

Original article

Association between farm exposure and atopy, according to the CD14 C-159T polymorphism

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Background: A higher exposure to bacterial compounds is purported to explain the lower prevalence of allergy in farm children, but responsiveness to bacterial compounds is modulated by genetic factors.

Objective: To assess whether the protective effect of farm exposure on atopy is influenced by a CD14 promoter functional polymorphism.

Methods: We administered a detailed questionnaire on farm exposure in childhood and genotyped the CD14 C-159T polymorphism in 2 French centers participating in the European Community Respiratory Health Survey (ECRHS)–II. **Results:** Six hundred randomly selected young adults provided blood samples for IgE measurements and had CD14 C-159T genotyped. Exposure to a farming environment in early life was associated with a reduced risk of nasal allergies (odds ratio [OR], 0.54; 95% CI, 0.29–1.00) and atopic sensitization (OR, 0.47; 95% CI, 0.24–0.93) in adulthood. A lower risk of allergic rhinitis and atopy was also observed in carriers of the CD14-159TT genotype compared with –159CC subjects (OR, 0.52; 95% CI, 0.30–0.88; and OR, 0.54; 95% CI, 0.31–0.92, respectively). When farm exposure and CD14 C-159T were considered together, the risk of nasal allergies and atopy was the most reduced in the subjects who combined both an early-life exposure to a farming environment and the –159TT genotype (OR, 0.26; 95% CI, 0.07–0.94; and OR, 0.21; 95% CI, 0.05–0.93, respectively, vs nonexposed –159CC+CT subjects). The results were consistent in the 2 centers, supporting the validity of the results.

Conclusion: A gene-by-environment interaction between CD14 C-159T and environmental exposure in childhood may modify the development of atopy.

Clinical implications: This polymorphism should be considered in interventions studies that use microbial stimuli to reduce sensitization. (*J Allergy Clin Immunol* 2006;■■■:■■■–■■■.)

Key words: Gene, asthma, allergic rhinitis, immunologic sensitization, epidemiologic studies

Epidemiologic studies have demonstrated that children brought up on a farm had a lower risk of atopy and hay fever than other children.^{1,2} In an earlier article, we showed that living on a farm in childhood was also associated with a lower risk of atopic sensitization and nasal allergies in adulthood.³ It has been suggested that increased exposure to bacterial compounds related to the presence of cattle in traditional farming environment would enhance the generation of T-cell responses protective against the development of allergy.^{4–8} High levels of endotoxin, a cell wall component of Gram-negative bacteria, have been measured in house and mattress dust samples from children with regular contact with farm animals, and an inverse relationship has been demonstrated between endotoxin exposure and the occurrence of hay fever, atopic asthma, and atopic sensitization.^{2,7–10}

If early exposure to endotoxin may have a protective role, one might expect that genetic factors that modify LPS (endotoxin) responsiveness would also influence the susceptibility to develop atopic diseases.^{11,12} In children recruited from a general population sample, a C to T transition at position -159 in the promoter of the gene encoding CD14, the major receptor for LPS, was associated with increased levels of serum circulating soluble CD14 (sCD14).¹³ It was proposed that decreased sCD14 could result in decreased IL-12 production that might prevent T_H1 polarization.^{11,13} Low sCD14 levels in amniotic fluid, breast milk, or plasma were found to be associated with an increased risk of developing atopic sensitization or wheezing at the age of 1 year in different birth cohorts.^{14,15} Among non-Hispanic white children with positive skin tests, carriers of the CD14-159TT genotype had the lowest levels of total IgE and lower number of positive skin tests.¹³ Consistent associations between the CD14 C-159T polymorphism and atopic sensitization or total IgE levels were found in Dutch adults,¹⁶ Czech patients,¹⁷ Chinese

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Abbreviations used

ECRHS: European Community Respiratory Health Survey

OR: Odds ratio

sCD14: Soluble CD14

children,¹⁸ and Australian children to the age of 18 years.¹⁹ However, no association was observed in other groups or subgroups of subjects,^{13,20-22} and conversely, the T allele was found to be overtransmitted to atopic individuals in an inbred population of Hutterites,²³ and was associated with nonatopic asthma and food allergy, eczema, and atopy in a few other studies.^{22,24,25} Because environment may greatly differ between the populations considered in these studies, it is suggested that gene-environment interactions might explain the discrepant results.^{11,12,20,22}

To assess a possible interaction between the CD14 gene and farm environment on the risk of atopy and asthma, we administered a detailed questionnaire on farm exposure in childhood in 2 French centers participating in the European Community Respiratory Health Survey (ECRHS)–II, and genotyped the CD14 C-159T polymorphism in all participants. Because TT homozygotes have been shown to have higher levels of sCD14 than carriers of the -159 C allele,^{13,21} we expected that farm exposure in early life would have a stronger protective effect on atopy in TT homozygotes than in CC or CT subjects.

METHODS

Data were collected in 2 French centers participating in ECRHS II. The methods of this international survey have been described elsewhere.²⁶

Briefly, in 1991 and 1992, 1500 men and 1500 women between 20 and 44 years old were randomly selected from electoral rolls in the 18th District of Paris and in the town of Grenoble. They were sent a self-administered questionnaire on asthma and asthmalike symptoms derived from the bronchial symptom questionnaire of the International Union Against Tuberculosis and Lung Disease. A subsample of 485 and 660 respondents attended the university clinic in Grenoble and Paris, respectively, for further testing including an extended interviewer-administered questionnaire (ECRHS I).

In 1999 and 2001 (ECRHS II), all ECRHS I participants who could be located were recontacted to determine symptom status and invited to the local clinic. They were administered a standardized questionnaire that included detailed questions on asthma and asthmalike symptoms, nasal allergies, smoking, and environment. In addition, subjects from Paris and Grenoble answered a short questionnaire on farm exposure in childhood, detailing whether this took place during the first year of life or after.

Genomic DNA was obtained centrally at the laboratory of Biochimie, Bichat Hospital, Paris, from EDTA-anticoagulated peripheral blood samples after extraction from blood leucocytes by a standard procedure with the QIAmp DNA blood Midi kit (Qiagen GmbH, Courtabouef Cedex, France), according to the manufacturer's instructions. Genotyping of the -159 C->T polymorphism in the 5' flanking region of the CD14 gene (also reported as C-260T, rs2569190, in the literature) was conducted by using an adapted method of DNA

amplification by PCR procedure with specific primers, as previously described.¹⁷ PCR products were digested by specific restriction enzymes and separated by appropriate electrophoresis.

Serum samples were sent to a central laboratory at the King's College of London for measurements of total IgE and specific IgE to house dust mite (*Dermatophagoides pteronyssinus*), cat, *Cladosporium*, and timothy grass, using the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden).

The protocol was approved by the local institutional review boards for human studies, and informed written consent, including consent for genetic studies, was obtained from all of the subjects before testing.

Analysis

Atopy was defined as positive specific IgE (≥ 0.35 kIU/L) to at least 1 of the 4 allergens tested. Sensitization to individual allergen was also considered in the analyses, except for sensitization to *Cladosporium*, because its prevalence was too low for statistical analysis. Contingency tables were analyzed by using χ^2 test (or Fisher exact test when there were too few subjects). Mantel-Haenszel χ^2 was used to test for trend. Logistic regressions were performed to estimate adjusted odds ratio (OR), taking potential confounders into account. We first computed the change in risk associated with exposure to farm, then the change in risk associated with CD14 genotype, and then considered farm and CD14 in a same model. The same variables included in the first regression for the effect of farm have been used in all models to allow possible comparison of the estimates. Because total IgE levels was not normally distributed, geometric means for total IgE level were calculated, and *P* values from Wilcoxon rank test for 2 group comparisons were computed. The level of total IgE was also analyzed as a categorical variable, with a cutoff at 100 IU/mL. The analysis was performed by using the SAS-PC statistical package (release 8.1; SAS Institute Inc, Cary, NC).

RESULTS

In Grenoble, 79 subjects completed the ECRHS II questionnaire at home, and 305 subjects came to the university clinic and answered the interviewer-administered questionnaire, of whom 293 subjects provided blood samples. Because of technical problems during transportation of samples from Grenoble to Paris, 25 samples could not be genotyped. Therefore, the analysis included 268 subjects who had the CD14 C-159T genotyped and complete information on farm exposure in childhood. In Paris, 80 subjects completed the questionnaire at home, and 360 came to the university clinic, of whom 332 had the CD14 C-159T genotyped and complete information on farm exposure in childhood. In Paris, the 332 participants included in the analysis more frequently reported asthma ever at the first study than nonparticipants in ECRHS II or subjects not included in the analysis (14.5% vs 9.5%). In both centers, subjects included in the analysis tended to be older than subjects not included. Otherwise, no difference in sex, allergic rhinitis, and IgE measurements at ECRHS I was observed between subjects included in the current analysis and those not included.

Characteristics of the study population are presented in Table I. More than 80% of the subjects never lived on a farm. One hundred four subjects reported that they had spent at least 3 months on a farm during childhood.

TABLE I. Description of the population

	Grenoble (N = 268)	Paris (N = 332)	P	Grenoble + Paris (N = 600)
Age (y), mean (SD)	45.3 (6.9)	44.7 (7.4)	.3	44.9 (7.2)
Women, %	45.9	55.1	.03	51.0
Tobacco smoking				
Never smokers, %	41.0	41.0		41.0
Ex-smokers, %	34.2	22.9		27.9
Current smokers, %	24.8	36.1	.005	31.1
Farm exposure in childhood				
Never lived on a farm, %	80.6	84.3		82.7
Lived on a farm in the first year of life, %	10.8	12.1		11.5
Lived on a farm later in childhood, %	8.6	3.6	.04	5.8
Pet in childhood, %	53.7	61.1	.07	57.8
Parental history of asthma or allergy, %	38.1	41.9	.3	40.2
Severe respiratory infection in childhood, %	6.5	8.9	.3	7.8
Asthma ever in life, %	13.1	16.0	.3	14.7
Nasal allergies, %	29.1	41.6	.002	36.0
Atopy, %	35.5	30.6	.2	32.8
Positive IgE to dust mites, %	20.1	22.7	.4	21.6
Positive IgE to cat, %	11.6	8.2	.2	9.7
Positive IgE to timothy grass, %	21.6	12.7	.004	16.6
Positive IgE to cladosporium, %	4.3	0.9	.008	2.4
Total IgE >100 IU/mL, %	26.3	32.7	.09	29.0
CD14-159				
CC, %	23.1	25.9		24.7
CT, %	54.5	49.7		51.8
TT, %	22.4	24.4	.5	23.5

Overall, 15% of the subjects ever had asthma. Nasal allergies were more frequently reported by subjects from Paris, whereas sensitization to at least 1 of the 4 allergens tested was slightly more frequent in Grenoble. There was no difference between the 2 centers in the distribution of the CD14-159 genotypes. Allele frequency for C was estimated at 50.6%, and the distribution was consistent with the expected Hardy-Weinberg equilibrium. The genotypes distribution in subjects who never lived on a farm was 25.4% for CC, 52.0% for CT, and 22.6% for TT, which was not significantly different from the corresponding distribution in subjects who ever lived on a farm (21.2%, 51.0%, and 27.9%).

Association between farm exposure in childhood and asthma and allergy

Only 35 subjects reported that they had spent at least 3 months on a farm in childhood, but after the first year of life. The prevalence of asthma, nasal allergies, or atopy was not significantly different in these subjects compared with subjects who never lived on a farm in childhood (results not shown). These subjects were excluded from the following analyses.

Twenty-nine subjects in Grenoble and 40 subjects in Paris lived on a farm during their first year of life. In Paris, as in Grenoble, no association could be found between farm exposure in the first year of life and the risk of asthma in adulthood (Table II). Otherwise, subjects who lived on a farm in the first year of life were less likely to report nasal allergies or to be sensitized to individual allergens than subjects who never lived on a farm, in both centers, with almost all of the ORs < 0.60, although not significantly so when the 2 centers were considered separately. Of note, the associations between farm exposure and nasal allergies and between farm exposure and atopy were very consistent in the 2 centers, and became significant after the data from the 2 centers were combined and adjusted for center and potential confounders (Table II, right column).

Association between the CD14 C-159T polymorphism and asthma and allergy

Although differences in prevalence were observed between the 2 centers, the trends in the association between the CD14 C-159T polymorphism and the frequency of allergy were similar in Paris and Grenoble (Fig 1). The CD14 C-159T polymorphism was not associated with the frequency of asthma. In both centers, carriers of the CT genotype had a slightly, although not significant, lower risk of having atopic sensitization than CC homozygotes (OR, 0.64; 95% CI, 0.34-1.22 in Grenoble; and OR, 0.65; 95% CI, 0.37-1.15 in Paris), and this risk was further decreased in T homozygotes compared with CC subjects (OR, 0.57; 95% CI, 0.26-1.27; $P = .17$ in Grenoble; and OR, 0.54; 95% CI, 0.27-1.07; $P = .08$ in Paris).

In Grenoble, C homozygotes had higher levels of total IgE than carriers of the CT genotype ($P = .05$) and T homozygotes ($P = .04$; geometric means, 66.0, 41.8, and 32.8 kU/L, respectively). In Paris center, the level of total IgE was similar in C homozygotes, CT and TT subjects (geometric means, 52.2, 52.5, and 49.0 kU/L, respectively). Similar associations between the level of total IgE and the CD14 C-159T polymorphism were observed when total IgE was analyzed as a categorical variable (Fig 1).

Because the results were consistent in Grenoble and Paris, the data from the 2 centers were combined for the multivariable analysis, with center included as a covariate (Table III). The ORs suggested a lower risk of nasal allergies and atopy in CT subjects compared with CC, but no association was significant. In contrast, TT subjects were at a significantly lower risk of nasal allergies, atopy, and cat sensitization than C homozygotes (Table III). Higher levels of total IgE were observed in C homozygotes than in carriers of the CT genotype ($P = .12$) and T homozygotes ($P = .07$; geometric means, 57.5, 47.4, and 41.8 kU/L, respectively).

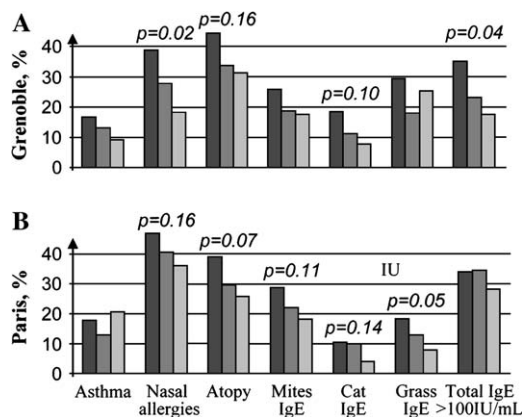
Effect of farm exposure in early life, stratified by the CD14 C-159T genotype

To assess whether farm exposure in early life would have a stronger effect in TT subjects, CC and CT subjects were combined into a single group. In the final logistic

TABLE II. ORs for the risk of having asthma, nasal allergies, atopic sensitization, or level of total IgE >100 IU/mL in subjects who lived on a farm during the first year of life compared with subjects who never lived on a farm

	Grenoble			Paris			Grenoble + Paris		
	OR	95% CI	P	OR	95% CI	P	Adjusted OR*	95% CI	P
Asthma	1.07	(0.35-3.32)	.9	0.92	(0.37-2.32)	.9	1.05	(0.49-2.21)	.9
Nasal allergies	0.49	(0.18-1.35)	.17	0.58	(0.28-1.19)	.13	0.54	(0.29-1.00)	.05
Atopy	0.25	(0.08-0.75)	.009	0.52	(0.23-1.18)	.12	0.47	(0.24-0.93)	.03
Dust mites IgE	0.26	(0.06-1.15)	.06	0.84	(0.37-1.90)	.7	0.70	(0.34-1.47)	.3
Cat IgE	0.50	(0.11-2.22)	.4	0.53	(0.12-2.34)	.6	0.69	(0.23-2.05)	.5
Timothy grass IgE	0.37	(0.11-1.26)	.10	0.51	(0.15-1.74)	.3	0.59	(0.24-1.45)	.3
Total IgE >100 IU/mL	0.32	(0.09-1.08)	.06	0.87	(0.42-1.78)	.7	0.73	(0.39-1.37)	.3

*Adjusted for center, age, sex, smoking habits, pets in childhood, severe respiratory infection in childhood, and parental history of asthma or allergy.

**FIG 1.** Prevalence of asthma, nasal allergies, atopic sensitization to any allergens, sensitization to house dust mites, sensitization to cat, and sensitization to timothy grass, and percentage of subjects with total IgE > 100 IU/mL, in CC (darker bars), CT and TT (lighter bars) subjects, from Grenoble (A) and Paris (B) centers, and P values for test for trend (only P values < .20 are reported).

regression including subjects with all data for covariates available, 4 groups were compared: 375 carriers of the CD14-159 CC or CT genotype who never lived on a farm during childhood, 110 TT subjects who did not live on a farm in childhood, 46 CC+CT subjects who lived on a farm before the age of 1 year, and 20 TT subjects brought up on a farm.

Farm exposure in early life was not associated with asthma, whatever the CD14-159 genotype (Table IV). The OR for the effect of farm exposure on nasal allergy and atopy was <0.70 in CC+CT subjects. No association reached the level of statistical significance when the analysis was run separately in each stratum of CD14 genotype, and the tests for interaction were also not significant. However, the relative sizes of the ORs suggest that farm exposure might have a substantially more marked protective effect in T homozygotes than in CC+CT subjects: the ORs suggesting a protective effect of farm exposure for the risk of nasal allergies and atopy estimated in CD14-159 TT subjects were half the ORs estimated in CC+CT subjects.

When the total sample was analyzed by using a single model, considering 4 groups of subjects according to whether they were exposed to farm in childhood and to

their CD14 gene (Table V), the risk of nasal allergy and atopy was reduced (although not statistically significantly so) in CC+CT subjects brought up on a farm, compared with CC+CT subjects who never lived on a farm (OR, 0.62; and OR, 0.58, respectively), and TT subjects brought up on a farm in early life had a substantially and significantly lower risk of having nasal allergies (OR, 0.26; $P = .04$) and sensitization to any allergens (OR, 0.21; $P = .04$) and a lower risk of having increased level of total IgE (OR, 0.42; $P = .18$) than carriers of the CD14-159 CC or CT genotype who never lived on a farm during childhood (Table V).

When the prevalence of sensitization to specific allergen was considered, no significant association with farm exposure was observed in the CC+CT group. Of the 21 TT subjects brought up on a farm, only 2 (10%) were sensitized to dust mites, and none (0%) were sensitized to either cat or timothy grass, compared respectively with 24%, 12%, and 18% in CC+CT subjects who never lived on a farm ($P = .033$ for sensitization to timothy grass, not significant otherwise).

DISCUSSION

To assess whether the protective effect of farm exposure on atopy might be influenced by genetic factors likely to modify responsiveness to bacterial compounds, we first investigated whether farm exposure was associated with the risk of asthma or allergy, and then assessed whether the protective effect of farm exposure was modified by the CD14 C-159T polymorphism. Farming exposure was associated with a reduced risk of nasal allergies and atopic sensitization in all subjects. However, the strongest protective effect was observed in subjects who combined both an early life exposure to a farming environment and the CD14-159TT genotype.

Our results on the effect of farming environment are in accordance with our first report based on ECRHS I international data set and with studies in children, which all have shown a protective effect of farm exposure on the risk of atopic sensitization and hay fever, whereas the association between farm exposure in early life and the risk of asthma or wheeze is not consistent.³ Regarding

TABLE III. Adjusted ORs for the association between CD14 C-159T polymorphism and asthma, nasal allergies, atopic sensitization, and total IgE level (data from Grenoble and Paris combined)

	OR*	95% CI	P
Asthma (n = 81)			
Model 1*			
CC	1.00	(Reference)	
CT	0.76	(0.42-1.38)	.4
TT	0.92	(0.47-1.80)	.8
Model 2*			
T allele	0.96	(0.68-1.36)	.8
Nasal allergies (n = 192)			
Model 1*			
CC	1.00	(Reference)	
CT	0.74	(0.48-1.15)	.18
TT	0.52	(0.30-0.88)	.02
Model 2*			
T allele	0.72	(0.55-0.94)	.02
Atopy (n = 179)			
Model 1*			
CC	1.00	(Reference)	
CT	0.67	(0.43-1.04)	.08
TT	0.54	(0.31-0.92)	.03
Model 2*			
T allele	0.73	(0.56-0.96)	.03
Dust mite IgE (n = 118)			
Model 1*			
CC	1.00	(Reference)	
CT	0.76	(0.46-1.26)	.3
TT	0.57	(0.31-1.05)	.08
Model 2*			
T allele	0.75	(0.55-1.03)	.07
Cat IgE (n = 54)			
Model 1*			
CC	1.00	(Reference)	
CT	0.76	(0.39-1.45)	.4
TT	0.30	(0.11-0.79)	.02
Model 2*			
T allele	0.59	(0.38-0.90)	.02
Timothy grass IgE (n = 91)			
Model 1*			
CC	1.00	(Reference)	
CT	0.58	(0.34-1.00)	.06
TT	0.52	(0.27-1.03)	.06
Model 2*			
T allele	0.71	(0.50-1.00)	.05
Total IgE >100 IU/mL (n = 159)			
Model 1*			
CC	1.00	(Reference)	
CT	0.86	(0.54-1.35)	.5
TT	0.65	(0.37-1.14)	.13
Model 2*			
T allele	0.81	(0.61-1.07)	.13

*ORs are adjusted for center, age, sex, smoking habits, pets in childhood, severe respiratory infection in childhood, and parental history of asthma or allergy. In model 1, no *a priori* assumption was made on the effect of T allele. In model 2, the CD14/-159 genotype is coded as a 3-level variable for the number of T allele. The total number of subjects included in the logistic regression was N = 551 when the risk of asthma and nasal allergies was modeled, and N = 542 in models for total or specific IgE.

the CD14/-159 genotype, our results are consistent with many other studies in which the T allele was found to be protective against atopy, whereas no association was observed for asthma. However, the opposite findings were observed in a few other studies, suggesting possible gene-by-environment interactions. A limitation of our study relates to the small number of subjects who lived on a farm in early life. The results were based on 2 French centers participating in the ECHRS. The 2 samples of subjects came from distinct areas, mostly urban, but geographically contrasted (urban 18th district of Paris, and industrialized town of Grenoble, at the border of the Alps). None of the subjects were currently living on a farm at the time of the study assessment, and none were involved in farm working. Although the results were generally not significant at the center level, the similar trends observed in the 2 centers add to the reliability of our finding. The lack of power in assessing the association between farm exposure and asthma preclude reaching any conclusion from our results on whether the farm might or might not be protective against asthma. In contrast, the indication of a protective effect of farm exposure on atopy, although not statistically significant in Paris, is observed in a consistent way in the 2 centers despite the low number of subjects brought up on a farm in each single center, which supports the existence of a real protective effect of farm exposure on atopy. In the same way, although no conclusion could be drawn from our study on the association between the CD14-159 genotype and the risk of asthma, the findings showing a decreasing trend in the risk of nasal allergies and atopy with increasing number of T alleles, which are consistently observed in both centers, do support an underlying association between the CD14 C-159T polymorphism and atopic sensitization. Finally, although we were not able to demonstrate a significant interaction between farm exposure and CD14 gene, the relative size of the estimates for the effect of farm in each stratum of the CD14-159 genotype suggests that the protective effect of farm exposure is strengthened in T homozygotes, in accordance with our hypothesis.

To our knowledge, this is the first study to show that the protective effect of farm exposure varies according to the CD14 C-159T polymorphism. Gern et al²⁷ have reported a gene-by-environment interaction, similar to the modifying effect we observed, showing a stronger protective effect of dog ownership on atopic dermatitis in children carrying the CD14-159TT genotype, compared with CC and CT subjects. In a former analysis of the Allergy and Endotoxin (ALEX) Study, Eder et al² showed an interaction between farm exposure in childhood and a polymorphism in the genes encoding Toll-like receptor 2, which is a pattern recognition receptor involved in the response of cell membrane components of either Gram-positive or Gram-negative bacteria, mycoplasma, mycobacteria, parasites, and yeasts. More recently, the same team reported a significant interaction between environment in childhood and the CD14/-260 polymorphism (also referred to as CD14/-159).²⁸ In this study, however, the C allele was indeed associated with more frequent atopic sensitization in

TABLE IV. Stratified ORs for the risk of having asthma, nasal allergies, atopic sensitization, or level of total IgE >100 IU/mL in subjects who lived on a farm during the first year of life compared with subjects who never lived on a farm, in CD14-159 CC+CT subjects, and in CD14-159 TT subjects

		Adjusted OR†	95% CI	P
Asthma				
60/421*	In CD14-159 CC+CT	1.02	(0.42-2.48)	.9
21/130*	In CD14-159 TT	1.01	(0.22-4.53)	.9
Nasal allergies				
155/421*	In CD14-159 CC+CT	0.62	(0.31-1.25)	.18
37/130*	In CD14-159 TT	0.42	(0.10-1.67)	.2
Atopy				
144/416*	In CD14-159 CC+CT	0.55	(0.26-1.18)	.13
35/126*	In CD14-159 TT	0.24	(0.05-1.20)	.08
Total IgE >100 IU/mL				
128/416*	In CD14-159 CC+CT	0.82	(0.40-1.69)	.6
31/126*	In CD14-159 TT	0.43	(0.11-1.78)	.3

*Number of cases and total number of subjects with complete data for variables included in the logistic regression.

†OR adjusted for center, age, sex, smoking habits, pets in childhood, severe respiratory infection in childhood, and parental history of asthma or allergy.

TABLE V. Adjusted OR for the risk of having asthma, nasal allergy, atopic sensitization, or increased level of total IgE, according to farm exposure in childhood and CD14 C-159T genotype

	Asthma (n = 81 cases)			Nasal allergy (n = 192 cases)			Atopy (n = 179 cases)			Total IgE > 100 IU/mL (n = 159 cases)		
	OR*	95% CI	P	OR*	95% CI	P	OR*	95% CI	P	OR*	95% CI	P
Nonfarming, CC+CT	1.00	(Reference)		1.00	(Reference)		1.00	(Reference)		1.00	(Reference)	
Nonfarming, TT	1.10	(0.61-2.01)	.8	0.68	(0.43-1.09)	.11	0.78	(0.48-1.25)	.3	0.76	(0.47-1.25)	.3
Farming, CC+CT	1.06	(0.44-2.57)	.9	0.62	(0.31-1.26)	.19	0.58	(0.27-1.22)	.15	0.81	(0.40-1.67)	.6
Farming, TT	1.10	(0.30-4.00)	.9	0.26	(0.07-0.94)	.04	0.21	(0.05-0.93)	.04	0.42	(0.12-1.51)	.18

*Adjusted for center, age, sex, tobacco smoking, pet in childhood, respiratory infection in childhood, and parental history of asthma or allergy. The total number of subjects included in the logistic regression was N = 551 when the risk of asthma and nasal allergies was modeled and N = 542 in models for total or specific IgE.

children with regular contact with pets, but an association in the opposite direction was found in children with regular contact with farm animals, as well as in children in the highest tertile of house dust endotoxin. The aim of this analysis by Eder et al²⁸ was to assess whether the association between the CD14 gene and IgE levels was modified according to environment. Although the results are not presented in a way to examine whether the effect of environment is modified according to the CD14, it seems that regular contact with farm animals is associated with a lower risk of atopy in C homozygotes, whereas no significant differences are observed in CT or TT subjects, which contrasts with our results. The authors suggested that the CD14 polymorphism might have a different effect in subjects exposed to moderate levels of endotoxin, compared with subjects with high levels of exposure.

Besides the level of exposure, the timing of exposure might also modify the association. In the Allergy and Endotoxin Study, some children with reported current exposure to stable animals have not been exposed to farm animals in the first year of life (first visit to stable before the age of first year of life was reported by 63% of farmers' children and 16% of nonfarmers' children in a previous report¹). In our study, there were very few subjects who lived on a farm in childhood after the age of 1 year, and a protective effect of farm exposure was demonstrated

only in subjects who were living on a farm during their first year of life. Riedler et al¹ showed a protective effect of consumption of farm milk and exposure to stables for children exposed in the first year of life, but not for children exposed later in life. Exposure to endotoxin, as well as genetic factors that would modify responsiveness to endotoxin exposure, might also have a dual effect depending on whether an atopic sensitization is established at the time of exposure.^{11,29} In workers with documented allergy to animals, working in one of the largest repository of mouse strains in the United States, higher levels of IgE have been reported in carriers of the CD14-159 T allele.³⁰ On the other hand, early exposure to endotoxin has been shown to have a protective effect on the induction of atopic sensitization. In a rat model, exposure to aerosolized LPS before ovalbumin sensitization and as long as 4 days after sensitization inhibited the rise in ovalbumin-specific IgE, and resulted in increased levels of ovalbumin-specific IgG.³¹ In contrast, exposure to LPS 6 days and more after ovalbumin sensitization did not result in a change in IgE or IgG production, but potentialized the allergen-induced inflammatory cell influx.³¹ In infants, increased house dust endotoxin concentrations correlated with increased proportions of IFN- γ -producing CD4 T cells.⁹ In an *ex vivo* human model of allergen-induced inflammation in explanted nasal mucosa, LPS administration

prevented the allergen-induced increase in T_H2 cytokines in young children, whatever the atopic status, but not in atopic adults.³²

In the current study, only subjects combining an exposure to a farming environment in early life and the CD14-159TT genotype were at significantly lower risk of atopic sensitization and nasal allergies. Because CD14-159TT subjects are likely to be more responsive to LPS stimulation because they have higher levels of soluble CD14, our results are consistent with the hypothesis that the protective effect of farm exposure might be explained by endotoxin or other bacterial wall-derived components stimulating the maturation of a T_H1 immune response in early life. An alternative model has been proposed suggesting that not only bacterial but also T_H2 -skewing parasite infections might inhibit inflammatory reactions to environmental allergens, through stimulation of regulatory T cells.³³ Another possibility might be a modified T_H2 -response (involving IgG production) that promotes tolerance to environmental allergens.³⁴ We do not have any data to investigate whether the protective effect of farm exposure may result from an enhanced maturation of T_H1 or a modified T_H2 response, or would involve regulatory T cells. Neither could we assess the relative effect of the level of respiratory endotoxin compared with the effect of other bacterial compounds, or other characteristics related to the farm environment that may confer protection against the development of atopy, such as differences in diet (which may result in different exposure to gastrointestinal endotoxin) or differences in physical activity.

In conclusion, our results support the hypothesis that environmental factors encountered in a farming environment in childhood and genetic factors that modify responsiveness to endotoxin may interact to have a lifelong protective effect against the development of allergy. Despite the low number of subjects brought up on a farm, to our knowledge this study is the first to show that the protective effect of farm environment on atopy may be modified by CD14-159 genotype. The consistency of the findings in the 2 centers supports the validity of the results, and suggests that it is worth further testing the interaction between childhood environment and polymorphisms of the CD14 gene or other genes likely to influence responsiveness to bacterial compounds, in greater samples from more heterogeneous populations.

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