from macro-

phages, and reduces the expression of major histocompatibility complex class II and costimulatory molecules. Interleukin-10 is a potent stimulator of natural killer cells and $CD8^+$ T cells (16). Moreover, interleukin-10 enhances B-cell proliferation and differentiation (17). In addition, it has been reported that the generation of autoantibodies is stimulated by interleukin-10, which might play a role in the aetiology of autoimmune diseases, such as Lupus erythematodes (18,19) or rheumatoid arthritis (20,21). The immunopathogenesis of these diseases shows mechanisms similar to that of periodontitis (22). It is assumed that auto-antibodies play a role in the etiology of periodontitis (23,24).

Approximately 50 to 80% (25,26) of interleukin-10 production can be explained by genetic factors. Three interleukin-10 single nucleotide polymorphisms in the promoter region have been associated with altered interleukin-10 production (17,27,28). The −1082 (−1087) single nucleotide polymorphism represents a G-to-A substitution. Functional analysis has shown that the −1082 region contains a putative ETS-like transcription factor-binding site and that polymorphisms in this region can influence transcriptional activity (28–30). Moreover, following concavalin A stimulation of peripheral blood leukocytes, interleukin-10-1082A negative individuals showed significantly higher interleukin-10 production compared to interleukin-10-1082A positive individuals (17). The −819 C > T single nucleotide polymorphism is situated in a DNA motif, forming a putative estrogen responsive element (18). The third polymorphism, at position −590 (−592), is a C-to-A substitution. It is located between consensus sequences for transcription factor Sp1 binding and binding by members of the ETS family. This polymorphism is associated with elevated total serum IgE in subjects who are heterozygous or homozygous for this base substitution (31). The three single nucleotide polymorphisms are in a linkage disequilibrium, resulting in three preference haplotypes: GCC, ACC and ATA (17,32). GCC has been associated with 'high' production of interleukin-10 (17) and production of autoantibodies (18). ATA has been identified as a 'low', and ACC as an 'intermediate', interleukin-10 producer (2,33). The genotypes ATA/ATA, ACC/ATA and ACC/ACC were classified as 'low', the genotypes GCC/ACC, GCC/ATA as 'intermediate' and GCC/GCC as 'high' interleukin-10 producer genotypes (34).

There is evidence that the degree of interleukin-10 production influences the etiology of periodontitis. Interleukin-10(–/–) mice (i.e. mice lacking the interleukin-10 gene) were found to have accelerated alveolar bone loss compared with interleukin-10-positive mice. It was assumed that this phenomenon is caused by an enhanced production of pro-inflammatory cytokines. Additionally, sera from interleukin-10(–/–) mice showed a stronger humoral response against peptides from periodontopathic bacteria in comparison to sera from interleukin-10(+/+) mice (35). Moreover, a limited number of studies have investigated interleukin-10 promoter polymorphisms at three positions [−1082 (−1087), −819 (−824) and −590 (−592, −597)], as well as at two microsatellites (interleukin-10.R and interleukin-10.G), in patients with periodontitis compared with healthy controls. The results were partially inconsistent. For instance, three previous studies (36–38) failed to demonstrate associations between periodontitis and polymorphisms in the interleukin-10 gene. By contrast, in patients of north European origin with severe chronic periodontitis, a higher frequency was found of the wild-type genotype GG at position −1082 (39). Moreover, in Brazilian Caucasian chronic periodontitis patients, the mutant allele T, at position −819, was more expressed (40).

In all previous studies on interleukin-10 polymorphisms in periodontal disease, no multivariate analyses, including established confounders for periodontitis, such as age, gender, plaque level, periodontopathic bacteria or smoking, were carried out to generate adjusted odds ratios for an interleukin-10 polymorphism. Moreover, to date, only one study of German patients suffering from aggressive or chronic periodontitis exists, in which only the interleukin-10 polymorphisms at positions −819 (−824) C > T and −590 (−597) C > A were investigated (38). Therefore, the aim of the present study was to examine the interleukin-10 promoter polymorphisms at positions −819 C > T, −590 C > A and, additionally, at position −1082 G > A in German unrelated Caucasian patients suffering from chronic periodontitis or aggressive periodontitis. Single nucleotide polymorphisms, genotypes and haplotypes were determined. Risk factor analyses were carried out considering established confounders for periodontitis, such as age, gender, smoking, plaque index and the occurrence of subgingival periodontopathic bacteria. A further aim of the present study was to investigate the influence of different interleukin-10 haplotypes on the subgingival colonization of periodontopathic bacteria.

Material and methods

Study population and clinical investigation

Twenty-seven patients with severe generalized chronic periodontitis, 32 patients with generalized aggressive periodontitis, as well as 34 periodontitis-free controls, were included. All participants were unrelated Germans of Caucasian descent.

During the anamnesis, the participants were questioned regarding the occurrence of general diseases, drug consumption and smoking status. A person who smoked at least one cigarette per day was considered a smoker. In order to assess a putative family history of periodontitis, all study participants were asked whether their parents, siblings or children had lost their teeth early because of periodontitis. Moreover, all periodontitis patients were asked whether symptoms of periodontitis, such as bleeding, swelling of the gingiva, pocket formation, increased tooth mobility, etc.,

occurred before they reached 35 years of age. The clinical assessment included determination of the approximal plaque index (in percentage) (41), pocket depth (in mm), and clinical attachment loss (in mm). Both the maximal clinical pocket depth (the distance between the gingival margin and the bottom of the pocket) and maximal clinical attachment loss (the distance between the cemento–enamel junction and the bottom of the pocket) for each tooth were derived by measuring six sites around each tooth and recording the maximum values. In order to determine the extent of periodontitis in one person, the percentage of teeth with pocket depth and clinical attachment loss values of > 4 mm were recorded.

Two periodontists (S.R. and J.K.) independently determined the clinical diagnosis of chronic periodontitis or aggressive periodontitis. Any differences were resolved by discussion. When necessary, a third periodontist (U.Z.) was consulted. Chronic periodontitis patients were selected if they showed an attachment loss in at least 30% of their teeth with a minimum pocket depth of 4 mm. The amount of attachment loss was consistent with the presence of mineralized plaque. More horizontal than vertical approximal bone loss was visible in the X-rays. Patients with aggressive periodontitis were included only if there was evidence (from dental history and/or old radiographs) that the onset of the disease occurred before the age of 35 years. These patients had an attachment loss of ≥4 mm in at least 30% of their teeth. In order to exclude localized aggressive periodontitis, at least three of the affected teeth had to be not first molars or incisors. In contrast to chronic periodontitis, the severity of attachment loss was inconsistent with the amount of mineralized plaque, and more vertical than horizontal approximal bone loss was visible in the X-rays. Periodontitis-free individuals were included if they were at least 30 years of age and did not show any attachment loss (probing depth ≤ 3.5 mm and no gingival recession because of periodontitis). Clinical attachment loss of > 3.5 mm as a consequence of traumatic tooth brushing, overhanging dental fillings, orthodontic therapy, etc., was not considered as a case of periodontitis.

Exclusion criteria for all participants were pregnancy, the use of antibiotics during the last 6 mo and general diseases that have associations with periodontitis and/or with interleukin-10 polymorphisms.

All participants gave their written consent to participate. The study was approved by the local ethics committee and was carried out in accordance with the ethical guidelines of the 'Declaration of Helsinki'.

Molecular assessment of periodontopathic bacteria

Microbial samples were taken before subgingival scaling, to avoid a reduction in the number of bacteria and consequently false-negative results. The microbial samples were taken from the deepest pocket of each quadrant by insertion of a sterile paper point for 20 s. Bacterial plaque samples of each patient were pooled in one tube. Preparation of bacterial DNA was carried out using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's manual. For specific amplification of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis* and *Treponema denticola*, the micro-Ident® test (Hain Lifescience, Nehren, Germany), based on an alkaline phosphatase-mediated staining reaction, was used. Polymerase chain reaction (PCR) was performed (5 min at 95°C; 10 cycles of 30 s at 95°C and 2 min at 58°C; 20 cycles of 25 s at 95°C, 40 s at 53°C and 40 s at 70°C; and 8 min at 70°C) in a personal cycler (Biometra, Göttingen, Germany).

The PCR products were hybridized to a strip containing DNA sequences of each bacterium as well as a positive control. The presence of bacteria was determined visually by means of colored bands. Two positive controls for the amplification reaction and for the conjugate were included in the test.

Genomic studies on three interleukin-10 promoter polymorphisms

Preparation of genomic DNA from fresh human venous EDTA-anticoagulated blood was carried out using the QIAamp® blood extraction kit (Qiagen), according to the manufacturer's manual. The detection of genotypes and haplotypes of interleukin-10 single nucleotide polymorphisms (−1082A > G, −819C > T and −590C > A) was executed using the CYTOKINE Genotyping array CTS-PCR-SSP Tray kit of the Collaborative Transplant Study, Department of Transplantation Immunology of the University Clinic of Heidelberg, Germany. For each PCR, a fragment of 89 bp of the globin gene was co-amplified as a positive control. The PCRs were performed using sequence-specific primers for detection of possible haplotypes, prepipetted and lyophilized in thin-walled plastic 96-well PCR trays. For each PCR, 10 μL of a Mastermix containing 1 U of *Taq*-Polymerase (Invitek, Berlin, Germany), 100 ng of genomic DNA, 5% glycerol and PCR reaction buffer, was added. PCR was performed on a Mastercycler Gradient (Eppendorf, Hamburg, Germany). The following cycling conditions were used: initial denaturation of 2 min at 94°C; 10 cycles of 15 s at 94°C, 1 min at 64°C and 30 s at 72°C; and 20 cycles of 15 s at 94°C, 50 s at 61°C and 30 s at 72°C. After agarose-gel electrophoresis, the results were evaluated visually. Bands of 305 and 530 bp correspond to the interleukin-10 single nucleotide polymorphisms at positions −1082, −819 and −592.

Statistical analysis

The genotype and allele frequencies were calculated by direct counting and then dividing by the number of subjects to produce genotype frequency, or by the number of chromosomes to produce allele frequency. Differences between patients and controls were determined by the chi-square test. If $n < 5$, Fisher's Exact test was performed. In general, p-values of ≤ 0.05

were accepted as statistically significant. Significant deviations were additionally tested by Bonferroni correction. Stepwise binary logistic regression (backwards elimination analysis) was used in order to determine adjusted odds ratios of interleukin-10 haplotypes for periodontitis, including the cofactors approximal plaque index, periodontopathic bacteria, gender, age and smoking. A variable was excluded if the significance of the chance from the previous step was greater than $p = 0.10$.

Results

Clinical differences between periodontitis and controls

In comparison with the healthy control group (age 42.4 ± 13.5 years, 58.5% women, 32.4% smokers), there were no significant differences between the aggressive periodontitis and chronic periodontitis groups with respect to age (aggressive periodontitis 36 ± 8.4 years, chronic periodontitis 47.8 ± 10.3 years), percentage of women (aggressive periodontitis 59.4%, chronic periodontitis 63.0%) and percentage of current smokers (aggressive periodontitis 37.5%, chronic periodontitis 18.5%). However, compared with controls, the patients with aggressive periodontitis reported significantly more frequent early tooth loss as a consequence of periodontitis among their relatives (43.8 vs. 5.9% $p = 0.001$). In both groups of patients, the mean percentage of teeth with pocket depth > 4 mm or clinical attachment loss was $\approx 70\%$, indicating generalized attachment loss. With the exception of *A. actinomycetemcomitans*, all the bacteria investigated occurred significantly more frequently in patients with chronic periodontitis in comparison with the periodontitis-free control group. In aggressive periodontitis, only *T. denticola* was not significantly increased (Table 1).

Allele, genotype and haplotype frequencies of three interleukin-10 single nucleotide polymorphisms

The mutant allele T at position −819 (29.7 vs. 16.2%, $p = 0.064$), and the mutant allele A at position −590 (31.3 vs. 17.6%, $p = 0.068$) occurred more frequently in patients with aggressive periodontitis than in healthy controls (Table 2). However, this difference was not statistically significant. Moreover, the mutant genotype TT at position −819 (15.6 vs. 0.0%, $p = 0.056$) was only detected in patients with aggressive periodontitis. There was a trend that the haplotype ATA (Table 3) was expressed more in patients with aggressive periodontitis (29.7 vs. 16.2%, $p = 0.064$). In patients with chronic periodontitis, the frequency of ATA was decreased compared with healthy controls (9.3 vs. 16.2%). This latter difference was not statistically significant. The rare haplotype GCA was found only in the healthy control group. The combination ATA/ATA (15.6 vs. 0.0%, $p = 0.023$) was found among patients with aggressive periodontitis (Table 4), but not in the groups of chronic periodontitis patients and control individuals. However, the latter association remained not significant after Bonferroni correction.

Subgingival colonization of five periodontopathic bacteria in dependence of the different interleukin-10 haplotypes

In the whole study group, the prevalence of *P. intermedia* was significantly decreased in subjects who were positive for ACC (41.3 vs. 66.7%, $p = 0.022$), ATA (33.3 vs. 57.1%, $p = 0.032\%$) and ACA/ATA (20 vs. 55.9%, $p = 0.022$). GCC/GCC-positive individuals, however, were more commonly infected with *P. intermedia* (86.7 vs. 42.3%, $p = 0.002$). The occurrence of the other bacteria investigated was not associated with interleukin-10 polymorphisms.

Regression analysis for periodontitis, and the occurrence of ATA, ACC and GCC in a given individual

In order to investigate the impact of the genetic background of the interleukin-10 haplotypes ATA, ACC and GCC, backwards binary logistic regression analysis was carried out. Established confounders for periodontitis, such as age, gender, smoking and plaque level, and the occurrence of the five investigated bacteria, were included. As the approximal plaque index was significantly positively correlated with the presence of *P. gingivalis* ($r = 0.374$, $p = 0.0001$), *T. forsythensis* ($r = 0.334$, $p = 0.001$) and *T. denticola* ($r = 0.334$, $p = 0.001$), in the first step only the approximal plaque index was taken into account. In the second step, instead of approximal plaque index, the detection of periodontopathic bacteria was included. When the approximal

Table 1. Individual proof of periodontopathic bacteria in dependence on diagnosis

	Chronic periodontitis ($n = 27$)	Aggressive periodontitis ($n = 32$)	Healthy controls ($n = 34$)	p-values CP vs. controls	p-values AP vs. controls
A. actinomycetemcomitans, *n* (%)	6 (22.2)	16 (50.0)	3 (8,8)	NS	< 0.001
P. gingivalis, *n* (%)	26 (96.3)	25 (78.1)	4 (11.8)	< 0.001	< 0.001
P. intermedia, *n* (%)	17 (63.0)	20 (62.5)	9 (26.5)	0.004	0.003
T. forsythensis, *n* (%)	26 (96.3)	28 (87.5)	19 (55.9)	< 0.001	0.005
T. denticola, *n* (%)	27 (100)	28 (87.5)	23 (67.6)	0.001	NS
P. gingivalis + *T. forsythensis* + *T. denticola*, *n* (%)	25 (92.6)	22 (68.8)	4 (11.8)	< 0.001	< 0.001

Table 2. Frequencies of interleukin-10 single nucleotide polymorphisms in individuals with chronic periodontitis (CP) and aggressive periodontitis (AP) compared with healthy controls

Single nucleotide polymorphism	Chronic periodontitis *n* (%)	Aggressive periodontitis *n* (%)	AP + CP *n* (%)	Healthy controls *n* (%)	*p*-values CP vs. controls	*p*-values AP vs. controls	*p*-values AP + CP vs. controls
−1082 Allele G > A	*n* = 54	*n* = 64	*n* = 118	*n* = 68	NS	NS	NS
G	27 (50.0)	43 (67.2)	70 (59.3)	38 (55.9)			
A	27 (50.0)	21 (32.8)	48 (40.7)	30 (44.1)			
−1082 Genotype	*n* = 27	*n* = 32	*n* = 59	*n* = 34	NS	NS	NS
GG	5 (18.5)	3 (9.4)	8 (13.6)	6 (17.6)			
AG	17 (63.0)	15 (46.9)	32 (54.2)	18 (52.9)			
AA	5 (18.5)	14 (43.8)	19 (32.2)	10 (29.4)			
AG + AA vs. GG	22 (81.5)	29 (90.6)	51 (86.4)	28 (82.4)	NS	NS	NS
−819 Allele C > T	*n* = 54	*n* = 64	*n* = 118	*n* = 68	NS	0.064	NS
C	47 (87.0)	45 (70.3)	92 (78.0)	57 (83.8)			
T	7 (13.0)	19 (29.7)	26 (22.0)	11 (16.2)			
−819 Genotype	*n* = 27	*n* = 32	*n* = 59	*n* = 34	NS	0.056	NS
CC	20 (74.1)	18 (56.3)	38 (64.4)	23 (67.6)			
CT	7 (25.9)	9 (28.1)	16 (27.1)	11 (32.4)			
TT	0	5 (15.6)	5 (8.5)	0			
CT + TT vs. CC	7 (25.9)	14 (43.8)	21 (35.6)	11 (32.4)	NS	NS	NS
−590 Allele C > A	*n* = 54	*n* = 64	*n* = 118	*n* = 68	NS	0.068	NS
C	47 (87.0)	44 (68.8)	91 (77.1)	56 (82.4)			
A	7 (13.0)	20 (31.3)	27 (22.9)	12 (17.6)			
−590 Genotype	*n* = 27	*n* = 32	*n* = 59	*n* = 34	NS	NS	NS
CC	20 (74.1)	18 (56.3)	38 (64.4)	23 (67.6)			
CA	7 (25.9)	8 (25.0)	15 (25.4)	10 (29.4)			
AA	0	6 (18.8)	6 (10.2)	1 (9.2)			
CA + AA vs. CC	7 (25.9)	14 (43.8)	21 (35.6)	11 (32.4)	NS	NS	NS

Table 3. Distribution of interleukin-10 haplotypes (arranged as allele frequencies) in individuals with chronic periodontitis (CP) and aggressive periodontitis (AP) compared with healthy controls

Haplotypes	Chronic periodontitis *n* (%)	Aggressive periodontitis *n* (%)	AP + CP *n* (%)	Healthy controls *n* (%)	*p*-values CP vs. controls	*p*-values AP vs. controls	*p*-values AP + CP vs. controls
−1082-819-590	*n* = 54	*n* = 64	*n* = 118	*n* = 68			
GCC vs.	27 (50.0)	22 (34.4)	49 (41.5)	29 (42.6)	NS	NS	NS
others	27 (50.0)	42 (65.6)	69 (58.5)	39 (57.4)			
ACC vs.	22 (40.7)	23 (35.9)	45 (38.1)	27 (39.7)	NS	NS	NS
others	32 (59.3)	41 (64.1)	73 (61.9)	41 (60.3)			
ATA vs.	5 (9.3)	19 (7,29)	24 (3,20)	11 (16.2)	NS	0.064	NS
others	49 (90.7)	45 (70.3)	94 (79.7)	57 (83.8)			

AP, aggressive periodontitis; CP, chronic periodontitis.

plaque index was included (Table 5, model 1) the occurrence of ATA in an individual increased the adjusted odds ratio for aggressive periodontitis (odds ratio = 3.71, confidence interval: 1.06–12.94, $p = 0.040$). Including the presence of periodontopathic bacteria (Table 5, model 2) did not yield any significant odds ratio for an interleukin-10 haplotype. Moreover, there was no significant influence of any of the interleukin-10 haplotypes in the chronic periodontitis and the whole periodontitis group.

Discussion

The present study analyzed three single nucleotide polymorphisms in the promoter region of the interleukin-10 gene in patients with aggressive periodontitis and chronic periodontitis in comparison to controls without periodontitis. In addition, possible associations between the individual expression of inter leukin-10 haplotypes and the occurrence of five periodontopathic bacteria were investigated. Patients with aggressive periodontitis and chronic periodontitis were diagnosed with respect to clinical, radiographic and historical findings. Patients with localized aggressive periodontitis were not included in the group with generalized aggressive periodontitis, as localized and generalized aggressive periodontitis could differ regarding disease-modifying genetic factors (e.g. genes that influence the IgG_2 response to periodontopathic peptides) (42). To date, there are no parameters that permit absolute distinction to be made between aggressive and chronic

Table 4. Distribution of interleukin-10 combinations (arranged as genotype frequencies) found in individuals with chronic periodontitis (CP) and aggressive periodontitis (AP) compared with healthy controls

Genotypes	Chronic periodontitis *n* (%)	Aggressive periodontitis *n* (%)	AP + CP vs. controls	Healthy controls *n* (%)	*p*-values CP vs. controls	*p*-values AP vs. controls	*p*-values AP + CP vs. controls
−1082-819-590	$n = 27$	$n = 32$	$n = 59$	$n = 34$			
GCC/GCC vs. others	5 (18.5)	4 (12.5)	9 (15.3)	6 (17.6)	NS	NS	NS
	22 (81.5)	28 (87.5)	50 (84.7)	28 (82.4)			
ACC/ACC vs.	1 (7.3)	5 (15.6)	0.6 (10.2)	3 (8.8)	NS	NS	NS
others	26 (96.3)	27 (84.4)	53 (89.8)	31 (91.2)			
ATA/ATA vs.	0	5 (15.6)	5 (8.5)	0	No statistic	0.023	NS
others	27 (100)	27 (84.4)	54 (91.5)	34 (100)			
GCC/ACC vs.	16 (59.3)	9 (28.1)	25 (42.4)	14 (41.2)	NS	NS	NS
others	11 (40.7)	23 (71.9)	34 (57.6)	20 (58.8)			
GCC/ATA vs.	1 (7.3)	5 (15.6)	6 (10.2)	3 (8.8)	NS	NS	NS
others	26 (96.3)	27 (84.4)	53 (89.9)	31 (91.2)			
ACC/ATA vs.	4 (14.8)	4 (12.5)	8 (13.6)	7 (20.6)	NS	NS	NS
others	23 (85.2)	28 (87.5)	51 (86.4)	27 (79.4)			

Table 5. Backwards stepwise binary logistic regression investigating the adjusted odds ratio of the interleukin-10 haplotype ATA for aggressive periodontitis under consideration of the cofactors age, gender, smoking and approximal plaque index (API) (model 1) or regarding the presence of A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythensis and T. denticola instead of API (model 2). Only cofactors which fit the models are shown

Significant variables	Regression coefficient	SE	*p*-value	Odds ratio	95% CI	
					Lower	Upper
Model 1						
API	0.033	0.012	0.005	1.034	1.01	1.06
ATA	1.310	0.638	0.040	3.705	1.06	12.94
Model 2						
Age	–0.085	0.043	0.049	0.919	0.84	1.00
A. actinom.comit.	2.133	0.963	0.027	8.443	1.28	55.73
P. gingivalis	4.166	1.014	< 0.001	5.036	8.83	470.26
ATA	1.617	0.912	0.076	5.036	0.84	30.11

API, approximal plaque index; CI, confidence interval; SE, standard error.

periodontitis. Hence, a misdiagnosis cannot be completely excluded. In spite of this fact, in the present study aggressive and chronic periodontitis differed clearly regarding their interleukin-10 polymorphisms. Moreover, in accordance with the new classification system for periodontal diseases (43) patients with aggressive periodontitis more often showed a familial aggregation of the disease in comparison with healthy controls. Additionally, patients with aggressive periodontitis were more often infected with *A. actinomycetemcomitans* (Table 1). In the chronic periodontitis group, we did not obtain such significant differences. Consequently, a sufficient distinction between patients with aggressive and chronic periodontitis can be assumed.

The microbial analysis showed that all bacteria investigated were also found in the control group. There was neither a significant difference in the number of chronic periodontitis patients and controls who were infected with *A. actinomycetemcomitans*, nor in the number of aggressive periodontitis patients and controls who were infected with *T. denticola* (Table 1). These results suggest that besides periodontopathic bacteria, the immune response and its genetic control could be important factors in the pathway of periodontitis.

The findings of this study indicate that the odds ratio for aggressive periodontitis is increased by the interleukin-10 promoter haplotype ATA, when adjusted for established confounders for periodontitis (Table 5, model 1). The calculated adjusted odds ratio for ATA was three times higher than for the approximal plaque index. However, there was no statistical significance when instead of the approximal plaque index, the five periodontopathic bacteria were included in the regression model (Table 5, model 2). Obviously, the presence of *A. actinomycetemcomitans* and *P. gingivalis* might represent a higher risk factor for developing aggressive periodontitis than certain interleukin-10 promoter polymorphisms.

In agreement with our results concerning the aggressive periodontitis group, there was a higher frequency of the haplotype ATA among Brazilian Caucasian periodontitis patients (40). However, this was only significant among female patients suffering from chronic periodontitis, with no significant results being obtained for patients with aggressive periodontitis. Moreover, the positive association of the genotype −1082GG to chronic periodontitis yielded among patients of north European origin (39) was not confirmed by the present study. A possible reason for these conflicting results might be based on ethnic differences in the prevalence of interleukin-10 polymorphisms. For instance, by contrast to a Caucasian group (17), among Japanese individuals (37) the wild-type haplotype GCC was completely absent (51 vs. 0%). Moreover, as well as polymorphisms in the interleukin-10 gene, other factors, such as

the lipopolysaccharide concentration, gender and smoking (22,33), are known to influence the interleukin-10 production.

The associations between ATA and aggressive periodontitis, revealed in the present study, could be explained by the following mechanisms. Compared with other genotypes, after lipopolysaccharide stimulation the genotypes containing ATA, (33) or homozygosity for ATA (20), were associated with lower interleukin-10 production. Low interleukin-10 levels lead to an enhanced release of pro-inflammatory cytokines, such as tumor necrosis factor-α (44), which have been implicated in alveolar bone loss (45). Moreover, interleukin-10 is a potent inhibitor of *in vitro* osteoclast formation (46). Both aspects could be important in the accelerated alveolar bone loss shown for interleukin-10-lacking mice (35). Hence, among ATA-positive individuals, the high susceptibility for aggressive periodontitis could be explained by both a high release of pro-inflammatory cytokines and enhanced formation of osteoclasts.

In the present study we obtained an association between interleukin-10 polymorphisms and the subgingival colonization of *P. intermedia*. *P. intermedia* was found to be decreased in ACC-, ATA- and ATA/ACC-positive individuals, but increased in GCC/GCC carriers. The reasons for these associations are not entirely clear. We assume that a decreased interleukin-10 level relates to a high local immune response against *P. intermedia*. This idea is supported by the fact that in the sera of interleukin-10(–/–) mice, an increased humoral immune response against subgingival bacteria, such as *P. intermedia*, was shown (35). However, this immune response could be hyperactive and could maintain local inflammation. This mechanism is shown in interleukin-10-deficient mice that developed a chronic colitis triggered by the commensal flora (47). Colitis and intestinal epithelial cell damage were caused by an aberrant expression of major histocompatibility complex class II molecules (47) and the formation of autoreactive T cells (48).

In summary, the results of this study suggest that the interleukin-10 promoter haplotype, ATA, is a putative risk indicator for aggressive periodontitis. Moreover, certain interleukin-10 haplotypes are associated with the occurrence of *P. intermedia*. However, we revealed that the presence of *A. actinomycetemcomitans* and *P. gingivalis* are stronger predictors for aggressive periodontitis than interleukin-10 gene polymorphisms.

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