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GENE--ENVIRONMENT INTERACTIONS INFLUENCE AIRWAYS FUNCTION IN LABORATORY ANIMAL WORKERS

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Abstract

Background—Most diseases, including asthma, result from the interaction between environmental exposures and genetic variants. Functional variants of CD14 negatively affect lung function in farm workers and children exposed to animal allergens and endotoxin.

Objective—We hypothesized that CD14 polymorphisms interact with inhaled endotoxin and/or allergen to decrease airways function in laboratory animal workers.

Methods—369 Caucasian workers completed a symptom and work exposure questionnaire, prick skin testing, and spirometry. Individual exposure estimates for endotoxin and mouse allergen were calculated by weighting task-based breathing zone concentrations by time reported for each task and length of time in current job. Real-time PCR was used to assess CD14/-1619, -550, and -159 alleles. Multiple linear regression predicting airways function included an interaction term between genotype and exposure.

Results—Workers at the highest quartile of the natural log transformed cumulative endotoxin exposure and with the endotoxin responsive CD14/-1619 G allele had significantly lower FEV₁ and FEF_{25–75} percent predicted compared to workers with an AA, with no significant differences noted at lower endotoxin levels for either genotype. The gene by environment effect was marked for atopic workers. Laboratory animal allergy, mouse allergen exposure, CD14/-159 or -550 genotype, and a gene-exposure interaction term for these genotypes and exposures did not predict changes in lung function.

Conclusions—A significant gene by environment interaction affects airways function in laboratory animal workers. More highly endotoxin exposed workers with CD14/-1619G alleles have significantly lower FEV₁ and FEF_{25–75} percent predicted than those with CD14/-1619AA. Atopic workers are particularly affected by cumulative endotoxin exposures.

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Keywords

CD14/-1619; occupational asthma; endotoxin; mouse allergen; CD14/-159; laboratory animal allergy

INTRODUCTION

Expressed primarily on monocytes, macrophages, and dendritic cells, CD14 is a functionally important surface receptor that operates at the intersection of innate and adaptive immunity. As such, it is critical in the pathogenesis of allergic diseases. CD14 is able to bind endotoxin via the lipopolysaccharide (LPS) binding protein, and helps mediate endotoxin effects by means of the TLR4 receptor. CD14 is also released from these cells, and circulates in blood in a soluble form able to bind LPS and stimulate cells that do not normally express this receptor. In addition, CD14 significantly modulates adaptive allergic immunity, with effects on asthma, measures of lung function, and allergic sensitization, with several functionally important polymorphisms in the promoter region of the gene (1). Informative data come from the study of two specific promoter polymorphisms: the -1619 A/G and -159 C/T sites, in which the G and T alleles respectively are associated with higher circulating levels of CD14 (2), and the CD14/-550 T allele which is associated with lower sCD14 levels in plasma (3). The health effects of these gene variants, however, appear to be highly dependent on the environment in which they are studied. When examined in the absence of an environmental context, the associations between specific alleles and asthmatic or allergic outcomes have been ambiguous (1).

Most research on associations between CD14, endotoxin, and risk for atopy and asthma has focused on the CD14/-159 C → T polymorphism. Several studies have shown that the C allele is associated with atopy and asthma in settings of lowest endotoxin exposures, but 'switches' and becomes protective against atopy in settings of highest endotoxin (4,5). Conversely, the CD14/-159 T allele is negatively associated with atopy in lower endotoxin exposures, but increases the risk of atopy in higher endotoxin environments. Multivariate regression models detected a significant gene-environment interaction between CD14/-159 genotype and endotoxin exposure that predicted higher serum IgE in adults (6) and children (7,8) with the C allele exposed to pets, and lower IgE in those with the C allele with higher endotoxin in home dust or exposed to stable animals. Supporting a gene-environment interaction is the lack of difference noted in serum IgE by CD14/-159 genotype when animal and microbial exposures were not considered (7,9). Whether the CD14/-1619 variant alleles have different effects on atopy depending on the endotoxin exposure has not yet been shown.

Airways function also appears to be affected by CD14 alleles in the context of farm or endotoxin exposures. LeVan and colleagues demonstrated significantly lower lung function, as measured by FEV1 and FEF₂₅₋₇₅, and increased prevalence of wheeze, in male farmers homozygous for CD14 alleles more responsive to endotoxin: CD14/-1619 GG and CD14/-159 TT, compared to farmers with the alternate alleles (10). A family based study in Barbados found that individuals with the CD14/-159 C allele had higher risks for asthma in low house dust endotoxin exposure, but much lower risks at high endotoxin levels (11). In contrast, a study of young Danish farmers found no association between CD14/-159 genotype and risk for asthma, but did not assess exposure differences between asthmatic cases and controls that might have interacted with genotype (12).

We and others have previously shown that laboratory researchers and animal handlers are exposed to airborne endotoxin and animal allergens in varying concentrations during the

course of normal work tasks (13–15). These workers frequently develop work-related respiratory symptoms and allergic disease. Whether endotoxin or allergen exposures affect lung function, alone or in combination, and if CD14 genes modulate this effect, are unknown. Our previous studies demonstrated that researchers and technicians with work-related respiratory symptoms, who were not allergic to mice, were exposed to significantly higher endotoxin concentrations than those without such symptoms (13). Other studies in endotoxin-exposed cotton textile workers followed over 20 years found more persistent respiratory symptoms and greater yearly declines in FEV1 and FVC than in silk workers exposed to dust, but no endotoxin. (16). The cumulative effects of endotoxin on lung function have not been evaluated previously in laboratory animal workers. We hypothesized that exposed laboratory animal workers with endotoxin responsive CD14 variants (CD14/-1619 G, CD14/-550 C, or CD14/-159 T) would have more frequent chest symptoms and lower indices of lung function, and that there would be a significant gene–environment interaction demonstrating decreased lung function for carriers of these alleles at higher endotoxin exposures.

METHODS

Study Subjects

Between 2003 and 2008, we recruited 475 research scientists and technicians ranging in age from 18 to 65, from two major research institutions. Subjects performed research or worked as animal handlers as previously reported (14). Our study population was predominantly Caucasian (78%), 15% Asian, 5% Hispanic, and 2% African American, reflecting the demographics of this population. For this study, we included only non-Hispanic Caucasians to limit population stratification, for a total of 369 workers. We conservatively excluded four highly exposed dirty cage wash workers from consideration in the multivariate models, as their measured workplace exposures were not representative of the cohort at large as they were orders of magnitude higher than the rest of the cohort. In addition, this helped eliminate the potential impact of short-term acute endotoxin exposures. The study was approved by the IRB from both institutions, and all subjects provided informed consent. Subjects completed a questionnaire containing questions on respiratory symptoms, work, and smoking history; skin or RAST testing for six common environmental allergens and five laboratory animal allergens; and spirometry meeting ATS criteria for quality and reproducibility. Subjects were preferentially evaluated on Thursday and Friday mornings to minimize day of the week and time of day effects on lung function. Atopy was defined as one or more positive skin tests or RASTs to common environmental allergens not including lab animals. Lab animal allergy was defined as a positive skin test to mouse, rat, rabbit, hamster, and/or guinea pig. Based on Venables et al (17), we defined asthma symptoms as one or more chest symptoms occurring in the past 4 weeks (13). Genotyping was performed on DNA extracted from peripheral blood cells, or on mouthwash samples from those who did not wish to donate blood.

Exposure assessment

Workers were initially categorized into one of the following four job classifications based on questionnaire self-report: 1) animal handler, which included technicians or clean cage wash personnel who worked primarily with animals or animal products in the animal facility; 2) research technician, which included other lab personnel who may have contact with animals in the lab or in the animal facility; 3) research scientist, which included investigators with advanced degrees working in laboratories with possible animal contact; or 4) research coordinator, which included epidemiologists, data analysts, or regulatory specialists who were not exposed to lab animals. To better characterize lab animal exposures, we identified job tasks based on a walk-through of the animal and research facilities by a certified

industrial hygienist (MVD), supplemented by worker interviews. Workers were asked to estimate the time spent per week in each task. Tasks consisted of cage change (transferring animals and feeding/watering), cage wash (dumping soiled cages and placing them on a wash conveyor), animal research work (experimental procedures on animals or animal tissue such as bleeding, tail-clipping, necropsy, etc.), and non-animal work (other laboratory or office work not involving laboratory animals) classified by location.

We measured endotoxin and mouse allergen concentrations for the major tasks identified, obtaining 173 personal breathing zone air samples from mouse-exposed workers in two academic animal care facilities and 27 university laboratories. Samples were collected over a three year period, but measurements did not differ significantly over time (data not shown). Samples were collected on 37 mm glass fiber filters previously depyrogenated by heat, using personal sampling pumps set at 12 lpm and a target sampling times of 90 minutes/task (mean 122 minutes/task). Filters were then shipped overnight on ice to the University of Iowa for analysis. Samples were eluted with 5 mL pyrogen-free water or PBS with 0.05% Tween-20. Endotoxin samples were analyzed using a modification of the kinetic chromogenic Limulus amoebocyte lysate (LAL) assay (BioWhittaker, Walkersville, MD) (18,19). Mus m1 was measured using a modified specific sandwich EIA (20) for mouse urinary protein (Indoor Biotechnology, Inc., Charlottesville, VA).

Using the task-based air concentrations and worker estimates of time spent performing each task, we calculated individual cumulative exposures using the sum of the arithmetic mean of the task-specific endotoxin (ET) or mouse allergen (MA) concentrations, multiplied by the percent time spent in each task, and the total number of months worked in the current job as reported on the questionnaire. Average exposures were calculated by dividing the cumulative exposure by the total number of months worked in the current job.

Genotyping of CD14 Alleles

We used a custom-designed real-time PCR assay (Applied Biosystems, Foster City, CA) to genotype CD14/-1619 and CD14/-550. We used allele-specific PCR to genotype 146 subjects for CD14/-159, changing to a pre-designed real-time genotyping assay (Applied Biosystems, Foster City, CA) for the remaining 228 subjects due to higher throughput capabilities. We compared results from both assays for accuracy in 17 (5%) randomly selected subjects and found no change in allele calls for the -159 polymorphism. Real-time assay reactions were performed in duplicate, and repeated in one sample whose duplicates were not concordant. Allele determination for all real-time assays utilized the DNA Engine Opticon 2 System version 2.02 software (MJ Research, Waltham, MA).

Statistical Analysis

All genotype frequencies were tested for Hardy-Weinberg equilibrium proportions using Chi-Square goodness-of-fit-tests. Comparison of exposures by job category were performed on the natural log transformed variables using Analysis of Variance (ANOVA) techniques and, if significant at the $p < 0.05$ level, Tukey's multiple range test was used to identify significant comparisons at the $p < 0.05$ level. The main outcome variables were FEV1/FVC ratio as a percent, and percent predicted (pp) FEV1, FVC, and FEF 25–75 based on Hankinson (21). We chose to use the percent predicted for spirometry as these variables accounted for height, age, sex and race. Linear regression was used to assess the influence of allele and genotypes on these outcome variables, along with individual estimates of the natural log transformed average and cumulative endotoxin and mouse allergen exposures, atopy, laboratory animal allergy, and smoking status. All variables were evaluated by univariable analysis, and those found to be significant at $p \leq 0.15$ were included in a backward regression model. Variables still significant at the $p \leq 0.10$ level were included in

the final model. Since our main interests were exposure and CD14 genotypes, these variables were forced into the backward regression model, and the significance of an interaction term between CD14 genotypes and environmental exposures was determined when other significant variables were included in the model. Statistical analysis was performed using SAS software (SAS Institute, Inc., Cary, NC version 9.2).

RESULTS

Demographics and characterization of the laboratory animal cohort

Most of the 369 subjects were women and never smokers, as noted in Table 1. Over half of our cohort was atopic, similar to the self-reported rate of physician-diagnosed allergy on the questionnaire. Of the atopic subjects enrolled, 30% were sensitized to laboratory animals. Sixty percent of our cohort reported currently working with laboratory animals (Table 2); of these, 18% (41/225) reported laboratory animal associated asthma symptoms (data not shown). For the entire cohort, 11% reported lab animal associated asthma symptoms. Current smoking rates were low (9% of the total population).

Table 2 shows the distribution of laboratory animal exposures, prevalence of lab animal associated asthma symptoms and allergy, and CD14 allele carrier frequencies among the four job classifications. Not surprisingly, animal handlers spent more hours per week (average 31.1 hours) working with laboratory animals than any of the other job classifications ($p < 0.0001$), although the length of time in the job did not differ between job categories. However, we did not assess worker turnover rates in the different job categories. The average number of hours per week spent working with lab animals did not differ significantly between research scientists (4.8 hours) and research technicians (8.4 hours). Average and cumulative endotoxin exposures were significantly higher for animal handlers (6.4 EU/m^3 , $77.5 \text{ EU/m}^3\text{-months}$ respectively) than for research scientists (1.7 EU/m^3 , $30.9 \text{ EU/m}^3\text{-months}$, $p < 0.05$). Log transformed average and cumulative mouse allergen exposures were also significantly higher for animal handlers (4.2 ng/m^3 , 51.4 ng/m^3) than for research scientists and research technicians (0.2 and 0.3 ng/m^3 , and 4.4 and $4.8 \text{ ng/m}^3\text{-months}$ respectively). Notably, the rates of lab animal allergy, lab animal associated asthma symptoms, and general asthma symptoms in the past month were similar across jobs. The distributions of CD14/-1619, -550, and -159 alleles in the study population were in Hardy Weinberg equilibrium and carrier frequencies were not statistically different across the four job categories (Table 2). There were no differences in carrier frequencies for any of the CD14 alleles among workers with atopy or lab animal allergy (data not shown).

Airflow is lower in subjects with physician diagnoses of asthma, but is not associated with CD14 genotypes alone

We evaluated whether CD14 genotypes, atopy, asthma symptoms, or endotoxin or mouse allergen exposures were independently associated with airways function (Table 3). The more endotoxin responsive CD14 genotypes were not associated with lung function in univariate analyses. Only a physician diagnosis of asthma was significantly associated with lower FEF_{25-75} pp and lower FEV1/FVC ratio. Spirometric measurements did not differ based on measures of atopy, lab animal allergy, lab animal-related asthma symptoms, or general asthma symptoms in the past month. Although the LN transformed endotoxin and mouse allergen exposures were significantly associated with airflow measurements ($p < 0.05$), the associations were not clearly meaningful with $r^2 = 0.02$ (data not shown).

CD14/-1619G interacts with cumulative endotoxin exposure to predict airflow

Based on our hypothesis that CD14 alleles are associated with airways function only when considered in the context of relevant exposures, we next analyzed predictors of four

spirometric measurements of airways function, FEV1 pp, FVC pp, FEV1/FVC ratio, and FEF 25–75 pp, in linear regression models that included an interaction term between the LN transformed endotoxin and mouse allergen exposure estimates and the CD14/-1619, -550, and -159 alleles of interest. As we found no significant predictors of FVC pp or FEV1/FVC ratio (data not shown), only the FEV1pp and FEF25–75 data are shown.

A physician diagnosis of allergy, atopy, lab animal allergy, lab animal related asthma symptoms, general asthma symptoms in the past month, and job classification were not associated with any spirometric measurements of airflow in univariable analyses and thus were not included in the final models. Smoking status did not contribute to univariate models of lung function. A physician diagnosis of asthma did not remain in the models once genotype and exposures were added. Of note, only the CD14/-1619 genotypes (AA vs. AG/GG) remained in the models with a significant interactive effect with endotoxin exposure. Neither of the other two CD14 variants, -550 and -159, were associated with spirometric outcomes in the multivariable analysis even when exposure variables were included in the models. The LN transformed average and cumulative mouse allergen exposure estimates also did not contribute to models predicting airflow in multivariable analyses. Although we did find an interactive effect between the LN transformed average endotoxin exposure and genotype on airflow (data not shown), the effect was most evident with the LN transformed cumulative endotoxin exposure. The LN transformed cumulative endotoxin was strongly predictive of both FEV1 pp and FEF25–75 pp, and a gene-environment interaction contributed significantly to the models. Effect estimates for variables predicting FEV1 pp and FEF25–75 pp are presented in Table 4.

Figure 1 displays the averages for FEV1 pp and FEF 25–75 pp for subjects with CD14/-1619 AA or AG/GG, comparing those at the 75th percentile of the LN transformed cumulative endotoxin exposure to those at the 25th percentile of the LN transformed cumulative endotoxin exposure. Workers with either the AA or AG/GG alleles at the 25th percentile of the LN transformed cumulative endotoxin exposure had similar FEV1 pp (104.7% vs. 103.8%) and FEF 25–75 pp (108.8% vs. 103.9%). Workers with the G allele at the 75th quartile of the LN transformed cumulative endotoxin exposure, however, had lower FEV1 pp (100.9% vs. 103.5%, $p=0.16$) and significantly lower FEF 25–75 pp (100.9% vs. 108.8%, $p<0.05$) compared to those with the AA genotype. Comparing only those workers with the CD14/-1619 G allele, the FEV1 pp was significantly lower in workers at the 75th percentile of the LN transformed endotoxin exposure (100.8%) compared to those at the 25th percentile (103.8%, $p=0.004$).

Because atopy is a significant risk factor for lab animal allergy and symptomatic disease, we evaluated this gene by environment interaction in atopic workers only, who constituted 63% of our total population. FEV1 pp and FEF 25–75 pp in atopic subjects did not differ by CD14/-1619 genotype when comparing workers at the 25th percentile of the LN transformed cumulative endotoxin exposure (Figure 2). At the 75th percentile of the LN transformed cumulative endotoxin exposure, however, atopic workers with the CD14/-1619 G allele had significantly lower FEV1 pp (99.9 % vs. 105.1%, $p=0.003$) and significantly lower FEF 25–75 pp (97.5 % vs. 107.8%, $p=0.039$) compared to atopic workers with an AA. Comparing only those atopic workers with a G allele, lung function was also significantly lower in those at the 75th percentile compared to those at the 25th percentile of the LN transformed cumulative endotoxin exposure (99.9% vs. 103.9%, $p=0.003$ for FEV1 pp, and 97.5% vs. 104.7%, $p=0.009$ for FEF 25–75 pp).

DISCUSSION

This study of endotoxin and allergen exposed laboratory animal workers demonstrates a significant CD14 gene by environment interaction associated with lung function. Workers with the CD14/-1619 G allele in the highest quartile of cumulative endotoxin exposure had significantly lower airways function than workers similarly exposed but with the AA genotype. As the CD14/-1619 G allele has been associated with higher circulating concentrations of sCD14, we speculate that the negative effects on lung function noted for the highest endotoxin exposures are due to enhanced endotoxin uptake and associated bronchoconstrictor responses.

Effects on lung function were particularly apparent in atopic workers, who showed significant differences in spirometric markers of airflow based on differing genotypes when exposed at the 75% of cumulative endotoxin exposure, as well as between workers with the G allele exposed at the 25% of cumulative endotoxin exposure. Atopic individuals in general are often more affected by irritant and inflammatory exposures (22), and it is possible that they already have a low level of lung inflammation that renders them more susceptible to the effects of inhaled endotoxin. The consequence of CD14's position at the intersection of innate and allergic immunity may be to render atopic individuals with the CD14/-1619 G allele, already more susceptible to CD14 mediated allergic effects, also more susceptible to endotoxin effects via higher circulating concentrations of sCD14.

Recent investigations have underscored the importance of evaluating the effects of genetic polymorphisms in the context of the environment in which they are active (5,23), as genes active in one exposure setting may not be functional in another. If the environment is not considered in the gene – health effect equation, then no association may be noted with the gene. In our population, polymorphisms at CD14 -1619, -550, and -159 did not significantly affect airways function when considered independent of exposure. Job titles or tasks alone did not adequately classify exposure effects on airways function. Only when average and cumulative individual exposure estimates were added to the model did the gene effect become apparent. Other studies of different populations and exposure settings have detected similar effects of endotoxin-responsive CD14 alleles and workplace endotoxin exposures on airways function (10). CD14 alleles are also known to affect allergic sensitization, and the influence of specific polymorphisms on atopy appears to be similarly modulated by the environment (9). This effect appears to be greatest in childhood (24), which may be why we did not find an association between any of the CD14 alleles we studied and risk for atopy or specific lab animal sensitization in our adult study subjects.

One of the strengths of this study was our ability to develop individual exposure estimates for all study participants. We categorized major job tasks based on participant interviews and site visit assessments by a trained industrial hygienist. We based our sampling strategy on tasks rather than job titles, and thus were able to estimate individual exposures by weighting task-based concentrations of airborne allergen and endotoxin using questionnaire derived information. Our ability to individually reconstruct endotoxin exposures helped delineate the gene-environment effects of CD14/-1619 and endotoxin on lung function. These estimates do not take into account use of personal protective equipment such as respirators that may affect individual exposure. However, since the majority of subjects did not use respiratory protection when working with animals in a laboratory setting, and used masks haphazardly when working with animals in the animal care facility, it is unlikely that respiratory protection had a major impact on our exposure estimates.

Our use of quantitative lung function measures as analysis endpoints, and treatment of variables as continuous rather than dichotomous, also enhanced our ability to detect

significant gene by environment interactions despite the relatively low levels of endotoxin exposure in these workplace settings. The spirometric differences we detected are small, which may reflect the low levels of endotoxin exposure experienced by most of the participating workers, and probably have limited clinical implications for individuals in the lower FEV₁ group as a whole. The fact that our study population is a healthy one with normal lung function and a very low prevalence of cigarette smoking may have also increased our ability to detect significant effects as we would be likely to have less confounding from smoking on lung function. As we were unable to assess job turnover in different job categories, it is possible that our study population could be a survivor population. That is, those with more severe effects on lung function may have left their jobs and thus would not have been included in the analysis; this would have reduced the difference in lung function findings. Nonetheless, the differences we found in lung function based on the interaction of lab animal exposure and genotype are noteworthy, and supports the role of endotoxin as a relevant exposure in the lab animal workplace. This may also suggest the potential for much more clinically significant lung function decrements among newly and/or more heavily exposed carriers of the CD14/-1619 G allele.

Although CD14 is known to interact with both endotoxin and allergen, we attribute the airways effects noted in our study primarily to endotoxin exposures for several reasons. The CD14/-1619 G allele is associated with higher circulating levels of CD14 and increased responsiveness to endotoxin (2), and is the genotype that demonstrated an interaction with endotoxin exposure in our models. In contrast, atopy, specific lab animal sensitization, and mouse allergen exposure were not associated in any univariate analyses nor in multivariate regression models. The negative effect of cumulative endotoxin exposure on FEV₁ in our cohort is intriguing, as is the fact that atopic workers were particularly affected. Adverse respiratory health effects of long term (20 year) endotoxin exposure have been reported in cotton textile workers, although atopic individuals were not separately considered in those analyses (16). Although the endotoxin exposures of laboratory animal workers are orders of magnitude lower than those of cotton textile workers, the cumulative effects on airways function appear to be significant, and are consistent with other reported outcomes of prolonged endotoxin exposure.

In summary, our study demonstrates a novel gene by environment effect on airways function in laboratory animal workers, and supports the necessity of considering exposure in assessing gene effects on lung function and allergic outcomes. We found that cumulative endotoxin exposures negatively impact lung function in laboratory animal workers, especially in the context of a specific genotype. While our study was cross-sectional in nature, longitudinal studies will help elucidate whether these effects are permanent and/or progressive, and whether different gene-exposure interactions may contribute to, or protect against, laboratory animal induced respiratory disease.

Key Messages (findings and implications)

- In exposed laboratory animal researchers and technicians, cumulative endotoxin exposures significantly interact with the endotoxin responsive CD14/-1619 G allele and are associated with lower FEV₁ and FEF₂₅₋₇₅ percent predicted.
- Atopic workers are particularly affected by this gene by environment interaction.

Capsule summary

Laboratory animal workers have significantly lower FEV₁ pp and FEF_{25–75} pp if they work in settings with higher endotoxin exposure, and have the endotoxin-responsive CD14/-1619 G allele, compared to those with an AA. This effect is greatest in atopic workers.

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Abbreviations

LA	laboratory animal
ET	endotoxin
MA	mouse allergen
LN	natural log
PP	percent predicted
SD	standard deviation

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Figure 1.

Gene by environment effect in all workers of CD14/-1619 alleles on FEV1 pp (a) and FEF 25-75 pp (b) as affected by cumulative endotoxin exposure.

(a) Mean FEV1 percent predicted is shown for the LN transformed cumulative endotoxin exposure at the 25th percentile (grey line) and 75% (black line). Comparison is between CD14/-1619 AA genotype on the left, and CD14/-1619 AG/GG on the right.

(b) Mean FEF 25-75 percent predicted is shown for the LN transformed cumulative endotoxin exposure at the 25th percentile (grey line) and 75% (black line). Comparison is between CD14/-1619 AA genotype on the left, and CD14/-1619 AG/GG on the right.

Figure 2.

Gene by environment effect in atopic workers of CD14/-1619 alleles on FEV1 pp (a) and FEF 25–75 pp (b) as affected by cumulative endotoxin exposure.

(a) Mean FEV1 percent predicted is shown for the LN transformed cumulative endotoxin exposure at the 25th percentile (grey line) and 75% (black line). Comparison is between CD14/-1619 AA genotype on the left, and CD14/-1619 AG/GG on the right.

(b) Mean FEF 25–75 percent predicted is shown for the LN transformed cumulative weekly endotoxin exposure at the 25th percentile (grey line) and 75% (black line). Comparison is between CD14/-1619 AA genotype on the left, and CD14/-1619 AG/GG on the right.

Table 1

Demographic findings, smoking status, and allergy history in 369 Caucasians workers.

Age mean years (range)		34.0 (18–65)
<hr/>		
Gender	n (%)	
Female	250 (68%)	
Male	119 (32%)	
<hr/>		
Smoking Status	n (%)	
Current	32 (9%)	
Never	244 (68%)	
Past	84 (23%)	
<hr/>		
Allergy and Asthma History	n (%)	
Physician diagnosed asthma	64 (17%)	
Physician diagnosed allergy	172 (47%)	
<hr/>		
Sensitization and symptoms	n (%)	
Atopic (1 or more PST+)	233 (63%)	
Lab animal allergy	78 (21%)	
Lab animal associated asthma symptoms	41 (11%)	

Table 2

Distribution of jobs tasks, sensitization to lab animals and CD14 alleles by job title.

Job Titles n (%)					
Work with laboratory animals		All n=369	Animal Handler 31 (8%)	Research Scientist 183 (50%)	Research Tech 135 (37%)
Current direct work with lab animals n (%)		224 (61%)	97%	61%	61%
Hours/week work with lab animals mean(SD)		9.7 (9.4)	31.1 (15.7)	4.8 (4.9)	8.4 (10.9)
Research Coord 20 (5%)					0
0					0
Geometric Mean (GSD)					
Distribution of exposures		All [†] GM (GSD)			
Months at current job		17.6 (4.9)	12.0 (3.6)	18.6 (5.0)	17.2 (5.1)
Average endotoxin exposure (EU/m3) *		2.0 (1.8)	6.4 (2.0)	1.7 (1.4) ***	1.8 (1.7) ***
Cumulative ET exposure (EU/m3-month) **		33.5 (4.9)	77.5 (3.3)	30.9 (4.9) ***	30.3 (5.5) ***
Average mouse allergen exposure (ng/m3) *		.31 (4.0)	4.2 (3.4)	0.2 (3.1) ***	0.3 (3.9) ***
Cumulative MA exposure (ng/m3-month) *		5.5 (8.2)	51.4 (3.4)	4.4 (1.2) ***	4.8 (1.2) ***
% in job group					
Sensitization/Symptoms		n (%)			
Physician diagnosed asthma		64 (17%)	6%	21%	15%
Atopic (1 or more PST+)		233 (63%)	68%	66%	60%
Lab animal allergy		78 (21%)	19%	19%	24%
Lab animal associated asthma symptoms		41 (11%)	13%	12%	10%
General asthma symptoms in past month		152 (41%)	42%	44%	39%
n (%)					
Distribution of CD14 alleles		n (%)			
CD14/-1619 AA		97 (26%)	4 (13%)	53 (29%)	34 (25%)
AG/GG		271 (74%)	29 (83%)	129 (71%)	101 (75%)
CD14/-550 CC		227 (62%)	17 (55%)	117 (64%)	83 (61%)
CT/TT		142 (38%)	14 (45%)	66 (36%)	52 (39%)
CD14/-159 CC		77 (21%)	7 (23%)	42 (23%)	24 (18%)
CT/TT		292 (79%)	24 (77%)	141 (77%)	111 (82%)

* p<0.0001
** p<0.05

AH vs other job category $p < .05$

Statistical comparisons were made on the natural log transformed data and are presented in this format for ease of interpretation.

Table 3

Distribution of spirometric variables by CD14 alleles and allergic history.

CD14 alleles (n)	FEV1 PP mean (SD)	p	FEF 25-75 PP mean (SD)	p	% FEV1/FVC mean (SD)	p
CD14/-1619 AA (97)	104.4(12.7)	0.15	106.9(28.7)	0.15	83.7 (6.6)	0.57
any G (271)	102.2 (12.4)		102.3 (26.2)		83.3 (6.4)	
CD14/-550 any T (142)	104.0 (12.3)	0.16	103.1 (28.0)	0.77	83.6 (6.6)	0.31
CC (227)	102.1 (12.6)		103.8 (26.3)		82.9 (6.1)	
CD14/-159 CC (77)	103.6(12.3)	0.52	105.0 (27.8)	0.60	83.1 (6.0)	0.64
any T (292)	102.6 (12.6)		103.2 (26.7)		83.4 (6.6)	

Atopy/Symptoms (n)	FEV1 PP mean (SD)	p	FEF 25-75 PP mean (SD)	p	% FEV1/FVC mean (SD)	p
Physician diagnosed asthma Yes (64)	100.9 (13.3)	0.19	96.3 (26.8)	.0179	81.4 (6.5)	.0068
No (305)	103.2 (12.3)		105.1 (26.7)		83.8 (6.3)	
MD diagnosis of allergies Yes (172)	101.9 (13.6)	0.19	101.8 (27.7)	0.25	82.8 (6.7)	0.14
No (196)	103.6 (11.4)		105.1 (26.2)		83.8 (6.2)	
Atopic (1 or more PST+) Yes (233)	102.4 (13.1)	0.45	102.1 (26.3)	0.17	83.2 (6.2)	0.56
No (136)	103.5 (12.4)		106.1 (27.9)		83.6 (6.8)	
Lab animal allergy Yes (78)	101.3 (15.3)	0.24	99.8 (32.5)	0.17	82.9 (6.4)	0.49
No (291)	103.2 (11.7)		104.6 (25.2)		83.5(6.5)	
Lab animal associated asthma symptoms Yes (41)	101.6 (13.9)	0.53	103.7 (29.7)	0.98	83.1 (5.3)	0.76
No (328)	103.0 (12.3)		103.5(26.6)		83.4 (6.6)	
General asthma symptoms in past month Yes (152)	102.6 (12.1)	0.76	102.2 (25.8)	0.41	83.4 (5.9)	0.99
No (217)	103.0 (12.8)		104.5 (27.7)		83.4 (6.8)	

(Definition of abbreviations: PP = percent predicted; SD = standard deviation)

p<0.05

0.05< p≤0.15

Table 4

Effect estimates for predictors of FEV1 pp and FEF25–75 pp

In All Subjects			
FEV1 PP	Estimate	Std Error	p-value
Intercept	107.02	1.80	<0.0001
LN cumulative endotoxin exposure	−1.34	.46	0.0036
CD14/-1619 AA	−.91	3.6	0.8008
LN cumulative endotoxin*CD14/-1619 AA	.78	.97	0.4221
FEF 25–75 PP	Estimate	Std Error	p-value
Intercept	107.19	3.88	<.0001
LN cumulative endotoxin exposure	−1.37	.99	0.1648
CD14/-1619 AA	−7.91	7.77	0.3091
LN cumulative endotoxin*CD14/-1619 AA	3.46	2.09	0.0979
In Atopics Only			
FEV1 PP	Estimate	Std Error	p-value
Intercept	108.33	2.45	0.0001
LN cumulative endotoxin exposure	−1.84	.61	0.0029
CD14/-1619 AA	−5.46	4.63	0.2395
LN cumulative endotoxin*CD14/-1619 AA	2.32	1.27	0.0688
FEF 25–75 PP	Estimate	Std Error	p-value
Intercept	112.74	5.07	<0.0001
LN cumulative endotoxin exposure	−3.34	1.26	0.0089
CD14/-1619 AA	−17.19	9.57	0.0735
LN cumulative endotoxin*CD14/-1619 AA	6.00	2.62	0.0230