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ORIGINAL ARTICLE

Serum sCD14, polymorphism of CD14^{-260} and periodontal infection

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BACKGROUND AND AIMS: CD14 is a co-receptor involved in the recognition of Gram-negative and positive bacteria. Infections are known to influence serum sCD14 levels, and CD14 gene promoter polymorphism (CD14 C−260T) has been reported to be associated with many infectious diseases. Our aim was to investigate whether serum sCD14 concentration is associated with periodontal infection and the CD14^{-260} genotype.

SUBJECTS AND METHODS: The periodontal status of 56 subjects with chronic periodontitis and 28 controls was clinically examined. Serum sCD14 concentration was analyzed using ELISA and CD14^{-260} genotype using polymerase chain reaction (PCR).

RESULTS: The mean concentration of sCD14 in serum was significantly higher in subjects with periodontitis than in control subjects (4.9 μg ml^{-1} *vs* 3.8 μg ml^{-1}, $P < 0.001$). Serum sCD14 concentration associated significantly with the extent of advanced periodontal disease. In a regression analysis including both subject groups, the CD14^{-260} genotype was a significant determinant for serum sCD14 concentration. After stratification by periodontal health status (periodontitis *vs* controls), the influence of the CD14^{-260} genotype on serum sCD14 concentration was seen only in the control group.

CONCLUSIONS: Periodontal infection is associated with the serum concentration of sCD14. Moderate to severe periodontal infection overshadows the influence of the genotype on serum sCD14 concentration.

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Introduction

Periodontitis is a multifactorial disease with microbial dental plaque as the initiator.

Besides the pathogenic microbial flora, a wide variety of other determinants including social, behavioral, and systemic factors as well as genetic and environmental factors influence the initiation and progression of the disease (Nunn, 2003). Among the bacterial species reported to be consistently and strongly associated with the progression of periodontitis, are the Gram-negative species *Porphyromonas gingivalis*, *Tannerella forsythia*, *and Treponema denticola* (Socransky and Haffajee, 2005). However, the major factors responsible for the destruction of the tooth supporting periodontal structures are the inflammatory mediators released by the host cells. High responders are individuals who, as a response to the bacterial antigens, produce elevated levels of inflammatory mediators and consequently experience more severe disease.

CD14 plays a central role in the recognition of Gram-negative and positive bacteria. It acts on the cell membrane by retaining LPS and other bacterial ligands and facilitating the ligand-Toll-like receptor interaction (Wright *et al*, 1999; Kaisho and Akira, 2001; da Silva Correia *et al*, 2001; Bas *et al*, 2004). Engagement of this complex results in the release of inflammatory cytokines from the cell (Medzhitov and Janeway, 2000). CD14 protein is found in two distinct forms: membrane CD14 (mCD14), expressed primarily on the surface of monocytes/macrophages and neutrophils, and a soluble form (sCD14) that lacks the glycosylphosphatidylinositol anchor. Soluble CD14 is shed from monocytes and secreted by liver cells, and it is found in normal serum in microgram concentrations (Bazil *et al*, 1986). Soluble CD14 mediates LPS-induced activation of non-CD14-expressing cells, such as endothelial and epithelial cells (LeVan *et al*, 2001). Even though CD14, in both the membrane bound and soluble forms, mainly causes enhanced inflammatory responses, some reports also

mention that sCD14 may act as a down-regulatory molecule, especially when present in high serum concentrations. It may compete with mCD14 for LPS binding and divert LPS from mCD14 and transfer it to plasma lipoproteins for LPS inactivation (Kitchens and Thompson, 2005).

Soluble CD14 is also known for its capability of modulating cellular and humoral immune responses by interacting directly with T and B cells. Accordingly, the inhibitory effect of sCD14 on both T cell proliferation and on the release of the Th1 and Th2 associated cytokines IL-2, IFN-γ, and IL-4 has been demonstrated (Rey Nores *et al*, 1999). A later study by the same group (Arias *et al*, 2000) reports of the capacity of sCD14 to bind to different B cell subsets. Human tonsillar B cells were stimulated under various conditions and the cultures were either supplemented with sCD14 or not. Irrespective of the stimulating condition, the IgG1 production was significantly increased in the presence of sCD14. On the contrary, sCD14 had an inhibitory effect on the production of IgE by activated tonsillar B cells. Furthermore, a significant increase in the production of IgG1 and a profound inhibition of IgE by antigen-stimulated PBMC were noted in the presence of sCD14.

The concentration of sCD14 in serum is elevated in many systemic inflammatory, immunologic, and infectious diseases, including Gram-negative septic shock (Landmann *et al*, 1995), HIV infection (Lien *et al*, 1998), rheumatoid arthritis (Yu *et al*, 1998), infectious neurological diseases (Nockher *et al*, 1999), atopic dermatitis (Wűthrich *et al*, 1992), and asthma (Garty *et al*, 2000; Martin *et al*, 2006).

Patients with periodontitis have been reported to have increased serum concentrations of sCD14 compared with periodontally healthy control subjects (Hayashi *et al*, 1999). According to a recent report by Isaza-Guzmán *et al* (2008), the salivary sCD14 levels of subjects with either chronic or aggressive periodontitis were significantly higher than those detected for healthy controls. Contradictory to the above, no statistically significant differences were found in the mean serum sCD14 concentration between patients with generalized aggressive periodontitis and periodontally healthy control subjects (Pietruska *et al*, 2006).

A single nucleotide polymorphism (SNP) in the proximal CD14 promoter at position −260 (corresponding to position −159) has been identified. Carriage of the T allele has been reported to be associated with myocardial infarction (Hubacek *et al*, 1999; Morange *et al*, 2005) and carotid atherosclerosis (Giacconi *et al*, 2007). The possible role of the CD14 polymorphism has also been analyzed in relation to the expression of periodontal disease, and carriage of the TT genotype of $CD14^{-260}$ has been associated with disease susceptibility (Yamazaki *et al*, 2003; Laine *et al*, 2005). According to our previous result, periodontitis patients carrying the T-containing genotype of $CD14^{-260}$ (CT/TT) have a higher extent of periodontal disease compared with those carrying the CC genotype (Tervonen *et al*, 2007). T allele carriage is reported to be connected to higher serum CD14 levels in either homozygote or heterozygote form (Baldini *et al*, 1999; Karhukorpi *et al*, 2002; Zhao *et al*, 2007; LeVan *et al*, 2008). To our knowledge, no data is available of the association of CD14 gene polymorphism and the expression of sCD14 in periodontal disease.

In this study, our aim was to investigate whether serum sCD14 concentration associates with the extent of periodontal infection and the $CD14^{-260}$ genotype.

Materials and methods

The study protocol was accepted by the Ethical Committee of the Faculty of Medicine, University of Oulu, Finland, and the informed consent of all the subjects was obtained.

Subjects

A total of 56 subjects of Caucasian origin and a mean age of 43.2 (±9.2) years (range 22–61 years) with chronic periodontitis (Armitage, 1999) participated in the study. In principle, the subjects were periodontally untreated and referred to specialist therapy at the Specialist Dental Health Care Unit, City of Oulu. The control group consisted of 28 systemically healthy control subjects, aged 40.0 (±10.7) years (range 20–58 years) (Table 1). The total number of sites measured in these subjects was 3068. PD ≥ 4 mm was detected in 0.9%, AL ≥ 4mm on 0.5%, and BL ≥ 6 mm on 3.9% of the measured sites per subject. All subjects were examined by the same specialist in periodontology (TR). Exclusion criteria included need for prophylactic antibiotic medication in conjunction with periodontal probing, use of immunosuppressive medication, presence of rheumatoid arthritis, diabetes mellitus, and

Table 1 Subject characteristics indicated as mean values (±s.d.) and numbers (percentages) of subjects

Parameter	*Periodontitis group,* n = *56*	*Control group,* n = *28*
Age		
Mean (±s.d.)	43.2 ± 9.2	40.0 ± 10.7
Range	22–61	20–58
Gender (%)		
Female	37 (66.1)	18 (64.3)
Male	19 (33.9)	10 (35.7)
CD14 genotype (%)		
CT/TT	27 (48.2)	10 (35.7)
CC	29 (51.8)	18 (64.7)
Smoking habits (%)		
Non-smoker	20 (35.7)	18 (64.3)
Smoker	36 (64.3)	10 (35.7)
Periodontal parameters (mean % of sites ± s.d.)		
BOP	80.6 ± 18.9	14.5 ± 10.1
PD ≥ 4 mm	51.3 ± 23.1	0.90 ± 2.16
PD ≥ 6 mm	17.3 ± 21.2	–
AL ≥ 4 mm	45.2 ± 28.3	0.51 ± 1.50
AL ≥ 6 mm	18.0 ± 23.5	–
BL ≥ 6 mm	31.2 ± 25.8	3.88 ± 7.15
BL ≥ 8 mm	15.4 ± 18.5	–

BOP, bleeding on probing, PD, probing depth; AL, attachment level; BL, bone level.

asthma, and use of antibiotics during the past four months. None of the subjects had been diagnosed with cardiovascular disease. There were altogether 20 non-smokers, who had never smoked, in the periodontitis group. The group of smokers comprised nine smokers who smoked either occasionally or <10 cigarettes per day and 27 who smoked ≥ 10 cigarettes per day. In the control group, 10 subjects smoked currently and 18 subjects had never smoked. The subjects' body mass index (BMI, weight of the subject divided by the square of the height, i.e., kg m^{-2}) and age (years) were recorded.

Clinical and radiographic parameters

Bleeding on probing (BOP), probing pocket depth (PD), and periodontal attachment level (AL) were measured at four sites (mesiobuccal, midbuccal, distobuccal, and midlingual) of all the teeth except the third molars. The alveolar bone level (BL) from the cementoenamel junction to the level of the alveolar ridge was measured from orthopantomograms at the mesial and distal sites of the teeth by one examiner (TT). A more detailed description of the clinical and radiological parameters is included in our previous report (Tervonen *et al*, 2007).

Serum sCD14 concentration

A 10 ml blood sample was taken into a non-coagulant containing tube for serum collection. Serum sCD14 concentrations of the samples were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (Human sCD14 Elisa test kit; Hycult biotechnology, Uden, the Netherlands). The minimum detection level of the test is 2 ng ml^{-1} and a measurable concentration range 2 to 100 ng ml^{-1}. The principle of the test is a sandwich ELISA, where the sample sCD14 is captured by the solid bound antibody. The bound sCD14 is detected by a second (tracer) antibody using a streptavidin-peroxidase conjugate. The wells are read using 450 nm wavelength in an ELISA reader (Multiskan; Labsystems, Turku, Finland). The amount of sCD14 in the samples tested is obtained using the standard curve of known amounts of sCD14 provided by the manufacturer (concentrations 1.6–100 ng ml^{-1}). The serum dilution of 1:400 was used for measurement, and the results were multiplied with the factor 400.

CD14 (C−260T) genotyping

Patient DNA was extracted from an EDTA blood sample (10 ml) using salt extraction method. The CD14 (C−260T) polymorphism was measured using allele-specific PCR method, developed, and validated by our group (Karhukorpi *et al*, 2002). Primers *cfors* (5′-CTC CAG AAT CCT TCC TGT TAC GAC-3′, Karhukorpi *et al*, 2002) and *cdp2* (5′-TTC TTT CCT ACA CAG CGG CAC CC-3′, Hubacek *et al*, 1999) were used for C allele detection. Primers *trevs* (5′-TGT AGG ATG TTT CAG GGA GGG GTA-3′, Karhukorpi *et al*, 2002) and *cdp1* (5′-TTG GTG CCA ACA GAT GAG GTT CAC-3′, Hubacek *et al*, 1999) were used for T allele detection. Primers *cfors* and *trevs* have a mismatch inserted at the penultimate 3′ nucleotide to increase the specificity for the allele (mismatch underlined). The primers were originally constructed to be used in the same tube for PCR amplification, as the primers amplify the specific allele (C *vs* T) and the length of PCR product shows the allele (allele T: 381 bp, allele C: 227 bp). In this study, we however did separate tubes for both alleles (Figure 1). Amplification conditions were: an initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, at 60°C for 30 s, and at 72°C for 1 min. The final extension step was at 72°C for 5 min. In each PCR series, samples with known genotypes were also included to show that the PCR works. In Figure 1, the second line in the heading shows which primers were used for amplification, and the visible band of the right size indicates the presence of the allele. Two parallel tubes are shown for each patient. The genotype is indicated in the first line of the figure heading. In the statistical evaluation, genotypes TT and CT (T-containing genotypes) *vs* genotype CC was estimated separately, as T-containing genotypes were reported to be associated with higher serum sCD14 values (Karhukorpi *et al*, 2002). The Hardy–Weinberg equilibrium criteria for CD14 genotypes were fulfilled in both subject groups.

Data analysis

The severity and the distribution of periodontal disease were expressed as the extent (percentages) of affected sites using two different threshold values for each variable (≥ 4 mm and ≥ 6 mm for PD and AL and ≥ 6 mm and ≥ 8 mm for BL). The serum sCD14 levels were normally distributed and the Student's *t*-test was used to assess statistical significance in the mean values of serum sCD14 between the periodontitis subjects and control subjects, and between the $CD14^{-260}$ genotypes. Associations between soluble sCD14 concentrations (used as a continuous variable) and periodontal infec-

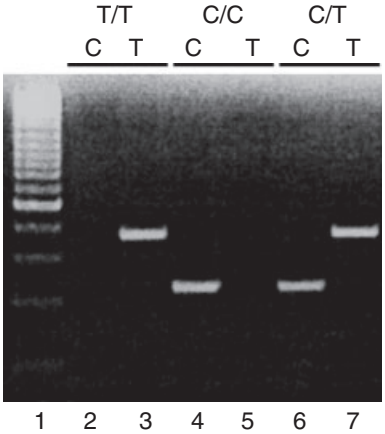

Figure 1 A gel electrophoresis image of CD14 (−260C/T) genotyping. The results of three patient samples are shown (patient 1, lanes 2–3; patient 2, lanes 4–5; patient 3, lanes 6–7). The first lane of each sample is an electrophoresis picture of the amplification with primers specific for allele C, and the second lane with primers specific for allele T. Allele C is indicated if there is a positive 227 bp band and T in case of a positive 381 bp band on the respective lanes. The genotype is marked in the first line of the heading of the picture. Lane 1: mw ladder

tion and the $CD14^{-260}$ genotype were analyzed using linear regression analyses adjusted for gender, smoking, age, and BMI. In the statistical analyses, the level of significance was set at $P < 0.05$. All calculations were carried out with the aid of statistical software (SPSS, version 16.0; SPSS Inc., Chicago, IL, USA).

Results

The characteristics of the subjects in the periodontitis and the control group are shown in Table 1.

The mean concentration of sCD14 (±s.d.) in serum was significantly higher in the patients with periodontitis than in the control subjects (4.9 ± 1.2 $\mu g\ ml^{-1}$ *vs* 3.8 ± 0.7 $\mu g\ ml^{-1}$, $P < 0.001$). Irrespective of their periodontal health status, the subjects carrying the CT/TT genotype presented higher sCD14 levels than did those carrying the CC genotype (4.9 ± 1.4 $\mu g\ ml^{-1}$ *vs* 4.3 ± 1.0 $\mu g\ ml^{-1}$, $P = 0.019$). The differences in the concentrations of sCD14 by genotype after stratification by periodontal health status (periodontitis *vs* control group) were significant ($P = 0.03$) only in the control subjects (Figure 2).

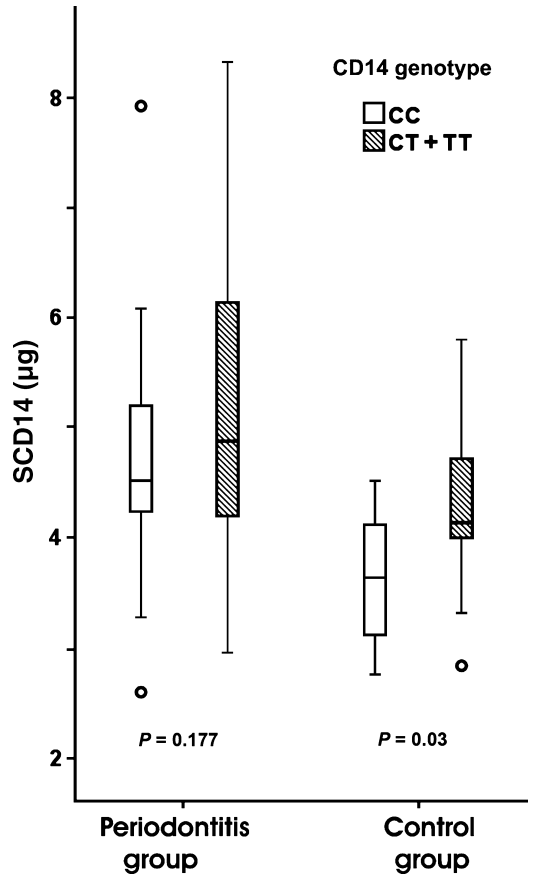

Figure 2 Box-plots representing concentrations of sCD14 ($\mu g\ ml^{-1}$) by $CD14^{-260}$ genotype in subjects with periodontitis and in the control subjects. The box represents the first and third quartiles (rectangular boxes); the line within the box is the median and the little circles represent atypical values that were plotted separately

Associations of serum sCD14 concentration (the outcome variable) with periodontal health status and the $CD14^{-260}$ genotype were analyzed by means of linear regression analysis, adjusting for age, gender, smoking, and BMI. Both the $CD14^{-260}$ genotype and the subject group (periodontitis *vs* control) were found to be significant determinants of serum sCD14 concentration (Table 2). Furthermore, after adjustments for age, gender, smoking, and BMI, the extent of advanced periodontal disease as indicated by the percentages of sites with AL ≥ 6 mm and BL ≥ 8 mm was associated with serum sCD14 concentration in subjects with periodontitis ($P = 0.002$ and $P = 0.007$, respectively, Table 3). The association between the extent of PD ≥ 6 mm and sCD14 concentration was significant at the level of $P = 0.067$.

Discussion

One of the main findings of this study was that periodontal infection was an independent determinant of serum sCD14 concentration. This is in line with the result of Hayashi *et al* (1999), who found increased serum concentrations of sCD14 in patients with periodontitis. Therefore, regardless of the local character of periodontal disease, it may be regarded equal to systemic inflammatory and infectious diseases that up-regulate serum sCD14 (Landmann *et al*, 1995; Lien *et al*, 1998; Yu *et al*, 1998; Garty *et al*, 2000; Martin *et al*, 2006).

Previously, the extent of bacteremia has been observed to be related to the severity of periodontal infection (The American Academy of Periodontology, 1998; Geerts *et al*, 2002). The total surface area of all periodontal lesions in an untreated patient with severe

Table 2 Associations between serum sCD14 level (the outcome variable) and $CD14^{-260}$ genotype (CT/TT *vs* CC) and subject group (periodontitis *vs* control group) adjusted for age, gender, smoking, and BMI

	B	*95% CI for B*	P-*value*
$CD14^{-260}$ genotype (CT/TT *vs* CC)	486.8	10.8–962.8	0.045
Subject group (Periodontitis *vs* controls)	887.6	354.4–1420.8	0.001

Both subject groups included ($n = 84$).

Table 3 Age, gender, smoking, and BMI adjusted associations between serum sCD14 level (the outcome variable) and the extent of periodontal disease (percentages of affected sites) in the periodontitis group

	B	*95% CI for B*	P-*value*
PD ≥ 6 mm	14.6	−1.1–30.0	0.067
AL ≥ 6 mm	21.8	8.2–35.5	0.002
BL ≥ 8 mm	25.0	7.0–42.9	0.007

PD, pocket depth; AL, attachment level; BL, bone level.

periodontitis may cover from 15 to 20 cm^2, and the ulcerated subgingival pocket epithelium allows bacterial cells or their products (*i.e.,* LPS) to enter the systemic circulation (Loos *et al*, 2005). The source of sCD14 in the circulation is either protease-mediated shedding of mCD14 from leukocytes (Bazil and Strominger, 1991), leakage from the inflamed area into the circulation, or production by hepatocytes (Hetherington *et al*, 1999; Bas *et al*, 2004). According to the results of this study, dose-dependence prevailed between the extent of periodontal infection and serum sCD14. It can be hypothesized that with an increasing systemic LPS challenge, higher amounts of sCD14 were produced by hepatocytes, and locally more sCD14 was dumped into the circulation from the inflamed periodontal area. In the control subjects of this study, both bleeding sites (14%) as well as occasional sites with deepened pockets (0.9% of the sites) were found (Table 1). Whether or not this minimal amount of inflammation had any influence on the sCD14 levels of the control subjects is not known.

In addition to the production of sCD14 by hepatocytes, it has been reported that CD14 is also secreted by serous acinar and intercalated duct cells of the human major salivary glands (Uehara *et al*, 2003). Apparently, the saliva CD14 plays a role in maintaining oral health, the concentration of soluble CD14 in parotid saliva being comparable with that in serum and 10-fold the amount in whole saliva. The soluble form of CD14 is expressed in the inflamed periodontal area and washed into saliva by gingival crevicular fluid (Jin and Darveau, 2001; Duncan *et al*, 2004). Isaza-Guzmán *et al* (2008) report a statistically significant correlation between salivary sCD14 concentration and the clinical periodontal parameters including the extent of periodontal disease and interpret it as increased production of sCD14 in cases with moderate to severe and generalized periodontal breakdown. That the extent of periodontal disease is associated significantly with the concentration of serum sCD14 level in our study is in line with the previous studies and indicates that CD14 amplifies the inflammatory response, resulting in periodontal attachment and bone loss.

Based on previous studies, it is likely that the $CD14^{-260}$ polymorphism alone is not sufficient to modulate serum sCD14 concentration, but rather sCD14 concentration is regulated by a combination of several SNPs (LeVan *et al*, 2006, 2008). Thus, regardless of the finding that a significantly higher serum sCD14 concentration was found in subjects carrying the CT/TT genotype than in those carrying the CC genotype of $CD14^{-260}$, we cannot ignore the possibility that SNPs other than $CD14^{-260}$ on their part modulated sCD14 concentration.

After stratification by periodontal health status, the between-genotype differences in serum sCD14 concentrations were statistically significant only in the control group. The reason for the lack of significant differences in the periodontitis group could be that in the case of moderate to severe disease, the infection had a dominant influence on the sCD14 level, overshadowing the significance of the genotype. LeVan *et al* (2008) recently evaluated the role of three different CD14 polymorphisms (including the $CD14^{-159}$polymorphism, in current nomenclature $CD14^{-260}$) in modulating the concentration of serum sCD14. Serum sCD14 concentration was measured both before and after purified *E. coli* LPS stimulation to understand the influence of CD14 polymorphism and endotoxin challenge on sCD14 levels. Homozygotes for the −159T alleles (and also for −1619G and −1359G) had higher baseline levels of sCD14. However, the association between CD14 polymorphisms and sCD14 levels was no longer present after the exposure, a situation that is analogous to the finding of our study.

The results of this study are based on the findings in fairly small sample sizes and therefore a need for verification of the results in larger samples is needed. However, we conclude that periodontal infection associates with the serum concentration of sCD14 in a dose-dependent way. Subjects carrying the T-containing genotype (CT/TT) of the $CD14^{-260}$ have higher concentrations of serum sCD14 than those with the CC genotype. Moderate to severe periodontal infection overshadows the influence of the genotype on serum sCD14 concentration observed in control subjects.

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References

Arias MA, Rey Nores JE, Vita N *et al* (2000). Cutting edge: human B cell function is regulated by interaction with soluble CD14: opposite effects on IgG1 and IgE production. *J Immunol* **164:** 3480–3485.

Armitage GC (1999). Development of a classification system for periodontal diseases and conditions. *Ann Periodontol* **4:** 1–6.

Baldini M, Lohman IC, Halonen M, Erickson R, Holt P, Martinez F (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. *Am J Respir Cell Mol Biol* **20:** 976–983.

Bas S, Gauthier BR, Spenato U, Stingelin S, Gabay C (2004). CD 14 is an acute-phase protein. *J Immunol* **172:** 4470–4479.

Bazil V, Strominger JL (1991). Shedding as a mechanism of down-modulation of CD14 on stimulated human monocytes. *J Immunol* **147:** 1567–1574.

Bazil V, Horejsi V, Baudys M *et al* (1986). Biochemical characterization of a soluble form of the 53-kDa monocyte surface antigen. *Eur J Immunol* **16:** 1583–1589.

Duncan L, Yoshioka M, Chandad F, Grenier D (2004). Loss of lipoplysaccharide receptor CD14 from the surface of human macrophage-like cells mediated by *Porphyromonas gingivalis* outer membrane vesicles. *Microb Pathog* **36:** 319–325.

Garty BZ, Monselise Y, Nitzan M (2000). Soluble CD14 in children with status asthmaticus. *Isr Med Assoc J* **2:** 104–107.

Geerts SO, Nys M, De MP *et al* (2002). Systemic release of endotoxins induced by gentle mastication: association with periodontitis severity. *J Periodontol* **73:** 73–78.

Giacconi R, Caruso C, Lio D *et al* (2007). CD 14 C (-260) T polymorphism, atherosclerosis, elderly: role of cytokines and metallothioneins. *Int J Cardiol* **120:** 45–51.

Hayashi J, Masaka T, Ishikawa I (1999). Increased levels of soluble CD14 in sera of periodontitis patients. *Infect Immun* **67:** 417–420.

Hetherington CJ, Kingsley PD, Crocicchio F *et al* (1999). Characterization of human endotoxin lipoplysaccharide receptor CD14 expression in transgenic mice. *J Immunol* **162:** 503–509.

Hubacek JA, Rothe G, Pitha J, Skodova Z, Stanek V, Poledne R (1999). C(-260) → T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. *Circulation* **99:** 3218–3220.

Isaza-Guzmán DM, Aristizábal-Cardona D, Martínez-Pabón MC, Velásquez-Echeverri H, Tobón-Arroyave SI (2008). Estimation of sCD14 levels in saliva obtained from patients with various periodontal conditions. *Oral Dis* **14:** 450–456.

Jin J, Darveau RP (2001). Soluble CD14 levels in gingival crevicular fluid of subjects with untreated adult periodontitis. *J Periodontol* **72:** 634–640.

Kaisho T, Akira S (2001). Dendritic-cell function in Toll-like receptor- and MyD88-knockout mice. *Trends Immunol* **22:** 78–83.

Karhukorpi J, Yan Y, Niemelä S *et al* (2002). Effects of CD14 promoter polymorphism and *H. pylori* infection and its clinical outcomes on circulating CD14. *Clin Exp Immunol* **128:** 326–332.

Kitchens RL, Thompson PA (2005). Modylatory effects of sCD14 and LPB on LPS-host cell interactions. *J Endotoxin Res* **11:** 225–229.

Laine ML, Morré SA, Murillo LS, van Winkelhof AJ, Pena AS (2005). CD14 and TLR4 gene polymorphisms in adult periodontitis. *J Dent Res* **11:** 1042–1046.

Landmann R, Zimmerli W, Sansano S *et al* (1995). Increased circulating soluble CD14 is associated with high mortality in gram-negative septic shock. *J Infect Dis* **171:** 639–644.

LeVan TD, Bloom JW, Bailey TJ *et al* (2001). A common single nucleotide polymorphism in the CD14 promoter decreases the affinity of Sp protein binding and enhances transcriptional activity. *J Immunol* **167:** 5838–5844.

LeVan TD, Guerra S, Klimecki W *et al* (2006). The impact of CD14 polymorphisms on the development of soluble CD14 levels during infancy. *Genes Immun* **7:** 77–80.

LeVan TD, Michel O, Dentener M *et al* (2008). Association between CD14 polymorphisms and serum soluble CD14 levels: effect of atopy and endotoxin inhalation. *J Allergy Clin Immunol* **121:** 434–440.

Lien E, Aukrust P, Sundan A, Műller F, Frøland S, Espevik T (1998). Elevated levels of serum-soluble CD 14 in human immunodeficiency virus type 1 (HIV-1) infection: correlation to disease progression and clinical events. *Blood* **92:** 2084–2092.

Loos BG, John RP, Laine ML (2005). Identification of genetic risk factors for periodontitis and possible mechanisms of action. *J Clin Periodontol* **32:** 159–179.

Martin AC, Laing IA, Khoo SK *et al* (2006). Acute asthma in children. Relationships among CD14 and CC16 genotypes, plasma levels and severity. *Am J Respir Crit Care Med* **173:** 617–622.

Medzhitov R, Janeway C Jr (2000). Innate immunity. *N Engl J Med* **343:** 338–344.

Morange PE, Saut N, Alessi MC *et al* (2005). Interaction between the C-260 T polymorphism of the CD14 gene and the plasma IL-6 concentration on the risk of myocardial infarction: the HIFMECH study. *Atherosclerosis* **179:** 317–323.

Nockher WA, Wick M, Pfister HW (1999). Cerebrospinal fluid levels of soluble CD14 in inflammatory and non-inflammatory diseases of the CNS: upregulation during bacterial infections and viral meningitis. *J Neuroimmunol* **101:** 161–169.

Nunn ME (2003). Understanding the etiology of periodontitis: an overview of periodontal risk factors. *Periodontol 2000* **32:** 11–23.

Pietruska M, Zak J, Pietruski J, Wysocka J (2006). Evaluation of mCD14 expression on monocytes and the blood level of sCD14 in patients with generalized aggressive periodontitis. *Adv Med Sci* **51:** 166–169.

Rey Nores JE, Bensussan A, Vita N *et al* (1999). Soluble CD14 acts as a negative regulator of human T cell activation and function. *Eur J Immunol* **29:** 265–276.

da Silva Correia J, Soldau K, Christen U, Tobias PS, Ulevitch RJ (2001). Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex: transfer from CD14 to TLR4 and MD-2. *J Biol Chem* **276:** 21129–21135.

Socransky S, Haffajee A (2005). Periodontal microbial ecology. *Periodontol 2000* **38:** 135–187.

Tervonen T, Raunio T, Knuuttila M, Karttunen R (2007). Polymorphisms in CD14 and IL-6 genes associated with periodontal disease. *J Clin Periodontol* **34:** 377–383.

The American Academy of Periodontology (1998). Periodontal disease as a potential risk factor for systemic diseases. *J Periodontol* **69:** 841–850.

Uehara A, Sugawara S, Watanabe K *et al* (2003). Constitutive expression of a bacterial pattern recognition receptor, CD14, in human salivary glands and secretion as a soluble form in saliva. *Clin Diagn Lab Immunol* **10:** 286–292.

Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC (1999). CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* **249:** 1431–1433.

Wűthrich B, Kägi MK, Joller-Jemelka H (1992). Soluble CD14 but not interleukin-6 is a new marker for clinical activity in atopic dermatitis. *Arch Dermatol Res* **284:** 339–342.

Yamazaki K, Ueki-Maruyama K, Oda T *et al* (2003). Single-nucleotide polymorphism in the CD14 promoter and periodontal disease expression in a Japanese population. *J Dent Res* **82:** 612–616.

Yu S, Nakashima N, Xu BH *et al* (1998). Pathological significance of elevated soluble CD14 production in rheumatoid arthritis: in the presence of soluble CD14, lipopolysaccharides at low concentrations activate RA synovial fibroblasts. *Rheumatol Int* **17:** 237–243.

Zhao D, Sun T, Zhang X *et al* (2007). Role of CD14 promoter polymorphisms in *Helicobacter pylori* infection- related gastric carcinoma. *Clin Cancer Res* **13:** 2362–2368.