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