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Long-Term Ambient Air Pollution Exposures and Circulating and Stimulated Inflammatory Mediators in a Cohort of Midlife Adults

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Background: Chronic exposure to air pollution may prime the immune system to be reactive, increasing inflammatory responses to immune stimulation and providing a pathway to increased risk for inflammatory diseases, including asthma and cardiovascular disease. Although long-term exposure to ambient air pollution has been associated with increased circulating markers of inflammation, it is unknown whether it also relates to the magnitude of inflammatory response.

Objectives: The aim of this study was to examine associations between chronic ambient pollution exposures and circulating and stimulated levels of inflammatory mediators in a cohort of healthy adults.

Methods: Circulating interleukin (IL)-6, C-reactive protein (CRP) ($n = 392$), and lipopolysaccharide stimulated production of IL-1β, IL-6, and tumor necrosis factor (TNF)-α ($n = 379$) were measured in the Adult Health and Behavior II cohort. Fine particulate matter [particulate matter with aerodynamic diameter less than or equal to 2.5 μm ($PM_{2.5}$)] and constituents [black carbon (BC), and lead (Pb), manganese (Mn), zinc (Zn), and iron (Fe)] were estimated for each residential address using hybrid dispersion land use regression models. Associations between pollutant exposures and inflammatory measures were examined using linear regression; models were adjusted for age, sex, race, education, smoking, body mass index, and month of blood draw.

Results: There were no significant correlations between circulating and stimulated measures of inflammation. Significant positive associations were found between exposure to $PM_{2.5}$ and BC with stimulated production of IL-6, IL-1β, and TNF-α. Pb, Mn, Fe, and Zn exposures were positively associated with stimulated production of IL-1β and TNF-α. No pollutants were associated with circulating IL-6 or CRP levels.

Discussion: Exposure to $PM_{2.5}$, BC, Pb, Mn, Fe, and Zn was associated with increased production of inflammatory mediators by stimulated immune cells. In contrast, pollutant exposure was not related to circulating markers of inflammation. These results suggest that chronic exposure to some pollutants may prime immune cells to mount larger inflammatory responses, possibly contributing to increased risk for inflammatory disease. https://doi.org/10.1289/EHP7089

Introduction

Exposure to airborne fine particulate matter [particulate matter with aerodynamic diameter less than or equal to 2.5 μm ($PM_{2.5}$)] has been associated with increased risk of cardiopulmonary diseases, and activation of innate immune mechanisms is hypothesized as a key mediating pathway (Brook et al. 2010; Cosselman et al. 2015; Guarnieri and Balmes 2014; Heinrich and Schikowski 2018; Libby 2006; Pope et al. 2004; Thurston et al. 2015). Some studies have reported associations between long-term exposure to $PM_{2.5}$ and circulating markers indicative of systemic inflammation (Dubowsky et al. 2006; Hajat et al. 2015; Hampel et al. 2015; Hoffmann et al. 2009; Ostro et al. 2014; Thompson et al. 2010) that have been associated with chronic inflammatory disease and premature mortality (Chung et al. 2009). However, little is known about potential effects of chronic $PM_{2.5}$ exposures on the innate immune response.

Local inflammatory responses play a key role in the pathogenesis of many diseases, including cardiometabolic disease, asthma, and autoimmune disease (McEwen et al. 1997). Epidemiological studies have reported associations between long-term exposure to $PM_{2.5}$ and circulating levels of inflammatory markers among healthy and vulnerable populations, including older, obese, diabetic, and hypertensive persons (Dubowsky et al. 2006; Zeka et al. 2006); for example, Ostro et al. (2014) found that $PM_{2.5}-CRP$ associations differed substantially by diabetic status; a 10-$\mu g/m^3$ increase in annual $PM_{2.5}$ was associated with a 72% increase in C-reactive protein [CRP; 95% confidence interval (CI): 2.9, 188%] among women with diabetes, compared with a 23% increase (95% CI: 7.1, 40%) in women without diabetes (Ostro et al. 2014).

Studies have reported inconsistent associations between chronic $PM_{2.5}$ and circulating levels of inflammatory mediators (e.g., Hajat et al. 2015), possibly owing to differences in population susceptibility (e.g., preexisting inflammatory conditions), $PM_{2.5}$ composition (Brook et al. 2010; Diez-Roux et al. 2006; Zeka et al. 2006), or to other time-varying factors that may influence inflammatory status at the time of blood draw (e.g., transient illness). Epidemiological studies, to date, have not focused on the influence of environmental exposures on stimulated production of cytokines. In contrast to circulating measures, which capture levels of inflammatory mediators at the time of blood draw, stimulated measures provide a unique indicator of the functional ability of immune cells to respond to challenge. An inflammatory response begins when monocytes/macrophages are activated by exposure to pathogens or tissue damage, resulting in the production and release of proinflammatory cytokines [e.g., tumor necrosis factor (TNF)-α, Il-1β, and IL-6] (Sternberg 2006). The magnitude of this response is critical; insufficient response may leave the organism vulnerable to infection, whereas excessive or prolonged activation of the inflammatory response can increase risk for chronic inflammatory diseases

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(e.g., cardiometabolic, autoimmune disease) (McEwen et al. 1997; Pavlov and Tracey 2004).

In vivo inflammation takes place locally in tissues, and individual differences in the magnitude of this response are difficult to assess. However, it is possible to assess the capacity of immune cells to respond to microbial challenge *in vitro* by stimulating whole blood with a bacterial product [lipopolysaccharide (LPS)], and quantifying proinflammatory cytokine production by the full array of immune cells acting in concert within their normal milieu (De Groote et al. 1992). These stimulated cytokine measures permit the quantification of individual differences in the propensity of the innate immune system to respond when stimulated (Ai et al. 2013; De Craen et al. 2005). Factors that contribute to differences in the magnitude of response remain unclear; initial evidence suggests that social adversity, including low socioeconomic status, associates with heightened stimulated inflammatory response (Chen et al. 2009). Here, we consider the possibility that chronic air pollution exposures may also contribute to plausibly heightened inflammatory responses.

Although no studies to date, to our knowledge, have systematically examined air pollution impacts on stimulated cytokine production across a cohort of healthy adults, Gawda et al. (2018), reported that macrophages exposed to PM *ex vivo* mounted larger proinflammatory (IL-6, TNF-α) responses to LPS challenge. A study of the impact of exposure to wildfire smoke in infancy on LPS-stimulated production of proinflammatory cytokines in adolescent rhesus monkeys reported that monkeys exposed to smoke as infants displayed lower stimulated production of IL-6 and IL-8 than nonexposed monkeys displayed (Black et al. 2017). There are very few prior studies combining air pollution exposures and stimulated cytokine measures, and those that have been performed used very different approaches, thereby complicating interpretability and comparability to our study. For example, Negherbon et al. (2017) compared stimulated responses to traffic-related particulate matter (TRPM) and LPS, respectively, among children with no asthma, controlled asthma, and uncontrolled asthma. The authors reported no significant differences among the groups in response to LPS-stimulation, but IL-8 responses following TRPM stimulation were lower among children with controlled or uncontrolled asthma than in nonasthmatic children. (Negherbon et al. 2017). Klümper et al. estimated effects of traffic-related air pollution (TRAP) on stimulated cytokines using blood samples from children with asthma ($n=27$) vs. children who did not have asthma ($n=59$), stimulated using the PM standard EHC-93 urban dust; higher chronic traffic-related air pollution (TRAP) exposures were associated with increased production of IL-6, IL-8, and TNF-α, only among children with asthma (Klümper et al. 2015).

Finally, an important area of investigation in environmental epidemiology has been the identification of specific components in $PM_{2.5}$ that may lead to adverse health outcomes. $PM_{2.5}$ constituents differ in toxicity (Bell et al. 2014; Franklin et al. 2008), and some prior studies have identified a heightened effect of steel-related airborne metals on inflammation (Ghio and Devlin 2001; Kumarathasan et al. 2018).

In the current study, we examined associations between annual average residence-based exposures to ambient $PM_{2.5}$, black carbon (BC), and metal components, with circulating and stimulated levels of proinflammatory mediators among healthy middle-aged adults in the Adult Health and Behavior project phase II (AHAB-II) cohort. To do so, we used hybrid-dispersion land use regression (LUR) models that we previously developed in Pittsburgh, Pennsylvania, to assess elevated concentrations of airborne metals related to steel emissions—lead (Pb), manganese (Mn), iron (Fe), and zinc (Zn)—across the urban area (Tripathy et al. 2019). Based on the existing evidence, we hypothesized that elevated exposures to $PM_{2.5}$, BC, Pb, Mn, Zn, and Fe would be associated with an increased inflammatory response to LPS, as indicated by LPS-stimulated production of cytokines (IL-6, IL-1β, and TNF-α).

Methods

AHAB-II Cohort

AHAB-II is a cohort study of generally healthy middle-aged adults in western Pennsylvania, developed to identify neural and biobehavioral correlates of physical and mental health in midlife. Participants were recruited between March 2008 and October 2011 through mass mailings of invitation letters to individuals randomly selected from voter registration and other public-domain lists. Eligible individuals were age 30–54 y who were working at least 25 h per week outside the home. Individuals were excluded if they: *a*) had a history of cardiovascular disease, schizophrenia, bipolar disorder, chronic hepatitis, renal failure, major neurological disorder, lung disease requiring treatment, or stage 2 hypertension (systolic blood pressure/diastolic blood pressure $\geq 160/100$); *b*) consumed more than five alcoholic drinks three to four times per week; *c*) took fish-oil supplements or used prescribed insulin, glucocorticoid, anti-arrhythmic, antihypertensive, lipid-lowering, psychotropic, or prescription weight-loss medications; *d*) were pregnant; or *e*) were shift workers. The University of Pittsburgh Institutional Board approved the study; all participants provided informed consent in accordance with its regulations and were remunerated for their participation (Marsland et al. 2017a).

Circulating Inflammatory Mediators

Blood samples were drawn between 0730 hours and 1235 hours ($M=9{:}16\pm0{:}54$ min) to determine levels of circulating IL-6 and CRP. Prior to the blood draw, participants were asked to fast for 8 h, avoid vigorous exercise for 12 h and alcohol for 24 h, and refrain from using tobacco products that morning. The blood draw was rescheduled if the participant reported symptoms of acute infection or use of antibiotics or antivirals in the previous 2 weeks. At the blood draw visit, a registered nurse completed a medical history and medication-use interview, obtained height and weight measurements to determine body mass index (BMI in kg/m^2), and drew a 40-mL blood sample. Plasma samples were collected from citrated tubes and frozen at −80 °C until analysis in batches. IL-6 levels were determined in duplicate by high-sensitivity quantitative sandwich enzyme immunoassay kit (R&D Systems; standard range = 0.156–10 pg/mL) run according to manufacturer's directions. CRP was measured at the University of Vermont's Laboratory of Clinical Biochemistry Research with the Dade Behring BNII nephelometer using a particle-enhanced immunonephelometric assay. Average inter- and intraassay coefficients of variation were <10% for both IL-6 and CRP.

Stimulated Cytokines

Whole blood was collected in heparin-treated vacutainer tubes and stimulated with LPS (serotype 026:B6; Sigma) at a final concentration of 2.5 μg/mL under sterile conditions, and incubated at 37°C with 5% CO_2 for 24 h. Tubes were then centrifuged at $1{,}000\times g$ for 10 min, and plasma was frozen at −80°C. Samples were assayed in one batch using a multiplex analysis system and Multiplex bead kits (BioSource), based on the principle of solid phase sandwich immunoassays. Stimulated levels of IL-6, IL-1β, and TNF-α were determined using Bio-Plex Manager Software (Bio-Rad Corp.), interpolating from the standard curve (Logistic-

5PL curve fit). Pooled plasma controls were included on all plates to determine assay reliability. Inter- and intraassay coefficients of variability were less than 10%. Unstimulated samples served as the baseline production of cytokines by immune cells. Stimulated cytokine production was quantified as the difference between stimulated levels and unstimulated samples (Prather et al. 2007).

Geocoding

AHAB-II participant addresses were geocoded using a three-tiered system in a Geographic Information System (GIS), following methods we have previously developed to maximize geocoding accuracy as shown in Figure 1 (Michanowicz et al. 2016). Briefly, addresses were first standardized using the U.S. Postal Service reference data set using ZP4 software (Semaphore Corp.). We excluded participants from the analysis with incomplete addresses, P.O. box numbers, and addresses outside Allegheny County, Pennsylvania. We first attempted to match addresses using an address point-based locator, which matches to exact building locations. Any addresses that were not geocoded were then matched to tax parcel centroids and, finally, remaining addresses were interpolated along a street segment using a street network locator.

Spatial Air Pollution Data

Pollutant concentrations were measured during a multipollutant air monitoring campaign in Allegheny County, as previously described (Shmool et al. 2014; Tripathy et al. 2019; Tunno et al. 2015). Our sampling domain, including both urban and suburban areas in the greater Pittsburgh region, was determined using a GIS (ArcMap 10.0–10.3; Redlands) to capture major industrial sources in Allegheny County (e.g., steel mills, coke works). A stratified random sampling design was used to select 36 monitoring sites based on cross-stratified classes of elevation, traffic density, and industrial emissions. Monitoring was completed during summer (June–July 2012), and the same sites were repeated in winter (January–March 2013). $PM_{2.5}$ samples were collected using Harvard Impactors (Air Diagnostics and Engineering Inc.) over 7 d. Eight sites were sampled per session. $PM_{2.5}$ concentrations were calculated based on gravimetric analysis of Teflon filters, and BC measured using an EEL43M Smokestain Reflectometer (Diffusion Systems). Elemental concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS) (Wisconsin State Laboratory of Hygiene) (Shmool et al. 2014; Tunno et al. 2015).

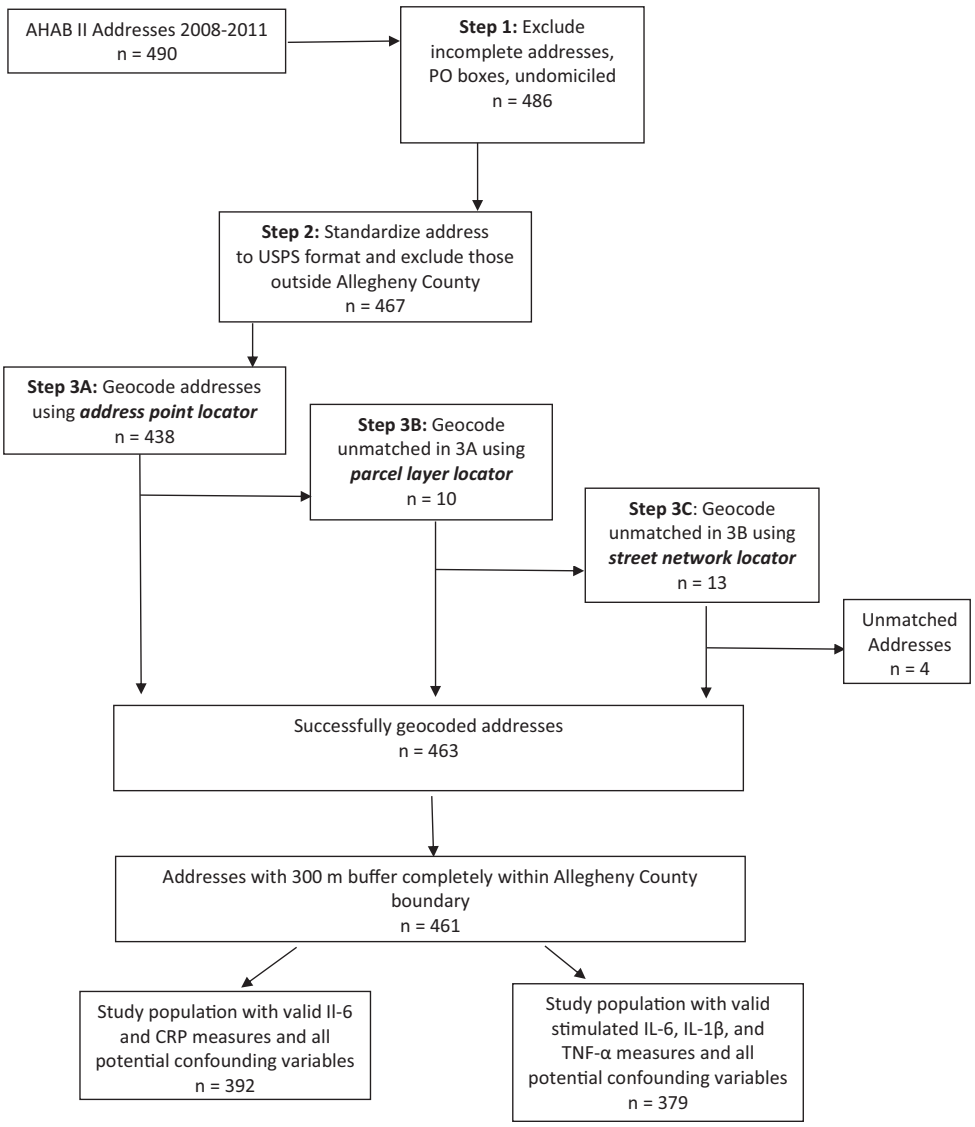

Figure 1. AHAB II participant exclusions and geocoding methodology.

Hybrid LUR Models

To estimate average one-year air pollution exposures at the homes of each AHAB II participant, we used pollution surfaces developed using hybrid LUR models for $PM_{2.5}$, BC, Pb, Mn, Fe, and Zn, detailed in (Tripathy et al. 2019). Briefly, spatial covariates were created using GIS to capture a variety of potential pollutant sources around monitoring locations, including traffic density indicators; transportation indicators; road-specific measures; land use/built environment; industrial emissions; population; and truck, bus, and diesel indicators (Tunno et al. 2015). Following our hybrid American Meteorological Society/Environmental Protection Agency Regulatory Model (AERMOD)-LUR modeling approach (Michanowicz et al. 2016; Tripathy et al. 2019), two additional covariates were developed using the AERMOD atmospheric dispersion model. One dispersion variable was built using $PM_{2.5}$ emissions profiles for 207 sources (industrial AERMOD term) and a second was developed using only the 14-point source profiles associated with the Edgar Thomson Steel Works (ETSW AERMOD term).

Hybrid LUR models were built using a manual forward stepwise approach combined with random forest analyses using SAS (version 9.3; SAS Institute, Inc.) and R (version 3.1.0; R Development Core Team) to select covariates that contributed the most variability in explaining pollutant distributions. A temporal term was incorporated using daily concentrations from a U.S. Environmental Protection Agency (U.S. EPA) Air Quality System (AQS) monitor maintained by the Allegheny County Health Department (ACHD), centrally located in the sampling domain in Lawrenceville, Pennsylvania (hereafter referred to as "Lawrenceville AQS monitor"). These models were used to predict pollutant concentrations across the monitoring domain using source layers in GIS (Tripathy et al. 2019). Model predictions were then extrapolated outside of the original sampling domain to include all of Allegheny County, where most AHAB-II participants lived.

Model results are described previously (Tripathy et al. 2019). The final Hybrid LUR models predicted 79% of the variability in $PM_{2.5}$; 59% of the variability in BC; and 35%, 55%, 55%, and 37% for Pb, Mn, Fe, and Zn, respectively. After performing a leave-one-out cross-validation (LOOCV), the difference $[R^2(\text{full model}) - R^2(\text{LOOCV model})]$ ranged from 0.03 to 0.07.

All models contained a temporal term and an AERMOD dispersion covariate. $PM_{2.5}$ models included industrial land use within a 500-m buffer and percent impervious surface within a 200-m buffer; BC models included commercial and industrial land use within a 200-m buffer; Zn, Fe, and Mn metal models included percent impervious surface within a 500-m buffer; and Pb, Mn, and Fe models included summed railroad length within a 300-m buffer (Tripathy et al. 2019).

Reference Site

To temporally adjust exposure estimates, we used daily data from a U.S. EPA AQS monitor (#420030008), centrally located within our sampling domain in Lawrenceville, Pittsburgh. We determined that data was available throughout the study period and verified that measurements were comparable to colocated repeated samples from our spatial monitoring campaign described above (Shmool et al. 2014; Tunno et al. 2015). Daily $PM_{2.5}$ data for this site were downloaded from the U.S. EPA air data website (U.S. EPA 2017), restricted to National Ambient Air Quality Standard (NAAQS)-compliant data. One daily monitor with only 176 missing days over the 10-y period was used as the primary data source. Concentrations for 141 additional missing days were filled with data from three other monitors (e.g., speciation monitors), leaving 35 d with missing data. To predict concentrations for missing days, we built a model using daily $PM_{2.5}$ concentrations as the dependent variable, and year, month, and day of the week as categorical variables in SAS (version 9.3). The resultant model was used to predict missing concentrations.

Exposure Assignment for Cohort Participants: Annual average $PM_{2.5}$, BC, Pb, Mn, Fe, and Zn pollutant exposures were estimated as mean concentrations within a 300-m buffer of each participant's geocoded address during the 12 months before blood draw, using data from our spatially refined LUR model with temporal adjustment to the specific time period of interest, consistent with previous studies in southwestern Pennsylvania (Talbott et al. 2015); New York, New York (Ross et al. 2013); and other locations (Brauer et al. 2008). Specifically, we corrected the LUR-derived pollutant exposure estimate for the residential buffer at the geocoded address for participant i (pollutant_i) using data from the reference monitor and the temporal term (β) from the LUR model as follows:

$$\begin{aligned}&\text{Temporally-adjusted pollutant}_i = \\ &\text{pollutant}_i - \beta \times \text{reference concentration during sampling year} + \\ &\beta \times \text{reference concentration during year prior to blood draw.}\end{aligned}$$

Our LUR model, which included summer and winter pollutant concentration data and covariates derived as annual averages, was optimized for estimating annual average exposures. However, we also estimated 3-month and 5-y average exposures before each participant's blood draw for use in sensitivity analyses, using reference monitor concentrations during the same time periods for temporal adjustment.

Statistical Analyses

We natural log-transformed outcomes and exposure estimates for metal pollutants after examination of scatter plots and histograms, to normalize distributions before analysis. Outcome and pollutant exposures were treated as continuous variables in all models. Bivariate linear regression models were run for each pollutant and inflammatory marker, followed by linear regression models adjusted for potential confounders of the association between chronic air pollution exposures and inflammatory mediators. Confounders included age (continuous), sex, race (White, Black, other), smoking status (current, former, never), BMI (continuous), education level (highest educational degree attained, categorized as ≤high school, some college/university, undergraduate degree, graduate degree), and month of blood draw (categorical with each month modeled as a separate group) to account for seasonal variation. All covariates were self-reported except month of blood draw and BMI, which was determined by measured weight and height at time of blood draw. Statistical analyses were generated using SAS (versions 9.3 and 9.4). Associations were classified as statistically significant by a p-value of <0.05. Given evidence that sex and obesity influence immune response (Dixon and Peters 2018; Fink and Klein 2015; Klein et al. 2015), we examined modification by both for significant results. We selected a BMI cut point of 30 based on the adverse relationship between obesity and inflammation (Dixon and Peters 2018; Rodríguez-Hernández et al. 2013). Potential interaction of pollutants by sex and BMI ≥30 were also tested as sensitivity analyses for adjusted models with pollutant–outcome relationships that were statistically significant. We included an interaction term between each pollutant and sex or BMI and report the p-value for the interaction.

Sensitivity Analyses

Bivariate linear regression models and linear regression models adjusting for confounders were run excluding participants who

did not live within the boundary demarcated by the original air monitoring domain (Shmool et al. 2014) to ensure results were not due to misclassification by pollutant sources that may not have been represented in the original sampling domain.

Although the main exposure period of interest for this study was 1-y pollutant exposure, we also tested 3-month and 5-y exposures for statistically significant 1-y pollutant outcome relationships. We chose 3-month exposures to account for potential differences by seasonally varying pollutant exposures. We also examined 5-y pollutant exposures to test whether longer-term exposures may have greater influence on inflammatory response.

Results

AHAB II Sample Size

The original AHAB II cohort included 490 participants. Twenty-nine participants were excluded due to an incomplete address, P.O. box numbers, or address outside of Allegheny County (Figure 1). Remaining addresses were geocoded using the composite locator, resulting in four unmatched addresses ($n = 463$). Two additional addresses with 300-m buffers extending outside of Allegheny County were excluded. Exposures were assigned at 461 geocoded locations using hybrid LUR air pollutant exposure surfaces and AQS data, as described above. A separate sample size was determined for circulating cytokines and stimulated cytokines because 393 participants had complete circulating cytokine data vs. only 379 for stimulated cytokines. One participant was excluded from the circulating cytokine analyses due to missing data on smoking status. Geocoded locations for final analytic cohorts are shown in Figure 2.

Sample Characteristics

Characteristics were similar for participants with valid circulating inflammatory mediators ($n = 392$) and stimulated cytokine data ($n = 379$) (Table 1). Both analytic cohorts had slightly more women than men, and the average age was approximately 43 y old. Participants were predominately White never smokers with undergraduate or graduate degrees.

Statistical Analyses

Average pollutant exposures were similar between participants with valid measures of circulating cytokines and participants with valid measures of stimulated cytokines (Table 2). Concentrations of circulating and stimulated inflammatory mediators (Table 3) were similar to measures in a previous cohort of healthy middle-aged adults in the greater Pittsburgh area (AHAB I) (Marsland et al. 2008; Prather et al. 2007). All pollutants were positively correlated, with correlation coefficients ≥ 0.63 (Table S1). Stimulated cytokines and circulating inflammatory mediators were not correlated with each other ($r \leq 0.06$). Stimulated cytokines were all positively correlated ($r > 0.57$). Circulating IL-6 and CRP were also positively correlated ($r = 0.47$) (Table S2).

None of the pollutants were significantly associated with circulating IL-6 or CRP, either before or after adjustment (Table 4).

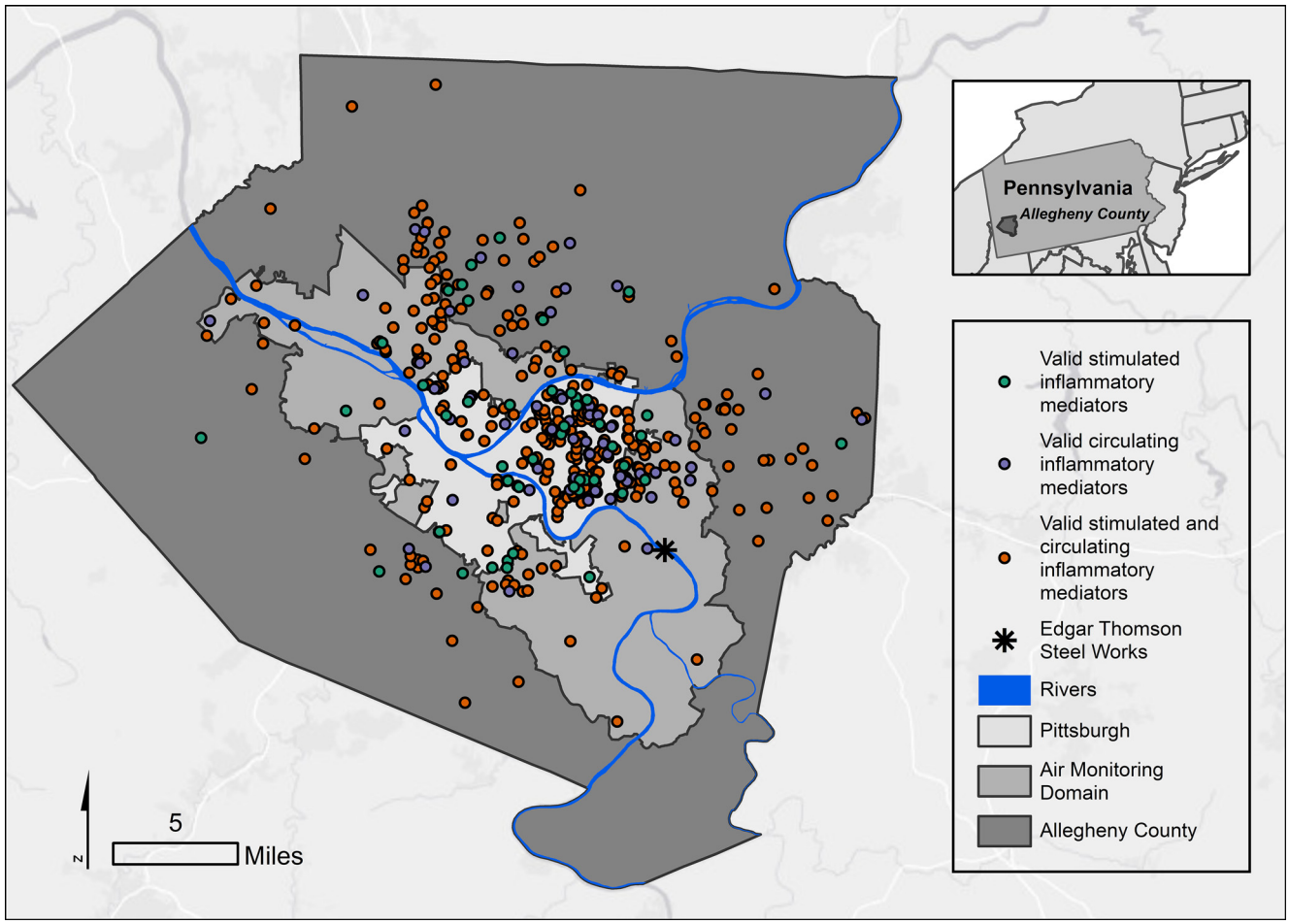

Figure 2. AHAB II geocoded addresses. Service Layer Credits: Esri, HERE, Garmin, (C) OpenStreetMap contributors, and the GIS user community.

Table 1. AHAB II Participant characteristics.

Sample characteristics	Participants with IL-6 and CRP ($n=392$)				Participants with stimulated IL-6, IL-1β, and TNF-α ($n=379$)			
	Mean	Min	Max	n (%)	Mean	Min	Max	n (%)
Age (y)	43.1	30	54	—	42.8	30	54	—
BMI (kg/m^2)	27.2	17.9	46.5	—	26.9	17.5	49.6	—
BMI ≥30				107 (27)				93 (25)
BMI <30				285 (73)				286 (75)
Sex								
Male				188 (48)				176 (46)
Female				204 (52)				203 (54)
Race								
White				320 (82)				312 (82)
Black				64 (16)				57 (15)
Other				8 (2)				10 (3)
Smoking status								
Former				80 (20)				80 (21)
Current				65 (17)				55 (15)
Never				247 (63)				244 (64)
Education level								
High school graduate or less				27 (7)				22 (6)
Some college				91 (23)				82 (22)
Undergraduate degree				140 (36)				141 (37)
Graduate degree				134 (34)				134 (35)
Month of blood draw								
January				27 (7)				24 (6)
February				31 (8)				36 (10)
March				55 (14)				53 (14)
April				49 (13)				51 (13)
May				34 (9)				33 (9)
June				26 (7)				27 (7)
July				34 (9)				30 (8)
August				30 (8)				29 (8)
September				30 (8)				35 (9)
October				32 (8)				25 (7)
November				20 (5)				16 (4)
December				24 (6)				20 (5)

Note: Data are complete for all variables shown. —, not applicable; BMI, body mass index; CRP, C-reactive protein; max, maximum; min, minimum.

In contrast, $PM_{2.5}$, BC, and all metal constituents were associated with significantly higher IL-1β and TNF-α production after LPS stimulation (Table 5). For example, an interquartile range (IQR) (1.88 μg/m^3) increase in $PM_{2.5}$ was associated with a 35% higher average stimulated IL-1β (95% CI: 26, 46%), and a 65% higher production of stimulated TNF-α (95% CI: 49, 84%), with associations of similar magnitude for an IQR increase (0.21 abs) in BC. Associations between a 1% increase in metals and stimulated IL-1β and TNF-α production were strongest for Pb (0.72%; 95% CI: 0.45, 0.99% and 1.18%; 95% CI: 0.76, 1.59%, respectively) and generally similar for Mn, Fe, and Zn (e.g., for a 1% increase in Mn, 0.28%; 95% CI: 0.13, 0.44% and 0.52%; 95% CI: 0.28, 0.76%, respectively). IQR increases in $PM_{2.5}$ and BC were also associated with significantly higher IL-6 production in response to stimulation (17%; 95% CI: 7.7, 26% and 21%; 95% CI: 7.4, 40%, respectively.) Associations between metals and stimulated IL-6 production were positive but weak in comparison with corresponding estimates for IL-1β and TNF-α, with the strongest association estimated for a 1% increase in Pb (0.26%; 95% CI: 0.01, 0.5%).

Sensitivity Analyses

Associations were similar to estimates for the study population as a whole after 103 participants who did not live within the original air pollution monitoring domain were excluded (resulting $n=276$, Table S3).

Positive associations of stimulated cytokines with $PM_{2.5}$, BC, and all metals were consistently stronger for women than for men (Table S4). However, differences between women and men were significant only for stimulated IL-6 in association with an IQR increase in BC (11% higher; 95% CI: –2.6, 34% for men vs. 32% higher; 95% CI: 11, 69% for women, p-interaction 0.04) and for stimulated IL-6 in association with 1% increases in Mn and Pb

Table 2. AHAB II 1-y residential pollutant exposure estimates.[a,b]

Exposure estimates	Participants with IL-6 and CRP ($n=392$)						Participants with stimulated IL-6, IL-1β, and TNF-α ($n=379$)					
	Median	Min	Max	25%	75%	IQR	Median	Min	Max	25%	75%	IQR
$PM_{2.5}$ (μg/m^3)	12.82	10.67	16.96	12.04	13.99	1.95	12.72	10.67	16.96	11.97	13.85	1.88
BC (abs)	0.94	0.70	1.53	0.85	1.06	0.21	0.93	0.70	1.53	0.84	1.05	0.21
Pb (ng/m^3)	2.82	1.63	9.74	2.43	3.35	0.93	2.82	1.63	9.74	2.43	3.29	0.85
Mn (ng/m^3)	2.90	1.18	19.26	2.06	3.78	1.72	2.86	1.18	19.26	2.05	3.70	1.65
Fe (ng/m^3)	66.81	28.32	390.07	48.41	86.91	38.50	66.30	28.32	390.07	48.19	84.35	36.16
Zn (ng/m^3)	18.71	7.80	42.60	12.86	24.00	11.14	18.70	7.80	42.60	12.73	23.53	10.80

[a]Pollutant exposures are average exposures during the 12 months before the date of the blood draw. BC, black carbon; CRP, C-reactive protein; Fe, iron; IQR, interquartile range; LUR, land use regression; Mn, manganese; max, maximum; min, minimum; Pb, lead; Zn, zinc.
[b]The spatial resolution for pollutant exposures are within a 300-m buffer surrounding individual's home from the LUR 100-m surface.

Table 3. AHAB II Outcomes. Circulating IL-6 and CRP were measured in whole blood samples. Stimulated IL-6, IL-1β, and TNF-α were measured from whole blood samples stimulated with LPS and quantified as the difference between stimulated levels and unstimulated samples.

Inflammatory marker	*n*	Median	Min	Max	25%	75%	IQR
IL-6 (pg/mL)	392	0.89	0.06	9.83	0.61	1.37	0.76
CRP (ng/mL)	392	0.88	0.16	9.90	0.45	2.06	1.61
Stimulated IL-6 (pg/mL)	379	44,421.76	2,510.77	254,583.25	31,001.40	63,333.57	32,332.17
Stimulated IL-1β (pg/mL)	379	10,478.80	248.36	46,733.32	7,005.78	15,874.56	8,868.78
Stimulated TNF-α (pg/mL)	379	5,893.26	33.44	39,857.35	2,869.74	9,794.29	6,924.55

Note: CRP, C-reactive protein; IQR, interquartile range; LPS, lipopolysaccharide; max, maximum; min, minimum.

[e.g., for Pb, 0.13%; –0.20, 0.46% in men vs. 0.40%; 95% CI: 0.05, 0.75%, *p*-interaction 0.03; (Table S4)]. Associations between the pollutant exposures and stimulated cytokines were similar for participants with BMI ≥30 in comparison with <30, with no significant differences between the two groups, and no consistent patterns of effects across pollutants (Table S5).

Point estimates for 3-month and 5-y average exposures were similar to or weaker than corresponding estimates for 1-y average exposures (Table S6).

Discussion

We found that air pollution exposures were significantly associated with higher production of proinflammatory cytokines in response to *ex vivo* stimulation with LPS among a cohort of adults living in Allegheny County. Average exposures to $PM_{2.5}$ and BC during the 12 months before each participant's blood draw were associated with significantly higher LPS-stimulated production of IL-1β, TNF-α, and IL-6, whereas 1-y average Pb, Mn, Fe, and Zn exposures were associated with significantly higher stimulated levels of TNF-α, and IL-1β. In contrast, there were no significant associations between any of the pollutants during the previous year and circulating levels of IL-6 or CRP.

Our results provide preliminary evidence that chronic air pollution exposure is associated with a stronger proinflammatory response by stimulated immune cells. This is consistent with Klümper et al. (2015), who reported that 1-y exposure to TRAP (including NO_2, NO_x, PM_{10}, $PM_{2.5}$, and $PM_{2.5\,absorbance}$) was associated with higher PM-stimulated production of IL-6 and TNF-α in whole blood samples collected from children with asthma (Klümper et al. 2015). These results raise the possibility that increased proinflammatory reactivity may contribute to inflammatory conditions associated with air pollution.

In contrast to previous findings, we did not estimate significant associations between pollutant exposures and circulating levels of IL-6 or CRP. Several factors may have influenced these results. Although we examined associations with $PM_{2.5}$, BC, Pb, Mn, Zn, and Fe components, distinct sources of $PM_{2.5}$ components may be differentially associated with systemic inflammation. For example, Zeka et al. (2006) found significant positive associations between traffic-related particles and inflammatory markers, but not with total $PM_{2.5}$ or sulphates (Zeka et al. 2006). Duration of the pollutant exposures and population susceptibility may also influence associations (Brook et al. 2010). Relatively few AHAB II participants lived in close proximity to the Edgar Thomson Steel Works, and thus more participants may have been exposed to other sources of $PM_{2.5}$, such as traffic-related sources, long-range traffic, or other industrial sources. A previous analysis of $PM_{2.5}$ constituents in the study area attributed Mn, Fe, Pb, and Zn to steel-related sources, whereas other metals (e.g., copper, chromium, aluminum) were attributed to traffic-related sources (Tunno et al. 2016). Temporality may also be of concern because we were unable to account for prenatal or early childhood exposures. Lack of association between pollutant exposure with

Table 4. Linear regression model estimates for associations of circulating IL-6 and CRP with residential exposures (300-m buffer) to $PM_{2.5}$ and $PM_{2.5}$ constituents during the 12-months prior to blood draw among AHAB II study participants ($n = 392$).

	Circulating IL-6			Circulating CRP		
Pollutant	Estimate	95% CI	*p*-Value	Estimate	95% CI	*p*-Value
$PM_{2.5}$[a]						
Unadjusted	−3.99	(−12.71, 3.80)	0.4	−7.71	(−21.26, 5.73)	0.3
Adjusted	0.45	(−7.37, 9.64)	0.9	−1.27	(−14.45, 13.63)	0.9
BC[b]						
Unadjusted	−4.60	(−10.15, 3.89)	0.2	−3.65	(−12.11, 12.94)	0.6
Adjusted	−1.15	(−7.61, 8.50)	0.8	3.90	(−7.87, 26.21)	0.6
Mn[c]						
Unadjusted	−0.0035	(−0.15, 0.15)	1.0	0.07	(−0.17, 0.31)	0.6
Adjusted	0.034	(−0.11, 0.18)	0.6	0.14	(−0.09, 0.37)	0.2
Pb[c]						
Unadjusted	−0.030	(−0.29, 0.23)	0.8	0.13	(−0.29, 0.56)	0.5
Adjusted	0.094	(−0.16, 0.35)	0.5	0.29	(−0.11, 0.70)	0.2
Fe[c]						
Unadjusted	−0.011	(−0.17, 0.14)	0.9	0.05	(−0.20, 0.30)	0.7
Adjusted	0.035	(−0.11, 0.18)	0.6	0.13	(−0.11, 0.37)	0.3
Zn[c]						
Unadjusted	0.0033	(−0.17, 0.18)	1.0	0.01	(−0.27, 0.29)	0.9
Adjusted	0.017	(−0.15, 0.18)	0.8	0.08	(−0.18, 0.35)	0.5

Note: Concentrations of IL-6 and CRP in whole blood samples were ln-transformed for analysis. $PM_{2.5}$ and BC concentrations were modeled as untransformed continuous variables, metal concentrations were ln-transformed for analysis. Adjusted models include age, sex, race (White, Black, other), smoking status (former, current, never), educational attainment (≤high school, some college, undergraduate degree, graduate degree), month of blood draw (categorical with each month modeled as a separate group) and BMI (continuous). BC, black carbon; CI, confidence interval; CRP, C-reactive protein; Fe, iron; IQR, interquartile range; Mn, manganese; Pb, lead; Zn, zinc.

[a]Estimates represent the estimated difference in mean IL-6 (pg/mL) and CRP (ng/mL) with an IQR (1.88 $\mu g/m^3$) increase in $PM_{2.5}$ during the 12 months prior to blood draw.

[b]Estimates represent the estimated difference in mean IL-6 (pg/mL) and CRP (ng/mL) with an IQR (0.21 absorbance units) increase in BC during the 12 months prior to blood draw.

[c]Estimates represent the estimated percent difference in mean IL-6 and CRP with a 1% increase in exposure during the 12 months prior to blood draw.

Table 5. Linear regression model estimates for associations of stimulated IL-6, IL-1β, and TNF-α with residential exposures (300-m buffer) to $PM_{2.5}$ and $PM_{2.5}$ constituents during the 12-months prior to blood draw among AHAB II study participants ($n = 379$).

	Stimulated IL-1β			Stimulated TNF-α			Stimulated IL-6		
Pollutant	Estimate	95% CI	*p*-Value	Estimate	95% CI	*p*-Value	Estimate	95% CI	*p*-Value
$PM_{2.5}$[a]									
Unadjusted	32.62	(23.97, 43.93)	<.0001	61.89	(46.26, 78.78)	<.0001	14.22	(5.73, 21.86)	0.001
Adjusted	34.84	(26.10, 46.26)	<.0001	65.4	(48.62, 84.17)	<.0001	16.99	(7.67, 26.10)	<.0001
BC[b]									
Unadjusted	47.07	(23.90, 81.98)	<.0001	169.8	(79.94, 342.04)	<.0001	17.95	(5.43, 36.08)	0.002
Adjusted	51.12	(26.21, 88.35)	<.0001	168.92	(77.94, 342.04)	<.0001	20.72	(7.35, 40.22)	0.001
Mn[c]									
Unadjusted	0.27	(0.12, 0.42)	0.0006	0.52	(0.28, 0.76)	<.0001	0.08	(−0.06, 0.22)	0.258
Adjusted	0.28	(0.13, 0.44)	0.0004	0.52	(0.28, 0.76)	<.0001	0.11	(−0.03, 0.25)	0.124
Pb[c]									
Unadjusted	0.67	(0.41, 0.92)	<.0001	1.15	(0.74, 1.55)	<.0001	0.21	(−0.03, 0.45)	0.09
Adjusted	0.72	(0.45, 0.99)	<.0001	1.18	(0.76, 1.59)	<.0001	0.26	(0.01, 0.50)	0.039
Fe[c]									
Unadjusted	0.32	(0.17, 0.47)	<.0001	0.61	(0.37, 0.85)	<.0001	0.11	(−0.03, 0.25)	0.138
Adjusted	0.33	(0.18, 0.49)	0.0001	0.61	(0.36, 0.85)	<.0001	0.14	(0.00, 0.28)	0.055
Zn[c]									
Unadjusted	0.29	(0.11, 0.47)	0.001	0.57	(0.29, 0.85)	<.0001	0.13	(−0.03, 0.30)	0.104
Adjusted	0.29	(0.11, 0.47)	0.001	0.56	(0.29, 0.84)	<.0001	0.16	(0.00, 0.32)	0.048

Note: Concentrations of stimulated cytokines in whole blood samples were ln-transformed for analysis. $PM_{2.5}$ and BC concentrations were modeled as untransformed continuous variables, $PM_{2.5}$ metal constituent concentrations were ln-transformed for analysis. Adjusted models include age, sex, race (White, Black, other), smoking status (former, current, never), educational attainment (high school graduate or less, some college, undergraduate degree, graduate degree), month of blood draw (categorical with each month modeled as a separate group), and BMI (continuous). BC, black carbon; BMI, body mass index; CI, confidence interval; Fe, iron; IQR, interquartile range; Mn, manganese; Pb, lead; Zn, zinc.

[a]Estimates represent the estimated difference in mean stimulated IL-6 (pg/mL), stimulated IL-1β (pg/ml), and stimulated TNF-α (pg/ml) with an IQR ($1.88\ \mu g/m^3$) increase in $PM_{2.5}$ during the 12 months prior to blood draw.

[b]Estimates represent the estimated difference in mean stimulated IL-6 (pg/mL), stimulated IL-1β (pg/mL), and stimulated TNF-α (pg/mL) with an IQR (0.21 absorbance units) increase in BC during the 12 months prior to blood draw.

[c]Estimates represent the estimated percent difference in mean stimulated IL-6, stimulated IL-1β, and stimulated TNF-α with a 1% increase in exposure during the 12 months prior to blood draw.

circulating inflammatory mediators in this study could also be related to the AHAB II cohort, which is composed of relatively healthy, middle-aged participants, with no history of clinical cardiovascular disease, angina, or claudication, and taking no cardiovascular medications. In this regard, a number of the studies that have found an association have examined less healthy populations such as individuals with obesity, diabetes, or hypertension (Dubowsky et al. 2006; Ostro et al. 2014; Zeka et al. 2006).

Circulating IL-6 levels reflect production by adipocytes, myocytes, and endothelial cells, as well as immune cells (Mohamed-Ali et al. 1997). For example, IL-6 is produced by muscle cells, and circulating IL-6 levels have been shown to increase dramatically following exercise (Pedersen and Febbraio 2008) and acute psychological stress (Marsland et al. 2017b). It has been estimated that up to 40% of circulating IL-6 is derived from fat cells (Mohamed-Ali et al. 1997). IL-6 responses may also affect circulating CRP levels, because IL-6 stimulates CRP production and release by the liver (Bode et al. 2012).Therefore, it may be difficult to identify the independent contribution of chronic effects of air pollution on circulating levels of these inflammatory mediators. In contrast, IL-6 production in response to stimulation is a functional measure of the ability of white blood cells to mount an acute inflammatory response. Individuals vary markedly in the magnitude of this response, and genetic factors may contribute to this variability (De Craen et al. 2005).

Our findings suggest that exposure to air pollution may also contribute to interindividual variability in immune responsiveness and that effects on immune responsiveness may help explain associations between air pollution and inflammatory diseases.

Local inflammatory responses play a key role in the pathogenesis of many diseases, including cardiometabolic disease, asthma, and autoimmune disease (McEwen et al. 1997). Epidemiological studies have not focused on the contribution of individual differences in stimulated production of cytokines for practical reasons; however, considerable literature shows a positive association between circulating levels of inflammatory markers and risk for a range of chronic inflammatory diseases and premature mortality among adults (Chung et al. 2009). Chronic activation of this immune pathway may result in increased susceptibility to inflammatory diseases (Pavlov and Tracey 2004), providing a pathway between exposure to air pollution and poor health.

Strengths and Limitations

A limitation of this study is the single assessment of stimulated cytokine levels. Although evidence suggests that stimulated cytokine production by peripheral leukocytes is stable over time for healthy volunteers (De Craen et al. 2005), multiple assessments over time would provide a more accurate indicator of interindividual variability and progression. Another limitation is the spatial extrapolation of predicted pollutant concentrations from the original monitoring domain to all of Allegheny County. However, results were similar when limited to participants residing within the original sampling domain.

Although blood samples were collected in the period 2008–2011, our exposure models were developed using pollutant concentrations measured in 2012 and 2013; therefore, predicted pollutant concentrations were temporally adjusted using daily regulatory data from the Lawrenceville AQS monitor. Associations with 3-month and 5-y average exposures were similar to or weaker than associations with 1-y average exposures, but our exposure models may be less accurate for earlier points in time or time periods less than 1 y. Exposures may also have been misclassified for participants who lived at a different address prior to the study. Other factors, such as diet, chronic stress, chronic noise exposures, neighborhood characteristics, and household chemical use, were not examined in this study but may be potential confounders.

The impact of chronic exposure to air pollution on the ability of immune cells to mount inflammatory responses remains to be fully determined, but these initial findings support the need for

additional research on the link between air pollution exposures and stimulated cytokine responses. AHAB-II was a predominately White, relatively healthy, and well-educated cohort; thus results may not be generalizable to other populations, including older populations, people with chronic diseases, more diverse populations, and populations in other geographic areas. Future research should also consider the role of toll-like receptor ligands such as TLR3, which plays a key role in the initial innate response against viruses and is relevant for asthma exacerbation (Schurman et al. 2018). In addition, additional work is needed to determine the clinical significance of differences in stimulated levels of cytokines.

In conclusion, our findings suggest that chronic exposure to air pollution may prime the innate immune system to be more reactive and increase inflammatory responses to immune stimulation. This pathway may serve as a link between pollution exposures and increased risk for inflammatory diseases, including allergies, asthma, and cardiovascular disease. Examining associations between airborne pollutants and both circulating and LPS-stimulated production of inflammatory mediators offers an opportunity to further examine unique inflammatory mechanisms that may contribute to pollution-related disease risk.

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