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Air Pollution During Pregnancy and Cord Blood Immune System Biomarkers

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Objectives: We aimed to determine whether average and trimester-specific exposures to ambient measures of nitrogen dioxide (NO_2) and particular matter ($PM_{2.5}$) were associated with elevated cord blood concentrations of immunoglobulin E (IgE) and two epithelial cell produced cytokines: interleukin-33 (IL-33) and thymic stromal lymphopoietin (TSLP). **Methods:** This study utilized data and biospecimens from the Maternal-Infant Research on Environmental Chemicals (MIREC) Study. There were 2001 pregnant women recruited between 2008 and 2011 from 10 Canadian cities. Maternal exposure to NO_2 and $PM_{2.5}$ was estimated using land use regression and satellite-derived models. **Results:** We observed statistically significant associations between maternal NO_2 exposure and elevated cord blood concentrations of both IL-33 and TSLP among girls but not boys. **Conclusions:** Maternal NO_2 exposure may impact the development of the newborn immune system as measured by cord blood concentrations of two cytokines.

Learning Objectives

- Summarize previous findings on the association between air pollution and childhood allergic disease, including the role of in utero versus childhood exposure.
- Discuss the new findings on maternal pollutant exposures associated with specific types of immune system biomarkers in umbilical cord blood.
- Identify other characteristics affecting the reported associations between pollution exposure and cord blood cytokine levels.

The role of maternal exposure to environmental contaminants on the developing fetal immune system is not clear. It has been suggested that fetal exposure to some environmental contaminants can promote life-long changes to the developing immune system that would have an effect on immune system responses resulting in an increased risk of an allergic phenotype in childhood and beyond.[1–3] Results from research related to the health effects of childhood exposures tend to be more conclusive than studies evaluating effects of fetal exposures. Authors of a comprehensive review concluded that "evidence is sufficient to support a causal association between traffic-related air pollution exposure and exacerbation of childhood asthma."[4] These authors also concluded that the evidence was suggestive but not sufficient to support a causal association between traffic exposure and incident asthma.[4] Authors of cohort studies in France,[5,6] Netherlands,[7,8] Sweden,[9] Germany,[10] China,[11] and North America,[12–15] have reported that childhood air pollution exposure is significantly associated with exacerbation or development of childhood asthma or allergic disease. Systematic reviews have reported that traffic-related air pollution is associated with severity of childhood asthma symptoms[16] and increased risk of incident asthma, allergies, and sensitization.[17]

The body of literature regarding air pollution exposure and childhood asthma and allergic disease has limited ability to disentangle the role of in utero versus childhood exposure in allergic disease etiology.[13,14] This distinction has relevance for identifying critical windows of exposure and effective prevention strategies. Recent studies have reported that NO_2 and $PM_{2.5}$ may affect placental function[18] and gene expression,[19] a process that may influence fetal development. Authors of birth cohort studies have reported associations between prenatal air pollution exposure and cord blood levels of immunoglobulin E (IgE),[20] interleukin-10 (IL-10),[21] and certain lymphocytes.[22,23] However, to date, no studies have examined the association between in utero air pollution exposure and epithelial cell derived cytokines. It has been observed that epithelial cells have important roles in the regulation of both innate and adaptive immunity.[24,25] As epithelial cells line the respiratory tract and skin, these cells may represent the point of first contact for many environmental contaminants.[26] Unlike lymphopoietic cell derived cytokines, epithelial cell derived cytokines function in the absence of a mature immune system.[27] In addition, common air pollutants such as NO_2 have been shown to impact epithelial cell function.[28,29]

IL-33 and thymic stromal lymphopoietin (TSLP) are two epithelial cell derived cytokines that participate in allergic disease and type 2 inflammation.[30] These mediators are known to have a critical role in the etiology of atopic dermatitis that is often the first manifestation of allergic disease in childhood.[31] These two cytokines may have different mechanisms, as TSLP is thought to have a role in childhood allergy and asthma,[32] whereas IL-33 has been linked to mechanisms underlying food allergy.[33,34] We have previously demonstrated that these biomarkers are detectable at birth and associated with self-reported traffic exposure.[35] Previous studies have examined the association between in utero environmental contaminant exposure and cord blood IgE concentrations as a biomarker for infant allergy[20,36,37] and this may also serve as an indicator of fetal susceptibility to air pollution.[20]

The aim of the present study was to investigate the association between maternal exposure to ambient measures of $PM_{2.5}$ and

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The JOEM editorial board and planners have no financial interest related to this research.

This work was funded by a Category A grant (grant #10012) from the Izaak Walton Killam (IWK) Health Centre. The MIREC Study was funded by the Chemicals Management Plan of Health Canada, the Canadian Institutes for Health Research (grant# MOP – 81285), and the Ontario Ministry of the Environment.

The authors report no conflicts of interest.

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DOI: 10.1097/JOM.0000000000000841

NO_2 and cord blood concentrations of IgE, TSLP, and IL-33. The secondary objective is to determine the nature of these associations by trimester of exposure.

METHODS

Study Population and Data Sources

Details on the study design as well as the subpopulation for the current analysis have been published elsewhere.[35,38] As described elsewhere, data and biospecimens were obtained from the Maternal-Infant Research on Environmental Chemicals (MIREC) Study, a trans-Canada prospective cohort study of pregnant women recruited in the first trimester from 10 Canadian cities between 2008 and 2011.[38] As previously described, cord blood samples that were determined to be contaminated with maternal blood based on an elevated immunoglobulin A (IgA) concentration ($\geq$10 μg/mL) were excluded from the analysis.[39] We excluded preterm infants and multiple births, as these infants have lower levels of the immune system biomarkers of interest in this study and children born preterm have been shown to have an increased risk of asthma in childhood.[40,41] All participants gave informed consent upon recruitment and research ethics board approval was obtained from Health Canada, St. Justine's Hospital (Montreal, QC), and the Izaak Walton Killam (IWK) Health Centre (Halifax, NS).

Air Pollution Exposure

Details regarding development of the air pollution models and methodology for determining prenatal exposure histories have been described previously.[42] Briefly, information on participant's residential location [based on the forward sortation area (FSA) (ie, the first three letters of the postal code)] was collected from questionnaires administered in the 1st and 3rd trimesters. Air pollution concentrations were based on the population-weighted geographical coordinates of the FSA in which the mothers lived during pregnancy. If a subject moved during pregnancy, pollution estimates were reassigned according to their new FSA.

Estimates of prenatal exposure to $PM_{2.5}$ were derived from monthly surfaces of a land use regression (LUR) model that was developed using satellite-based $PM_{2.5}$ estimates as well $PM_{2.5}$ measurements from fixed-site monitoring stations.[43,44] Our estimates of NO_2 were derived from a national LUR model developed using satellite NO_2 estimates and geographic predictors[43] scaled temporally with ground monitors from the National Air Pollution Surveillance (NAPS) network.[45] This scaling was done by first calculating a location-specific scaling factor at each fixed monitoring location for each month of the study period. A spatially resolved scaling surface was then created for each month within the study period through spatial interpolating of these location-specific monthly scaling factors using an inverse distance weighting (IDW) interpolation. This approach was applied to create monthly NO_2 estimates for FSA locations within 25 km of a NAPS station that measured NO_2 during the study period. Monthly scaling surfaces were then combined with annual LUR estimates to create monthly NO_2[46] and to estimate mean pregnancy and trimester-specific NO_2 exposure. We used a 25 km buffer for temporal adjustment with ground-based monitoring stations based on prior literature.[46] We conducted a sensitivity analysis by using the NO_2 estimates from a national NO_2 LUR model[43,44] (without the inclusion of the temporally scaled NAPS data). The national model had an R^2 of 0.73 and a root mean square error of 2.9 parts per billion (ppb). Pregnancy and trimester-level exposure estimates were calculated from monthly estimates for each calendar year on the basis of the proportion of pregnancy or trimester in that year. We used the temporally scaled exposure assessment model as our primary means of exposure assessment, as it facilitated trimester-specific model estimates.

Immune System Biomarkers

As previously described, immune system biomarkers were measured in the plasma of umbilical cord blood samples (details in).[35] TSLP concentrations were measured using a commercial antibody kit (Biolegend, San Diego, CA). IL-33 concentrations were analyzed using antibodies from an R & D systems duoset (Minneapolis, MN). Total IgE and total IgA concentrations were analyzed using ELISA kits (EBioscience, San Diego, CA).

Statistical Analysis

On the basis of our previous work, the percentage of detectable samples was 42%, 52%, and 18% for TSLP, IL-33, and IgE, respectively. As a result, each immune system biomarker was categorized as binary variables. A composite variable was developed to identify samples with elevated concentrations of both TSLP and IL-33 (IL-33/TSLP), as these cytokines are highly correlated (Spearman correlation coefficient = 0.8). TSLP and IL-33 were categorized as elevated at the 80th percentile (TSLP = 554 pg/mL; IL-33 = 879 pg/mL) because there are no pre-existing thresholds. Elevated concentrations of the composite IL-33/TSLP variable were defined as those samples that had elevated concentrations ($\geq$80%ile) of both TSLP and IL-33. In recognition of the arbitrary nature of the 80th percentile threshold, we also conducted analyses between average pollutant exposure and IL-33 and TSLP dichotomized at the level of detection (LOD). On the basis of established cut-off values for IgE where odds of childhood allergic disease are increased, values at least 1.2 ng/mL (0.5 kU/L) were considered elevated.[47,48]

Mixed effect logistic regression models were used to determine associations between average prenatal exposure to ambient air pollutants and binary measures of immune system biomarkers. This model accounted for potential correlations and other characteristics that distinguish subjects within the same recruitment site and FSAs. FSA and study recruitment site were included in the model as nested random effects. Ambient concentrations of $PM_{2.5}$ and NO_2 were evaluated as continuous variables (1 unit increases) and as quartiles in logistic regression analysis. Confounders were a priori identified as maternal age and income on the basis of our previous analysis of predictors of the immune system biomarkers.[35] Due to the strong association between maternal allergy and the immune system biomarkers, and to avoid overadjustment, we did not adjust for this variable. In addition, though cigarette smoking is a risk factor for allergic disease, it was not associated with the immune system biomarkers, possibly due to the low prevalence of smoking in the MIREC population, and, therefore, not included in the multivariate models. Therefore, we conducted sensitivity analyses excluding mothers with a history of maternal allergy and excluding current smokers. We tested for effect modification by sex by evaluating the P value of the sex–exposure interaction term and stratifying results by sex.[49] We also examined the influence of including both pollutants in a model on effect estimates. Results are presented for average exposure throughout pregnancy as well as by trimester. In the trimester-specific analysis, only the joint IL-33/TSLP results are shown, as the individual IL-33 and TSLP results were similar.

Restricted cubic spline analyses were performed on all associations with significant results in analyses by both average pregnancy and trimester-specific exposure. This component of the analysis facilitated examination of dose–response relationships using continuous measures of exposure. Knots were specified at the 25th, 50th, and 75th percentiles; Akaike information criterion values were comparable to default knot choices and offered the advantage of comparison with the quartile results. Analyses were performed using SAS v. 9.3 (SAS Institute Inc., Cary, NC) and R v. 3.2.2 (R Foundation, Vienna, Austria).

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TABLE 1. Study Participant Characteristics, MIREC Study, Canada, 2008–2011 ($n = 1253$)*

Characteristic	*n* (%)
Maternal age, yrs	
≤29	328 (26.2)
30–34	451 (36.0)
≥35	474 (37.8)
Household income ($CAD)	
≤30,000	90 (7.5)
30,001–50,000	117 (9.7)
50,001–100,000	513 (42.6)
>100,000	485 (40.3)
Smoking	
Never or quit before pregnancy	1102 (88.0)
Quit when pregnancy confirmed	90 (7.2)
Current	61 (4.9)
Pre-pregnancy BMI†	
Underweight (<18.5)	26 (2.2)
Normal (18.5–24.9)	708 (60.5)
Overweight (25–29.9)	268 (22.9)
Obese (≥30)	168 (14.4)
Maternal allergy‡	
No	1199 (95.7)
Yes	54 (4.3)
Parity	
Nulliparous	524 (41.9)
Primiparous	511 (40.9)
Multiparous	216 (17.3)
Infant sex	
Male	671 (62.2)
Female	582 (46.5)
Birth weight, g	
≤2500	11 (0.9)
2501–4000	1050 (83.8)
>4000	192 (15.3)

*Subgroup total may not equal 1253 due to missing data.
†World Health Organization Classification (WHO, 2006).
‡Defined as use of maternal allergy medication.

RESULTS

The number of cord blood samples that met the inclusion criteria (term, singleton birth) was 1253. We excluded 171 samples from the NO_2 analysis due to residence beyond 25 km of a NAPs station. Baseline maternal and infant characteristics are summarized in Table 1. Only a small number of participants used allergy medications (4.3%) or smoked during pregnancy (4.9%). Most participants were 30 years of age or over and had a household income of greater than $50,000 (Table 1). Median $PM_{2.5}$ levels for the duration of pregnancy were 8.34 $\mu g/m^3$ with an interquartile range (IQR) of 3.22 $\mu g/m^3$. Median NO_2 levels for the duration of pregnancy were 14.15 ppb (IQR 12.99). Average NO_2 and $PM_{2.5}$ were moderately correlated (Spearman correlation coefficient $r = 0.40$). Correlations among the trimester-specific measures of each pollutant were moderate ($PM_{2.5}$ ranged from 0.59 to 0.62; NO_2 ranged from 0.79 to 0.83). Trimester-specific and average measures were highly correlated for each pollutant (0.82 to 0.86 for $PM_{2.5}$ and 0.88 to 0.95 for NO_2). Effect modification by infant sex was observed in the NO_2 and IL-33/TSLP model (product term $P = 0.01$) and the $PM_{2.5}$ and IgE model (product term $P = 0.05$). Although no effect modification by sex was observed between NO_2 and IgE or $PM_{2.5}$ and IL-33/TSLP (both $P > 0.10$), we present all results stratified by infant sex.

Among boys, no statistically significant associations were observed between NO_2 or $PM_{2.5}$ and any of the immune system biomarkers; odds ratios (ORs) were close to the null value and not suggestive of an inverse or positive relation (Table 2). Among girls, statistically significant associations were observed between the top two quartiles of NO_2 (>14.16 ppb) and elevated IL-33, TSLP, and TSLP/IL-33 (Table 3). Compared with women with NO_2 exposure levels in the lowest quartile, those in the highest quartile (>20.76 ppb) had an elevated odds of high cord blood IL-33/TSLP [OR = 3.42, 95% confidence interval (95% CI): 1.61 to 7.27], TSLP (OR = 3.00; 95% CI: 1.55 to 5.82), and IL-33 concentrations (OR = 3.20; 95% CI: 1.60 to 6.40). One unit (ppb) increases in NO_2 were also statistically significantly associated with IL-33, TSLP, and IL-33/TSLP (OR of elevated IL-33/TSLP per 1 ppb increase in $NO_2 = 1.05$, 95% CI: 1.02 to 1.08). When IL-33 and TSLP were dichotomized at the LOD, the associations with mean NO_2 were attenuated [fourth quartile NO_2: IL-33 OR = 1.25 (95% CI: 0.76 to 2.05); TSLP OR = 1.38 (95% CI: 0.84 to 2.29)]. No statistically significant associations were observed between $PM_{2.5}$ and IL-33 or TSLP whether measured independently or jointly. Elevated (fourth quartile) $PM_{2.5}$ exposure (>9.23 $\mu g/m^3$) was associated with significantly increased odds of IgE concentrations among girls (OR = 2.57, 95% CI: 1.24 to 5.32). Maternal $PM_{2.5}$ exposure, measured as a continuous variable, was positively associated with elevated IgE (OR per 1 $\mu g/m^3$ $PM_{2.5} = 1.14$, 95% CI: 1.00 to 1.31). Inclusion of both pollutants in one model did not produce any notable changes in results.

When stratified by trimester of exposure, results were similar for boys with no significant associations and effect estimates tending to be close to the null value (Table 4). Among girls, the magnitude of the association between NO_2 and IL-33/TSLP was strongest when exposure was measured during the third trimester (third trimester NO_2 >20.76 ppb, OR IL-33/TSLP = 4.39, 95% CI: 2.00 to 9.64). First trimester $PM_{2.5}$ concentrations in the fourth quartile (>9.23 $\mu g/m^3$) were significantly associated with elevated levels of IgE (OR = 2.37, 95% CI: 1.23 to 4.58) among girls. Second trimester third quartile $PM_{2.5}$ exposure was associated with statistically significantly increased odds of high IL-33/TSLP (OR = 2.16, 95% CI: 1.03 to 4.53) among girls (Table 5).

In the cubic spline analysis, the overall association between the NO_2 and high IL33/TSLP among girls was significant ($P = 0.004$). The P value testing the null hypothesis of linearity was 0.09. The spline curve plateaus at approximately the 75%ile (20.76 ppb), though CIs are wide and crossing the null value at this concentration (Fig. 1). The spline curve of the relation between $PM_{2.5}$ and IgE among girls did not have a significant overall association (P overall association = 0.15) and did not significantly deviate from linearity ($P = 0.77$) (Fig. 2).

In the sensitivity analysis, excluding women with a history of maternal allergy or current smokers had no impact on the results (data not shown in tables). In addition, using the LUR model of NO_2 exposure assessment did not materially change the ORs from the mean pregnancy exposure models. Trimester-specific values estimated from the LUR model were highly correlated. As a result, trimester-specific ORs were similar to each other (data not shown).

DISCUSSION

This study was conducted to determine the association between maternal exposure to ambient air pollutants and umbilical cord blood concentrations of IgE, TSLP, and IL-33. There is a body of literature supporting a causal association between childhood exposure to air pollutants and exacerbation of asthma and literature that is suggestive of an association between air pollution and development of asthma[4] as well as recent immunological literature demonstrating the integral role of these immune system biomarkers in the mechanisms underlying childhood allergy.[30]

We observed that maternal NO_2 exposure was associated with significantly increased odds of high cord blood IL-33 and TSLP concentrations among girls. This finding was consistent in analyses

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TABLE 2. Mixed-Level Logistic Regression Analysis of Average Ambient Air Pollutants and Cord Blood Immune System Biomarkers Among Boys, Odds Ratios, and 95% Confidence Intervals ($n = 574$)*

Pollutant†	IgE ≥0.5 ku/L	IL-33 ≥80%ile	TSLP ≥80%ile	IL-33/TSLP ≥80%ile
NO_2, ppb				
≤7.77	1.0	1.0	1.0	1.0
7.78 to ≤14.15	1.26 (0.61–2.62)	0.80 (0.43–1.49)	1.00 (0.54–1.84)	0.91 (0.46–1.80)
14.16 to ≤20.76	0.90 (0.41–1.99)	0.80 (0.44–1.45)	0.93 (0.51–1.69)	0.86 (0.44–1.67)
>20.76	1.10 (0.50–2.43)	0.90 (0.50–1.64)	0.71 (0.38–1.34)	0.95 (0.49–1.86)
1	1.00 (0.96–1.04)	1.01 (0.98–1.04)	0.99 (0.96–1.02)	1.01 (0.98–1.04)
$PM_{2.5}$, $\mu g/m^3$				
≤6.01	1.0	1.0	1.0	1.0
6.02 to ≤8.34	1.44 (0.74–2.79)	0.86 (0.48–1.55)	0.91 (0.51–1.63)	0.90 (0.48–1.69)
8.44 to ≤9.23	0.81 (0.36–1.80)	1.58 (0.90–2.77)	1.41 (0.80–2.48)	1.53 (0.83–2.81)
>9.23	0.72 (0.33–1.60)	1.32 (0.75–2.32)	0.99 (0.55–1.76)	0.94 (0.50–1.78)
1	0.95 (0.81–1.10)	1.09 (0.98–1.21)	1.03 (0.92–1.14)	1.02 (0.91–1.15)

IgE, immunoglobulin E; IL, interleukin; TSLP, thymic stromal lymphopoietin.
*Adjusted for maternal age, household income (center and FSA are random effects).
†Pollutant concentrations are average exposure levels throughout pregnancy.

of categorical and continuous exposure variables and persisted whether IL-33 and TSLP were analyzed individually or jointly. This association was not, however, observed in analyses wherein TSLP and IL-33 were dichotomized at the LOD. In light of literature suggesting that IL-33 and TSLP are cross-regulated and that IL-33 can induce TSLP production, our finding of an association between NO_2 and two cytokines together is not surprising.[25] When stratified by trimester of exposure, this association was most pronounced during the third trimester. Given the wide CIs in the NO_2 effect estimates as well as the novelty of these findings, replication in further studies is warranted.

As this study is the first of its kind, it was not feasible to conduct direct comparisons with previous epidemiological studies. However, the present findings are consistent with results regarding relations among NO_2, epithelial cells, and TSLP. NO_2 exposure may induce epithelial cell injury[29] and promote release of pro-inflammatory mediators.[28] Furthermore, diesel exhaust, which may contain NO_2, has been shown to upregulate epithelial cell production of TSLP.[50]

The present findings are also consistent with literature from animal models reporting that in utero exposure to pollutants, including diesel and particulate matter, promotes susceptibility to asthma and allergic sensitization[51–53] and induces the release of pro-inflammatory mediators, such as IL-8, from lung tissue.[54] Maternal air pollution exposure may also stimulate placental production of pro-inflammatory cytokines and subsequently influence fetal immune system development.[55] We therefore speculate that the observed association between NO_2 and IL-33 and TSLP is mediated by air pollution induced inflammatory responses in maternal airway tissue. These maternal responses, which can include release of pro-inflammatory mediators, may promote fetal epithelial cell cytokine production. Given the stronger magnitude of association in the third trimester, it is possible that later gestational NO_2 exposure is the critical window for influencing TSLP and IL-33 concentrations. Experimental evidence has suggested that third trimester exposure to the heavy metal lead may be more likely to promote Th2 cytokine response than exposure earlier in pregnancy.[56] However, authors of a birth cohort study in the Czech Republic reported that air pollution ($PM_{2.5}$ and polycyclic aromatic hydrocarbons) exposure during the first trimester was associated with increases in T lymphocytes that may promote autoimmune responses.[22] Scientific understanding regarding the critical window of gestational exposure for each pollutant and specific immunological endpoint is limited. Replication of the present findings regarding third trimester NO_2 exposure and pro-inflammatory cytokine responses will help address this knowledge gap.

TABLE 3. Mixed-Level Logistic Regression Analysis of Average Ambient Air Pollutants and Cord Blood Immune System Biomarkers Among Girls, Odds Ratios, and 95% Confidence Intervals ($n = 507$)*

Pollutant†	IgE ≥0.5 ku/L	IL-33 ≥80%ile	TSLP ≥80%ile	IL-33/TSLP ≥80%ile
NO_2, ppb				
≤7.77	1.0	1.0	1.0	1.0
7.78 to ≤14.15	1.28 (0.63–2.61)	2.31 (1.14–4.65)	2.25 (1.16–4.38)	1.91 (0.86–4.21)
14.16 to ≤20.76	1.93 (0.93–3.99)	2.51 (1.19–5.28)	2.87 (1.43–5.76)	2.77 (1.24–6.20)
>20.76	1.02 (0.47–2.17)	3.20 (1.60–6.40)	3.00 (1.55–5.82)	3.42 (1.61–7.27)
1	1.01 (0.98–1.04)	1.04 (1.04–1.08)	1.04 (1.01–1.07)	1.05 (1.02–1.08)
$PM_{2.5}$, $\mu g/m^3$				
≤6.01	1.0	1.0	1.0	1.0
6.02 to ≤8.43	1.97 (0.93–4.20)	1.26 (0.61–2.60)	1.52 (0.78–2.97)	1.35 (0.64–2.82)
8.44 to ≤9.23	1.37 (0.64–2.92)	1.53 (0.79–2.98)	1.44 (0.75–2.78)	1.44 (0.70–2.95)
>9.23	2.57 (1.24–5.32)	1.25 (0.57–2.70)	1.14 (0.55–2.37)	1.04 (0.47–2.31)
1	1.14 (1.00–1.31)	1.07 (0.93–1.23)	1.03 (0.89–1.18)	1.02 (0.88–1.18)

IgE, immunoglobulin E; IL, interleukin; TSLP, thymic stromal lymphopoietin.
*Adjusted for maternal age, household income (center and FSA are random effects).
†Pollutant concentrations are average exposure levels throughout pregnancy.

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TABLE 4. Mixed-Level Logistic Regression Analysis of Quartiles of Ambient Air Pollutants by Trimester of Exposure and Cord Blood Immune System Biomarkers Among Boys, Odds Ratio, and 95% Confidence Intervals*

Pollutant	*N* (%)	IgE ≥0.5 ku/L	IL-33/TSLP ≥80%ile
NO_2, ppb			
Trimester 1			
≤7.77	81 (15.5)	1.0	1.0
7.78 to ≤14.15	142 (27.2)	0.95 (0.42–2.20)	1.08 (0.52–2.26)
14.16 to ≤20.76	133 (25.4)	1.13 (0.47–2.69)	0.82 (0.38–1.78)
>20.76	167 (31.9)	1.31 (0.57–3.03)	0.91 (0.43–1.91)
Trimester 2			
≤7.77	90 (16.6)	1.0	1.0
7.78 to ≤14.15	138 (25.5)	1.12 (0.53–2.36)	1.17 (0.56–2.45)
14.16 to ≤20.76	183 (33.8)	0.67 (0.30–1.47)	0.70 (0.33–1.49)
>20.76	131 (24.2)	0.54 (0.22–1.30)	1.18 (0.56–2.52)
Trimester 3			
≤7.77	125 (22.2)	1.0	1.0
7.78 to ≤14.15	135 (24.0)	1.30 (0.64–2.64)	0.63 (0.30–1.31)
14.16 to ≤20.76	174 (31.0)	0.59 (0.26–1.35)	1.05 (0.55–2.00)
>20.76	128 (22.8)	0.79 (0.33–1.89)	0.99 (0.50–1.97)
$PM_{2.5}$, $\mu g/m^3$			
Trimester 1			
≤6.01	178 (26.5)	1.0	1.0
6.02 to ≤8.43	203 (30.3)	1.09 (0.59–2.02)	0.79 (0.44–1.41)
8.44 to ≤9.23	115 (17.1)	1.50 (0.72–3.13)	0.93 (0.48–1.79)
>9.23	175 (26.1)	0.93 (0.46–1.89)	0.98 (0.55–1.75)
Trimester 2			
≤6.01	193 (28.8)	1.0	1.0
6.02 to ≤8.43	167 (24.9)	1.21 (0.64–2.28)	0.79 (0.43–1.47)
8.44 to ≤9.23	124 (18.5)	0.97 (0.46–2.06)	1.08 (0.58–2.03)
>9.23	187 (27.9)	0.60 (0.29–1.27)	1.19 (0.67–2.09)
Trimester 3			
≤6.01	194 (28.9)	1.0	1.0
6.02 to ≤8.43	198 (29.5)	1.31 (0.72–2.40)	1.75 (0.99–3.09)
8.44 to ≤9.23	123 (18.3)	0.81 (0.38–1.73)	1.00 (0.50–2.02)
>9.23	156 (23.2)	1.05 (0.52–2.09)	1.34 (0.72–2.48)

IgE, immunoglobulin E; IL, interleukin; TSLP, thymic stromal lymphopoietin.
*Adjusted for maternal age and income (center and FSA are random effects).

We hypothesize that it is unlikely that the cytokines measured in the present study are produced by maternal epithelial cells. IL-33 is a member of the IL-1 family and previous research has reported that IL-1α and IL-1β do not readily cross the placenta.[57,58] TSLP has been shown to be produced by trophoblasts during early gestation.[59] However, it is unlikely that maternal TSLP crosses the placenta due to its large molecular weight, which is of comparable size to IL-1, and the absence of known active transport processes.

The observed sex-dependent nature of the association between NO_2, IL-33, and TSLP is also consistent with previous research. Studies examining relations between traffic-related pollutants, including NO_2, and childhood asthma have shown stronger associations among girls than boys.[13,60,61] This potential early life air pollution susceptibility among girls is of particular interest, as boys tend to have a higher prevalence of asthma at younger ages.[62] The mechanism underlying these observed sex-dependent differences and susceptibilities at birth warrants further investigation.

Our finding of a stronger, more consistent association with NO_2 than $PM_{2.5}$ may be attributable to the fact that the NO_2 exposure estimates are an approximation of traffic-related air pollution exposure, whereas the satellite-derived PM2.5 estimates are reflective of both anthropogenic and biogenic sources of $PM_{2.5}$. Traffic-related air pollution and correlated exposures,[63,64] rather than NO_2, therefore may be the underlying causal agent. These correlated exposures include diesel exhaust, polycyclic aromatic hydrocarbons, and certain volatile organic compounds (eg, benzene, toluene, ethylbenzene, and xylene), all of which have been linked to related adverse respiratory and allergic outcomes.[65,66] Literature regarding $PM_{2.5}$ and NO_2 exposure has not demonstrated consistent results regarding the relative contributions of these pollutants to childhood allergic disease outcomes. Authors of a prospective birth cohort study in Germany reported that NO_2 but not $PM_{2.5}$ was associated with childhood eczema, whereas $PM_{2.5}$ but not NO_2 was associated with asthmatic bronchitis.[10] Authors of another cohort study reported that the association between early life air pollution exposure and certain types of asthma was stronger with NO_2 than $PM_{2.5}$.[13]

We observed statistically significant associations between $PM_{2.5}$ exposure, particularly during the first trimester, and IgE among girls. One identified birth cohort study from the Czech Republic reported a statistically significant positive association between $PM_{2.5}$ exposure during the sixth month of gestation and cord blood IgE; exposure during all other time periods was of a null or inverse relation.[20] Herr et al[20] examined effect estimates of elevated IgE per 25 $\mu g/m^3$ increase in $PM_{2.5}$, which reflects a notably higher ambient concentration than measured in the MIREC cohort (median = 8.34; maximum = 11.57 $\mu g/m^3$). It is possible that ambient $PM_{2.5}$ concentrations in the Czech Republic study were sufficiently high to suppress first trimester β-cell production of IgE, whereas the lower concentrations observed in the present study did not result in a similar immunologic response. Replication of the present finding is necessary to determine whether the observed associations between $PM_{2.5}$ and IgE among girls are a result of chance, confounding, or reflective of a true association.

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TABLE 5. Mixed-Level Logistic Regression Analysis of Air Pollutants by Trimester of Exposure and Cord Blood Immune System Biomarkers Among Girls, Odds Ratios, and 95% Confidence Intervals*

Pollutant	*N* (%)	IgE ≥0.5 ku/L	IL-33/TSLP ≥80%ile
NO_2, ppb			
Trimester 1			
≤7.77	118 (24.9)	1.0	1.0
0.78 to ≤14.15	133 (28.1)	1.81 (0.80–4.07)	0.82 (0.35–1.95)
14.16 to ≤20.76	102 (21.6)	2.39 (1.03–5.55)	1.36 (0.56–3.29)
>20.76	120 (25.4)	1.67 (0.72–3.86)	1.89 (0.84–4.27)
Trimester 2			
≤7.77	118 (24.3)	1.0	1.0
7.78 to ≤14.15	145 (29.9)	1.56 (0.69–3.48)	1.27 (0.58–2.76)
14.16 to ≤20.76	105 (21.6)	3.37 (1.49–7.62)	2.45 (1.13–5.32)
>20.76	117 (24.1)	1.13 (0.47–2.74)	1.90 (0.88–4.08)
Trimester 3			
≤7.77	146 (29.4)	1.0	1.0
7.78 to ≤14.15	136 (27.4)	1.45 (0.71–2.97)	2.41 (1.08–5.37)
14.16 to ≤20.76	113 (22.8)	1.43 (0.67–3.05)	2.71 (1.19–6.16)
>20.76	131 (20.4)	1.63 (0.77–3.47)	4.39 (2.00–9.64)
$PM_{2.5}$, μg/m^3			
Trimester 1			
≤6.01	169 (29.1)	1.0	1.0
6.02 to ≤8.43	158 (27.2)	1.47 (0.72–2.96)	0.93 (0.45–1.90)
8.44 to ≤9.23	98 (16.9)	1.26 (0.55–2.86)	0.81 (0.35–1.85)
>9.23	155 (26.7)	2.37 (1.23–4.58)	1.34 (0.66–2.70)
Trimester 2			
≤6.01	148 (25.5)	1.0	1.0
6.02 to ≤8.43	166 (28.6)	1.84 (0.89–3.78)	1.59 (0.79–3.19)
8.44 to ≤9.23	113 (19.5)	1.75 (0.80–3.85)	2.16 (1.03–4.53)
>9.23	153 (26.4)	1.92 (0.92–4.00)	1.10 (0.51–2.36)
Trimester 3			
≤6.01	170 (29.3)	1.0	1.0
6.02 to ≤8.43	166 (28.6)	1.05 (0.53–2.08)	1.04 (0.52–2.09)
8.44 to ≤9.23	123 (21.2)	1.52 (0.76–3.03)	1.72 (0.85–3.48)
>9.23	121 (20.9)	1.60 (0.80–3.18)	1.27 (0.62–2.61)

IgE, immunoglobulin E; IL, interleukin; TSLP, thymic stromal lymphopoietin.
*Adjusted for maternal age and income (center and FSA are random effects).

Strengths of this study included the relatively large sample size in the MIREC study, the inclusion of many potential confounding factors, and the availability of novel immune system biomarkers. The use of spatiotemporal models for NO_2 and $PM_{2.5}$

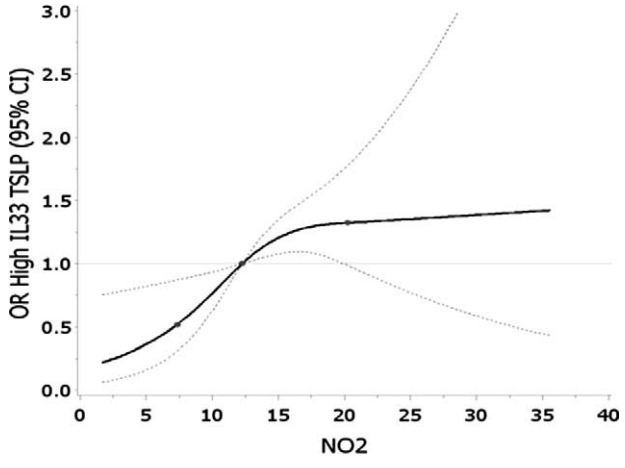

FIGURE 1. Restricted cubic spline analysis of average NO_2 (ppb) and elevated IL-33 and TSLP among girls (≥80th%ile). Adjusted for maternal age and income. Knots set at 25th, 50th, and 75th percentiles. Error bars indicate 95% confidence intervals.

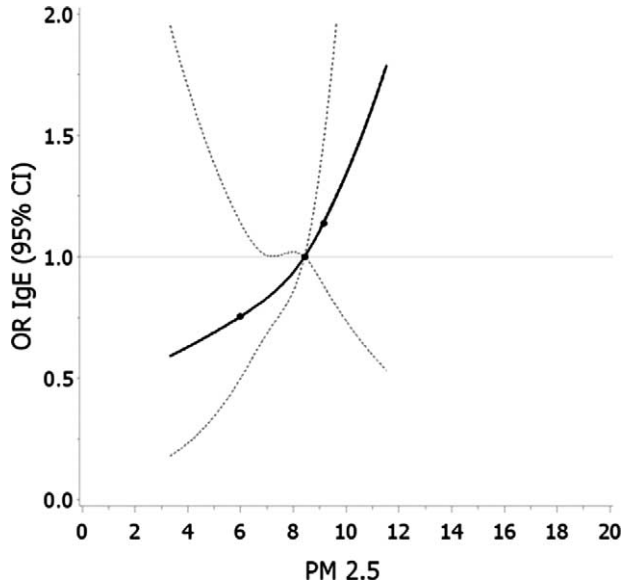

FIGURE 2. Restricted cubic spline analysis of average $PM_{2.5}$ (μg/m^3) and elevated IgE (≥0.5 ku/L) among girls. Adjusted for maternal age and income. Knots set at 25th, 50th, and 75th percentiles. Error bars indicate 95% confidence intervals.

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enabled us to develop average exposure measures over the time span of a pregnancy that captured both detailed spatial and temporal variations in pollutant concentrations.

A primary limitation of our study is the potential exposure misclassification of the air pollutants resulting from the use of three-digit postal codes rather than full residential address. We did not have information on work location, time spent at home, or indoor pollutants. Further, the use of different exposure estimation methods for the two pollutants precluded direct comparison of results for the two pollutants. A second limitation is the lack of clinical outcome data. Although IL-33 and TSLP have been implicated in allergic disease,[31] literature on the longitudinal relation from birth to childhood is lacking. Thus, it is not possible to draw definitive conclusions about the risk of developing childhood allergic disease on the basis of these findings. Determining the impacts of the observed results on childhood allergic disease requires further follow-up of the MIREC cohort. Third, considering that high NO_2 concentrations are found in regions of high population density, it is possible that the observed results are subject to residual confounding due to co-occurring pollutants or characteristics unique to this subpopulation.

CONCLUSIONS

We report a positive, statistically significant association between maternal NO_2 exposure and elevated ($\geq$80th percentile) cord blood concentrations of the epithelial cell derived cytokines TSLP and IL-33. We also observed a positive association between elevated overall average and first trimester $PM_{2.5}$ exposure ($>9.23\ \mu g/m^3$) and high IgE among girls (first trimester exposure OR = 2.37, 95% CI: 1.23 to 4.58), although these associations were not statistically significant in spine analysis. These findings suggest that maternal NO_2 exposure may impact newborn immune system development as measured by cord blood concentrations of two cytokines. Replication of these observed findings, particularly in a cohort of differing socioeconomic and ethnic background than the MIREC population, will help clarify the public health implications of low-level in utero NO_2 exposure. Studies that further elucidate the observed dose–response relationships, the role of timing of exposure, and the physiologic mechanisms will be a valuable contribution to understanding early life influences on immune system function, potential subsequent risk of allergic disease, and differential responses by child sex.

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