Published in final edited form as:
Ann Allergy Asthma Immunol. 2008 February ; 100(2): 128–136.

Gene-environment interactions between *CD14* C-260T and endotoxin exposure on Foxp3$^+$ and Foxp3$^-$ CD4$^+$ lymphocyte numbers and total serum IgE levels in early childhood

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Abstract

Background—Innate immune system stimuli, such as endotoxin, seem to affect allergy risk. Previously, we described gene-environment interactions between the endotoxin receptor polymorphism C-260T of the *CD14* gene and endotoxin exposure on total serum IgE level; however, the mechanism of this interaction is not known.

Objective—To examine whether this gene-environment interaction affects early CD4$^+$Foxp3$^-$ or CD4$^+$Foxp3$^+$ lymphocyte numbers.

Methods—Participating children were part of a birth cohort in the Detroit metropolitan area. Participants were genotyped for the *CD14* C-260T polymorphism. Endotoxin exposure was estimated from dust measured in the home when children were 6 months old. Intracellular Foxp3 protein expression, a regulatory T-cell marker, was used to characterize CD4$^+$ lymphocytes in blood samples collected at the age of 12 months; total serum IgE level was also measured at this time. Because race/ethnicity may confound or modify genetic associations, all analyses were stratified by race/ethnicity.

Results—We observed a significant gene-environment interaction between *CD14* C-260T genotype and endotoxin exposure on CD4$^+$ lymphocyte numbers, particularly CD4$^+$Foxp3$^-$ lymphocytes. Stratified analyses suggest effect modification by race/ethnicity on CD4$^+$Foxp3$^+$ lymphocyte numbers, with a significant interaction in African American children but not in white children. The interaction between *CD14* C-260T genotype and endotoxin exposure on total IgE levels was opposite that observed for CD4$^+$ lymphocyte numbers, suggesting reciprocal relationships.

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Disclosures: Authors have nothing to disclose.

Conclusions—A gene-environment interaction between endotoxin and *CD14* C-260T genotype on IgE levels may be the result of an upstream, opposing effect on $CD4^+Foxp3^+$ and $CD4^+Foxp3^-$ lymphocyte numbers. Race/ethnicity may affect which of these cell populations is affected by this gene-environment interaction.

INTRODUCTION

Strong innate immune system stimuli may reduce the risk of atopic conditions. For example, our group[1] previously showed that the frequency and intensity of fevers, a marker of innate immune stimulation, between 6 and 12 months old are associated with a lower likelihood of allergic sensitization, asthma, and asthma with allergic sensitization at 6 to 7 years old.

Endotoxin, a potent stimulator of inflammation through the innate immune system, has also been shown to have a protective association with allergic sensitization and asthma in some observational studies.[2,3] As a component of gram-negative bacteria cell walls, endotoxin is ubiquitous in the environment, but levels are particularly high with farming and livestock contact.[4] However, even in metropolitan settings endotoxin levels may be sufficient to affect atopy.[5]

The relationship between endotoxin exposure and IgE levels seems to be affected by a common single nucleotide polymorphism (SNP) in the promoter region of *CD14*, a receptor involved in endotoxin recognition.[6] Although this apparent gene-environment interaction between a cytosine (C) to thymine (T) transition at base pair −260 (C-260T) of *CD14* and endotoxin exposure has now been described for IgE levels[6–8] and asthma severity,[9] the immunologic mechanism of this interaction is not known.

Innate stimuli, such as endotoxin, may affect the expansion of regulatory T cells (T_{reg} cells),[10] which, in turn, regulate the expression of atopy through their immunosuppressive properties.[11] Were endotoxin to affect T_{reg}-cell development in vivo, we would also expect to see a gene-environment interaction between *CD14* C-260T genotype and endotoxin exposure on T_{reg}-cell expansion.

Studying children enrolled in the Wayne County Health, Environment, Atopy & Asthma Longitudinal Study (WHEALS), an ongoing longitudinal birth cohort, we examined the interactive relationship between endotoxin exposure at the age of 6 months and *CD14* C-260T genotype on IgE levels and $CD4^+$ lymphocyte numbers at the age of 1 year. This included the number of $CD4^+Foxp3^+$ lymphocytes, because $Foxp3^+$ expression is considered to be specific for T_{reg} cells.[12,13] The interval was selected a priori based on previous observations of febrile infections.[1] Because this is a racially diverse cohort, we stratified analyses by race/ethnicity to minimize confounding due to population stratification.[14] Furthermore, race/ethnicity-specific gene-environment effects have previously been described for this polymorphism.[15]

METHODS

Study Population

This study was approved by the institutional review board at Henry Ford Health System and was compliant with its Health Insurance Portability and Accountability Act of 1996 policy. A full description of recruitment, data collection, and endotoxin measurements appears in an earlier publication.[6] In brief, enrollment in the WHEALS birth cohort began in August 2003 and is planned to continue through 2007. To be eligible, pregnant women in their second or third trimester had to be at least 21 years old, live in a geographically defined area of urban and suburban Detroit, and attend 1 of 5 clinics for their prenatal care. A mother's signed consent was required for both mother and child to participate. Compared with US census figures, the

health system from which participants were recruited serves a population that is broadly representative of the Detroit metropolitan area in age, sex, and race/ethnicity. The intent of this longitudinal cohort is to followup newborns and parents to identify environmental and genetic factors associated with the development of atopy and associated conditions. The present analysis involves children enrolled in the study.

Data Collection

The mean ± SD age of the children included in this analysis at the "6-month" visit was 7.1 ± 1.2 months; the mean ± SD age at the "12-month" visit was 13.8 ± 2.0 months; and the mean ± SD time difference between the 2 visits was 6.7 ± 2.6 months. For the reasons mentioned in the "Introduction," we were interested in endotoxin exposure at the age of 6 months; this exposure was estimated in dust collected from the home at this time. Blood collected from the child at approximately 1 year old was analyzed for $CD4^+$ lymphocyte populations, total serum IgE levels, and total white blood cell (WBC) count. Genomic DNA was isolated from blood samples using a DNA kit (FlexiGene; Qiagen, Valencia, California).

Analysis of Total Serum IgE Levels

Plasma was separated, frozen, and shipped in batches for analysis of total IgE levels. Total IgE levels were measured using Pharmacia CAP (Pharmacia Diagnostics AB, Portage, Michigan) according to the manufacturer's protocols.

CD14 C-260T Genotyping

Genotyping of children's DNA for the *CD14* C-260T polymorphism was performed by means of allelic discrimination using a SNP genotyping assay (TaqMan; Applied Biosystems, Foster City, California). Inconclusive results were adjudicated by direct sequencing. The *CD14* SNP analyzed, rs2569190, has recently been redesignated C-260T[16]; it is also referred to as C-159T in several publications.[15,17]

Endotoxin Assay

The endotoxin activity in each house dust sample was measured using the fluorescent microplate assay based on recombinant *Limulus* factor C (PyroGene Recombinant Factor C; Cambrex Bio Science, Walkersville, Maryland) and an endotoxin standard (Cambrex Bio Science). A full description of the collection and assay procedure can be found in a previous publication.[6]

Quantifying $CD4^+Foxp3^-$ and $CD4^+Foxp3^+$ Lymphoctyes

Blood samples were collected in heparinized tubes and were processed within 18 hours of initial collection following the Becton Dickinson FastImmune protocol (BD Biosciences, San Jose, California). Samples were then stored at −80°C until staining and analysis. Within 6 months of collection, frozen samples were analyzed for $CD4^+Foxp3^-$ and $CD4^+Foxp3^+$ lymphocytes. We used mouse anti–human chlorophyll protein-cyanine (PerCP-Cy5.5)–conjugated anti-CD4 antibody (BD Biosciences) for surface molecule staining. After permeablizing the cells, phycoerythrin-conjugated rat anti–human monoclonal antibody against intracellular Foxp3 (eBioscience, San Diego) was added.

Cell populations were identified using a 3-laser benchtop flow cytometer (BD LSR; BD Biosciences). On each batch of samples run, quadrant markers were set according to the isotype control antibody results. CellQuest Pro v4.0.2 software (BD Biosciences) was used to gate on the $CD4^+$ cell population. Among the $CD4^+$ lymphocytes, scatterplots then quantified the proportion of $Foxp3^+$ cells (ie, the proportion of $CD4^+$ lymphocytes exhibiting the highest mean fluorescence intensity in the FL2 channel used to evaluate intracellular Foxp3 staining).

We estimated the absolute number of $CD4^+Foxp3^+$ cells by multiplying the total WBC count by the proportion of $CD4^+$ lymphocytes in all WBCs and the proportion of $Foxp3^+$ cells in all $CD4^+$ cells. Similar calculations were performed to quantify the number of $CD4^+Foxp3^-$ cells. Additional information on the processing and staining of cells is available on request.

Statistical Analysis

We evaluated differences in the characteristics of children with vs without complete information at the age of 1 year using a 2-sample *t* test and χ^2 tests. Endotoxin levels measured in dust collected from the floors of mothers' bedrooms were used as a proxy for overall endotoxin exposure.[6] Pearson correlation coefficients were used to evaluate correlations between dust endotoxin exposure at the age of 6 months and the following outcomes at the age of 1 year: total serum IgE levels, number of $CD4^+$ lymphocytes, number of $CD4^+$ $Foxp3^+$ lymphocytes, and number of $CD4^+Foxp3^-$ lymphocytes. In these analyses, dust endotoxin levels, serum IgE levels, and lymphocyte counts were $\log_e$ transformed to normalize their distribution and reduce the effect of large observations. Analyses were stratified by *CD14* C-260T genotype.

Linear regression was used to estimate the interactive relationship between *CD14* C-260T genotype and endotoxin exposure on each of the following $\log_e$-transformed variables: total serum IgE levels and $CD4^+$, $CD4^+Foxp3^+$, and $CD4^+Foxp3^-$ lymphocyte numbers. Based on the observed correlations, we modeled *CD14* genotype as an ordinal variable (ie, an additive genetic relationship). Adjusting for child's age, child's sex, being breastfed, number of siblings, pet exposure, parental smoke exposure, and maternal atopy did not substantively affect the relationships between *CD14* C-260T genotype and endotoxin exposure on IgE levels or $CD4^+$ lymphocyte numbers (data not shown); therefore, these variables were not included in the final models.

To our knowledge, this is the first effort to describe a gene-environment interaction effect on $CD4^+$ lymphocyte numbers. As such, we did not have a precedent with which to estimate effect size. However, because we hypothesized that these changes occur upstream of effects on IgE, we estimated that the effect size for $CD4^+$ lymphocyte numbers would be larger than that previously seen for IgE.[6] With a sample size of 90, we had 90% power to detect a medium effect size of 0.15. Herein, effect size is defined as $r_{all} - r_{reduce}/1 - r_{all}$, where r_{all} is the squared correlation for the model with all the variables and r_{reduce} is the squared correlation with the model variables excluding the interaction term.[18] The power to detect interactions despite the small sample size was due, in part, to the cohort study design and the use of continuous outcome measures (ie, quantitative phenotypes).[18]

The frequency of the *CD14* C-260T genotype has been shown to vary by race/ethnicity, and these differences could confound the results through population stratification.[15] To address potential confounding and effect modification by race/ethnicity, we stratified the regression models for African American and white patients. Race/ethnicity categories were based on mothers' reports. Finally, we reran the regression models substituting endotoxin measures from the 6-month visit with those from the 1-month visit. All statistical analyses were performed using a software program (SAS v9.1; SAS Institute Inc, Cary, North Carolina). $P < .05$ was considered statistically significant.

RESULTS

Study Population

At the time of this study, recruitment and follow-up in WHEALS were ongoing. Between August 1, 2003, and May 31, 2005, 476 children enrolled in WHEALS. However, data for the

primary analysis, the relationship between *CD14* C-260T genotype and dust endotoxin levels at the age of 6 months on $CD4^+$ lymphocyte counts ($Foxp3^+$ and $Foxp3^-$) at the age of 1 year, were available on 90 children. These 90 children are compared with the 386 excluded children in Table 1. These groups did not differ significantly in the characteristics examined. In the analytic group, the geometric mean ± SD number of $CD4^+Foxp3^-$ lymphocytes was $294.8 \times 10^7 \pm 1.6 \times 10^7$ cells/L and of $CD4^+Foxp3^+$ lymphocytes was $14.9 \pm 2.1 \times 10^7$ cells/L (ie, approximately 4.8% of all $CD4^+$ lymphocytes were $Foxp3^+$). In the analytic group and in children not included, the *CD14* C-260T genotype frequencies were in Hardy-Weinberg equilibrium. Complete data for the analysis between *CD14* C-260T genotype and dust endotoxin levels at the age of 6 months on total IgE levels at the age of 1 year were available on 72 children. The geometric mean ± SD of the total serum IgE level in this group was 15.4 ± 4.0 IU/mL.

Gene-Environment Interactions on Total Serum IgE Levels

We observed a possible gene-environment interaction between *CD14* C-260T genotype and endotoxin exposure at the age of 6 months on total IgE levels at the age of 1 year. Increasing endotoxin exposure at the age of 6 months was associated with significantly lower IgE levels at 1 year in children with the *CD14* C-260T *CC* genotype ($P = .02$), nonsignificantly lower IgE levels in children with the *CT* genotype ($P = .75$), and nonsignificantly higher IgE levels at 1 year in children with the *TT* genotype ($P = .75$) (Fig 1). The interaction between *CD14* C-260T genotype and endotoxin exposure was borderline significant ($P = .08$) (Table 2). To address potential confounding by race/ethnicity (ie, population stratification) or the possibility of a race/ethnicity-specific effect, we stratified these analyses for African American and white patients (Table 2). Parameter estimates were similar for both groups, suggesting that the observed relationships were due to neither population stratification nor an effect in only 1 race/ethnic group.

Gene-Environment Interactions on $CD4^+$ Lymphocyte Numbers

We also observed gene-environment interactions between children's *CD14* C-260T genotype and dust endotoxin exposure on total numbers of $CD4^+$, $CD4^+Foxp3^+$, and $CD4^+Foxp3^-$ lymphocytes at the age of 1 year. In general, these patterns were opposite those seen for total IgE levels. In children with the *CC* genotype at *CD14* C-260T, increasing endotoxin exposure was associated with lower total serum IgE values at the age of 1 year (Fig 1) but higher numbers of $CD4^+Foxp3^+$ and $CD4^+Foxp3^-$ cells (Fig 2). This relationship was statistically significant for $CD4^+Foxp3^-$ lymphocytes, where endotoxin exposure seemed to account for 20% of the variation in these cell numbers at the age of 1 year.

We explicitly tested an interaction between endotoxin exposure and *CD14* C-260T genotype on $CD4^+$ lymphocyte numbers in the regression models (Table 2). These suggested a significant gene-environment interaction on $CD4^+$ lymphocyte numbers overall ($P = .005$) and, in particular, on $CD4^+Foxp3^-$ lymphocyte numbers ($P = .005$). A significant gene-environment interaction was not evident for $CD4^+Foxp3^+$ lymphocyte numbers. The implications of these regression equations for the interaction between endotoxin exposure and *CD14* C-260T genotype on total serum IgE levels and $CD4^+$ lymphocyte numbers are shown in Figure 3.

To again address the possibility of confounding or effect modification by race/ethnicity, we stratified these analyses for African American and white patients (Table 2). Parameter estimates for both populations were similar for models predicting $CD4^+$ lymphocyte numbers overall and $CD4^+Foxp3^-$ lymphocyte numbers but were quite different for $CD4^+Foxp3^+$ lymphocyte numbers. Specifically, these models suggested that the gene-environment interaction between *CD14* C-260T genotype and endotoxin exposure on $CD4^+Foxp3^+$ lymphocyte numbers was limited to African American patients ($P = .03$). The correlation

coefficients between endotoxin exposure and $CD4^+Foxp3^+$ lymphocyte numbers in African American children were positive ($\rho = 0.32$; $P = .18$), neutral ($\rho = -0.04$; $P = .84$), and negative ($\rho = -0.61$; $P = .20$) for children with the *CD14* C-260T *CC* genotype, *CT* genotype, and *TT* genotype, respectively.

Timing of Endotoxin Exposure on Outcomes

Given previous work on febrile infections,[1] we hypothesized that endotoxin exposure as evaluated at 6 months would have the largest effect on study outcomes at the age of 1 year compared with earlier exposure. When we reanalyzed the data looking at an earlier time point for endotoxin exposure (ie, age of 1 month), we did not find a significant gene-environment interaction between *CD14* C-260T genotype and endotoxin exposure on total IgE levels or $CD4^+$ lymphocyte numbers (data not shown). This suggested that outcomes were the result of recent endotoxin exposure (ie, approximately 6 months of age) and not earlier exposures. This is in keeping with the hypothesis of an important window for innate immune system stimuli in the later half of the first year of life.[1]

DISCUSSION

We show for the first time a gene-environment interaction between *CD14* C-260T genotype and endotoxin exposure on $CD4^+$ lymphocytes numbers, including putative T_{reg} cells. In children with the *CD14* C-260T *CC* genotype, increasing endotoxin exposure at the age of 6 months was associated with lower IgE levels and higher numbers of $CD4^+$ lymphocytes, particularly $CD4^+Foxp3^-$ lymphocytes, for which endotoxin exposure accounted for 20% of the variance. We also show potential effect modification by race/ethnicity whereby *CD14* C-260T genotype and endotoxin exposure seem to interact to affect T_{reg}-cell numbers in African American children but not in white children.

Consistent with previous work looking at the timing of innate stimuli, such as febrile infections,[1] we also found that endotoxin exposure at the age of 6 months, but not at the age of 1 month, was associated with IgE levels and $CD4^+$ lymphocyte numbers at the age of 1 year. Together, these findings provide evidence of the importance of innate immune system stimuli and their timing on adaptive immune system responses (ie, $CD4^+$ lymphocyte numbers and IgE levels). Although the present study does not settle the question as to whether the effect of innate stimuli on allergy risk results from T_{reg}-cell expansion or immune deviation (ie, T_H1-cell expansion),[19,20] both processes may be involved, and the degree to which each process is involved may be genotype and race/ethnicity specific. For example, we observed that the numbers of T_{reg} cells (ie, $CD4^+Foxp3^+$ lymphocytes) and effector T-helper cells (ie, $CD4^+Foxp3^-$ lymphocytes) rose and fell in parallel, particularly in African American children. This finding is consistent with others that T_{reg}-cell number expansion requires interleukin 2 (IL-2), and this cytokine indexes the T_{reg}-cell population to the number of IL-2–producing $CD4^+$ T cells.[21,22]

Recently, a mechanism relating innate immune system stimulation to adaptive immune responses has become better understood (Fig 4). Innate stimuli, such as endotoxin, can induce antigen-presenting cells (APCs) to up-regulate co-stimulatory molecules (eg, CD80 and CD86) and at high levels can produce proinflammatory cytokines (eg, IL-6 and IL-12).[23,24] In the context of antigen presentation, these stimuli may promote T_H2-type responses at low levels of endotoxin exposure[25] but T_H1-cell differentiation at high levels of exposure.[26] Production of IL-6 by APCs can also promote effector cell proliferation by blocking T_{reg}-cell suppression[27]; APCs matured through toll-like receptor (TLR) stimulation may also induce the conversion of naïve $CD4^+$ cells into T_{reg} cells. Similarly, interferon-γ production by T_H1 cells may induce Foxp3 production in $CD4^+CD25^-$ T cells.[28] Last, direct stimulation of TLRs located on T_{reg} cells can result in regulatory cell proliferation and affect suppressive function.

[10,29–32] Although this mechanism explains the observed relationship between endotoxin exposure and increasing $CD4^+$ lymphocyte numbers in children with the *CD14* C-260T *CC* genotype, it does not explain the seemingly opposite relationship observed for children with the *TT* genotype.

CD14, an accessory receptor that facilitates the binding of endotoxin to TLR-4,[33] exists as a membrane bound receptor and in soluble form (sCD14). The *C* to *T* transition at *CD14* position −260 has been associated with increased transcriptional activity[34] and increased levels of sCD14.[17,35–37] These differences in sCD14 levels by *CD14* C-260T genotype may be seen as early as the age of 3 months.[38] However, the disparate effects of increasing endotoxin exposure by *CD14* C-260T genotype suggest that the effect of this polymorphism may also differ by level of endotoxin exposure.

Unlike earlier work that showed a race/ethnicity-specific interaction between *CD14* C-260T genotype and environmental tobacco exposure on total IgE levels in Puerto Rican patients but not in Mexican patients,[15] we did not find a race/ethnicity-specific interaction between *CD14* C-260T genotype and endotoxin exposure on total IgE levels in African American and white patients. However, we observed potential effect modification by race/ethnicity on T_{reg}-cell numbers. African American children, but not white children, demonstrated a significant interaction between *CD14* C-260T genotype and endotoxin exposure on T_{reg}-cell numbers. These findings need to be confirmed in other diverse cohorts.

This study has several limitations. First, there were limited numbers of children with data available for both 6 months and 1 year for outcomes of interest. Although this prohibited us from looking in detail at changes across time, we examined the a priori hypothesis between exposures at the age of 6 months and outcomes at the age of 1 year. Despite limited numbers, the findings were consistent with the *CD14* C-260T genotype and endotoxin interaction that has been described for total IgE,[6] allergen specific IgE,[7] and asthma severity[9] in other populations. In these studies, the *CD14* C-260T *CC* and *TT* genotypes seemed to have opposite effects on outcomes, and these relationships were reversed at low and high levels of endotoxin exposure. Second, we identified T_{reg} cells by intracellular Foxp3 expression and not by surface CD25 expression because Foxp3 expression seems to better delineate $CD4^+$ T_{reg} cells, at least in animal models, compared with CD25 expression.[13,39] In addition to the limited specificity of CD25, we could not further distinguish between thymus-generated natural T_{reg} cells and allergen-specific adaptive T_{reg} cells, because both may be characterized by Foxp3 expression.[40] We also did not attempt to identify other T_{reg} cells, such as those in $CD8^+$ and natural killer T-cell populations or those characterized by IL-10 secretion.[41] Third, although we enumerated $CD4^+Foxp3^+$ lymphocytes, we did not evaluate T_{reg}-cell function. As mentioned already, endotoxin may affect the suppressive function of T_{reg} cells,[10] which may be relevant to allergic diseases, because individuals with atopy have been shown to have T_{reg} cells with diminished suppressive function.[42] Next, we used endotoxin measured on mothers' bedroom floors as a proxy of exposure in the first year of life. It is unknown whether this is the most biologically relevant location or method of measuring exposure in infants. However, these findings were consistent with previous observations,[6] suggesting that this location was a reasonable proxy for biologically relevant exposure. The absence of an association between earlier endotoxin levels and study outcomes suggested that the relationships shown herein were related to recent exposure, supporting the a priori hypothesis of a postnatal period of increased susceptibility.[1] Last, we did not specifically delineate whether $CD4^+Foxp3^-$ lymphocytes had a T_H1 or T_H2 phenotype. This is important because it could help clarify the relative importance of endotoxin exposure on T_H1/T_H2 differentiation vs T_{reg}-cell expansion and its subsequent effects on IgE levels. We hope to include this in future studies.

Although this study was small, it provides some interesting directions for further research. We identified that the gene-environment interaction between *CD14* C-260T genotype and endotoxin exposure may have reciprocal effects on total serum IgE levels and $CD4^+$ lymphocyte numbers (ie, $CD4^+Foxp3^+$ and $CD4^+Foxp3^-$). Although this may provide a simple immunologic explanation for the observed gene-environment interaction on IgE levels, research is needed to determine the upstream effects of this polymorphism on the innate immune response and antigen presentation and why this promoter SNP seems to have disparate effects at high and low endotoxin levels. Given well-described disparities in atopic conditions by race/ethnicity,[43–45] it will be important to clarify whether the early race/ethnicity differences observed herein for T_{reg} cells contribute to later disparities. These findings provide a rationale for further research into the early determinants of these disparities.

Acknowledgements

We acknowledge the contributions of all the WHEALS staff, whose dedication to the study made this article possible.

Funding Sources: This work was supported by grants from the Fund for Henry Ford Hospital and the National Institute of Allergy and Infectious Diseases (AI61774, AI50681, AI59415) and the National Heart, Lung, and Blood Institute (HL79055), National Institutes of Health.

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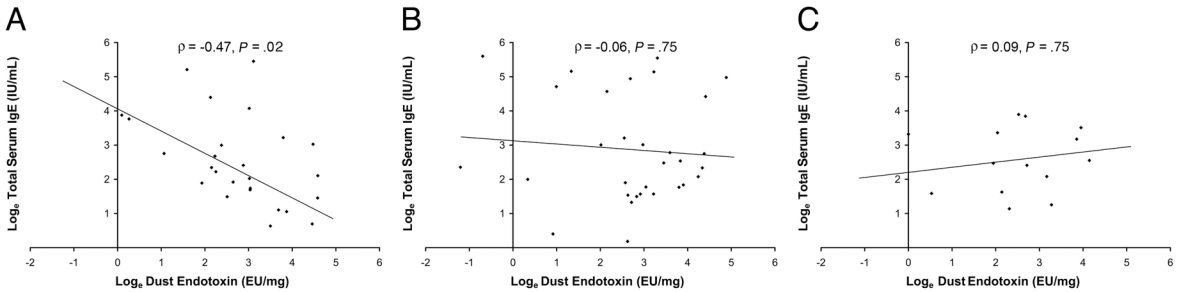

Figure 1.

Relationship between endotoxin exposure at the age of 6 months and total serum IgE levels at the age of 1 year stratified by *CD14* C-260T genotype: *CC* (A), *CT* (B), and *TT* (C). *CC* denotes individuals homozygous for cytosine at both alleles (n = 26); *CT*, individuals heterozygous for the transition (n = 32); and *TT*, individuals homozygous for the cytosine (C) to thymine (T) transition at position −260 of *CD14* (n = 14).

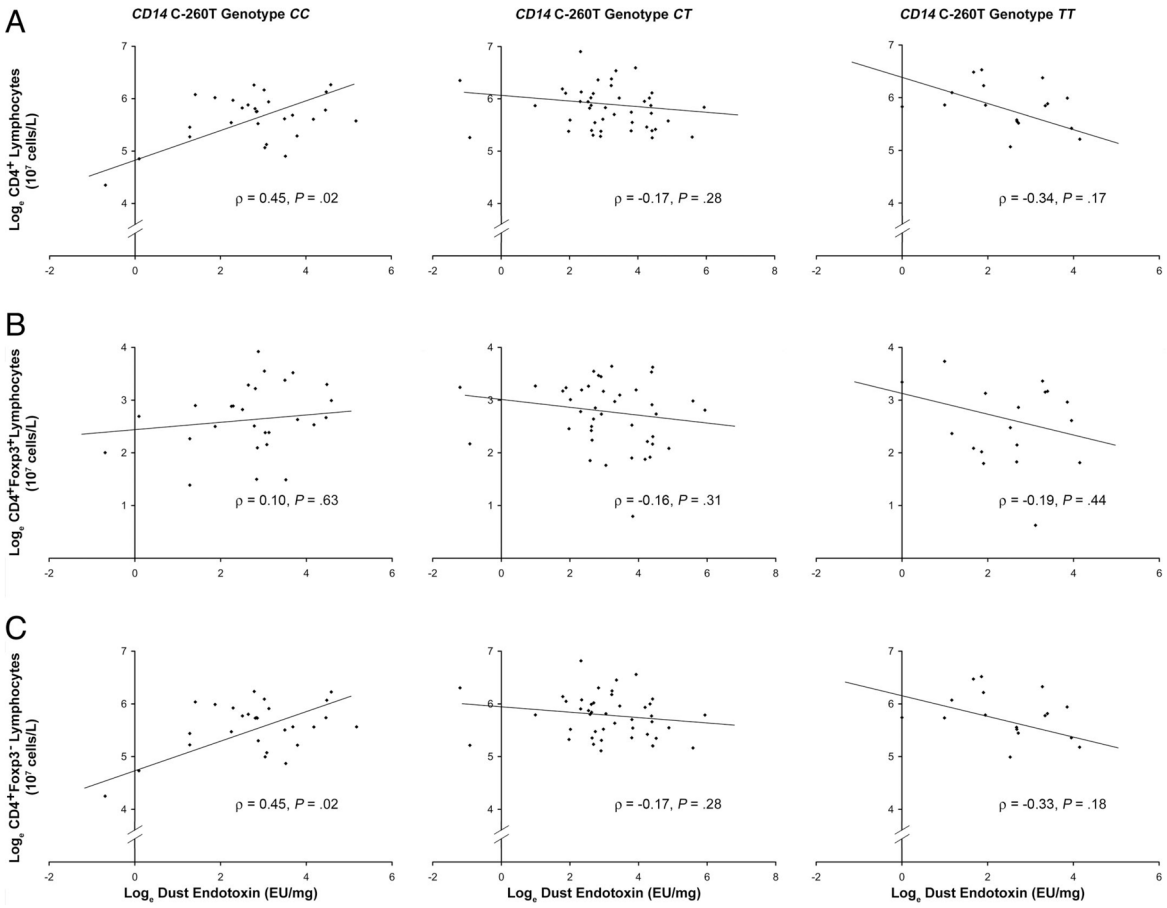

Figure 2.

Relationship between endotoxin exposure at the age of 6 months and total $CD4^+$ (A), $CD4^+Foxp3^+$(B), and $CD4^+Foxp3^-$ (C) lymphocyte numbers at the age of 1 year stratified by *CD14* C-260T genotype. *TT* denotes individuals homozygous for the cytosine (C) to thymine (T) transition at position −260 of *CD14* (n = 18); *CT*, individuals heterozygous for the transition (n = 44); and *CC*, individuals homozygous for cytosine at both alleles (n = 28).

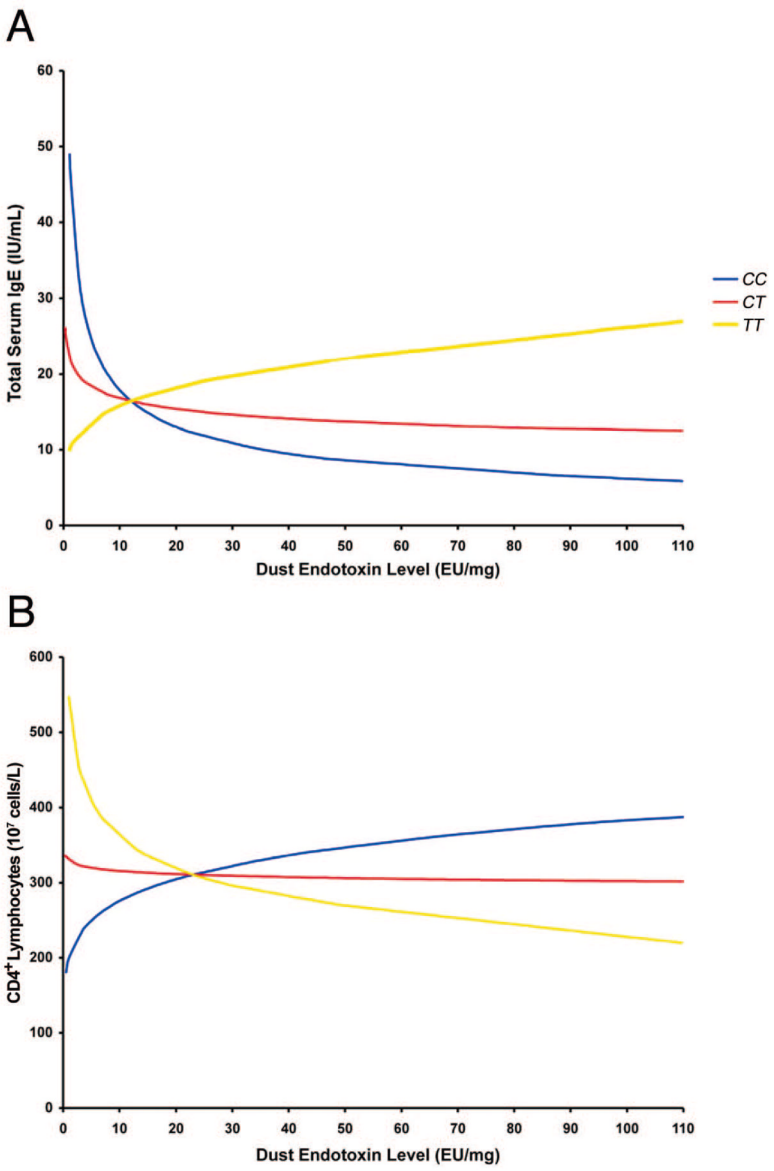

Figure 3.

Interaction between *CD14* C-260T genotype and dust endotoxin levels on total serum IgE levels (A) and $CD4^+$ lymphocyte numbers (B) as implied by the regression models in Table 2, which included the variables *CD14* C-260T genotype, endotoxin levels, and their interaction term for all study patients (n = 90). *TT* denotes individuals homozygous for the cytosine (C) to thymine (T) transition at position −260 of CD14; *CT*, individuals heterozygous for the transition; and *CC*, individuals homozygous for cytosine at both alleles.

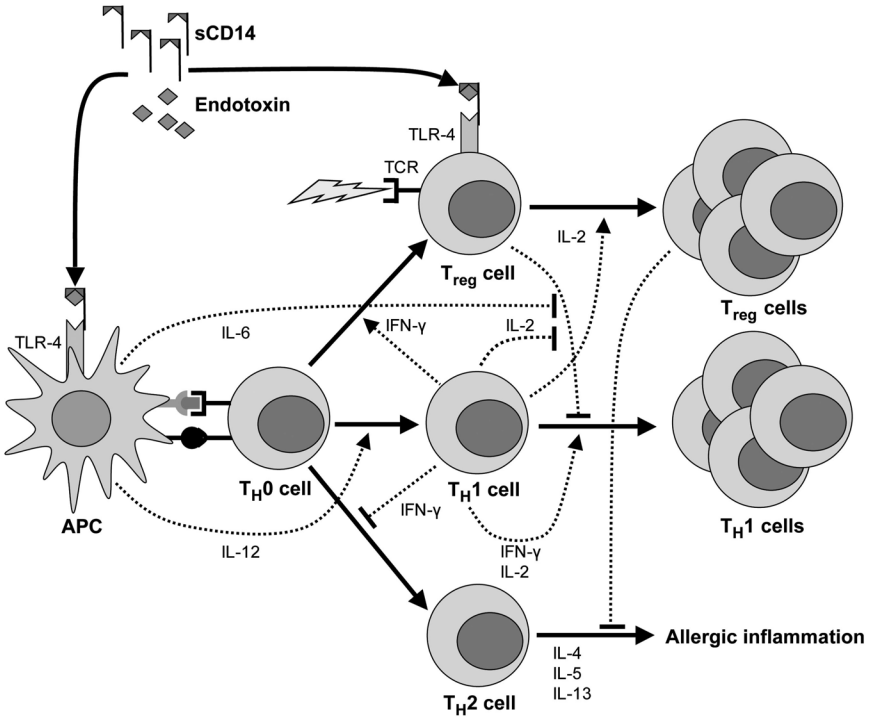

Figure 4.

Hypothetical model for the relationships among endotoxin exposure, regulatory and effector T-cell expansion, and allergic disease. Dashed lines ending in arrows indicate stimulation, whereas dashed lines ending in a vertical line indicate inhibition. Endotoxin binds to soluble *CD14* (sCD14) and other binding proteins. Endotoxin then interacts with the toll-like receptor 4 (TLR-4) receptor complex, which stimulates antigen-presenting cells (APCs) to express co-stimulatory molecules at the time of antigen presentation. Interleukin 6 (IL-6) and IL-12 produced by APCs inhibit regulatory T-cell (T_{reg}-cell) suppression and promote the differentiation of naïve T cells (T_H0 cells) into T_H1 cells, respectively. Interferon-γ (IFN-γ) and IL-2 promote the expansion of T_{reg} cells and T_H1 cells. The TLR-4 stimulation may also promote T_H0-cell differentiation into T_{reg} cells or their expansion. Both T_{reg} cells and T_H1 cells suppress allergy, promoting T_H2 cells through cell-to-cell contact or cytokine production. Low levels of endotoxin exposure in the setting of antigen presentation may also promote T_H2 responses if the former is sufficient to promote APC maturation but insufficient to induce inflammatory cytokine production. TCR indicates T-cell receptor.

Table 1

Characteristics of Patients Included and Not Included in the Analysis[a]

Characteristic	Included (n = 90)	Not included (n = 386)	*P* value
Male sex	48/90 (53.3)	192/386 (49.7)	.54
Race/ethnicity			.76
African American	48/90 (53.3)	201/386 (52.1)	
White	34/90 (37.8)	132/386 (34.2)	
Other/unknown	8/90 (8.9)	53/386 (13.7)	
Household income, mean ± SD, $[b]	61,039 ± 41,883	63,668 ± 41,337	.61
Parents reporting smoking	15/78 (19.2)	57/365 (15.6)	.50
CD14 C-260T genotype[c]			.34
CC	28/90 (31.1)	82/205 (40.0)	
CT	44/90 (48.9)	89/205 (43.4)	
TT	18/90 (20.0)	34/205 (16.6)	
Dust endotoxin level, geometric mean ± SD, EU/mg[d]	18.2 ± 3.8	15.2 ± 3.3	.26
Total serum IgE level at the age of 1 y, geometric mean ± SD, IU/mL[e]	15.4 ± 4.0	15.1 ± 3.9	.93

[a] Data are given as number/total number (percentage) except where indicated otherwise.

[b] The numbers of children with data are 77 for included and 368 for not included.

[c] *CD14* C-260T denotes a single nucleotide polymorphism at position −260 resulting in a cytosine (C) to thymine (T) transition.

[d] The numbers of children with data are 90 for included and 200 for not included.

[e] The numbers of children with data are 72 for included and 51 for not included.

Table 2

Gene-Environment Interaction Between Dust Endotoxin Exposure at the Age of 6 Months and *CD14* C-260T[a] Genotype on Total Serum IgE Levels and $CD4^+$ Lymphocyte Numbers at the Age of 1 Year[b]

Outcome variable at 1 y of age	Independent variable	All patients (n = 90)		African American patients (n = 48)		White patients (n = 34)	
		Independent variable parameter estimate	*P* value	Independent variable parameter estimate	*P* value	Independent variable parameter estimate	*P* value
Total IgE[c]	Endotoxin	−0.46	.03	−0.39	.22	−0.45	.17
	CD14 C-260T genotype	−0.83	.14	−0.62	.56	−0.67	.38
	Endotoxin × *CD14* interaction	0.33	.08	0.42	.24	0.21	.42
$CD4^+$ cells (No.)	Endotoxin	0.14	.02	0.17	.04	0.22	.05
	CD14 C-260T genotype	0.51	.002	0.50	.04	0.47	.048
	Endotoxin × *CD14* interaction	−0.16	.005	−0.20	.02	−0.14	.08
$CD4^+Foxp3^+$ cells (No.)	Endotoxin	0.04	.71	0.24	.06	−0.03	.88
	CD14 C-260T genotype	0.19	.47	0.87	.03	−0.24	.55
	Endotoxin × *CD14* interaction	−0.08	.39	−0.29	.03	0.01	.94
$CD4^+Foxp3^-$ cells (No.)	Endotoxin	0.15	.02	0.16	.04	0.23	.04
	CD14 C-260T genotype	0.52	.002	0.48	.05	0.51	.04
	Endotoxin × *CD14* interaction	−0.16	.005	−0.20	.02	−0.15	.07

[a] *CD14* C-260T denotes a single nucleotide polymorphism at position −260 resulting in a cytosine (C) to thymine (T) transition.

[b] Regression equations are of the form ln(outcome variable) = β_1(endotoxin) + β_2(*CD14* genotype) + β_3(endotoxin)(*CD14* genotype) *or* outcome variable = $e^{\beta 1(\text{endotoxin}) + \beta 2(CD14\ \text{genotype}) + \beta 3(\text{endotoxin})(CD14\ \text{genotype})}$, where β represents the individual parameter estimates given in the table. Endotoxin is included as the $\log_e$ transformation of measured value in endotoxin units per milligram of dust, and *CD14* C-260T genotype is categorized into *CC* (=0, referent), *CT* (=1), and *TT* (=2).

[c] For total IgE, the numbers of children with data are 72, 28, and 39, for all patients, African American patients, and white patients.