

The -159 C→T polymorphism of *CD14* is associated with nonatopic asthma and food allergy

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Background: CD14, the receptor for LPS, plays an important role in innate immunity. A polymorphism in the promotor for CD14, -159 C→T, has been implicated in atopy.

Objective: We explored the relationship of this polymorphism with both atopic and nonatopic asthma, as well as with food allergy.

Methods: Patients with asthma and food allergy were recruited along with nonatopic, nonasthmatic control subjects. The -159 C→T polymorphism was genotyped by using the PCR-based RFLP assay.

Results: The -159 T allele was more common among patients with nonatopic asthma and food allergy than among control subjects ($\chi^2 = 6.03$, $P = .01$ and $\chi^2 = 4.94$; $P = .03$, respectively). Patients with food allergy had a 4-fold increased odds of having the TT genotype versus carriers of the C allele compared with control subjects (odds ratio [OR] = 3.9, 95% CI = 1.5-10.3), whereas patients with nonatopic asthma had a 3-fold increased odds of having the TT genotype (OR = 3.1 [95% CI = 1.1-9.1]). Controlling for sex differences between groups did not alter this relationship, which remained significant for patients with food allergy (OR = 3.7 [95% CI = 1.4-10.1]) or nonatopic asthma (OR = 2.7 [95% CI = 0.9-8.0]). We performed a stratified analysis, limited to white patients, to reduce population stratification. The relationship with the TT genotype was stronger in white patients with nonatopic asthma (OR = 4.4 [95% CI = 1.3-14.8]) and patients with food allergy (OR = 5.1 [95% CI = 1.6-16.2]), even adjusting for sex differences (OR = 3.9 [95% CI = 1.1-13.5] and OR = 4.6 [95% CI = 1.4-14.8], respectively).

Conclusions: The TT genotype of -159 C→T *CD14* is associated with nonatopic asthma and food allergy, particularly in white subjects. Thus *CD14* is a candidate gene specifically for nonatopic asthma and not for asthma in general. This indicates that atopic and nonatopic asthma might be distinct con-

ditions in their genetic predisposition, despite the fact that they are very similar once they have been established. (J Allergy Clin Immunol 2003;112:438-44.)

Key words: Asthma, allergy, genetics, food hypersensitivity, toll receptors, CD14, LPS

Allergic disorders, including asthma and food allergy, are major and growing public health issues. In the United States alone asthma affects 15 million people,¹ many of whom experience asthma independent of atopy, and food allergy affects nearly 8% of children.^{2,3} Although environmental influences are clearly important in the development of these disorders, there is a strong genetic predisposition.⁴⁻⁶

There is considerable evidence for linkage of asthma and atopy with genes located on chromosome 5q31.⁷⁻¹¹ Several positional candidates in this region have been explored, including the cytokine gene cluster that includes *IL4*, *IL5*, *IL9*, *IL13*, and other genes, such as *CD14*. CD14 is a pattern-recognition receptor and binds LPS, as well as other bacterial components. Engagement of CD14 by LPS results in activation of antigen-presenting cells, including macrophages and dendritic cells, and subsequent release of pro-inflammatory cytokines and mediators.¹² Recently, the *CD14* gene has been reported to contain several polymorphisms in both the coding and promoter regions. One polymorphism, a C to T transition at position -159 (-159 C→T), has been investigated as a candidate gene for atopy.^{13,14} The functional relevance of this polymorphism was also explored. The -159 T allele was found to correlate with the level of soluble CD14, such that TT homozygotes¹³ or carriers of the T allele¹⁵ had higher soluble CD14 levels than those with CC genotypes. Soluble and membrane-bound CD14 might modulate the T_H1/T_H2 balance indirectly by inducing IL-12 production in response to LPS, resulting in the production of IFN- γ , which antagonizes IgE production.^{12,16} Therefore the -159 T allele, resulting in higher soluble CD14 levels, should theoretically be associated with reduced IgE levels and a lower risk of atopy. Studies aimed at examining these associations have generally supported the association of the T allele with lower IgE levels. Carriers of the -159 T allele were found to have lower serum IgE levels, but the differences were significant only among those with positive skin test responses in one study¹³ and, conversely, in subjects with negative RAST results in another study of British subjects.¹⁷ In another study CC homozygotes were found to have a

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Supported by NIH R01AI46652-01A1 (Dr Hershey). Dr Woo was supported by a National Institute of Environmental Health Sciences' Molecular Epidemiology in Children's Environmental Health Training Grant, T32-ES 10957.

Received for publication January 8, 2003; revised May 1, 2003; accepted for publication May 2, 2003.

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0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.1634

Abbreviations used

ATS: American Thoracic Society
OR: Odds ratio
TLR: Toll-like receptor

higher number of positive skin test responses and higher IgE levels among individuals with positive skin test responses.^{14,17} In these studies –159 C→T in *CD14* did not correlate with the presence or absence of atopy but did correlate with the level of IgE. Thus this polymorphism has been suggested to act as a severity marker (or disease modifier) for allergy.¹⁴ The role of *CD14* in atopy is not straightforward, as evidenced by a recent study that found higher levels of soluble *CD14* in asthmatic children compared with control subjects, possibly exacerbating allergic inflammation in asthmatic subjects.¹⁸ Kopelman et al¹⁴ also report an association between the *CD14* –159 CC genotype and asthma severity.

Because *CD14* is important in the innate immune response, we hypothesized that the –159 *CD14* single nucleotide polymorphism (snp) might be more important in nonatopic or intrinsic asthma. The pro-inflammatory response stimulated by LPS through *CD14* might contribute to the development of these conditions. Food allergy often occurs early in life before the onset of other allergies, and we hypothesized that innate immunity in early childhood might also be important in the development of food allergy. Despite the fact that asthma and atopy frequently co-occur, no studies to date have explored whether *CD14* affects asthma with atopy different from asthma independent of atopy or whether the *CD14* –159 polymorphism affects predisposition to food allergies. To further explore the relationship between *CD14* and atopy, we investigated both patients with atopic and nonatopic asthma, as well as patients with food allergy, relative to nonasthmatic nonatopic control subjects.

METHODS

Subjects

One hundred seventy-five unrelated adult asthmatic patients were prospectively recruited sequentially from allergy offices affiliated with the University of Cincinnati Medical Center if they met the American Thoracic Society (ATS) criteria for the diagnosis of asthma. Patients were recruited from 1998 to 2002. Asthma was diagnosed in accordance with the ATS criteria by demonstrating a 12% or greater increase in FEV₁ after a bronchodilator or after a 2-week trial of oral corticosteroids.^{19,20} Pulmonary function testing was performed according to the 1994 revised ATS guidelines by using Pneumedics Dataloop (Norwalk, Conn).²⁰ Recruited subjects all underwent skin prick testing, including positive and negative controls, to a panel of 14 relevant environmental antigens indigenous to the Ohio valley (A.L.K. Laboratories Inc, Wallingford, Conn). They were instructed to discontinue antihistamines before skin testing in accordance with the published guidelines.²¹ Patients were divided into atopic and nonatopic groups on the basis of the results of the skin tests. Those with positive reactions (>3 mm wheal with erythema) to one or more antigens tested were designated as atopic. Baseline FEV₁ values were used to classify the patients into

mild, moderate, and severe asthma groups. As outlined in the Guidelines for the Diagnosis and Management of Asthma, Expert Panel Report 2,¹ those with a baseline FEV₁ of greater than or equal to 80% of predicted value were classified as having mild asthma, those with a baseline FEV₁ of between 60% and 80% of predicted value were classified as having moderate asthma, and those with a baseline FEV₁ of less than 60% of predicted value were classified as having severe asthma. There were equal proportions (24%) of smokers (past or present history) in the atopic and nonatopic asthma groups. Seventy-seven patients with food allergy were included in the study. Patients were recruited from 1998 to 2002. Seventy patients were recruited sequentially to the study from the Allergy Clinic at the Cincinnati Children's Hospital Medical Center, 5 patients were referred to the study from the office of a practicing allergist, and 2 patients responded to an advertisement to the local food allergy support group. Patients were asked to participate in the study if they had a history of an immediate reaction to a food and positive antigen test responses (either by means of skin prick tests or RASTs). Open food challenges were done as indicated by the clinical presentation and judged by the treating physician and, when acceptable, performed on the patients, parents, or both. A number of food challenges were done to confirm the history, and others were done at a time when repeat skin tests or RASTs predicted that the food allergy would be clinically resolved. Of the 77 patients, 71 had a history of an immediate adverse reaction to one or more foods and a positive skin prick test response to one or more foods. Fifty-seven of these patients also had positive RAST results to one or more foods. Five patients had a history of immediate adverse reactions to one or more foods that was confirmed on the basis of RASTs only, and one patient was given a diagnosis of food allergy on the basis of the history alone. Thirty-one patients underwent open food challenges in the office to at least one food. Twenty-three patients had an immediate allergic reaction to a food challenge.

For the nonatopic, nonasthmatic control group, healthy, unrelated volunteers were prospectively recruited from the employee pool of University of Cincinnati Medical Center and Children's Hospital Medical Center, Cincinnati. Individuals were included in this group if they had no history of allergies, including food allergy, asthma, chronic cough, chronic obstructive pulmonary disease, or smoking. They underwent skin prick testing as outlined above, and those who demonstrated no positive reactions (excluding histamine) were included in the control group. Informed consent was obtained from all participants in these studies. These studies were approved by the Children's Hospital Medical Center Institutional Review Board.

PCR-based RFLP assay for detection of –159 C and –159 T alleles

Genomic DNA was isolated from EDTA-anticoagulated whole blood according to the GenomicPrep kit from Pharmacia Biotech (Piscataway, NJ) and was then analyzed for the presence of –159 C or –159 T alleles by means of PCR. Primers used were as follows: sense, 5'-GTGCCAACAGATGAGGTTTCAC-3'; and antisense 5'-GCCTCTGACAGTTTATGTAATC-3'. These primers amplified a 497-bp segment of the *CD14* promoter from –517 to –19. An *Ava*II restriction site exists at position –159, such that the T allele is cut, resulting in bands of 144 and 353 bp, whereas the C allele remains uncut at 497 bp. After PCR amplification, the reaction volume was digested with 10 U of *Ava*II (New England Biolabs, Beverly, Mass), and restriction fragments were resolved on a 2% agarose gel.

Statistical analysis

All statistical analysis was conducted with the SAS system, version 8.0 (SAS Institute, Cary, NC). Associations of genotypes or alleles with patient groups versus nonatopic control subjects were

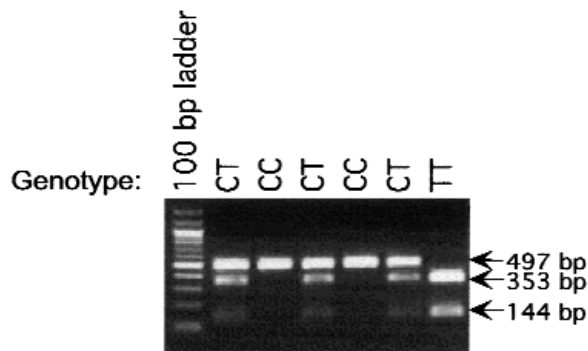


FIG 1. Identification of -159 C and -159 T *CD14* allelic variants. Patients were genotyped with a PCR-based RFLP assay (see the "Methods" section). The C allele is uncut at 497 bp, whereas the T allele is cut, resulting in bands of 144 and 353 bp. Lane 1, 100-bp ladder; lanes 2-7, genotypes noted above lane.

determined by using χ^2 analysis with appropriate *df*. Mantel-Haenszel odds ratios (ORs) and 95% CIs were calculated with standard methods. To determine differences in asthma severity (mean FEV₁ values) by patient group, we conducted an ANOVA. A *P* value of less than .05 was considered significant.

To explore the contribution of the -159 genotype independent of demographic characteristics, we conducted both logistic regression analyses and a stratified analysis, focusing on our largest racial group, white subjects. Logistic regression models were used to adjust for sex differences between the patient and control groups. To reduce the effects of population stratification in our multiracial sample, we conducted a stratified analysis including only white patients and control subjects. The white subsets were then also analyzed by using logistic regression procedures to adjust for sex differences between groups.

Three genetic models were tested. First, we tested a codominance model, which compared genotype frequency across all genotypes, CC versus CT versus TT. Then 2 recessive models were tested: (1) a model in which the C allele acts recessively, comparing the CC homozygotes versus carriers of the T allele (CT or TT), and (2) a model in which the T allele acts recessively, comparing the TT homozygotes versus carriers of the C allele (CT or CC).

RESULTS

-159 T *CD14* is significantly associated with nonatopic asthma and food allergy

To explore the relationship of the *CD14* -159 C $\rightarrow$ T polymorphism on allergy and asthma, we recruited and genotyped 175 adult asthmatic patients, of whom 128 had atopic asthma and 47 had nonatopic asthma. In addition, we recruited and genotyped 77 patients with food allergy, primarily children, and 61 nonatopic, nonasthmatic control adults. The demographic characteristics of the study groups, including age, sex, and race, are summarized in Table I. The racial distribution of the population mirrors that of the Greater Cincinnati area, from which the patients and control subjects were drawn.²²

Representative results from the restriction digest assays are shown in Fig 1. The uncut CC genotype can be seen in lanes 3 and 5; the CT genotype in lanes 2, 4, and 6; and the TT genotype in lane 7. All patient populations, including the subsets of atopic and nonatopic asthma,

were in Hardy-Weinberg equilibrium with respect to the -159 C $\rightarrow$ T polymorphism (data not shown).

We first examined the allele frequencies of each group relative to that of the control subjects, as summarized in Table II. Considering all asthmatic patients, we found no significant difference in the proportion of -159 T alleles between patients and control subjects (*P* > .1). Similarly, the subset of atopic asthmatic patients was no different from the subset of nonatopic, nonasthmatic control subjects (*P* > .3). However, both nonatopic asthmatic patients and patients with food allergy had an increased proportion of T alleles relative to control subjects (OR = 2.0 [95% CI = 1.1-2.8] and OR = 1.7 [95% CI = 1.1-2.8], *P* = .01 and *P* = .03, respectively). This indicates a 2-fold and 1.7-fold increased odds of finding the T allele among nonatopic asthmatic patients and patients with food allergy, respectively.

Table III summarizes the codominance model of the -159 C $\rightarrow$ T polymorphism, comparing the relative frequencies of CC, CT, and TT genotypes between patients and control subjects. Both nonatopic asthmatic patients and patients with food allergy had significantly different distributions of genotypes compared with control subjects (χ^2 = 7.35, *P* = .03 and χ^2 = 8.24, *P* = .02, respectively).

To explore the dominant-recessive character of the -159 C $\rightarrow$ T polymorphism, we tested the relevant homozygote with the heterozygotes and alternate homozygote combined by using the χ^2 test with 1 *df*. We present the Mantel-Haenszel case-control ORs in Table III. Tests of the recessiveness of the C allele (eg, CC vs CT/TT) revealed that the CC genotype conferred protection from nonatopic asthma (OR = 0.4 [95% CI = 0.1-0.9], *P* = .03) but not other conditions studied. All other tests of the C allele recessiveness were nonsignificant (*P* > .3). In contrast, the TT genotype occurred significantly more often in both nonatopic asthmatic patients and patients with food allergy (OR = 3.1 [95% CI = 1.1-9.1] and OR = 3.9 [95% CI = 1.5-10.3], *P* = .03 and *P* = .004, respectively) than in control subjects. The proportion of TT genotypes in the atopic asthmatic patients was similar to that in the control subjects (*P* > .1).

Association of -159 T *CD14* with nonatopic asthma and food allergy is not due to sex differences

Asthma is generally more common in female subjects, and this is also true in our population (Table I). Therefore we explored the effect of the -159 C $\rightarrow$ T polymorphism after adjusting for sex differences between patient and control groups by using logistic regression models. Sex differences between the groups were significant in models for 3 of the study groups (*P* = .02 for all asthma, *P* = .02 for allergic asthma, and *P* = .02 for food allergy). When adjusting for sex, the estimates of effect for the -159 C $\rightarrow$ T polymorphism were consistent with previous findings but somewhat less robust. In all analyses testing the CC genotype versus others, sex was an important independent risk factor (*P* = .01 for all asthma, *P* = .02 for allergic asthma, *P* = .04 for nonallergic asthma, and *P*

TABLE I. Demographic characteristics of the atopic asthmatic, nonatopic asthmatic, food allergy, and nonatopic control populations

Population	Age $\pm$ SD (range)*	Male sex (%)	Race (%)			
			White	African American	Other	Unknown
Asthma (n = 175)	48.2 $\pm$ 16.9 (11-89)	34	82	17	0	1
Atopic asthma (n = 128)	44.6 $\pm$ 16.3 (11-76)	34	78	20	0	2
Nonatopic asthma (n = 47)	58.1 $\pm$ 14.3 (24-89)	32	91	9	0	0
Food allergy (n = 77)	5.2 $\pm$ 5.3 (0.8-33.9)	74	83	12	5	0
Control (n = 61)	29.9 $\pm$ 7.4 (20-51)	53	80	3	10	7

*Age at time of study when available (n = 161 for all asthma, n = 118 for atopic asthma, n = 43 for non-atopic asthma, n = 70 for food allergy, n = 39 for nonatopic control populations).

TABLE II. Frequencies, *P* values and ORs of *CD14* –159 C and T alleles

Population	No. of alleles	Alleles (%)		T vs C	
		–159 C	–159 T	<i>P</i> value*	OR (95% CI)†
All asthma	350	186 (53)	164 (47)	.11	1.4 (0.9-2.1)
Atopic asthma	256	144 (56)	112 (44)	.34	1.2 (0.8-1.9)
Nonatopic asthma	94	42 (45)	52 (55)	.01	2.0 (1.1-3.4)
Food allergy	154	74 (48)	80 (52)	.03	1.7 (1.1-2.8)
Control	122	75 (61)	47 (39)	—	1.0 (reference)

**P* values from χ^2 test with 1 *df* comparing the ratio of T alleles versus C alleles between patients and control subjects.

†ORs and 95% CIs are reported as Mantel-Haenszel ORs.

TABLE III. *CD14* –159 genotype frequency, *P* values and ORs under 3 genetic models

Population	Genotypes (%)			CC vs CT vs TT	CC vs others		TT vs others	
	CC	CT	TT	<i>P</i> value*	<i>P</i> value	OR (95% CI)†	<i>P</i> value	OR (95% CI)
All asthma (n = 175)	46 (26)	94 (54)	35 (20)	.18	.33	0.7 (0.4-1.4)	.07	2.3 (0.9-5.8)
Atopic asthma (n = 128)	39 (30)	66 (52)	23 (18)	.35	.75	0.9 (0.5-1.7)	.15	2.0 (0.8-5.2)
Nonatopic asthma (n = 47)	7 (15)	28 (60)	12 (26)	.03	.03	0.4 (0.1-0.9)	.03	3.1 (1.1-9.1)
Food allergy (n = 77)	20 (26)	34 (44)	23 (30)	.02	.38	0.7 (0.3-1.5)	.004	3.9 (1.5-10.3)
Control (n = 61)	20 (33)	35 (57)	6 (10)	—	—	1.0 (reference)	—	1.0 (reference)

**P* values from the χ^2 test with 2 *df* for CC versus CT versus TT and 1 *df* for TT or CC versus other genotypes.

†ORs and 95% CIs are reported as Mantel-Haenszel ORs.

= .02 for food allergy). The model for the recessive C allele was supported only among nonatopic asthmatic patients (OR = 0.37, 95% CI = 0.14-0.98) after adjusting for sex. Similarly, in all analyses testing the recessive model for the T allele, sex was again a significant risk factor (*P* = .02 for all asthma, *P* = .02 for allergic asthma, *P* = .06 for nonatopic asthma, and *P* = .02 for food allergy). After adjusting for sex, the TT genotype showed a nonsignificant trend toward increased risk for nonatopic asthma (OR = 2.7, 95% CI = 0.9-8.0, *P* = .07) but remained significantly associated with increased risk for food allergy (OR = 3.7, 95% CI = 1.4-10.1, *P* = .009).

Association of –159 T *CD14* with nonatopic asthma and food allergy is not due to racial differences

We considered whether population stratification or underlying differences in allele frequencies in different ethnic populations could account for our results. There-

fore the sample was limited to white subjects, and a stratified analysis was conducted. As shown in Tables IV and V, nearly all relationships between the T allele or the TT genotype were strengthened in white subjects. The T allele was approximately twice as common among all asthmatic patients, nonatopic asthmatic patients, and patients with food allergy compared with control subjects. The distribution of the CC, CT, and TT genotypes was different from that in control subjects in all asthmatic patients, nonatopic asthmatic patients, and patients with food allergy (*P* = .04, *P* = .01, and *P* = .01, respectively). The recessive model of the C allele had support among both all asthmatic patients and the subset of nonatopic asthmatic patients (OR = 0.5 [95% CI = 0.2-1.0] and OR = 0.3 [95% CI = 0.1-0.9], *P* = .04 and *P* = .02, respectively). However, the TT genotype was significant and 4.4-fold more common among nonatopic asthmatic patients and 5-fold higher among patients with food allergy compared with control subjects. Because the asthma subsets and all asthmatic patients considered

TABLE IV. Analysis of *CD14* –159 C and T allele frequency, white subjects only

Population	No. of alleles	Alleles (%)		T vs C	
		–159 C	–159 T	<i>P</i> value*	OR (95% CI)†
All asthma	284	141 (50)	143 (50)	.02	1.7 (1.1-2.8)
Atopic asthma	198	104 (53)	94 (47)	.08	1.6 (0.9-2.6)
Nonatopic asthma	86	37 (43)	49 (57)	.006	2.3 (1.3-4.1)
Food allergy	128	62 (48)	66 (52)	.03	1.8 (1.1-3.1)
Control	98	62 (63)	36 (37)	—	1.0 (reference)

**P* values from χ^2 test with 1 *df* comparing the ratio of T alleles versus C alleles between patients and control subjects.

†ORs and 95% CIs are reported as Mantel-Haenszel ORs.

TABLE V. Stratified analysis of *CD14* –159 genotype frequencies, *P* values, and ORs under 3 genetic models, white subjects only

Population	Genotypes (%)			CT vs CC vs others		TT vs others		
	CC	CT	TT	CC vs TT <i>P</i> value*	<i>P</i> value	OR (95% CI)†	<i>P</i> value	OR (95% CI)
All asthma (n = 142)	29 (20)	83 (58)	30 (16)	.04	.04	0.5 (0.2-1.0)	.04	3.0 (1.0-9.0)
Atopic asthma (n = 99)	23 (23)	58 (59)	18 (18)	.15	.14	0.6 (0.3-1.2)	.09	2.5 (0.8-7.8)
Nonatopic asthma (n = 43)	6 (14)	25 (58)	12 (28)	.01	.02	0.3 (0.1-0.9)	.01	4.4 (1.3-14.8)
Food allergy (n = 64)	18 (28)	26 (41)	20 (31)	.01	.45	0.7 (0.3-1.6)	.003	5.1 (1.6-16.2)
Control (n = 49)	17 (35)	28 (57)	4 (8)	—	—	1.0 (reference)	—	1.0 (reference)

**P* values from the χ^2 test with 2 *df* for CC versus CT versus TT and 1 *df* for TT or CC versus other genotypes.

†ORs and 95% CIs are reported as Mantel-Haenszel ORs.

together are not independent, we suspect that the support for a role of *CD14* –159 in all asthma is directly due to the strong relationship among nonatopic asthmatic patients. Furthermore, among white subjects, sex was not a significant predictor of nonatopic asthma ($P = 0.15$). The association of the TT genotype with nonatopic asthma remained strong after adjusting for sex (OR = 3.9 [95% CI = 1.1-13.5], $P = .03$). Among white patients with food allergy, sex was an important independent risk factor ($P = .01$) and support for the recessive T model remained significant after adjusting for sex differences (OR = 4.6 [95% CI = 1.4-14.8], $P = .01$).

***CD14* –159 C→T is not associated with asthma severity**

In addition to affecting the predisposition toward disease, we investigated whether the *CD14* –159 C→T polymorphism influenced the severity of asthma. We measured severity by using FEV₁ (percent predicted) measurements, as discussed in the “Methods” section. We found no relationship between genotype and asthma severity (defined by mean FEV₁), either for all asthmatic patients ($P = .43$) or for the subsets of atopic ($P = .58$) and nonatopic asthmatic ($P = .41$) patient groups. In addition, FEV₁ severity for those with the TT genotype was not significantly different than that for the combined CC/CT groups in the all-asthma group ($P > .8$) or the atopic or nonatopic asthma subsets ($P > .5$ for both).

DISCUSSION

Elucidating the genetics of atopy is complicated by the presence of multiple genes, disease-modifying environmental and genetic factors, and population heterogeneity. Furthermore, asthma is a heterogeneous disorder that is almost completely related to allergies in some individuals while being independent of allergy or atopy in others. Many of the genes that have been implicated in asthma play some role in IgE production or IgE effector function, including the genes encoding MHC class II,^{23,24} FcεR1β,²⁵ IL-4,⁹ IL-13,^{26,27} and IL-9.²⁸ However, some of the genes important in the development of nonatopic asthma are likely to be distinct from those important in the development of atopy or atopic asthma. Our data herein demonstrate for the first time that a snp in the promoter for *CD14* is associated with nonatopic but not atopic asthma, supporting the hypothesis that the molecular mechanisms underlying the development of nonatopic asthma are distinct from those underlying atopic asthma. We next examined the association of this *CD14* snp in food allergy, a condition that often occurs early in life and precedes the development of environmental allergies. Our data demonstrate for the first time that *CD14* –159 C→T is strongly associated with food allergy and support a role for this pathway in the pathogenesis of food allergy.

The importance of endotoxin or LPS in the pathogenesis of asthma has been supported by epidemiologic

studies in children demonstrating that endotoxin exposure might be protective for later development of asthma.²⁹ Presumably, this is due to activation of pathways that contribute to T_H1-type responses. LPS, a complex glycolipid, is an integral component of the cell wall of gram-negative bacteria. A complex of LPS and LPS-binding protein in the serum initiate signals in monocytes and macrophages through membrane CD14. However, soluble CD14 can also initiate signals, and thus cells lacking CD14 can also respond to LPS. Toll-like receptor 4 (TLR4) and MD-2 are part of the receptor complex for LPS and are required for signaling.¹² Mutations in TLR4 have been shown to associate with endotoxin hyporesponsiveness.³⁰ LPS, acting through its receptor complex, including CD14 and TLR4, induces maturation of antigen-presenting cells, including dendritic cells and macrophages. This results in IL-12 production and upregulation of costimulatory molecules³¹ and might contribute to T_H1 polarization. The -159 C *CD14* allele has been associated with increased IgE levels^{13,14} and with decreased soluble CD14 levels in patients with positive skin test responses.¹³ These data have led to speculation that decreased soluble CD14 would result in decreased IL-12 production and thus T_H2 skewing and increased IgE production.^{32,33} However, other studies have yielded conflicting results whereby soluble CD14 levels were increased in children with asthma.³⁴ Thus the precise roles of membrane and soluble CD14 in the pathogenesis of atopic conditions are far from clear.

The studies discussed above support a role for *CD14* as a disease-modifying gene (or severity marker) for atopy in that it was associated with IgE levels and the number of positive skin test responses in individuals who had positive skin test responses. However, the studies herein found that *CD14* is not an atopy-susceptibility gene; that is, it was not associated with the presence of atopy. We hypothesized that innate immunity might be more important in the pathogenesis of atopic conditions that occur independent of environmental allergies. Our data demonstrate that the -159 T *CD14* allele is associated with nonatopic asthma and food allergy but not with atopic asthma. We did not find an association of the -159 *CD14* snp with asthma severity; however, studies in larger populations will be necessary to address this. Recently, the T allele was shown to result in approximately 32% increased transcriptional activity of the promoter when compared with the C allele, and the mechanism of this association was related to alterations in transcription factor binding to this region.³⁵ Studies have found that soluble CD14 levels are higher in asthmatic patients than in nonasthmatic control subjects.³⁴ This supports our finding that the T allele, which is associated with increased transcriptional activity, is found with increased frequency in patients with nonatopic asthma. Studies examining levels of soluble CD14 in atopic versus nonatopic asthmatic patients are currently underway in our laboratory. The more important question is why an allele associated with an increase in transcription of CD14 correlates with the presence of nonatopic asthma. Increased CD14

expression would result in increased LPS binding and increased production of pro-inflammatory mediators by monocytes and macrophages, including prostaglandins, reactive oxygen and nitrogen intermediates, IL-1, IL-6, IL-8, TNF- α , and IL-12, which are thought to generally antagonize allergic inflammation but might sometimes play an important role in asthma, especially nonallergic asthma. Studies have demonstrated the importance of T_H1 cytokines and pathways in asthma.³⁶ Furthermore, upsetting the balance between soluble and membrane CD14 might have more complex effects that are not yet recognized. Our data support that *CD14* is a candidate gene for only nonatopic asthma and not for asthma. This is a key observation and represents the only candidate gene thus far that is related exclusively to nonatopic asthma. This suggests that atopic and nonatopic asthma are developmentally distinct conditions, despite the fact that they are very similar once they have been established.

The association that we have found between *CD14* and food allergy is also very novel. Food allergy clearly has a genetic component.³⁷ Although some candidate genes have been examined,³⁸ the association with *CD14* appears to be the strongest. The mechanism for this association might be that the LPS in foods acts essentially as an adjuvant and increases the specific IgE response to foods in susceptible individuals. This is different than total IgE because many patients with food allergy do not have increased total IgE levels. Alternatively, an increased pro-inflammatory response in the gut caused by the presence of the *CD14* T allele, increased CD14 production, and resultant increased inflammatory cytokine production might be associated with an enhanced sensitization to food allergens, especially early in life.

We are grateful to Drs Grace Lemasters and Christopher Karp for critical review of this manuscript. We thank Connie Pettit for excellent secretarial support.

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