Evaluation of the CD14/-260 polymorphism and house dust endotoxin exposure in the Barbados Asthma Genetics Study

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Background: Both a functional promoter polymorphism in the gene encoding CD14 (C-260T) and exposure to endotoxin are believed to play key roles in modulating the immune response and expression of atopic disease.

Objective: We aimed to evaluate the role of the CD14 C-260T polymorphism in a population of African descent and to test for interaction between this genotype and house dust endotoxin (HDE) exposure on atopic phenotypes.

Methods: Asthmatic probands and their families were recruited as part of the Barbados Asthma Genetics Study. The C-260T polymorphism and two additional *CD14* promoter markers (G-1461T, C-1721T) were genotyped. Endotoxin was measured in house dust samples.

Results: Using a Family-Based Association Test, the C-260T allele appeared to be protective against asthma ($z = -2.444$; $P = .015$) and asthma severity ($z = -2.615$; $P = .009$) under a recessive model. No significant associations were observed for the G-1461T and C-1721T markers both individually and in haplotypes. In a case-control analysis, the CD14 TT genotype was found to reduce risk of asthma compared with the CD14 CC/CT genotypes (odds ratio [OR], 0.26; 95% CI, 0.14-0.49) and was associated with lower asthma severity scores ($P < .002$). The TT genotype might protect against asthma for individuals with low HDE (OR, 0.09; 95% CI, 0.03-0.24), but may be a risk factor for individuals with high HDE (OR, 11.66; 95% CI, 1.03-131.7), suggesting a gene-environment interaction.

Conclusion: These data suggest that the CD14-260 polymorphism may play a role in controlling risk to atopic disease and underscore the importance of incorporating key environmental exposures into studies of genetic risk factors. (J Allergy Clin Immunol 2005;115:1203-9.)

Key words: *CD14, endotoxin, LPS, asthma, allergy, atopy, asthma severity, IgE, genetics, gene-environment interaction*

Endotoxin or LPS is a potent proinflammatory agent capable of inducing severe airway inflammation via multiple mechanisms.[1,2] Inhalation studies have shown that endotoxin can induce bronchoconstriction and airway hyperresponsiveness in subjects with and without asthma.[3-5] It has been suggested that subjects with asthma and/or atopic individuals may be more sensitive to endotoxin compared with individuals without allergy.[6-9]

CD14 is a pattern recognition receptor that plays a key role in the immune response to LPS. The *CD14* gene has received significant attention as a candidate gene for allergic disease as well as a modifier of innate host defense mechanisms. Potential downstream effects of variants in the *CD14* gene (as well as other genes involved in this pathway) are substantial and include controlling release of inflammatory cytokines and the upregulation of accessory molecules, features that are both critical in directing the adaptive immune response. In 1999, Baldini et al[10] identified a single nucleotide polymorphism (SNP) involving a substitution of T>C at position -159 upstream from the *CD14* transcription start site (later determined to be at position -260). The variant T allele was positively associated with increased levels of circulating soluble CD14 (sCD14) and was negatively associated with total serum IgE (tIgE). Since that time, several groups have examined the relationship between this CD14-260 SNP and allergic disease,[11-16] but none have tested this association in populations of African descent. Limited work has been published on the relationship of *CD14* promoter polymorphisms other than the C-260T variant and asthma/allergic disease,[17] but no significant associations have been identified. Finally, it has been hypothesized that the effects of variants in the *CD14* gene on clinical phenotypes may be modified by environmental endotoxin exposure, yet joint effects of endotoxin exposure and CD14-260 genotype on risk of asthma and its clinical severity have not been considered. The aim of this study was to test for an interactive effect of the CD14-260 genotype

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Abbreviations used
ASQ: Asthma severity questionnaire
EU: Endotoxin unit
FBAT: Family-Based Association Test
FVC: Forced vital capacity
HDE: House dust endotoxin
LD: Linkage disequilibrium
OR: Odds ratio
sCD14: Soluble CD14
SNP: Single nucleotide polymorphism
tIgE: Total IgE

with current house dust endotoxin (HDE) exposure on asthma severity in a family study of genetics in Barbados. Two additional variants (C-1461T, A-1721G) with reasonable frequency in populations of African descent were included in this study to investigate potential linkage disequilibrium and to provide additional coverage of markers for tests of association in the promoter region.

METHODS

Study population

The population of Barbados is primarily of African descent with approximately 25% European admixture, similar to that observed in African Americans.[18] Families were recruited in Barbados as a part of an ongoing asthma genetics study initiated in 1993.[19,20] Briefly, asthmatic probands were recruited from all regions of the island, including 6 polyclinics, 2 private clinics, and the Accident and Emergency Department of Queen Elizabeth Hospital. Patients with a positive family history of asthma or 1 or more siblings with asthma were referred by physicians cooperating with on-site study investigators at the University of the West Indies. First-degree relatives (eg, nuclear families) were recruited and extended whenever possible. Informed consent was obtained from all subjects per a protocol approved by the Johns Hopkins Medical Institutions' Joint Committee on Clinical Investigations and the Ministry of Health in Barbados. From the initial enrollment, and of those with *CD14* genotype data (n = 747), total IgE levels were available for 93% of subjects (n = 697). Extended phenotype data (skin test sensitization and spirometry) were available on a limited subset of the study population (n = 287). For this endotoxin study, asthmatic probands and their families were recontacted to assess current asthma severity and to collect samples of house dust. From a total of 157 eligible independent nuclear families, 122 were recontacted, whereas 35 were either unreachable or declined to participate. Asthma severity questionnaires (ASQs) were collected from these 122 nuclear families including 254 individuals (122 probands, 101 siblings, 18 mothers, 13 fathers). Of the 122 recontacted nuclear families, house dust samples and endotoxin data were available on 92 (75.4%) independent households.

Genotype data were available for 747 individuals from 125 pedigrees (327 nuclear families). The Genetic Analysis System program version 2.0 (http://users.ox.ac.uk/~ayoung/gas.html) was used to detect mendelian inconsistencies. When inconsistencies could not be resolved, the family was excluded; however, this resulted in excluding only 1 pedigree.

Clinical phenotyping

The evaluation protocol for asthma included (1) administration of a standardized and validated Respiratory Health Questionnaire[20] and (2) administration of an ASQ, if appropriate. Subjects were also skin tested for a panel of relevant allergens, and serum was collected for total and specific IgE measurements. The operational definition of current asthma included (1) a documented history of asthma using the Respiratory Health Questionnaire, which includes questions about respiratory symptoms (shortness of breath, cough, wheeze, chest tightness) and a history of physician-diagnosed asthma (past/current); and (2) confirmation of asthma by an interview with a primary diagnostician in Barbados.

Allergic sensitization. The puncture skin test was performed by using 12 standardized, commercially available allergens: *Paspalum notatum*, *Sorghum helepense*, *Blomia tropicalis*, *Dermatophagoides pteronyssinus*, *D farinae*, grass mix, olive pollen, Bermuda grass, cat, dog, cockroach mix (*Blattella germanica, Periplaneta americana*), and *Alternaria alternata* (Greer Laboratories, Lenoir, NC). PBS and histamine were negative and positive controls, respectively. Response was measured 15 minutes after application of the extract on the forearm and puncture with a bifurcated needle by transferring an imprint (3M Transpore tape; 3M HealthCare, St Paul, Minn) of the wheal and the erythema perimeter onto paper for documentation. The 2 cross-diameters were measured to determine independently the size of the wheal and erythema. A positive test was defined as an average wheal diameter $\geq$3 mm above the saline control.

Asthma severity. Asthma severity was measured by using an ASQ developed by Togias et al[21] and modified for this Barbados population (Barnes et al, in preparation). The ASQ assesses asthma severity on the basis of symptoms in the past year, broken down into 3 relevant seasons in Barbados (dry, summer, rainy). The number of emergency department visits, hospitalizations and intubations as well as the number of oral steroid courses during the past year and the average daily number of β-agonist inhalations was also recorded. This instrument records any impairment in physical activities and daily routine responsibilities in a quantifiable manner, with each answer scored on a validated scale. The total asthma severity score obtained can be used as a continuous variable, or subjects can be categorized into 5 severity groups: mild, mild to moderate, moderate, moderate to severe, and severe.

Routine spirometry. Spirometry was performed according to American Thoracic Society guidelines[22] by using a KOKO (Pulmonary Data Services, Inc, Louisville, Colo) pneumotach connected to a laptop computer. Measures included FEV_1, forced vital capacity (FVC), FEV_1/FVC, and maximum midexpiratory flow, or the forced expiratory flow at 25% to 75% of forced vital capacity. Values were expressed as percent predicted by using the formula published by Goldman and Becklake[23] for FVC, derived from the data of others for FEV_1[24-27] and from the values published by Leuallen and Fowler[28] for maximum midexpiratory flow. Only FEV_1 and FVC were used here.

Laboratory methods

Serum CD14 levels. Serum sCD14 levels were measured by using a commercially available ELISA kit supplied by Biosource (Europe, South America). The sCD14–enzyme amplified sensitivity immunoassay is a solid-phase enzyme amplified sensitivity immunoassay performed on a microtiter plate on the basis of the oligoclonal system in which several mAbs directed against distinct epitopes of sCD14 are used. The assay was performed as recommended by the manufacturer on human serum diluted 1/101 with diluent. The concentration of each sample (unknown) is determined by extrapolation from a standard curve estimated from a panel of standards of known concentrations.

Serum tIgE measurements. tIgE concentrations were measured as previously described[19] using the chemilluminometric Magic Lite immunoassay (ALK, Copenhagen, Denmark; CIBA-Corning, Medfield, Mass). All measurements were repeated in duplicate in an

independent assay, and any pair of values differing by >10% was retested until the coefficient of variation was ≤15%. Values were age-adjusted and sex-adjusted. Because of skewness, total IgE levels were log-transformed.

SNP genotyping. Genomic DNA was extracted from peripheral (whole) blood samples obtained by venipuncture using the QIAmp Blood midi kit (QIAGEN, Inc, Valencia, Calif). Genotyping for the CD14-260 variant (rs2569190) was performed through PCR amplification and restriction enzyme digest as previously described.[10] Known samples were included as positive controls. Two additional SNPs in the CD14 promoter region (rs3138078, G-1461T; rs2915863, C-1721T) using the TaqManR probe-based, 5′ nuclease assay with minor groove binder chemistry were genotyped. Genotyping of both SNPs was performed by using Assays-by-Design (Applied Biosystems, Foster City, Calif) as follows: -300 bp of flanking sequence on both sides of the SNPs were imported into the File Builder AB software (Applied Biosystems) and the probes and primers were synthesized by Applied Biosystems.

Environmental assessment

Dust sampling. Endotoxin levels were measured in samples of house dust by using a conventional approach for allergen collection as previously described.[29] This is the most common and feasible method for determining endotoxin levels in household environments and is highly reproducible.[30,31] Living room composite dust samples were obtained from the households of 92 independent asthmatic probands and their families.

Measurement of HDE levels. Endotoxin content from the living room composite dust sample was measured by using a kinetic limulus amebocyte lysate assay as previously described.[32] Endotoxin results were expressed as endotoxin units (EU) per square meter of the area sampled (load). All observed endotoxin levels were within the limits of detection of this assay. High endotoxin exposure was defined as ≥75th percentile (44,000 EU/m^2), and low HDE was defined as <44,000 EU/m^2. This cutpoint was chosen based on a natural break in the distribution as well as published levels defining high exposure in farming communities.[33]

Statistical analysis

Continuous variables were tested for normality by using the Shapiro-Wilk test. When variables were not normally distributed or when numbers of participants were small, nonparametric analyses were performed (eg, Wilcoxon rank-sum test). For differences in proportions, the Fisher exact test was used.

Both departures from Hardy-Weinberg equilibrium proportions at each locus were tested among the founders to compare observed versus expected genotype frequencies, and pairwise linkage disequilibrium (LD) was measured by using STATA 8.2 (StataCorp, College Station, Tex).[34] The Family-Based Association Test (FBAT)[35,36] was used to test for linkage in the presence of association (disequilibrium). The empirical variance option was used for most tests to account for the correlated nature of genotypes among offspring within the same nuclear family or nuclear families within the same pedigree. Using the empirical variance option ensures an asymptotically correct test statistic regardless of pedigree structure or previous evidence of linkage around the marker.[37] When analyzing sCD14, this empirical variance option was not used because data were available for independent asthmatic probands only. For the family-based analysis of the haplotypes, FBAT was also used. The approach implemented in FBAT assumes no recombination between markers, and thus the haplotype set becomes analogous to the single-locus, multiallelic statistic described by Rabinowitz and Laird[36] allowing for weighting all phased genotypes (haplotypes) possible within each family. An expectation-maximization algorithm maximizes the likelihood of the phased genotype (haplotype) frequencies on the basis of all of the observed family genotypes and is used to estimate haplotype frequencies from which the weights are determined. All analyses using FBAT were first conducted under the robust 3-genotype model and subsequently under additive or recessive models as suggested by the general model.

To confirm the results of the family-based analyses as well as to test for gene-environment interaction, a case-control design was used. For these analyses, cases were defined as independent asthmatic probands with available endotoxin data (n = 221). Controls (n = 230) consisted primarily of siblings without asthma. Analyses were performed by using multivariate logistic and linear regression models as implemented in the STATA software package (version 8.0, 2003, #2798; StataCorp). The cluster function of STATA was used to allow for dependence between subjects within the same family.

RESULTS

Subject characteristics and genotype data

Baseline characteristics of the study population by asthma status (and ignoring family structure) are summarized in Table I. There was no sex difference between subjects with and without asthma; however, subjects without asthma were, on average, twice as old as subjects with asthma. Given that this sample consists of families recruited through children with asthma and their parents, such an age difference is expected. Also as expected, subjects with asthma had a significantly higher asthma severity score than did subjects without asthma; however, some subjects without asthma (N = 6) registered a score >0. A third (33.3%) of subjects without asthma were atopic and/or reported allergic rhinitis. Total IgE levels (available for 93.3% of individuals with DNA) differed significantly between subjects with and without asthma ($P < .0001$). More extensive phenotype data (skin test sensitization and pulmonary function testing) were available only on a subset (primarily asthma probands and their siblings; n = 287). These associated phenotypes, including spirometry measurements, also differed significantly between subjects with and without asthma ($P = .0001$–$.0064$; Table I). FVC% predicted was relatively low among subjects without asthma, but this was not surprising given that the families were selected via an asthmatic proband. There were no meaningful differences in the distribution of *CD14* genotypes or in log-transformed tIgE levels for the subset of individuals with extensive phenotyping versus those without these data. There was no statistically significant difference in mean sCD14 levels or HDE exposure between subjects with and without asthma. Smoking, as a variable, was not included as a confounder in this study because the prevalence of smoking is extremely low in Barbados (<10%), especially among families of patients with asthma.

HDE levels

House dust endotoxin levels for living room composite samples were available for 498 people from 122 households. The geometric mean of endotoxin load was 23,144 EU/m^2 (95% CI, 20,832-25,713) with a range of 236 to 3,773,750 EU/m^2 (data not shown). To determine whether

TABLE I. Subject characteristics for entire family-based sample by asthma status

Characteristic	N	Subjects with asthma (N = 293)	Subjects without asthma (N = 454)	*P* value
Current age, y, mean (SD)	747	24.1 (13.0)	40.5 (15.4)	<.0001
Male, %	747	51.2%	47.9%	.3780
CD14-260 genotype, %	747			
CC	336	47.1%	43.5%	.3341
CT	343	46.8%	45.3%	.6879
TT	68	6.1%	11.2%	.0185
CD14-1461 genotype, %	692			
GG	26	3.41%	3.52%	.9361
GT	203	27.30%	27.09%	.9498
TT	463	61.77%	62.11%	.9255
CD14-1721 genotype, %	729			
CC	25	2.05%	4.19%	.1125
CT	250	29.01%	36.34%	.0382
TT	454	66.21%	57.27%	.0145
Serum total IgE,* ng/mL, geometric mean (95% CI)	696	1031.1 (871.9-1219.4)	444.65 (383.3-515.8)	<.0001
Skin test positivity (≥1 positive skin prick test), %	287	59.8%	35.6%	<.0001
Number of positive skin tests, median (range)	287	1.0 (0-6)	0.0 (0-4)	<.0001
FEV_1 % predicted, mean (SD)	287	80.7 (14.7)	86.8 (20.6)	.0064
FVC % predicted, mean (SD)	287	77.1 (16.9)	70.7 (13.8)	.0004
Asthma Severity Score, median (range)	254	6.0 (0-62)	0.0 (0-21)	.0141
sCD14, ng/uL, geometric mean (95% CI)	174	5386.6 (4707.8-6163.3)	4974.7 (3104.8-7970.7)	.2256
High HDE load, EU/m^2	498	25.5%	24.02%	.4380

*Adjusted for age and sex, centered around the predicted mean for 20-year-old male patients.

these HDE levels changed over time, dust sampling was repeated an average of 15.8 months after initial sampling for 12 households. No statistically significant difference in endotoxin load (EU/m^2) between these repeated measurements was found (Wilcoxon signed-rank test resulted in $P = .114$; data not shown), indicating HDE concentrations are stable over time in this population. High HDE exposure (living room composite ≥ 44,000 EU/m^2) was not associated ($P > .05$) with increased risk of asthma, higher tIgE, or higher asthma severity scores in this population (data not shown). When expressing endotoxin levels as EU per milligram of dust, similar results were obtained.

Association of the CD14 polymorphisms with asthma-associated outcomes

Allele frequencies for the CD14(-260) SNP were 68.1% and 31.9% for the C and T alleles, respectively. Genotype frequencies agreed with expectations under Hardy-Weinberg equilibrium. Overall, the distribution of CD14-260 genotypes did not differ between subjects with and without asthma ($P = .446$); however, the TT genotype was nearly twice as common among subjects without asthma, suggesting a protective effect (Table I, $P = .0185$).

Family-Based Association Test results testing for linkage and association between the T allele and asthma and atopy-associated outcomes are presented in Table II. Among these phenotypes, the strongest evidence for an association with CD14-260 was observed under a recessive model (data not shown for other models). We observed a statistically significant association between the T allele and lower asthma severity scores ($P = .0089$). There was also evidence for association of asthma with this polymorphism ($P = .0145$). Fewer TT homozygotes among subjects with asthma were observed than expected under the null hypothesis, suggesting that the C allele may be associated with a higher risk of asthma. None of the individual atopy phenotypes showed significant association individually with the CD14-260T variant ($P > .05$). We also found no statistically significant association between the T allele and sCD14 levels. However, these families did suggest that the TT genotype might be protective for all other asthma-associated outcomes.

Two additional SNPs in the *CD14* promoter region were typed in the study population: G-1461T and C-1721T, with the former positioned 1201 bp upstream of CD14-260 and the latter 258 bp further upstream. Associations for these SNPs were tested for asthma and age-adjusted and sex-adjusted log-transformed tIgE in FBAT. Allele frequencies for the 2 additional *CD14* markers in the full Barbados population were as follows: C-1721T results were 20.6% and 79.3%, respectively, and G-1461T results were 18.4% and 81.6%, respectively, which is consistent with other reports of frequencies of these variants in a population of African descent (http://innateimmunity.net/IIPGA/IIPGA2/PGAs/InnateImmunity/CD14; Table I). Genotypes in the population were consistent with Hardy-Weinberg Equilibrium proportions (at $\alpha = 0.01$). The Lewontin D'[38] was found to be 0.88 between C-1721T and G-1461T and 0.93 and 0.89 for pairwise comparisons between G-1461T, and CD14-260 and C-1721T, respectively, reflecting high pairwise linkage disequilibrium among these three markers. Single marker analyses using

FBAT under a recessive model indicated no significant associations ($P > .05$) for any of the phenotypes for either marker (data not shown). In a haplotype analysis in which all 3 *CD14* markers (C-1721T, G-1461T, C-260T) were included, the 2-marker haplotype (-1461T/-260T) was marginally significant ($P = .0642$); however, the addition of the third marker (C-1721T) eliminated this apparent effect, lending supporting evidence for a protective role of the CD14-260T allele.

These family data were also evaluated with a case-control design to determine whether a more traditional epidemiological approach would support these findings. Asthma, asthma severity, and total IgE were evaluated as outcomes. The TT genotype was associated with a greater than 4-fold reduction in risk of asthma compared with the CC/CT genotypes (odds ratio [OR], 0.26; 95% CI, 0.14-0.49; data not shown). For asthma severity score, individuals with the TT genotype showed significantly lower levels compared with those with CT or CC genotypes ($P = .0002$).

Interactive effect of current endotoxin exposure and CD14(-260)C>T

Table III shows age-adjusted ORs for the association between asthma and CD14-260 genotype, stratified by high versus low HDE load. Among individuals with low HDE exposure, the TT genotype appeared protective for asthma (OR, 0.09; 95% CI, 0.03-0.27). However, TT individuals with high HDE exposure were >11 (95% CI, 1.03-131.7) times more likely to have asthma than individuals with the CC genotype, although this result is based on a small sample of TTs in the population. Table IV shows the association between CD14-260 genotypes and both tIgE and asthma severity score by high versus low HDE load. TT homozygotes had, on average, lower tIgE regardless of HDE exposure; however, the effect was stronger in the low exposure category. We also observed a strong association between TT genotype and lower asthma severity scores, but only when HDE exposure was also low ($P < .001$). For individuals with low HDE exposure, 82.5% of TT homozygotes (n = 33) had an asthma severity score of zero.

We hypothesized that the association between the CD14-260 SNP and asthma outcomes would be further modified by atopic sensitization; however, we could not assess this fully because of limited numbers of patients with asthma with a negative skin test result and the rarity of the TT genotype. There was some evidence from our limited analyses (data not shown) that the protective effect afforded by the TT genotype in individuals with low HDE exposure may only exist among subjects with a negative skin prick test result; however, the number of subjects in each category was quite small.

DISCUSSION

Individuals of African descent are at increased risk for asthma and atopic disease compared with European and European-American populations, and they have a more severe disease, even after controlling for factors such as access to medical care and socioeconomic status. Differences in genetic risk factors may contribute to these health disparities. We genotyped 747 individuals from 125 pedigrees (327 nuclear families) for the CD14-260 polymorphism and found the frequency of the -260T allele was markedly lower in this Afro-Caribbean population,[10-12] even after considering the selection bias for asthma in our study. The TT genotype was very rare in this population, with only 6.1% of subjects with asthma and 11.2% of subjects without asthma homozygous for the -260T allele. In contrast, the 2 additional promoter variants studied (G-1461T and C-1721T), which were in strong LD with the C-260T variant (D' = 0.88-0.93), were very common in this population (83% and 77%, respectively), although no associations with asthma or tIgE were detected.

Because this study population was enriched for asthma, we hypothesized that the T allele (and TT genotype, in particular) would be less frequent if, indeed, the C allele were associated with asthma. However, the relative rarity of the TT genotype in our study sample compared with previous studies of subjects with asthma[10-13] remains compelling.

This study in a population of African descent provides additional evidence for an association between the CD14-260 TT genotype and lower tIgE and is the first report of an inverse association between the CD14-260 TT genotype and risk of asthma. In addition, the -260T allele appeared to be associated with decreased asthma severity. Koppelman et al[11] provide the only other study that addressed this relationship by looking at the effect of CD14-260 on basic spirometry and lung challenge parameters; however, none of their associations was statistically significant. It should be noted that objective pulmonary measures show short-term phenotypic variability that could easily obscure modest effects of genotype. In contrast, we hypothesized that there may be an association between CD14-260 and a more global measure of asthma severity. The lack of association for the traits tested and 2 additional promoter variants in strong LD with C-260T suggest that the functional -260T variant may indeed be the causal variant in asthma and/or allergic disease.

Tests of association showing an inverse association between CD14-260 TT genotype and asthma and asthma

TABLE II. FBAT for the CD14(-260T) variant and asthma-associated phenotypes under a recessive model

Phenotype	*z* Score	FBAT *P* value
Asthma (yes/no)	−2.444	.0145
Asthma severity	−2.615	.0089
tIgE (ng/mL)*	−0.848	.3966
Positive SPT (yes/no)	−1.021	.3072
Number of positive SPT	−1.665	.0958
sCD14 (ng/mL)	−1.606	.1084

SPT, Skin prick test.
*Adjusted for age and sex.

TABLE III. Age-adjusted ORs and 95% CIs for CD14(C-260T) genotype and asthma, stratified by high versus low HDE load*

CD14-159 genotype	Low HDE (N = 332)	High HDE (N = 111)
CC (N = 182)	1.0 (N = 133)	1.0 (N = 49)
CT (N = 205)	0.84 (0.39-1.82) (N = 150)	0.77 (0.27-2.23) (N = 55)
TT (N = 56)	0.09 (0.03-0.27) (N = 49)	11.66 (1.03-31.7) (N = 7)

*Defined according to a living room composite endotoxin load ≥(high) or < (low) 44,000 EU/m^2.

TABLE IV. Mean total IgE and Asthma Severity Score stratified by CD14-260 genotype and HDE exposure level

	Low HDE				High HDE			
	TT	CT	CC	*P* value*	TT	CT	CC	*P* value*
Adjusted tIgE†	436 (n = 41)	638 (n = 143)	733 (n = 129)	.059	324 (n = 7)	570 (n = 55)	818 (n = 47)	.279
Asthma Severity Score	2.40 (n = 40)	11.90 (n = 68)	10.89 (n = 64)	<.001	10.17 (n = 6)	9.07 (n = 21)	5.44 (n = 27)	.468

**t* Test comparing TT versus CT/CC.
†Geometric means for age-adjusted and sex-adjusted log IgE, centered around the predicted mean for 20-year-old male patients.

severity (both in family-based and case-control analyses), as well as tIgE (in case-control analyses), suggest that factors enhancing a T_H1 response may decrease T_H2-type inflammation and expression of allergic disease. We further investigated the role of this CD14-260 SNP in allergic disease by considering the joint effects of house dust endotoxin (HDE or LPS) exposure and genotype on asthma, total IgE, and asthma severity. In our preliminary tests for gene-environment interaction, we observed an interesting trend for all 3 phenotypes. Specifically, at low HDE exposure, the CD14-260 TT genotype appeared to be protective compared with the CC and CT genotypes, but at high HDE exposure, any protective effect of TT genotype disappeared, and the risk factor for asthma itself seemed to be greater. It has been suggested that enhanced ability to bind endotoxin (vis-à-vis increased transcription of *CD14*) may protect against T_H-2 type inflammation by upregulating innate immune mechanisms (and activation of T_H1-type pathways). In other words, TT homozygotes may mount a response at lower levels of endotoxin exposure than CC or CT individuals, affording some protective effect. However, there appears to be a threshold level at which the TT genotype may actually be associated with increased risk and severity of disease. The threshold at which the TT genotype becomes a risk factor for asthma and allergic disease remains uncertain and may vary between populations. Nevertheless, this concept is consistent with studies in which high sCD14 has been associated with mortality caused by sepsis[37] and the CD14-260T allele, specifically, has been shown to be associated with both septic shock and death caused by sepsis.[39] In addition, we observed further modification of the association between the CD14-260 SNP and asthma outcomes by allergic sensitization; however, the rarity of the TT genotype precluded making a strong inference from our findings.

Because of the relative rarity of the T allele (and TT genotype) in this population of African descent, the results of our study must be interpreted with caution. Small sample sizes, particularly when stratified by HDE exposure, limit the precision of the tests. Consideration of HDE levels in this study poses additional limitations. Because the study is cross-sectional, the temporal relationship between observed exposure and disease remains uncertain. However, in this population, individuals can remain in the same dwelling for years, and our data suggest that HDE levels are relatively constant over time in this tropical environment. The absence of additional environmental data is another limitation of our study. If endotoxin concentrations in house dust were correlated with other constituents of house dust (eg, dust mite levels or peptidoglycan), then there could be confounding between HDE exposure and asthma outcomes. However, because *CD14* is the LPS receptor and the literature strongly supports a role for endotoxin exposure in the pathogenesis of asthma and allergic disease, we contend that the association with endotoxin per se is reasonable.

In conclusion, our data support a role for CD14-260 as an intrinsic immune modifying locus whose effects both change and are changed by the dynamic state of the T_H1-T_H2 balance. Important cofactors such as variation in other modifying/susceptibility loci, environmental exposures, disease status, and presence of comorbid conditions contribute to a dynamic state in which the effects of *CD14* may vary both quantitatively and qualitatively. Additional studies will need to focus on well defined populations with well characterized phenotypes and comprehensive environmental assessment to allow better refinement of our understanding of how genes such as *CD14* influence risk to allergic diseases. Additional key environmental cofactors will be needed to confirm the findings presented here.

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REFERENCES

1. Reed CE, Milton DK. Endotoxin-stimulated innate immunity: a contributing factor for asthma. J Allergy Clin Immunol 2001;108:157-66.
2. Cella M, Sallusto F, Lanzavecchia A. Origin, maturation and antigen presenting function of dendritic cells. Curr Opin Immunol 1997;9:10-6.
3. Rizzo M, Arruda LK, Naspitz CK. Endotoxins and asthma in Brazil. Allergy Clin Immunol Int 1999;11:153-6.
4. Michel O. Systemic and local airways inflammatory response to endotoxin. Toxicology 2000;152:25-30.
5. Rylander R, Bake B, Fischer JJ, Helander IM. Pulmonary function and symptoms after inhalation of endotoxin. Am Rev Respir Dis 1989;140: 981-6.
6. Rylander R. Evaluation of the risks of endotoxin exposures. Int J Occup Environ Health 1997;3:S32-6.
7. Michel O, Ginanni R, Le Bon B, Content J, Duchateau J, Sergysels R. Inflammatory response to acute inhalation of endotoxin in asthmatic patients. Am Rev Respir Dis 1992;46:352-7.
8. Michel O, Ginanni R, Sergysels R. Relation between the bronchial obstructive response to inhaled lipopolysaccharide and bronchial responsiveness to histamine. Thorax 1992;47:288-91.
9. Peden DB, Tucker K, Murphy P, Newlin-Clapp L, Boehlecke B, Hazucha M, et al. Eosinophil influx to the nasal airway after local, low-level LPS challenge in humans. J Allergy Clin Immunol 1999;104: 388-94.
10. Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. 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 1999;20:976-83.
11. Koppelman GH, Reijmerink NE, Colin Stine O, Howard TD, Whittaker PA, Meyers DA, et al. Association of a promoter polymorphism of the CD14 gene and atopy. Am J Respir Crit Care Med 2001;163:965-9.
12. Gao PS, Mao XQ, Baldini M, Roberts MH, Adra CN, Shirakawa T, et al. Serum total IgE levels and CD14 on chromosome 5q31. Clin Genet 1999;56:164-5.
13. Ober C, Tsalenko A, Parry R, Cox NJ. A second-generation genomewide screen for asthma-susceptibility alleles in a founder population. Am J Hum Genet 2000;67:1154-62.
14. Sengler C, Haider A, Sommerfeld C, Lau S, Baldini M, Martinez F, et al. Evaluation of the CD14 C-159 T polymorphism in the German Multicenter Allergy Study cohort. Clin Exp Allergy 2003;33:166-9.
15. Heinzmann A, Dietrich H, Jerkic SP, Kurz T, Deichmann KA. Promoter polymorphisms of the CD14 gene are not associated with bronchial asthma in Caucasian children. Eur J Immunogenet 2003;30: 345-8.
16. Woo JG, Assa'ad A, Heizer AB, Bernstein JA, Hershey GK. The -159 C–>T polymorphism of CD14 is associated with nonatopic asthma and food allergy. J Allergy Clin Immunol 2003;112:438-44.
17. Vercelli D, Baldini M, Stern D, Lohman IC, Halonen M, Martinez F. CD14: a bridge between innate immunity and adaptive IgE responses. J Endotoxin Res 2001;7:45-8.
18. Nickel RG, Willadsen SA, Freidhoff LR, Huang S-K, Caraballo L, Naidu RP, et al. Determination of Duffy-genotypes in three populations of African descent using PCR and sequence specific oligonucleotides. Hum Immunol 1999;60:738-42.
19. Barnes KC, Neely JD, Duffy DL, Freidhoff LR, Breazeale DR, Schou C, et al. Linkage of asthma and total serum IgE concentration to markers on chromosome 12q: evidence from Afro-Caribbean and Caucasian populations. Genomics 1996;37:41-50.
20. Barnes KC, Freidhoff LR, Horowitz EM, Mathias RA, Mulkern DM, Bonacum J, et al. Physician-derived asthma diagnoses made on the basis of questionnaire data are in good agreement with interview-based diagnoses and are not affected by objective tests. J Allergy Clin Immunol 1999;104:791-6.
21. Togias A, Horowitz E, Joyner D, Guydon L, Malveaux F. Evaluating the factors that relate to asthma severity in adolescents. Int Arch Allergy Immunol 1997;113:87-95.
22. Standardization of spirometry, 1994 update. American Thoracic Society. Am J Respir Crit Care Med 1995;152:1107-36.
23. Goldman HI, Becklake MR. Respiratory function tests: normal values at median altitudes and the prediction of normal results. Am Rev Respir Dis 1959;79:457-67.
24. Pemberton J, Flanagar E. Vital capacity and timed vital capacity in normal men over forty. J Appl Physiol 1956;9:291-6.
25. Gaensler EA. Analysis of the ventilatory defect by timed capacity measurements. Am Rev Tuberc 1951;64:256-78.
26. Kory RC, Callshan R, Boren HG, Syner JC. The Veterans Administration-Army cooperative study of pulmonary function, I: clinical spirometry in normal medicine. Am J Med 1961;3C:243-58.
27. Miller WF, Johnson RL, Wu N. Relationship between fast vital capacity and various timed expiratory capacities. J Appl Physiol 1959;14:157-63.
28. Leuallen EC, Fowler WS. Maximal midexpiratory flow. Am Rev Tuberc 1955;72:783-800.
29. Barnes KC, Fernández-Caldas E, Trudeau WL, Milne DE, Brenner RJ. Spatial and temporal distribution of house dust mite (Astigmata: Pyroglyphidae) allergens Der p 1 and Der f 1 in Barbadian homes. J Med Entomol 1997;34:212-8.
30. Akashi S, Ogata H, Kirikae F, Kirikae T, Kawasaki K, Nishijima M, et al. Regulatory roles for CD14 and phosphatidylinositol in the signaling via toll-like receptor 4-MD-2. Biochem Biophys Res Commun 2000;268: 172-7.
31. Gereda JE, Leung DY, Thatayatikom A, Streib JE, Price MR, Klinnert MD, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. Lancet 2000;355:1680-3.
32. Chun DT, Chew V, Bartlett K, Gordon T, Jacobs R, Larsson BM, et al. Second inter-laboratory study comparing endotoxin assay results from cotton dust. Ann Agric Environ Med 2002;9:49-53.
33. von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlermann S, Maisch S, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. Clin Exp Allergy 2000; 30:1230-4.
34. StataCorp. Stata statistical software: release 8. College Station (TX): StataCorp LP; 2003.
35. Horvath S, Wei E, Xu X, Palmer LJ, Bauer M. Family-based association test method: age of onset traits and covariates. Genet Epidemiol 2001; 21(suppl 1):S403-8.
36. Rabinowitz D, Laird N. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. Hum Hered 2000;50:211-23.
37. Lake SL, Blacker D, Laird NM. Family-based tests of association in the presence of linkage. Am J Hum Genet 2000;67:1515-25.
38. Lewontin R. On measures of gametic disequilibrium. Genetics 1988;120: 849-52.
39. Landmann R, Zimmerli W, Sansano S, Link S, Hahn A, Glauser MP, et al. Increased circulating soluble CD14 is associated with high mortality in gram-negative septic shock. J Infect Dis 1995;171:639-44.