

Association between *CD14* polymorphisms and serum soluble *CD14* levels: Effect of atopy and endotoxin inhalation

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Background: A prerequisite for activation of the innate immune response by endotoxin is its binding to *CD14*.

Objective: The aim of this study was to evaluate the role of *CD14* polymorphisms, atopy, and inhaled endotoxin in modulating serum *CD14* levels.

Methods: Healthy volunteers ($n = 88$) were genotyped for *CD14* polymorphisms at the -1619 , -1359 , and -159 loci, relative to the transcription start site. Subjects inhaled $20 \mu\text{g}$ of endotoxin, and white blood cell, C-reactive protein, LPS-binding protein, and soluble *CD14* (s*CD14*) levels were measured before and after exposure.

Results: Homozygotes for the -1619G , -1359G , and -159T alleles had higher baseline levels of s*CD14* than carriers of the *CD14*/ -1619AA ($P = .015$), -1359GT/TT ($P = .015$), or -159CC ($P = 0.017$) genotypes. s*CD14* levels increased within 24 hours of endotoxin inhalation ($P < .0001$ for all biomarkers); however, the association between *CD14* polymorphisms and s*CD14* levels was no longer present after exposure. The atopic status of an individual did not alter these associations. *CD14* polymorphisms were not associated with levels of white blood cells, C-reactive protein, and LPS-binding protein before or after endotoxin challenge.

Conclusion: These data suggest that *CD14* promoter polymorphisms and inhaled endotoxin modulate s*CD14* levels. **Clinical implications:** This might represent a mechanism by which *CD14* polymorphisms and endotoxin exposure differentially result in disease status. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: *CD14* antigen, endotoxin, single nucleotide polymorphism, inflammation, innate immunity, atopy, lipopolysaccharide, exposure

Endotoxin, with its purified derivative LPS from gram-negative bacteria, is a commonly encountered air contaminant in occupational (swine pens, poultry farms, cotton mills, breweries, and waste-processing plants)¹ and domestic² settings (house dust) that has been linked to classic respiratory symptoms in adults: reversible airflow obstruction, airway and systemic inflammation, airway hyperactivity, and accelerated decrease in airflow. In healthy subjects inhalation of endotoxin induces dose-related symptoms, a decrease in lung function, and both bronchial and systemic inflammatory responses.^{3,4} The airway symptoms (airway responsiveness and bronchial inflammation) are similar in healthy and atopic subjects in response to inhaled endotoxin, whereas the systemic inflammatory response is attenuated in atopic individuals.⁵ There is much variability between individuals in the amplitude of both the clinical and inflammatory responses to endotoxin,^{3,6,7} suggesting that endotoxin sensitivity, in part, is genetically determined. Subsequent studies in mice and human subjects with Toll-like receptor (TLR) 4 have indeed found this to be true.^{8,9}

CD14 is a pattern-recognition receptor that plays a critical role in the innate immune response to endotoxin.¹⁰⁻¹² Through interaction with both *CD14* and LPS-binding protein (LBP), endotoxin binds to the membrane-bound TLR4/MD-2 signaling complex and initiates innate host defense mechanisms, such as release of proinflammatory cytokines.¹³ We reasoned that if genetic variations in *CD14* could alter the structure of the *CD14* receptor or the amount of *CD14* available for interaction with endotoxin, the potential to influence clinical and inflammatory responses could be substantially modified.¹⁴ As part of

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Abbreviations used

CRP: C-reactive protein
 LBP: LPS-binding protein
 LD: Linkage disequilibrium
 sCD14: Soluble CD14
 SNP: Single nucleotide polymorphism
 SPT: Skin prick test
 WBC: White blood cell

the Innate Immunity Program in Genomic Applications, the *CD14* gene was screened for polymorphisms.¹⁵ No missense mutations were found in the coding region of the gene, but several were found in the 5' genomic region. In particular, a genetic variant, *CD14*−159 C→T (relative to the transcription start site) or *CD14*−260 C→T (relative to the translation start site), was found to be associated with higher levels of serum soluble CD14 (sCD14) expression in our Tucson studies.^{16–18} Functional genomic studies of *CD14*−159 and sCD14 expression supported this epidemiologic evidence. Increased transcriptional activity in luciferase reporter assays for the T allele was found, and this change in transcriptional activity could be attributed to altered affinity between this genomic region and SP1 and SP3 transcription factors.¹⁹

The aim of this study was to evaluate the role of *CD14* polymorphisms, inhaled endotoxin, and atopy in modulating the concentration of systemic inflammatory markers and serum sCD14 levels. Two additional tag polymorphisms²⁰ (*CD14*−1359 and *CD14*−1619 relative to transcription start site) with minor allele frequencies of greater than 10% and a between linkage disequilibrium (LD) of less than 0.8 were included in this study to provide further coverage of the *CD14* promoter polymorphisms.

METHODS

Study population

Volunteer subjects were recruited from Brussels, Belgium, and were healthy nonsmoking adults between the ages of approximately 18 and 60 years. All subjects were nonasthmatic and free from regular use of medication. The Free University of Brussels and the University of Arizona Institutional Review Boards approved the study. Written informed consent was obtained from all subjects.

Endotoxin challenge

Subjects were exposed to 20 µg of endotoxin by means of inhalation, as described by Michel et al.⁴ Briefly, purified LPS (*Escherichia coli* O26:B6, Lot-2654; Sigma Chemical Co, St Louis, Mo) was prepared in saline at a final concentration of 1 mg/mL. The LPS solution was aerosolized in dosimetric nebulizers (Mefar MB3 Inc, Bovezzi, Italy) delivering 5 µL per aerosol spray over 0.6 seconds. Four such doses were given, yielding a total inhaled dose of 20 µg. In this study, as well as in our previous studies, this dose was shown to cause an inflammatory response in the absence of significant adverse symptoms.^{3,5} Saline controls were not performed in these experiments because our previous studies showed no effect of inhaled saline on lung function or serum concentration of inflammatory markers.^{3–5}

Clinical evaluation

A venous blood sample was taken before and after (6 and 24 hours) endotoxin inhalation. These time intervals were chosen based on previous time-course studies.⁵ Enumeration of white blood cells (WBCs) was determined at 6 and 24 hours after exposure by using an automated H1 counter (Technicon Instruments, Tarrytown, NY). Serum concentration of C-reactive protein (CRP) was determined at 6 and 24 hours after exposure by means of immunonephelometry with antibody-coated polystyrene particles (N Latex High Sensitivity CRP; Dade Behring, Marburg, Germany). Serum LBP²¹ and sCD14 (R&D Systems, Minneapolis, Minn) levels were measured by means of ELISA at baseline and 24 hours after endotoxin challenge. LBP and sCD14 levels were not measured at 6 hours after endotoxin inhalation because of sample limitations.

Skin prick tests (SPTs) were conducted with extracts of dust mite, cat, birch, and grass pollen. Subjects were considered to have positive skin test responses and to be atopic, with a wheal diameter 4 mm or larger for 1 or more allergens.

Molecular genetic analysis

The complete coding region of *CD14*, the intronic sequence, and approximately 6 kb of 5' genomic and 2 kb of 3' genomic area were resequenced from DNA obtained from 23 European American subjects as part of the Innate Immunity Program in Genomic Applications.^{15,22} A total of 17 single nucleotide polymorphisms (SNPs) were identified, 15 of which had a minor allele frequency of greater than 10%. From the European American-specific catalog of polymorphic sites, a tagging strategy based on bins of polymorphic sites that exceeded 10% minor allele frequency and a within-bin LD exceeding 0.8 was examined with publicly available software, as recently described.²⁰ This algorithm resulted in 4 tagging SNPs, 3 of which were included in this study (rs2569190, −159; rs3138078, −1359; and rs2915863, −1619; nomenclature relative to the transcription start site). The *CD14*−159 polymorphism is the only polymorphism in this gene that has been shown to be functional.¹⁹ PCR and restriction digests were performed as described by Guerra et al.¹⁷

Statistical analyses

Because the distribution of serum sCD14, CRP, and LBP values were skewed, the values were log-transformed before performing parametric statistical tests. Standard procedures (Pearson correlation, paired *t* test, *t* test, and ANOVA) were used to assess statistical significance by using SPSS Windows 15.0 (SPSS, Inc, Chicago, Ill). In the ANOVA post-hoc analysis, the *P* value for pairwise comparisons is reported by using the Bonferroni correction. An α level of significance of .05 was chosen for all the performed statistical tests.

Multiple regression was done by using a univariate model. Exploratory analysis tested the significance of each independent variable (ethnicity, age, sex, and SPT results) on the dependent variables, baseline sCD14 levels and sCD14 levels, after endotoxin challenge. These independent variables were then added to the models *CD14* polymorphisms and baseline sCD14 and *CD14* polymorphisms and sCD14 after endotoxin challenge in a step-up approach.

LD (r^2) was calculated by using Linkage Disequilibrium Analyzer software 1.0. Because the gametic phase of the genotypic data was unknown, we could not uniquely define the DNA phase of subjects heterozygous for more than 1 polymorphism. Therefore putative haplotypes were inferred by using Phase software,²³ with a probability threshold set to 95%. In the haplotype analysis results from each subject were associated with each of the 2 haplotypes carried by the same subject. Consequently, statistical analyses were performed by chromosomes rather than by subject.²⁴

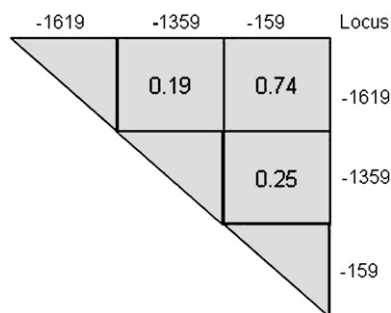


FIG 1. Pairwise r^2 values between the *CD14* polymorphisms –1619, –1359, and –159. Values of r^2 were calculated by using the software Linkage Disequilibrium Analyzer 1.0 and are shown in shaded boxes. The LD statistic r^2 describes the similarity of patterns between pairs of polymorphic sites.

RESULTS

LD and haplotype structure of the *CD14* proximal promoter

The following SNPs were genotyped in the cohort: –1619, –1359, and –159 relative to the transcription start site or –1720, –1460, and –260 relative to the translation start site. All of these polymorphisms had a minor allele frequency of greater than 10% (*CD14*/–1619A→G = 0.455; *CD14*/–1359G→T = 0.262; *CD14*/–159T→C = 0.472) and were similar in frequency to the European American reference population used for the Program in Genomic Applications (<http://innateimmunity.net>). The genotype frequencies showed no significant deviation from a population in Hardy-Weinberg equilibrium (data not shown). We found no significant differences in allele frequency among white versus nonwhite individuals at each locus (data not shown), although the number of nonwhite subjects was small. The strength of pairwise LD between the 3 polymorphisms is shown in Fig 1. LD was particularly strong between *CD14*/–1619 and *CD14*/–159 ($r^2 = 0.74$). Six haplotypes were identified in the population. Their estimated frequencies were 43.8% for the GGT haplotype (–1619, –1359, and –159), 23.9% for the ATC haplotype, 23.3% for the AGC haplotype, 6.8% for the AGT haplotype, 1.7% for the GTT haplotype, and 0.6% for the ATT haplotype. Because of their low frequency, the GTT and ATT haplotypes were not considered further.

Study population demographics

Study population demographics and concentration of WBCs, LBP, CRP, and sCD14 before and after endotoxin challenge are presented in Table I. Eighty-eight nonsmoking subjects with a mean age of 33.8 years (range, 18–56 years) were studied. The population was mainly white (87.5%). Of note, only a portion of subjects were assessed for WBC (n = 73), LBP (n = 73), and sCD14 (n = 60) levels because of sample limitations; however, there were no demographic differences (age, ethnicity, sex, and SPT) among those subjects with or without samples (data not shown). There was a significant increase in

TABLE I. Endotoxin inhalation study: Demographics and concentration of systemic inflammatory markers

Subjects (n)*	88
Mean age (y [range])	33.8 (18–56)
Ethnicity (% white)	87.5
Male sex (%)	50.0
Positive SPT response (%)†	35.2
Mean WBC t = 0 (cells/L [SEM])	5996.0 (205.3)
Mean WBC t = 6 h (cells/L [SEM])	10616.2 (351.8)
Geometric mean LBP t = 0 (mg/L [95% CI])	23.3 (20.5–26.5)
Geometric mean LBP t = 24 h (mg/L [95% CI])	40.1 (35.5–45.3)
Geometric mean CRP t = 0 (mg/L [95% CI])	1.7 (1.3–2.3)
Geometric mean CRP t = 24 h (mg/L [95% CI])	8.9 (7.2–10.9)
Geometric mean sCD14 t = 0 (μg/mL [95% CI])	4.89 (4.64–5.12)
Geometric mean sCD14 t = 24 h (μg/mL [95% CI])	5.49 (5.22–5.64)

*Only a portion of subjects were assessed for WBC (n = 73),

LBP (n = 73), or sCD14 (n = 60) levels.

†A positive SPT response reflects 1 or more positive test results to birch, grass, dust mite, or cat allergen.

serum levels of WBC (paired *t* test, $P < .0001$), LBP (paired *t* test, $P < .0001$), CRP (paired *t* test, $P < .0001$), and sCD14 (paired *t* test, $P < 0.0001$) within 24 hours of endotoxin inhalation, indicating systemic stimulation by endotoxin (Table I). Correlations between these systemic inflammatory markers before and after endotoxin challenge are presented in Table II. CRP and LBP levels were significantly correlated at both time points. Interestingly, there was no significant correlation between sCD14 levels and either LBP or CRP levels.

Association of *CD14* polymorphisms with systemic inflammatory markers

There was no association between *CD14* polymorphisms and either baseline concentration of LBP (n = 73; –1619, ANOVA, $P = .8$; –1359, ANOVA, $P = .4$; –159, ANOVA, $P = .9$) or concentration of LBP at 24 hours after endotoxin inhalation (n = 73; –1619, ANOVA, $P = .9$; –1359, ANOVA, $P = .8$; –159, ANOVA, $P = 0.8$; data not shown). Similar results were found for *CD14* haplotypes (t = 0: ANOVA, $P = 0.93$; t = 24 hours: ANOVA, $P = .89$; Fig 2). We also confirmed our previous findings⁵ that LBP levels were higher for non-atopic versus atopic subjects (t = 0: geometric mean of 25.9 vs 19.2 mg/L, $P = .02$; t = 24 hours: geometric mean of 42.7 vs 35.5 mg/L, $P = .15$), respectively, although at 24 hours, the difference did not reach statistical significance.

There was no association between *CD14* polymorphisms and the concentration of CRP at baseline (t = 0, n = 86; –1619, ANOVA, $P = .8$; –1359, ANOVA, $P = .3$; –159, ANOVA, $P = .4$) or at 24 hours after endotoxin inhalation (n = 86; –1619, ANOVA, $P = .8$; –1359, ANOVA, $P = .6$; –159, ANOVA, $P = .9$; data

TABLE II. The Pearson correlation between systemic inflammatory markers before and after endotoxin challenge

		sCD14 (t = 24 h)	LBP (t = 0)	LBP (t = 24 h)	CRP (t = 0)	CRP (t = 24 h)
sCD14 (log, $\mu\text{g/mL}$) (t = 0)	<i>r</i>	0.45	−0.08	−0.04	−0.14	−0.14
	<i>P</i> value	<.001	.6	.7	.3	.3
	N	60	60	60	60	60
sCD14 (log, $\mu\text{g/mL}$) (t = 24 h)	<i>r</i>	—	−0.06	−0.01	0.12	0.02
	<i>P</i> value	—	.7	.9	.4	.9
	N	—	60	60	60	60
LBP (log, mg/L) (t = 0)	<i>r</i>	—	—	0.58	0.61	0.32
	<i>P</i> value	—	—	<.001	<.001	.006
	N	—	—	73	73	73
LBP (log, mg/L) (t = 24 h)	<i>r</i>	—	—	—	0.43	0.59
	<i>P</i> value	—	—	—	<.001	<.001
	N	—	—	—	73	73
CRP (log, mg/L) (t = 0)	<i>r</i>	—	—	—	—	0.51
	<i>P</i> value	—	—	—	—	<.001
	N	—	—	—	—	86

not shown). Similar results were found for *CD14* haplotypes (t = 0: ANOVA, $P = .93$; t = 6 hours: ANOVA, $P = .94$; t = 24 hours: ANOVA, $P = .56$; Fig 2). As found previously,⁵ CRP levels were not associated with the atopic status (negative SPT response vs positive SPT response) of the subject (t = 0: geometric mean of 1.73 vs 1.74 mg/L, $P = .9$; t = 24 hours, 9.00 vs 8.81 mg/L, $P = .9$).

WBC counts increased significantly from baseline to 6 hours after endotoxin inhalation (n = 73; paired *t* test, $P < .0001$; Table I) and then decreased back to baseline values. There was no association between *CD14* polymorphisms and WBC concentrations at baseline (n = 73; −1619, ANOVA, $P = .21$; −1359, ANOVA, $P = .34$; −159, ANOVA, $P = .23$; data not shown). At 6 and 24 hours after endotoxin inhalation, the association between WBC concentration and *CD14* genotype was inconsistent (data not shown). However, haplotype analysis showed a significant relationship between *CD14* haplotypes and levels of WBC at 6 hours (n = 144 chromosomes, $P = .025$) and 24 hours (n = 144 chromosomes, ANOVA, $P = .046$; Fig 2). Subjects with the AGT or ATC haplotype had significantly higher WBC values than those with the GGT or AGC haplotype at both time points.

Association of *CD14* polymorphisms with serum sCD14 levels before and after endotoxin inhalation

sCD14 was analyzed before and after endotoxin inhalation to understand the influence of *CD14* polymorphisms and endotoxin challenge on sCD14 levels. There was a significant association among the *CD14*/−1619 genotypes and baseline levels of sCD14 (n = 60; ANOVA, $P = .007$; Fig 3). Subjects homozygous for the *CD14*/−1619 G allele had significantly higher sCD14 levels at baseline than those with the AG genotype (Bonferroni, $P = .013$) or those with the AA genotype (Bonferroni, $P = .015$). Also, there was an association of *CD14* genotypes at the −1359 (n = 60;

t test, $P = .015$) and −159 (n = 60; ANOVA, $P = .007$) loci and baseline sCD14 levels (Fig 3). Of note, subjects with the *CD14*/−1359 GT or TT genotype were combined for analysis because of the low number of subjects with the TT genotype (n = 3). At the −159 locus, individuals with the TT genotype had higher baseline levels of sCD14 than those with the CT (Bonferroni, $P = .016$) or CC (Bonferroni, $P = .017$) genotype. Haplotype results were in concordance with those observed at each individual locus and differed significantly at baseline (n = 119 chromosomes; ANOVA, $P = .011$; Fig 3). Pairwise comparisons indicated that serum sCD14 levels associated with the GGT haplotype (−1619, −1359, and −159) were higher than those associated with the ATC haplotype (Bonferroni, $P = .006$). These data indicate that *CD14* polymorphisms are associated with serum levels of sCD14 at baseline before endotoxin inhalation.

In contrast, 24 hours after endotoxin inhalation, there was no association between *CD14* genotypes at the −1619 (n = 60; ANOVA, $P = .5$), −1359 (n = 60; *t* test, $P = .7$), and −159 (n = 60; ANOVA, $P = .08$) loci and sCD14 levels (Fig 3). Haplotype analysis also yielded no significant relationship with sCD14 levels (n = 119 chromosomes; ANOVA, $P = .2$) 24 hours after endotoxin inhalation. Thus the association between *CD14* polymorphisms and sCD14 levels was no longer present after endotoxin inhalation.

Association of ethnicity, age, sex, and atopic sensitization with serum sCD14 levels before and after endotoxin inhalation

Serum sCD14 levels at baseline or 24 hours after endotoxin challenge were not significantly associated with ethnicity, age (tertiles), or sex ($P > .2$ for all variables, Table III). Male subjects tended to have higher sCD14 levels than female subjects, although this association did not reach statistical significance. At baseline, subjects with positive SPT responses had lower serum sCD14

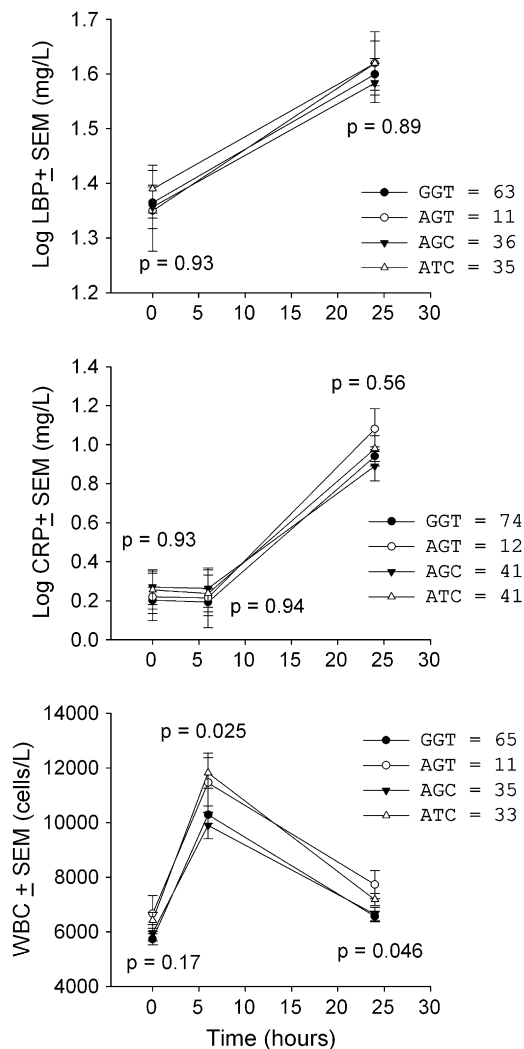


FIG 2. Association of *CD14* haplotypes with systemic inflammatory markers before and after endotoxin inhalation. The graphs represent results for LBP (top), CRP (middle), and WBCs (bottom). Mean log LBP level ± SEM (in milligrams per liter), mean log CRP level ± SEM (in milligrams per liter), and mean WBC level ± SEM (in cells per liter) are on the y-axis, and time after endotoxin inhalation is on the x-axis. *CD14* haplotypes are defined by loci -1619/-1359/-159. *P* values were calculated by using ANOVA at each time point. *n*, Number of chromosomes.

levels than subjects with negative SPT responses (*t* test, *P* = .042). In contrast, 24 hours after endotoxin inhalation, no association was found between sCD14 levels and skin test sensitivity (*t* test, *P* = .9). Of note, *CD14* polymorphisms were significantly associated with atopic sensitization in this population (see Fig E1 in this article's Online Repository at www.jacionline.org).

Nevertheless, to rule out potential confounding, we re-evaluated the effect of *CD14* genotypes/haplotypes on serum sCD14 levels before and after endotoxin challenge after adjusting for ethnicity, age, sex, and SPT responses in a multivariate analysis. These adjustments did not

change the relations of *CD14* genotypes/haplotypes to sCD14 levels before or after endotoxin inhalation, as described above.

DISCUSSION

In 1990, Wright et al¹⁰ first described CD14 as a receptor involved in the recognition of LPS and other bacterial wall components. It was not until 1999 that an association between *CD14* polymorphisms and sCD14 levels was described.¹⁶ Baldini et al¹⁶ found that *CD14*/-159TT homozygotes had significantly higher sCD14 levels than did carriers of the CC and CT genotypes. Although several studies have confirmed this association between various *CD14* polymorphisms and sCD14 levels,^{17,18,25-28} virtually none have considered the effect of endotoxin exposure on this relationship. In this study we found that the relationship between *CD14* polymorphisms and circulating sCD14 levels is modulated by endotoxin inhalation. Before endotoxin inhalation, subjects homozygous for *CD14*/-1619GG, *CD14*/-1359GG, or *CD14*/-159TT had significantly higher baseline levels of sCD14 compared with those carrying the A, T, or C alleles, respectively. However, in the same individuals after endotoxin inhalation, there was no association between *CD14* polymorphisms and circulating sCD14 levels.

The molecular mechanism or mechanisms that explain this effect of inhaled endotoxin on the association between *CD14* polymorphisms and sCD14 levels remain to be elucidated. However, in the absence of endotoxin, *CD14*/-159T was previously demonstrated *in vitro* to be more transcriptionally active than the C allelic variant.¹⁹ This increase in activity was paralleled by an altered affinity of the SP family of transcription factors in the *CD14* promoter region that contained the *CD14*/-159 polymorphism. This is in concordance with our findings of a positive association between the T allele and sCD14 levels at baseline and hence strongly implicates the functional polymorphism at *CD14*/-159. It should also be noted that this study provides data on the combined effect of 3 *CD14* polymorphisms on sCD14 levels by using haplotype analysis. Previous studies on the effect of genetic variants on sCD14 levels have investigated, with few exceptions,^{17,18,29-31} only *CD14*/-159. Our haplotype findings suggest that the *CD14*/-159 polymorphism alone is not sufficient to modulate sCD14 levels, and therefore the combined effects of different SNPs in affecting sCD14 levels should be taken into account in future functional genomic studies. To date, no functional genomic studies have been performed for the *CD14*/-1619 and *CD14*/-1359 polymorphisms or other SNPs in LD.

Even though we observed a differential effect of inhaled endotoxin on the association between *CD14* polymorphisms and sCD14 levels, there was no relation between *CD14* polymorphisms and the acute-phase reaction, as measured by means of LBP, CRP, and WBC levels. This

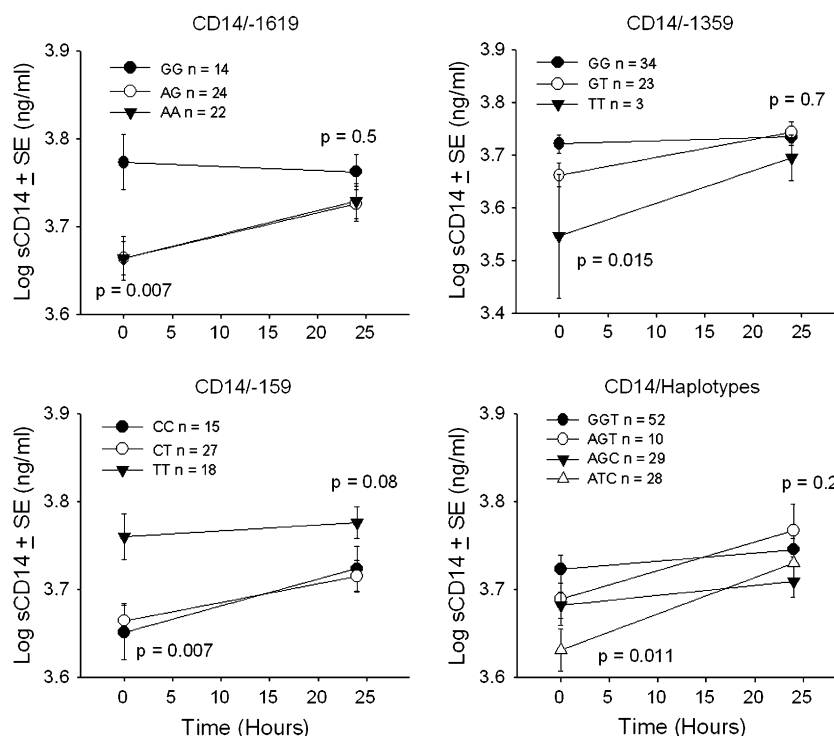


FIG 3. Association of *CD14* polymorphisms with serum sCD14 levels before and after endotoxin inhalation. The graphs represent results for *CD14*–1619 (top left), *CD14*–1359 (top right), *CD14*–159 (bottom left), and *CD14*/haplotypes (bottom right). Serum sCD14 levels are shown as mean log sCD14 ± SEM in nanograms per milliliter on the y-axis, and time after endotoxin inhalation is shown on the x-axis. *CD14* haplotypes are defined by loci –1619/–1359/–159. *P* values for *CD14*–1619, *CD14*–159, and *CD14*/haplotypes were calculated by using ANOVA for data at time 0 and time 24 hours. *P* values for *CD14*–1359 were calculated by using the *t* test for GG versus GT/TT. Genotype results: *n*, number of subjects; haplotype results: *n*, number of chromosomes.

TABLE III. Association of ethnicity, age, sex, and atopic sensitization with sCD14 level before and after endotoxin challenge

	Geometric mean sCD14 (μg/mL [95% CI]), t = 0	Geometric mean sCD14 (μg/mL [95% CI]), t = 24 h
Ethnicity		
Total population, n = 60	4.89 (4.56-5.25)	5.43 (5.15-5.75)
White only, n = 51	4.86 (4.49-5.27)	5.49 (5.18-5.82)
Age (y)		
<27, n = 19	4.85 (4.35-5.42)	5.43 (5.02-5.88)
28-40, n = 19	4.72 (4.29-5.18)	5.18 (4.59-5.84)
>40, n = 19	5.09 (4.35-5.94)	5.68 (5.16-6.26)
Sex		
Male, n = 30	5.10 (4.54-5.75)	5.62 (5.24-6.02)
Female, n = 30	4.69 (4.33-5.07)	5.27 (4.82-5.76)
SPT response		
Positive, n = 20	4.43 (3.97-5.03)	5.40 (4.87-5.99)
Negative, n = 40	5.14 (4.74-5.58)*	5.45 (5.09-5.84)

*Significantly different from negative SPT response (*P* = .042).

might seem paradoxical in light of the numerous articles showing an association between *CD14* polymorphisms and inflammatory diseases^{28,32-36}; however, these results could be explained by several mechanisms.

First, the inflammatory effect at the exposure site itself (ie, the lung) was not measured. Our previous studies have shown that the systemic and airway inflammatory responses are not necessarily linked, especially in atopic individuals, on endotoxin challenge.⁵

Second, genetically driven differences in systemic inflammatory markers might be seen with higher or lower doses of endotoxin or in shorter or longer time periods. It has been speculated that there are different local and systemic effects with low dose versus higher-dose endotoxin exposure.³⁷

Lastly, it is noteworthy that the cell types producing serum sCD14, LBP, and CRP are likely to be different. sCD14 is a class 2 acute-phase protein produced by hepatocytes in response to IL-6 and inhibited by IL-1β.³⁸ Monocytes and alveolar macrophages are responsive to endotoxin^{39,40}; therefore sCD14 levels might be directly stimulated by endotoxin or might be transcriptionally activated by a cytokine-dependent mechanism.^{41,42} Another cell source of sCD14 is the protease-mediated shedding of membrane CD14 from monocytes.⁴³ In contrast, LBP

and CRP are class 1 acute-phase proteins produced mainly by hepatocytes in response to IL-1 β and in synergy with IL-6.⁴⁴ Their similar mode of stimulation might explain why LBP and CRP were significantly correlated in this study. In contrast, there was no correlation between LBP/CRP and sCD14 levels, which suggests that their pathway of stimulation, regulation, or both is different and cell specific.

sCD14 levels at baseline were modulated by the atopic status of the individual. We observed lower levels of sCD14 among atopic subjects compared with those among nonatopic subjects before endotoxin inhalation. In contrast, this relationship was lost after endotoxin inhalation. Several studies have investigated the relationship between atopy and sCD14 levels, with conflicting results. Studies from Sweden and Japan found that individuals with positive SPT responses or specific IgE to house dust mite, respectively, had lower levels of sCD14 than nonatopic individuals.^{31,45} In contrast, 2 other studies found no relation.^{29,46} Based on our study, it is tempting to speculate that these conflicting results can be explained by differential endotoxin exposure in their respective environments; that is, individuals from Sweden and Japan were exposed to low doses of environmental endotoxin compared with the other 2 studies. Unfortunately, environmental endotoxin was not measured in these previous studies.

Many reports are available that describe the association between novel polymorphisms and phenotypes of the gene (ie, transcription, expression, or both). A common theme of these studies is that the polymorphic variants exhibit relatively modest differences in transcription or expression compared with their wild-type counterparts. This paradigm is consistent with the idea that these polymorphisms might be insufficient to cause disease when taken individually. However, they might induce significant modulation of regulatory pathways when taking into account gene-by-gene interactions, gene-by-environment interactions, or both. Thus complex phenotypes, polygenic diseases, or both would not be expected to be an all-or-none event but rather to result from a combination of small quantitative effects. We could envision that small changes in serum sCD14 levels could affect the equilibrium between LBP and sCD14,⁴⁷ thus altering the availability of LPS for cell activation through TLR4/MD-2.

In conclusion, serum sCD14 levels were strongly influenced by polymorphisms in the 5' flanking region of the encoding *CD14* gene, yet no relation was observed after endotoxin exposure. This might be an important link in our understanding of the biologic mechanism by which genetic variation in the *CD14* gene and endotoxin exposure differentially results in disease status. Further studies are necessary to identify important cofactors, such as other environmental exposures and bacterial infections, that might further influence the relation of *CD14* polymorphisms and gene expression.

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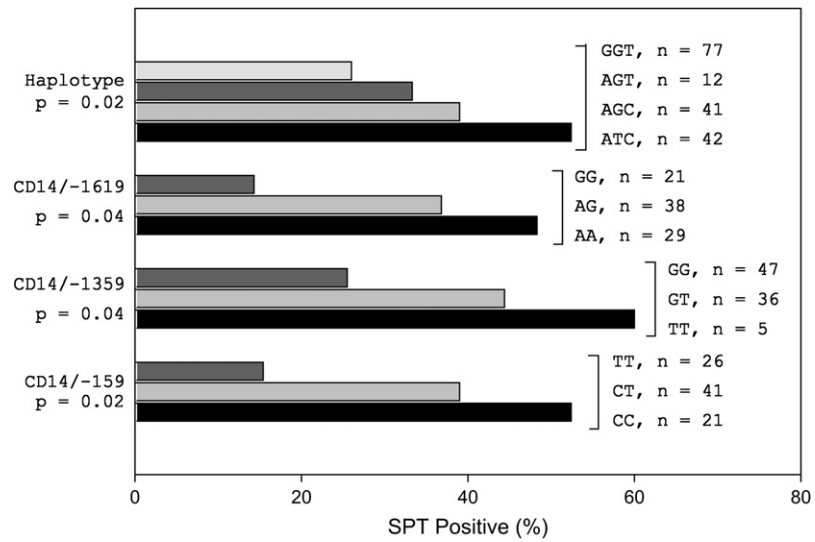


FIG E1. Association between *CD14* polymorphisms and positive SPT responses. Criteria for positive SPT response are described in the Methods section. *CD14* haplotypes are defined by loci -1619/-1359/-159. *P* values for -1619, -159, and haplotypes were derived by using ANOVA. *P* values for -1359 were calculated by using *t* tests for GG versus GT/TT. Genotype results: *n*, number of subjects; haplotype results: *n*, number of chromosomes.