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Role of IL-6, TNF-A and LT-A variants in the modulation of the clinical expression of plaque-induced gingivitis

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Abstract

Aim: The purpose of the present study was to assess the association of interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-A) and lymphotoxin alpha (LT-A) gene polymorphisms with the clinical parameters of gingivitis in a large experimental gingivitis trial and with each of two subgroups, ''high responder'' (HR, $n = 24$) and ''low responder'' (LR, $n = 24$), with distinct susceptibility to gingivitis.

Material and Methods: Ninety-six systemically and periodontally healthy non-smokers, 46 males (mean age: 23.9 ± 1.7) and 50 females (mean age: 23.3 ± 1.6), were included in a randomized split-mouth localized 21-day experimental gingivitis trial. Plaque index, gingival index, gingival crevicular fluid volume and angulated bleeding score were recorded. HR and LR subgroups were characterized by substantially different severities of gingival inflammation despite a similar plaque accumulation rate. All subjects were genetically characterized for IL-6^{-174}, IL-6^{-597}, TNF-A^{-308} and LT-A^{+252} polymorphisms.

Results: None of the variants analysed, either as single polymorphisms or as a combined genotype, was associated with the clinical parameters in the overall population. For the polymorphisms studied, genotypic distributions in HR and LR subjects were not significantly different.

Conclusions: The present results suggest an absence of association between IL-6, TNF-A and LT-A polymorphisms and subject-based clinical behaviour of the gingiva in response to de novo plaque accumulation.

Key words: gene polymorphisms; gingivitis/genetics; IL-6; LT-α; periodontal diseases/gingivitis; TNF-α

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Epidemiological data suggest that over 50% of adults have gingivitis in the United States (Oliver et al. 1998). Despite this high prevalence, studies on the significance of gingivitis as a precursor disease state in the development of periodontitis and on the possible genetic factors determining or modulating subject-dependent variability in susceptibility to gingivitis are scarce (Jepsen et al. 2003, Tatakis & Trombelli 2004, Scapoli et al. 2005).

In contrast, numerous genetic factors have been studied in relation to periodontitis (Hart & Kornman 1997, Michalowicz et al. 2000). Among them, polymorphisms of interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-A) genes have received considerable attention, because of the significant pathophysiologic role of these cytokines in inflammation, in general (Waterer & Wunderink 2003), and in periodontitis, in particular (Loos et al. 2000, Endo et al. 2001, Amar et al. 2003, Fassmann et al. 2003, D'Aiuto et al. 2004, Takashiba & Naruishi 2006).

TNF-α is a critical cytokine in the inflammatory response to infection (Beutler & Grau 1993). Accordingly, any genetic variability in the production of TNF-α after an infectious stimulus could have a significant impact on the

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degree of inflammatory response and could potentially influence the clinical outcome (Holmes et al. 2003). In vitro studies have consistently found marked individual variation in TNF-α production after a variety of inflammatory stimuli (Aguillon et al. 2001). A significant amount of evidence supports the biological importance of polymorphisms within the TNF-A promoter region. A guanine (G) to adenine (A) transition at TNF-A^{-308} is perhaps the best-studied cytokine polymorphism and the one for which the best evidence of functional significance exists. Carriage of the A allele of TNF-A^{-308} has been associated with an increased risk for many diseases, including septic shock (Tang et al. 2000), ulcerative colitis and Crohn's disease (Sykora et al. 2006), paediatric-onset inflammatory bowel disease (Cucchiara et al. 2007) and periodontitis (Galbraith et al. 1999, Fassmann et al. 2003).

Complicating the assessment of TNF-A polymorphisms is the high degree of linkage disequilibrium (LD) between TNF-A promoter polymorphisms and between other polymorphisms within other nearby genes including lymphotoxin-α (LT-A). LD with the TNF-A^{-308}*G allele and the LT-A^{+252}*A allele (Heesen et al. 2003) suggests that these polymorphisms should be jointly assessed. Carriage of the A allele of LT-A^{+252} has been associated with increased TNF-α production both in vitro (Messer et al. 1991) and in vivo (Heesen et al. 2003, Majetschak et al. 2005), although the mechanism by which this mutation impacts on TNF-α production is unknown.

Within LT-A (formerly known as tumour necrosis factor-β) an A–G transition in the first intron has been identified as a potentially influential variant under many inflammatory conditions. In a landmark study of 40 patients with septic shock (Stuber et al. 1996), the carriage of the LT-A^{+252} AA genotype resulted in a substantially greater risk for death. Subsequent studies by the same group suggested that carriage of LT-A^{+252}*A is also a risk factor for the development of septic shock, in post-trauma patients (Majetschak et al. 1999) and in patients with community-acquired pneumonia (Waterer et al. 2001).

IL-6 has been demonstrated to be a marker of the severity and outcome of sepsis by a number of studies, and a haplotype involving at least four single nucleotide polymorphisms (SNPs) within the promoter has been identified and appears to influence the rate of IL-6 transcription (Terry et al. 2000). One of the first IL-6 promoter variants investigated was a biallelic polymorphism localized at position −174 of the promoter region of the IL-6 gene. Functional in vitro studies associated the two alleles −174 G and −174 C with low and high IL-6 production, respectively; the homozygous GG genotype results in a lower IL-6 expression after an inflammatory stimulus compared with the CC genotype; this is supported by the in vitro observation that IL-6 levels are higher in normal subjects with the CC genotype, compared with GC or GG subjects (Fishman et al. 1998). Recently, two other functional SNPs in the IL-6 promoter at positions −597 and −572 were identified (Kitamura et al. 2002, Villuendas et al. 2002). It has been shown that these three SNPs (−174, −572 and −597) of the IL-6 promoter do not act independently in the regulation of IL-6 transcription (Terry et al. 2000, Rivera-Chavez et al. 2003, Muller-Steinhardt et al. 2004). A positive association between IL-6 polymorphisms and periodontitis has been reported (Brett et al. 2005, Babel et al. 2006).

In an effort to identify and characterize factors, be they local, systemic, genetic, acquired, etc., which determine subject differences in gingivitis susceptibility, we initiated a series of experimental gingivitis studies aiming to first identify individuals with different gingival responses to similar plaque accumulation (Tatakis & Trombelli 2004, Trombelli et al. 2004b). This was accomplished with the identification of two subgroups of subjects, ''high responders'' (HR) and ''low responders''(LR), who, respectively, exhibited high and low gingival inflammatory responses to similar rates and levels of plaque accumulation (Trombelli et al. 2004b). The purpose of the present study was to test the association of two polymorphisms of the IL-6 gene, i.e. −174 G/C, −597 G/A promoter variants, and of two selected polymorphisms respectively, one in the TNF gene (namely molecular variants G-308A of the TNF-A) and the second the *Nco*I RFLP (A+252G) in the LT-α gene, with clinical parameters of gingivitis in a large experimental gingivitis trial and with each of the two subgroups (HR, LR) with distinct susceptibility to gingivitis.

Material and Methods

Experimental design and study population

The overall experimental design has been detailed previously (Trombelli et al. 2004b), and the clinical analysis of the examined population, consisting of 96 systemically and periodontally healthy non-smokers, 46 males (mean age: 23.9 ± 1.7) and 50 females (mean age: 23.3 ± 1.6), has already been described (Trombelli et al. 2004a, b). Briefly, the randomized split-mouth localized 21-day experimental gingivitis clinical trail was conducted with volunteers among current and permanent residents of the Ferrara area. Two subgroups were defined from the entire study population, HR and LR groups, on the basis of clinical signs of gingivitis, standardized for plaque exposure. The HR group comprised of 13 males and 11 females (mean age: 24.1 ± 1.6), and the LR group comprised of 11 males and 13 females (mean age: 23.4 ± 1.9) (Trombelli et al. 2004b).

The descriptive statistics and statistical comparisons for the clinical parameters recorded in the test quadrant on day 21 measured in LR and HR subjects have been reported previously (Trombelli et al. 2004b), and those data demonstrated the significantly different clinical gingival response between two subgroups of subjects exposed to similar plaque levels.

For all 96 subjects, cytokine gene polymorphisms were assessed by means of analysis of DNA purified from peripheral blood leucocytes. The study design was approved by the local ethical committee and was found to conform to the requirements of the ''Declaration of Helsinki'' as adopted originally and revised subsequently (www.wma.net/e/policy/b3.htm) (The World Medical Association Declaration 2004). All participants provided written informed consent.

Clinical parameters

The following clinical parameters were assessed, as detailed previously (Trombelli et al. 2004b): gingival index (GI), plaque index (PlI), gingival crevicular fluid volume (GCF) and angulated bleeding score (AngBS). A derived parameter, ''cumulative plaque exposure'' (CPE), was also calculated (Trombelli et al. 2004b). In each test and control quadrant, clinical parameters were recorded on the following

three maxillary teeth: lateral incisor; first pre-molar (if missing, replaced by the second pre-molar); and first molar (if missing, replaced by the second pre-molar). For each tooth, clinical parameters were evaluated on two sites: the buccal and the mesiobuccal aspect. All clinical parameters were recorded on days 0, 7, 14 and 21 (at completion of the experimental gingivitis period), by two trained and calibrated examiners with good to excellent intra- and inter-examiner agreement (Trombelli et al. 2004b).

DNA extraction

Peripheral blood (20 ml) was collected in EDTA-containing tubes and stored at − 20°C until analysis. Genomic DNA was extracted by a salting-out method as described (Miller et al. 1988), aliquoted in distilled water and stored at 4°C. Genomic DNA integrity was tested by electrophoresis (0.8% agarose gel, ethidium bromide stain).

Cytokine genotyping

The following cytokine gene polymorphisms were analysed by polymerase chain reaction (GeneAmp PCR System 9700 thermocycler, Perkin Elmer, Wellesley, MA, USA), primers (Promega, Milan, Italy) and conditions as listed in Table 1.

The size of the amplified DNA fragments was determined electrophoretically (3% agarose gel, ethidium bromide stain) using GeneRuler DNA Ladder Plus (Promega) size standards.

TNF-A (G-308A)

The 117 base pair (bp) region of the TNF-A gene was amplified by PCR (Galbraith et al. 1997). Genotypes of the TNF-A^{-308} (allele1: − 308G; allele 2: − 308A) polymorphism were determined using the restriction enzyme *Nco*I (Promega). *Nco*I on allele 1 results in two fragments of 97 bp and 20 bp, while on allele 2 it results in a single fragment of 117 bp. All three fragments were present in heterozygous subjects. All 96 subjects were successfully typed for this polymorphism.

IL-6 (G-174C)

The PCR targeted the 496 bp region of $IL6^{-174}$ (Pascual et al. 2000), and genotypes of the $IL6^{-174}$ restriction fragment length polymorphism (RFLP) (allele 1: − 174G; allele 2: − 174C) were determined with Hsp92II (Promega). Two fragments of 169 bp and 327 bp were observed in subjects homozygous for allele 1, while on allele 2 three fragments of 47, 122 and 327 bp were observed. All four fragments were present in heterozygous subjects; all 96 subjects were successfully typed for this polymorphism.

IL-6 (G-597A)

The 597 bp region on the promoter of the IL6 gene was amplified (Pascual et al. 2000), and genotypes of the $IL6^{-597}$ RFLP (allele 1: − 597G; allele 2: − 597A) were determined with *Fok*I (GE Healthcare, Amersham Place, UK). Two fragments of 468 and 57 bp was observed in subjects homozygous for allele 2 and a single fragment of 597 bp in subjects homozygous for allele 1. All 96 subjects were successfully typed for this polymorphism.

LT-A (A+252G)

The 368 bp region on the first intron of LT-A was examined by PCR-RFLP (Stanulla et al. 2001). The genotypes of the LT-A^{+252} (allele 1: 252A; allele 2: 252G) were determined with *Nco*I (Promega). A single fragment of 368 bp was observed in subjects homozygous for allele 1, while two fragments of 133 and 235 bp in subjects homozygous for allele 2; 92 subjects were successfully typed for this polymorphism.

Table 1. Polymerase chain reaction: primers and conditions

TNF-A^{-308}	(F) 5′-AGG CAA TAG GTT TTG AGG GCC AT-3′
	(R) 5′-ACA CTC CCC ATC CTC CCG GCT-3′
	95°C 2 min. (95°C 15 s; 60°C 3 s) × 35; 72°C 5 min.
LT-A^{+252}	(F): 5′-CTC CTG CAC CTG CTG CCT GGA TC-3′
	(R): 5′-GAA GAC ACG TTC AGG TGG TGT CAT-3′
	92°C 50 s (92°C 30 s; 65°C 40 s; 72°C 40 s) × 31; 72°C 2 min.
$IL-6^{-174}$	(F): 5′-GGA GTC ACA CAC TCC ACC T-3′
$IL-6^{-597}$	(R): 5′-CTG ATT GGA AAC CTT ATT AAG-3′
	94°C 5 min. (94°C 54 s; 57°C 30 s; 72°C 10 s) × 30; 72°C 10 min.

IL-6, interleukin-6; LT-A, lymphotoxin alpha; TNF-A, tumour necrosis factor alpha.

Statistical analysis

The subject was regarded as the statistical unit. For each clinical parameter, the recordings from the six selected sites for either the test or the control quadrants were added and divided by 6 to give the mean value for each subject. Therefore, for each parameter at each observational period, the subject was represented by a single test and a single control value. Data were expressed by either median and inter-quartile range (IR) for non-parametric variables (GI, AngBS), or mean ± standard deviation (SD) for parametric variables (PlI, CPE, GCF).

χ^2 analysis was used to test for deviation of genotype frequencies from Hardy–Weinberg expectations for biallelic systems. To test the effect of different genotypes on response clinical variables, one-way parametric ANOVA and Kruskal–Wallis non-parametric test were used. Post hoc comparisons were performed to explore differences among groups. To compare genotype/allele distributions among HR and LR subjects, the maximum likelihood (ML) χ^2 has been estimated through log–linear analysis.

The estimate of LD between IL-6 gene variants and TNF-A and LT-A polymorphisms has been carried out using Arlequin software (Release 3.1) (Excoffier et al. 2005). Besides individual gene polymorphisms, combined genotypes were also tested. Specifically, the combined genotype of $IL\text{-}6^{-174}$ ∗2–$IL\text{-}6^{-597}$∗2, as reported by Muller-Steinhardt et al. (2004), and the combined genotype of $TNF\text{-}A^{-308}$∗2–$LT\text{-}A^{+252}$∗2, as reported by Fassmann et al. (2003), were used. For all data analysis, the level of significance was set at 5%.

Results

Study population genetic polymorphisms

The genotype frequencies of the examined polymorphisms are shown in Table 2.

When the genotypic and allelic distributions observed in the study population were compared with published control Caucasian populations from southern and eastern European countries (Fedetz et al. 2001, Fassmann et al. 2003, Landi et al. 2003), no significant differences emerged between the present and the referring population for either genotypes or alleles (data not

shown). All the polymorphisms analysed were in Hardy–Weinberg equilibrium. In contrast, when genotypic frequencies were compared with distributions of CEPH population of the HapMap Project, a significant difference ($p < 0.001$ for all comparisons) between our study group and the HapMap-CEU collection was found.

From the genotype distribution comparisons, an association emerged between IL-6^{-174} and IL-6^{-597} variants ($\chi^2_{[4]} = 124.7$, $p \ll 0.001$). In the present sample, the two markers in the promoter region of IL-6 show a strong association, due to the strong LD existing between these two variants as already described in the literature (Fassmann et al. 2003, Muller-Steinhardt et al. 2004). However, in our population, the LD is high, but not complete as observed by other authors ($r^2 = 0.76$; $\chi^2_{[1]} = 174.00$, $p < 0.0001$).

Genotypic and allelic distributions, evaluated for each gender, did not differ in males and females (p value range: 0.07–0.73 for genotypes, and 0.07–0.92 for alleles).

Table 2. Genotype frequencies of TNF-A, LT-A, IL-6 single nucleotide polymorphisms

Gene	Genotype			Total
	11	12	22	
TNF-A^{-308}	76	18	2	96
LT-A^{+252}	60	31	1	92*
IL-6^{-597}	48	40	8	96
IL-6^{-174}	50	36	10	96

*Four subjects non successfully genotyped.
IL-6, interleukin-6; LT-A, lymphotoxin alpha; TNF-A, tumour necrosis factor alpha.

Relationship between genetic polymorphisms and clinical parameters

The descriptive statistics for day-21 test quadrant clinical parameters according to genotype distributions for genetic variants analysed are provided in (Table 3). No statistical associations were observed.

HR and LR individuals and genetic polymorphisms

No differences in either genotypic or allelic distributions of TNF-A^{-308}, LT-A^{+252} or IL-6 variants were observed between HR and LR subjects (Table 4).

Combined genotypes

The relationship between clinical parameters and genotype-positive/-negative individuals, for both (IL-6^{-174}*2–IL-6^{-597}*2) and (TNF-A^{-308}*2–LT-A^{+252}*2) combined genotypes, was assessed in the present data set. No significant association between the clinical parameters and any of the two combined genotypes was observed. The prevalence of positive genotype (IL-6^{-174}*2–IL-6^{-597}*2) as well as the prevalence of the combined genotype (TNF-A^{-308}*2–LT-A^{+252}*2) were the same in HR and LR groups ($\chi^2_{[1]} = 0.0$, $p = 1.0$ and $\chi^2_{[1]} = 0.12$, $p = 0.73$, respectively).

Discussion

Findings from examination of dizygotic and monozygotic twins (Michalowicz et al. 1991, 2000) indicated (i) that between 38% (regarding probing attachment loss) and 82% (regarding gingivitis) of the population variance could be attributed to genetic factors (Michalowicz et al. 1991) and (ii) that

Table 3. Descriptive statistics for clinical parameters recorded in test quadrant on day 21 for the overall population subdivided according to TNF-A, LT-A and IL-6 genotype distributions

	N	Mean ± SD			Median (IR)*	
		PII	CPE	GCF (μl)	GI	AngBS
All subjects	96	1.69 ± 0.35	27.20 ± 4.97	0.33 ± 0.12	0.67 (0.42–0.83)	0.50 (0.17–0.92)
TNF-A^{-308}						
11	76	1.67 ± 0.35	27.12 ± 4.85	0.33 ± 0.11	0.67 (0.33–0.83)	0.50 (0.25–0.92)
12	18	1.78 ± 0.35	27.94 ± 5.52	0.34 ± 0.15	0.58 (0.50–0.83)	0.50 (0.17–0.83)
22	2	1.42 ± 0.12	23.63 ± 5.36	0.36 ± 0.23	0.25 (0.00–0.50)	0.75 (0.17–1.33)
ANOVA		$F = 1.31$	$F = 0.72$	$F = 0.19$	$H = 2.53$	$H = 0.29$
		$p = 0.274$	$p = 0.489$	$p = 0.829$	$p = 0.282$	$p = 0.866$
LT-A^{+252}						
11	60	1.68 ± 0.35	27.04 ± 4.98	0.34 ± 0.12	0.67 (0.42–0.83)	0.50 (0.17–0.66)
12	31	1.67 ± 0.37	27.27 ± 5.10	0.32 ± 0.14	0.67 (0.33–0.83)	0.83 (0.17–1.00)
22	1	2.00 ± –	30.92 ± –	0.28 ± –	0.83 –	1.00 –
ANOVA		$F = 0.43$	$F = 0.31$	$F = 0.24$	$H = 0.78$	$H = 3.39$
		$p = 0.650$	$p = 0.737$	$p = 0.786$	$p = 0.676$	$p = 0.184$
IL-6^{-597}						
11	48	1.71 ± 0.34	27.25 ± 4.88	0.33 ± 0.12	0.67 (0.33–0.83)	0.50 (0.17–1.00)
12	40	1.64 ± 0.37	26.76 ± 5.13	0.32 ± 0.12	0.58 (0.50–0.83)	0.50 (0.17–0.75)
22	8	1.79 ± 0.26	29.09 ± 4.77	0.40 ± 0.15	0.50 (0.50–0.83)	0.83 (0.42–1.17)
ANOVA		$F = 0.86$	$F = 0.74$	$F = 1.61$	$H = 0.03$	$H = 2.27$
		$p = 0.427$	$p = 0.482$	$p = 0.206$	$p = 0.985$	$p = 0.322$
IL-6^{-174}						
11	50	1.72 ± 0.37	27.28 ± 5.22	0.33 ± 0.12	0.67 (0.33–1.00)	0.50 (0.17–1.00)
12	36	1.62 ± 0.32	26.61 ± 4.78	0.31 ± 0.12	0.50 (0.50–0.75)	0.50 (0.17–0.75)
22	10	1.75 ± 0.27	28.93 ± 4.24	0.39 ± 0.13	0.50 (0.50–1.00)	0.83 (0.33–1.00)
ANOVA		$F = 1.06$	$F = 0.87$	$F = 1.84$	$H = 1.80$	$H = 3.18$
		$p = 0.352$	$p = 0.422$	$p = 0.165$	$p = 0.406$	$p = 0.204$

*Interquartile range.
AngBS, angulated bleeding score; CPE, cumulative plaque exposure; GCF, gingival crevicular fluid; GI, gingival index; IL-6, interleukin-6; LT-A, lymphotoxin alpha; PI, plaque index; SD, Standard deviation; TNF-A, tumour necrosis factor alpha.

Table 4. Comparisons of genotypic (A) and allelic (B) distribution of TNF-A, LT-A and IL-6 single nucleotide polymorphisms in HR ($n = 24$) and LR ($n = 24$) subjects

	ML $\chi^2_{[2]}$	p
(A)		
TNF-A^{-308}	0.55	0.760
LT-A^{+252}	2.66	0.264
IL-6^{-597}	1.62	0.446
IL-6^{-174}	2.94	0.230
	ML $\chi^2_{[1]}$	p
(B)		
TNF-A^{-308}	0.38	0.539
LT-A^{+252}	0.06	0.803
IL-6^{-597}	0.84	0.361
IL-6^{-174}	2.61	0.106

HR, high responder; IL-6, interleukin-6; LR, low responder; LT-A, lymphotoxin alpha; ML, maximum likelihood; TNF-A, tumour necrosis factor alpha.

chronic periodontitis has about 50% heritability (Michalowicz et al. 2000).

Growing evidence suggests that gingival inflammation may represent a true risk factor for attachment loss and tooth loss (Suda et al. 2000, Schatzle et al. 2003, 2004), and that an association may exist between susceptibility to gingivitis and susceptibility to periodontitis (Trombelli 2004, Dietrich et al. 2006).

In this context, it is feasible that genes implicated in the regulation of inflammatory process of periodontal tissues associated with plaque accumulation may play a role in explaining the individual variability in the severity of both plaque-induced gingivitis and destructive periodontitis. Therefore, the purpose of the present study was to investigate whether and to what extent gene polymorphisms of inflammatory cytokines that had been putatively associated with periodontitis, such as TNF-α, IL-6 and LT-α, may be involved in the subject susceptibility to plaque-induced gingivitis in a Caucasian Italian population. Our results suggest that the IL-6, TNF-A and LT-A gene polymorphisms analysed do not appear to be involved in determination of the clinical expression of plaque-induced gingivitis in our population. Moreover, when we separately analysed two subgroups of subjects with a different inflammatory response to similar plaque accumulation, no differences in genotype and allelic distribution for any investigated polymorphisms could also be detected.

One of the significant features of a complex disease is the modest contribution of each susceptibility gene to the onset of the disease (Lohmueller et al. 2003). According to this idea, sample sizes must be quite large to have enough power to achieve even nominal significance ($p < 0.05$), often in the 1000–10,000 range, unreasonable numbers for experimental gingivitis trials. Thus, although the lack of a significant association between the investigated polymorphisms and the clinical parameters of gingivitis may reflect false-negative results due to a limited sample size and consequently low statistical power, our findings seem to indicate that these candidate polymorphisms may not represent a major factor affecting gingival inflammatory response to plaque.

In 1998, Kornman & Di Giovine (1998) reported a higher TNF-A^{-308}*2 allelic frequency in Caucasian patients with chronic periodontitis than in healthy individuals. This difference was correlated with the severity of the disease. Similarly, Galbraith et al. (1999) demonstrated that the TNF-A^{-308}*2 allele is a risk factor for the severity of adult chronic periodontitis, as its presence is detected in the most severe cases of the disease (Galbraith et al. 1998). More recently, Lin et al. (2003) demonstrated an increased frequency for the TNF-A^{-308}*2 allele in Chinese patients with chronic periodontitis. Fassmann et al. (2003) demonstrated, in a Czech population, that the TNF-A (−308G/A) polymorphism itself showed no association with chronic periodontitis, whereas the frequency distribution of the LT-A (+252A/G) genotypes showed statistically significant differences between diseased cases and healthy controls. Moreover, a significant difference in the frequencies of the combined genotypes (TNF-A and LT-A) between the control and the patient groups was found. Recently the association between periodontitis and TNF-A^{-308} gene polymorphism has been questioned by two independent reviews (Kinane & Hart 2003, Takashiba & Naruishi 2006). Consistently, our analyses indicate an absence of the influence of TNF-A^{-308} and LT-A^{+252} gene polymorphisms, studied both as a single SNPs and as combined genotypes, on the clinical expression of plaque-induced gingivitis.

Yoshie et al. (2007) reviewed the role of IL-6 polymorphisms in periodontitis. They reported that the G–C SNP at the (− 174) position correlated with chronic periodontitis susceptibility in Brazilian Caucasians but not in Czech Caucasian populations. With regard to the other IL-6 SNP locations, the Czech study suggested that the (− 572) G/C polymorphism of the IL-6 gene may be one of the protective factors associated with lower susceptibility to chronic periodontitis (Hollà et al. 2004). Several studies indicate the importance of considering the cooperative influence of the IL-6 promoter variants, (− 174) G/C, (− 190) C/T and (− 597) G/A, in determining the response to disease (Terry et al. 2000, Muller-Steinhardt et al. 2004). Our material suggests that the IL-6 gene polymorphisms analysed are not implicated in the subject variability in the clinical expression of plaque-induced gingivitis, either as single SNPs or as combined genotypes. Furthermore, no genetic differences have been observed between the two subgroups with different gingivitis susceptibilities.

Studies on the possible genetic factors determining or modulating susceptibility to gingivitis are limited (Goodson et al. 1982, Reuland-Bosma et al. 1986, Preshaw et al. 1998, Jepsen et al. 2003, Scapoli et al. 2005). It has been shown that children with Down' syndrome manifest more extensive and severe gingival inflammation at an earlier age than age- and gender-matched healthy controls, despite having no differences in plaque accumulation rates (Reuland-Bosma et al. 1986). This study represents the only definitive report of a genetic condition associated with susceptibility to plaque-induced gingivitis. Recently, we have demonstrated an association between IL-1RN polymorphism and subject-based clinical behaviour of the gingiva in response to de novo plaque accumulation, as well

as a possible association between IL-1B $^{-511}$ polymorphism and gingivitis susceptibility (Scapoli et al. 2005). In contrast, the results emerging from other studies on IL-1 polymorphisms on different ethnic groups (Goodson et al. 1982, Jepsen et al. 2003) and from our investigation on polymorphisms in TNF-A, LT-A and IL-6 genes seem to indicate that, if there is a role for any of these variants in the inflammatory process involved in gingivitis, it will be either population specific and/or may exert a minor effect on the clinical expression of plaque-induced gingivitis.

The low prevalence of certain alleles or genotypes in a particular ethnic/patient group may limit the reliability of the conclusions on genotype associations with clinical findings. Ample evidence pointing to significant ethnic differences in gene polymorphisms and their association with periodontal disease (Yoshie et al. 2007) calls for studies in individual ethnic groups.

A major conceptual weakness of many studies is the assumption that a genuine association between a particular gene and disease will be simple, with one or a few major mutations accounting for the extent of the genetic load. It is reasonable to assert that gingivitis as well as periodontitis are not single-gene but polygenic diseases. The concept of ''complex diseases'' implies that in some cases, dozens or even hundreds of gene variants could be interacting on a population-wide basis to result in a given condition. The fact that many diseases show a widely varying course indicates that several subgroups of patients may exist, each with a particular genetic profile. Similar to other complex diseases, it is estimated that between 10 and 50 genes with several major master genes may be involved in periodontitis (Suzuki et al. 2004).

It is feasible that a concept such as the ''susceptibility profile'', described for other diseases (McGeer & McGeer 2001), may also pertain to both gingivitis and periodontitis, where several relatively common high-risk polymorphisms could be inherited by an individual, giving him a cumulative high-susceptibility profile.

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Clinical Relevance

Scientific rationale for the study: Genes implicated in the regulation of inflammatory process of periodontal tissues associated with plaque accumulation may play a role in explaining the individual variability in the severity of both plaque-induced gingivitis and destructive periodontitis. Therefore, we investigated the association between gene polymorphisms in inflammatory cytokines, TNF-A, IL-6 and LT-A and gingivitis.

Principal findings: These polymorphisms do not appear to be involved in the clinical expression of plaque-induced gingival inflammation.

Practical implications: Our findings suggest the possibility that these candidate polymorphisms may be population-specific and/or may exert a minor effect on clinical expression of plaque-induced gingivitis.