A Section 508–conformant HTML version of this article
is available at https://doi.org/10.1289/EHP661.

House Dust Endotoxin and Peripheral Leukocyte Counts: Results from Two Large Epidemiologic Studies

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Background: The peripheral leukocyte count is a biomarker of inflammation and is associated with human all-cause mortality. Although causes of acute leukocytosis are well-described, chronic environmental determinants of leukocyte number are less well understood.

Objectives: We investigated the relationship between house dust endotoxin concentration and peripheral leukocyte counts in human subjects.

Methods: The endotoxin–leukocyte relationship was evaluated by linear regression in the National Health and Nutrition Examination Survey (NHANES) 2005–2006 ($n=6{,}254$) and the Agricultural Lung Health Study (ALHS; $n=1{,}708$). In the ALHS, we tested for a gene [Toll-like Receptor 4 (*TLR4*), encoding the endotoxin receptor]-by-environment interaction in the endotoxin–leukocyte relationship using regression models with an interaction term.

Results: There is a statistically significant, positive association between endotoxin concentration and total leukocyte number [estimated change, $0.186\times10^3/\mu L$ (95% CI: 0.070, $0.301\times10^3/\mu L$) per 10-fold change in endotoxin; $p=0.004$) in the NHANES. Similar positive associations were found for monocytes, lymphocytes, and neutrophils. Stratified analyses revealed possible effect modification by asthma and chronic obstructive pulmonary disease. We observed similar associations in the ALHS. For total leukocytes, there was suggestive evidence in the ALHS of a gene-by-environment interaction for minor allele carrier status at the *TLR4* haplotype defined by rs4986790 and rs4986791 (interaction $p=0.15$).

Conclusions: This is, to our knowledge, the first report of an association between house dust endotoxin and leukocyte count in a national survey. The finding was replicated in a farming population. Peripheral leukocyte count may be influenced by residential endotoxin exposure in diverse settings. https://doi.org/10.1289/EHP661

Introduction

Leukocytes are effectors and biomarkers of inflammation. The total count of white blood cells (WBCs) in the circulation is associated with coronary, cancer, and all-cause mortality in human subjects (de Labry et al. 1990; Grimm et al. 1985). Although peripheral WBC count is well-known in clinical medicine to rise acutely during infection, tissue injury, and certain toxic/occupational exposures (Chabot-Richards and George 2014), there is relatively less information available regarding chronic environmental exposures that determine WBC levels in healthy human populations.

Endotoxin [i.e., lipopolysaccharide (LPS)], a glycolipid shed from the outer cell wall of Gram-negative bacteria and detected by Toll-like receptor 4 (TLR4) on the surface of mammalian cells, is arguably the prototypical environmental stimulus of inflammation (Park and Lee 2013). On the high end of the range of human exposure, endotoxin inhalation is thought to underlie the pathogenesis of respiratory and systemic illness in textile fiber and animal confinement workers, among other industries (Liebers et al. 2006). Controlled human exposure studies have revealed that acute endotoxin inhalation not only induces robust neutrophilic airway inflammation (Alexis et al. 2001; Sandström et al. 1992) but also increases peripheral WBC count and other inflammatory biomarkers, such as serum C-reactive protein (CRP) (Dillon et al. 2011; Michel et al. 1992, 1995, 1997).

Low-level endotoxin is ubiquitous in the environment. Studies focused on pediatric populations have shown that house dust endotoxin is associated with reduced sensitization (Gereda et al. 2000) and atopy (Braun-Fahrländer et al. 2002). House dust endotoxin has also been linked to worsened asthma symptoms (Thorne et al. 2005, 2015). To our knowledge, no reports have investigated whether there is a relationship between house dust endotoxin and peripheral WBC count or other inflammatory biomarkers. Given that house dust endotoxin exposure can be reduced by simple interventions (Chen et al. 2012; Gereda et al. 2001; Ownby et al. 2013; Thorne et al. 2009) and that WBC count has been linked to several human diseases (Grimm et al. 1985), confirmation of an endotoxin–WBC relationship might have public health implications.

The National Health and Nutrition Examination Survey (NHANES) is a biennial, cross-sectional population-based survey of the noninstitutionalized, primarily urban and suburban U.S. population. Our group quantified endotoxin levels in dust samples collected from nearly 7,000 households in NHANES 2005–2006 using extreme quality assurance (QA) measures (Thorne et al. 2015). We hypothesized that endotoxin would be positively related to peripheral WBC count and serum CRP. Given that cigarette smoke contains endotoxin and potentiates endotoxin signaling (Hasday et al. 1999; Kulkarni et al. 2007; Pace et al. 2008) and that asthma modifies the acute response to inhaled endotoxin (Hernandez et al. 2012), we hypothesized that smoking and inflammatory lung diseases would modify the relationship of endotoxin to these inflammatory outcome measures.

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Supplemental Material is available online (https://doi.org/10.1289/EHP661).
The authors declare they have no actual or potential competing financial interests.
Received 15 June 2016; Revised 13 September 2016; Accepted 27 September 2016; Published 31 May 2017.

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To test the generalizability of our findings, we also evaluated the relationship of endotoxin to WBC count and differential in the Agricultural Lung Health Study (ALHS), a sub-study of the Agricultural Health Study (AHS), an adult U.S. farming cohort (Alavanja et al. 1996).

Methods

Study Populations

The NHANES 2005–2006 (CDC 2005) used a complex multistage design to assess the health and nutritional status of the civilian, noninstitutionalized U.S. population and was approved by the National Center for Health Statistics Research Ethics Review Board (Fessler et al. 2009, 2013; Jaramillo et al. 2013). All NHANES participants provided informed consent. To ensure adequate sample sizes of certain population subgroups, NHANES oversampled low-income persons, adolescents (12–19 y), elderly subjects (≥ 60 y), African Americans, and Mexican Americans, among others. A detailed description is posted at http://www.cdc.gov/nchs/nhanes.htm. Of 6,963 participants aged ≥ 1 year with household endotoxin data, blood samples were collected from 6,254 (89.8%). WBC differentials were not available for 19 (0.3%) participants, resulting in a WBC differential study population of 6,235.

The ALHS is a case–control study of current asthma nested within the AHS, a prospective cohort of licensed pesticide applicators ($n = 52{,}395$) and their spouses ($n = 32{,}347$) originally enrolled from 1993–1997 in North Carolina and Iowa, approved by the Institutional Review Board at the National Institute of Environmental Health Sciences (Alavanja et al. 1996). All AHS participants provided informed consent. The ALHS enrolled 3,301 individuals identified during a follow-up telephone interview administered from 2005–2010 as either asthma cases ($n = 1{,}223$) or noncases ($n = 2{,}078$). A subset of participants had bedroom dust samples collected for endotoxin analysis (2,485 independent households). Complete blood cell count with differential data were available for 1,941 participants. After exclusion of 216 samples with $>20\%$ smudge cells and 17 samples with platelet clumping, the final study population with house dust endotoxin and blood cell count data was $n = 1{,}708$. The analysis used the following releases of AHS data: P3REL201209.00, P1REL201209.00, and AHSREL201304.00.

Endotoxin Analysis

In both studies, combined bed and bedroom floor dust samples were collected at each participant's home and were sieved before being frozen according to a common protocol (Thorne et al. 2015). Endotoxin was extracted and analyzed at four dilutions using the kinetic chromogenic Limulus amebocyte lysate assay at the same laboratory, adopting multiple quality assurance (QA) measures (Thorne et al. 2015; Vojta et al. 2002). The lower limit of detection (LOD) was 0.000488 endotoxin units per milligram of dust.

Hematologic Measurements

In NHANES, a Beckman Coulter MAXM was used for blood cell analysis, and standard procedures were followed. Platelet count was derived from the platelet histogram and multiplied by a calibration constant. Erythrocyte mean corpuscular volume (MCV) was derived from the erythrocyte histogram and used to compute hematocrit as follows: $\text{erythrocyte count} \times \text{MCV}/10$. Hemoglobin concentration was determined by absorbance found through photocurrent transmittance. WBC differential was determined using VCS technology. CRP was measured by latex-enhanced nephelometry. In the ALHS, peripheral blood smears were prepared for each sample and were stained with a modified Romanowsky stain using a Wescor Aerospray® Hematology Slide Stainer Cytocentrifuge; automated total WBC counts were obtained using a Drew Scientific HEMAVET Multispecies Hematology Analyzer, and manual differentials were counted on peripheral blood smears. Smudge cell percentages were calculated by counting the number of ruptured or smudge cells present per 100 intact WBCs. Platelet clumping was also recorded.

Covariates and Effect Modification Variables

In NHANES, covariates were obtained from the questionnaire (age, race/ethnicity, gender, smoking, asthma, COPD, wheeze, antiinflammatory medication usage), from laboratory analyses (cotinine, atopy), and from physical examination (height, weight). Participants with asthma were defined as those who answered in the affirmative to the question "Has a doctor ever told you that you had asthma?" Current asthmatics additionally replied in the affirmative to "Do you still have asthma?" For assessment of wheezing, participants were asked "Have you had wheezing or whistling in your chest at any time in the past 12 mo?" We have previously used "wheeze" from the NHANES questionnaire as an alternate, symptom-based phenotypic categorization of lung disease that captures a broad base of diagnosed and undiagnosed respiratory disorders (Fessler et al. 2009; Thorne et al. 2015). Participants with chronic obstructive pulmonary disease (COPD) were defined as those who answered in the affirmative to a question asking whether "a physician or health care professional has told you that you have emphysema or chronic bronchitis" and/or to coughing up phlegm on most days for ≥ 3 consecutive months for ≥ 2 consecutive years in the context of a ≥ 10 pack-year history of cigarette smoking. Medications defined as antiinflammatory included nonsteroidal antiinflammatory drugs (NSAIDs), systemic and inhaled glucocorticoids, cytotoxic drugs (e.g., methotrexate, mercaptopurine, hydroxyurea, azathioprine, leflunomide, cyclosporine), biologic agents (e.g., etanercept), and leukotriene inhibitors. Atopy was defined as $\geq$detectable (≥ 0.35 kU/L) serum allergen-specific immunoglobulin E (IgE), which was measured using a Pharmacia Diagnostics ImmunoCAP 1000 system, now known as Thermo Scientific™ ImmunoCAP Specific IgE, as previously reported (Jaramillo et al. 2013). Serum cotinine was measured by isotope dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry.

In the ALHS, current asthma cases of three categories were pooled for analysis in order to be inclusive: self-reported current doctor-diagnosed asthma (based on positive responses to "have you ever been diagnosed with asthma?" and "do you still have asthma?") without a self-reported previous diagnosis of COPD or emphysema ($n = 876$), possible undiagnosed current asthma based on symptoms and asthma medication usage in nonsmokers or former smokers (<10 pack-years) who did not report current asthma or COPD ($n = 309$), and doctor-diagnosed current asthma plus COPD among subjects with <10 pack-years of smoking ($n = 38$). Individuals randomly selected from the AHS who reported neither current asthma nor asthma symptoms (i.e., wheeze or being awakened by respiratory symptoms) or use of asthma medications in the past 12 mo were considered noncases.

Gene-by-Environment (G×E) Interaction Analysis

To determine if the relationship of house dust endotoxin to WBC count depends on genotype, we performed a gene by environment interaction analysis within the ALHS, focusing on two SNPs in *TLR4*. The SNPs were selected from previous reports showing

attenuated *in vivo* proinflammatory responses, including peripheral leukocytosis, after endotoxin inhalation in human volunteers (Arbour et al. 2000; Michel et al. 2003). The selected SNPs, rs4986790 and rs4986791 [located in *TLR4* (Toll-like receptor 4, OMIM: 603030)], were genotyped on the UK Biobank Axiom Array (Axiom_UKB_WCSG) by Affymetrix Axiom Genotyping Services (Affymetrix, Inc.) using DNA extracted from blood or saliva collected from ALHS participants during the home visit. Both SNPs passed all quality control metrics including Hardy–Weinberg Equilibrium, missing rate $<5\%$, and a minor allele frequency $>5\%$. All samples had a missing call rate $<5\%$, and gender discrepancies, population outliers, and unexpected duplicates were excluded. For statistical modeling, we used a haplotype approach, combining both SNPs and categorizing individuals as carriers (≥ 1 copy of a minor allele) and noncarriers (homozygous for both major alleles) as previously described (Arbour et al. 2000; Werner et al. 2003). The final data set included 1,646 individuals with genotype and house dust endotoxin data.

Statistical Analysis

To account for the complex sampling design used in NHANES and to assure unbiased variance estimates, sample weights were used in all analyses, and standard errors (SEs), confidence intervals (CIs), and *p*-values were developed in accordance with the complex survey design using Taylor series linearization methods. All analyses were conducted using SAS Survey statistical software (v9.4; SAS Institute Inc.) survey procedures (SURVEYFREQ, SURVEYMEAN, SURVEYREG). Descriptive statistics were generated (means or percentages and associated standard errors). All blood parameters were assessed for normality. Linear regression analyses were performed to assess the change in blood cell count and $\log_{10}$CRP (NHANES only) per $\log_{10}$ change in endotoxin (Thorne et al. 2015). Covariates in the NHANES analysis included age, sex, race/ethnicity, poverty-to-income ratio (PIR), body mass index (BMI), and cotinine.

Covariates in the ALHS analysis included age, sex, race/ethnicity, smoking status (current/former/never), pack-years, asthma status, BMI, season, and state (IA vs. NC). Interactions between house dust endotoxin and age [juvenile (<18 y old) vs. adult (≥ 18 y old)], sex, race/ethnicity, smoking status (current, former, never), and cotinine level [10 ng/mL cut point; a threshold that has been associated with active smoking (Pirkle et al. 1996)] were tested by including a product term in the model. The relationship between house dust endotoxin and WBC count was rendered graphically as a weighted penalized B-spline (SAS v9.4) to facilitate depiction of the WBC trend throughout the full range of house dust endotoxin concentrations. G×E interaction analyses were performed using linear regression models, including an interaction term and main effects (SNP and $\log_{10}$ endotoxin EU/mg) with WBC count as the outcome, and were adjusted for asthma status, age, sex, state, smoking (never/past/current), pack-years, season, and BMI (categorical variable). Statistical significance was set at $p < 0.05$ (main effects) or $p < 0.10$ (interactions).

Results

As shown in Table S1, the NHANES 2005–2006 study population with available endotoxin and total WBC data ($n = 6{,}254$) was approximately equally divided between the sexes, with a mean $\pm$ SE age of 37.7 ± 0.6 years (range, $1-85$), and was predominantly (68.9%) non-Hispanic white, with the remainder represented by non-Hispanic black, Mexican American, and Other categories. Approximately half of subjects ≥ 20 years old were never smokers, one-quarter were former smokers, and the remainder were current smokers. Current asthma was reported by 8.9% of the participants, COPD by 7.5%, and antiinflammatory medication usage by 8.4%. The overall geometric mean endotoxin concentration (weighted) was 15.5 EU/mg [median $= 16.2$ EU/mg, interquartile range (IQR) $= 8.2 - 34.3$ EU/mg, minimum $= 0.0003$ EU/mg (adjusted LOD), maximum $= 9089.1$ EU/mg].

The mean value for peripheral WBC count in the overall NHANES study population was $7.43 \times 10^3/\mu l$. Bivariate analysis (see Table S2) revealed that current smokers, subjects with cotinine ≥ 10 ng/mL [a threshold that has been associated with active smoking (Pirkle et al. 1996)], and subjects with COPD all had increased WBC and that the latter two groups had increased serum CRP, whereas current asthmatics had an increase in CRP but not in WBC count. All WBC subtypes [monocytes, lymphocytes, neutrophils, eosinophils, and basophils; data available for 6,235 (89.5%) of participants with endotoxin data] were increased in current smokers and in subjects with cotinine ≥ 10 ng/mL, whereas in subjects with COPD, all WBC subtypes were increased with the exception of lymphocytes, which were decreased (see Table S3). Atopic subjects had equivalent WBC and CRP values to those in nonatopic subjects but had increased eosinophils, as expected.

As shown in Table 1, linear regression revealed a statistically significant, positive relationship between endotoxin and total WBC count in the overall population [estimated change (β coefficient) in leukocytes, $0.186 \times 10^3/\mu L$ (95% CI: 0.070, $0.301 \times 10^3/\mu L$) per 10-fold change in endotoxin; $p = 0.004$]. A spline fit of the WBC count along the full range of endotoxin concentrations revealed a possible plateauing of WBC toward the upper end of the endotoxin distribution but no evident no-effect threshold (Figure 1). Stratified analyses of the endotoxin–WBC relationship were performed to examine COPD, atopy, current asthma, and wheeze for possible effect modification (Table 1). Although the endotoxin–WBC relationship was negligible in the relatively small COPD and nonatopic asthma groups, there were no statistically significant interactions. Additional analyses revealed no significant relationship of endotoxin to hematocrit, hemoglobin concentration, or platelet count (data not shown). As shown in Table 1, a positive endotoxin–CRP relationship was also noted that was of borderline statistical significance ($p = 0.07$ in the overall population).

In a sensitivity analysis of the endotoxin–WBC association, we excluded subjects ($n = 479$) who reported usage of antiinflammatory medications during the month before blood collection and found no material changes in the association (see Table S4). Although we found no relationship of endotoxin concentration to the WBC differential [i.e., WBC percentage of neutrophils, monocytes, lymphocytes, eosinophils, and basophils (data not shown)], linear regression revealed a statistically significant, positive relationship of endotoxin to absolute counts of monocytes, lymphocytes, and neutrophils in the overall study population (Table 2).

We also tested for possible effect modification by demographic and cigarette smoking–related factors of the relationship of endotoxin to WBC count and serum CRP. Neither age, gender, nor race/ethnicity affected the endotoxin–WBC and endotoxin–CRP relationships (see Table S5). The relationship of endotoxin to leukocyte subtypes, stratified by demographic characteristics, is shown in Table S6.

Cigarette smoke exposure, evaluated either by questionnaire (i.e., current/former/never smoker) or by serum cotinine concentration (cut point, 10 ng/mL), did not modify the endotoxin–WBC or endotoxin–CRP relationship (data not shown). However, a complex reciprocal interaction was noted between endotoxin and smoking in the relationship to eosinophil and basophil counts

Table 1. Linear regression of the change in white blood cells and serum C-reactive protein associated with a 10-fold increase in house dust endotoxin concentration, in the overall NHANES 2005–2006 study population and within disease/symptom strata.

	White blood cells (1000 cells/μL)				C-reactive protein[e]				
Participant Subgroup[a]	*n*	β	95% CI	*p*-Value	*n*	β	95% CI	10^{β}	*p*-Value
Overall	5,474	0.186	(0.070, 0.301)	0.004	5,486	0.031	(−0.003, 0.065)	1.073	0.07
COPD									
Yes	265	−0.099	(−0.815, 0.617)	0.77	266	0.104	(−0.027, 0.235)	1.271	0.11
No	5,209	0.205	(0.093, 0.317)	0.001	5,220	0.023	(−0.013, 0.059)	1.054	0.20
Atopy[b]									
Yes	2,612	0.210	(−0.002, 0.422)	0.05	2,619	0.039	(−0.009, 0.086)	1.093	0.10
No	2,745	0.158	(0.013, 0.304)	0.04	2,750	0.024	(−0.034, 0.083)	1.058	0.39
Current asthma									
Yes	513	0.184	(−0.424, 0.792)	0.53	514	0.093	(0.008, 0.178)	1.240	0.03
No[c]	4,943	0.186	(0.098, 0.275)	<0.001	4,954	0.021	(−0.016, 0.059)	1.051	0.24
Current asthma type[d]									
Atopic	344	0.282	(−0.525, 1.090)	0.47	345	0.071	(−0.037, 0.178)	1.176	0.18
Non-atopic	158	−0.019	(−0.619, 0.581)	0.95	158	0.094	(−0.015, 0.204)	1.243	0.09
No asthma	4,838	0.178	(0.090, 0.266)	0.001	4,849	0.024	(−0.018, 0.065)	1.056	0.25
Wheeze									
Atopic	431	0.312	(−0.170, 0.793)	0.19	431	0.074	(−0.028, 0.175)	1.185	0.14
Non-atopic	300	0.353	(−0.236, 0.943)	0.22	300	0.192	(0.085, 0.300)	1.557	0.002
No wheeze	4,625	0.140	(0.046, 0.234)	0.006	4,637	0.012	(−0.031, 0.054)	1.028	0.56

Note: 10β expected ratio increase in C-reactive protein for every 10-fold increase in HDE concentration; COPD, chronic obstructive pulmonary disease; NHANES, National Health and Nutrition Examination Survey.

[a]Adjusted for age, sex, race/ethnicity, cotinine, poverty-to-income ratio (PIR), and body mass index (BMI). Overall data include participants with missing stratification status.

[b]Sensitized to ≥1 allergen-specific immunoglobulin E (IgE).

[c]Includes participants who either answered "no" to doctor-diagnosed asthma, or who answered "yes" to doctor-diagnosed asthma but "no" to "Do you still have asthma?"

[d]Captures the same participants as the "Current asthma" categorization above with the exception that it only includes participants with nonmissing data for atopic status.

[e]Linear analysis performed on $\log_{10}$–transformed C-reactive protein values (raw values in milligrams/deciliter).

(Table 3). Subjects with cotinine ≥10 ng/mL had positive endotoxin–basophil and inverse endotoxin–eosinophil associations, whereas the opposite directionality for both cell types was noted for subjects with low cotinine. Supporting the robustness of this finding, consistent findings were noted using smoking status by questionnaire in place of cotinine. However, given the low values for counts of both eosinophils, these findings must be interpreted with caution.

To test the generalizability of our primary finding, that of the positive association between endotoxin and peripheral WBC count, we repeated the analysis in the ALHS. The ALHS is a case–control study of current asthma nested within the AHS, an adult U.S. farming cohort of pesticide applicators (mostly farmers) and their spouses. Endotoxin concentrations in dust collected from the same household locations as in NHANES (bedding surface and bedroom floor) were measured by the same laboratory as used for the NHANES analysis and using identical analytic procedures.

Characteristics of the ALHS study population of 1,708 subjects ($n = 645$ asthma cases, $n = 1{,}063$ controls) that had endotoxin and

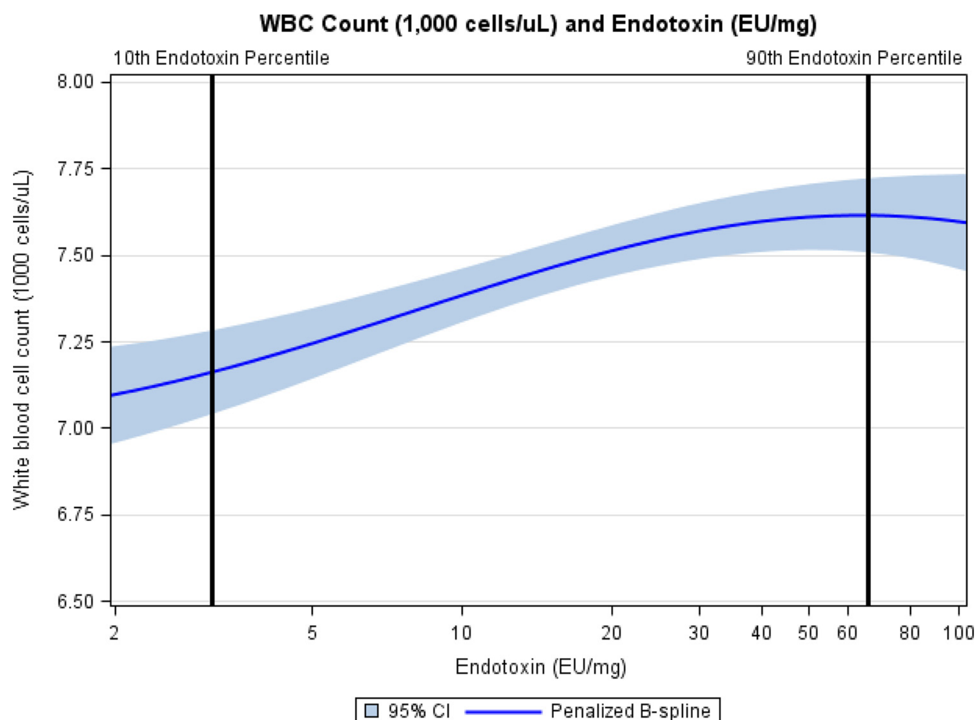

Figure 1. Weighted penalized B-spline of white blood cell (WBC) count through the full range of household dust endotoxin concentration in the National Health and Nutrition Examination Survey (NHANES) 2005–2006 study population.

Table 2. Linear regression of the change in absolute count of WBC subtypes associated with a 10-fold increase in house dust endotoxin concentration in the NHANES 2005–2006 study population.

Absolute cell count[a]	n	β	95% CI	p-Value
Monocyte (1,000/μL)	5,457	0.015	(0.005, 0.026)	0.006
Lymphocyte (1,000/μL)	5,457	0.074	(0.025, 0.123)	0.006
Eosinophil (1,000/μL)	5,457	0.006	(−0.010, 0.022)	0.43
Basophil (1,000/μL)	5,457	−0.00002	(−0.004, 0.004)	0.99
Neutrophil (1,000/μL)	5,457	0.094	(0.011, 0.177)	0.03

Note: CI, confidence interval; NHANES, National Health and Nutrition Examination Survey; WBC, white blood cell.
[a]Adjusted for age, gender, race/ethnicity, cotinine, poverty-to-income ratio (PIR), and body mass index (BMI).

peripheral blood cell count data are shown in Tables S7–S8. Compared with the NHANES 2005–2006 study population, the ALHS study population by design had a higher percentage of asthma cases and was restricted to adults (99% were ≥ 40 years old). The study population was also composed much more predominantly of white subjects and had a lower frequency of current smokers. House dust endotoxin concentrations were higher in the ALHS than in the NHANES study population, with an overall geometric mean endotoxin concentration of 30.7 EU/mg [median $=$ 45.0 EU/mg, IQR $=$ 20.1–76.3 EU/mg, minimum $=$ 0.0003 EU/mg (adjusted LOD), maximum 4,452 EU/mg]. As shown in Table 4, a statistically significant, positive association was found between endotoxin concentration and total WBC count. As in NHANES, there was no relationship of endotoxin to the percentages (i.e., differential) of any of the WBC subtypes (data not shown). However, a statistically significant, positive association was observed between endotoxin and absolute neutrophil count (Table 4). The magnitude of the endotoxin–WBC relationship was similar between the two studies (β values in Tables 1 and 4), and the endotoxin–neutrophil relationship was virtually identical between the two studies (β values in Tables 2 and 4).

Minor allele status at two *TLR4* loci that are in high linkage disequilibrium, rs4986790 and rs4986791, confers reduced responsiveness to inhaled LPS, including attenuated leukocytosis (Arbour et al. 2000; Michel et al. 2003). Thus, we hypothesized that these variants may modify the association between endotoxin and total WBC count. Individuals in the ALHS study population ($n = 1{,}646$) were categorized as noncarriers (89%) if they were homozygous for both major alleles or as a variant carrier (11%) if one of the minor alleles was present, as

Table 3. Linear regression of the change in count of leukocyte subtypes in NHANES 2005–2006 study population associated with a 10-fold increase in house dust endotoxin concentration within strata of smoking status and serum cotinine.

Cell count[a]	Participant strata	n	β	95% CI	p-Value	Interaction p-value[b]
Monocytes (1,000/μL)	Current smokers[c]	717	0.013	(−0.015, 0.041)	0.34	0.71
	Former smokers[c]	752	0.024	(−0.005, 0.054)	0.10	
	Never smokers[c]	1,539	0.005	(−0.014, 0.024)	0.59	
	Current smokers	721	0.014	(−0.014, 0.041)	0.30	0.63
	Former smokers	757	0.026	(−0.004, 0.055)	0.08	
	Never smokers	1,550	0.004	(−0.015, 0.023)	0.67	
	Cotinine <10 ng/mL	4,432	0.012	(−0.000, 0.023)	0.05	0.33
	Cotinine ≥ 10 ng/mL	1,025	0.030	(0.006, 0.054)	0.02	
Lymphocytes (1,000/μL)	Current smokers[c]	717	0.027	(−0.089, 0.143)	0.63	0.85
	Former smokers[c]	752	0.073	(−0.098, 0.244)	0.38	
	Never smokers[c]	1,539	0.025	(−0.116, 0.167)	0.71	
	Current smokers	721	0.036	(−0.083, 0.155)	0.53	0.81
	Former smokers	757	0.069	(−0.101, 0.239)	0.40	
	Never smokers	1,550	0.027	(−0.117, 0.171)	0.70	
	Cotinine <10 ng/mL	4,432	0.069	(0.004, 0.134)	0.04	0.93
	Cotinine ≥ 10 ng/mL	1,025	0.066	(−0.008, 0.140)	0.08	
Eosinophils (1,000/μL)	Current smokers[c]	717	−0.019	(−0.044, 0.006)	0.12	0.24
	Former smokers[c]	752	0.007	(−0.010, 0.024)	0.38	
	Never smokers[c]	1,539	0.005	(−0.020, 0.031)	0.67	
	Current smokers	721	−0.019	(−0.044, 0.006)	0.13	0.26
	Former smokers	757	0.007	(−0.010, 0.023)	0.42	
	Never smokers	1,550	0.005	(−0.020, 0.030)	0.67	
	Cotinine <10 ng/mL	4,432	0.012	(−0.005, 0.029)	0.16	0.03
	Cotinine ≥ 10 ng/mL	1,025	−0.014	(−0.033, 0.004)	0.12	
Basophils (1,000/μL)	Current smokers[c]	717	0.003	(−0.005, 0.011)	0.43	0.22
	Former smokers[c]	752	−0.000	(−0.011, 0.011)	0.98	
	Never smokers[c]	1,539	−0.000	(−0.006, 0.006)	0.89	
	Current smokers	721	0.003	(−0.005, 0.011)	0.41	0.28
	Former smokers	757	0.000	(−0.012, 0.012)	0.99	
	Never smokers	1,550	−0.000	(−0.006, 0.006)	0.98	
	Cotinine <10 ng/mL	4,432	−0.002	(−0.007, 0.004)	0.50	0.03
	Cotinine ≥ 10 ng/mL	1,025	0.005	(−0.001, 0.011)	0.10	
Neutrophils (1,000/μL)	Current smokers[c]	717	0.097	(−0.144, 0.337)	0.40	0.27
	Former smokers[c]	752	0.278	(0.041, 0.515)	0.02	
	Never smokers[c]	1,539	0.023	(−0.134, 0.181)	0.76	
	Current smokers	721	0.135	(−0.139, 0.408)	0.31	0.19
	Former smokers	757	0.304	(0.060, 0.549)	0.02	
	Never smokers	1,550	0.018	(−0.130, 0.166)	0.80	
	Cotinine <10 ng/mL	4,432	0.063	(−0.012, 0.138)	0.09	0.19
	Cotinine ≥ 10 ng/mL	1,025	0.216	(−0.019, 0.451)	0.07	

Note: CI, confidence interval; NHANES, National Health and Nutrition Examination Survey.
[a]Adjusted for age, sex, race, poverty-to-income ratio (PIR), and body mass index (BMI).
[b]p-Value of interaction term in nonstratified model.
[c]Model also adjusted for cotinine.

Table 4. Linear regression of the change in absolute count of total WBC and WBC subtypes associated with a 10-fold increase in house dust endotoxin concentration in the ALHS.

Absolute cell count[a]	n	β	95% CI	p-Value
WBC (1,000/μL)	1,659	0.127	(0.019, 0.235)	0.02
Monocytes (1,000/μL)	1,659	0.011	(−0.005, 0.027)	0.19
Lymphocytes (1,000/μL)	1,659	0.022	(−0.029, 0.073)	0.39
Eosinophils (1,000/μL)	1,659	−0.0004	(−0.012, 0.011)	0.95
Neutrophils (1,000/μL)	1,659	0.093	(0.001, 0.185)	0.046

Note: ALHS, Agricultural Lung Health Study; CI, confidence interval; WBC, white blood cell.
[a]Adjusted for age, sex, race, asthma status, state (NC vs. IA), smoking (current/former/never), pack-years, season, and body mass index (BMI). An analysis of the endotoxin–basophil relationship was not possible because all subjects had 0% basophils. $n=1{,}659$ for analysis because covariate data were missing for 49 subjects.

described previously (Werner et al. 2003). Using this model, we found suggestive evidence that a G×E interaction may exist because a positive endotoxin–WBC relationship was found only in *TLR4* variant noncarriers (interaction p-value $=0.15$; Table 5).

Discussion

The peripheral WBC count is an established predictor of human all-cause mortality (de Labry et al. 1990; Grimm et al. 1985). In addition, peripheral leukocyte counts have been associated with multiple clinical outcomes, including obesity (Schwartz and Weiss 1991), acute myocardial infarction (Liebetrau et al. 2015), and chronic kidney disease (Erlinger et al. 2003). Beyond being mere biomarkers, several leukocyte subtypes—neutrophils, monocytes, and monocyte-derived macrophages, in particular—have proven causal roles in atherosclerosis, cancer, and other major chronic diseases (Fredman and Spite 2013; Kim and Bae 2016). Thus, identification of common and potentially mitigable environmental determinants of leukocyte number and activation may reveal new features of human leukocyte biology and may also suggest potential strategies for public health intervention.

When we analyzed the U.S. NHANES survey, we found that residential exposure to endotoxin, as quantified by endotoxin concentration, is positively associated with the total count of circulating WBCs. Consistent with household endotoxin influencing systemic inflammatory state in human subjects, a positive relationship of endotoxin to plasma CRP of borderline statistical significance was also noted. The endotoxin–WBC relationship was not meaningfully affected by a sensitivity analysis restricted to subjects not reporting usage of antiinflammatory medications, suggesting that the association is not an artifact of drugs that can alter leukocyte survival (Saffar et al. 2011). The relationship was also comparable among current/former/never smokers and between subjects with cotinine levels above versus below 10 ng/mL.

Our finding that cigarette smoke but not endotoxin is associated with elevated eosinophils and basophils suggests that these two exposures may operate through distinct biological mechanisms. A cotinine cut point of 10 ng/mL revealed opposite effects of cigarette exposure on the endotoxin–eosinophil and endotoxin–basophil relationships, suggesting complex, reciprocal interactions between cigarette smoke and endotoxin on these two leukocyte subtypes. In subjects with cotinine ≥ 10 ng/mL, endotoxin was related positively to basophils and inversely to eosinophils, whereas the opposite directionality of the relationships between endotoxin and both cell types was found in subjects with cotinine <10 ng/mL. Although a specific underlying biological mechanism for this reciprocal effect is not revealed by our analyses, these two cell types differentiate from a common progenitor, and growth factors have been identified that induce selective commitment of this progenitor to the basophil versus eosinophil lineage (Ohmori et al. 2009; Tanno et al. 1987). Thus, future studies may be warranted to define whether smoking "switches" endotoxin-induced basophil/eosinophil commitment preference, perhaps through modification of the cytokine milieu.

The magnitude of the endotoxin relationship to WBC count we report is modest. Whereas in bivariate comparison, current smokers in NHANES had a mean WBC count of $8.3\times10^3/\mu L$ compared with $7.2\times10^3/\mu L$ in former smokers, smoothed analysis of the endotoxin–WBC relationship indicated a point estimate of $\sim 7.1\times10^3/\mu L$ for WBC toward the low end of the endotoxin range compared with $\sim 7.6\times10^3/\mu L$ toward the upper end (Figure 1). Although this range of $\sim 0.5\times10^3/\mu L$ is relatively small, it may not be biologically insignificant given that a difference of $1\times10^3/\mu L$ in WBC count, even within the normal range, has been associated with a 14% change in coronary heart disease death (Grimm et al. 1985) and a 20% change in all-cause mortality (de Labry et al. 1990).

Several genetic variants have been identified in humans that putatively modify LPS responsiveness. In particular, the *TLR4* polymorphism rs4986790 (Asp299Gly) has been shown in direct functional studies to reduce cellular proinflammatory functions triggered by LPS, perhaps because of reduced recruitment of intracellular adaptor proteins to the receptor (Figueroa et al. 2012; Long et al. 2014). Consistent with a prior report that subjects with a minor allele at rs4986790 (and rs4986790, which is in high linkage disequilibrium) have an attenuated increase in peripheral WBCs after experimental LPS inhalation (Michel et al. 2003), in the ALHS, we found suggestive evidence that minor allele carriers at these loci do not exhibit an increase in WBC count with increasing endotoxin concentration. In recent years, a growing number of additional genetic polymorphisms have been identified in the TLR4 signaling pathway that are associated with an altered biological response to LPS (Netea et al. 2012). Variants have also been identified outside the TLR4 pathway that associate with an exaggerated *in vivo* inflammatory response to LPS, including the *GSTM1*-null genotype (Dillon et al. 2011) and the *APOε4* allele (Gale et al. 2014). Given this information, it is possible that a more comprehensive evaluation might identify other

Table 5. Gene by environment interaction analysis of WBC count and $\log_{10}$ house dust endotoxin in the ALHS study population.

Gene/SNP	Minor allele frequency	Haplotype-stratified β[a] (95% CI) AA/CC $n=1{,}463$	G-/T- $n=183$	Interaction p-value[b]
TLR4				
rs4986790	G: 0.05	0.17 (0.05, 0.29)*	−0.20 (−0.57, 0.17)	0.15
rs4986791	T: 0.06			

Note: ALHS, Agricultural Lung Health Study; CI, confidence interval; SNP, single nucleotide polymorphism; TLR4, Toll-like receptor 4; WBC, white blood cell.
*$p<0.01$.
[a]Model: Haplotype is coded as noncarrier (participant was homozygous for both major alleles; AA/CC frequency: 0.89) versus carrier. Recessive is coded 0,1 based on being homozygous for the minor allele. Linear regression model of estimated change in WBC count per $\log_{10}$ endotoxin (linear variable), adjusted for asthma status, age, sex, state (IA vs. NC), smoking (never/past/current), pack-years, season, and body mass index (BMI) (categorical variable), and stratified by *TLR4* genotype (nonvariant carrier vs. variant carrier).
[b]Interaction p-value generated from linear regression models of WBC count and $\log_{10}$ endotoxin with a gene-by-environment interaction term adjusted for asthma status, age, gender, state (IA vs. NC), smoking (never/past/current), pack- years, season, and BMI (categorical variable).

genes involved in differential susceptibility of WBC count to endotoxin exposure. In addition, it has recently been shown that LPS and other exposures can induce epigenetic modifications (i.e., histone modification, DNA methylation, microRNA induction) that reprogram inflammatory responsiveness to LPS (Chiariotti et al. 2016). Taken together, as more genetic and epigenetic determinants of the LPS response become catalogued by the field, future studies aiming to correlate environmental endotoxin exposure to *in vivo* biologic measures such as WBC count will likely benefit from patient-level measurement of these determinants and perhaps from patient-level functional measurement of LPS responsiveness (e.g., using *ex vivo* cell-based assays).

As in NHANES 2005–2006, a positive endotoxin–WBC association was observed in the ALHS, an occupationally and geographically distinct cohort with a higher endotoxin concentration. Unlike the NHANES, in which significant endotoxin relationships to absolute counts of neutrophils, lymphocytes, and monocytes were observed, in the ALHS, endotoxin concentration was related only to neutrophil count, suggesting that the WBC relationship in the ALHS was largely driven by this cell type. Because peripheral neutrophil count is a highly sensitive biomarker of LPS inhalation in humans (Michel et al. 1997), the concordance of both studies on this finding is not unexpected. Future studies are warranted to address potential interactions of environmental endotoxin with other components of the exposome in inducing biological effects. Given that inhalation of different agricultural grain dusts induces distinct effects on peripheral lymphocyte number despite comparable endotoxin contamination (Clapp et al. 1993), it appears likely that environmental coexposures may modify the host response to endotoxin. Pesticides have recently been identified that attenuate the proinflammatory response of macrophages to LPS (Helali et al. 2016). The detailed collection of pesticide exposure data in the AHS will likely facilitate future molecular epidemiology addressing these issues (Hofmann et al. 2015).

Our study has limitations. Despite the strong biological plausibility of the endotoxin effect on WBC number, the cross-sectional design of the NHANES precludes inferences of causality. Endotoxin could be a proxy for other environmental microbial exposures, such as Gram-positive bacterial and fungal components (Sordillo et al. 2011), that drive leukocytosis. The endotoxin–WBC relationship persisted after adjustment for multiple covariates; nonetheless, we cannot exclude the possibility of residual confounding. Genetic data were not collected in NHANES 2005–2006, nor were measurements of inflammatory biomarkers other than WBC count and CRP. Several studies have shown poor correlation between dust endotoxin and airborne endotoxin levels (Mazique et al. 2011; Park et al. 2000, 2001) and have suggested that portable endotoxin monitors may be superior to stationary monitors for judging individual exposure (Rabinovitch et al. 2005). However, compared with other sampling sites for household endotoxin, bed dust, one of the primary sources of dust for our analysis in both NHANES and the ALHS, has been shown to have superior (reduced) within-home variance (Park et al. 2000).

Conclusions

We have used national survey data in a largely urban/suburban study population to report for the first time that house dust endotoxin is positively associated with peripheral WBC count and serum CRP. The endotoxin–WBC association was also observed in a cohort of adult farmers and their spouses, with possible effect modification by a *TLR4* polymorphism. Taken together with the existing literature on experimental human endotoxin inhalation, these findings suggest the provocative postulate that leukocyte number and systemic inflammatory state in healthy human subjects may be measurably influenced by "real-world" residential exposure to endotoxin in diverse settings.

Acknowledgments

We thank the numerous study staff at Social & Scientific Systems, Inc. who played a role in the data collection for the Agricultural Lung Health Study. We also thank M. Richards, J. Jin, and S. Long of Westat, Inc. for expert programming assistance.

This research was supported in part by the Intramural Research Program of the National Institutes of Health (NIH), National Institute of Environmental Health Sciences (NIEHS) (Z01 ES102005, Z01 ES025041, Z01 ES049030, Z01 ES102385) and the National Cancer Institute (Z01 CP010119). P.S.T. was supported by NIEHS (P30 ES005605) and the Centers for Disease Control and Prevention (CDC) (200-2010-34238 NCE1).

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