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Polymorphic cytokine genotypes as markers of disease severity in adult periodontitis

Gillian M. P. Galbraith[1,2], Thomas M. Hendley[2], John J. Sanders[2], Yuko Palesch[3] and Janardan P. Pandey[1]
Departments of [1]Microbiology and Immunology, [2]Stomatology and [3]Biometry, Medical University of South Carolina, Charleston, SC, USA

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Abstract. The distributions of the bi-allelic interleukin-1β^{+3953} and tumor necrosis factor-α^{-308} genotypes were determined in 20 patients with advanced adult periodontitis, 20 patients with plaque associated gingivitis, and 45 referent population subjects. A significant increase in IL-1β^{+3953} allele 2 frequency was found in patients with advanced periodontitis compared to referent subjects (the Fisher exact test; $p=0.013$). Furthermore, the frequency of TNF-α^{-308} allele 1 was significantly greater in patients with advanced disease compared to those with plaque associated gingivitis (the Fisher exact test; $p=0.014$). No significant correlation was observed between genotype and cytokine production in these patient populations.

Key words: periodontal disease; TNF-α; IL-1β; polymorphisms; genotypes

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Periodontal diseases include a broad spectrum of inflammatory and destructive responses to oral microbes in the susceptible human host. While uncomplicated gingivitis is a common concomitant of inadequate oral hygiene, progression to severe periodontitis with loss of supporting structures is influenced by several factors, including genetic predisposition (Hassel & Harris 1995, Hart & Kornman 1997). The search for genetic markers of periodontitis has been generally disappointing, since no consistent disease associations have emerged. However, recent studies of cytokine gene polymorphisms in patients with periodontitis have yielded more encouraging results.

The rationale for the examination of cytokine genotypes in periodontal disease is intuitively attractive, since it is recognized that cytokines such as interleukin-1α and β (IL-1α and IL-1β) and tumor necrosis factor-α (TNF-α) may play a pivotal role in the pathogenesis of periodontitis (reviewed by Offenbacher 1996), and several of the known polymorphisms of the IL-1 and TNF gene complexes appear to influence gene regulation. For example, the bi-allelic restriction fragment length polymorphism (RFLP) due to a single base exchange at position +3953 within the fifth exon of the IL-1β gene (IL-1β^{+3953}) has been associated with the level of IL-1β production (Pociot et al. 1992); similarly, the single base polymorphism within the promoter region of the TNF-α locus at position −308 (TNF-α^{-308}) influences the level of TNF-α gene transcription (Wilson et al. 1997, Kroeger et al. 1997).

The first study of cytokine gene polymorphisms in periodontal disease was reported by Kornman et al., who found a significant association between a composite IL-1 genotype and advanced adult periodontitis (1997). This genotype included the less common allele 2 of the IL-1β^{+3953} polymorphism and allele 2 of a single base polymorphism at position −889 of the IL-1α gene (IL-1α^{-889}). Moreover, this association was demonstrable only in patients who were non-smokers. In contrast, studies in our laboratory revealed a significant association between the IL-1β^{+3953} allele 2 alone and severity of adult periodontitis in a small patient population that included smokers (Gore et al. 1998). In a subsequent study of the same population, we were unable to detect significant differences in the distribution of TNF-α^{-308} genotypes, although oral neutrophils from patients with the uncommon allele 2 of this polymorphism were found to produce greater levels of TNF-α (Galbraith et al. 1998).

The purpose of the study reported here was to further examine the distributions of the IL-1β^{+3953} and TNF-α^{-308} genotypes in a larger number of patients with adult periodontitis of strictly defined severity and a referent population. In addition, the influence of genotype on cytokine synthesis by oral and peripheral blood polymorphonuclear leukocytes (PMN) was examined.

Material and Methods

Subjects

This study was approved by the Institutional Review Board for Human Research. Patients were selected according to the guidelines published by the American Academy of Periodontology (1996). The patient population included 20 subjects with plaque-associated gin-

givitis with no attachment loss, and 20 patients with adult periodontitis characterized by advanced loss of periodontal support and at least 3 sites with probing depths equal to or greater than 7 mm and attachment loss greater than 5 mm. Diagnosis was made on the basis of physical examination, medical and dental history, probing depth, assessment of attachment loss, tooth mobility, observation of bleeding on probing, and full mouth radiography. All patients selected for study were between the ages of 35 and 65 years. The gingivitis group (age 48.4±9.2 years, mean±SD) included 7 males and 13 females, and three smokers. The group with advanced disease (age 46.8±8.9 years) was comprised of 6 males and 14 females, and 15 smokers. The sample was limited to Caucasian subjects, since racial differences are common in polymorphic systems (Mourant et al. 1976). All patients were in good health apart from their periodontal disease.

The referent population consisted of 45 unrelated Caucasian subjects in good health, residing in the same geographic area as the patients studied, and of unknown periodontal health status. After informed consent was obtained, patients provided an oral rinse sample and a peripheral blood sample; referent subjects donated only peripheral blood.

Isolation of oral PMN

Oral PMN were obtained by a standardized mouth rinse procedure as described elsewhere (Hendley et al. 1995, Galbraith et al. 1997). The resulting cell population included >90% PMN of >95% viability as assessed by trypan blue dye exclusion; mononuclear leukocytes were very rarely seen. The PMN were resuspended in a solution containing 10 mM glucose, 150 mM NaCl, 3 mM KCl, 5 mM Hepes, 2 mM $CaCl_2$, and 100 μg/ml ampicillin, pH 7.4, and incubated at 37°C for 3 h. Culture supernatants were stored at −70°C for subsequent measurement of IL-1β and TNF-α content.

Isolation of peripheral blood PMN

PMN were obtained from heparinized peripheral venous blood by dextran sedimentation and density gradient centrifugation as previously described (Galbraith et al. 1997), and resuspended in RPMI medium 1640 containing 10% fetal calf serum (both from Sigma Chemicals, St. Louis, MO) at a concentration of 2×10^7/ml. Cells were cultured for 5 h at 37°C in the presence or absence of 1 μg/ml lipopolysaccharide (LPS, *E. coli* serotype 111:B4, Sigma Chemicals); these culture conditions had been previously determined to be optimum for stimulation of cytokine synthesis by PMN. Supernatants were stored at −70°C until measurement of IL-1β and TNF-α levels.

Quantitation of IL-1β and TNF-α in cell supernatants

This was determined in duplicate samples by an enzyme-linked immunosorbant assay (ELISA) (CYTImmune Sciences, Inc., College Park, MD). This employed an antibody sandwich method, using microtiter plates coated with monoclonal murine antibody to the appropriate human cytokine, a second polyclonal rabbit antibody to the cytokine, and an alkaline phosphatase-labeled goat antibody to rabbit immunoglobulin. Following the addition of a color generating system, the optical densities of the reaction mixtures were read at 492 nm and analysed using a Bio-kinetics microplate reader (Bio-Tek Instruments, Winooski, VT).

Isolation of genomic DNA

Mononuclear cells were obtained from the interface of the density gradient (above). Genomic DNA was isolated from these samples using Puregene (Gentra Systems, Minneapolis, MN). The DNA was resuspended in Puregene hydration solution at a concentration of 100 μg/ml and stored at −20°C until analysed for IL-1β^{+3953} and TNF-α^{-308} genotypes.

IL-1β^{+3953} genotyping

Genotypes of the IL-1β^{+3953} RFLP were determined by polymerase chain reaction (PCR) as previously described (Gore et al. 1998), using GeneAmp PCR kit (Perkin Elmer, Norwalk, CT) and the following primers: 5′-CTCAGGTGTCCTCCAAGAAATCAAA-3′ and 5′-GCTTTTTTGCTGTGAGTCCCG-3′. The 194 bp region of the IL-1β gene was amplified for 35 cycles using the Perkin Elmer model 9600 thermocycler at 95°C for 15 s and 60°C for 30 s. Taq1 restriction digestion of the PCR product and subsequent electrophoresis on a 10% polyacrylamide gel resulted in two fragments of 86 bp and 108 bp in subjects homozygous for allele 1 and a single fragment of 194 bp in subjects homozygous for allele 2. All three fragments were present in heterozygous subjects.

TNF-α^{-308} genotyping

TNF-α^{-308} genotypes were determined as described elsewhere (Galbraith et al. 1998). In brief, the 117 bp region of the TNF-α gene was amplified using the following primers: 5′-AGGCAATAGGTTTTGAGGGCCAT-3′ and 5′-ACACTCCCCATCCTCCCGGCT-3′. PCR was performed for 35 cycles of 95°C and 60°C for 15 and 30 s, respectively. Nco1 digestion of the amplified product and subsequent electrophoresis revealed the two alleles of TNF-α^{-308}: T1 (fragments of 97 bp and 20 bp), and T2 (117 bp).

Analysis of data

The Fisher's 2-tailed exact test was used to examine differences in distribution of IL-1β and TNF-α genotypes and alleles in patients with the two presentations of periodontal disease and the referent population, using the SAS software (SAS Institute, Inc. 1990). Because we had previously found evidence of association between IL-1β^{+3953} allele 2 and severity of adult periodontal disease (Gore et al. 1998), the need for Bonferroni correction for experiment-wise error rate was obviated. Supernatant cytokine levels did not fall within a normal distribution; therefore the data were transformed by inversion before analysis by the *t*-test. In all instances, the alpha level of 0.05 determined the statistical significance of the results.

Results

The distributions of IL-1β and TNF-α genotypes in patients and referent subjects are shown in Table 1. The frequency of IL-1β^{+3953} 1,2 and 2,2 genotypes (allele 2 carriage rate) was significantly elevated in the total patient group compared to the referent population ($p=0.043$, Fisher's exact test); this reflected the increased frequency in patients with advanced adult periodontitis compared to referent subjects ($p=0.048$). No statistically significant difference in the allele 2 carriage rate was found between patients with gingivitis

Table 1. Distribution of IL-1β and TNF-α genotypes in adult periodontitis

Genotype	Adult periodontitis		Referent population (n=45)
	Gingivitis (n=20)	Advanced (n=20)	
IL-1β^{+3953} a)			
1/1	11 (55%)	9 (45%)	33 (73.3%)
1/2	8 (40%)	7 (35%)	9 (20%)
2/2	1 (5%)	4 (20%)	3 (6.7%)
TNF-α^{-308} b)			
1/1	13 (65%)	18 (95%)	34 (75.6%)
1/2	5 (25%)	1 (5%)	11 (24.4%)
2/2	2 (10%)	0	0

a) The proportion of genotypes containing IL-1β^{+3953} allele 2 was significantly increased in patients with advanced adult periodontitis compared to the referent group (p=0.048, the Fisher exact test; odds ratio 3.36, 95% confidence interval (1.12, 10.11)).

b) The proportion of the TNF-α^{-308} 1,1 genotype was significantly increased in patients with advanced disease compared to those with gingivitis (p=0.044, The Fisher exact test; odds ratio 10.20, 95% confidence interval (1.12, 90.91)); also increased compared to referent subjects (p=0.086, not significant). Data not available for one subject with advanced periodontitis.

and those with advanced disease, or between patients with gingivitis and the referent group. Further analysis revealed that the IL-1β^{+3953} allele 2 frequency was even more significantly increased in patients with advanced disease compared to referent subjects (p=0.013).

Examination of the TNF-α^{-308} polymorphism in these populations revealed a significant increase in the 1,1 genotype in patients with advanced periodontitis compared to those with gingivitis (p=0.044), and an even more significant increase in allele 1 frequency between the two patient groups (p=0.014). The increase in both the 1,1 genotype and the allele 1 frequency in patients with advanced disease compared to referent subjects failed to reach significance.

Studies of cytokine production showed that IL-1β synthesis by oral PMN from patients with advanced periodontitis was significantly greater than that of patients with gingivitis (57.4±17 pg/2×10^6 PMN versus 36.8±9.7 pg/2×10^6 PMN, respectively, mean±SE; p=0.04, t-test of transformed data). No significant differences in IL-1β production by LPS-activated peripheral blood PMN, or in TNF-α synthesis by either oral or peripheral PMN were found (data not shown).

Analysis of cytokine synthesis by PMN of patients with the different cytokine genotypes revealed no statistically significant correlations between genotype and level of production. However, there was a trend towards increased IL-1β production by oral PMN in patients with advanced disease who possessed the IL-1β^{+3953} allele 2 compared to those lacking allele 2 (74±29.2 pg/2×10^6 PMN versus 37.1±11.6 pg/2×10^6 PMN, respectively).

Discussion

IL-1β and TNF-α are proinflammatory cytokines that have received a considerable amount of attention as potentially pathogenic factors in periodontal disease because of their effects on bone and fibroblast metabolism (Takeshita et al. 1992, DuFour et al. 1993, Manolagas 1995, Offenbacher 1996, Wondimu & Modeer 1997). Numerous reports have described increased levels of these cytokines in crevicular fluid and periodontal tissues of patients with periodontitis (Masada et al. 1990, Rossomando et al. 1990, Stashenko et al. 1991, Tokoro et al. 1996, Roberts et al. 1997), and there is evidence that antagonists of IL-1 and TNF can inhibit bone loss associated with induced periodontitis in animal models (Assuma et al. 1998). The source of these cytokines in the inflamed periodontium includes several cell types (Agarwal et al. 1995, Hendley et al. 1995, Hillmann et al. 1995, Takahashi et al. 1995, Galbraith et al. 1997). In our studies we have focused on cytokine production by PMN, since these cells constitute the largest population in gingival crevicular fluid, and synthesize significant amounts of both IL-1β and TNF-α in situ (Hendley et al. 1995, Galbraith et al. 1997).

We hypothesized that if these cytokines are indeed involved in the pathogenesis of periodontitis, then their increased synthesis could play a role in disease progression. Of the many factors that regulate cytokine gene function, gene polymorphisms are of particular interest in the context of diseases known to have a genetic predisposition. The IL-1 and TNF genes include several single base transition polymorphisms, in addition to microsatellite and variable number tandem repeat polymorphisms (Jongeneel et al. 1991, Pociot et al. 1992, Wilson et al. 1992, Tarlow et al. 1993, McDowell et al. 1995). The bi-allelic IL-1β^{+3953} and TNF-α^{-308} RFLPs are examples of polymorphisms involved with regulation of gene function; in each instance the less common allele 2 has been found to be associated with increased cytokine production (Pociot et al. 1992, Kroeger et al. 1997, Wilson et al. 1997, Louis et al. 1998).

The inevitable search for disease associations with these polymorphic markers has resulted in a flurry of positive findings. For example, the frequency of IL-1β^{+3953} allele 2 was increased in patients with myasthenia gravis (Huang et al. 1998), and the TNF-α^{-308} allele 2 was identified as a marker of severity in infectious diseases such as cerebral malaria, mucocutaneous leishmaniasis, meningococcal disease, and lepromatous leprosy (McGuire et al. 1994, Cabrera et al. 1995, Nadel et al. 1996, Roy et al. 1997). Kornman et al. (1997) first reported that IL-1β^{+3953} allele 2, in combination with IL-1α^{-889} allele 2, was significantly increased in patients with advanced adult periodontitis compared to those with early disease. In the same study, these authors found no differences between the two patient groups in the distribution of TNF-α^{-308} alleles. In contrast, we previously reported an association between severity of periodontal disease and IL-1β^{+3953} allele 2 alone, in a small sample population (Gore et al. 1998).

One of the most notable findings of the study reported here was the confirmation that the IL-1β^{+3953} allele 2 frequency was significantly increased in patients with advanced adult periodontitis compared to the referent population, and subjects possessing this allele were more than 3 times more likely to have advanced disease. These findings differ from those of Kornman et al. (1997) in several ways. First, the association with IL-1β^{+3953} allele 2 was not dependent on the simultaneous presence of IL-1α^{-889} allele 2. However,

we have previously shown that IL-1β^{+3953} allele 2 and IL-1α^{-889} allele 2 are in significant linkage disequilibrium, which may account for this discrepancy (Gore et al. 1998). Second, the composite genotype association reported by Kornman et al. was only demonstrable in non-smoking patients with advanced disease. Smoking is a major risk factor for periodontitis (Salvi et al. 1997); indeed, in the present study, the majority (75%) of patients with severe periodontitis were smokers, as opposed to only 15% of the subjects with plaque associated gingivitis. Of the five non-smokers in the advanced disease group, two possessed the IL-1β^{+3953} allele 2.

No significant correlation between IL-1β^{+3953} genotype and IL-1β production was found in this study, although a trend to increased synthesis by oral PMN was observed in patients with IL-1β^{+3953} allele 2. A recurrent problem with studies of cytokine production is the wide variation between individuals, as previously reported by us and others (Galbraith et al. 1997, Mølvig et al. 1988, McFarlane et al. 1990, Kjeldsen et al. 1995). However, although original studies by Pociot et al. (1992) showed increased IL-1β production in vitro in association with IL-1β^{+3953} allele 2, a more recent study by Santtila et al. (1998) failed to confirm this finding. This aspect must be further investigated using a considerably larger population sample size.

An unexpected finding in the current study was the significant increase in frequency of the TNF-α^{-308} 1,1 genotype in advanced periodontitis compared to gingivitis, although the odds ratio of 10.2 must be viewed with caution because of the wide confidence intervals. As mentioned above, this was not found by Kornman et al. (1997). Furthermore, in our previous studies, which included a small number of patients with advanced disease, we were unable to detect a significant association (Galbraith et al. 1998). The association between the TNF-α^{-308} 1,1 genotype and severe disease, and, conversely, the association between the 1,2 genotype and gingivitis, is difficult to interpret. The majority of previous reports have suggested that allele 2 is the marker of disease severity, as discussed above, and this has been ascribed to the upregulatory influence of allele 2 on TNF-α production (Kroeger et al. 1997, Wilson et al. 1997). Interestingly, in our previous studies of alopecia areata, a common inflammatory skin disease, we also found an association of the 1,2 genotype with the less severe form of disease (Galbraith & Pandey 1995). However, the TNF-α^{-308} allele 1 has been shown to be significantly associated with HLA DR 4 and 6 alleles [whereas allele 2 is in linkage disequilibrium with the DR3 allele (Wilson et al. 1993)]. In this context, it should be noted that although few HLA association studies have been performed in adult periodontitis, Katz et al. (1987) reported the presence of HLA DR4 in eight of ten patients with rapidly progressing periodontitis. Further studies will be necessary to determine if the TNF-α^{-308} allele 1 homozygosity is associated with DR4 expression in severe adult periodontitis.

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Zusammenfassung

Polymorphe Cytokin-Genotype als Marker der Erkrankungsschwere bei der adulten Parodontitis

Die Verteilungen des biallenen Interleukin-1β^{+3953} und Tumornekrosefaktors α^{-308} Genotyps wurden bei 20 Patienten mit fortgeschrittener Erwachsenen-Parodontitis, bei 20 Patienten mit plaque-assoziierter Gingivitis und bei 45 Referenzpersonen bestimmt. Eine signifikante Erhöhung der IL-1β^{+3953} Allel2-Frequenz wurde bei Patienten mit fortgeschrittener Parodontitis im Vergleich zu den Referenzpersonen gefunden (Fisher's exakter Test; $p=0.013$). Zusätzlich war die Häufigkeit des TNF-α^{-308} Allel1 signifikant größer bei Patienten mit fortgeschrittener Erkrankung, wenn mit den Personen mit plaque-assoziierter Gingivitis verglichen wurde (Fisher exakter Test; $p=0.014$). Zwischen dem Genotyp und der Cytokinproduktion bei diesen Patienten wurde keine signifikante Korrelation beobachtet.

Résumé

Génotypes de cytokine polymorphiques en tant que marqueurs de la sévérité de la maladie dans la parodontite de l'adulte

Les répartitions de l'interleucine-1β^{+3953} biallélique et des génotypes facteur α^{-308} de nécrose tumorale ont été déterminées chez 20 patients avec parodontite adulte avancée, 20 autres avec gingivite due à la plaque dentaire et 45 sujets de référence. Une augmentation significative de la fréquence de l'allèle d'IL-1β^{+3953} a été trouvée chez les patients avec parodontite avancée comparés aux sujets de référence (test exact de Fisher; $p=0.013$). De plus la fréquence de l'allèle 1TNF-α^{-308} était significativement plus importante chez les patients avec maladie avancée comparés à ceux qui avaient une gingivite associée à la plaque dentaire (test exact de Fisher; $p=0.014$). Aucune relation significative n'a été observée entre le génotype et la production de cytokine dans ces groupes de patients.

References

Agarwal, S., Baran, C., Piesco, N. P., Quintero, J. C., Langkamp, H. H., Johns, L. P. & Chandra, C. S. (1995) Synthesis of proinflammatory cytokines by human gingival fibroblasts in response to lipopolysaccharides and interleukin-1-beta. *Journal of Periodontal Research* **30**, 382–389.

Assuma, R., Oates, T., Cochran, D., Amar, S. & Graves, D. T. (1998) IL-1 and TNF antagonists inhibit the inflammatory response and bone loss in experimental periodontitis. *Journal of Immunology* **160**, 403–409.

Cabrera, M., Shaw, M. A., Sharples, C., Williams, H., Castes, M., Convit, J. & Blackwell, J. M. (1995) Polymorphism in tumor necrosis factor genes associated with mucocutaneous leishmaniasis. *Journal of Experimental Medicine* **182**, 1259–1264.

DuFour, A., Baran, C., Langkamp, H. L., Piesco, N. P. & Agarwal, S. (1993) Regulation of differentiation of gingival fibroblasts and periodontal ligament cells by rhIL-1β and rhTNF-α. *Journal of Periodontal Research* **28**, 566–568.

Galbraith, G. M. P. & Pandey, J. P. (1995) Tumor necrosis factor alpha (TNF-α) gene polymorphism in alopecia areata. *Human Genetics* **96**, 433–436.

Galbraith, G. M. P., Hagan, C., Steed, R. B., Sanders, J. J. & Javed, T. (1997) Cytokine production by oral and peripheral blood neutrophils in adult periodontitis. *Journal of Periodontology* **68**, 832–838.

Galbraith, G. M. P., Steed, R. B., Sanders, J. J. & Pandey, J. P. (1998) Tumor necrosis factor alpha production by oral leukocytes: influence of tumor necrosis factor genotype. *Journal of Periodontology* **69**, 428–433.

Gore, E. A., Sanders, J. J., Pandey, J. P., Palesch, Y. & Galbraith, G. M. P. (1998) Interleukin-1β^{+3953} allele 2: association with disease status in adult periodontitis. *Journal of Clinical Periodontology* **25**, 781–785.

Hart, T. C. & Kornman, K. S. (1997) Genetic factors in the pathogenesis of periodontitis. *Periodontology 2000* **14**, 202–215.

Hassel, T. M. & Harris, E. L. (1995) Genetic influences in caries and periodontal diseases. *Critical Reviews in Oral Biology and Medicine* **6**, 319–342.

Hendley, T. M., Steed, R. B. & Galbraith, G.

M. P. (1995) Interleukin-1*β* gene expression in human oral polymorphonuclear leukocytes. *Journal of Periodontology* **66**, 761–765.

Hillmann, G., Hillmann, B. & Geurtsen, W. (1995) Immunohistological determination of interleukin-1 beta in inflamed gingival epithelium. *Archives of Oral Biology* **40**, 353–359.

Huang, D., Pirskanen, R., Hjelmstrom, P. & Lefvert, A. K. (1998) Polymorphisms in IL-1-beta and IL-1 receptor antagonist associated with myasthenia gravis. *Journal of Neuroimmunology* **81**, 76–81.

Jongeneel, C. V., Briant, L., Udalova, I. A., Sevin, A., Nedospasov, S. A. & Cambon-Thomsen, A. (1991) Extensive genetic polymorphism in the human tumor necrosis factor region and relation to extended HLA haplotypes. *Proceedings of the National Academy of Sciences USA* **88**, 9717–9721.

Katz, J., Goultschin, J., Benoliel, R. & Brautbar, C. (1987) Human leukocyte antigen (HLA) DR4. Positive association with rapidly progressing periodontitis. *Journal of Periodontology* **58**, 607–610.

Kjeldsen, M., Holmstrup, P., Lindemann, R. A. & Bendtzen, K. (1995) Bacterial-stimulated cytokine production of peripheral mononuclear cells from patients of various periodontitis categories. *Journal of Periodontology* **66**, 139–144.

Kornman, K. S., Crane, A., Wang, H. Y., di Giovine, F. S., Newman, M. G., Pirk, F. W., Wilson Jr., T. G., Higginbottom, F. L. & Duff, G. W. (1997) The interleukin-1 genotype as a severity factor in adult periodontal disease. *Journal of Clinical Periodontology* **24**, 72–77.

Kroeger, K. M., Carville, K. S. & Abraham, L. J. (1997) The −308 tumor necrosis factor-alpha promoter polymorphism affects transcription. *Molecular Immunology* **34**, 391–399.

Louis, E., Franchimont, D., Piron, A., Gevaert, Y., Schaaf-Lafontaine, N., Roland, S., Mahieu, P., Malaise, M., de Groote, D., Louis, R. & Belaiche, J. (1998) Tumour necrosis factor (TNF) gene polymorphism influences TNF-*α* production in lipopolysacchraride (LPS)-stimulated whole blood cell culture in healthy humans. *Clinical Experimental Immunology* **113**, 401–406.

Manolagas, S. C. (1995) Role of cytokines in bone resorption. *Bone* **17**, 63S–67S.

Masada, M. P., Persson, R., Kenney, J. S., Lee, S. W., Page, R. C. & Allison, A. C. (1990) Measurement of interleukin-1*α* and -1*β* in gingival crevicular fluid: Implications for the pathogenesis of periodontal disease. *Journal of Periodontal Research* **26**, 156–163.

McDowell, T. L., Symons, J. A., Ploski, R., Forre, O. & Duff, G. W. (1995) A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1*α* polymorphism. *Arthritis and Rheumatism* **38**, 221–228.

McFarlane, C. G., Reynolds, J. J. & Meikle, M. C. (1990) The release of interleukin-1*β*, tumor necrosis factor-*α* and interferon-*γ* by cultured peripheral blood mononuclear cells from patients with periodontitis. *Journal of Periodontal Research* **25**, 207–214.

McGuire, W., Hill, A. V. S., Allsop, C. E. M., Greenwood, B. M. & Kwiatkowski, D. (1994) Variation in the TNF-*α* promoter region associated with susceptibility to cerebral malaria. *Nature* **371**, 508–511.

Mølvig, J., Baek, L., Christensen, P., Manogue, K. R., Vlassara, H., Platz, P., Nielsen, L. S., Svejgaard, A. & Nerup, J. (1988) Endotoxin-stimulated human monocyte secretion of interleukin-1, tumour necrosis factor alpha, and prostaglandin E2 shows stable interindividual differences. *Scandinavian Journal of Immunology* **27**, 705–716.

Mourant, A. E., Kopec, A. C. & Domaniewska-Sobczak, K. (1976) In: *The distribution of the human blood groups and other polymorphisms*. Oxford: Oxford University Press.

Nadel, S., Newport, M. J., Booy, R. & Levin, M. (1996) Variation in the tumor necrosis factor-alpha gene promoter region may be associated with death from meningococcal disease. *Journal of Infectious Diseases* **174**, 878–880.

Offenbacher, S. (1996) Periodontal diseases: pathogenesis. *Annals of Periodontology* **1**, 821–878.

Pociot, F., Molvig, J., Wogensen, L., Worsaae, H. & Nerup, J. (1992) A Taq I polymorphism in the human interleukin-1beta (IL-1*β*) gene correlates with secretions in vitro. *European Journal of Clinical Investigation* **22**, 396–402.

Roberts, F. A., Mccaffery, K. A. & Michalek, S. M. (1997) Profile of cytokine mRNA expression in chronic adult periodontitis. *Journal of Dental Research* **76**, 1833–1839.

Rossomando, E. F., Kennedy, J. E. & Hadjimichael, J. (1990) Tumour necrosis factor alpha in gingival crevicular fluid as a possible indicator of periodontal disease in humans. *Archives of Oral Biology* **35**, 431–434.

Roy, S., McGuire, W., Mascietaylor, C. G. N., Saha, B., Hazra, S. K., Hill, A. V. S. & Kwiatkowski, D. (1997) Tumor necrosis factor promoter polymorphism and susceptibility to lepromatous leprosy. *Journal of Infectious Diseases* **176**, 530–532.

Salvi, G. E., Lawrence, H. P., Offenbacher, S. & Beck, J. D. (1997) Influence of risk factors on the pathogenesis of periodontitis. *Periodontology 2000* **14**, 173–201.

Santtila, S., Savinainen, K. & Hurme, M. (1998) Presence of the IL-1ra allele 2 (IL1RN*2) is associated with enhanced IL-1-beta production in vitro. *Scandinavian Journal of Immunology* **47**, 195–198.

SAS Institute, Inc. (1990) In: *SAS/STAT user's guide*. SAS Institute, Cary, Version 6, 4th edition, vols 1, 2.

Stashenko, P., Jandinski, J. J., Fujiyoshi, P., Rynar, J. & Socransky, S. S. (1991) Tissue levels of bone resorptive cytokines in periodontal disease. *Journal of Periodontology* **62**, 504–509.

Takahashi, K., Poole, I. & Kinane, D. F. (1995) Detection of interleukin-1-beta mRNA-expressing cells in human gingival crevicular fluid by in situ hybridization. *Archives of Oral Biology* **40**, 941–947.

Takeshita, A., Zhon, G. N., Hanazawa, S., Takara, I., Higuchi, H., Katayama, I. & Kitano, S. (1992) Effect of interleukin-1*β* on gene expressions and functions of fibroblastic cells derived from human periodontal ligament. *Journal of Periodontal Research* **27**, 250–255.

Tarlow, J. K., Blakemore, A. I. F., Lennard, A., Solari, R., Hughes, H. N., Steinkasserer, A. & Duff, G. W. (1993) Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. *Human Genetics* **91**, 403–404.

The American Academy of Periodontology. (1996) *Parameters of care*. Chicago.

Tokoro, Y., Yamamoto, T. & Hara, K. (1996) IL-1*β* mRNA as the predominant inflammatory cytokine transcript: correlation with inflammatory cell infiltration into human gingiva. *Journal of Oral Pathology and Medicine* **25**, 225–231.

Wilson, A. G., di Giovane, F. S., Blakemore, A. I. F. & Duff, G. W. (1992) Single base polymorphism in the human tumour necrosis factor alpha (TNF*α*) gene detectable by Nco1 restriction of PCR product. *Human Molecular Genetics* **1**, 353.

Wilson, A. G., de Vries, N., Pociot, F., Di Giovane, F. S., Van der Putte, L. B. A. & Duff, G. W. (1993) An allelic polymorphism within the human tumor necrosis factor *α* promoter region is strongly associated with HLA A1, B8, and DR3 alleles. *Journal of Experimental Medicine* **177**, 557–560.

Wilson, A. G., Symons, J. A., McDowell, T. L., McDevitt, H. O. & Duff, G. W. (1997) Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proceedings of the National Academy of Sciences USA* **94**, 3195–3199.

Wondimu, B. & Modeer, T. (1997) Cyclosporin A upregulates prostaglandin E_2 production in human gingival fibroblasts challenged with tumor necrosis factor alpha in vitro. *Journal of Oral Pathology and Medicine* **26**, 11–16.

Address:

Gillian M. P. Galbraith
Department of Microbiology and Immunology
Medical University of South Carolina
173 Ashley Avenue, PO Box 250504
Charleston, SC 29425, USA

Fax: +1 843 792 2464
e-mail: galbragm@musc.edu