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Association of the − 159 CD14 gene polymorphism and lack of association of the − 308 TNFA and Q551R IL-4RA polymorphisms with severe chronic periodontitis in Swedish Caucasians

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Abstract

Background: Severe forms of periodontitis are suggested to have a genetic basis.

Objective: The aim of the present investigation was to study the association of gene polymorphisms related to some immune regulation components (G-308A *TNFA*, Q551R *IL-4RA* and C-159T *CD14*) with severe chronic periodontitis.

Materials and Methods: Sixty patients (aged 36–74 years; mean 54.5 ± 8.5) with severe and generalized chronic periodontitis were included. The patients exhibited bone loss >50% at all teeth. Thirty-nine periodontally healthy subjects between 35 and 78 years of age (mean 51.0 ± 10.9) were recruited as controls. DNA was isolated from peripheral blood cells and genotyping was performed by combination of PCR and restriction endonuclease mapping.

Results: While gene polymorphisms for *TNFA* and *IL-4RA* did not show any association with severe chronic periodontitis, the analysis of the − 159 *CD14* gene polymorphism revealed significant differences between test and control groups. The proportion of subjects that exhibited the TT genotype was significantly smaller in the group with severe periodontitis than in periodontal healthy group ($p = 0.028$; Fisher's exact test). The C allele carriage was 90% in the periodontitis group and significantly higher than in the healthy control group (72%).

Conclusion: It is suggested that the − 159 *CD14* gene polymorphism is associated with chronic periodontitis in Caucasian subjects of a north European origin.

Key words: allele; genotype; periodontal disease; receptor

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The most common form of periodontitis has been reported to affect about 30–40% of an adult population and approximately 10% of the subjects exhibit severe disease (Papapanou & Lindhe 2003). Michalowicz et al. (1991, 1994, 2000) estimated that genetic factors may account for 50% of the variation seen in periodontal disease expression in humans.

The aetiopathogenesis of periodontitis appears to be multifactorial and involves

a series of events of host response to microbial challenge.

The specific inflammatory and immunological reactions that occur as a response to the challenge from predominantly Gram-negative microorganisms are of specific interest and critical parts of the immune control have been analysed in subjects with advanced periodontitis (Kinane et al. 2003).

The tumour necrosis factor (TNF) is an important mediator in inflammatory reactions and appears to play a central role in the pathogenesis of severe chronic inflammatory diseases (Beutler & Cerami 1989). Differences in the rate of production of TNF have been demonstrated and a familial ability to produce higher or lower cytokine levels seems to exist (Pociot et al. 1993). The TNF synthesis may be influenced by the presence of certain gene polymorphisms (Duff 1994, Wilson & di Giovine 1995) and conflicting results have been reported regarding the association of −308 TNFA gene polymorphism and periodontitis (Kornman et al. 1997, Galbraith et al. 1998, 1999, Craandijk et al. 2002).

Findings from previous studies have revealed that subjects with severe chronic periodontitis exhibit large proportions of B cells (B-2 and B-1) in the gingival lesion and in peripheral blood (Afar et al. 1992, Berglundh et al. 2002). The cytokines related to B-cell proliferation and activation may be of importance in the development of chronic periodontitis (Berglundh et al. 2003).

One of the cytokines, interleukin-4 (IL-4), is produced by T helper 2 cells and binds to the IL-4 receptor (IL-4R) of B cells (for a review see Gemmell et al. 1997, 2002). Variations in the *IL-4RA* gene have been associated with different human disorders (Hershey et al. 1997, Kruse et al. 1999, Mitsuyasu et al. 1999). The gene polymorphism at the position 551 at the promoter region was shown to be associated also with Systemic lupus erythematosus and primary Sjögren's syndrome (Youn et al. 2000). No data are available concerning the possible association of *IL-4RA* gene polymorphisms and periodontal disease.

The CD14 receptor (mCD14) is a glycoprotein that is expressed primarily on the surface of monocytes, macrophages, neutrophils and gingival fibroblasts, and is involved in the process of cellular response to bacterial lipopolysaccharides (LPS). A soluble form of CD14 (sCD14) is present in serum and derives both from enzymatically cleaved glycosyl-phosphatidylinositol-anchored mCD14 and from secretion of CD14 (Haziot et al. 1988, Ulevitch & Tobias 1995). Furthermore, the LPS-induced upregulation of CD14 seems to elicit an increased production of IL-12 by antigen-presenting cells (Cleveland et al. 1996).

The aim of the present investigation was to study the association of some immune regulation gene polymorphisms (G-308A *TNFA*, Q551R *IL-4RA* and C-159T *CD14*) with severe chronic periodontitis.

Material and Methods

Two groups of Caucasian subjects were included. The test group consisted of 60 patients (28 females and 32 males, aged 36–74 years; mean 54.5 ± 8.5) with generalized, severe chronic periodontitis (Consensus Report 1999). The patients were recruited from the Clinic of Periodontics, Gothenburg and exhibited bone loss >50% at all teeth. Prior to periodontal therapy they all had probing pocket depths >6 mm and bleeding on probing at >80% of the proximal sites. Sets of intra-oral radiographs were obtained using a standardized parallel technique (Eggen 1969). In the radiographs, the distance between the cemento-enamel junction (CEJ) and the most coronal level of the bone crest (BC) was assessed at the mesial and distal aspects of each tooth. For details regarding the radiographic measurements, see Berglundh et al. (1998).

Thirty-nine periodontally healthy subjects (24 females and 15 males) between 35 and 78 years of age (mean 51.0 ± 10.9) were also recruited (Control group). The subjects in this group demonstrated normal radiographic bone levels, i.e. a distance of <3 mm between the CEJ and BC at >95% of the proximal tooth sites.

The study protocol was approved by the local human review board and prior to enrollment the subjects of the two groups received information regarding the purpose of the study. None of the subjects had a known systemic disorder that could have affected the periodontal conditions. Smoking habits with regard to present smoker and never smoked were recorded in both groups.

Blood samples, DNA separation and genotype detection

Samples of peripheral blood were obtained by venipuncture from the arm vein of each subject. The 20 ml blood sample collected in EDTA tubes was given a code and was stored in −70°C until further processing.

DNA from EDTA blood was extracted by a modification of a previously described method (Kawasaki 1990) or by ''salting-out'' method.

Genotyping for the C-159T CD14 and for the Q551R and V75I IL-4RA polymorphisms was performed by restriction endonuclease mapping. Briefly, in PCR mixture with 1.25 U of AmpliTaq Gold and 2 mM of $MgCl_2$ (Applied Biosystems, Foster City, CA, USA) 5–30 ng of template DNA was added and amplification was run using the GeneAmp PCR System 9600 (Perkin Elmer, Norwalk, CT, USA) with 12 min. 94°C hot start, 30 s 94°C, 30 s 60°C and 60 s of 72°C (35 cycles for CD14 and 30 cycles for IL-4RA), followed by 5 min. of extension at 72°C. Resulting PCR product was digested by *HpyCH4 III* (for C-159T CD14) or *Msp*I (for Q551R). Each method includes conservative recognition sites for control digestion. Sequences of the primers may be obtained from the authors on request. Electrophoresis in polyacrylamide gel was performed exactly as previously reported (Padyukov et al. 2001a). Detection of −308 TNFA polymorphism was described earlier (Hahn-Zoric et al. 2003).

Statistical analysis

The distribution of frequencies of genotypes for each group was compared using the chi-squared test, while the frequencies of allele carriage and the allele frequency in the test and control group were compared using Fisher's exact test. All analyses, except for the allele frequency assessment, were performed using the subject as the statistical unit, i.e. $n = 60$ (test; severe chronic periodontitis) and $n = 39$ (control), respectively. Comparisons of allele frequencies were performed using the chromosome as the statistical unit.

All genetic markers in the present study were found to be in Hardy–Weinberg equilibrium.

Results

The amount of the bone loss assessed in the radiographs from the periodontitis group has already been reported in Berglundh et al. (2003). In brief, the bone

loss, varied between 7.7 and 13.6 mm (mean 9.0 ± 1.4 mm).

TNFA genotype frequencies

The results from the assessment of G-308A *TNFA* genotype frequencies for the subjects in both groups are reported in Table 1. The distribution of the different genotypes within test group did not differ from that in the controls ($p = 0.653$; chi-squared). The homozygous TNF1/TNF1 genotype was detected in 78.3% (47 out of 60) of the subjects with advanced periodontitis and in 82% (32 out of 39) of the periodontally healthy individuals. The homozygous TNF2/TNF2 genotype was not detected in any of the groups. Heterozygous state TNF1/TNF2 occurred in 21.7% of the test group and in 18% of the control group.

Similarly, the distribution of genotypes within the fraction of non-smokers (Table 1) did not differ between the two groups of subjects ($p = 0.953$; chi-squared test). Almost similar proportions of patients in the test and control group exhibited the homozygous TNF1/TNF1 genotype (80% *versus* 79.4%) and the heterozygous TNF1/TNF2 genotype (20% *versus* 20.6%).

IL-4RA genotype frequencies

The results from the assessment of IL-4RA genotype frequencies for all subjects in both groups are reported in Table 2. Three samples (two test and one control) out of 99 samples could not be analysed regarding the IL-4RA genotypes. The distribution of the different genotypes in the remaining samples did not differ between test and controls ($p = 0.455$; chi-squared). Similar observations were made with respect to the fraction of non-smoking subjects (Table 2) ($p = 0.710$).

CD14 genotype frequencies

The results from the assessment of *CD14* genotype frequencies for all subjects and for non-smokers in the two groups are presented in Table 3. The CC genotype was found in 33% (20 out of 60) of the subjects with severe periodontitis and in 23% (nine out of 39) of periodontally healthy individuals. The CT genotype occurred in 56% of test subjects and in 48% of controls. The subjects with severe periodontitis exhibited the homozygous TT genotype in 10% of the cases while in the periodontally healthy subjects the corresponding frequency was 28%. The analysis of the distribution of genotypes in the fraction of non-smokers revealed similar findings as made for all subjects ($p = 0.053$ and 0.058; chi-squared test, respectively).

Frequencies of the allele carriage in TNFA gene

In Table 4 the frequencies of TNF2 allele carriage, i.e. the proportion of the subjects in the two groups carrying at least one TNF2 allele are reported.

Since no TNF2/TNF2 genotype were detected in both the groups, the data that show the frequencies of the TNF2 allele carriage are similar to those describing the distribution of the TNFA genotypes (Table 1). No statistical differences were detected for all subjects ($p = 0.799$; Fisher's exact test) or for non-smokers ($p = 1.00$; Fisher's exact test).

Frequencies of IL-4RA allele carriage

The frequencies of Q allele carriage, i.e. the proportion of subjects in the two groups carrying at least one Q allele, are reported in Table 5. The majority of the subjects were QQ or QR genotypes in both groups (96.5% and 92.1%, respectively) and no statistical differences were found between the test ($n = 60$) and control group ($n = 39$) for the entire cohort ($p = 0.381$; Fisher's exact test) or for the non-smoking category of subjects ($p = 1.00$; Fisher's exact test). The frequencies of the R allele carriage are reported in Table 6. The differences between test and control subjects were not statistically significant ($p = 0.676$, all subjects; $p = 0.616$, non-smokers).

Frequencies of CD14 allele carriage

The frequencies of the C allele carriage are described in Table 7. The percentage of subjects within the periodontitis group that had no allele C (i.e. TT genotype) was 10.0%. The corresponding figure for the control group was 28.2%. This difference was statistically significant ($p = 0.028$) and was consistent with the corresponding comparison made within the fraction of non-smokers ($p = 0.025$). On the other hand, the

Table 1. Distribution of G-308A *TNFA* genotypes for all subjects and the fraction of non-smokers in the periodontitis and control groups; number and (%) of subjects

Genotype	All subjects		Non-smokers	
	$n = 60$, periodontitis	$n = 39$, control	$n = 30$, periodontitis	$n = 34$, control
TNF1/TNF1	47 (78.3)	32 (82.0)	24 (80.0)	27 (79.4)
TNF1/TNF2	13 (21.7)	7 (18.0)	6 (20.0)	7 (20.6)

All subjects: $p = 0.653$ (chi-squared test); non-smokers: $p = 0.953$ (chi-squared test).

Table 2. Distribution of Q551R *IL-4RA* genotypes for all subjects and the fraction of non-smokers in the periodontitis and control groups; number and (%) of subjects

Genotype	All subjects		Non-smokers	
	$n = 60$, periodontitis	$n = 39$, control	$n = 30$, periodontitis	$n = 34$, control
QQ	32 (55.2)	23 (60.5)	16 (53.3)	20 (60.6)
QR	24 (41.4)	12 (31.6)	12 (40.0)	10 (30.3)
RR	2 (3.4)	3 (7.9)	2 (6.7)	3 (9.1)

All subjects: $p = 0.455$ (chi-squared test); non-smokers: $p = 0.710$ (chi-squared test).

Table 3. Distribution of C-159T *CD14* genotypes for all subjects and the fraction of non-smokers in the periodontitis and control groups; number and (%) of subjects

Genotype	All subjects		Non-smokers	
	$n = 60$, periodontitis	$n = 39$, control	$n = 30$, periodontitis	$n = 34$, control
CC	20 (33.3)	9 (23.1)	8 (26.7)	9 (26.5)
CT	34 (56.7)	19 (48.7)	20 (66.7)	15 (44.1)
TT	6 (10.0)	11 (28.2)	2 (6.6)	10 (29.4)

All subjects: $p = 0.058$ (chi-squared test); non-smokers: $p = 0.053$ (chi-squared test).

Table 4. G-308A *TNFA* allele carriage: number and (%) of subjects carrying any or no TNF2 allele in the periodontitis and control groups (all subjects and the fraction of non-smokers)

Allele	All subjects		Non-smokers	
	$n = 60$, periodontitis	$n = 39$, control	$n = 30$, periodontitis	$n = 34$, control
Any TNF2 allele (TNF2/TNF2 or TNF1/TNF2 genotypes)	13 (21.7)	7 (18.0)	6 (20.0)	7 (20.6)
No TNF2 allele (TNF1/TNF1 genotype)	47 (78.3)	32 (82.0)	24 (80.0)	27 (79.4)

All subjects: $p = 0.799$ (Fisher's exact test); non-smokers: $p = 1.00$ (Fisher's exact test).

Table 5. Q551R *IL-4RA* allele carriage: number and (%) of subjects carrying any or no allele Q in the periodontitis and control groups (all subjects and fractions of non-smokers)

Allele	All subjects		Non-smokers	
	$n = 58$, periodontitis	$n = 38$, control	$n = 30$, periodontitis	$n = 33$, control
Any Q allele (QQ or QR genotypes)	56 (96.5)	35 (92.1)	28 (93.3)	30 (90.9)
No Q allele (RR genotype)	2 (3.5)	3 (7.9)	2 (6.7)	3 (9.1)

All subjects: $p = 0.381$ (Fisher's exact test); non-smokers: $p = 1.00$ (Fisher's exact test).

Table 6. Q551R *IL-4RA* allele carriage: number and (%) of subjects carrying any or no allele R in the periodontitis and control groups (all subjects and non-smokers)

Allele	All subjects		All subjects	
	$n = 58$, periodontitis	$n = 38$, control	$n = 30$, periodontitis	$n = 33$, control
Any R allele (RR or QR genotypes)	26 (44.8)	15 (39.5)	14 (46.7)	13 (39.4)
No R allele (QQ genotype)	32 (55.2)	23 (60.5)	16 (53.3)	20 (60.6)

All subjects: $p = 0.676$ (Fisher's exact test); non-smokers: $p = 0.616$ (Fisher's exact test).

Table 7. C-159T *CD14* allele carriage: number and (%) of subjects carrying any or no allele C in the periodontitis and control groups (all subjects and non-smokers)

Allele	ALL sujects		Non-smokers	
	$n = 60$, periodontitis	$n = 39$, control	$n = 30$, periodontitis	$n = 34$, control
Any C allele (CC or CT genotypes)	54 (90.0)	28 (71.8)	28 (93.3)	24 (70.5)
No C allele (TT genotype)	6 (10.0)	11 (28.2)	2 (6.7)	10 (29.5)

All subjects: $p = 0.028$ (Fisher's exact test); non-smokers: $p = 0.025$ (Fisher's exact test).

Table 8. C-159T *CD14* allele carriage: number and (%) of subjects carrying any or no allele T in the periodontitis and control groups (all subjects and non-smokers)

Allele	All subjects		Non-smokers	
	$n = 60$, periodontitis	$n = 39$, control	$n = 30$, periodontitis	$n = 34$, control
Any T allele (TT or CT genotypes)	40 (66.7)	30 (76.9)	22 (73.3)	25 (73.5)
No T allele (CC genotype)	20 (33.3)	9 (23.1)	8 (26.7)	9 (26.5)

All subjects: $p = 0.366$ (Fisher's exact test); non-smokers: $p = 1.0$ (Fisher's exact test).

analysis of the T allele carriage (Table 8) revealed no differences between the groups.

Allele frequency (C and T) in CD14 genes

The C allele frequency for the entire cohort was 61.7% in the test and 47.5% in the control group (Table 9). The corresponding figures for the non-smoking fraction of subjects were 60.0% and 48.5%, respectively. T allele occurred in 38.3% of the test subjects and in 52.4% of all control individuals. This difference was statistically significant ($p = 0.049$). Non-smokers exhibited T allele frequencies of 40.0% in the periodontitis group and 51.5% in the control group.

Discussion

The present investigation was carried out to study the association of some immune regulation gene polymorphisms with severe chronic periodontitis. It was demonstrated that gene polymorphisms for *TNFA* and *IL-4RA* did not show any association with severe chronic periodontitis. The analysis of the -159 *CD14* gene polymorphism, however, revealed significant differences between test and control groups. Thus, the proportion of subjects that exhibited the TT genotype was significantly smaller in the group with severe periodontitis than in periodontal healthy group. Further, the C allele carriage was 90% in the periodontitis group and significantly higher than in the healthy control group (72%).

It is therefore suggested that the -159 *CD14* gene polymorphism is associated with chronic periodontitis in Caucasian subjects of a north European origin.

TNF-A and IL-4RA gene polymorphisms

Galbraith et al. (1998) described TNF-α genotypes of three bi-allelic polymorphisms in 32 Caucasian patients with adult periodontitis and in 32 healthy matched controls. No differences in the distribution of TNFA alleles of the -238, -308 or $+252$ gene polymorphisms were observed between patients and controls or between patients with different disease severity. Craandijk et al. (2002) analysed the occurrence of four bi-allelic polymorphisms in the *TNFA* gene (at positions -376, -308, -238 and $+489$) in 90 periodontitis patients

Table 9. Allele frequency for − 159 CD14 polymorphism: number and (%) of chromosomes positive for the allele T and C in the periodontitis and control groups (all subjects and non-smokers)

Allele	All subjects		Non-smokers	
	$n = 120$, periodontitis	$n = 78$, control	$n = 60$, periodontitis	$n = 68$, control
Allele C	74 (61.7)	37 (47.4)	36 (60.0)	33 (48.5)
Allele T	46 (38.3)	41 (52.6)	24 (40.0)	35 (51.4)

All subjects: $p = 0.057$ (Fisher's exact test); non-smokers: $p = 0.216$ (Fisher's exact test); all subjects: $p = 0.049$ (chi-squared test); non-smokers: $p = 0.194$ (chi-squared test).

and in 264 reference controls selected from the blood bank samples. No differences between test and control subjects were found for any of the four *TNFA* polymorphisms studied. The lack of association between *TNFA* polymorphism and periodontitis reported by Galbraith et al. (1998) and Craandijk et al. (2002) is consistent with observations made in the present investigation.

In the current study it was also demonstrated that no differences in the occurrence of the Q551R *IL-4RA* polymorphism was found between subjects with severe chronic periodontitis and healthy controls. It has been suggested that the lack of IL-4 may lead to continuous accumulation of macrophages in periodontitis lesions and that this recruitment of cells is regulated by the expression of IL-4R (Yamamoto et al. 1996, 1997). Apparently, previously demonstrated B-cell proliferation and activation seem not to be associated with the IL-4RA polymorphism.

CD14 studies

It has been demonstrated that the C to T transition at position − 159 is related to the production of the sCD14 (Baldini et al. 1999). Baldini et al. (1999) reported that subjects with the homozygous TT genotype exhibited significantly higher sCD14 levels than individuals with CC and CT genotypes. This finding indicates that variations in the promoter region of the CD14 gene may change the production of CD14 and, also influence the balance between Th2 and Th1 cells in the response to a bacterial challenge.

Holla et al. (2002) studied two CD14 gene polymorphisms (at positions − 159 and − 1359) in relation to the severity of chronic periodontitis in subjects of a Czech ethnicity. It was reported that the distribution of the genotypes did not differ between periodontitis subjects and healthy controls. Holla et al. (2002) further reported that the frequency of the C allele carriage (TT *versus* CT+CC genotypes) tended to be larger in subjects with severe periodontitis than in subjects with moderate periodontitis. This finding is not in agreement with observations made in the present study. Thus, the distribution of genotypes in the current material tended to differ between test and control subjects with regard to all subjects ($p = 0.058$) as well as in the non-smoking fraction of subjects ($p = 0.052$). In other words, a higher frequency of the TT genotype was found in the control group than in the periodontitis group. Furthermore, the analysis of the present samples revealed a statistically significant difference between test and control regarding the carriage of allele C, once again demonstrating that the TT genotype occurred in a larger proportion of control subjects (28.2%) than in subjects with severe chronic periodontitis (10.0%).

The reasons for the conflicting results in the present study and in the study by Holla et al. (2002) are not presently understood but may be related to the inclusion criteria of subjects in the test and control groups as well as differences in genetic structure of the populations.

Yamazaki et al. (2003) reported on the occurrence of the − 159 CD14 gene polymorphism in a Japanese population. It was demonstrated that genotype distribution (CT, CC or TT) and the allele frequency did not differ between periodontitis patients and control subjects. Also the results reported by Yamazaki et al. (2003) regarding the − 159 CD14 gene polymorphism are in contrast with the findings made in the present investigation. In particular, a smaller proportion of the subjects in the test group in the current study exhibited the TT genotype as compared with the test subjects in the study by Yamazaki et al. (2003). While differences may exist with respect to inclusion criteria in test and control groups in the present material and the study by Yamazaki et al. (2003), other reasons, such as ethnic origin of subjects must be considered. In the current sample Caucasians of a north European origin were included, while the participants in the study by Yamazaki et al. (2003) were Japanese. Recently, cytokine selected gene polymorphisms were analysed in an Asian population (Hong Kong Chinese) (Padyukov et al. 2001b). It was demonstrated that an allele in a certain position was found to be very rare in the Chinese population, while in the Swedish Caucasian population this allele was more frequently represented. This means that the interpretation of findings regarding gene polymorphisms must also be related to the ethnic origin of the population.

The results obtained in the present study revealed an association of the − 159 CD14 gene polymorphism and severe chronic periodontitis in Swedish Caucasians. On the other hand, no associations with the − 308 *TNFA* and the 551 *IL-4RA* gene and chronic periodontitis were demonstrated.

Further studies on CD14 gene polymorphisms and the biological functions related to the interactions between LPS and CD14 or toll-like receptor 4 are needed in order to elucidate the influence of this gene polymorphism in the pathogenesis of periodontal disease.

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References

Afar, B., Engel, D. & Clark, E. A. (1992) Activated lymphocyte subsets in adult periodontitis. *Journal of Periodontal Research* **27**, 126–133.

Baldini, M., Lohman, I. C., Halonen, M., Erickson, R. P., Holt, P. G. & Martinez, F. D. (1999) A polymorphism* in the 5′ flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *American Journal of Respiratory Cell and Molecular Biology* **20**, 976–983.

Berglundh, T., Donati, M., Hahn-Zoric, M., Hanson, L. A. & Padyukov, L. (2003) Association of the − 1087 IL 10 gene polymorphism with severe chronic periodontitis in

Swedish Caucasians. *Journal of Clinical Periodontology* **30**, 249–254.

Berglundh, T., Lijenberg, B., Tarkowski, A. & Lindhe, J. (1998) Local and systemic TCR V gene expression in advanced periodontal disease. *Journal of Clinical Periodontology* **25**, 125–134.

Berglundh, T., Liljenberg, B., Tarkowski, A. & Lindhe, J. (2002) Local and systemic autoreactive B cells in advanced periodontitis. *Journal of Clinical Periodontology* **29**, 281–286.

Beutler, B. & Cerami, A. (1989) The biology of TFN, a primary mediator of the host response. *Annual Review of Immunology* **7**, 625–655.

Cleveland, M. G., Gorham, J. D., Murphy, T. L., Toumanen, E. & Murphy, K. M. (1996) Lipoteichooic acid preparations of gram-positive bacteria induce interleukin-12 through a CD14-dependent pathway. *Infection and Immunity* **64**, 1906–1912.

Consensus Report (1999) Chronic periodontitis. *Annals of Periodontology 1999* **4**, 38–39.

Craandijk, J., van Krugten, M. V., Verweij, C. L., van der Velden, U. & Loos, B. G. (2002) Tumor necrosis factor-alpha gene polymorphisms in relation to periodontitis. *Journal of Clinical Periodontology* **29**, 28–34.

Duff, G. W. (1994) Molecular genetics of cytokines. In: Thomson, A. (ed). *The Cytokine Handbook*, 2nd edition, pp. 21–30. London: Academic Press.

Eggen, S. (1969) Standardiserad intraoral röngtenteknik. *Sveriges Tandläkarförbunds Tidningen* **17**, 867–872.

Galbraith, G. M., Hendley, T. M., Sanders, J. J., Palesch, Y. & Pandey, J. P. (1999) Polymorphic cytokine genotypes as markers of disease severity in adult periodontitis. *Journal of Clinical Periodontology* **26**, 705–709.

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

Gemmell, E., Marshall, R. I. & Seymour, G. J. (1997) Cytokine and prostaglandins in immune homeostasis and tissue distraction in periodontal disease. *Periodontology 2000* **14**, 112–143.

Gemmell, E., Yamazaki, K. & Seymour, G. J. (2002) Destructive periodontitis lesions are determined by the nature of the lymphocytic response. *Critical Reviews in Oral Biology and Medicine* **13**, 17–34.

Hahn-Zoric, M., Hytönen, A. M., Hanson, L. Å., Nilsson, L. Å. & Padyukov, L. (2003) Association of −1087 IL10 and −308 TNFA gene polymorphisms with serological markers of coeliac disease. *Journal of Clinical Immunology* **23**, 291–296.

Haziot, A., Chen, S., Ferrero, E., Low, M., Silber, R. & Goyert, S. (1988) The monocyte differentiation antigen, CD14, is anchored to the cell membrane by a glycophosphatidyl linkage. *Journal of Immunology* **4**, 547–552.

Hershey, G. K. K., Friedrich, M. F., Esswein, L. A., Thomas, M. L. & Chatila, T. A. (1997) The association of atopy with a gain-of-function mutation in the subunit of the interleukin-4 receptor. *The New England Journal of Medicine* **337**, 1720–1725.

Holla, L. I., Buckova, D., Fassmann, A., Halabala, T., Vasku, A. & Vacha, J. (2002) Promoter polymorphisms in the CD14 receptor gene and their potential association with the severity of chronic periodontitis. *Journal of Medical Genetics* **39**, 844–848.

Kawasaki, E. S. (1990) Sample preparation from blood, cells and other fluids. In: Innis, M., Gelfand, D., Swinsky, J. & White, T. (eds.) *PCR Protocols: A Guide to Methods and Applications*, pp. 146–152. New York: Academic Press.

Kinane, D. F., Berglundh, T. & Lindhe, J. (2003) Host-parasite interactions in periodontal disease. In: Lindhe, J., Karring, T. & Lang, N. P. (eds). *Clinical Periodontology and Implant Dentistry*, 4th edition. UK: Blackwell Munksgaard.

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.

Kruse, S., Japha, T., Tedner, M., Sparholt, S. H., Forster, J., Kuehr, J. & Deichmann, K. A. (1999) The polymorphisms S503P and Q551R in the interleukin-4 receptor alpha gene are associated with atopy and influence the signal transduction. *Immunology* **96**, 365–371.

Michalowicz, B. S. (1994) Genetic and heritable risk factors in periodontal disease. *Journal of Periodontology* **65** (Suppl.), 479–488.

Michalowicz, B. S., Aeppli, D., Virag, J. G., Klump, D. G., Hinrichs, J. E., Segal, N. L., Bouchard, T. J. Jr & Pihlstrom, B. L. (1991) Periodontal findings in adult twins. *Journal of Periodontology* **62**, 293–299.

Michalowicz, B. S., Diehl, S. R., Gunsolley, J. C., Sparks, B. S., Brooks, C. N., Koertge, T. E., Califano, J. V., Burmeister, J. A. & Schenkein, H. A. (2000) Evidence of a substantial genetic basis for risk of adult periodontitis. *Journal of Periodontology* **71**, 1699–1707.

Mitsuyasu, H., Yanagihara, Y., Mao, X.-Q., Gao, P.-S., Arinobu, Y., Ihara, K., Takabayashi, A., Hara, T., Enomoto, T., Sasaki, S., Kawai, M., Hamasaki, N., Shirakawa, T., Hopkin, J. M. & Izuhara, K. (1999) Dominant effect of Ile50Val variant of the human IL-4 receptor-chain in IgE synthesis. *Journal of Immunology* **16**, 1227–1231.

Padyukov, L., Hahn-Zoric, M., Blomqvist, S., Ulanova, M., Welch, S. G., Feeney, A. J., Lau, Y. L. & Hanson, L. Å. (2001a) Distribution of human kappa locus *IGKV2-29* and *IGKV2D-29* alleles in Swedish Caucasians and Hong Kong Chinese. *Immunogenetics* **53**, 22–30.

Padyukov, L., Hahn-Zoric, M., Lau, Y. L. & Hanson, L. (2001b) Different allelic frequencies of several cytokine genes in Hong Kong Chinese and Swedish Caucasians. *Genes and Immunity* **2**, 280–283.

Papapanou, P. N. & Lindhe, J. (2003) Epidemiology of periodontal disease. In: Lindhe, J., Karring, T. & Lang, N. P. (eds). *Clinical Periodontology and Implant Dentistry*, 4th edition. UK: Blackwell Munksgaard.

Pociot, F., Briant, L., Jongeneel, C. V., Molvig, J., Worsaae, H., Abbal, M., Thomsen, M., Neurp, J. & Cambon-Thomsen, A. (1993) Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-a and TNF-b by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. *European Journal of Immunology* **23**, 224–231.

Ulevitch, R. & Tobias, P. (1995) Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annual Reviews of Immunology* **13**, 437–457.

Wilson, A. G. & di Giovine, F. S. (1995) Genetics of TNF-alpha in autoimmune, infectious and neoplastic disease. *Journal of Inflammation* **45**, 1–12.

Yamamoto, M., Fujihashi, K., Hiroi, T., McGhee, J. R., Van Dyke, T. E. & Kiyono, H. (1997) Molecular and cellular mechanisms for periodontal diseases: role of Th1 and Th2 type cytokines in induction of mucosal inflammation. *Journal of Periodontal Research* **32**, 115–119.

Yamamoto, M., Kawabata, K., Fujihashi, K., McGhee, J. R., Van Dyke, T. E., Bamberg, T. V., Hiroi, T. & Kiyono, H. (1996) Absence of exogenous IL-4 induced apoptosis of gingival macrophages may contribute to chronic inflammation in periodontal disease. *American Journal of Pathology* **148**, 331–339.

Yamazaki, K., Ueki-Maruyama, K., Oda, T., Tabeta, K., Shimada, Y., Tai, H., Nakajima, T., Yoshie, H., Herawati, D. & Seymour, G. J. (2003) Single-nucleotide polymorphism in the CD14 promoter and periodontal disease expression in a Japanese population. *Journal of Dental Research* **82**, 612–616.

Youn, J., Hwang, S., Cho, C., Min, J., Kim, W., Park, S. & Kim, H. (2000) Association of the interleukin-4 receptor α variant Q551R with Th1/Th2 imbalance in connective tissue disease. *Immunogenetics* **51**, 743–746.

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