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Lack of association between the TNFα G $-$ 308 A promoter polymorphism and periodontal disease

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Abstract

Background: Immunorelevant gene polymorphisms might influence the susceptibility for periodontal disease. The present study assessed the frequency of a promoter polymorphism (− 308G-to-A) of the tumour necrosis factor (TNF)α gene in patients with periodontitis and controls.

Methods: Eighty-one patients with generalized chronic periodontitis and 80 healthy controls were genotyped for the − 308 polymorphism of the TNFα gene by PCR amplification and subsequent restriction fragment length polymorphism analysis. The diagnosis of chronic periodontitis was made for each subject on basis of standardized clinical and radiographic criteria.

Results: In patients with peridontitis and controls, the frequency of the TNFα − 308 A allele was comparable (19.1% [31/162] versus 13.8% [22/160]; $p = 0.193$).

Conclusion: The present study revealed no association between the − 308 TNFα gene polymorphism and periodontal disease.

Key words: cytokine; destruction; proinflammatory; tissue; tumour necrosis factor α

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Chronic periodontitis is an inflammatory disease that results from the interaction of periodontopathogenic bacteria and host immune response mechanisms. This disease entity is characterized by an inflammatory reaction involving the supporting tissues of the teeth, comprising the marginal gingiva, periodontal attachment fibres and the alveolar bone (Page 1999). The local inflammatory response is initiated by several bacteria-derived virulence factors, e.g. lipopolysaccharides (Socransky et al. 1998). These virulence factors stimulate and amplify the production of numerous proinflammatory cytokines, i.e. interleukin-1β, interferon-γ, and tumour necrosis factor (TNF)α, which subsequently leads to the destruction of soft tissue and bone (Graves et al. 2000).

The results of previous studies support the model that variations in the immunological control of the periodontopathogenic infection influence the individual host susceptibility for periodontal disease. According to this concept either an inappropriate or exaggerated immune response against a given bacterial stimulus leads to a different susceptibility and/or severity of periodontitis (Page et al. 1997). The factors contributing to variations of the immune response include race, gender, and genetic predisposition (Hassell & Harris 1995). Genetic factors were estimated to account for even 50% of the increased susceptibility for periodontitis (Michalowicz et al. 1991).

Several genetic factors that may contribute to an enhanced susceptibility have been identified previously (Kornman et al. 1997, Gwinn et al. 1999, Hennig et al. 1999). Recent studies have particularly focused on the role of polymorphisms in genes encoding proinflammatory cytokines as genetic markers for periodontitis (Kornman & di Giovine 1998). Some of these genetic polymorphisms were suggested to result in changes of transcriptional activity and, hence, of cytokine production within the inflamed tissue (Bidwell et al. 1999). In fact, different levels of proinflammatory cytokines, i.e. TNFα, were found clinically in the gingival crevicular fluid and gingival tissues of patients with and without periodontal disease (Rossomando et al. 1990, Ebersole et al. 1993). Several polymorphisms in the promoter region of the human TNFα gene have been identified, which were suspected to

cause changes in the clinical expression of TNFα (Kinane et al. 1999). Although the influence of the different TNFα genotypes on the phenotypic cytokine production is not fully established, thus far, it was previously suggested that specifically the polymorphism at the − 308 base leads to changes in the TNFα production (Kroeger et al. 2000).

The purpose of the present study was to compare the allele distribution of the − 308 polymorphism of the TNFα gene in a population with chronic generalized periodontitis with that in a representative control group.

Materials and Methods

Study population

Periodontitis patients

Eighty-one consecutive periodontitis patients from the Department of Periodontology, Ludwig-Maximilians University (Munich, Germany), participated in this study. Within the periodontitis group, the age ranged from 19 to 74 years and the median age was 54 years (SD ± 12.4). The male to female ratio was 55–45%. All patients were adult Caucasians and presented with the diagnosis of generalized chronic periodontitis. The diagnosis was made for each subject employing a standardized periodontal evaluation procedure which comprised (1) the assessment of probing pocket depth measured with a Michigan-type ''O'' probe at six locations on each tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual, disto-lingual), (2) bleeding on probing registered as present or absent, (3) examination of furcation involvement by means of a Naber-type probe, and (4) bone loss estimated by orthopantomographs. The probing pocket depth was determined as the distance from the base of the periodontal pocket to the free gingival margin keeping the probe in line with the long axis of the tooth. The furcation defects were assessed by horizontal probing from the furcation entrance to the base of the defect. The evaluation of the furcation involvement was done on basis of the classification of Nyman & Lindhe (1997).

Each patient had to present the following characteristics to be classified as having generalized periodontitis: (1) at least 15 teeth remaining in situ, (2) probing pocket depth of ⩾5 mm and/or a furcation involvement ⩾class II at one or more locations at ⩾8 teeth, (3) a minimum of one-third of remaining teeth are periodontally involved, and (4) alveolar bone loss defined as a distance of ⩾3 mm between the alveolar crest and the cemento-enamel junction around the clinically affected teeth. Individuals included into this group were assessed on basis of the abovementioned criteria as having mild, moderate, or severe chronic periodontitis according to the classification of Flemmig (1999). Subjects providing clinical, radiographic, and/or microbiologic symptoms of aggressive periodontitis according to the classification of Tonetti & Mombelli (1999) were excluded from the study.

Patients completed personal and family history questionnaires, and were excluded for a history of severe medical disorders including diabetes mellitus, immunological disorders, increased risk for bacterial endocarditis, and pregnancy.

Control group

The referent population group comprised a sample of 80 unrelated, ethnically matched, healthy Caucasian individuals. The periodontal status of the control subjects had to meet the following criteria: (1) at least 22 teeth in situ, (2) not more than one site with probing pocket depth = 3 mm, and (3) no furcation involvement at any tooth. The median age within the control group was 45 years (SD ± 9.5) and the age ranged from 35 to 73 years.

Written informed consent was obtained from all study participants. The study protocol was approved by the ethics committee of the medical faculty of the Ludwig-Maximilians University (No. 290/01).

DNA isolation and genotyping of the − 308G-to-A polymorphism

Genomic DNA was obtained from peripheral venous blood samples (9 ml) by standard venipuncture. All blood samples were collected in sterile tubes containing potassium EDTA solution. Genomic DNA was either isolated from buffy coats using a commercially available kit (QIAamp® Blood Midi Kit, Qiagen, Hilden, Germany) or by a simple salting out procedure (Miller et al., 1988). Genotyping of the G-to-A polymorphism in the promoter region at position − 308 of the TNFα gene was carried out by PCR amplification and subsequent restriction fragment length polymorphism analysis according to Wilson et al. (1993). Briefly, a 107 bp PCR product was generated employing the primers 5′-AGGCAATAGGTTTT-GAGGGCCAT-3′ and 5′-TCCTCCCT-GCTCCGATTCCG-3′. The underlined base in the forward primer is different from the original sequence and serves for introduction of a recognition site for the restriction enzyme *Nco*I. A hot-start PCR was carried out using HotStar Taq® DNA Polymerase (Qiagen). The PCR conditions comprised an initial denaturation step of 15 min at 95°C, 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 52°C, and extension for 30 s at 72°C, and a final extension step for 10 min at 72°C. The final concentration of $MgCl_2$ was 3 mM. The total volume of the PCR was 50 μl, 30 μl of this product were digested with *Nco*I at 37°C overnight and analysed by electrophoresis on 2.5% agarose gels. The − 308 G allele is digested by *Nco*I resulting in a fragment of 87 and 20 bp for an individual homozygous for this allele. In the case of an individual homozygous for the − 308 A allele, which is not digested by *Nco*I, only the full-length PCR product of 107 bp is present. A heterozygous individual displays all three fragments of 107, 87, and 20 bp length (Fig. 1).

Statistical analysis

The distribution of the different genotypes of the − 308 TNFα polymorphism was analysed employing Pearson's χ^2 test. The allele frequencies were compared between the periodontitis and the controls using the Fisher's exact test. For all statistical procedures the significance was set at 5% ($p < 0.05$).

Results

The GA and AA genotypes were present in 33.3% and 2.5% of patients with periodontitis, respectively, whereas in the control group frequencies were 27.5% and 0% ($p = 0.228$) (Table 1). There was also no significant difference observed between patients and controls with respect to the frequency of the TNFα − 308 A allele (19.1% [31/162] versus 13.8% [22/160]; $p = 0.193$) (Table 2).

Discussion

TNFα is a proinflammatory cytokine of paramount importance for various in-

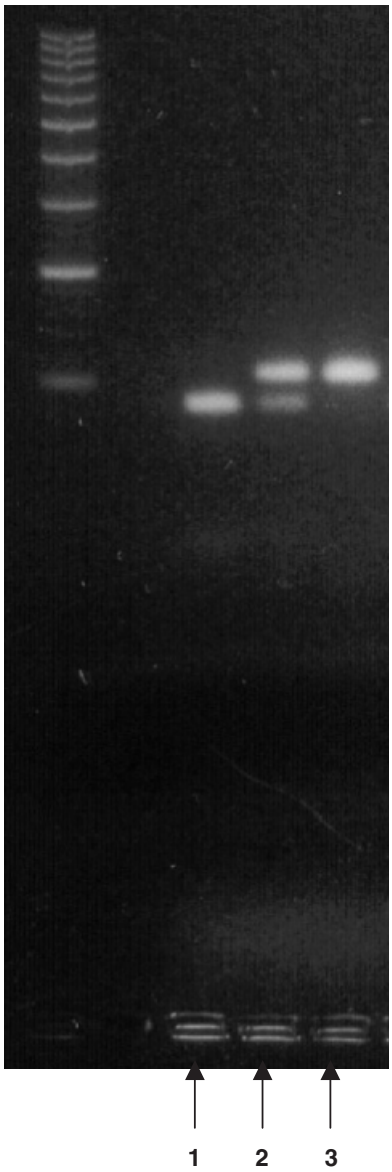

Fig. 1. Agarose gel electrophoresis of the −308 tumour necrosis factor (TNF)α gene polymorphism. Lane 1: homozygous GG individual (80/20 bp); lane 2: heterozygous individual GA (107/80/20 bp); lane 3: individual homozygous for the TNFα −308 A allele (107 bp).

Table 1. Distribution of the different genotypes of the −308 TNFα polymorphism as obtained in the periodontitis and the representative control group

	Periodontitis (%)	Control (%)
GG	52 (64.2)	58 (72.5)
GA	27 (33.3)	22 (27.5)
AA	2 (2.5)	0

The differences between groups were not significant as analysed with Pearson's χ^2 test ($p = 0.228$). TNFα, tumour necrosis factor α.

Table 2. Frequency of the alleles of the −308 TNFα gene polymorphism among periodontitis subjects and representative control individuals

	Periodontitis (%)	Control (%)
TNFα −308 G	131 (80.9)	138 (86.2)
TNFα −308 A	31 (19.1)	22 (13.8)

The differences between groups were not significant as analysed with Fisher's exact test ($p = 0.193$). TNF, tumour necrosis factor α.

flammatory conditions (for an overview, see Verweij 1999) that has recently also gained attention in periodontal disease due to its effects on bone and soft tissue metabolism (for an overview, see Graves & Cochran 2003). For example it was shown that TNFα has a strong potential to increase bone resorption (Stashenko et al. 1987, Nair et al. 1996) and is involved in the degradation of connective tissue (Delima et al. 2001). On basis of these observations and the generally accepted hypothesis that periodontitis has a genetic background (Page et al. 1997), it was assumed that the individual susceptibility for periodontitis might be related to genetically determined differences in the TNFα production (Kornman et al. 1997, Shapira et al. 2001, Craandijk et al. 2002).

Several promoter polymorphisms of the TNFα gene have been identified, which were found to cause changes of the transcriptional activity (Galbraith et al. 1998, Bidwell et al. 1999, Endo et al. 2001). Although there exists only limited evidence on this issue, it was proposed that differences of the transcriptional activity might directly result in changes of the phenotypic expression of TNFα (Kroeger et al. 2000).

The prevalence of the various polymorphisms of the TNFα gene promoter region shows considerable ethnic differences. The −308 and the −238 TNFα gene polymorphism were found in comparably high prevalence in Caucasians whereas several other polymorphisms were almost exclusively observed in Japanese populations (Endo et al. 2001). Among the polymorphisms that are more prevalent in Caucasian subjects, particularly the transition in the −308 position was postulated to cause changes in the phenotypic expression of TNFα.

As reported previously the guanine to adenine change at position −308 leads to two- to threefold higher transcriptional activity of TNFα upon stimulation with bacterial lipopolysaccharide (Kroeger et al. 2000). Consistently, individuals who are homozygous for the TNFα −308 A allele (adenine in position −308) have higher circulating TNFα levels than homozygotes for the TNFα −308 G allele (Bouma et al. 1996). Moreover, oral neutrophils from periodontitis patients with the transition to adenine in the −308 position produced significantly higher amounts of TNFα as compared with wild-type individuals (Galbraith et al. 1998). The increased release of TNFα in response to a given bacterial stimulus might probably amplify the inflammatory and, thus, the destructive immune process within the infected periodontal tissue of these individuals. Although the biological significance of these findings are yet not fully established, it was proposed that this genotype could be a prognostic marker in periodontitis.

In the present study, a significant difference regarding the allele or genotype distribution in controls and patients was not observed and results presented herein are in accordance with the majority of literature data (Galbraith et al. 1999, Shapira et al. 2001, Craandijk et al. 2002). However, some studies revealed a higher frequency of the TNFα −308 A allele for both, periodontitis patients and control subjects (Kornman et al. 1997, Galbraith et al. 1998). Although the latter studies did not refer specifically to this issue, these differences could at least partially be attributed to ethnic differences between study populations. Galbraith et al. (1999) described a significantly different allele carriage rate for patients with advanced periodontitis as compared with healthy individuals. Interestingly, in this study the TNFα −308 G allele (guanine in position −308) was found more frequent in periodontitis. Commonly the TNFα −308 A allele is considered to be the marker of disease susceptibility due to the upregulatory influence of this allele on the TNFα production.

Different reasons might have contributed to the lack of association between the −308 TNFα polymorphism and periodontal disease. Although it is generally accepted that chronic periodontitis has a genetic background epigenetic factors also might be of pathogenetic importance as well (Kinane et al. 1999) resulting in a multifactorial complex disease entity. Furthermore, the inheritance of periodontitis does not follow

simple Mendelian rules and appears to involve multiple genes. Thus, the analysis of single genetic polymorphisms might be an insufficient tool aiming on the delineation of the genetic background. As recently described in other complex genetic disorders such as Crohn's disease linkage analysis might provide a reasonable approach for future studies.

Furthermore, as mentioned above, the TNFα −308 A allele was shown to be related with a considerable higher transcriptional activity (Kroeger et al. 2000), but there is only limited evidence thus far that this in fact causes differences in the production of TNFα (Louis et al. 1998). Linkage between the −308 TNFα polymorphism and periodontal disease seems only plausible if different alleles lead not only to changes in the transcriptional activity but also in the expression of TNFα within the inflamed periodontal tissue.

Finally, also the selection of patients for both the study groups might have influenced the results as found herein. It is generally accepted that there are only insufficient clinical and radiographic criteria available to reliably define and differentiate between various periodontal disease entities, i.e. chronic and aggressive periodontitis (Tonetti & Mombelli 1999). Moreover, specifically chronic periodontitis was recommended to be classified on basis of the disease severity since this parameter was proposed to be a highly relevant prognostic factor (Flemmig 1999). Individuals as included in the periodontitis group of this study showed different degrees of disease severity. Hence, despite the use of commonly accepted criteria for the diagnosis of periodontitis, the selection of individuals for the both study groups might comprise a certain limitation of this study. However, since the alleles and genotypes of the −308 TNFα polymorphism were almost equally distributed among periodontitis patients as compared with healthy control individuals, it can be assumed that further investigations using different selection criteria might also not reveal any kind of association between the −308 TNFα polymorphism and periodontitis.

In conclusion, within the limitations of this study, the allele frequency for the −308 TNFα gene polymorphism was similar for patients with chronic periodontitis and healthy control subjects. Hence, this particular polymorphism appears not to be associated with the susceptibility for periodontitis.

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