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CD14 Monocyte Receptor, Involved in the Inflammatory Cascade, and Insulin Sensitivity

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Soluble CD14 (sCD14), detectable at high concentrations constitutively present in the circulation, plays a key role in the neutralization of lipopolysaccharide, one of the most potent biologic response modifiers currently recognized and involved in the regulation of the inflammatory cascade. We tested whether circulating sCD14 was linked to inflammatory parameters and to insulin resistance in apparently healthy subjects.

Serum sCD14 concentration did not significantly correlate with body mass index (BMI), waist to hip ratio, systolic or diastolic blood pressure, serum glucose, fasting insulin, fasting insulin resistance index [homeostasis model assessment (HOMA)], or serum uric acid among 123 subjects. The association between sCD14 and fasting insulin (r = −0.19, *P* = 0.08) and between sCD14 and HOMA (r = −0.21, *P* = 0.06) tended toward statistical significance among men. Unexpectedly, sCD14 correlated positively with fasting triglycerides (TG) in all the subjects (r = 0.22, *P* = 0.014), and this association was most significant in men (r = 0.34, *P* = 0.002). After controlling for TG, the relationships between sCD14 levels and fasting insulin (r = −0.25, *P* = 0.029), HOMA (r = −0.28, *P* = 0.014), and uric acid (r = −0.30, *P* = 0.006) were significant in men. Among nonsmoking men (n = 44), sCD14 significantly correlated with waist diameter (r = −0.30, *P* = 0.03), diastolic blood pressure (r = −0.34, *P* = 0.022), and HOMA (r = −0.30, *P* = 0.03). In a multiple linear regression analysis, BMI (*P* < 0.00001), TG (*P* = 0.003), and sCD14 (*P* = 0.04) (but not age, sex, waist, or smoking status) independently contributed to 26% of HOMA variance.

A polymorphism of the CD14 gene, a C-to-T transition at bp −159 from the major transcription start site, seems to play a significant role in regulating serum sCD14 levels. In a subsample of 33 healthy subjects, carriers of the T allele were similar (in age, sex, BMI, fat mass, waist to hip ratio, blood pressure, and fasting glucose and insulin levels) to C/C homozygotes. In the former, integrated area under the curve for serum glucose concentrations after an oral glucose tolerance test was significantly lower (*P* = 0.02), and insulin sensitivity (SI) index (minimal model analysis) significantly higher (*P* = 0.036), than in C/C homozygotes. Among 32 type 2 diabetic subjects, carriers of the T allele also showed a significantly higher SI index (*P* = 0.03) and had significantly lower C-reactive protein (*P* = 0.03) and lower circulating soluble intercellular adhesion molecule-1 concentrations (*P* = 0.01) than did C/C homozygotes.

To our knowledge, this is the first study to suggest an effect of a genetic polymorphism on both SI (healthy subjects and type 2 diabetic patients) and endothelial dysfunction (sICAM-1 levels) in type 2 diabetes mellitus. (*J Clin Endocrinol Metab* 88: 1780–1784, 2003)

INFLAMMATORY PROCESSES ARE being increasingly recognized as important actors in triggering and modulating insulin resistance and atherosclerosis and in their complications. Genetically determined variations in the inflammatory response contribute to different susceptibility for developing increased fat mass (FM), dyslipidemia, hypertension, and insulin resistance among healthy individuals, ultimately leading to atherosclerosis (1–9). The attachment of monocytes to the endothelium, followed by their migration into the intima, is a crucial step in the development of atherosclerotic lesions. Maturation and activation of monocytes in the subendothelial space is a complex process in which a myriad of molecules are involved. CD14 is a multifunctional receptor, a 55-kDa glycoprotein, constitutively expressed in considerable amounts on the surface of mature monocytes, macrophages, and neutrophils (10). CD14 has specificity for lipopolysaccharides (LPS) and other bacterial wall-derived components (11). The stimulation of monocytes/macrophages by LPS induces overexpression of certain cytokines and inflammatory mediators that amplify and diversify the LPS signals (12).

Mechanisms that regulate responses to LPS are very important for the host. LPS is well established to provoke severe endothelial dysfunction and shows a variety of proatherogenic properties (12). The buffering of LPS is crucial not only during acute inflammatory and infectious processes. In normal humans, triglyceride (TG)-rich lipoproteins contain detectable levels of endogenous LPS that are presumably scavenged *in vivo* (13).

A soluble form of CD14, sCD14, is abundant in serum and is apparently derived both from secretion of CD14 and from enzymatically cleaved glycosyl-phosphatidylinositol-anchored tissue CD14 (14, 15). Plasma lipoproteins promote the release of bacterial LPS from the monocyte cell surface, and sCD14 is involved in this process. Neutralization of LPS by reconstituted lipoprotein particles is accelerated more than 30-fold by addition of sCD14 (16, 17).

A polymorphism of the CD14 gene, a C-to-T transition at

Abbreviations: AUC, Area under the curve; BMI, body mass index; DBP, diastolic blood pressure; FM, fat mass; HOMA, homeostasis model assessment; LPS, lipopolysaccharide(s); OGTT, oral glucose tolerance test; SBP, systolic blood pressure; sCD, soluble CD; SI, insulin sensitivity; sICAM, soluble intercellular adhesion molecule; sTNFR, soluble TNF receptor; TG, triglycerides; WHR, waist to hip ratio.

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bp −159 from the major transcription start site, seems to play a significant role in regulating serum sCD14 levels (18). We hypothesized that the sensitivity of individuals to infection and inflammation runs together with susceptibility to developing insulin resistance and endothelial dysfunction, ultimately leading to atherosclerosis. To address this issue, we evaluated insulin resistance and inflammatory markers in apparently healthy individuals and in patients with type 2 diabetes mellitus according to CD14 gene polymorphism and circulating sCD14 concentration.

Subjects and Methods

Inclusion and exclusion criteria

Healthy subjects. None of the subjects were taking any medication or had any evidence of metabolic disease other than obesity. All subjects were of Caucasian origin and reported that their body weight had been stable for at least 3 months before the study. Inclusion criteria were: 1) body mass index (BMI; weight in kilograms divided by the square of height in meters) less than 40 kg/m^2; 2) absence of any systemic disease; and 3) absence of any infections in the previous month. Smoker was defined as any person consuming at least 1 cigarette a day in the previous 6 months.

Type 2 diabetic patients. All patients were of Caucasian origin and underwent a full medical history. The diabetic patients were prospectively recruited from diabetes outpatient clinics, on the basis of the criteria that excluded diseases associated with overt inflammation (4). Informed written consent was obtained after the purpose, nature, and potential risks were explained to the subjects. The experimental protocol was approved by the Hospital Ethics Committee. Alcohol, caffeine, and all medications (including sulfonylurea, metformin, and insulin) were withheld within 12 h of different tests.

Measurements

BMI, waist to hip ratio (WHR), FM and fat-free mass (bioelectric impedance; Holtain BC Analyzer, Cambridge, UK), and blood pressure were measured as previously described (1, 7). Tympanic membrane temperature (infrared tympanic thermometer; Omron Electric Co., Kyoto, Japan) was also measured in 28 consecutive healthy subjects.

Study of insulin sensitivity (SI)

In a subset of 33 healthy subjects, approximately half of the C/C homozygote group (n = 9, 5 women) and half of the carriers of the T allele (n = 24, 10 women) were selected for determination of glucose tolerance and SI (all nonsmokers). Mean age (36.6 ± 7 *vs.* 36.6 ± 7.5 yr) and mean BMI (31.2 ± 4.4 *vs.* 30.1 ± 5.4) were comparable between C/C homozygotes and carriers of the T allele. An oral glucose tolerance test (OGTT) and a frequently sampled iv glucose tolerance test to calculate SI were performed as previously described (5, 19, 20). Homeostasis model assessment (HOMA) value was calculated using the formula: glucose (mM) × insulin (mU/liter)/22.5 (21). In type 2 diabetic subjects, SI was calculated from an insulin tolerance test, as previously reported (22).

Genotyping of CD14 polymorphism

Genetic analyses involved the genotyping of a polymorphism in the promotor region of CD14 receptor (located on chromosomic band 5q31.1) characterized by a C to T transition at −159. This was accomplished by restriction fragment length polymorphism analyses. DNA was extracted from cellular blood components by the salting-out method. Genomic DNA was amplified using the following primer pair: forward, 5′-GTGCCAACAGATGAGGTTCAC-3′; and reverse, 5′-GCCTCTGACAGTTTATGTAATC-3′. The PCR was carried out in a final volume of 50 μl, containing 1 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate (Roche Molecular Biochemicals, Mannheim, Germany), 0.2 μM of each primer, and 1 U Taq DNA polymerase (Biotherm; Gene Craft, Munster, Germany). After an initial denaturation of 5 min at 94 C, the samples were subjected to 35 cycles at 94 C for 30 sec, 60.9 C for 30 sec, and 72 C for 30 sec, with a final extension of 7 min at 72 C. The 497-bp product was restricted with Eca 471 (Ava II; Fermentas, Amherst, NY) overnight at 37 C. The unrestricted 497-bp product represents the C allele (wild type), whereas a T allele was cut into 144- and 353-bp fragments. The three genotypes were scored after running on a 2.5% agarose gel and staining with ethidium bromide at a concentration of 10 mg/ml. A T/T homozygote was included in PCR reaction as a positive control of digestion. Additionally, genotyping in some patients was confirmed by sequence analysis using an ABI 310 PRISM Genetic Analyzer (PE Applied Biosystems).

Measurement of serum sCD14 levels

The sCD14-EASIA (Biosource Technologies, Inc. Europe S.A., Fleunes, Belgium) is a solid-phase enzyme-amplified sensitivity immunoassay performed on a microtiter plate. The minimum detectable concentration is estimated to be 1 ng/ml and is defined as the sCD-14 concentration corresponding to the average OD of 20 replicates of the zero SDs. The intra- and interassay coefficients of variation were less than 5.2% and 7.8%, respectively. No plasma proteins showed any cross-reactivity in this assay.

Analytical methods

Serum glucose and insulin concentrations and soluble TNF receptor 1 (sTNFR1) and receptor 2 (sTNFR2) levels were measured as previously described. HbA_{1c} (L-9100; Hitachi Scientific Instruments, Inc.), uric acid and C-reactive protein (Beckman, Fullerton, CA), and white blood total and differential cell count (Coulter Electronics, Hialeah, FL) were measured using routine laboratory tests. Soluble intercellular adhesion molecule (sICAM)-1 in citrated plasma samples was measured using a commercially available ELISA kit (sICAM-1 ELISA; BenderMedSystems Diagnostics, Vienna, Austria) according to the manufacturer's recommendations.

Statistical methods

Descriptive results of continuous variables are expressed as mean ± SD. Before statistical analysis, normal distribution and homogeneity of the variances were tested. Parameters that did not fulfill these tests (SI, sTNFR1, sTNFR2) were log transformed. The relations between variables were analyzed by unpaired *t* test, simple correlation (Pearson's r), and multiple regression in a stepwise manner. CD14 gene polymorphism was entered as 1 (C/C homozygotes) and 2 (carriers of the T allele). Levels of statistical significance were set at $P < 0.05$.

Results

To study the relationship between sCD14 and insulin-resistance-related phenotypes, we evaluated 123 healthy subjects. Eighty were men [age (mean ± SD), 40.2 ± 11 yr; BMI, 25.3 ± 3.9 kg/m^2; WHR, 0.97 ± 0.05]. Forty-three were women (age, 37.5 ± 9 yr; BMI, 24.1 ± 4.4 kg/m^2; WHR, 0.87 ± 0.056). When all subjects were considered as a whole, plasma sCD14 levels did not significantly correlate with BMI, WHR, systolic blood pressure (SBP) or diastolic blood pressure (DBP), serum glucose, fasting insulin, HOMA, or serum uric acid, a putative marker for insulin resistance. However, the association between sCD14 and fasting insulin (r = −0.19, $P = 0.08$) and between sCD14 and HOMA (r = −0.21, $P = 0.06$) tended toward statistical significance among men. Unexpectedly, sCD14 correlated positively with fasting TG (r = 0.22, $P = 0.014$), and this association was most significant in men (r = 0.34, $P = 0.002$). After controlling for TG, the relationships between sCD14 levels and serum insulin (r = −0.25, $P = 0.029$), FIRI (r = −0.28, $P = 0.014$), and uric acid (r = −0.30, $P = 0.006$) became significant in men, and a tendency was observed between sCD14 and SBP (r = −0.20, $P = 0.07$) and DBP (r = −0.21, $P = 0.058$). Smoking status also

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affected these associations. Among nonsmoking men (n = 44), sCD14 significantly correlated with waist diameter (r = −0.30, P = 0.03), HOMA (r = −0.30, P = 0.03), SBP (r = −0.32, P = 0.029), and DBP (r = −0.34, P = 0.022). Serum sCD14 concentration was also associated with body temperature, even within the narrow range of temperatures observed in healthy subjects (35.0–36.7 C) and correlated negatively with circulating sTNFR1 (r = −0.44, P = 0.01) and sTNFR2 (r = −0.43, P = 0.01) as markers of inflammation. In a multiple linear regression analysis to predict HOMA, BMI ($P < 0.00001$), TG ($P = 0.003$), and sCD14 ($P = 0.04$) (but not age, sex, waist, or smoking status) independently contributed to 26% of HOMA variance.

To evaluate whether these associations could be attributed to CD14 promoter gene polymorphism, 61 out of the 123 healthy subjects, whose characteristics are shown in Table 1, were genotyped. This subset did not differ significantly from the remaining subjects. They were divided into 2 groups on the basis of the CD14 gene polymorphism. Forty-one of these subjects had a T at position −159 of the CD14 gene: 25 were heterozygotes (C/T) and 16 homozygotes (T/T). The alleles were in Hardy-Weinberg equilibrium. Healthy carriers of the T allele were similar in age, sex, BMI, FM, WHR, blood pressure, and fasting glucose and insulin levels, in comparison with C/C homozygotes (Table 1). No significant differences in serum sCD14 concentration were found between healthy carriers and noncarriers of the T allele. A subset of 33 subjects, approximately half of the carriers of the T allele (n = 24, 10 women) and half of the C/C homozygote group (n = 9, 5 women), were selected for determination of glucose tolerance and SI (all nonsmokers). Integrated area under the curve (AUC) for serum glucose after OGTT (AUC glucose) was significantly lower in healthy carriers of the T allele in the presence of nonsignificantly different integrated insulin levels (Table 1). Carriers of the T allele also showed a significantly higher SI index (as measured during the OGTT or using the frequently sampled iv glucose tolerance test with minimal model analysis) than C/C homozygotes (Table 1).

TABLE 1. Anthropometric and biochemical variables of control subjects

Variable	C/C	C/T and TT	P
n	20	41	
Men/women	13/7	25/16	NS
Age (years)	40.1 ± 8.5	38.7 ± 9.5	NS
BMI (kg/m^2)	27.7 ± 4.7	27.1 ± 5.2	NS
FM (kg)	21.7 ± 12.6	19.6 ± 13.1	NS
Fat-free mass (kg)	60.1 ± 10.8	59.4 ± 11.8	NS
WHR			
Men	0.96 ± 0.03	0.98 ± 0.04	NS
Women	0.91 ± 0.1	0.92 ± 0.09	NS
SBP (mm Hg)	124.1 ± 13.6	124.7 ± 10.7	NS
DBP (mm Hg)	74.1 ± 10	73.1 ± 9.6	NS
Tympanic temperature (C) (n = 28)	35.6 ± 0.4	35.7 ± 0.4	NS
White blood cell count (× 10^9/ml)	6926 ± 1194	6897 ± 1751	NS
sTNFR1 (ng/ml)	1.93 ± 0.57	1.84 ± 0.50	NS
sTNFR2 (ng/ml)	3.58 ± 0.86	3.45 ± 1.07	NS
Fasting glucose (mM)	5.2 ± 1.04	5.1 ± 0.8	NS
Fasting insulin (mU/liter)	11.2 ± 6.6	9.3 ± 4.7	NS
sCD14 (μg/ml)	3.57 ± 1.2	3.55 ± 1.7	NS
AUC glucose[a]	10.5 ± 3.6	7.9 ± 2.4	0.022
During OGTT (mM)			
AUC insulin[a]	90.8 ± 61.5	81.1 ± 60.1	NS
During OGTT (mU/liter)			
SI OGTT[a] (mg·liter^2/mmol·mU·min)	35.2 ± 10.2	47.7 ± 15.4	0.033
SI[a] (min^{-1}/mU/liter)	1.42 ± 1.1	2.64 ± 1.5	0.036

NS, Not significant.

[a] n = 33 (9 C/C homozygotes and 24 carriers of the T allele. See text).

Last, we studied type 2 diabetic patients (n = 32; 8 women). Carriers of the T allele (n = 23; 6 women) were similar (as regards age, sex, BMI, WHR, SBP, DPB, fasting glucose, HbA_{1c}, years of evolution of diabetes, type of antidiabetic treatment, and chronic diabetic complications) to C/C homozygous subjects (n = 9; 2 women). Type 2 diabetic subjects with the T allele had significantly lower peripheral white blood cell count (6714 ± 1600 *vs.* 8501 ± 2291; P = 0.03), lower C-reactive protein (0.38 ± 0.27 *vs.* 1.43 ± 1.1; P = 0.03), lower serum concentration of sTNFR1 (0.83 ± 0.56 *vs.* 1.64 ± 0.9; P = 0.02), lower circulating sICAM-1 (174.2 ± 47.2 *vs.* 246.2 ± 77.1; P = 0.01), and higher SI index (3.65 ± 2.1 *vs.* 1.75 ± 0.95; P = 0.03) than C/C homozygotes. Because the usually increased BMI of type 2 diabetic patients is a well known confounding factor for sICAM-1 levels, we constructed a multiple linear regression analysis. Both BMI (P = 0.04) and CD14 gene polymorphism (P = 0.04) independently contributed to 41% of sICAM-1 variance. Furthermore, T/T homozygotes with type 2 diabetes showed significantly increased sCD14 levels, compared with C/T heterozygotes and C/C homozygotes (5.7 ± 1.7 *vs.* 4.6 ± 1.4 and 4.8 ± 1.3, respectively; P = 0.02).

Discussion

We describe here that sCD14 is associated with several insulin-resistance-related phenotypes (fasting insulin, HOMA, serum uric acid concentration), particularly in healthy men and type 2 diabetic patients. Furthermore, in nonsmoking men, sCD14 additionally correlated with waist diameter, SBP, and DBP. The sexual dimorphism in the relationship between SI and sCD14 is also observed with a myriad of other genetic and environmental factors. For instance, we have previously shown that SI was associated with serum IL-6 concentration in men but not in women (23).

Unexpected by us, sCD14 was directly associated with TG. We have not found any study evaluating sCD14 according to the lipoprotein profile in healthy volunteers. Interestingly, patients with chronic heart failure had simultaneously increased TG and sCD14 (24).

LPS, one of the most potent biologic response modifiers currently recognized, circulates in normal humans attached to TG-rich lipoproteins (13, 25). LPS is extraordinarily ubiquitous in nature, being present in food and water and in normal indoor environments as a constituent of house dust (26) and of cigarette smoke (27). LPS promotes the initiation of the inflammatory cascade and stimulates the production and release of cytokines. sCD14, detectable at high concentrations and constitutively present in the circulation (15), is believed to play a key role as intermediate in the neutralization of LPS under physiological conditions. sCD14 accelerates the transfer between

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LPS micelles and lipoproteins by acting as a carrier. sCD14 also enhances the release of monocyte-bound LPS, transferring LPS into plasma and into lipoproteins (16, 17) and, thus, decreases cellular responses to LPS, such as induction of TNF-α and interleukin 6 synthesis (16).

Endogenous LPS is continually produced within the gut, by the death of gram-negative bacteria, and is absorbed into intestinal capillaries. Low-grade portal venous LPS has been claimed to be the *status quo* in humans (28) and to tonically prime pancreatic insulin secretion in rats (29). In addition to monocytes, sCD14 is also produced by hepatocytes (30) and adipocytes in mice and humans (31, 32). Because both of these cells can also produce TNF-α, it is tempting to speculate that synthesis of this cytokine in the liver and adipose tissue is the result of the balance between LPS absorbed from the gut and locally produced sCD14. Decreased efficiency in neutralizing LPS-induced responses would lead to a chronic proinflammatory response and insulin resistance. This is further supported, in this study, by negative correlations between serum sCD14 and circulating sTNFR1 and sTNFR2 concentrations in healthy subjects. The soluble TNF-α receptors (sRNFRs) are quite stable in healthy individuals and are thought to reflect the inflammatory milieu (33). sCD14 levels were also negatively associated with body temperature. In fact, cytokines play a significant role in maintaining physiological functions, such as body temperature (34).

Type 2 diabetic carriers of the T allele showed decreased sTNFR1 levels, decreased white blood cell count, and decreased serum concentration of C-reactive protein, a major marker of inflammation. In fact, diabetic carriers of the T allele, probably associated with increased production of sCD14 in the long-term, exhibited a tightly regulated concentration of C-reactive protein in contrast to C/C homozygotes. Decreased circulating sICAM-1 concentrations [another marker of inflammation and endothelial dysfunction (35)] was also found in diabetic carriers of the T allele. sICAM-1 has been described to be independently associated with atherosclerosis and coronary artery disease (35). In fact, the T allele was found to be a protective factor for ischemic heart disease, because it occurs more frequently in older patients who had survived myocardial infarction (36).

In this sample, there was no differences in sCD14 levels among healthy subjects with different CD14 genotypes. However, it has been reported that T/T homozygotes have higher levels of circulating sCD14 (18), and we have observed similar associations in the subset of diabetic patients. Moreover, we show here that both CD14 genotype and sCD14 were associated with SI in both healthy subjects and type 2 diabetic patients. Although the reasons why we were not able to demonstrate a relationship between CD14 genotype and phenotype in healthy subjects are unclear, if not accounted for by the small number of subjects, we speculate that the higher SI of T carriers are linked to day-to-day increased efficiency in neutralizing LPS caused by constitutively increased sCD14 concentration.

To our knowledge, this is the first study to suggest an effect of a genetic polymorphism on both SI and endothelial dysfunction (serum sICAM-1 concentration) in type 2 diabetes mellitus. CD14 adds to TNF and adipoQ [as a close homolog of the complement protein Clq (37)] to the list of primordial molecules implicated in the recognition of microbial surfaces that are involved in immune system function and in the regulation of energy balance. Our findings further support that insulin resistance is, in fact, a chronic inflammatory condition (9).

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